" Acid Base Balance"

Acid-base balance

Blood hydrogen ion concentration lies within the range pH 7.36-7.44. The terms acidosis and alkalosis in clinical practice indicate a change or a tendency to a change in the pH of the blood in a particular direction. In acidosis, there is an accumulation of acid or a loss of a base causing a fall or a tendency to a fall in the pH. The converse occurs in alkalosis.

Buffering systems

The pH of the blood is regulated and controlled by various buffering systems essentially consisting of:

1- Weak acids and bases, of which the most important is the bicarbonate:carbonic acid ratio HCO_3:H2CO_3.

2- The removal of carbon dioxide by the lungs and

3- The excretion of both acids and bases by the kidneys.

The ratio of bicarbonate to carbonic acid is normally 20:1. Alteration in this ratio alters the pH. A decrease in the ratio leads to increased acidity and vice versa.

The bicarbonate level can be altered by metabolic factors, while the carbonic acid level is subject to alteration by respiratory factors. Alteration of one is followed automatically by a compensatory alteration in the other, so that the ratio (HCO_3:H_2CO_3) and therefore the pH of the blood remains constant.

Terms and normal values

PCO_2 is a measurement of the tension or partial pressure of carbon dioxide in the blood. The normal arterial PCO_2 is 31-42 mmHg. PO_2 is a measurement of the tension or partial pressure of oxygen in the blood. The normal arterial PO_2 is 80-110 mmHg.

Standard bicarbonate is the concentration of the serum bicarbonate .Normal levels are 22-25 mmol/litre.

Alkalosis

Metabolic alkalosis

Metabolic alkalosis, a condition of base excess or a deficit of any acid other than H_2CO_3, can be caused by:

1) Excessive ingestion of absorbable alkali. This is common in patients who take proprietary indigestion remedies without medical supervision;

2) Loss of acid from the stomach by repeated vomiting or aspiration;

3) Cortisone excess, usually the result of over-administration of adrenal corticoids, but occasionally due to Cushing's syndrome.

Compensation is effected by:

(a) Retention of carbon dioxide by the lungs; and

(b) Excretion of bicarbonate base by the kidneys (alkaline urine).

Clinical features:

Alkalosis due to loss of acid from the stomach is the most common and most important. In its most typical form, it is seen in patients with pyloric stenosis in whom the loss of acid by repeated vomiting is often increased by the taking of medicines containing sodium bicarbonate. The most striking feature of severe alkalosis is apnea lasting from 5 to 30 seconds. Subclinical degrees of alkalosis are recognizable only by a raised standard bicarbonate concentration. Severe alkalosis may result in renal epithelial damage and consequent renal insufficiency.

Treatment:

Metabolic alkalosis without hypokalaemia seldom requires direct treatment. The cause of the alkalosis should be removed where possible and a high urinary output encouraged.

Hypokalaemic alkalosis

Hypokalaemic alkalosis is seen in patients who have lost potassium and acid owing to repeated vomiting from pyloric stenosis. The low serum potassium causes potassium to leave the cell and be replaced by Na^+ and H^+ ions. The shift of H^+ ion into the cell causes intracellular acidosis and increases the cellular acidosis of the kidney cells.

Treatment: When hypokalaemia is sufficient to cause a metabolic alkalosis, the losses can be massive (> 1000 mmol). Replacement can be achieved gradually and relatively safely by supplementing intravenous fluids with 40 mmol/litre of KCl if the urine output is adequate.

Respiratory alkalosis

Respiratory alkalosis, a condition where the arterial PCO_2 is below the normal range of 31-42 mmHg, is caused most commonly in surgical practice by excessive pulmonary ventilation carried out upon an anesthetised patient. Other causes are hyperventilation, hyperpyrexia. Compensation, which depends on increased renal excretion of bicarbonate, usually is inadequate. During anaesthesia alkalosis is accompanied by pallor and a fall in blood pressure. In severe cases respiratory arrest follows.

Treatment: Respiratory suppression due to alkalosis is treated by insufflation of carbon dioxide.

Acidosis

Metabolic acidosis

Metabolic acidosis, a condition where there is a deficit of base or an excess of any acid other than H_2CO_3, occurs as a result of:

1-Increase in fixed acids due to the formation of ketone bodies as in diabetes or starvation, the retention of metabolites in renal insufficiency, and the rapid increase of lactic and pyruvic acids by anaerobic tissue metabolism, following cardiac arrest.

2-Loss of bases such as occurs in sustained diarrhea, ulcerative colitis, gastrocolic fistula, a high intestinal fistula or prolonged intestinal aspiration.

Clinical features:

In severe acidosis, the leading sign is rapid, deep, noisy breathing. The hyperpnea is due to over stimulation of the respiratory center by the reduction in pH of the blood, and the physiological purpose of overbreathing is to eliminate as much as possible of the acid substance H_2CO_3.The urine is strongly acidic.

Treatment: The commonest cause of an acute peroperative metabolic acidosis is tissue hypoxia and the correct treatment is restoration of adequate tissue perfusion. Treatment with bicarbonate solutions will correct the measured metabolic acidosis but not treat the problem. Indeed, as bicarbonate is rapidly converted into carbon dioxide intracellular acidosis may, in fact, get worse. The acute acidosis seen in prolonged cardiac arrest may require the infusion of 50 mmol of 8.4 per cent sodium bicarbonate solution.

Respiratory acidosis

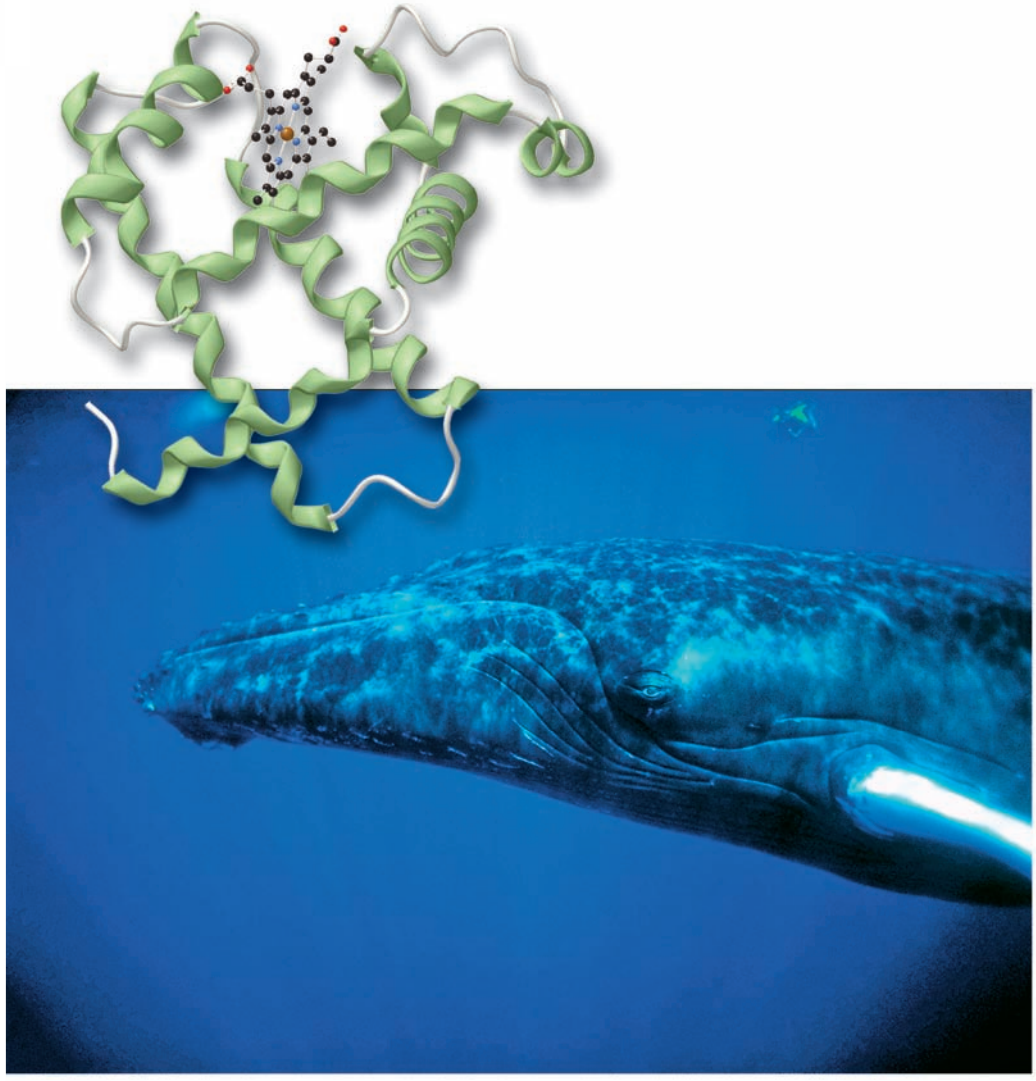
Respiratory acidosis, a condition where the PCO_2 is above the normal range, is caused by impaired alveolar ventilation. This problem most commonly occurs when there is inadequate ventilation of the anesthetised patient. There is also a risk of respiratory acidosis when the patient undergoing surgery already has pre-existing pulmonary disease (e.g. chronic bronchitis or emphysema).

The anion gap

The anion gap measures the difference or gap between the negatively and positively charged electrolytes in the blood. If the anion gap is too high, the blood is more acidic than normal. If the anion gap is too low, the blood is not acidic enough. Therefore, it is used to establish the cause of a

metabolic acidosis. Anion gap = (Na + K) ¬ (HCO3 + Cl). The normal anion gap is 10-16 mmol/litre. An increased anion gap acidosis is seen in metabolic acidosis due to ketoacidosis, lactic acidosis, poisoning (salicylates) and renal failure. A normal anion gap acidosis is seen in gastrointestinal or renal bicarbonate losses.

28 Amino Acids and Proteins



Myoglobin is a globular protein that contains 153 amino acids joined together, as well as a non-protein portion called a heme unit. The heme group consists of a large nitrogen heterocycle complexed with the Fe^{2+} cation. The Fe^{2+} cation binds oxygen in the blood and stores it in tissues. Whales have a particularly high myoglobin concentration in their muscles. It serves as an oxygen reservoir for the whale while it is submerged for long periods of time. In Chapter 28, we discuss the properties of proteins and the amino acids from which they are synthesized.

Of the four major groups of biomolecules—lipids, carbohydrates, nucleic acids, and proteins—proteins have the widest array of functions. **Keratin** and **collagen,** for example, are part of a large group of structural proteins that form long insoluble fibers, giving strength and support to tissues. Hair, horns, hooves, and fingernails are all made up of keratin. **Collagen** is found in bone, connective tissue, tendons, and cartilage. **Enzymes** are proteins that catalyze and regulate all aspects of cellular function. **Membrane proteins** transport small organic molecules and ions across cell membranes. **Insulin,** the hormone that regulates blood glucose levels, **fibrinogen** and **thrombin,** which form blood clots, and **hemoglobin,** which transports oxygen from the lungs to tissues, are all proteins.

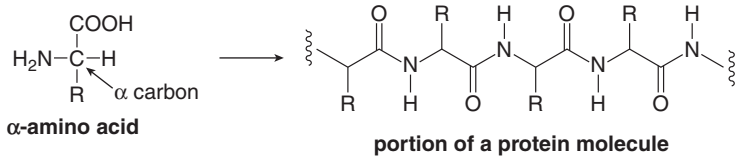
In Chapter 28 we discuss proteins and their primary components, the amino acids.

28.1 Amino Acids

Amino acids were first discussed in Section 19.14.

Naturally occurring amino acids have an amino group (NH_2) bonded to the α carbon of a carboxy group (COOH), and so they are called **α-amino acids.**

- **All proteins are polyamides formed by joining amino acids together.**



28.1A General Features of α-Amino Acids

The 20 amino acids that occur naturally in proteins differ in the identity of the R group bonded to the α carbon. The R group is called the **side chain** of the amino acid.

The simplest amino acid, called glycine, has R = H. **All other amino acids (R ≠ H) have a stereogenic center on the α carbon.** As is true for monosaccharides, the prefixes **D** and **L** are used to designate the configuration at the stereogenic center of amino acids. Common, naturally occurring amino acids are called **L-amino acids.** Their enantiomers, D-amino acids, are rarely found in nature. These general structures are shown in Figure 28.1. According to *R,S* designations, all L-amino acids except cysteine have the ***S* configuration.**

All amino acids have common names. These names can be represented by either a one-letter or a three-letter abbreviation. Figure 28.2 is a listing of the 20 naturally occurring amino acids, together with their abbreviations. Note the variability in the R groups. A side chain can be a simple alkyl group, or it can have additional functional groups such as OH, SH, COOH, or NH_2.

- **Amino acids with an additional COOH group in the side chain are called acidic amino acids.**
- **Those with an additional basic N atom in the side chain are called basic amino acids.**
- **All others are neutral amino acids.**

Figure 28.1
The general features of an α-amino acid

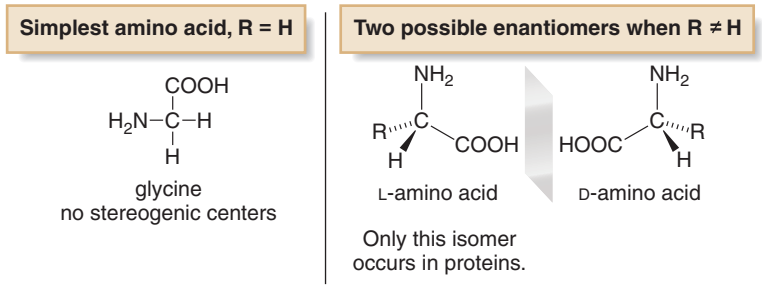
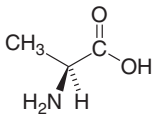
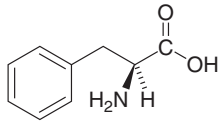
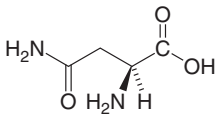
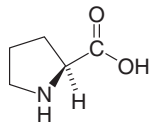
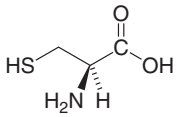
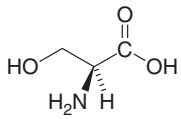
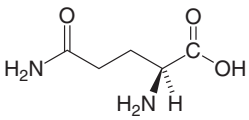
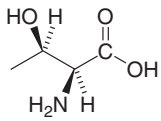
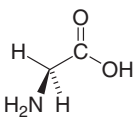
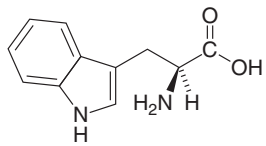
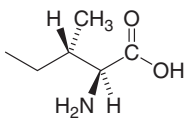
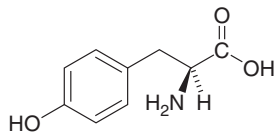
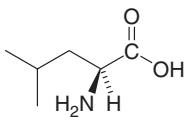
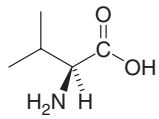
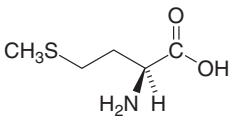
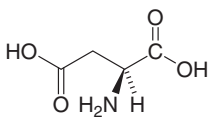
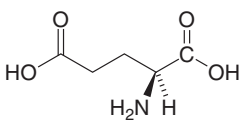


Figure 28.2 The 20 naturally occurring amino acids

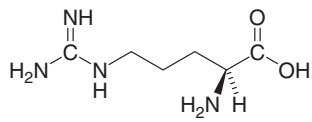
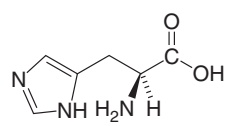
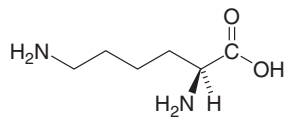
Neutral amino acids

Name	Structure	Abbreviations	Name	Structure	Abbreviations
Alanine		Ala A	Phenylalanine*		Phe F
Asparagine		Asn N	Proline		Pro P
Cysteine		Cys C	Serine		Ser S
Glutamine		Gln Q	Threonine*		Thr T
Glycine		Gly G	Tryptophan*		Trp W
Isoleucine*		Ile I	Tyrosine		Tyr Y
Leucine*		Leu L	Valine*		Val V
Methionine*		Met M			

Acidic amino acids

Name	Structure	Abbreviations
Aspartic acid		Asp D
Glutamic acid		Glu E

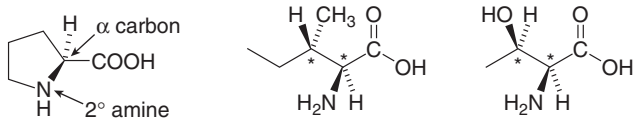
Basic amino acids

Name	Structure	Abbreviations
Arginine*		Arg R
Histidine*		His H
Lysine*		Lys K

Essential amino acids are labeled with an asterisk (*).

Look closely at the structures of proline, isoleucine, and threonine.

- **All amino acids are 1° amines except for proline,** which has its N atom in a five-membered ring, making it a **2° amine.**
- **Isoleucine** and **threonine** contain an additional stereogenic center at the β carbon, so there are four possible stereoisomers, only one of which is naturally occurring.

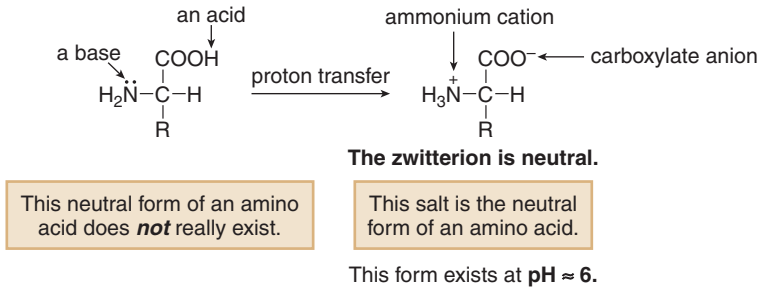


Humans can synthesize only 10 of these 20 amino acids. The remaining 10 are called **essential amino acids** because they must be obtained from the diet. These are labeled with an asterisk in Figure 28.2.

Problem 28.1 Draw the other three stereoisomers of L-isoleucine, and label the stereogenic centers as *R* or *S*.

28.1B Acid–Base Behavior

Recall from Section 19.14B that an amino acid has both an acidic and a basic functional group, so proton transfer forms a salt called a **zwitterion.**



- **Amino acids do not exist to any appreciable extent as uncharged neutral compounds. They exist as salts, giving them high melting points and making them water soluble.**

Amino acids exist in different charged forms, as shown in Figure 28.3, depending on the pH of the aqueous solution in which they are dissolved. For neutral amino acids, the overall charge is +1, 0, or –1. Only at pH ~6 does the zwitterionic form exist.

The $-COOH$ and $-NH_3^+$ groups of an amino acid are ionizable, because they can lose a proton in aqueous solution. As a result, they have different pK_a values. The pK_a of the $-COOH$ group is typically ~2, whereas that of the $-NH_3^+$ group is ~9, as shown in Table 28.1.

Some amino acids, such as aspartic acid and lysine, have acidic or basic side chains. These additional ionizable groups complicate somewhat the acid–base behavior of these amino acids. Table 28.1 lists the pK_a values for these acidic and basic side chains as well.

Figure 28.3
How the charge of a neutral amino acid depends on the pH

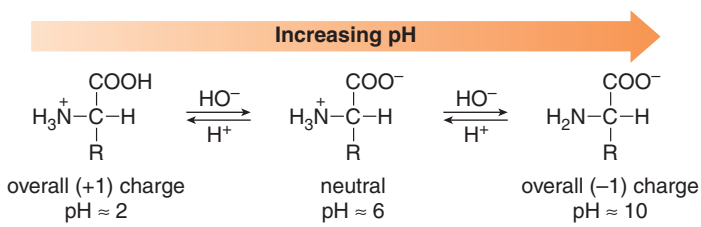


Table 28.1 pK_a Values for the Ionizable Functional Groups of an α-Amino Acid

Amino acid	α-COOH	α-NH_3^+	Side chain	p*I*
Alanine	2.35	9.87	—	6.11
Arginine	2.01	9.04	12.48	10.76
Asparagine	2.02	8.80	—	5.41
Aspartic acid	2.10	9.82	3.86	2.98
Cysteine	2.05	10.25	8.00	5.02
Glutamic acid	2.10	9.47	4.07	3.08
Glutamine	2.17	9.13	—	5.65
Glycine	2.35	9.78	—	6.06
Histidine	1.77	9.18	6.10	7.64
Isoleucine	2.32	9.76	—	6.04
Leucine	2.33	9.74	—	6.04
Lysine	2.18	8.95	10.53	9.74
Methionine	2.28	9.21	—	5.74
Phenylalanine	2.58	9.24	—	5.91
Proline	2.00	10.00	—	6.30
Serine	2.21	9.15	—	5.68
Threonine	2.09	9.10	—	5.60
Tryptophan	2.38	9.39	—	5.88
Tyrosine	2.20	9.11	10.07	5.63
Valine	2.29	9.72	—	6.00

Table 28.1 also lists the isoelectric points (p*I*) for all of the amino acids. Recall from Section 19.14C that the **isoelectric point is the pH at which an amino acid exists primarily in its neutral form,** and that it can be calculated from the average of the pK_a values of the α-COOH and α-NH_3^+ groups (for neutral amino acids only).

Problem 28.2 What form exists at the isoelectric point of each of the following amino acids: (a) valine; (b) leucine; (c) proline; (d) glutamic acid?

Problem 28.3 Explain why the pK_a of the $-NH_3^+$ group of an α-amino acid is lower than the pK_a of the ammonium ion derived from a 1° amine (RNH_3^+). For example the pK_a of the $-NH_3^+$ group of alanine is 9.7 but the pK_a of $CH_3NH_3^+$ is 10.63.

28.2 Synthesis of Amino Acids

Amino acids can be prepared in a variety of ways in the laboratory. Three methods are described, each of which is based on reactions learned in previous chapters.

28.2A S_N2 Reaction of α-Halo Acids with NH_3

The most direct way to synthesize an α-amino acid is by **S_N2 reaction of an α-halo carboxylic acid with a large excess of NH_3.**

General reaction

$$\text{R}-\underset{\text{Br}}{\text{CH}}\text{COOH} \xrightarrow[\text{(large excess)}]{\text{NH}_3} \text{R}-\underset{\text{NH}_2}{\text{CH}}\text{COO}^-\text{NH}_4^+ + \text{NH}_4^+\text{Br}^-$$

Example

$$(\text{CH}_3)_2\text{CH}-\underset{\text{Br}}{\text{CH}}\text{COOH} \xrightarrow[\text{(large excess)}]{\text{NH}_3} (\text{CH}_3)_2\text{CH}-\underset{\text{NH}_2}{\text{CH}}\text{COO}^-\text{NH}_4^+ + \text{NH}_4^+\text{Br}^-$$

S_N2

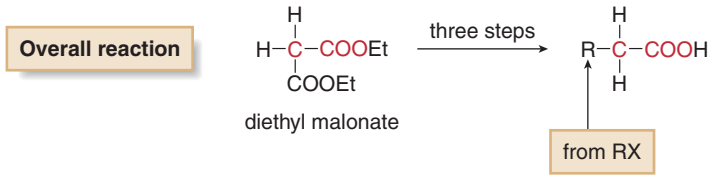
valine

Although the alkylation of ammonia with simple alkyl halides does not generally afford high yields of 1° amines (Section 25.7A), this reaction using α-halo carboxylic acids does form the desired amino acids in good yields. In this case, the amino group in the product is both less basic and more sterically crowded than other 1° amines, so that a single alkylation occurs and the desired amino acid is obtained.

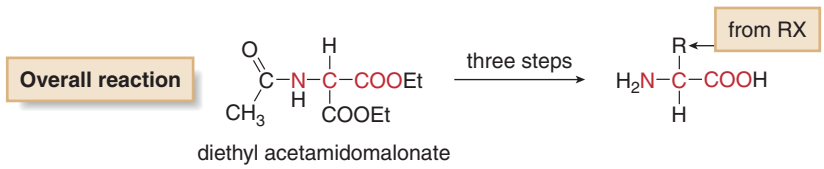
Problem 28.4 What α-halo carbonyl compound is needed to synthesize each amino acid: (a) glycine; (b) isoleucine; (c) phenylalanine?

28.2B Alkylation of a Diethyl Malonate Derivative

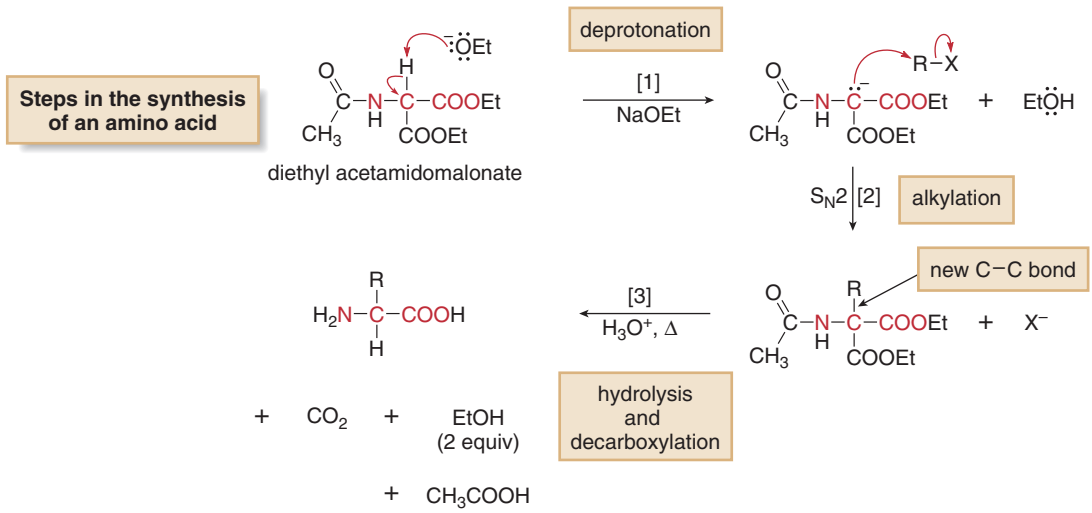
The second method for preparing amino acids is based on the malonic ester synthesis. Recall from Section 23.9 that this synthesis converts diethyl malonate to a carboxylic acid with a new alkyl group on its α carbon atom.



This reaction can be adapted to the synthesis of α-amino acids by using a commercially available derivative of diethyl malonate as starting material. This compound, **diethyl acetamidomalonate,** has a nitrogen atom on the α carbon, which ultimately becomes the NH_2 group on the α carbon of the amino acid.



The malonic ester synthesis consists of three steps, and so does this variation to prepare an amino acid.



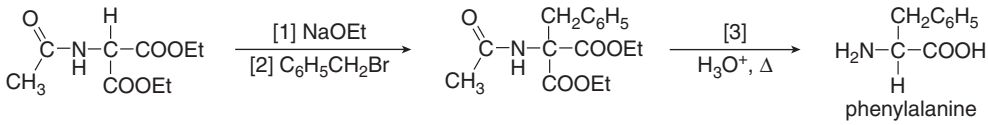
[1] **Deprotonation** of diethyl acetamidomalonate with NaOEt forms an enolate by removal of the acidic proton between the two carbonyl groups.

[2] **Alkylation** of the enolate with an unhindered alkyl halide (usually CH_3X or RCH_2X) forms a substitution product with a new R group on the α carbon.

[3] Heating the alkylation product with aqueous acid results in **hydrolysis** of both esters and the amide, followed by **decarboxylation** to form the amino acid.

Phenylalanine, for example, can be synthesized as follows:

Example



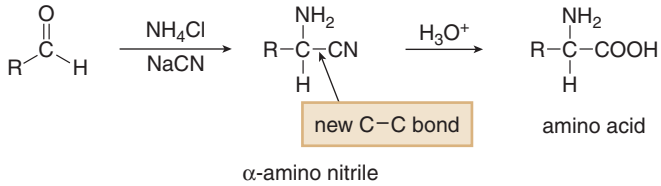
Problem 28.5 The enolate derived from diethyl acetamidomalonate is treated with each of the following alkyl halides. After hydrolysis and decarboxylation, what amino acid is formed: (a) CH_3I; (b) $(CH_3)_2CHCH_2Cl$; (c) $CH_3CH_2CH(CH_3)Br$?

Problem 28.6 What amino acid is formed when $CH_3CONHCH(CO_2Et)_2$ is treated with the following series of reagents: [1] NaOEt; [2] $CH_2=O$; [3] H_3O^+, Δ?

28.2C Strecker Synthesis

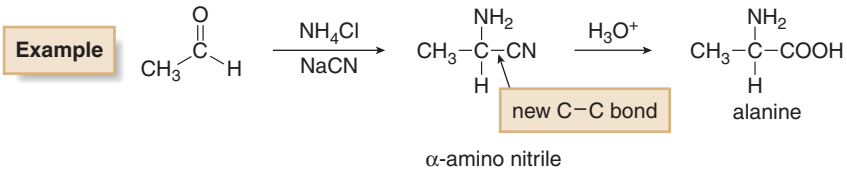
The third method, the **Strecker amino acid synthesis,** converts an aldehyde into an amino acid by a two-step sequence that adds one carbon atom to the aldehyde carbonyl. Treating an aldehyde with NH_4Cl and NaCN first forms an **α-amino nitrile,** which can then be hydrolyzed in aqueous acid to an amino acid.

Strecker synthesis



The Strecker synthesis of alanine, for example, is as follows:

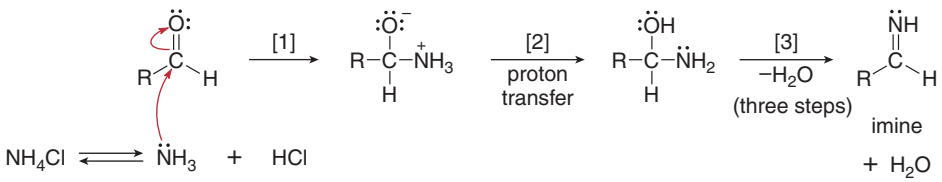
Example



Mechanism 28.1 for the formation of the α-amino nitrile from an aldehyde (the first step in the Strecker synthesis) consists of two parts: **nucleophilic addition of NH_3** to form an imine, followed by **addition of cyanide** to the C=N bond. Both parts are related to earlier mechanisms involving imines (Section 21.11) and cyanohydrins (Section 21.9).

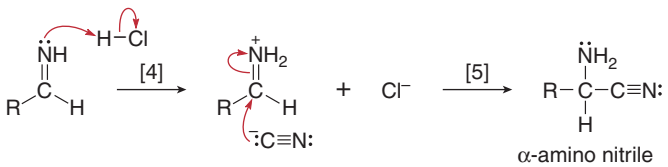
Mechanism 28.1 Formation of an α-Amino Nitrile

Part [1] Nucleophilic attack of NH_3 to form an imine



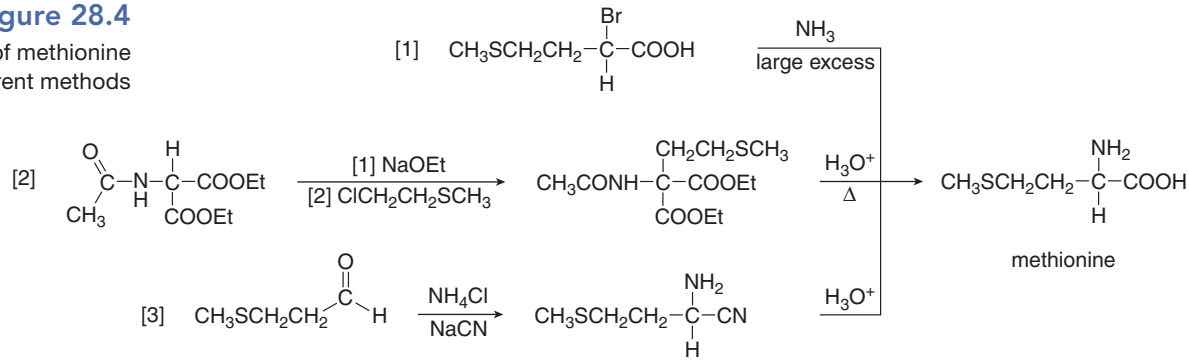
- **Part [1]** Nucleophilic attack of NH_3 followed by proton transfer and loss of H_2O forms an imine. Loss of H_2O occurs by the same three-step process outlined in Mechanism 21.5.

Part [2] Nucleophilic attack of ^-CN to form an α-amino nitrile



- **Part [2]** Protonation of the imine followed by nucleophilic attack of ^-CN gives the α-amino nitrile.

Figure 28.4
The synthesis of methionine by three different methods



Three methods of amino acid synthesis:

[1] S_N2 reaction using an α-halo carboxylic acid
[2] Alkylation of diethyl acetamidomalonate
[3] Strecker synthesis

The details of the second step of the Strecker synthesis, the hydrolysis of a nitrile (RCN) to a carboxylic acid (RCOOH), have already been presented in Section 22.18A.

Figure 28.4 shows how the amino acid methionine can be prepared by all three methods in Section 28.2.

Problem 28.7 What aldehyde is needed to synthesize each amino acid by the Strecker synthesis: (a) valine; (b) leucine; (c) phenylalanine?

Problem 28.8 Draw the products of each reaction.

a. $BrCH_2COOH \xrightarrow[\text{large excess}]{NH_3}$

b. $CH_3CONH{-}C(H)(COOEt)_2 \xrightarrow[\text{[2] } (CH_3)_2CHCl\text{ [3] } H_3O^+,\ \Delta]{\text{[1] NaOEt}}$

c. $CH_3CH_2CH(CH_3)CHO \xrightarrow[\text{[2] } H_3O^+]{\text{[1] } NH_4Cl,\ NaCN}$

d. $CH_3CONH{-}C(H)(COOEt)_2 \xrightarrow[\text{[2] } BrCH_2CO_2Et\text{ [3] } H_3O^+,\ \Delta]{\text{[1] NaOEt}}$

28.3 Separation of Amino Acids

No matter which of the preceding methods is used to synthesize an amino acid, all three yield a racemic mixture. Naturally occurring amino acids exist as a single enantiomer, however, so the two enantiomers obtained must be separated if they are to be used in biological applications. This is not an easy task. Two enantiomers have the same physical properties, so they cannot be separated by common physical methods, such as distillation or chromatography. Moreover, they react in the same way with achiral reagents, so they cannot be separated by chemical reactions either.

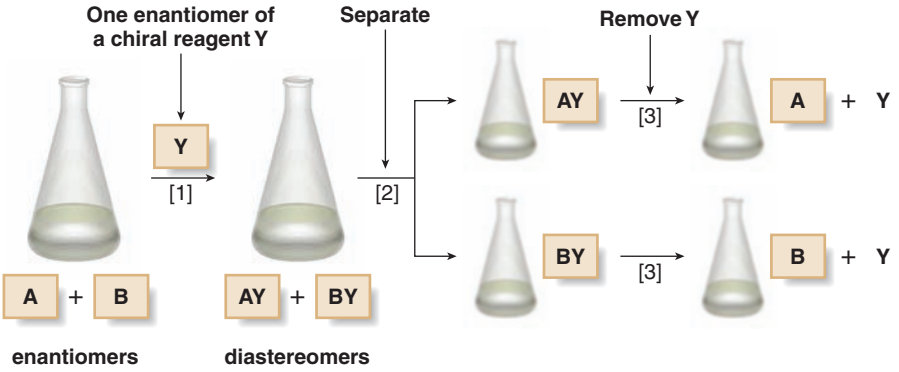
Nonetheless, strategies have been devised to separate two enantiomers using physical separation techniques and chemical reactions. We examine two different strategies in Section 28.3. Then, in Section 28.4, we will discuss a method that affords optically active amino acids without the need for separation.

- **The separation of a racemic mixture into its component enantiomers is called *resolution.* Thus, a racemic mixture is *resolved* into its component enantiomers.**

28.3A Resolution of Amino Acids

The oldest, and perhaps still the most widely used method to separate enantiomers exploits the following fact: **enantiomers have the *same* physical properties, but diastereomers have**

Figure 28.5
Resolution of a racemic mixture by converting it to a mixture of diastereomers



Enantiomers A and B can be separated by reaction with a single enantiomer of a chiral reagent, Y. The process of resolution requires three steps:

[1] Reaction of enantiomers **A** and **B** with **Y** forms two diastereomers, **AY** and **BY.**

[2] Diastereomers **AY** and **BY** have different physical properties, so they can be separated by physical methods such as fractional distillation or crystallization.

[3] **AY** and **BY** are then re-converted to **A** and **B** by a chemical reaction. The two enantiomers **A** and **B** are now separated from each other, and resolution is complete.

***different* physical properties.** Thus, a racemic mixture can be resolved using the following general strategy.

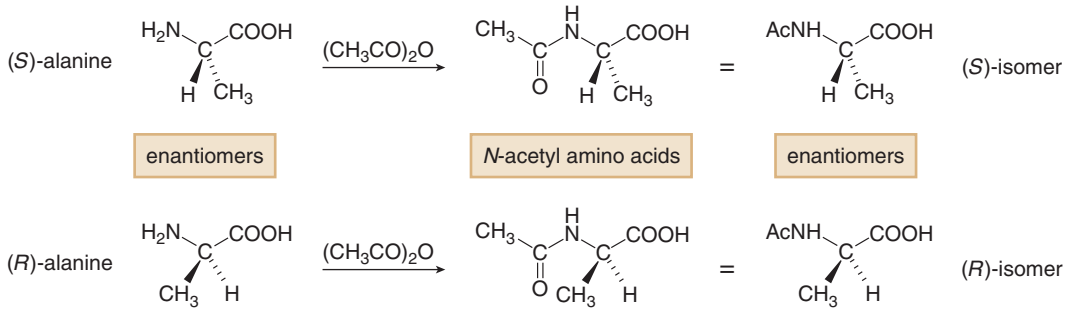
[1] **Convert a pair of enantiomers into a pair of diastereomers,** which are now separable because they have different melting points and boiling points.

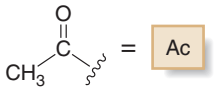
[2] **Separate the diastereomers.**

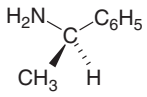
[3] **Re-convert each diastereomer into the original enantiomer,** now separated from the other.

This general three-step process is illustrated in Figure 28.5.

To resolve a racemic mixture of amino acids such as (*R*)- and (*S*)-alanine, the racemate is first treated with acetic anhydride to form ***N*-acetyl amino acids.** Each of these amides contains one stereogenic center and they are still enantiomers, so they are *still inseparable.*




= Ac

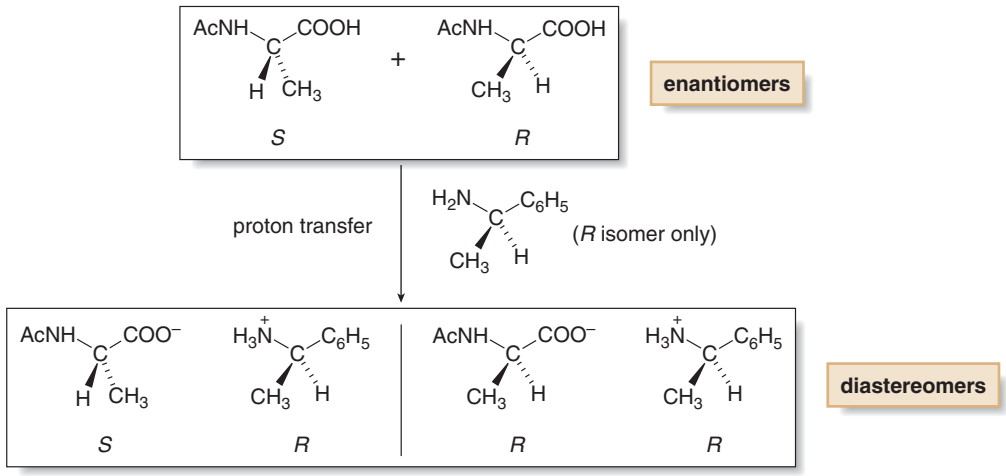

(*R*)-α-methylbenzylamine

a resolving agent

Both enantiomers of *N*-acetyl alanine have a free carboxy group that can react with an amine in an acid–base reaction. **If a chiral amine is used, such as (*R*)-α-methylbenzylamine, the two salts formed are diastereomers, *not* enantiomers.** Diastereomers can be physically separated from each other, so the compound that converts enantiomers into diastereomers is called a **resolving agent.** Either enantiomer of the resolving agent can be used.

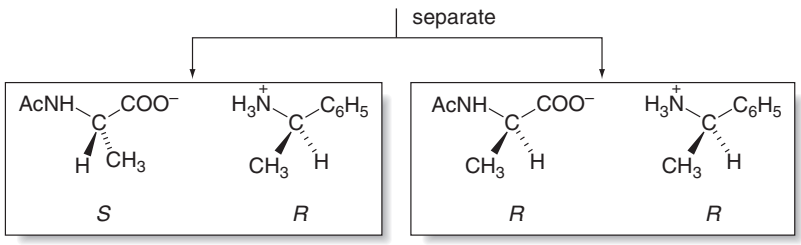
HOW TO Use (*R*)-α-Methylbenzylamine to Resolve a Racemic Mixture of Amino Acids

Step [1] React both enantiomers with the *R* isomer of the chiral amine.

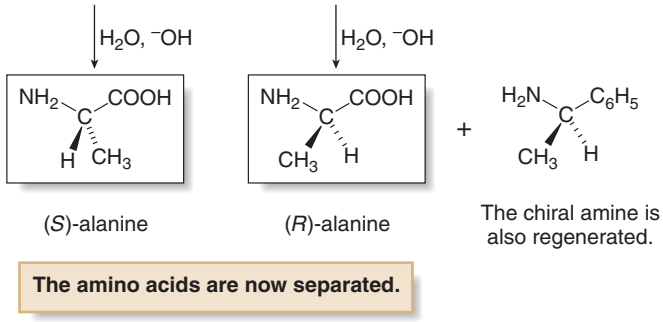


These salts have the *same* configuration around one stereogenic center, but the *opposite* configuration about the other stereogenic center.

Step [2] Separate the diastereomers.



Step [3] Regenerate the amino acid by hydrolysis of the amide.

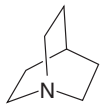
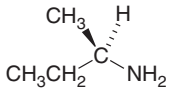
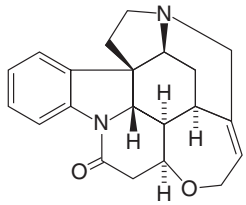


Step [1] is just an acid–base reaction in which the racemic mixture of *N*-acetyl alanines reacts with the same enantiomer of the resolving agent, in this case (*R*)-α-methylbenzylamine. The salts that form are **diastereomers, *not* enantiomers,** because they have the same configuration about one stereogenic center, but the opposite configuration about the other stereogenic center.

In **Step [2],** the diastereomers are separated by some physical technique, such as crystallization or distillation.

In **Step [3],** the amides can be hydrolyzed with aqueous base to regenerate the amino acids. The amino acids are now separated from each other. The optical activity of the amino acids can be measured and compared to their known rotations to determine the purity of each enantiomer.

Problem 28.9 Which of the following amines can be used to resolve a racemic mixture of amino acids?

a. $C_6H_5CH_2CH_2NH_2$ b.  c.  d. 

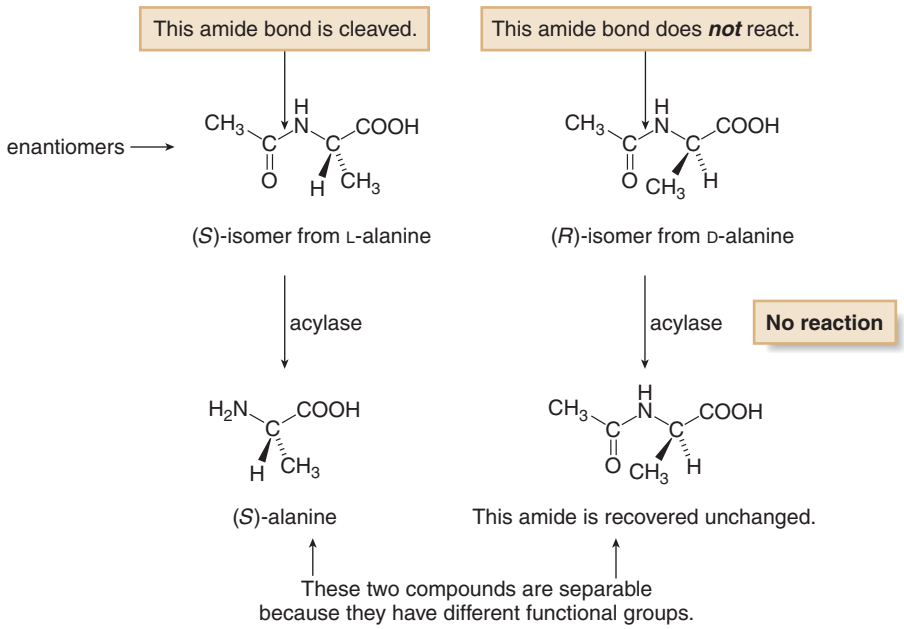
strychnine
(a powerful poison)

Problem 28.10 Write out a stepwise sequence that shows how a racemic mixture of leucine enantiomers can be resolved into optically active amino acids using (*R*)-α-methylbenzylamine.

28.3B Kinetic Resolution of Amino Acids Using Enzymes

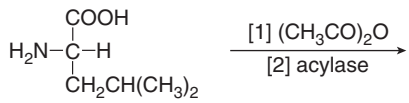
A second strategy used to separate amino acids is based on the fact that two enantiomers react differently with chiral reagents. An **enzyme** is typically used as the chiral reagent.

To illustrate this strategy, we begin again with the two enantiomers of *N*-acetyl alanine, which were prepared by treating a racemic mixture of (*R*)- and (*S*)-alanine with acetic anhydride (Section 28.3A). Enzymes called **acylases** hydrolyze amide bonds, such as those found in *N*-acetyl alanine, but only for amides of L-amino acids. Thus, when a racemic mixture of *N*-acetyl alanines is treated with an acylase, only the amide of L-alanine (the *S* stereoisomer) is hydrolyzed to generate L-alanine, whereas the amide of D-alanine (the *R* stereoisomer) is untouched. The reaction mixture now consists of one amino acid and one *N*-acetyl amino acid. Because they have different functional groups with different physical properties, they can be physically separated.



- **Separation of two enantiomers by a chemical reaction that selectively occurs for only one of the enantiomers is called *kinetic resolution.***

Problem 28.11 Draw the organic products formed in the following reaction.



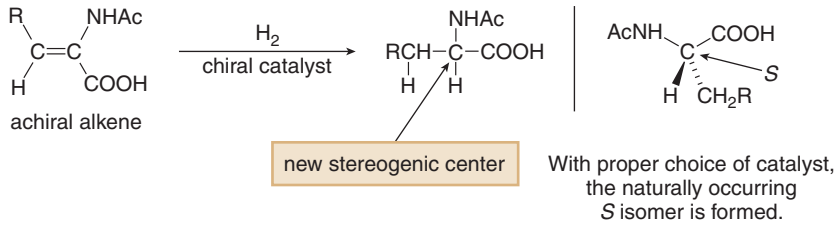
(mixture of enantiomers)

28.4 Enantioselective Synthesis of Amino Acids

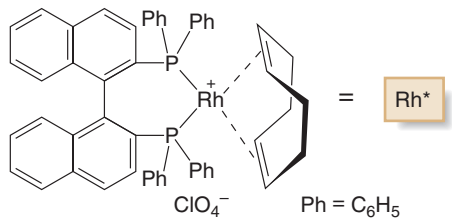
Although the two methods introduced in Section 28.3 for resolving racemic mixtures of amino acids make enantiomerically pure amino acids available for further research, half of the reaction product is useless because it has the undesired configuration. Moreover, each of these procedures is costly and time-consuming.

If we use a chiral reagent to synthesize an amino acid, however, it is possible to favor the formation of the desired enantiomer over the other, without having to resort to a resolution. For example, single enantiomers of amino acids have been prepared by using **enantioselective (or asymmetric) hydrogenation reactions.** The success of this approach depends on finding a chiral catalyst, in much the same way that a chiral catalyst is used for the Sharpless asymmetric epoxidation (Section 12.15).

The necessary starting material is an alkene. Addition of H_2 to the double bond forms an *N*-acetyl amino acid with a new stereogenic center on the α carbon to the carboxy group. With proper choice of a chiral catalyst, the naturally occurring *S* configuration can be obtained as product.



Several chiral catalysts with complex structures have now been developed for this purpose. Many contain **rhodium** as the metal, complexed to a chiral molecule containing one or more phosphorus atoms. One example, abbreviated simply as **Rh*,** is drawn below.

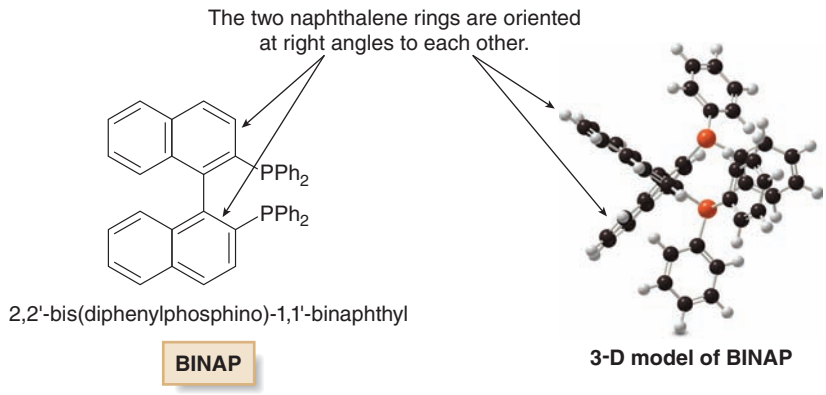


chiral hydrogenation catalyst

Ryoji Noyori shared the 2001 Nobel Prize in Chemistry for developing methods for asymmetric hydrogenation reactions using the chiral BINAP catalyst.

This catalyst is synthesized from a rhodium salt and a phosphorus compound, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl **(BINAP).** It is the BINAP moiety (Figure 28.6) that makes the catalyst chiral.

Figure 28.6
The structure of BINAP

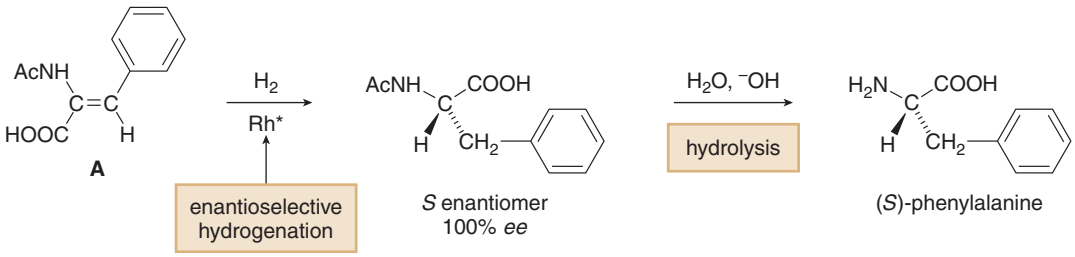


Twistoflex and helicene (Section 17.5) are two more aromatic compounds whose shape makes them chiral.

BINAP is one of a small number of molecules that is chiral even though it has no tetrahedral stereogenic centers. Its shape makes it a chiral molecule. The two naphthalene rings of the BINAP molecule are oriented at almost 90° to each other to minimize steric interactions between the hydrogen atoms on adjacent rings. This rigid three-dimensional shape makes BINAP nonsuperimposable on its mirror image, and thus it is a chiral compound.

The following graphic shows how enantioselective hydrogenation can be used to synthesize a single stereoisomer of phenylalanine. Treating achiral alkene **A** with H_2 and the chiral rhodium catalyst Rh* forms the *S* isomer of *N*-acetyl phenylalanine in 100% *ee*. Hydrolysis of the acetyl group on nitrogen then yields a single enantiomer of phenylalanine.

Example



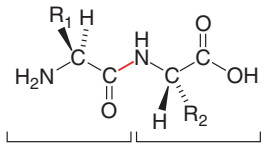
Problem 28.12 What alkene is needed to synthesize each amino acid by an enantioselective hydrogenation reaction using H_2 and Rh*: (a) alanine; (b) leucine; (c) glutamine?

28.5 Peptides

When amino acids are joined together by amide bonds, they form larger molecules called **peptides** and **proteins.**

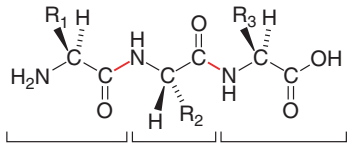
- A *dipeptide* has two amino acids joined together by *one* amide bond.
- A *tripeptide* has three amino acids joined together by *two* amide bonds.

Dipeptide



Two amino acids joined together.

Tripeptide



Three amino acids joined together.

[Amide bonds are drawn in red.]

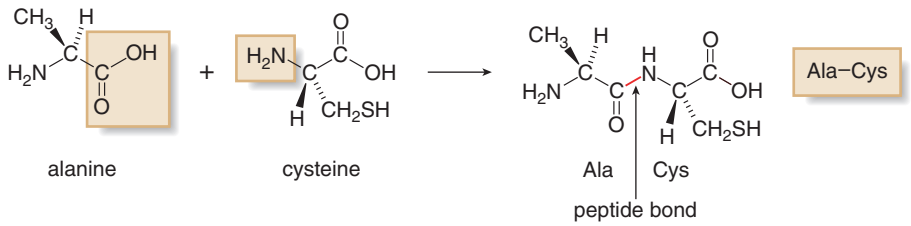
Polypeptides and **proteins** both have many amino acids joined together in long linear chains, but the term **protein** is usually reserved for polymers of more than 40 amino acids.

- The amide bonds in peptides and proteins are called *peptide bonds.*
- The individual amino acids are called *amino acid residues.*

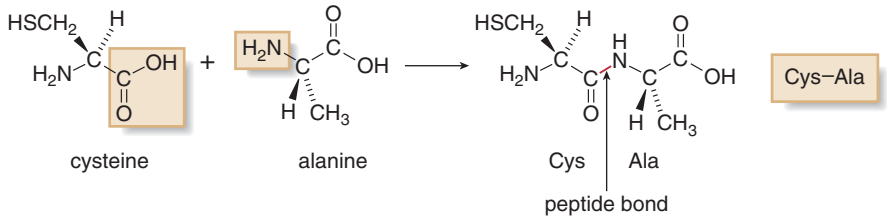
28.5A Simple Peptides

To form a dipeptide, the amino group of one amino acid forms an amide bond with the carboxy group of another amino acid. Because each amino acid has both an amino group and a carboxy group, **two different dipeptides can be formed.** This is illustrated with alanine and cysteine.

[1] **The COOH group of alanine can combine with the NH_2 group of cysteine.**



[2] **The COOH group of cysteine can combine with the NH_2 group of alanine.**



These compounds are **constitutional isomers** of each other. Both have a free amino group at one end of their chains and a free carboxy group at the other.

- The amino acid with the free amino group is called the *N-terminal amino acid.*
- The amino acid with the free carboxy group is called the *C-terminal amino acid.*

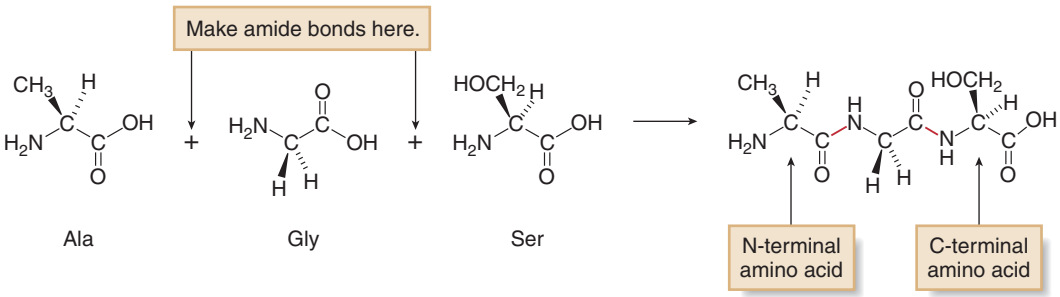
By convention, **the N-terminal amino acid is always written at the left end of the chain and the C-terminal amino acid at the right.** The peptide can be abbreviated by writing the one- or three-letter symbols for the amino acids in the chain from the N-terminal to the C-terminal end. Thus, Ala–Cys has alanine at the N-terminal end and cysteine at the C-terminal end, whereas Cys–Ala has cysteine at the N-terminal end and alanine at the C-terminal end. Sample Problem 28.1 shows how this convention applies to a tripeptide.

Sample Problem 28.1

Draw the structure of the following tripeptide, and label its N-terminal and C-terminal amino acids: Ala–Gly–Ser.

Solution

Draw the structures of the amino acids in order from **left to right,** placing the COOH of one amino acid *next* to the NH_2 group of the adjacent amino acid. Always draw the **NH_2 group on the *left*** and the **COOH group on the *right.*** Then, join adjacent COOH and NH_2 groups together in amide bonds to form the tripeptide.



tripeptide **Ala–Gly–Ser**
[The new peptide bonds are drawn in red.]

The N-terminal amino acid is **alanine,** and the C-terminal amino acid is **serine.**

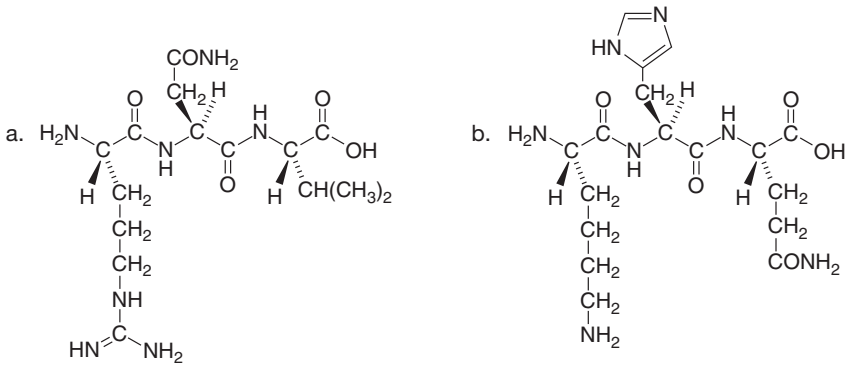
The tripeptide in Sample Problem 28.1 has one N-terminal amino acid, one C-terminal amino acid, and two peptide bonds.

- No matter how many amino acid residues are present, there is only *one* N-terminal amino acid and *one* C-terminal amino acid.
- For *n* amino acids in the chain, the number of amide bonds is *n* – 1.

Problem 28.13 Draw the structure of each peptide. Label the N-terminal and C-terminal amino acids and all amide bonds.

a. Val–Glu b. Gly–His–Leu c. M–A–T–T

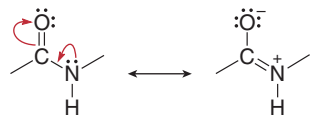
Problem 28.14 Name each peptide using both the one-letter and the three-letter abbreviations for the names of the component amino acids.



Problem 28.15 How many different tripeptides can be formed from three different amino acids?

28.5B The Peptide Bond

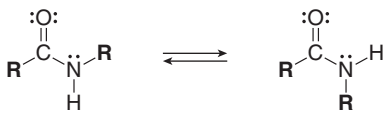
The carbonyl carbon of an amide is ***sp*^{2} hybridized** and has **trigonal planar** geometry. A second resonance structure can be drawn that delocalizes the nonbonded electron pair on the N atom. Amides are more resonance stabilized than other acyl compounds, so the resonance structure having the C=N makes a significant contribution to the hybrid.



two resonance structures for the peptide bond

Resonance stabilization has important consequences. Rotation about the C–N bond is restricted because it has partial double bond character. As a result, there are two possible conformations.

Two conformations of the peptide bond



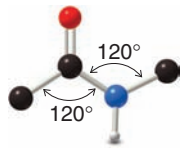
s-trans *s*-cis

Recall from Section 16.6 that 1,3-butadiene can also exist as *s*-cis and *s*-trans conformations. In 1,3-butadiene, the *s*-cis conformation has the two double bonds on the same side of the single bond (dihedral angle = 0°), whereas the *s*-trans conformation has them on opposite sides (dihedral angle = 180°).

- The *s*-trans conformation has the two R groups oriented on *opposite* sides of the C – N bond.
- The *s*-cis conformation has the two R groups oriented on the *same* side of the C – N bond.
- The *s*-trans conformation of a peptide bond is typically more stable than the *s*-cis, because the *s*-trans has the two bulky R groups located farther from each other.

A second consequence of resonance stabilization is that **all six atoms involved in the peptide bond lie in the same plane.** All bond angles are ~120° and the C=O and N–H bonds are oriented 180° from each other.

The planar geometry of the peptide bond is analogous to the planar geometry of ethylene (or any other alkene), where the double bond between sp^2 hybridized carbon atoms makes all of the bond angles ~120° and puts all six atoms in the same plane.

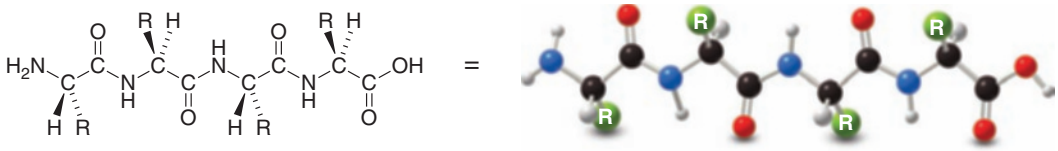


These six atoms lie in a plane.

The structure of a tetrapeptide illustrates the results of these effects in a long peptide chain.

- **The *s*-trans arrangement makes a long chain with a zigzag arrangement.**
- **In each peptide bond, the N−H and C=O bonds lie parallel and at 180° with respect to each other.**

A tetrapeptide



Problem 28.16 Draw the *s*-cis and *s*-trans conformations for the dipeptide formed from two glycine molecules.

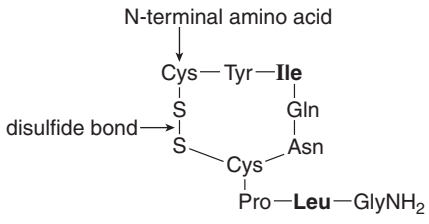
28.5C Interesting Peptides

Even relatively simple peptides can have important biological functions. **Bradykinin,** for example, is a peptide hormone composed of nine amino acids. It stimulates smooth muscle contraction, dilates blood vessels, and causes pain. Bradykinin is a component of bee venom.

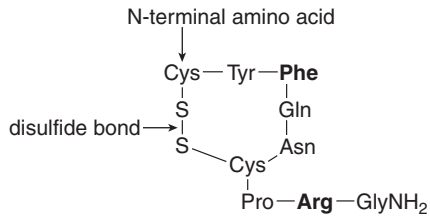
Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg

bradykinin

Oxytocin and **vasopressin** are nonapeptide hormones, too. Their sequences are identical except for two amino acids, yet this is enough to give them very different biological activities. Oxytocin induces labor by stimulating the contraction of uterine muscles, and it stimulates the flow of milk in nursing mothers. Vasopressin, on the other hand, controls blood pressure by regulating smooth muscle contraction. The N-terminal amino acid in both hormones is a cysteine residue, and the C-terminal residue is glycine. Instead of a free carboxy group, both peptides have an NH_2 group in place of OH, so this is indicated with the additional NH_2 group drawn at the end of the chain.



oxytocin



vasopressin

The structure of both peptides includes a **disulfide bond,** a form of covalent bonding in which the −SH groups from two cysteine residues are oxidized to form a sulfur–sulfur bond. In oxytocin and vasopressin, the disulfide bonds make the peptides cyclic. Three-dimensional structures of oxytocin and vasopressin are shown in Figure 28.7.

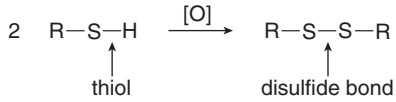
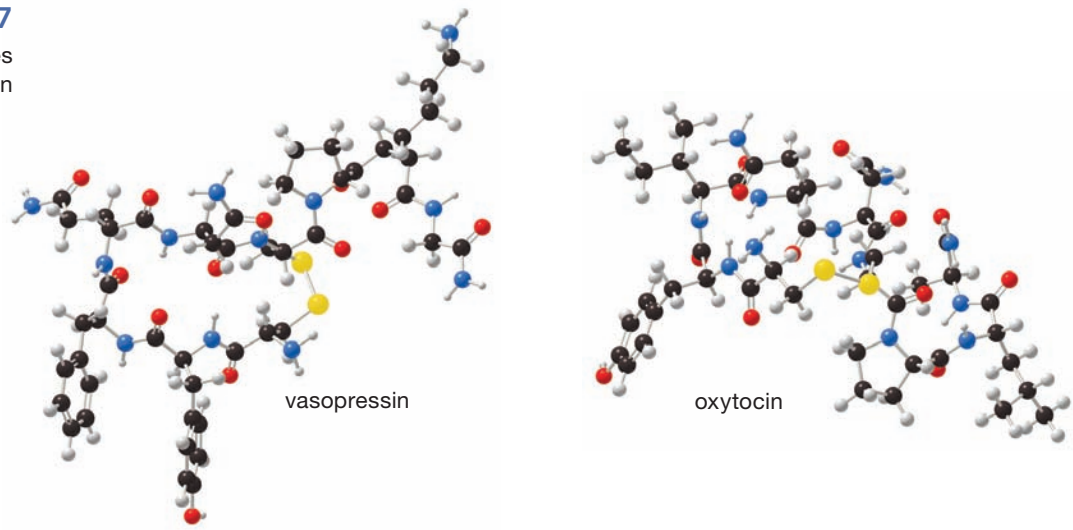
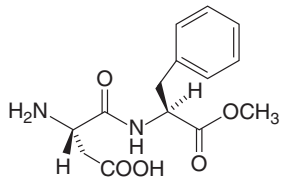


Figure 28.7
Three-dimensional structures of vasopressin and oxytocin



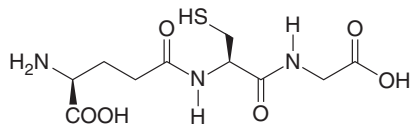
The artificial sweetener **aspartame** (Figure 27.11) is the methyl ester of the dipeptide Asp–Phe. This synthetic peptide is 180 times sweeter (on a gram-for-gram basis) than sucrose (common table sugar). Both of the amino acids in aspartame have the naturally occurring L-configuration. If the D-amino acid is substituted for either Asp or Phe, the resulting compound tastes bitter.



aspartame
the methyl ester of Asp–Phe
a synthetic artificial sweetener

Problem 28.17 Draw the structure of leu-enkephalin, a pentapeptide that acts as an analgesic and opiate, and has the following sequence: Tyr–Gly–Gly–Phe–Leu. (The structure of a related peptide, met-enkephalin, appeared in Section 22.6B.)

Problem 28.18 Glutathione, a powerful antioxidant that destroys harmful oxidizing agents in cells, is composed of glutamic acid, cysteine, and glycine, and has the following structure:



glutathione

a. What product is formed when glutathione reacts with an oxidizing agent?
b. What is unusual about the peptide bond between glutamic acid and cysteine?

28.6 Peptide Sequencing

To determine the structure of a peptide, we must know not only what amino acids comprise it, but also the sequence of the amino acids in the peptide chain. Although mass spectrometry has become an increasingly powerful method for the analysis of high molecular weight proteins (Section 13.4), chemical methods to determine peptide structure are still widely used and presented in this section.

28.6A Amino Acid Analysis

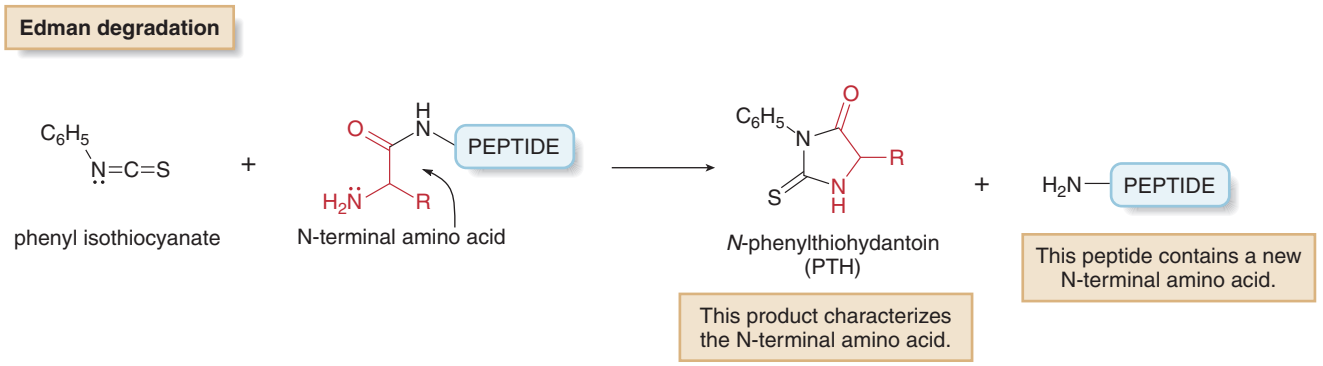
The structure determination of a peptide begins by analyzing the total amino acid composition. The amide bonds are first hydrolyzed by heating with hydrochloric acid for 24 h to form the individual amino acids. The resulting mixture is then separated using high-performance liquid chromatography (HPLC), a technique in which a solution of amino acids is placed on a column and individual amino acids move through the column at characteristic rates, often dependent on polarity.

This process determines both the identity of the individual amino acids and the amount of each present, but it tells nothing about the order of the amino acids in the peptide. For example, complete hydrolysis and HPLC analysis of the tetrapeptide Gly–Gly–Phe–Tyr would indicate the presence of three amino acids—glycine, phenylalanine, and tyrosine—and show that there are twice as many glycine residues as phenylalanine or tyrosine residues. The exact order of the amino acids in the peptide chain must then be determined by additional methods

28.6B Identifying the N-Terminal Amino Acid—The Edman Degradation

To determine the sequence of amino acids in a peptide chain, a variety of procedures are often combined. One especially useful technique is to identify the N-terminal amino acid using the **Edman degradation.** In the Edman degradation, amino acids are cleaved one at a time from the N-terminal end, the identity of the amino acid determined, and the process repeated until the entire sequence is known. Automated sequencers using this methodology are now available to sequence peptides containing up to about 50 amino acids.

The Edman degradation is based on the reaction of the nucleophilic NH_2 group of the N-terminal amino acid with the electrophilic carbon of phenyl isothiocyanate, $C_6H_5N{=}C{=}S$. When the N-terminal amino acid is removed from the peptide chain, two products are formed: **an *N*-phenylthiohydantoin (PTH) and a new peptide with one *fewer* amino acid.**

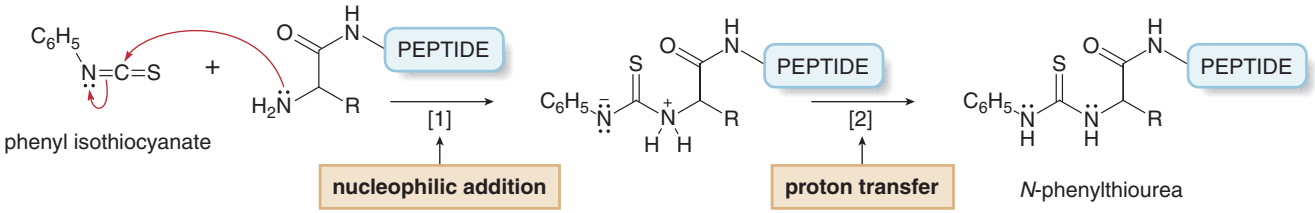


The *N*-phenylthiohydantoin derivative contains the atoms of the N-terminal amino acid. This product identifies the N-terminal amino acid in the peptide because the PTH derivatives of all 20 naturally occurring amino acids are known and characterized. The new peptide formed in the Edman degradation has one amino acid fewer than the original peptide. Moreover, it contains a new N-terminal amino acid, so the process can be repeated.

Mechanism 28.2 illustrates some of the key steps of the Edman degradation. The nucleophilic N-terminal NH_2 group adds to the electrophilic carbon of phenyl isothiocyanate to form an *N*-phenylthiourea, the product of nucleophilic addition (Part [1]). Intramolecular cyclization followed by elimination results in cleavage of the terminal amide bond in Part [2] to form a new peptide with one fewer amino acid. A sulfur heterocycle, called a thiazolinone, is also formed, which rearranges by a multistep pathway (Part [3]) to form an *N*-phenylthiohydantoin. The R group in this product identifies the amino acid located at the N-terminal end.

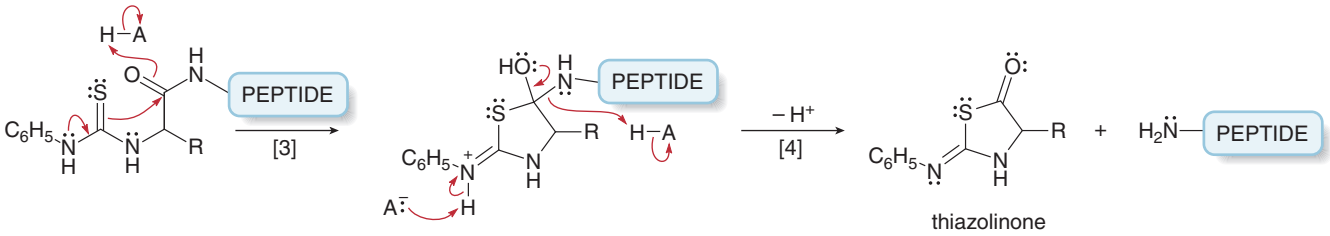
Mechanism 28.2 Edman Degradation

Part [1] Formation of an *N*-phenylthiourea



- Addition of the free amino group from the N-terminal amino acid to the electrophilic carbon of phenyl isothiocyanate followed by proton transfer forms an *N*-phenylthiourea.

Part [2] Cleavage of the amide bond from the N-terminal amino acid



- Nucleophilic addition in Step [3] followed by loss of the amino group in Step [4] forms two products: a five-membered thiazolinone ring and a peptide chain that contains one fewer amino acid than the original peptide.

Part [3] Rearrangement to form an *N*-phenylthiohydantoin (PTH)

- Under the conditions of the reaction, the thiazolinone rearranges by a multistep pathway to form an *N*-phenylthiohydantoin (PTH). This product contains the original N-terminal amino acid.

In theory a protein of any length can be sequenced using the Edman degradation, but in practice, the accumulation of small quantities of unwanted by-products limits sequencing to proteins having fewer than approximately 50 amino acids.

Problem 28.19 Draw the structure of the *N*-phenylthiohydantoin formed by initial Edman degradation of each peptide: (a) Ala–Gly–Phe–Phe; (b) Val–Ile–Tyr.

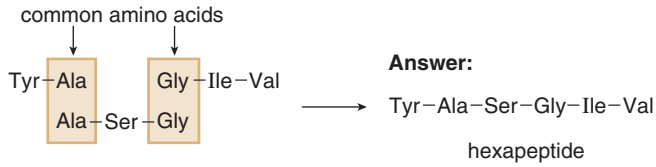
28.6C Partial Hydrolysis of a Peptide

Additional structural information can be obtained by cleaving some, but not all, of the amide bonds in a peptide. Partial hydrolysis of a peptide with acid forms smaller fragments in a random fashion. Sequencing these peptides and identifying sites of overlap can be used to determine the sequence of the complete peptide, as shown in Sample Problem 28.2.

Sample Problem 28.2 Give the amino acid sequence of a hexapeptide that contains the amino acids Ala, Val, Ser, Ile, Gly, Tyr, and forms the following fragments when partially hydrolyzed with HCl: Gly–Ile–Val, Ala–Ser–Gly, and Tyr–Ala.

Solution

Looking for points of overlap in the sequences of the smaller fragments shows how the fragments should be pieced together. In this example, the fragment Ala–Ser–Gly contains amino acids common to the two other fragments, thus showing how the three fragments can be joined together.



Problem 28.20 Give the amino acid sequence of an octapeptide that contains the amino acids Tyr, Ala, Leu (2 equiv), Cys, Gly, Glu, and Val, and forms the following fragments when partially hydrolyzed with HCl: Val–Cys–Gly–Glu, Ala–Leu–Tyr, and Tyr–Leu–Val–Cys.

Peptides can also be hydrolyzed at specific sites using enzymes. The enzyme carboxypeptidase catalyzes the hydrolysis of the amide bond nearest the C-terminal end, forming the C-terminal amino acid and a peptide with one fewer amino acid. In this way, carboxypeptidase is used to identify the C-terminal amino acid.

Other enzymes catalyze the hydrolysis of amide bonds formed with specific amino acids. For example, trypsin catalyzes the hydrolysis of amides with a carbonyl group that is part of the basic amino acids arginine and lysine. Chymotrypsin hydrolyzes amides with carbonyl groups that are part of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Table 28.2 summarizes these enzyme specificities used in peptide sequencing.

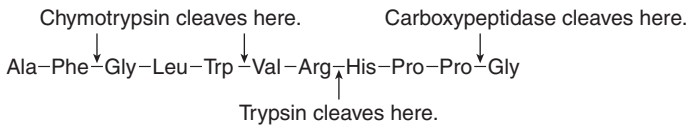


Table 28.2 Cleavage Sites of Specific Enzymes in Peptide Sequencing

Enzyme	Site of cleavage
Carboxypeptidase	Amide bond nearest to the C-terminal amino acid
Chymotrypsin	Amide bond with a carbonyl group from Phe, Tyr, or Trp
Trypsin	Amide bond with a carbonyl group from Arg or Lys

Problem 28.21 (a) What products are formed when each peptide is treated with trypsin? (b) What products are formed when each peptide is treated with chymotrypsin?

[1] Gly–Ala–Phe–Leu–Lys–Ala
[2] Phe–Tyr–Gly–Cys–Arg–Ser
[3] Thr–Pro–Lys–Glu–His–Gly–Phe–Cys–Trp–Val–Val–Phe

Sample Problem 28.3 Deduce the sequence of a pentapeptide that contains the amino acids Ala, Glu, Gly, Ser, and Tyr, from the following experimental data. Edman degradation cleaves Gly from the pentapeptide, and carboxypeptidase forms Ala and a tetrapeptide. Treatment of the pentapeptide with chymotrypsin forms a dipeptide and a tripeptide. Partial hydrolysis forms Gly, Ser, and the tripeptide Tyr–Glu–Ala.

Solution

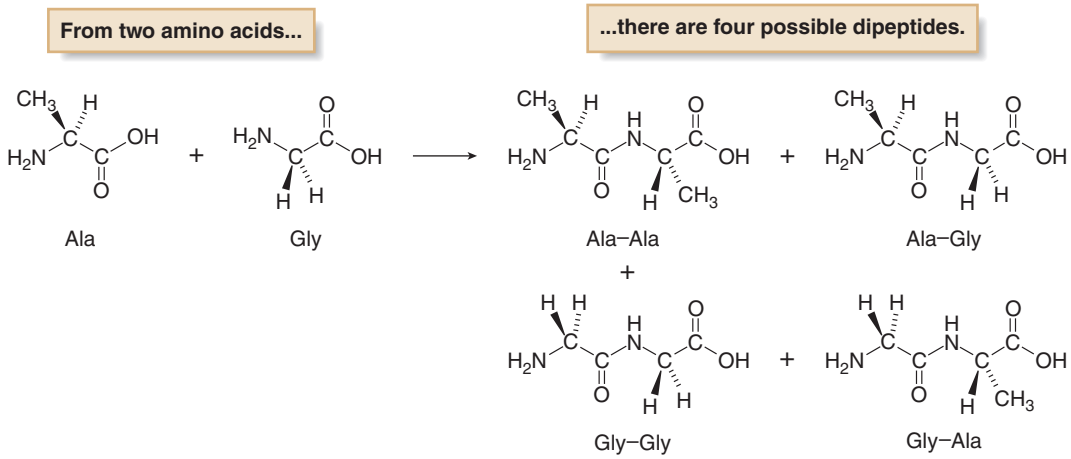
Use each result to determine the location of an amino acid in the pentapeptide.

Experiment		Result
• Edman degradation identifies the N-terminal amino acid—in this case, Gly.	→	Gly– _ – _ – _ – _
• Carboxypeptidase identifies the C-terminal amino acid (Ala) when it is cleaved from the end of the chain.	→	Gly– _ – _ – _ –Ala
• Chymotrypsin cleaves amide bonds that contain a carbonyl group from an aromatic amino acid—Tyr in this case. Because a dipeptide and tripeptide are obtained after treatment with chymotrypsin, Tyr must be the C-terminal amino acid of either the di- or tripeptide. As a result, Tyr must be either the second or third amino acid in the pentapeptide chain.	→	Gly–Tyr– _ – _ –Ala or Gly– _ –Tyr– _ –Ala
• Partial hydrolysis forms the tripeptide Tyr–Glu–Ala. Because Ala is the C-terminal amino acid, this result identifies the last three amino acids in the chain.	→	Gly– _ –Tyr–Glu–Ala
• The last amino acid, Ser, must be located at the only remaining position, the second amino acid in the pentapeptide, and the complete sequence is determined.	→	Gly–Ser–Tyr–Glu–Ala

Problem 28.22 Deduce the sequence of a heptapeptide that contains the amino acids Ala, Arg, Glu, Gly, Leu, Phe, and Ser, from the following experimental data. Edman degradation cleaves Leu from the heptapeptide, and carboxypeptidase forms Glu and a hexapeptide. Treatment of the heptapeptide with chymotrypsin forms a hexapeptide and a single amino acid. Treatment of the heptapeptide with trypsin forms a pentapeptide and a dipeptide. Partial hydrolysis forms Glu, Leu, Phe, and the tripeptides Gly–Ala–Ser and Ala–Ser–Arg.

28.7 Peptide Synthesis

The synthesis of a specific dipeptide, such as Ala–Gly from alanine and glycine, is complicated because both amino acids have two functional groups. As a result, four products—namely, Ala–Ala, Ala–Gly, Gly–Gly, and Gly–Ala—are possible.

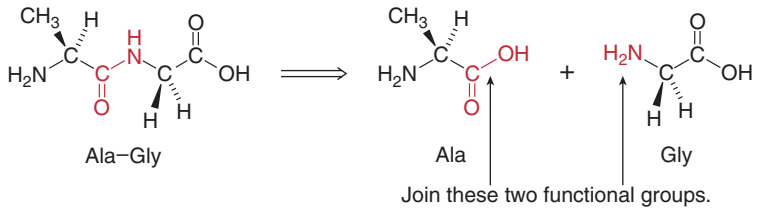


How do we selectively join the COOH group of alanine with the NH_2 group of glycine?

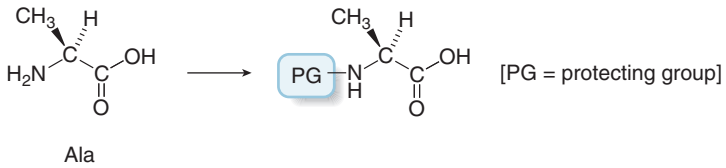
- **Protect the functional groups that we don't want to react, and then form the amide bond.**

HOW TO Synthesize a Dipeptide from Two Amino Acids

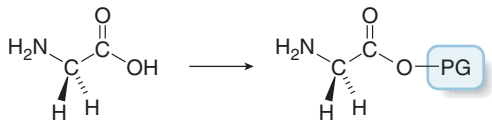
Example



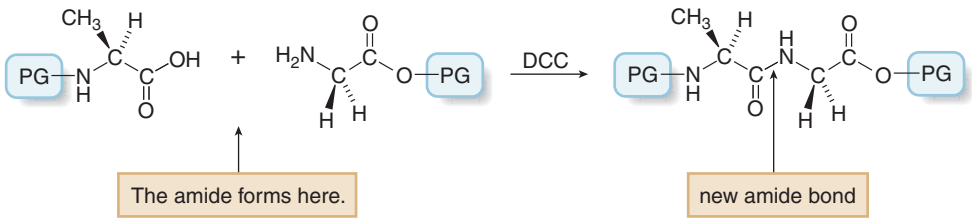
Step [1] Protect the NH_2 group of alanine.



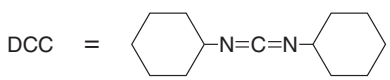
Step [2] Protect the COOH group of glycine.



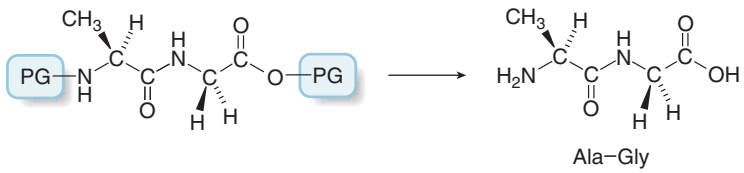
Step [3] Form the amide bond with DCC.



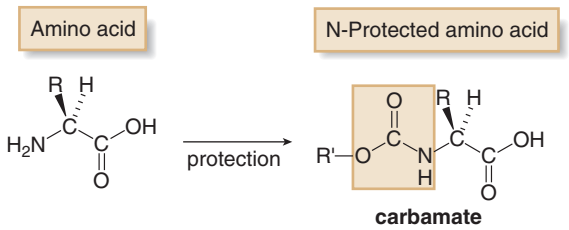
Dicyclohexylcarbodiimide (**DCC**) is a reagent commonly used to form amide bonds (see Section 22.10D). DCC makes the OH group of the carboxylic acid a better leaving group, thus **activating the carboxy group toward nucleophilic attack.**



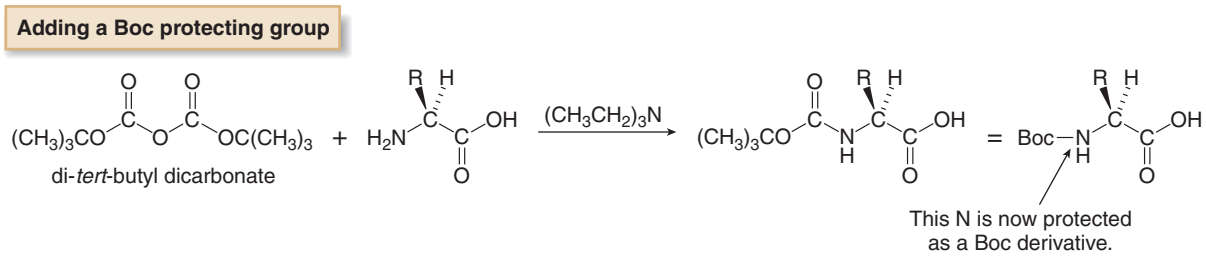
Step [4] Remove one or both protecting groups.

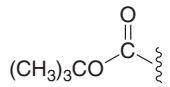


Two widely used amino protecting groups convert an amine into a **carbamate,** a functional group having a carbonyl bonded to both an oxygen and a nitrogen atom. Since the N atom of the carbamate is bonded to a carbonyl group, the protected amino group is no longer nucleophilic.

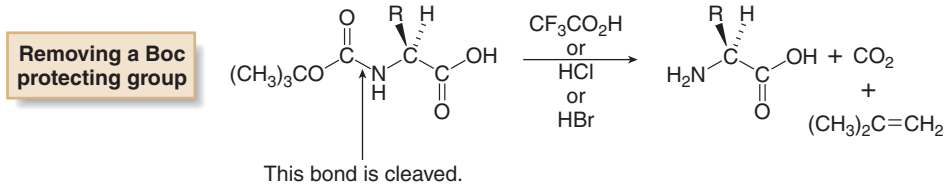


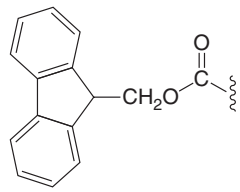
For example, the ***tert*-butoxycarbonyl protecting group,** abbreviated as **Boc,** is formed by reacting the amino acid with di-*tert*-butyl dicarbonate in a nucleophilic acyl substitution reaction.



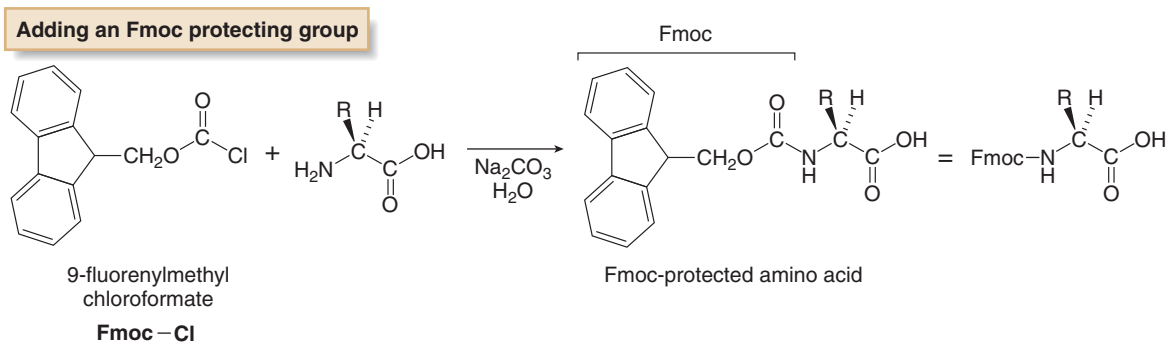


To be a useful protecting group, the Boc group must be removed under reaction conditions that do not affect other functional groups in the molecule. It can be removed with an acid such as **trifluoroacetic acid, HCl,** or **HBr.**

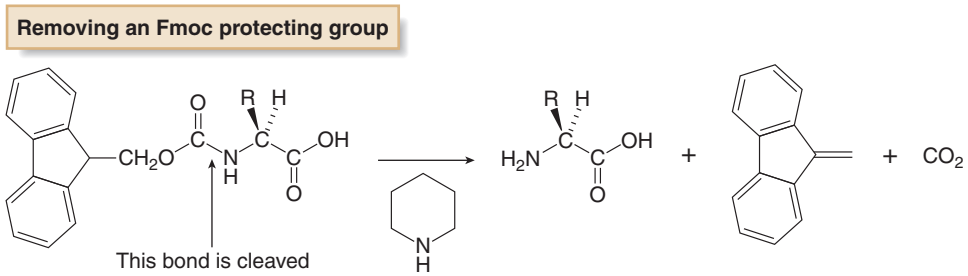




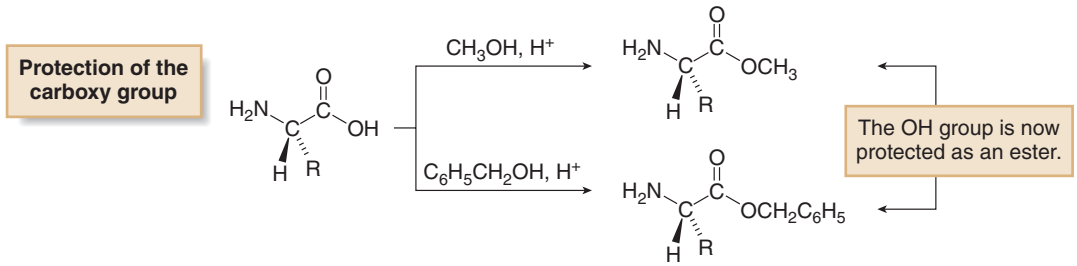
A second amino protecting group, the **9-fluorenylmethoxycarbonyl protecting group,** abbreviated as **Fmoc,** is formed by reacting the amino acid with 9-fluorenylmethyl chloroformate in a nucleophilic acyl substitution reaction.



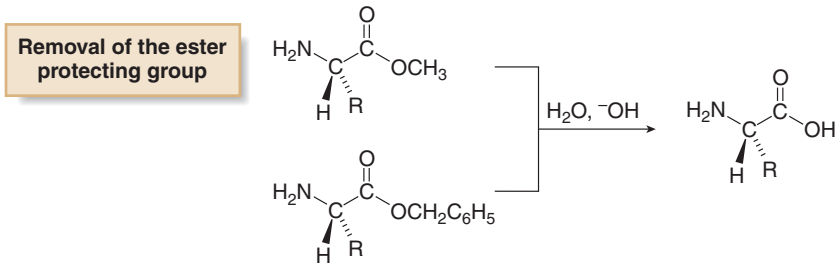
While the Fmoc protecting group is stable to most acids, it can be removed by treatment with base (NH_3 or an amine), to regenerate the free amino group.



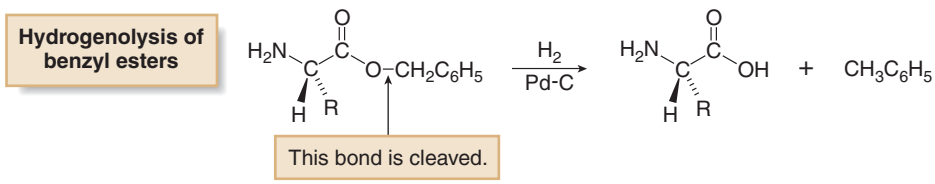
The carboxy group is usually protected as a **methyl** or **benzyl ester** by reaction with an alcohol and an acid.



These esters are usually removed by hydrolysis with aqueous base.



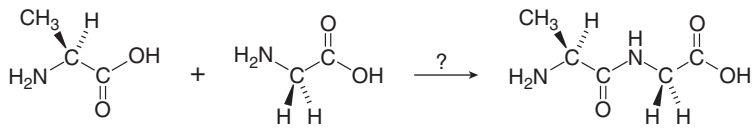
One advantage of using a benzyl ester for protection is that it can also be removed with H_2 in the presence of a Pd catalyst. This process is called **hydrogenolysis.** These conditions are especially mild, because they avoid the use of either acid or base. Benzyl esters can also be removed with HBr in acetic acid.



The specific reactions needed to synthesize the dipeptide Ala–Gly are illustrated in Sample Problem 28.4.

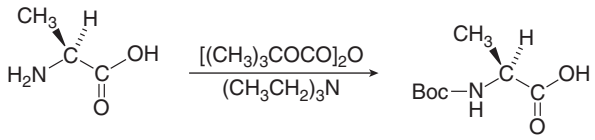
Sample Problem 28.4

Draw out the steps in the synthesis of the dipeptide Ala–Gly.

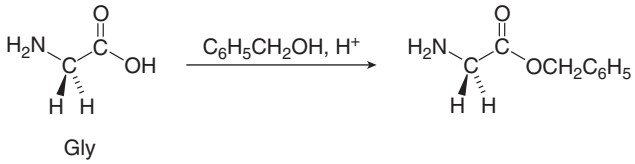


Solution

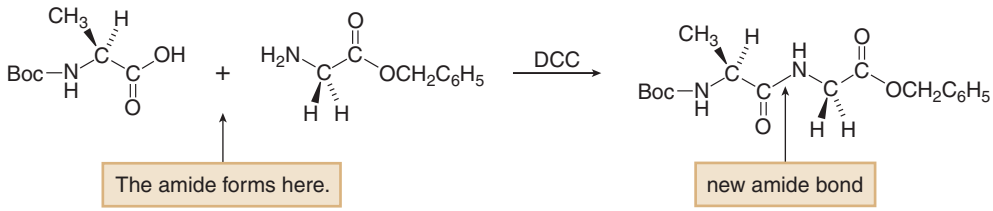
Step [1] Protect the NH_2 group of alanine using a Boc group.



Step [2] Protect the COOH group of glycine as a benzyl ester.

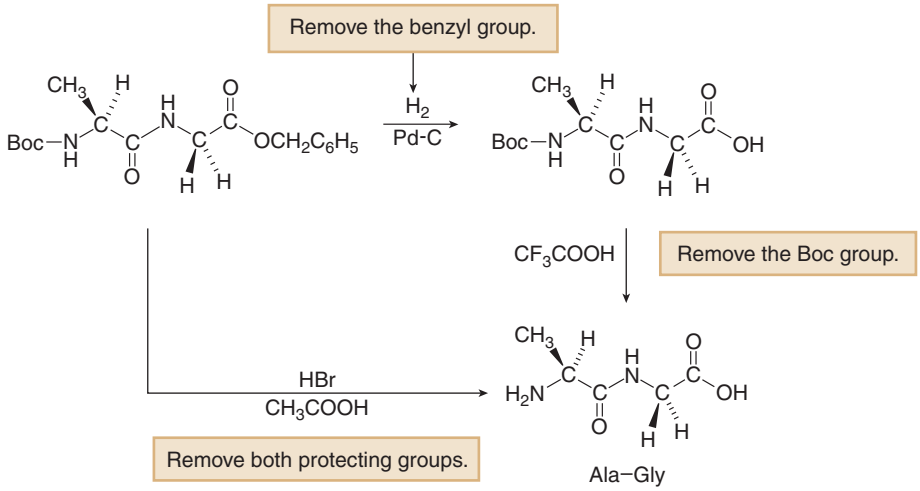


Step [3] Form the amide bond with DCC.

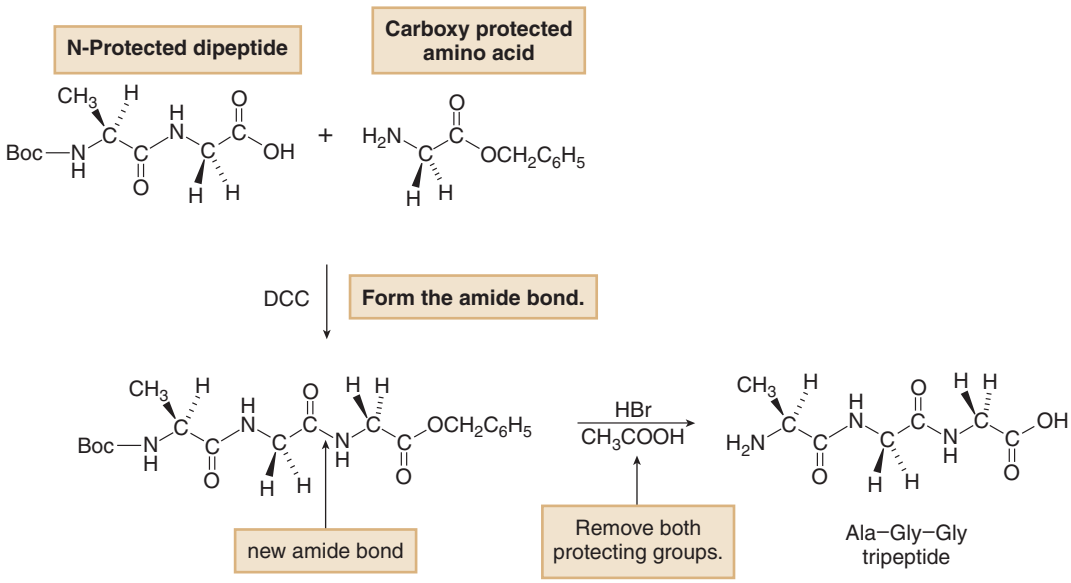


Step [4] Remove one or both protecting groups.

The protecting groups can be removed in a stepwise fashion, or in a single reaction.



This method can be applied to the synthesis of tripeptides and even larger polypeptides. After the protected dipeptide is prepared in Step [3], only one of the protecting groups is removed, and this dipeptide is coupled to a third amino acid with one of its functional groups protected, as illustrated in the following equations.



Problem 28.23 Devise a synthesis of each peptide from amino acid starting materials: (a) Leu–Val; (b) Ala–Ile–Gly; (c) Ala–Gly–Ala–Gly.

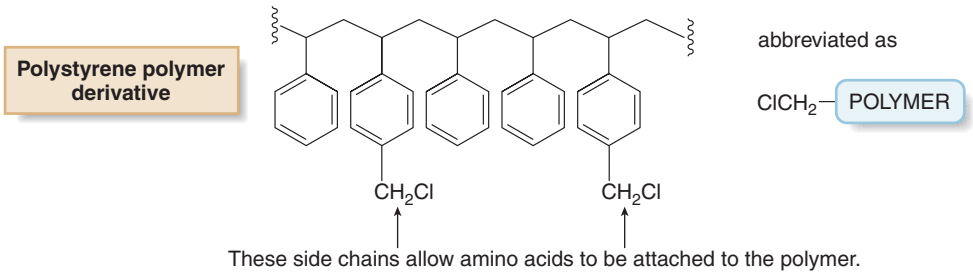
28.8 Automated Peptide Synthesis

The method described in Section 28.7 works well for the synthesis of small peptides. It is extremely time-consuming to synthesize larger proteins by this strategy, however, because each step requires isolation and purification of the product. The synthesis of larger polypeptides is usually accomplished by using the **solid phase technique** originally developed by R. Bruce Merrifield of Rockefeller University.

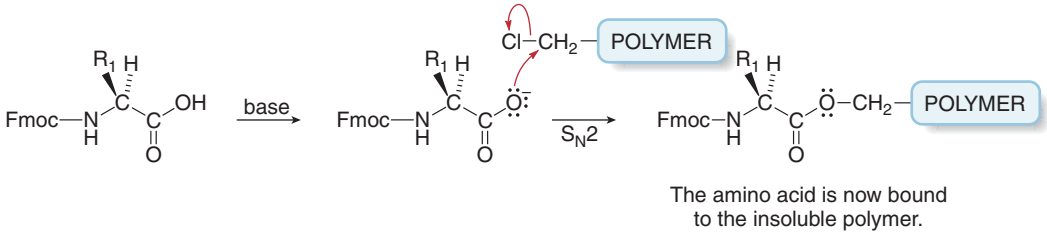
Development of the solid phase technique earned Merrifield the 1984 Nobel Prize in Chemistry and has made possible the synthesis of many polypeptides and proteins.

In the **Merrifield method** an amino acid is attached to an **insoluble polymer.** Amino acids are sequentially added, one at a time, thereby forming successive peptide bonds. Because impurities and by-products are not attached to the polymer chain, they are removed simply by washing them away with a solvent at each stage of the synthesis.

A commonly used polymer is a **polystyrene derivative** that contains $-CH_2Cl$ groups bonded to some of the benzene rings in the polymer chain. The Cl atoms serve as handles that allow attachment of amino acids to the chain.



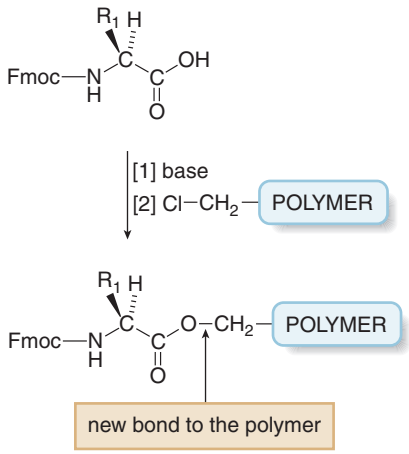
An Fmoc-protected amino acid is attached to the polymer at its carboxy group by an S_N2 reaction.



Once the first amino acid is bound to the polymer, additional amino acids can be added sequentially. The steps of the solid phase peptide synthesis technique are illustrated in the accompanying scheme. In the last step, HF cleaves the polypeptide chain from the polymer.

HOW TO Synthesize a Peptide Using the Merrifield Solid Phase Technique

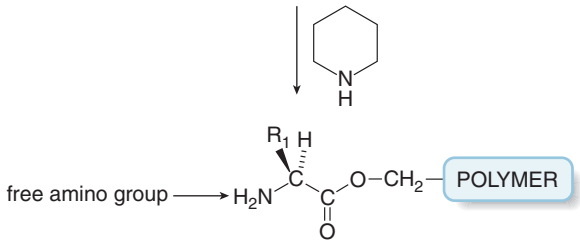
Step [1]
Attach an Fmoc-protected amino acid to the polymer.



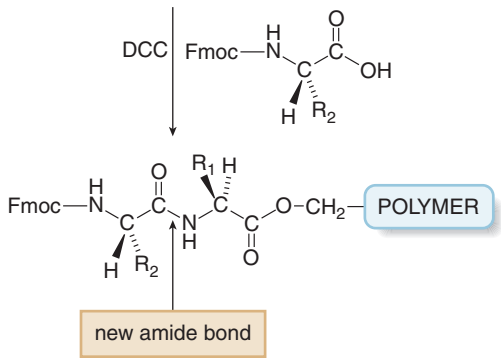
—Continued

HOW TO, continued . . .

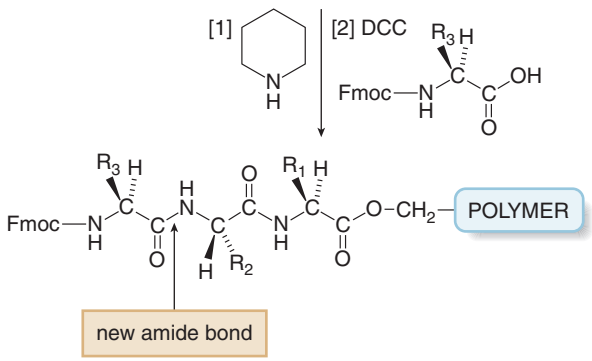
Step [2]
Remove the protecting group.



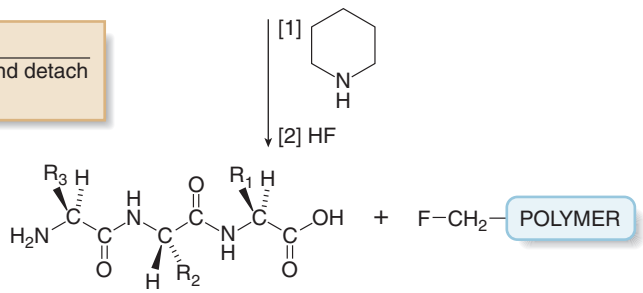
Step [3]
Form the amide bond with DCC.



Step [4]
Repeat Steps [2] and [3].



Step [5]
Remove the protecting group and detach the peptide from the polymer.



tripeptide

The Merrifield method has now been completely automated, so it is possible to purchase peptide synthesizers that automatically carry out all of the above operations and form polypeptides in high yield in a matter of hours, days, or weeks, depending on the length of the chain of the desired product. The instrument is pictured in Figure 28.8. For example, the protein ribonuclease, which contains 128 amino acids, has been prepared by this technique in an overall yield of 17%. This remarkable synthesis involved 369 separate reactions, and thus the yield of each individual reaction was > 99%.

Problem 28.24 Outline the steps needed to synthesize the tetrapeptide Ala–Leu–Ile–Gly using the Merrifield technique.

Figure 28.8
Automated peptide synthesizer

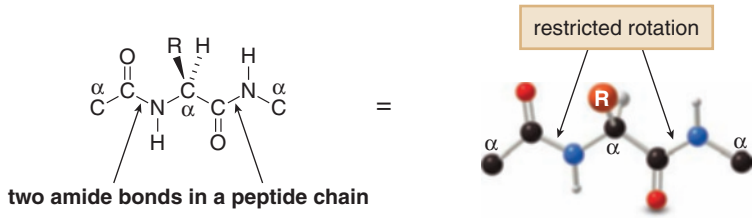
28.9 Protein Structure

Now that you have learned some of the chemistry of amino acids, it's time to study proteins, the large polymers of amino acids that are responsible for so much of the structure and function of all living cells. We begin with a discussion of the **primary, secondary, tertiary, and quaternary structure** of proteins.

28.9A Primary Structure

The *primary structure* of proteins is the particular sequence of amino acids that is joined together by peptide bonds. The most important element of this primary structure is the **amide bond.**

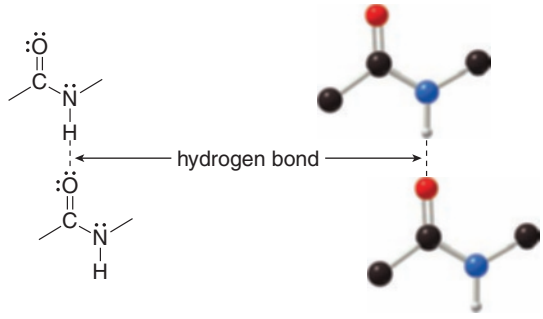
- Rotation around the amide C–N bond is *restricted* because of electron delocalization, and the s-trans conformation is the more stable arrangement.
- In each peptide bond, the N–H and C=O bonds are directed 180° from each other.



Although rotation about the amide bonds is restricted, **rotation about the other σ bonds in the protein backbone is not.** As a result, the peptide chain can twist and bend into a variety of different arrangements that constitute the secondary structure of the protein.

28.9B Secondary Structure

The three-dimensional conformations of localized regions of a protein are called its secondary structure. These regions arise due to hydrogen bonding between the N–H proton of one amide and C=O oxygen of another. Two arrangements that are particularly stable are called the **α-helix** and the **β-pleated sheet.**



α-Helix

The **α-helix** forms when a peptide chain twists into a right-handed or clockwise spiral, as shown in Figure 28.9. Four important features of the α-helix are as follows:

[1] **Each turn of the helix has 3.6 amino acids.**

[2] **The N−H and C=O bonds point along the axis of the helix.** All C=O bonds point in one direction, and all N−H bonds point in the opposite direction.

[3] **The C=O group of one amino acid is hydrogen bonded to an N−H group four amino acid residues farther along the chain.** Thus, hydrogen bonding occurs between two amino acids ***in the same chain.*** Note, too, that the hydrogen bonds are parallel to the axis of the helix.

[4] The **R groups of the amino acids extend outward** from the core of the helix.

An α-helix can form only if there is rotation about the bonds at the α carbon of the amide carbonyl group, and not all amino acids can do this. For example, proline, the amino acid whose nitrogen atom forms part of a five-membered ring, is more rigid than other amino acids, and its $C_\alpha-N$ bond cannot rotate the necessary amount. Additionally, it has no N−H proton with which to form an intramolecular hydrogen bond to stabilize the helix. Thus, proline cannot be part of an α-helix.

Both the myosin in muscle and α-keratin in hair are proteins composed almost entirely of α-helices.

β-Pleated Sheet

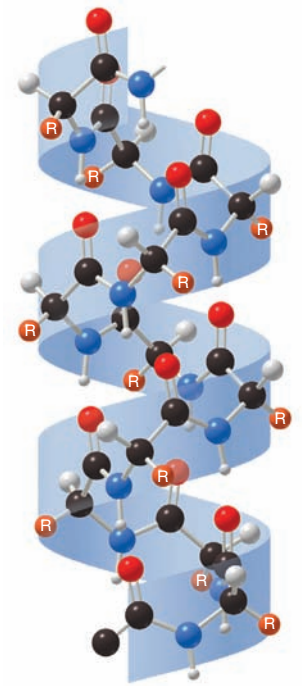
The **β-pleated sheet** secondary structure forms when two or more peptide chains, called **strands,** line up side-by-side, as shown in Figure 28.10. All β-pleated sheets have the following characteristics:

[1] **The C=O and N−H bonds lie in the plane of the sheet.**

[2] **Hydrogen bonding often occurs between the N−H and C=O groups of nearby amino acid residues.**

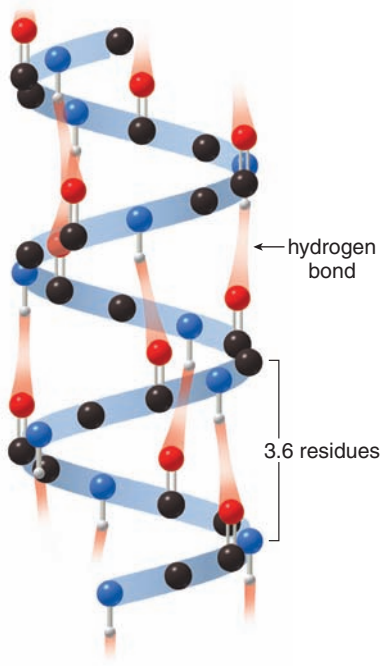
Figure 28.9 Two different illustrations of the α-helix

a. The right-handed α-helix



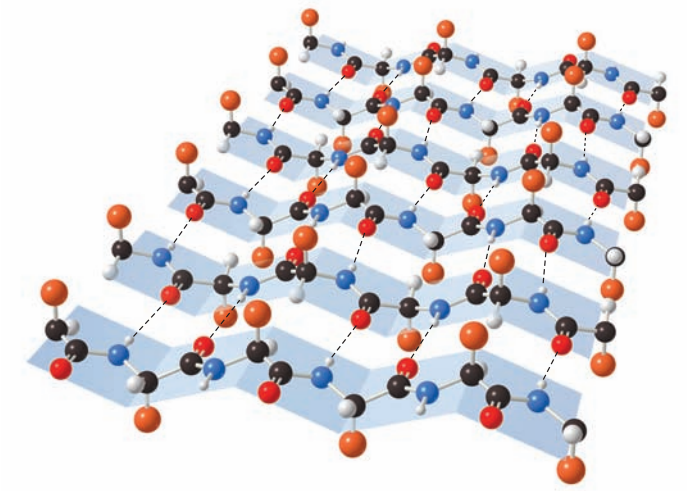
All atoms of the α-helix are drawn in this representation. All C=O bonds are pointing up and all N−H bonds are pointing down.

b. The backbone of the α-helix



Only the peptide backbone is drawn in this representation. The hydrogen bonds between the C=O and N−H of amino acids four residues away from each other are shown.

Figure 28.10
Three-dimensional structure of the β-pleated sheet



- The β-pleated sheet consists of extended strands of the peptide chains held together by hydrogen bonding. The C=O and N–H bonds lie in the plane of the sheet, and the R groups (shown as orange balls) alternate above and below the plane.

[3] The **R groups are oriented above and below the plane** of the sheet, and alternate from one side to the other along a given strand.

The β-pleated sheet arrangement most commonly occurs with amino acids with small R groups, like alanine and glycine. With larger R groups steric interactions prevent the chains from getting close together and so the sheet cannot be stabilized by hydrogen bonding.

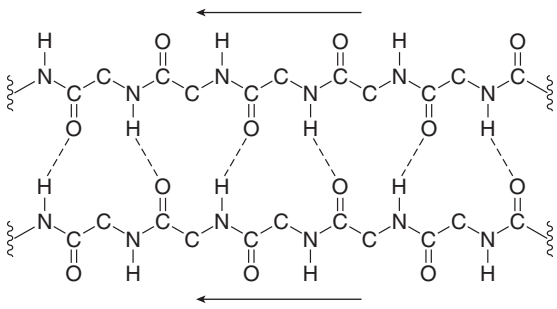
The peptide strands of β-pleated sheets can actually be oriented in two different ways, as shown in Figure 28.11.

- In a *parallel* β-pleated sheet, the strands run in the *same* direction from the N- to C-terminal amino acid.
- In an *antiparallel* β-pleated sheet, the strands run in the *opposite* direction.

Most proteins have regions of α-helix and β-pleated sheet, in addition to other regions that cannot be characterized by either of these arrangements. Shorthand symbols are often used to indicate regions of a protein that have α-helix or β-pleated sheet. A **flat helical ribbon** is used for

Figure 28.11 The parallel and antiparallel forms of the β-pleated sheet

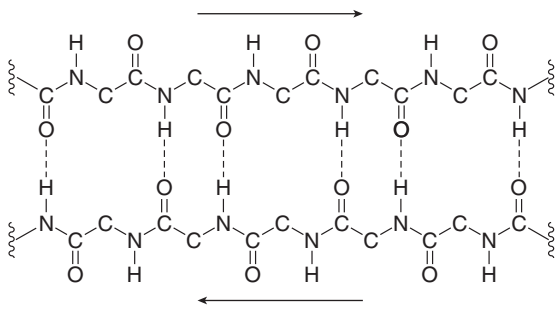
Parallel β-pleated sheet



The two peptide chains are arranged in the same direction. Hydrogen bonds occur between N–H and C=O bonds in adjacent chains.

[Note: R groups on the carbon chain are omitted for clarity.]

Antiparallel β-pleated sheet



The two peptide chains are arranged in opposite directions. Hydrogen bonding between the N–H and C=O groups still holds the two chains together.

the α-helix, and a **flat wide arrow** is used for the β-pleated sheet. These representations are often used in **ribbon diagrams** to illustrate protein structure.

α-helix shorthand β-pleated sheet shorthand

Proteins are drawn in a variety of ways to illustrate different aspects of their structure. Figure 28.12 illustrates three different representations of the protein lysozyme, an enzyme found in both plants and animals. Lysozyme catalyzes the hydrolysis of bonds in bacterial cell walls, weakening them, often causing the bacteria to burst.

Spider dragline silk is a strong yet elastic protein because it has regions of β-pleated sheet and regions of α-helix (Figure 28.13). α-Helical regions impart elasticity to the silk because the peptide chain is twisted (not fully extended), so it can stretch. β-Pleated sheet regions are almost fully extended, so they can't be stretched further, but their highly ordered three-dimensional structure imparts strength to the silk. Thus, spider silk suits the spider by comprising both types of secondary structure with beneficial properties.

Problem 28.25 Suggest a reason why antiparallel β-pleated sheets are generally more stable than parallel β-pleated sheets.

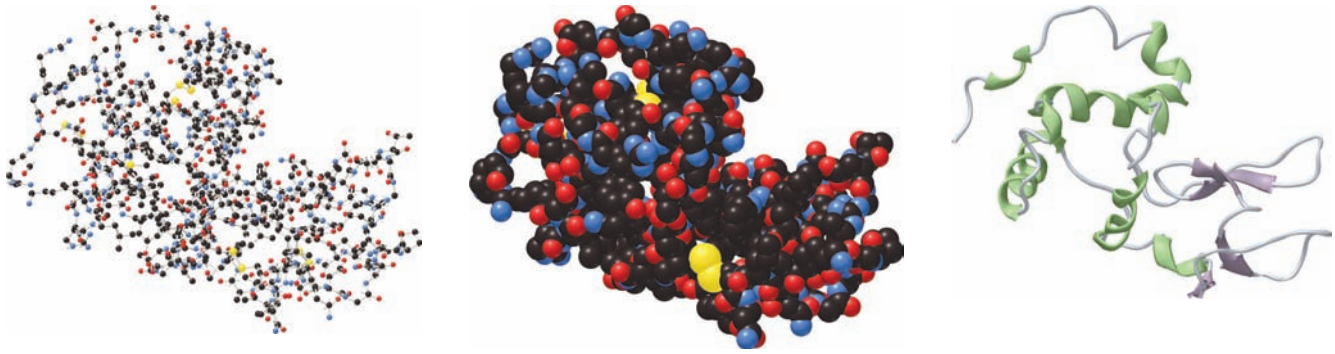
Problem 28.26 Consider two molecules of a tetrapeptide composed of only alanine residues. Draw the hydrogen bonding interactions that result when these two peptides adopt a parallel β-pleated sheet arrangement. Answer this same question for the antiparallel β-pleated sheet arrangement.

28.9C Tertiary and Quaternary Structure

The three-dimensional shape adopted by the entire peptide chain is called its tertiary structure. A peptide generally folds into a conformation that maximizes its stability. In the aqueous environment of the cell, proteins often fold in such a way as to place a large number of polar and charged groups on their outer surface, to maximize the dipole–dipole and hydrogen bonding interactions with water. This generally places most of the nonpolar side chains in the interior of the protein, where van der Waals interactions between these hydrophobic groups help stabilize the molecule, too.

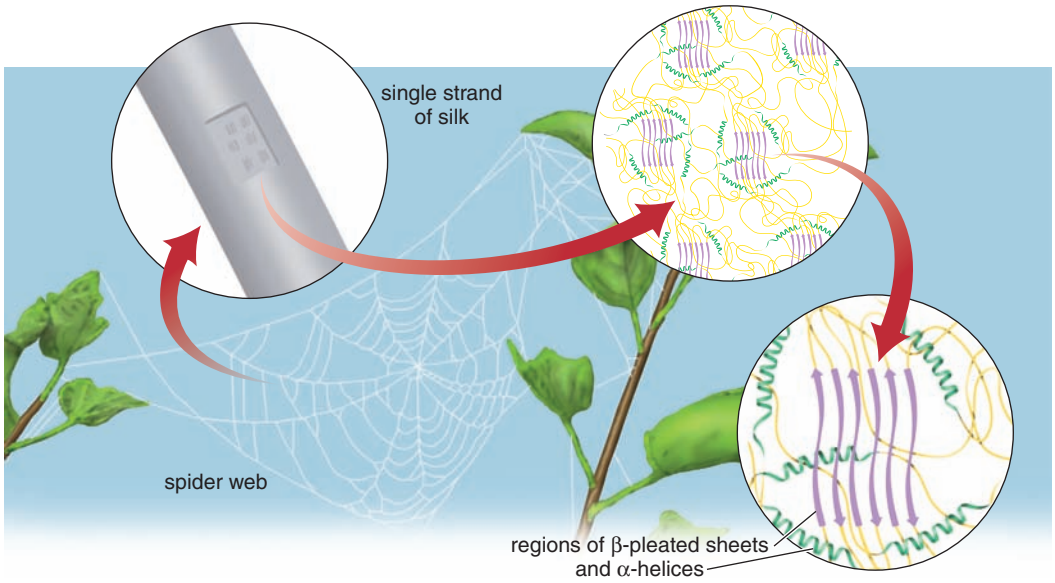
Figure 28.12 Lysozyme

a. Ball-and-stick model b. Space-filling model c. Ribbon diagram



(a) The ball-and-stick model of lysozyme shows the protein backbone with color-coded C, N, O, and S atoms. Individual amino acids are most clearly located using this representation. (b) The space-filling model uses color-coded balls for each atom in the backbone of the enzyme and illustrates how the atoms fill the space they occupy. (c) The ribbon diagram shows regions of α-helix and β-sheet that are not clearly in evidence in the other two representations.

Figure 28.13
Different regions of secondary structure in spider silk



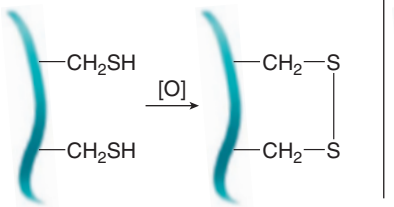
Spider silk has regions of α-helix and β-pleated sheet that make it both strong and elastic. The green coils represent the α-helical regions, and the purple arrows represent the β-pleated sheet regions. The yellow lines represent other areas of the protein that are neither α-helix nor β-pleated sheet.

In addition, polar functional groups hydrogen bond with each other (not just water), and amino acids with charged side chains like $-COO^-$ and $-NH_3^+$ can stabilize tertiary structure by electrostatic interactions.

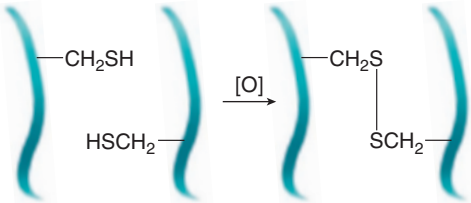
Finally, **disulfide bonds are the only covalent bonds that stabilize tertiary structure.** As previously mentioned, these strong bonds form by oxidation of two cysteine residues either on the same polypeptide chain or another polypeptide chain of the same protein.

Disulfide bonds can form in two different ways.

Between two SH groups on the same chain.



Between two SH groups on different chains.



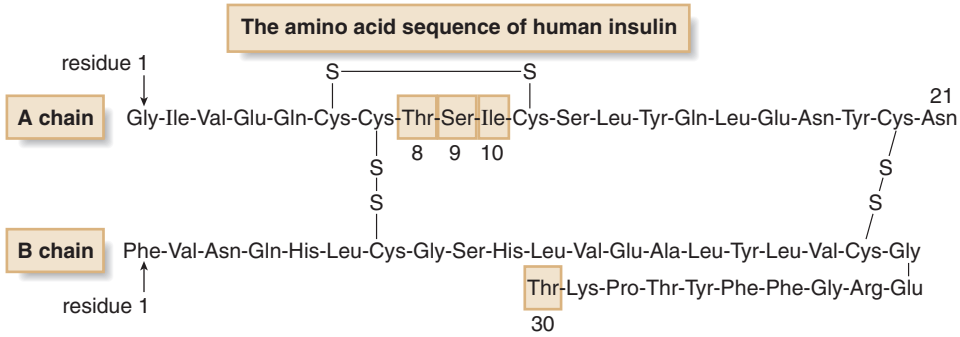
The nonapeptides **oxytocin** and **vasopressin** (Section 28.5C) contain intramolecular disulfide bonds. **Insulin,** on the other hand, consists of two separate polypeptide chains (**A** and **B**) that are covalently linked by two intermolecular disulfide bonds, as shown in Figure 28.14. The **A** chain, which also has an intramolecular disulfide bond, has 21 amino acid residues, whereas the **B** chain has 30.

Figure 28.15 schematically illustrates the many different kinds of intramolecular forces that stabilize the secondary and tertiary structures of polypeptide chains.

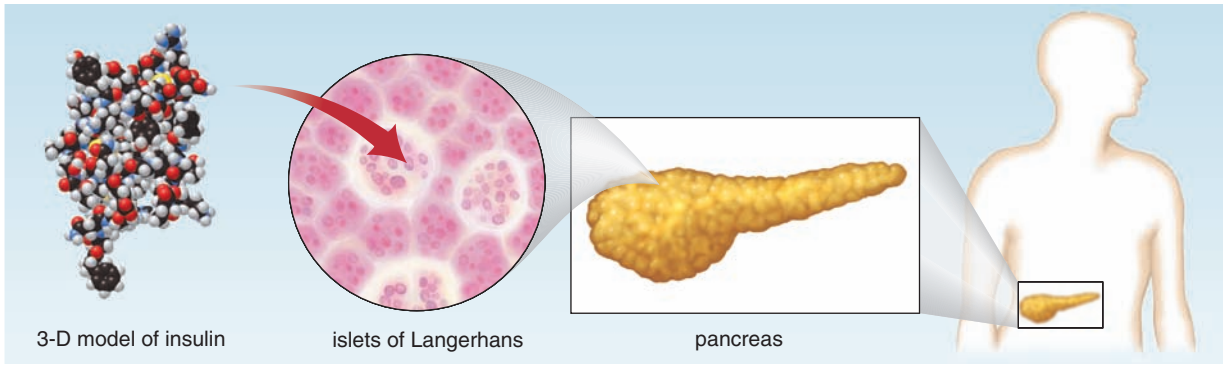
The shape adopted when two or more folded polypeptide chains aggregate into one protein complex is called the **quaternary structure** of the protein. Each individual polypeptide chain is called a **subunit** of the overall protein. **Hemoglobin,** for example, consists of two α and two β subunits held together by intermolecular forces in a compact three-dimensional shape. The unique function of hemoglobin is possible only when all four subunits are together.

The four levels of protein structure are summarized in Figure 28.16.

Figure 28.14
Insulin



Insulin is a small protein consisting of two polypeptide chains (designated as the **A** and **B** chains) held together by two disulfide bonds. An additional disulfide bond joins two cysteine residues within the **A** chain.



Synthesized by groups of cells in the pancreas called the islets of Langerhans, insulin is the protein that regulates the levels of glucose in the blood. Insufficiency of insulin results in diabetes. Many of the abnormalities associated with this disease can be controlled by the injection of insulin. Until the availability of human insulin through genetic engineering techniques, all insulin used by diabetics was obtained from pigs and cattle. The amino acid sequences of these insulin proteins is slightly different from that of human insulin. Pig insulin differs in one amino acid only, whereas bovine insulin has three different amino acids. This is shown in the accompanying table.

	Chain A			Chain B
Position of residue →	**8**	**9**	**10**	**30**
Human insulin	Thr	Ser	Ile	Thr
Pig insulin	Thr	Ser	Ile	Ala
Bovine insulin	Ala	Ser	Val	Ala

Problem 28.27 What types of stabilizing interactions exist between each of the following pairs of amino acids?

a. Ser and Tyr b. Val and Leu c. Two Phe residues

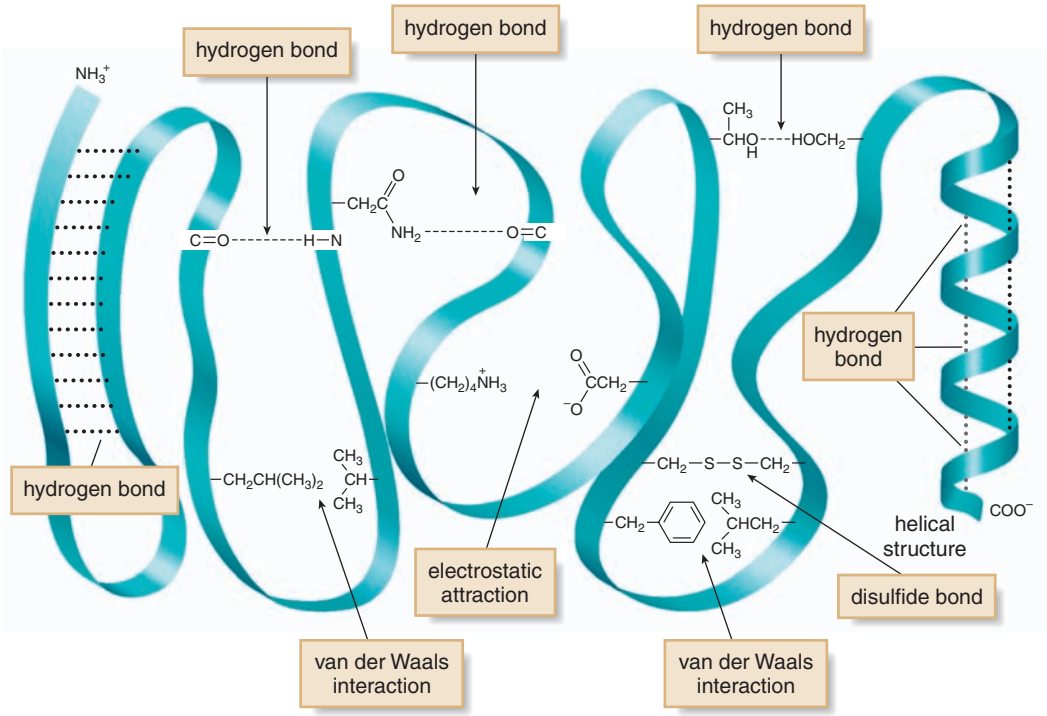
Problem 28.28 The fibroin proteins found in silk fibers consist of large regions of β-pleated sheets stacked one on top of another. (a) Explain why having a glycine at every other residue allows the β-pleated sheets to stack on top of each other. (b) Why are silk fibers insoluble in water?

28.10 Important Proteins

Proteins are generally classified according to their three-dimensional shapes.

- **Fibrous proteins** are composed of long linear polypeptide chains that are bundled together to form rods or sheets. These proteins are insoluble in water and serve structural roles, giving strength and protection to tissues and cells.
- **Globular proteins** are coiled into compact shapes with hydrophilic outer surfaces that make them water soluble. Enzymes and transport proteins are globular to make them soluble in the blood and other aqueous environments in cells.

Figure 28.15
The stabilizing interactions in secondary and tertiary protein structure



28.10A α-Keratins

α-Keratins are the proteins found in hair, hooves, nails, skin, and wool. They are composed almost exclusively of long sections of α-helix units, having large numbers of alanine and leucine residues. Because these nonpolar amino acids extend outward from the α-helix, these proteins are very water insoluble. Two α-keratin helices coil around each other, forming a structure called

Figure 28.16 The primary, secondary, tertiary, and quaternary structure of proteins

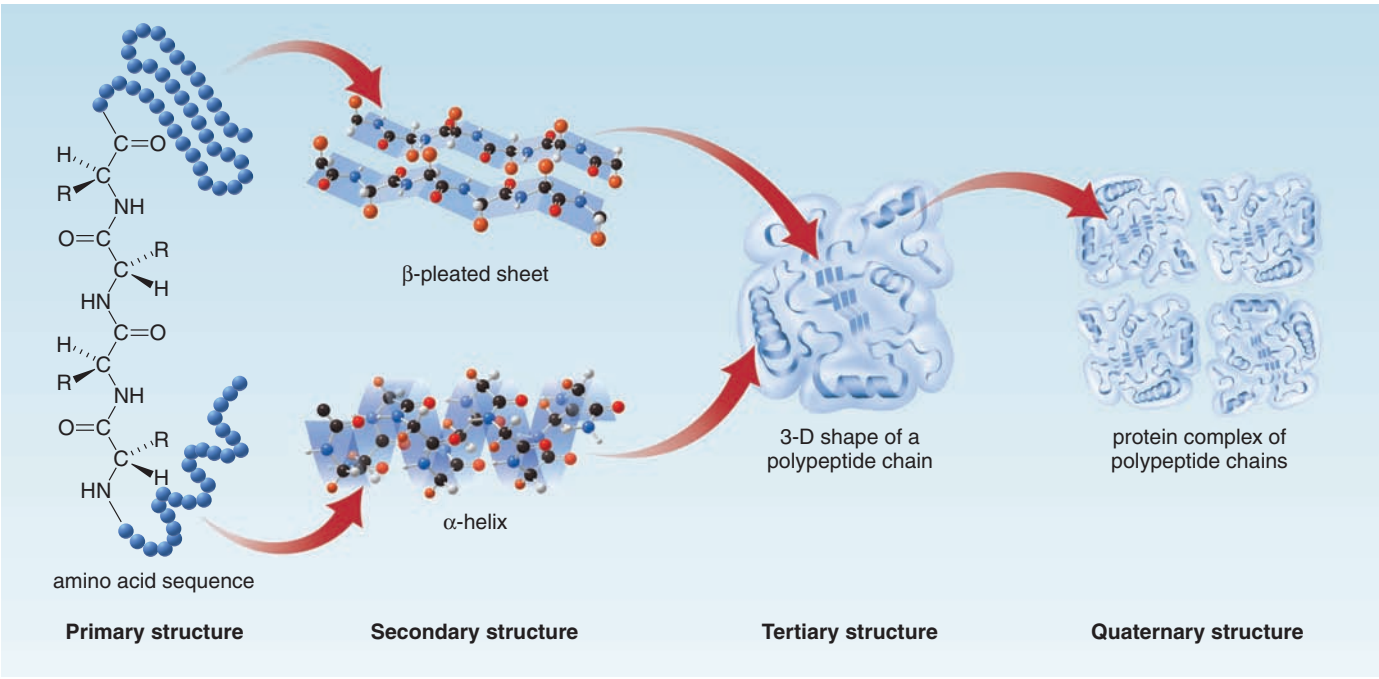
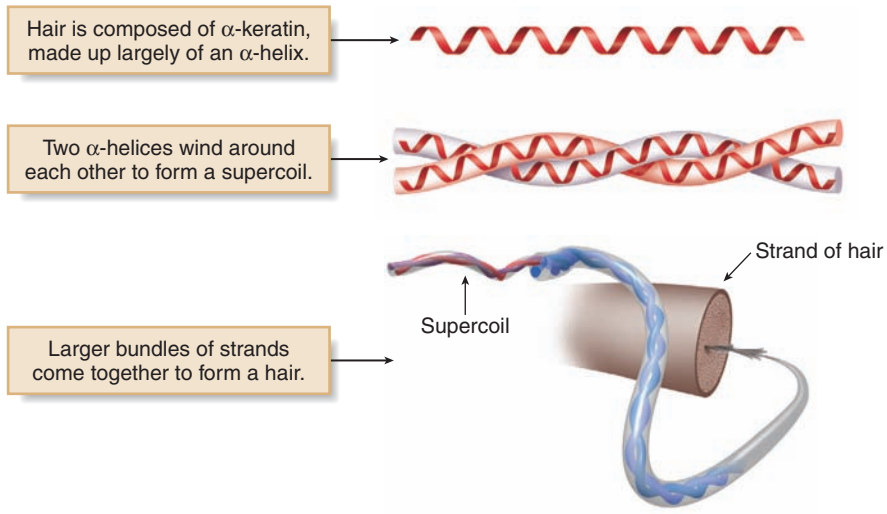


Figure 28.17
Anatomy of a hair—It begins with α-keratin.



a **supercoil** or **superhelix.** These, in turn, form larger and larger bundles of fibers, ultimately forming a strand of hair, as shown schematically in Figure 28.17.

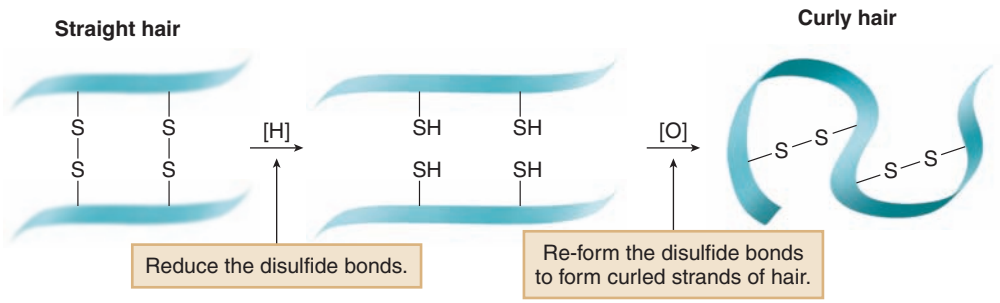
α-Keratins also have a number of cysteine residues, and because of this, disulfide bonds are formed between adjacent helices. The number of disulfide bridges determines the strength of the material. Claws, horns, and fingernails have extensive networks of disulfide bonds, making them extremely hard.

Straight hair can be made curly by cleaving the disulfide bonds in α-keratin, and then rearranging and re-forming them, as shown schematically in Figure 28.18. First, the disulfide bonds in the straight hair are reduced to thiol groups, so the bundles of α-keratin chains are no longer held in their specific "straight" orientation. Then, the hair is wrapped around curlers and treated with an oxidizing agent that converts the thiol groups back to disulfide bonds, now with twists and turns in the keratin backbone. This makes the hair look curly and is the chemical basis for a "permanent."

28.10B Collagen

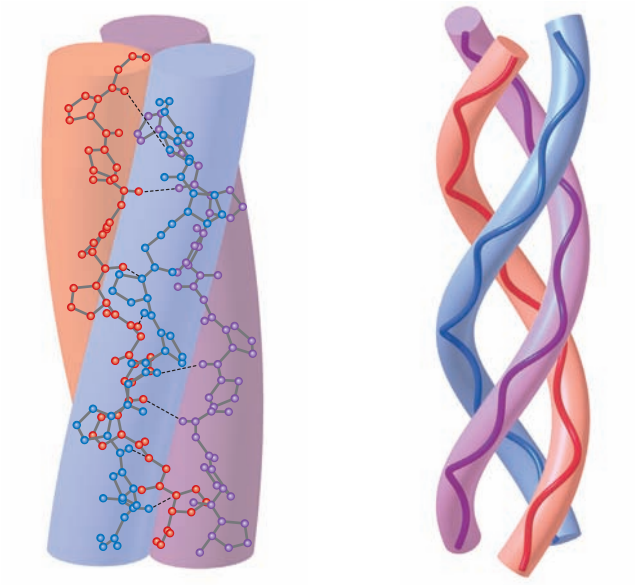
Collagen, the most abundant protein in vertebrates, is found in connective tissues such as bone, cartilage, tendons, teeth, and blood vessels. Glycine and proline account for a large fraction of its amino acid residues, whereas cysteine accounts for very little. Because of the high proline content, it cannot form a right-handed α-helix. Instead, it forms an elongated left-handed helix, and then three of these helices wind around each other to form a right-handed **superhelix** or **triple helix.** The side chain of glycine is only a hydrogen atom, so the high glycine content allows the

Figure 28.18
The chemistry of a "permanent"—Making straight hair curly



To make straight hair curly, the disulfide bonds holding the α-helical chains together are cleaved by reduction. This forms free thiol groups (–SH). The hair is turned around curlers and then an oxidizing agent is applied. This re-forms the disulfide bonds in the hair, but between different thiol groups, now giving it a curly appearance.

Figure 28.19
Two different representations for the triple helix of collagen



- In collagen, three polypeptide chains having an unusual left-handed helix wind around each other in a right-handed triple helix. The high content of small glycine residues allows the chains to lie close to each other, permitting hydrogen bonding between the chains.

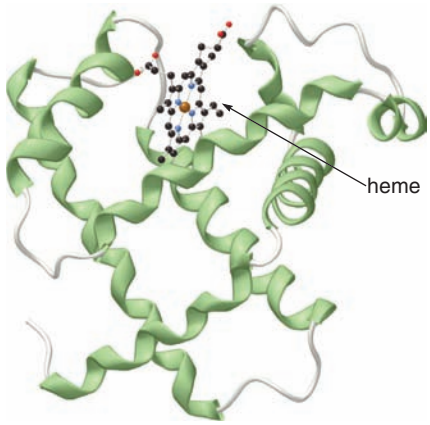
collagen superhelices to lie compactly next to each other, thus stabilizing the superhelices via hydrogen bonding. Two views of the collagen superhelix are shown in Figure 28.19.

28.10C Hemoglobin and Myoglobin

Hemoglobin and **myoglobin,** two globular proteins, are called **conjugated proteins** because they are composed of a protein unit and a nonprotein molecule called a **prosthetic group.** The prosthetic group in hemoglobin and myoglobin is **heme,** a complex organic compound containing the Fe^{2+} ion complexed with a nitrogen heterocycle called a **porphyrin.** The Fe^{2+} ion of hemoglobin and myoglobin binds oxygen in the blood. Hemoglobin, which is present in red blood cells, transports oxygen to wherever it is needed in the body, whereas myoglobin stores oxygen in tissues. Ribbon diagrams for myoglobin and hemoglobin are shown in Figure 28.20.

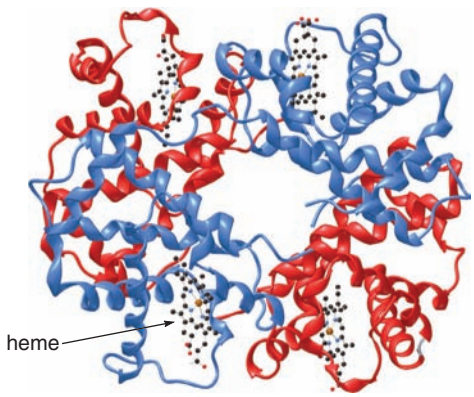
Figure 28.20
Protein ribbon diagrams for myoglobin and hemoglobin

a. Myoglobin

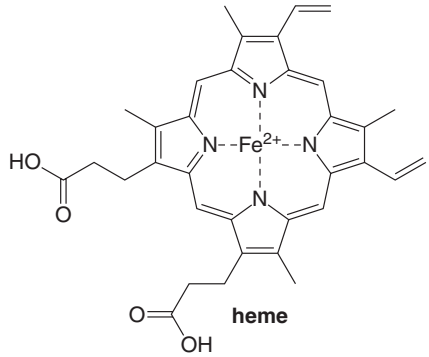


Myoglobin consists of a single polypeptide chain with a heme unit shown in a ball-and-stick model.

b. Hemoglobin

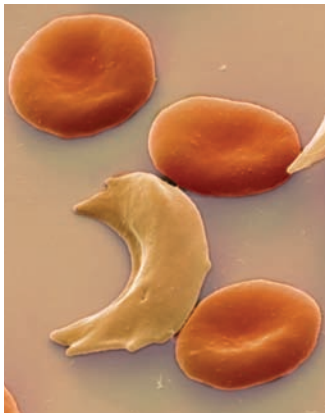


Hemoglobin consists of two α and two β chains shown in red and blue, respectively, and four heme units shown in ball-and-stick models.



heme

Myoglobin, the chapter-opening molecule, has 153 amino acid residues in a single polypeptide chain. It has eight separate α-helical sections that fold back on one another, with the prosthetic heme group held in a cavity inside the polypeptide. Most of the polar residues are found on the outside of the protein so that they can interact with the water solvent. Spaces in the interior of the protein are filled with nonpolar amino acids. Myoglobin gives cardiac muscle its characteristic red color.



When red blood cells take on a "sickled" shape in persons with sickle cell disease, they occlude capillaries (causing organ injury) and they break easily (leading to profound anemia). This devastating illness results from the change of a single amino acid in hemoglobin. Note the single sickled cell surrounded by three red cells with normal morphology.

Hemoglobin consists of four polypeptide chains (two α subunits and two β subunits), each of which carries a heme unit. Hemoglobin has more nonpolar amino acids than myoglobin. When each subunit is folded, some of these remain on the surface. The van der Waals attraction between these hydrophobic groups is what stabilizes the quaternary structure of the four subunits.

Carbon monoxide is poisonous because it binds to the Fe^{2+} of hemoglobin more strongly than does oxygen. Hemoglobin complexed with CO cannot carry O_2 from the lungs to the tissues. Without O_2 in the tissues for metabolism, cells cannot function, so they die.

The properties of all proteins depend on their three-dimensional shape, and their shape depends on their primary structure—that is, their amino acid sequence. This is particularly well exemplified by comparing normal hemoglobin with **sickle cell hemoglobin,** a mutant variation in which a single amino acid of both β subunits is changed from glutamic acid to valine. The replacement of one acidic amino acid (Glu) with one nonpolar amino acid (Val) changes the shape of hemoglobin, which has profound effects on its function. Deoxygenated red blood cells with sickle cell hemoglobin become elongated and crescent shaped, and they are unusually fragile. As a result, they do not flow easily through capillaries, causing pain and inflammation, and they break open easily, leading to severe anemia and organ damage. The end result is often a painful and premature death.

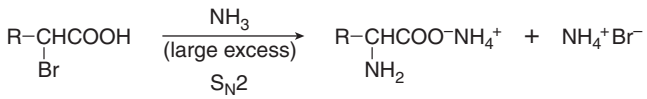
This disease, called **sickle cell anemia,** is found almost exclusively among people originating from central and western Africa, where malaria is an enormous health problem. Sickle cell hemoglobin results from a genetic mutation in the DNA sequence that is responsible for the synthesis of hemoglobin. Individuals who inherit this mutation from both parents develop sickle cell anemia, whereas those who inherit it from only one parent are said to have the sickle cell trait. They do not develop sickle cell anemia and they are more resistant to malaria than individuals without the mutation. This apparently accounts for this detrimental gene being passed on from generation to generation.

KEY CONCEPTS

Amino Acids and Proteins

Synthesis of Amino Acids (28.2)

[1] From α-halo carboxylic acids by S_N2 reaction



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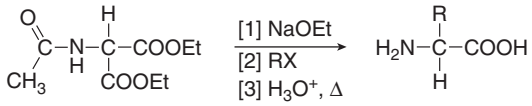
[2] By alkylation of diethyl acetamidomalonate



- Alkylation works best with unhindered alkyl halides—that is, CH_3X and RCH_2X.

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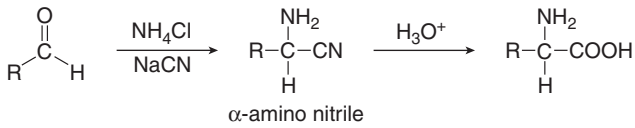
[3] Strecker synthesis



Preparation of Optically Active Amino Acids

[1] Resolution of enantiomers by forming diastereomers (28.3A)

- Convert a racemic mixture of amino acids into a racemic mixture of *N*-acetyl amino acids [(*S*)- and (*R*)-$CH_3CONHCH(R)COOH$].
- Treat the enantiomers with a chiral amine to form a mixture of diastereomers.
- Separate the diastereomers.
- Regenerate the amino acids by protonation of the carboxylate salt and hydrolysis of the *N*-acetyl group.

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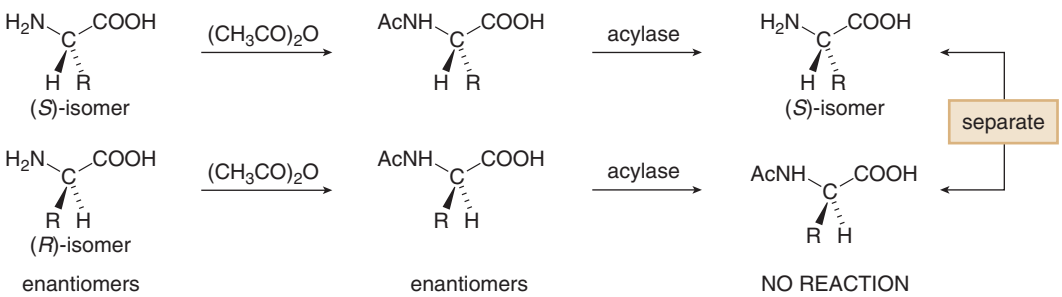
[2] Kinetic resolution using enzymes (28.3B)



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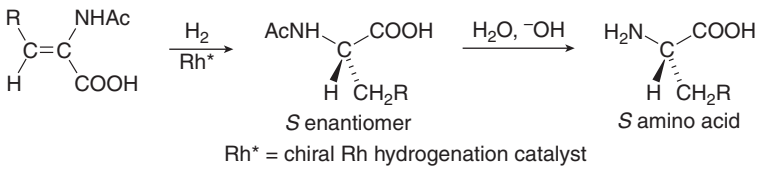
[3] By enantioselective hydrogenation (28.4)

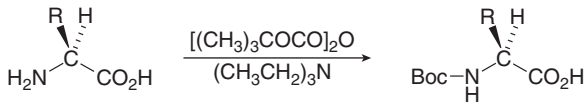


Summary of Methods Used for Peptide Sequencing (28.6)

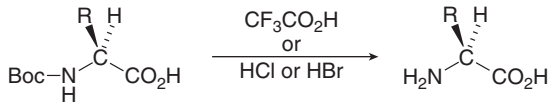
- Complete hydrolysis of all amide bonds in a peptide gives the identity and amount of the individual amino acids.
- Edman degradation identifies the N-terminal amino acid. Repeated Edman degradations can be used to sequence a peptide from the N-terminal end.
- Cleavage with carboxypeptidase identifies the C-terminal amino acid.
- Partial hydrolysis of a peptide forms smaller fragments that can be sequenced. Amino acid sequences common to smaller fragments can be used to determine the sequence of the complete peptide.
- Selective cleavage of a peptide occurs with trypsin and chymotrypsin to identify the location of specific amino acids (Table 28.2).

Adding and Removing Protecting Groups for Amino Acids (28.7)

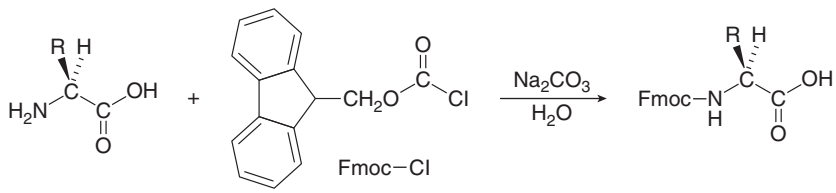
[1] Protection of an amino group as a Boc derivative



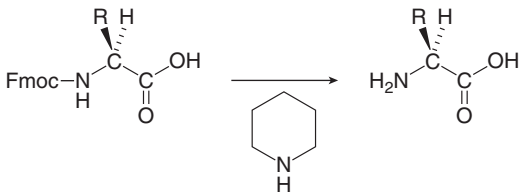
[2] Deprotection of a Boc-protected amino acid



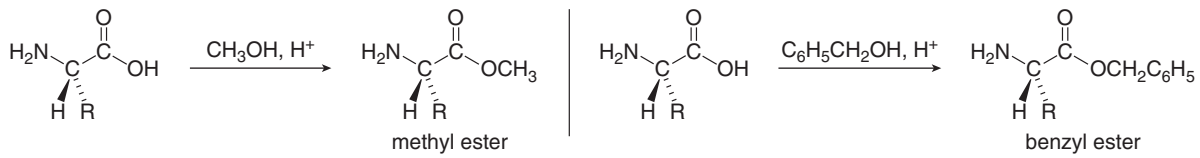
[3] Protection of an amino group as an Fmoc derivative



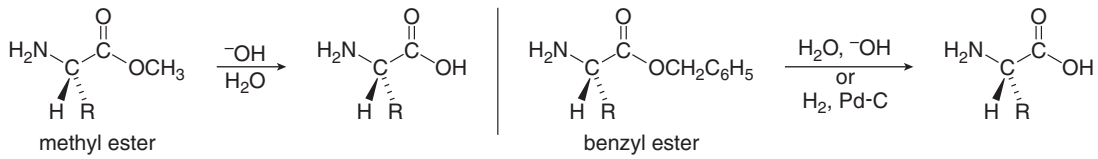
[4] Deprotection of an Fmoc-protected amino acid



[5] Protection of a carboxy group as an ester

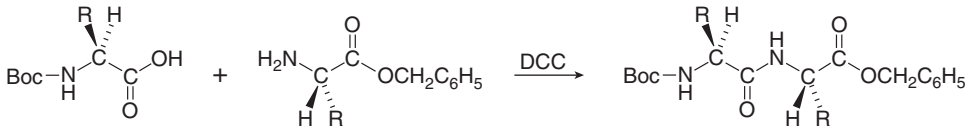


[6] Deprotection of an ester group



Synthesis of Dipeptides (28.7)

[1] Amide formation with DCC



[2] Four steps are needed to synthesize a dipeptide:

a. **Protect** the amino group of one amino acid with a Boc or Fmoc group.
b. **Protect** the carboxy group of the second amino acid as an ester.
c. Form the amide bond with **DCC.**
d. **Remove both protecting groups** in one or two reactions.

Summary of the Merrifield Method of Peptide Synthesis (28.8)

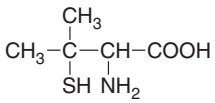
[1] Attach an Fmoc-protected amino acid to a polymer derived from polystyrene.
[2] Remove the Fmoc protecting group.
[3] Form the amide bond with a second Fmoc-protected amino acid by using DCC.
[4] Repeat steps [2] and [3].
[5] Remove the protecting group and detach the peptide from the polymer.

PROBLEMS

Amino Acids

28.29 Explain why L-alanine has the *S* configuration but L-cysteine has the *R* configuration.

28.30



penicillamine

a. (*S*)-Penicillamine, an amino acid that does not occur in proteins, is used as a copper chelating agent to treat Wilson's disease, an inherited defect in copper metabolism. (*R*)-Penicillamine is toxic, sometimes causing blindness. Draw the structures of (*R*)- and (*S*)-penicillamine.
b. What disulfide is formed from oxidation of L-penicillamine?

28.31 Explain why amino acids are insoluble in diethyl ether but *N*-acetyl amino acids are soluble.

28.32 Histidine is classified as a basic amino acid because one of the N atoms in its five-membered ring is readily protonated by acid. Which N atom in histidine is protonated and why?

28.33 Tryptophan is not classified as a basic amino acid even though it has a heterocycle containing a nitrogen atom. Why is the N atom in the five-membered ring of tryptophan not readily protonated by acid?

28.34 What is the structure of each amino acid at its isoelectric point: (a) alanine; (b) methionine; (c) aspartic acid; (d) lysine?

28.35 To calculate the isoelectric point of amino acids having other ionizable functional groups, we must also take into account the pK_a of the additional functional group in the side chain.

For an acidic amino acid (one with an additional acidic OH group):

$$pI = \frac{pK_a\ (\alpha\text{-COOH}) + pK_a\ (\text{second COOH})}{2}$$

For a basic amino acid (one with an additional basic NH group):

$$pI = \frac{pK_a\ (\alpha\text{-}NH_3^+) + pK_a\ (\text{side chain NH})}{2}$$

a. Indicate which pK_a values must be used to calculate the pI of each of the following amino acids: [1] glutamic acid; [2] lysine; [3] arginine.
b. In general, how does the pI of an acidic amino acid compare to that of a neutral amino acid?
c. In general, how does the pI of a basic amino acid compare to the pI of a neutral amino acid?

28.36 What is the predominant form of each of the following amino acids at pH = 1? What is the overall charge on the amino acid at this pH? (a) threonine; (b) methionine; (c) aspartic acid; (d) arginine

28.37 What is the predominant form of each of the following amino acids at pH = 11? What is the overall charge on the amino acid? (a) valine; (b) proline; (c) glutamic acid; (d) lysine

28.38 a. Draw the structure of the tripeptide A–A–A, and label the two ionizable functional groups.
b. What is the predominant form of A–A–A at pH = 1?
c. The pK_a values for the two ionizable functional groups (3.39 and 8.03) differ considerably from the pK_a values of alanine (2.35 and 9.87; see Table 28.1). Account for the observed pK_a differences.

Synthesis and Reactions of Amino Acids

28.39 Draw the organic product formed when the amino acid leucine is treated with each reagent.

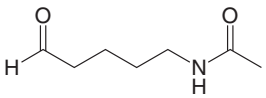
a. CH_3OH, H^+
b. CH_3COCl, pyridine
c. $C_6H_5CH_2OH$, H^+
d. Ac_2O, pyridine
e. HCl (1 equiv)
f. NaOH (1 equiv)
g. C_6H_5COCl, pyridine
h. $[(CH_3)_3COCO]_2O$, $(CH_3CH_2)_3N$
i. The product in (d), then $NH_2CH_2COOCH_3$ + DCC
j. The product in (h), then $NH_2CH_2COOCH_3$ + DCC
k. Fmoc–Cl, Na_2CO_3, H_2O
l. $C_6H_5N{=}C{=}S$

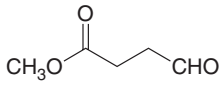
28.40 Answer Problem 28.39 using phenylalanine as a starting material.

28.41 Draw the organic products formed in each reaction.

a. $(CH_3)_2CHCH_2\underset{\displaystyle Br}{\underset{|}{C}}HCOOH \xrightarrow[\text{excess}]{NH_3}$

b. $CH_3CONHCH(COOEt)_2 \xrightarrow[\text{[2] } CH_3\text{-}\underset{\displaystyle CH_3}{\underset{|}{C}}(=O)\text{-}O\text{-}C_6H_4\text{-}CH_2Br]{\text{[1] NaOEt}}$ then [3] H_3O^+, Δ

c.  $\xrightarrow[\text{[2] } H_3O^+]{\text{[1] } NH_4Cl,\ NaCN}$

d.  $\xrightarrow[\text{[2] } H_3O^+]{\text{[1] } NH_4Cl,\ NaCN}$

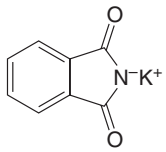
e. $CH_3CONHCH(COOEt)_2 \xrightarrow[\text{[2] } ClCH_2CH_2CH_2CH_2NHAc]{\text{[1] NaOEt}}$ then [3] H_3O^+, Δ

28.42 What alkyl halide is needed to synthesize each amino acid from diethyl acetamidomalonate: (a) Asn; (b) His; (c) Trp?

28.43 Devise a synthesis of threonine from diethyl acetamidomalonate.

28.44 Devise a synthesis of each amino acid from acetaldehyde (CH_3CHO): (a) glycine; (b) alanine.

28.45 Identify the lettered intermediates in the following reaction scheme. This is an alternative method to synthesize amino acids, based on the Gabriel synthesis of 1° amines (Section 25.7A).

$$CH_2(COOEt)_2 \xrightarrow[CH_3COOH]{Br_2} \mathbf{A} \xrightarrow{\text{  }} \mathbf{B} \xrightarrow[\text{[2] } ClCH_2CH_2SCH_3]{\text{[1] NaOEt}} \mathbf{C} \xrightarrow[\text{[2] } H_3O^+,\ \Delta]{\text{[1] NaOH, } H_2O} \mathbf{D}$$

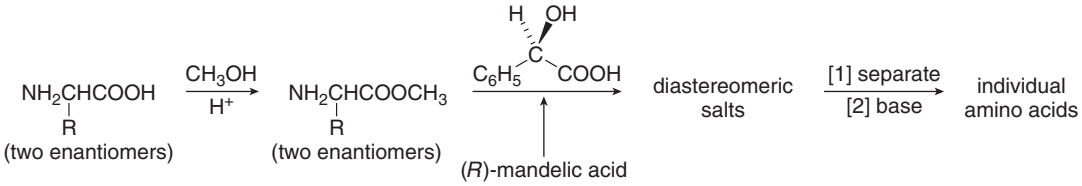
28.46 Glutamic acid is synthesized by the following reaction sequence. Draw a stepwise mechanism for Steps [1]–[3].

$$CH_3CONHCH(COOEt)_2 \xrightarrow[\text{[2] } CH_2{=}CHCOOEt\ \text{[3] } H_3O^+]{\text{[1] NaOEt}} CH_3CONH\text{-}\underset{\displaystyle CH_2CH_2COOEt}{\underset{|}{C}}(COOEt)\text{-}COOEt \xrightarrow[\Delta]{H_3O^+} \underset{\text{glutamic acid}}{H_2N\text{-}\underset{\displaystyle CH_2COOH}{\underset{|}{CH_2}}\text{-}CHCOOH}$$

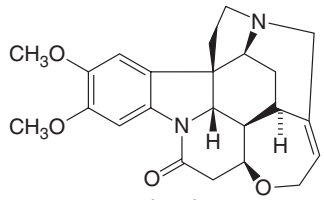
Resolution; The Synthesis of Chiral Amino Acids

28.47 Write out a scheme for the resolution of the two enantiomers of racemic lactic acid [$CH_3CH(OH)COOH$] using (*R*)-α-methylbenzylamine as resolving agent.

28.48 Another strategy used to resolve amino acids involves converting the carboxy group to an ester and then using a *chiral carboxylic acid* to carry out an acid–base reaction at the free amino group. The general plan is drawn below using (*R*)-mandelic acid as resolving agent. Using a racemic mixture of alanine enantiomers and (*R*)-mandelic acid as resolving agent, write out the steps showing how a resolution process would occur.

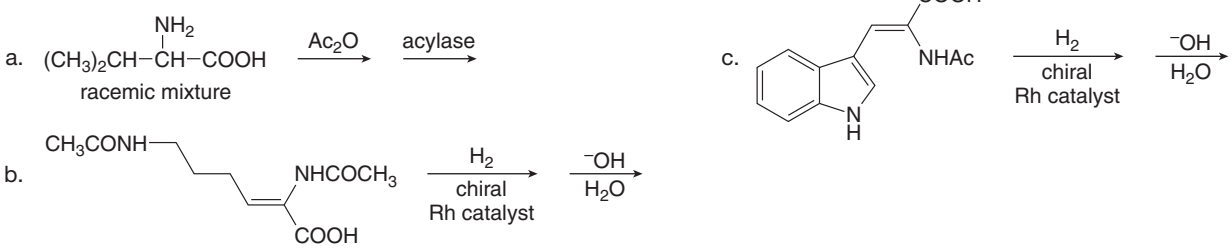


28.49 Brucine is a poisonous alkaloid obtained from *Strychnos nux vomica,* a tree that grows in India, Sri Lanka, and northern Australia. Write out a resolution scheme similar to the one given in Section 28.3A, which shows how a racemic mixture of phenylalanine can be resolved using brucine.

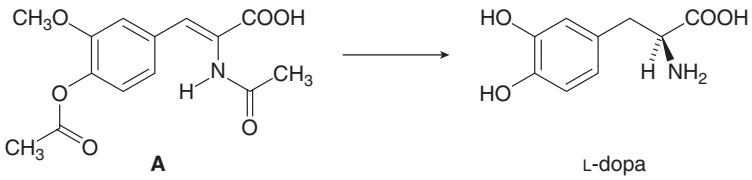


brucine

28.50 Draw the organic products formed in each reaction.



28.51 What two steps are needed to convert **A** to L-dopa, an uncommon amino acid that is effective in treating Parkinson's disease? These two steps are the key reactions in the first commercial asymmetric synthesis using a chiral transition metal catalyst. This process was developed at Monsanto in 1974.



A L-dopa

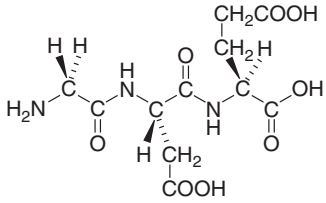
Peptide Structure and Sequencing

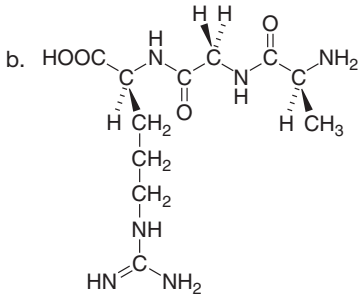
28.52 Draw the structure for each peptide: (a) Phe–Ala; (b) Gly–Gln; (c) Lys–Gly; (d) R – H.

28.53 For each tetrapeptide [1] Ala–Gln–Cys–Ser; [2] Asp–Arg–Val–Tyr:

a. Name the peptide using one-letter abbreviations.
b. Draw the structure.
c. Label all amide bonds.
d. Label the N-terminal and C-terminal amino acids.

28.54 Name each peptide using both the three-letter and one-letter abbreviations of the component amino acids.

a. 

b. 

28.55 Explain why a peptide C–N bond is stronger than an ester C–O bond.

28.56 Draw the *s*-trans and *s*-cis conformations of the peptide bond in the dipeptide Ala–Ala.

28.57 Draw the amino acids and peptide fragments formed when the decapeptide A–P–F–L–K–W–S–G–R–G is treated with each reagent or enzyme: (a) chymotrypsin; (b) trypsin; (c) carboxypeptidase; (d) $C_6H_5N{=}C{=}S$.

28.58 Give the amino acid sequence of each peptide using the fragments obtained by partial hydrolysis of the peptide with acid.

a. A tetrapeptide that contains Ala, Gly, His, and Tyr, which is hydrolyzed to the dipeptides His–Tyr, Gly–Ala, and Ala–His.
b. A pentapeptide that contains Glu, Gly, His, Lys, and Phe, which is hydrolyzed to His–Gly–Glu, Gly–Glu–Phe, and Lys–His.

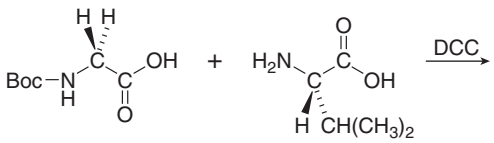
28.59 Angiotensin is an octapeptide that narrows blood vessels, thereby increasing blood pressure. ACE inhibitors are a group of drugs used to treat high blood pressure by blocking the synthesis of angiotensin in the body. Angiotensin contains the amino acids Arg (2 equiv), His, Ile, Phe, Pro, Tyr, and Val. Determine the sequence of angiotensin using the following fragments obtained by partial hydrolysis with acid: Ile–His–Pro–Phe, Arg–Arg–Val, Tyr–Ile–His, and Val–Tyr.

28.60 Use the given experimental data to deduce the sequence of an octapeptide that contains the following amino acids: Ala, Gly (2 equiv), His (2 equiv), Ile, Leu, and Phe. Edman degradation cleaves Gly from the octapeptide, and carboxypeptidase forms Leu and a heptapeptide. Partial hydrolysis forms the following fragments: Ile–His–Leu, Gly, Gly–Ala–Phe–His, and Phe–His–Ile.

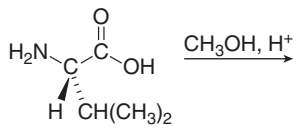
28.61 An octapeptide contains the following amino acids: Arg, Glu, His, Ile, Leu, Phe, Tyr, and Val. Carboxypeptidase treatment of the octapeptide forms Phe and a heptapeptide. Treatment of the octapeptide with chymotrypsin forms two tetrapeptides, **A** and **B.** Treatment of **A** with trypsin yields two dipeptides, **C** and **D.** Edman degradation cleaves the following amino acids from each peptide: Glu (octapeptide), Glu (**A**), Ile (**B**), Glu (**C**), and Val (**D**). Partial hydrolysis of tetrapeptide **B** forms Ile–Leu in addition to other products. Deduce the structure of the octapeptide and fragments **A–D.**

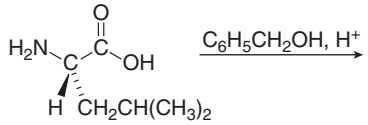
Peptide Synthesis

28.62 Draw all the products formed in the following reaction.



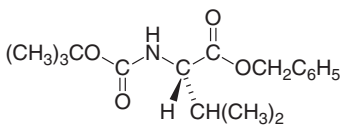
28.63 Draw the organic products formed in each reaction.

a. 

b. 

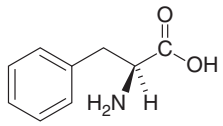
c. NH_2CH_2COOH $\xrightarrow[(CH_3CH_2)_3N]{[(CH_3)_3COCO]_2O}$

d. product in (b) + product in (c) $\xrightarrow{DCC}$

e. 

f. starting material in (e) $\xrightarrow[CH_3COOH]{HBr}$

g. product in (e) $\xrightarrow{CF_3COOH}$

h.  + Fmoc–Cl $\xrightarrow[H_2O]{Na_2CO_3}$

28.64 Draw all the steps in the synthesis of each peptide from individual amino acids: (a) Gly–Ala; (b) Phe–Leu; (c) Ile–Ala–Phe.

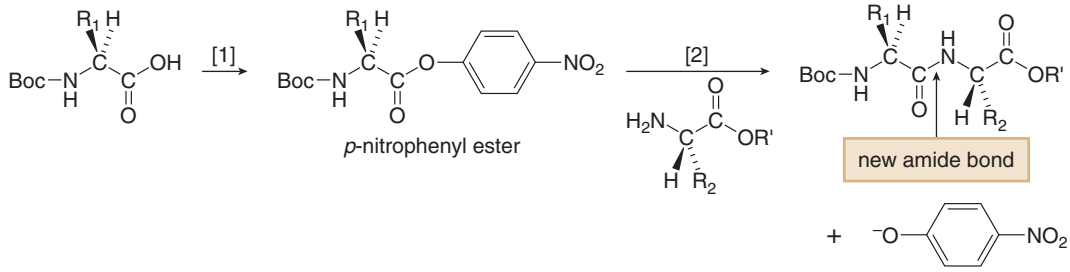
28.65 Write out the steps for the synthesis of each peptide using the Merrifield method: (a) Ala–Leu–Phe–Phe; (b) Phe–Gly–Ala–Ile.

28.66 An amino acid [$RCH(NH_2)COOH$] can readily be converted to an *N*-acetyl amino acid [$RCH(NHCOCH_3)COOH$] using acetic anhydride. Why can't this acetyl group be used as an amino protecting group, in place of the Boc group, for peptide synthesis?

28.67 Another method to form a peptide bond involves a two-step process:

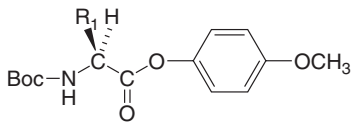
[1] Conversion of a Boc-protected amino acid to a *p*-nitrophenyl ester.

[2] Reaction of the *p*-nitrophenyl ester with an amino acid ester.



a. Why does a *p*-nitrophenyl ester "activate" the carboxy group of the first amino acid to amide formation?

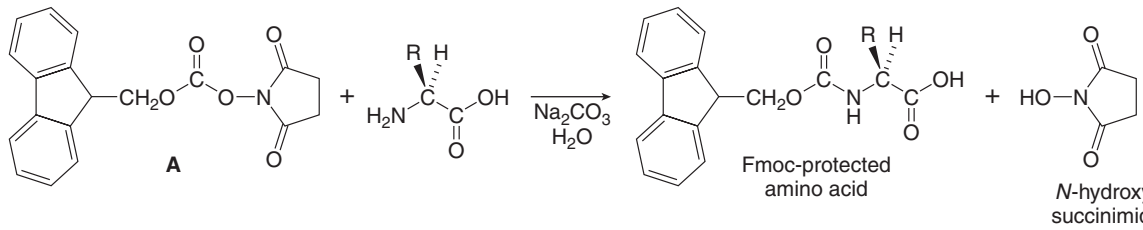
b. Would a *p*-methoxyphenyl ester perform the same function? Why or why not?



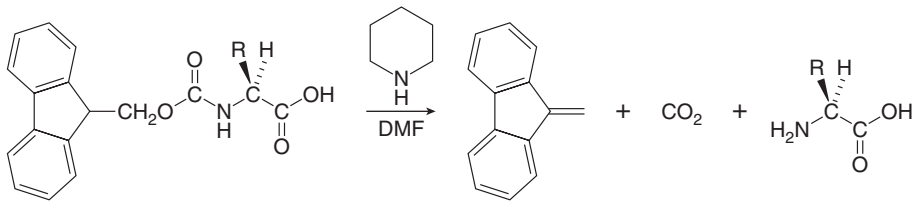
p-methoxyphenyl ester

28.68 In addition to forming an Fmoc-protected amino acid using Fmoc–Cl, an Fmoc protecting group can also be added to an amino group using reagent **A.**

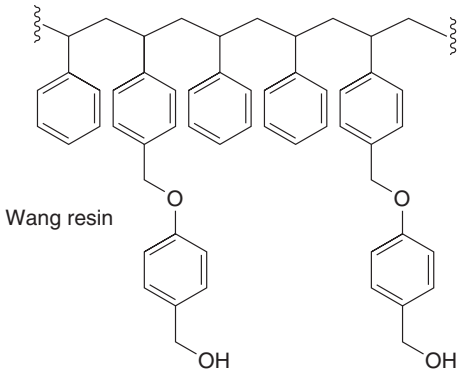
a. Draw the mechanism for the following reaction that adds an Fmoc group to an amino acid.



b. Draw the mechanism for the reaction that removes an Fmoc group from an amino acid under the following conditions:



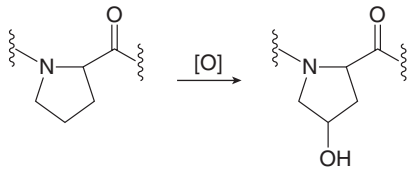
28.69 Many different insoluble polymers, called resins, are currently available for automated peptide synthesis. For example, the Wang resin contains benzene rings substituted with $-CH_2OH$ groups that serve as sites of attachment for amino acids. Propose reaction conditions that would bind an Fmoc-protected amino acid to a Wang resin. What reaction conditions could be used to remove the polypeptide from the resin after the synthesis is complete?



Proteins

28.70 Which of the following amino acids are typically found in the interior of a globular protein, and which are typically found on the surface: (a) phenylalanine; (b) aspartic acid; (c) lysine; (d) isoleucine; (e) arginine; (f) glutamic acid?

28.71 After the peptide chain of collagen has been formed, many of the proline residues are hydroxylated on one of the ring carbon atoms. Why is this process important for the triple helix of collagen?

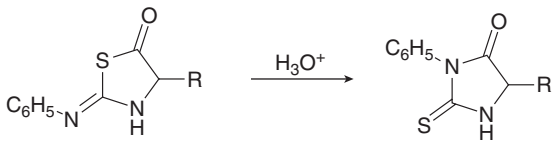


Challenge Problems

28.72 Devise a stepwise synthesis of the tripeptide Val–Leu–Val from 3-methylbutanal $[(CH_3)_2CHCH_2CHO]$ as the only organic starting material. You may also use any required inorganic or organic reagents.

28.73 Besides asymmetric hydrogenation (Section 28.4), several other methods are now available for the synthesis of optically active amino acids. How might a reaction like the Strecker synthesis be adapted to the preparation of chiral amino acids?

28.74 As shown in Mechanism 28.2, the final steps in the Edman degradation result in rearrangement of a thiazolinone to an *N*-phenylthiohydantoin. Draw a stepwise mechanism for this acid-catalyzed reaction.



thiazolinone *N*-phenylthiohydantoin

Carbohydrate Metabolism

Objectives

- Study utilization of glucose and other carbohydrates in the body
- Study the various mechanisms and fate of glucose in the body
- Study the energetics of the various mechanisms

*Fates of dietary glucose

The major source of dietary carbohydrate for humans is starch from consumed plant materials. This is supplemented with a small amount of glycogen from animal tissue, disaccharides such as sucrose from products containing refined sugar and lactose in milk.

Digestion in the gut converts all carbohydrate to monosaccharides which are transported to the liver and converted to glucose. The liver has a central role in the storage and distribution within the body of all fuels, including glucose.

Glucose in the body undergoes one of three metabolic fates :

1- It is catabolised to produce ATP

This occurs in all peripheral tissues, particularly in brain, muscle and kidney.

2- It is stored as glycogen

This storage occurs in liver and muscle.

3- It is converted to fatty acids

Once converted to fatty acids, these are stored in adipose tissue as triglycerides.

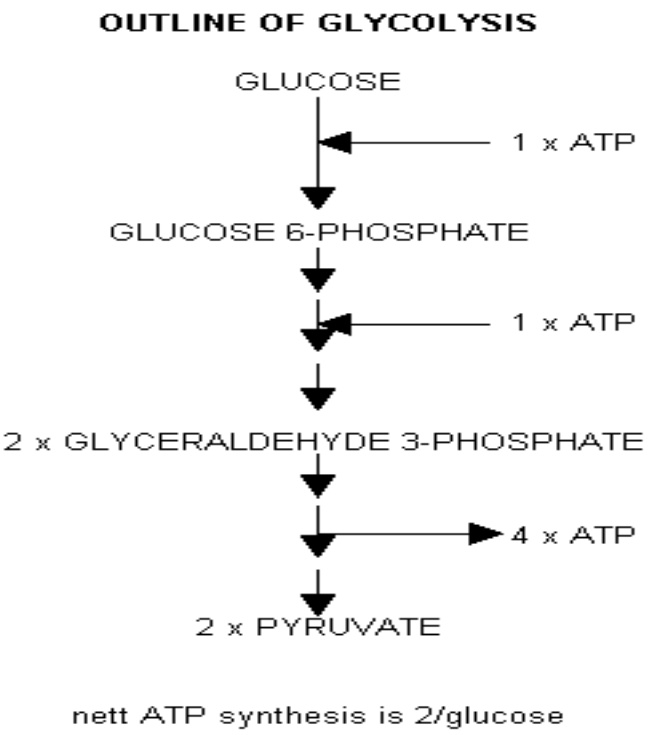
Glucose Metabolism

Glucose will be oxidized by all tissues to synthesis ATP. The first pathway which begins the complete oxidation of glucose is called **glycolysis.**

Glycolysis:

This pathway cleaves the six carbon glucose molecule ($C_6H_{12}O_6$) into two molecules of the three carbon compound pyruvate ($C_3H_3O_3^-$). This oxidation is coupled to the net production of two molecules of ATP/glucose.

The diagram below shows an outline of glycolysis.



One oxidation reaction occurs in the latter part of the pathway. It uses NAD as the electron acceptor. This cofactor is present only in limited amounts and once reduced to NADH, as in this reaction, it must be re-oxidized to NAD to permit continuation of the pathway.

This re-oxidation occurs by one of two methods :

Anaerobic glycolysis

pyruvate which resulted from glycolysis is reduced to a compound called lactate

This single reaction occurs in the absence of oxygen (anaerobically) and is ideally suited to utilization in heavily exercising muscle where oxygen supply is often insufficient to meet the demands of aerobic metabolism. The reduction of pyruvate to lactate is coupled to the oxidation of NADH to NAD.

The formation of lactate as an end product from glucose extracts only a relatively small amount of the bond energy contained in glucose. Accumulation of lactate (actually lactic acid) also causes a reduction in intracellular pH.

The lactate formed is removed to other tissues and dealt with by one of two mechanisms :

1- It is converted back to pyruvate

2- It is converted back to glucose in the liver.

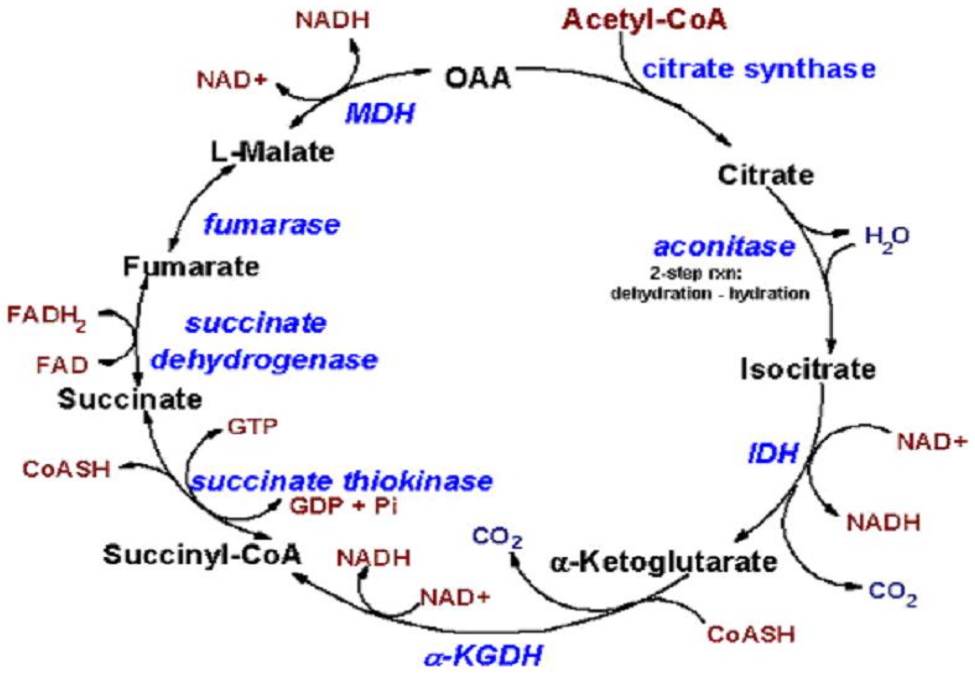
* Aerobic metabolism of glucose

pyruvate is transported inside **mitochondria** and oxidized to a compound called **acetyl coenzyme A** (abbreviated to "acetyl CoA"). This is an oxidation reaction and uses NAD as an electron acceptor.

By a further series of reactions collectively called the **citric acid cycle (CAC)** or also known tricarboxylic acid cycle (TCA), acetyl CoA is oxidized ultimately to CO_2.

Citric acid cycle is a chain of reactions occurring in the mitochondria, through which almost all living cells produce energy in aerobic respiration. It uses oxygen and gives out water and carbon dioxide as products.

These reactions are coupled to a process known as the **electron transport chain** which has the role of harnessing chemical bond energy from a series of oxidation/reduction reactions to the synthesis of ATP and simultaneously re-oxidizing NADH to NAD.



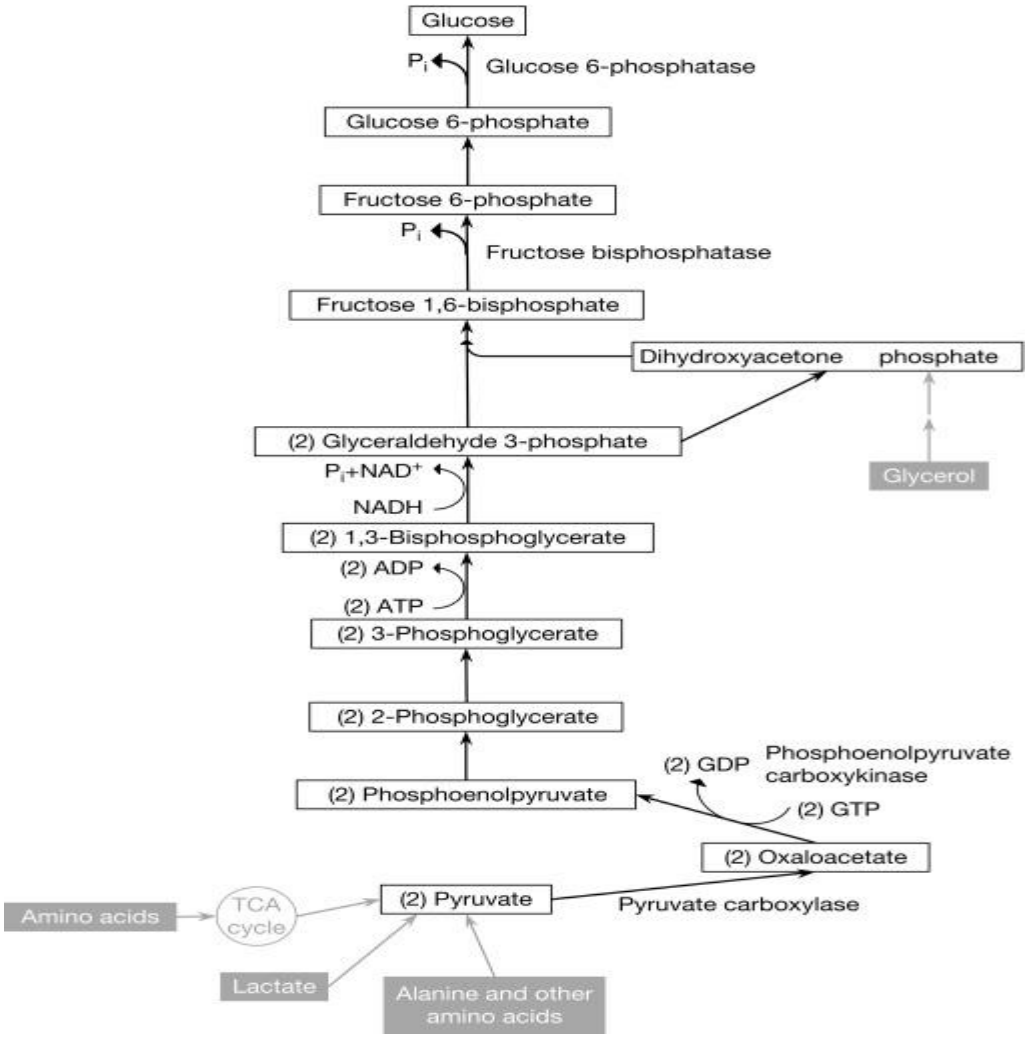
Gluconeogenesis

Gluconeogenesis refers to **synthesis of new glucose from noncarbohydrate precursors, provides glucose when dietary intake is insufficient or absent**. It also is essential in the regulation of acid-base balance, amino acid metabolism, and synthesis of carbohydrate derived structural components.

During a prolonged fast or vigorous exercise, glycogen stores become depleted, and glucose must be synthesized by gluconeogenesis principally in the liver and kidneys in order to maintain blood glucose levels from precursors such as glycerol, lactate, pyruvate, and glucogenic

amino acids. Gluconeogenesis is stimulated by the diabetogenic hormones (glucagon, growth hormone, epinephrine, and cortisol).

Insulin and glucagon are the most important hormones regulating hepatic gluconeogenesis. Hepatic glucose production is a sum of gluconeogenesis, which is the formation of glucose from pyruvate or other 3- or 4-carbon compounds, and glycogenolysis, which is the breakdown of glycogen to glucose.

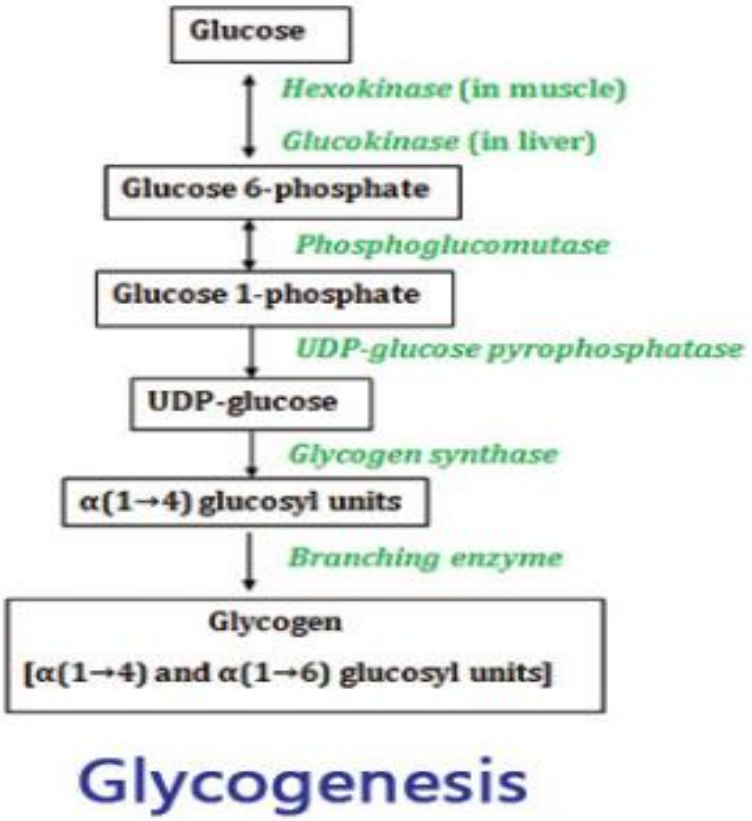


glycogenesis

glycogenesis, the formation of glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, from glucose. Glycogenesis takes place when blood glucose levels are sufficiently high to allow excess glucose to be stored in liver and muscle cells.

When the cells have depleted all their glucose and are not receiving more from the body, they can turn to their stores of glycogen. Muscle cells, for example, commonly use glycogenesis to provide energy while exercising, because the blood glucose concentrations are not sufficient.

The process usually occurs in the liver. It is important to note that the process of glycogenesis can also be activated by the peptide hormone insulin in order to respond to relatively high glucose levels in the body.

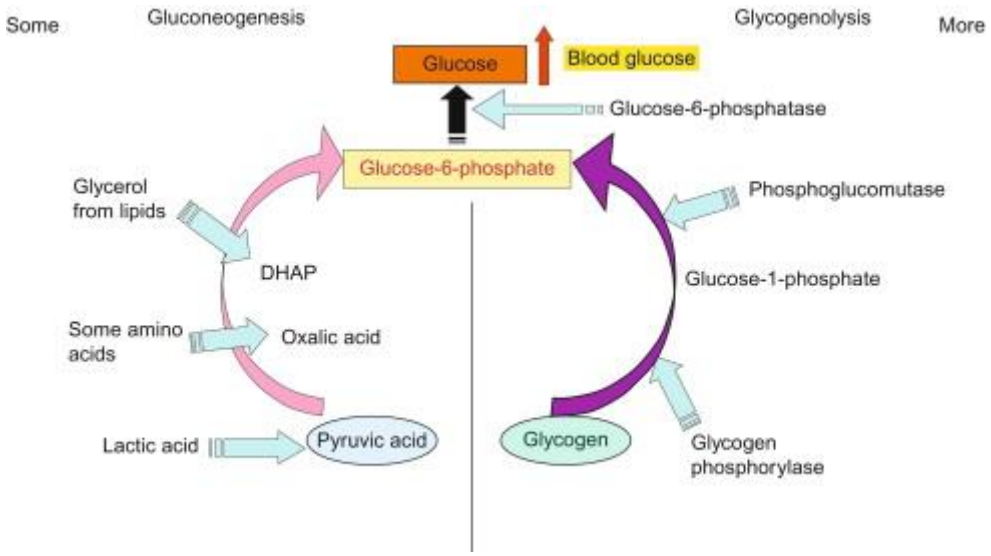


Glycogenesis

Glycogenolysis

Glycogenolysis is the biochemical pathway in which glycogen breaks down into glucose-1-phosphate and glucose. The reaction takes place in the hepatocytes and the myocytes. The process is under the regulation of two key enzymes: phosphorylase kinase and glycogen phosphorylase.

Low levels of ATP within live cells trigger the glycogenolysis process. When the cells detect a low level of ATP, the liver and muscles liberate glycogen and break it down into glucose or simple sugars, which are then used to produce ATP.



Dr. Salim.J.Kh.

/ /

CARBOHYDRATES

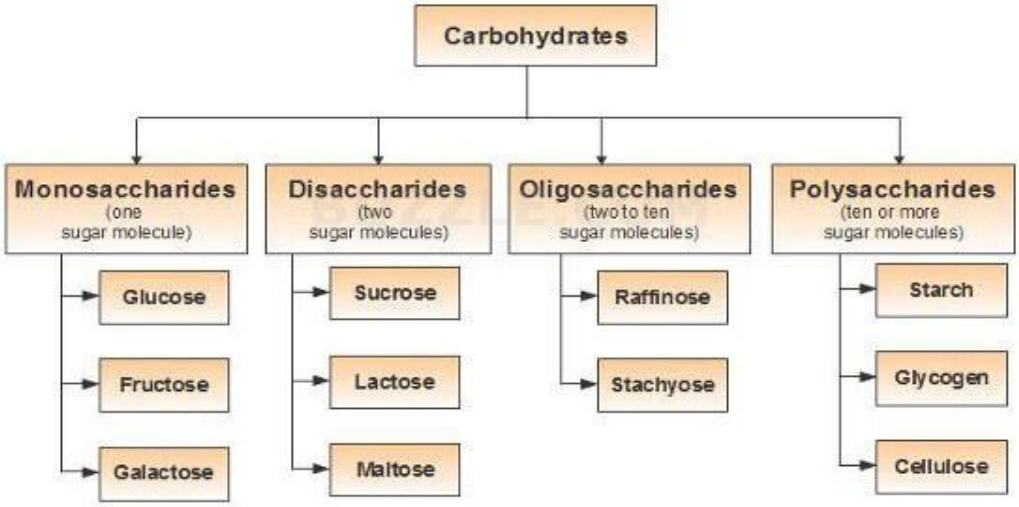
Carbohydrates are the most abundant biomolecules on earth. Oxidation of carbohydrates is the central energy-yielding pathway in most non-photosynthetic cells.

Definition: Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. carbohydrates have the formula $(CH_2O)_n$.

Biological Importance of carbohydrates

- Carbohydrates act as energy reserves and metabolic intermediates.
- Ribose and deoxyribose sugars forms the structural frame of the genetic material, RNA and DNA.
- Polysaccharides like cellulose are the structural elements in the cell walls of bacteria and plants.
- Carbohydrates are linked to proteins and lipids that play important roles in cell interactions.
- Carbohydrates are intermediates in biosynthesis of fats and proteins.
- In animals they are important constituent of connective tissues.
- They participate in biological transport, cell-cell communication and activation of growth factors.
- Carbohydrates that are rich in fiber content help to prevent constipation.

There are four major classes of carbohydrates:

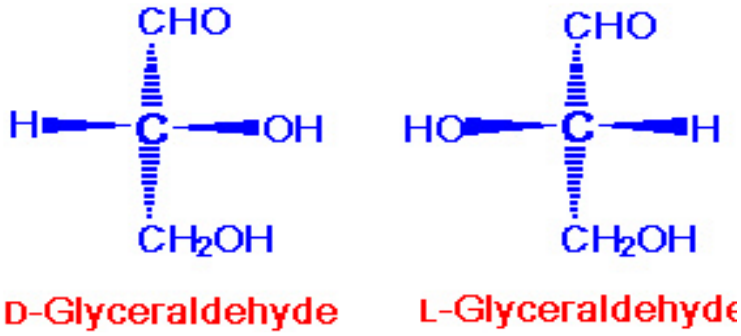


1. Monosaccharides

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most monosaccharide in nature is the six-carbon sugar D- glucose, sometimes referred to as dextrose. The word “Monosaccharides” derived from the Greek word “Mono” means Single and “saccharide” means sugar. They contain 3 to 7 carbon atoms, 2 or more hydroxyl (OH) groups and one aldehyde (CHO) or one ketone (CO) group.

Physical Properties of monosaccharides

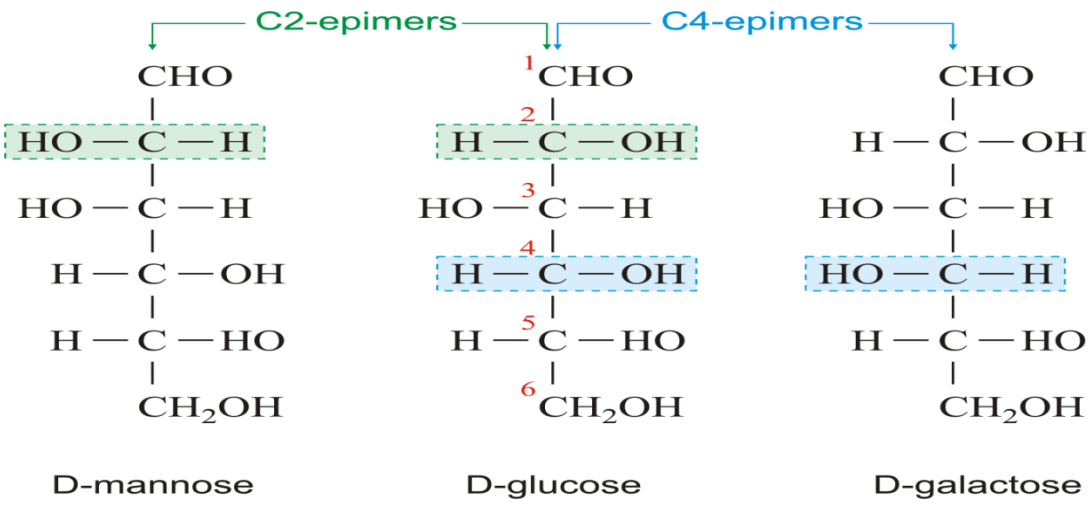
They are colorless, crystalline compounds, readily soluble in water. Their solutions are optically active. Carbohydrates spontaneously change between the α and β configuration.



When the - OH group around the carbon atom adjacent to the terminal primary alcohol carbon is on the right, the sugar is a member of the D-series, when it is on the left, it is a member of the L-series. These D and L configuration.

Optical Activity - The presence of asymmetric carbon atom causes optical activity. When a beam of plane polarized light is passed through a solution of carbohydrate it will rotate the light either to right or to left. Depending on the rotation, molecules are called dextrorotatory (+) (d) or levorotatory (-) (l).

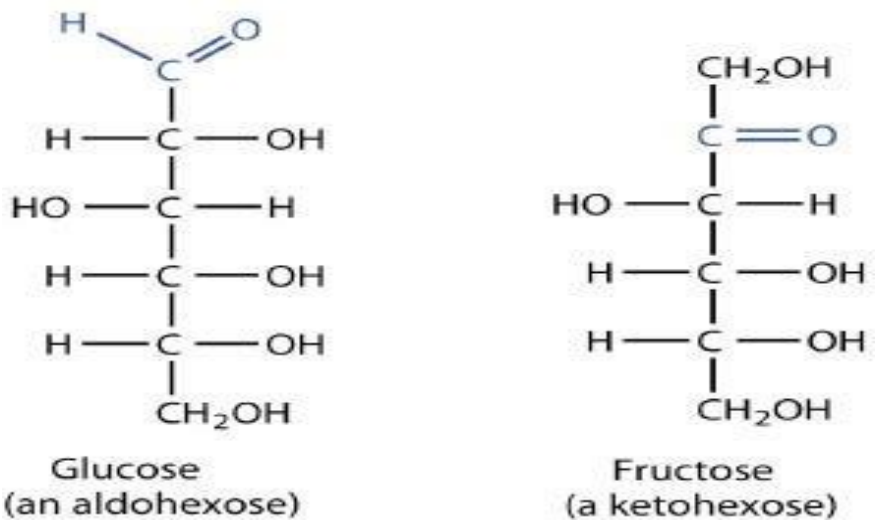
Epimers- When sugars are different from one another, only in a single carbon atom (around one carbon atom) they are called **epimers** of each other. For example glucose and mannose are epimers. They differ only in configuration around C2. Mannose and Galactose are epimers of Glucose.



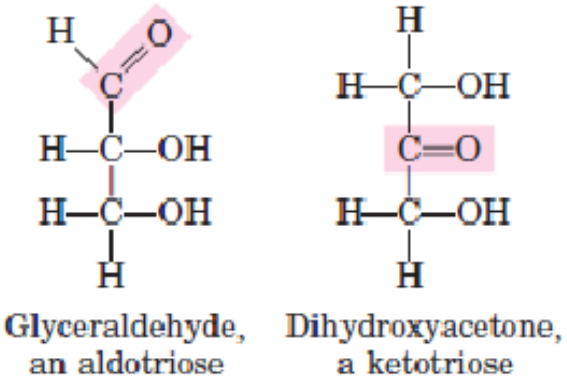
Classification of Monosaccharides

Monosaccharides are classified in two ways. (a) First of all, based on the number of carbon atoms present in them and (b) secondly based on the presence of carbonyl group.

The naturally occurring monosaccharides contain three to seven carbon atoms per molecule. For example, the terms *triose*, *tetrose*, *pentose*, and *hexose* signify monosaccharides with, respectively, three, four, five, and six carbon atoms. Monosaccharides are also classified as aldoses or ketoses. Those monosaccharides that contain an aldehyde functional group are called aldoses; those containing a ketone functional group on the second carbon atom are ketoses.



Name	Formula	Aldose	Ketose
Triose	$C_3H_6O_3$	Glycerose	Dihydroxy acetone
Tetrose	$C_4H_8O_4$	Erythrose	Erythrulose
Pentose	$C_5H_{10}O_5$	Ribose	Ribulose
Hexose	$C_6H_{12}O_6$	Glucose	Fructose
Heptose	$C_7H_{14}O_7$	Glucoheptose	Sedo heptulose



Glyceraldehyde, an aldotriose — Dihydroxyacetone, a ketotriose

Hexoses

Hexoses are “Monosaccharides” containing 6 carbon atoms. The molecular formula of Hexose is C6H12O6

Characteristics

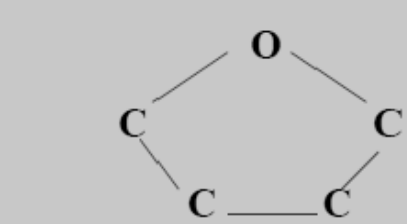
- Hexoses are simple sugars
- Hexoses are soluble in water
- They are sweet in taste .
- They are *crystalline* forms.
- The pentoses may contain an aldehyde group (aldohexose) or a ketone group (ketohexose).

Structure of Monosaccharides

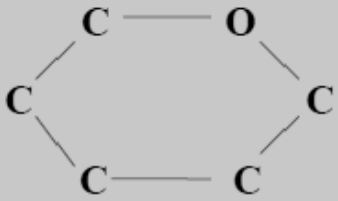
1. **Straight or Open Chain Structure**: Here 6 carbon atoms of glucose are arranged in a straight line. It is also called open chain structure because the two ends remain separate and they are not linked.

2. **Cyclic or Ring Structure:** Here the atoms are arranged in the form of a ring. Haworth (1929) proposed this formula and hence the name Haworth's Projection Formula. The sugar molecules exist in two type of rings which are as follows – (a) Furanose Ring – 5 membered ring

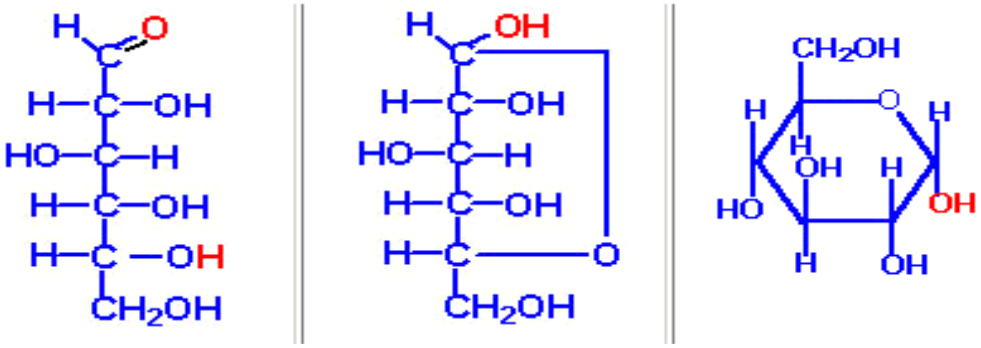
(b) Pyranose Ring- 6 membered ring



Furanose Ring



Pyranose Ring



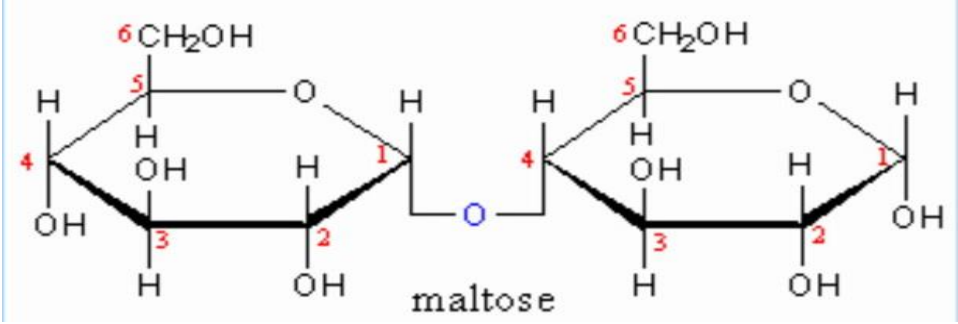
Open chain D-glucose α-D –glucose α-D –glucose
(Fisher formula) (Haworth formula)

2- Disaccharides

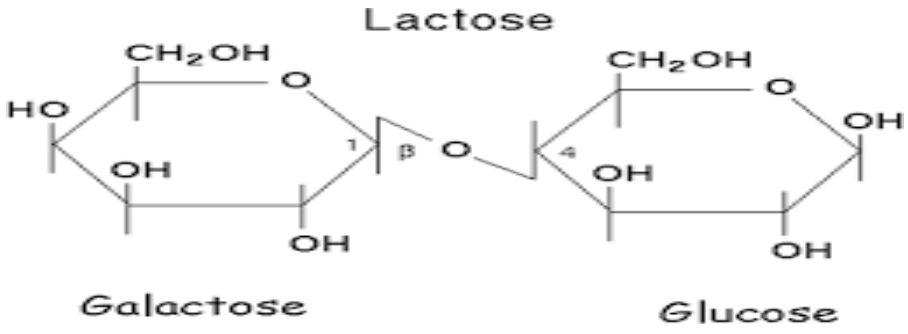
Disaccharides consist of two sugars joined by an glycosidic bond, like sucrose, lactose and maltose.

The disaccharides can be classified into:

1. Homodisaccharides



2. Heterodisaccharides: are formed of 2 different monosaccharide units like sucrose composed of glucose and fructose , lactose composed of glucose and galactose.



3- Oligosaccharides

Oligosaccharides consist of short chains of monosaccharide units (3-9), or residues, joined by characteristic linkages called glycosidic bonds. Common oligosaccharides include raffinose, and stachyose. These oligosaccharides can be found in relatively abundant levels in legumes, whole grains, some cruciferous vegetables, and some fruits.

4- Polysaccharides

Polysaccharides contain hundreds or thousands of carbohydrate units. The anomeric carbons are connected through glycosidic linkages.

Polysaccharides are of two types based on their function and composition.

A. Storage polysaccharide - starch.
B. Structural polysaccharide - cellulose.

Homopolysaccharide: a polysaccharide is made up of **one type** of monosaccharide unit.

Starch

- Starch is a polymer consisting of D-glucose units.
  Starches (and other glucose polymers) are usually insoluble in water because of the high molecular weight, but they can form thick colloidal suspensions with water.
- Starch is a **storage** compound in plants, and made of glucose units
- It is a homopolysaccharide made up of two components: **amylose** and **amylopectin**.
- Most starch is 10-30% amylose and 70-90% amylopectin.
- **Amylose** – a straight chain structure formed by **1,4 glycosidic bonds** between **α-D-glucose** molecules.

Glycogen

- Glycogen is the main storage polysaccharide of animal cells (Animal starch).
- - It is present in liver and in skeletal muscle.
- - Like amylopectin glycogen is a branched polysaccharide of D-glucose units in α - (1, 4) linkages, but it is highly branched.

- - The branches are formed by α -(1,6) glycosidic linkage that occurs after every 8 - 12 residues. Therefore liver cell can store glycogen within a small space. Multiple terminals of branch points release many glucose units in short time.
- Like amylopectin, glycogen gives a red-violet color with iodine.

Cellulose

- Cellulose is the most abundant structural polysaccharide in plants. It is fibrous, tough, water insoluble. Cellulose is a linear unbranched homopolysaccharide of 10,000 or more D- glucose units connected by β-(1, 4) glycosidic bonds. Humans cannot use cellulose because they lack of enzyme (cellulase) to hydrolyze the β-(1-4) linkages.
- Cellulose is an important structural polysaccharide, and is the single most abundant organic compound on earth. It is the material in plant cell walls that provides strength and rigidity; wood is 50% cellulose.
- Cellulose is also important industrially, from its presence in wood, paper, cotton, nitrocellulose, photographic films (cellulose acetate), etc.

Digestion and absorption

Digestion is the process by which food is broken down into simple chemical compounds that can be absorbed and used as nutrient substances the body can use for energy, tissue growth, and repair or eliminated by the body.

Absorption is the process by which the products of digestion are absorbed by the blood to be supplied to the rest of the body. During absorption, the digested products are transported into the blood or lymph through the mucous membrane.

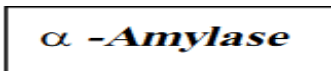
Digestion and absorption occur in the digestive tract. After the nutrients are absorbed, they are available to all cells in the body and are utilized by the body cells in metabolism. The digestive system prepares nutrients for utilization by body cells through six activities, or functions.

Digestion and absorption of carbohydrates

Dietary carbohydrates principally consist of the polysaccharides: starch and glycogen. It also contains disaccharides: sucrose, lactose, maltose and in small amounts monosaccharides like fructose and pentoses. Liquid food materials like milk, soup, fruit juice escape digestion in mouth as they are swallowed, but solid foodstuffs are masticated thoroughly before they are swallowed.

1. Digestion in mouth

Digestion of carbohydrates starts at the mouth, where they come in contact with saliva during mastication. Saliva contains a carbohydrate splitting enzyme called salivary amylase (ptyalin).



Starch or glycogen ⟶ Glucose, Maltose and Maltotriose

2. Digestion in Stomach

No carbohydrate splitting enzymes are available in gastric juice. HCl may hydrolyze some dietary sucrose to equal amounts of glucose and fructose.

3. Digestion in small intestine

Food reaches the small intestine from stomach where it meets the pancreatic juice. Pancreatic juice contains a carbohydrate-splitting enzyme pancreatic amylase. Other criteria and end products of action are similar of ptyalin. Also action of other enzymes like Lactase, Maltase, Sucrase.

Absorption of carbohydrates

Products of digestion of dietary carbohydrates are practically completely absorbed almost entirely from the small intestine.

Absorption from proximal jejunum is three times greater than that of distal ileum. It is also proved that some disaccharides, which escape digestion, may enter the cells of the intestinal lumen by “pinocytosis” and are hydrolyzed within these cells. No carbohydrates higher than the monosaccharides can be absorbed directly in to the blood stream.

Mechanism of absorption: Two mechanisms are involved:

1. Simple Diffusion

This is dependent on sugar concentration gradients between the intestinal lumen, mucosal cells and blood plasma. All the monosaccharides are probably absorbed to some extent by simple 'passive' diffusion.

2. "Active "Transport Mechanisms

- Glucose and galactose are absorbed very rapidly and hence it has been suggested that they are absorbed actively and it requires energy.

- Fructose absorption is also rapid but not so much as compared to glucose and galactose.

Digestion of lipids

Lipid digestion and absorption pose some special challenges. Triglycerides are large molecules, and unlike carbohydrates and proteins, they're not water-soluble. Because of this, they like to cluster together in large droplets when they're in a watery environment like the digestive tract. The digestive process has to break those large droplets of fat into smaller droplets and then enzymatically digest lipid molecules using enzymes called lipases.

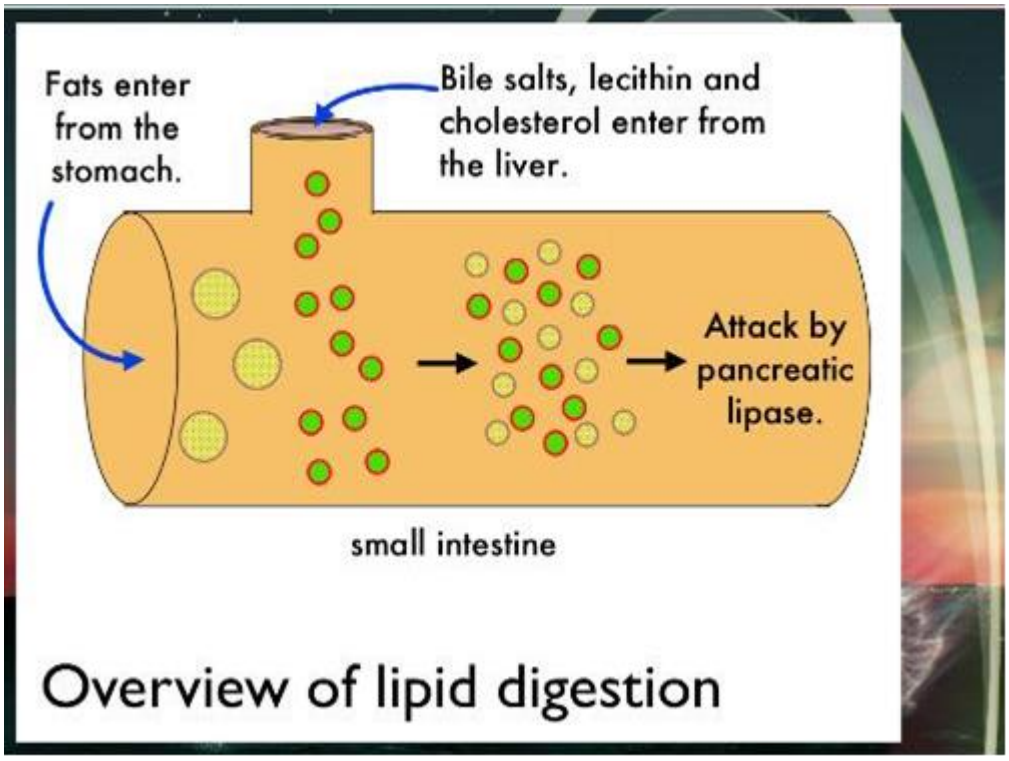
1- Lipid digestion in mouth and stomach

The digestion of lipids begins in the oral cavity through exposure to lingual lipases, which are secreted by glands in the tongue to begin the process of digesting triglycerides. Digestion continues in the stomach through the effects of both lingual and gastric enzymes (gastric lipase), these two lipases play only a minor role in fat digestion.

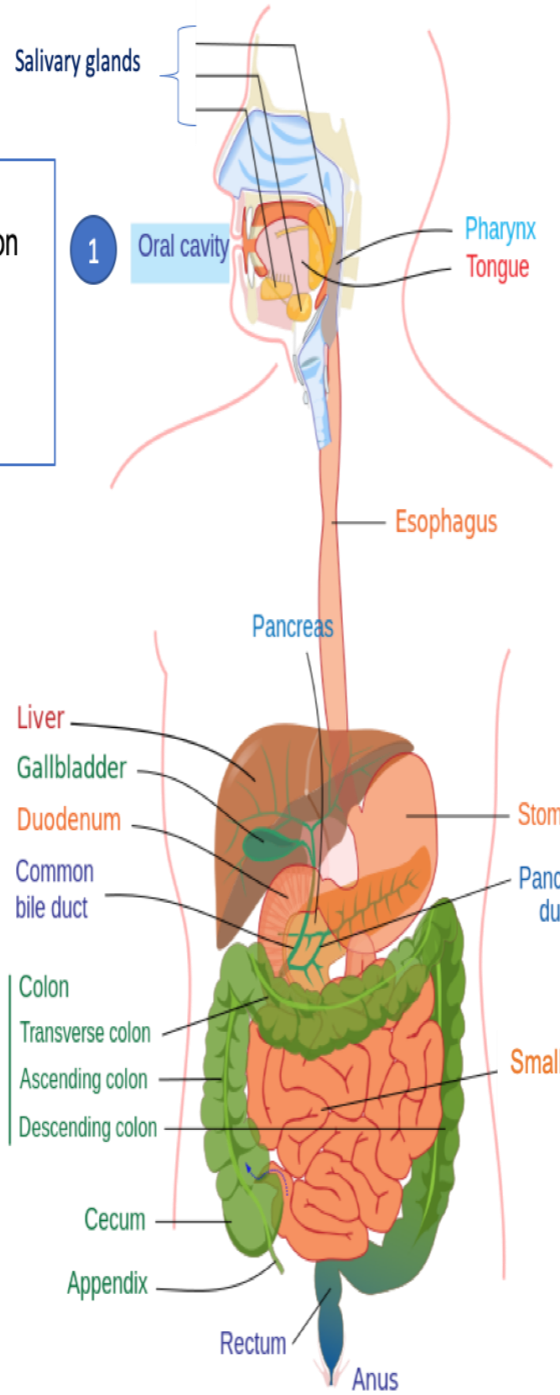
2- Lipid digestion in small intestine

As the stomach contents enter the small intestine, most of the dietary lipids are undigested and clustered in large droplets. **Bile**, which is made in the liver and stored in the gallbladder, is released into the duodenum, the first section of the small intestine. Bile salts have both a hydrophobic and a hydrophilic side, so they are attracted to both fats and water. This makes them effective emulsifiers, meaning that they break large fat globules into smaller droplets. Emulsification makes lipids more accessible to digestive enzymes by increasing the surface area for them to act.

The pancreas secretes ***pancreatic lipases*** into the small intestine to enzymatically digest triglycerides. Triglycerides are broken down to fatty acids, monoglycerides (glycerol backbone with one fatty acid still attached), and some free glycerol. Cholesterol and fat-soluble vitamins do not need to be enzymatically digested.



1. MOUTH
- mechanical digestion
- mixing with saliva
- limited enzymatic digestion (*lingual lipase*)



2. STOMACH
- mixing/churning
- limited enzymatic digestion (*gastric lipase*)

3. SMALL INTESTINE
- emulsification (bile)
- enzymatic digestion (*pancreatic lipases*)
- micelles help with absorption

Absorption of lipids

Next, those products of fat digestion (fatty acids, monoglycerides, glycerol, cholesterol, and fat-soluble vitamins) need to enter into the circulation so that they can be used by cells around the body. Again, bile helps with this process. Bile salts cluster around the products of fat digestion to form structures called micelles, which help the fats get close enough to the microvilli of intestinal cells so that they can be absorbed. The products of fat digestion diffuse across the membrane of the intestinal cells, and bile salts are recycled back to do more work emulsifying fat and forming micelles.

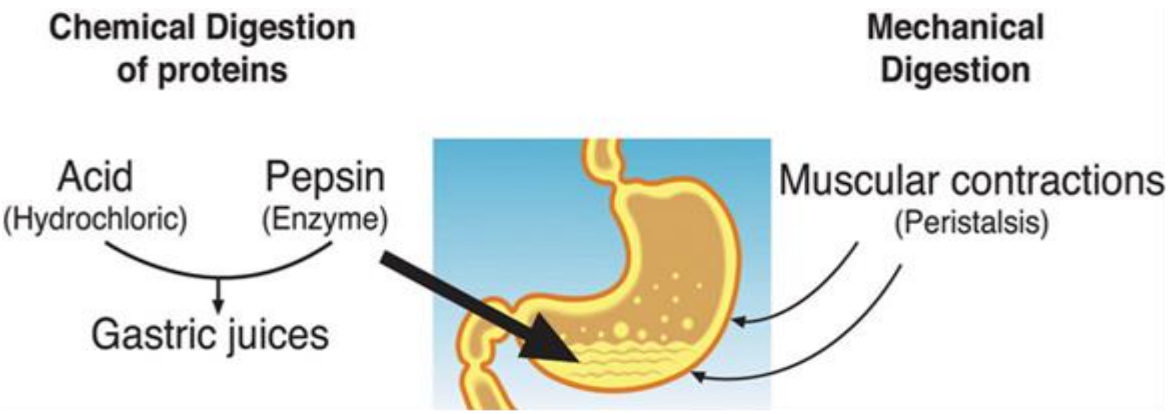
Digestion and absorption of proteins

Both mechanical and chemical digestion take place in the stomach. The stomach releases gastric juices containing **hydrochloric acid** and the enzyme, **pepsin**, which initiate the chemical digestion of protein. Muscular contractions, called peristalsis, also aid in digestion. The powerful stomach contractions churn the partially digested protein into a more uniform mixture, which is called **chyme**.

Because of the hydrochloric acid in the stomach, it has a very low pH of 1.5-3.5. The acidity of the stomach causes food proteins to denature, unfolding their three-dimensional structure to reveal just the polypeptide chain. This is the first step of chemical digestion of proteins. Recall that the three-dimensional structure of a protein is essential to its function, so denaturation in the stomach also destroys protein function. (This is why a protein such as insulin can't be taken as an oral medication).

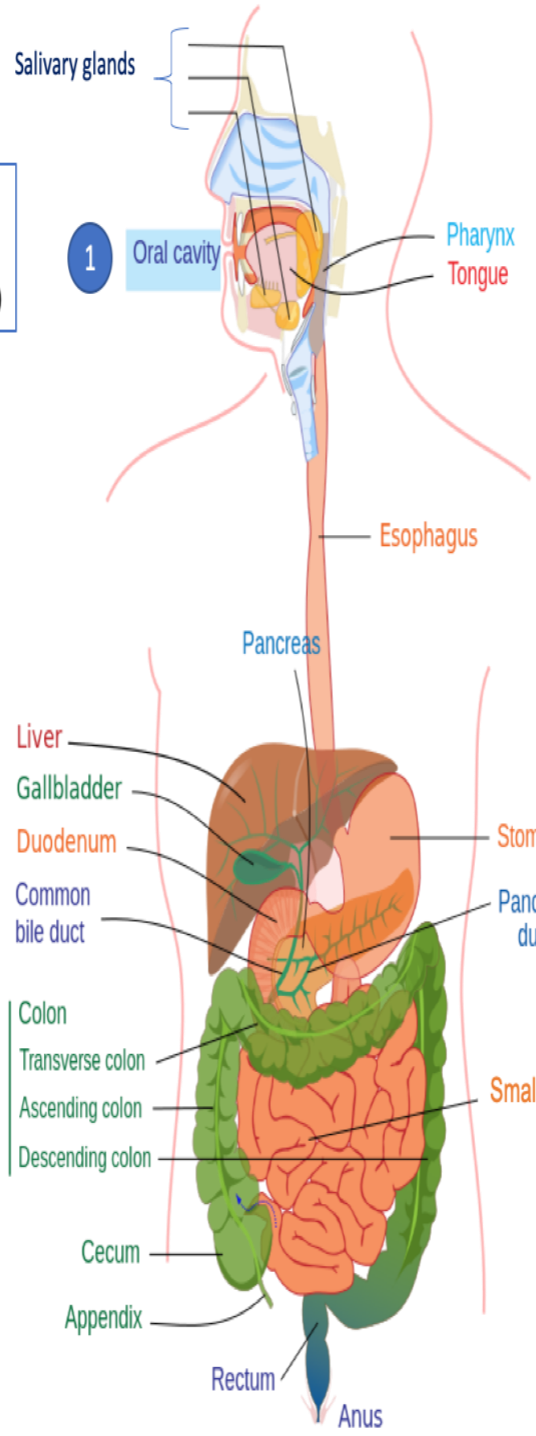
Once proteins are denatured in the stomach, the peptide bonds linking amino acids together are more accessible for enzymatic digestion. That

process is started by pepsin, an enzyme that is secreted by the cells that line the stomach and is activated by hydrochloric acid.



The two major pancreatic enzymes that digest proteins in the small intestine are ***chymotrypsin*** and ***trypsin***. Trypsin activates other protein-digesting enzymes called ***proteases***, and together, these enzymes break proteins down to tripeptides, dipeptides, and individual amino acids. The cells that line the small intestine release additional enzymes that also contribute to the enzymatic digestion of polypeptides.

1. MOUTH
- mechanical digestion (chewing)



2. STOMACH
- denaturation (HCl)
- enzymatic digestion (pepsin)

3. SMALL INTESTINE
- enzymatic digestion (chymotrypsin, trypsin, proteases)
- absorption of amino acids, di- and tripeptides

ENZYMES

General Properties

Enzymes are protein catalysts for chemical reaction in biological systems. They increase the rate of chemical reactions taking place within living cells without changing themselves.

Nature of Enzymes

Most enzymes are protein in nature. Depending on the presence and absence of a non-protein component with the enzyme, enzymes can exist as; simple enzyme or holoenzyme:

1. Simple enzyme: It is made up of only protein molecules not bound to any non-proteins. Example: Pancreatic Ribonuclease.

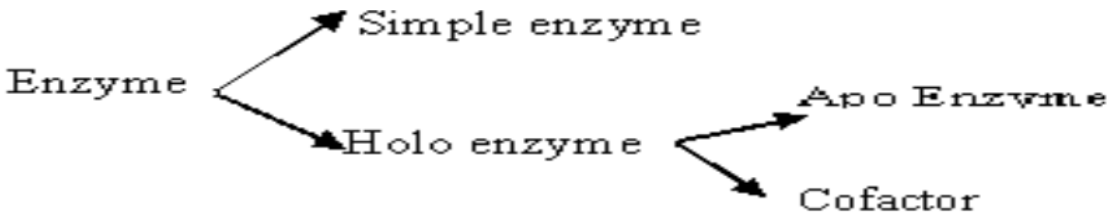
2. Holo enzyme is made up of protein groups and non-protein component.

-The protein component of this holo enzymes is called apoenzyme

-The non-protein component of the holo enzyme is called a cofactor.

If this cofactor is an organic compound like vitamins it is called a coenzyme and if it is an inorganic groups it is called activator (Fe^{2+}, Mn^{2+}, or Zn^{2+} ions).

If the cofactor is bound so tightly to the apoenzyme and is difficult to remove without damaging the enzyme it is sometimes called a **prosthetic group**



COENZYMES

Coenzymes are derivatives of vitamins without which the enzyme cannot exhibit any reaction. One molecule of coenzyme is able to convert a large number of substrate molecules with the help of enzyme. (Substrates are molecules that enzyme act on and converted it to product).

-Coenzyme accepts a particular group removed from the substrate or donates a particular group to the substrate.

-Coenzymes are called cosubstrate because the changes that take place in substrates are complimentary to the changes in coenzymes.

-The coenzyme may participate in forming an intermediate enzyme-substrate complex

Example: NAD, FAD, Coenzyme A

Metal ions in enzymes

Many enzymes require metal ions like Ca^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+} for their activity.

Metal-activated enzymes form only loose and easily dissociable complexes with the metal and can easily release the metal without denaturation. Metalloenzymes hold the metal tightly on the molecule and do not release it even during extensive purification.

Metal ions promote enzyme action by

a. Maintaining or producing the active structural conformation of the enzyme (e.g. glutamine synthase).

b. Promoting the formation of the enzyme-substrate complex (example: enolase and carboxypeptidase A).

c. Acting as electron donors or acceptors (example: Fe-S proteins and cytochromes).

d. Causing distortions in the substrate or the enzyme, example: phosphotransferases).

General terminology

Activation energy: It is usually considered to be the energy required for a molecule to form an activated complex which is in the transition of making or breaking a chemical bond. In an enzyme-catalyzed reaction, this corresponds to the formation of the activated enzyme-substrate complex.

Activator: An effector molecule that increases the catalytic activity of an enzyme when it binds to a specific site.

Active site: The part of enzyme at which the initial binding of substrate and enzyme occurs to form the intermediate enzyme-substrate complex and at which location further chemical change characteristic of the catalyzed reaction takes place.

Allostery: A phenomenon whereby the conformation of an enzyme is altered by combination, at a site other than the substrate-binding site, with a small molecule, referred to as an effector, which results in either increased or decreased activity by the enzyme.

Apoenzyme: The protein part of an enzyme without the cofactor necessary for catalysis. The cofactor can be a metal ion, an organic molecule (coenzyme), or a combination of both.

Catalyst: A catalyst is a substance that speed up or increases the rate of a reaction and its unchanged at the end of the process.

Denaturation: The partial or total alteration of the structure of a protein without change in covalent structure by the action of certain physical procedures (heating) or chemical agents. Denaturation is the result of the disruption of tertiary bonding, which causes the opening of the folded structure of a protein and the loss of characteristic physiologic, enzymatic, or physicochemical properties; it can be either reversible or irreversible.

Holoenzyme: An active enzyme consisting of the apoenzyme and coenzyme.

Induction: In enzymology, induction is a biological process which results in an increased biosynthesis of an enzyme thereby increasing its apparent activity.

Inhibitor: An inhibitor is a substance that diminishes the rate of a chemical reaction; the process is called inhibition.

Isoenzyme: One of a group of related enzymes catalyzing the same reaction but having different molecular structures and characterized by varying physical, and biochemical properties. Like creatine Kinase(CK) (CK-BB, CK-BM, CK-MM)

- **Enzyme unit:** the amount of enzyme required for transformation one micromole of substrate (reactant molecules) in one minute.
- **Turnover number:** the number of substrate molecules transformed per one minute by a single enzyme molecule.
- **Specific activity:** number of units of enzyme present in one mg of protein.

Properties of Enzyme

A. Active site

Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid chains that create a three-dimensional surface complementary to the substrate. The active site binds the substrate, forming an enzyme-substrate (ES) complex.

ES is converted to enzyme-product (EP); which subsequently dissociates to enzyme and product. For the combination with substrate, each enzyme is said to possess one or more active sites where the substrate can be taken up.

It is also possible that the active site (Catalytic site) is different from the binding site in which case they are situated closely together in the enzyme molecule.

B. Catalytic efficiency/ Enzyme turnover number

Most enzyme- catalyzed reactions are highly efficient proceeding from 10^3 to 10^8 times faster than uncatalyzed reactions. Typically each enzyme molecule is capable of transforming 100 to 1000 substrate molecule in to product each second.

Enzyme turn over number refers to the amount of substrate converted per unit time (carbonic anhydrase is the fastest enzyme).

C. Specificity

Enzymes are specific for their substrate. Specificity of enzymes are divided into:

1. **Absolute specificity:**- this means one enzyme catalyzes or acts on only one substrate. For example: Urease catalyzes hydrolysis of urea but not thiourea.

2. **Stereo specificity**- some enzymes are specific to only one isomer even if the compound is one type of molecule:

For example: glucose oxidase catalyzes the oxidation of β-D-glucose but not α-D-glucose, and arginase catalyzes the hydrolysis of L-arginine but not D-arginine.

*Maltase catalyzes the hydrolysis of α- but not β –glycosides.

3- Bond Specificity : enzymes that are specific for a bond or linkage such as ester, peptide or glycosidic belong to this group

Examples:

a. Esterases- acts on ester bonds

b. Peptidases-acts on peptide bonds

c. Glycosidases- acts on glycosidic bonds.

D. Regulation

Enzyme activity can be regulated that is, enzyme can be, activated or inhibited so that the rate of product formation responds to the needs of the cell.

E. Zymogens (- inactive form of enzyme)

Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes are called Zymogens (Proenzymes).

Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group

Examples: Pepsinogen - This zymogen is from gastric juice. When required Pepsinogen converts to Pepsin

Trypsinogen - This zymogen is found in the pancreatic juice, and when it is required gets converted to trypsin.

* The activation is brought about by specific ions or by other enzymes that are proteolytic.

Pepsinogen + H^+ ⟶ Pepsin

Trypsinogen $\xrightarrow{\text{Enteropeptidase}}$ Trypsin

Zymogen forms of enzymes a protective mechanism to prevent auto digestion of tissue producing the digestive enzymes and to prevent intravascular coagulation of blood.

*** Classification of Enzymes**

Enzymes are usually named in terms of the reactions they catalyze, by adding the suffix " ase " to a major part of the substrate acted upon like urease act on urea, and tyrosinase on tyrosine. There are some trivial names like pepsin and trypsin which are proteases.

Enzymes are classified on the basis of the reactions they catalyze. Each enzyme is assigned a four-digit classification number and a systematic name, which identifies the reaction catalyzed.

The international union of Biochemistry and Molecular Biology developed a system of nomenclature on which enzymes are divided into six major classes, each with numerous sub groups. Enzymes are classified based on the reactions they catalyze. Each enzyme is characterized by a code number comprising four digits separated by points. The four digits characterize class, sub-class, sub-sub-class, and serial number of a particular enzyme.

Class I. Oxidoreducatases- Enzymes catalyzing oxidation reduction reactions.

Example: Lactate-dehydrogenase

1- Lactic acid + NAD^+ ⟶ Pyruvic acid + $NADH+H^+$

Class II. Transferases:

Enzymes catalyzing a transfer of a group other than hydrogen (methyl, acyl, amino or phosphate groups)

Example: Enzymes catalyzing transfer of phosphorus containing groups.

ATP: D-hexose-6 phosphotransferase (Hexokinase)

ATP+D-Hexose ⟶ ADP+D-hexose-6-phosphate

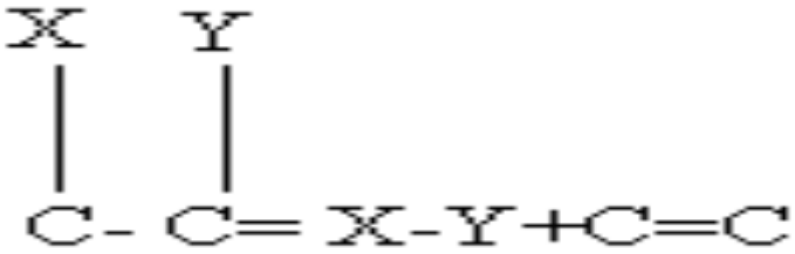
Class III. Hydrolases: Enzymes catalyzing hydrolysis of ester, ether, peptide, glycosyl, acid-anhydride, or C-C bonds by utilizing water.

Example: Enzymes action on glycosyl compounds

β-D- Galactoside galactohydrolase (β -Galactosidase)

β -D- Galactoside+H_2O ⟶ alcohol +D-Galactose

Class IV. Lyases: Enzymes that catalyze removal of groups from substances by mechanisms other than hydrolysis, leaving double bonds.



Enzymes acting on C-C, C-O, C-N, and C-S bonds.

Example : Carbon-Oxygen lyases

Malate hydrolyase (Fumarase)

Class V. Isomerases:

Includes all enzymes catalyzing interconversion of optical, geometric, or positional isomers.

Example: Enzymes catalyzing interconversion of aldose and ketoses

Glyceraldehyde-3- phosphate ketoisomerase (triosephosphate isomerase)

Glyceraldehyde-3phosphate ⟶ Dihydroxyacetone phosphate.

Class VI. Ligases or synthetases.

Enzymes catalyzing the linking together of 2 compounds coupled to the breaking of a pyrophosphate bond in ATP or similar trinucleotides: GTP, UTP etc. included are enzymes catalyzing reactions forming C-O, C-S, C-N, and C-C bonds.

Example: Enzymes catalyzing formation of C-C bonds

Acetyl-CoA: CO_2 ligase (ADP) [acetyl-CoA carboxylase] ATP+ Acetyl-COA+CO_2 ⟶ Malonyl-CoA + ADP + phosphate.

MECHANISM OF ACTION OF ENZYMES

Emil Fischer's model **lock and key model** 1890. Lock: Key model of enzyme action implies that the active site of the enzyme is complementary in shape to that of its substrate, i.e. the shape of the enzyme molecule and the substrate molecule should fit each other like a lock and Key

In 1958, Daniel Koshland, postulated another model, **induced-fit model**; which implies that the shapes & the active sites of enzymes are complementary to that of the substrate only after the substrate is bound.

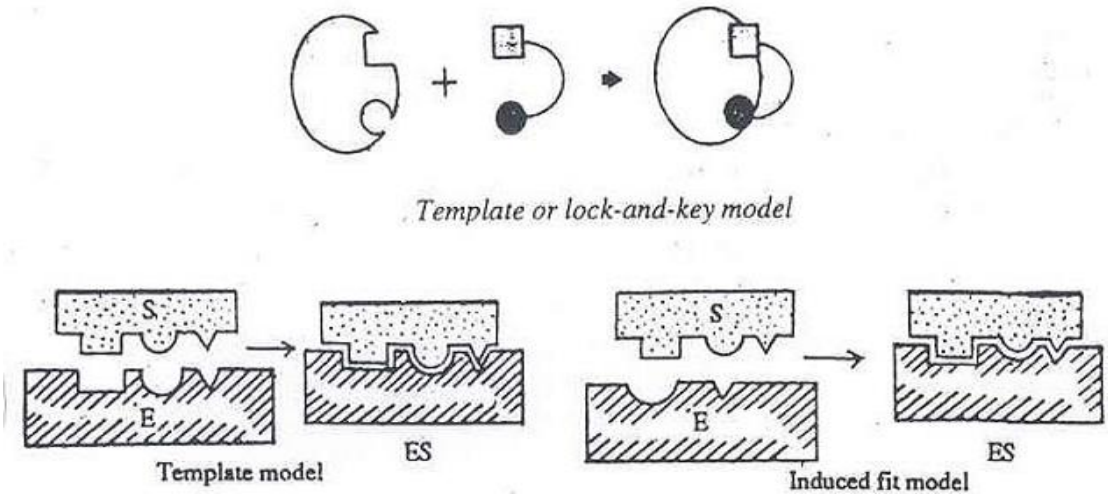


Figure: Models of enzyme- substrate interactions

Michaels and Menten have proposed a hypothesis for enzyme action, which is most acceptable. According to their hypothesis, the enzyme molecule (E) first combines with a substrate molecule (S) to form an enzyme substrate (ES) complex which further dissociates to form product (P) and enzyme (E) back. Enzyme once dissociated from the complex is free to combine with another molecule of substrate and form product in a similar way.

Enzymes Lowering Free Energy of Activation

Enzymes bind temporarily to one or more of the reactants of the reaction they catalyze, and this lead to lower the amount of activation energy needed and thus speed up the reaction.

A chemical reaction $S \longrightarrow P$ (where S is the substrate and P is the product or products) will take place when a certain number of S molecules at any given instant possess enough energy to attain an activated condition called the **"transition state"**, in which the probability of making or breaking a chemical bond to form the product is very high.

The transition state is the top of the energy barrier separating the reactants and products.

A rise in temperature, by increasing thermal motion and energy, causes an increase in the number of molecules on the transition state and thus accelerates a chemical reaction. The enzyme combines transiently with the substrate to produce a transient state having a lower energy of activation than that of substrate alone. This results in acceleration of the reaction.

Activation energy is defined as the energy required to convert all molecules in one mole of reacting substance from the ground state to the transition state.

Enzyme are said to reduce the magnitude of this activation energy.

* During the formation of an ES complex, the substrate attaches itself to the specific active sites on the enzyme molecule by Reversible interactions formed by Electrostatic bonds, Hydrogen bonds, Vanderwaals forces, Hydrophobic interactions.

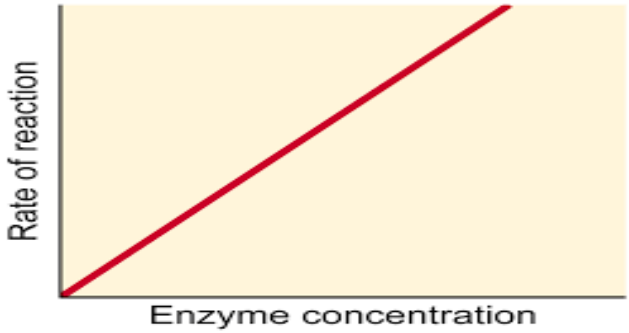
*** Factors Affecting Enzyme Activity**

Physical and chemical factors are affecting the enzyme activity. These include

1. Enzyme concentration
2. Temperature
3. pH
4. Substrate concentration.

1- **Enzyme concentration**

the enzyme activity increased with the increase of enzyme concentration, until reach all the reactant substances (substrates) will convert to products.



2.Temperature

Starting from low temperature as the temperature increases to certain degree the activity of the enzyme increases because the temperature increase the total energy of the chemical system. There is an optimal temperature at which the reaction is most rapid (maximum).

Above this the reaction rate decreases sharply, mainly due to denaturation of the enzyme by heat.

The temperature at which an enzyme shows maximum activity is known as the optimum temperature for the enzyme. For most body enzymes the optimum temperature is around 37^0C, which is body temperature.

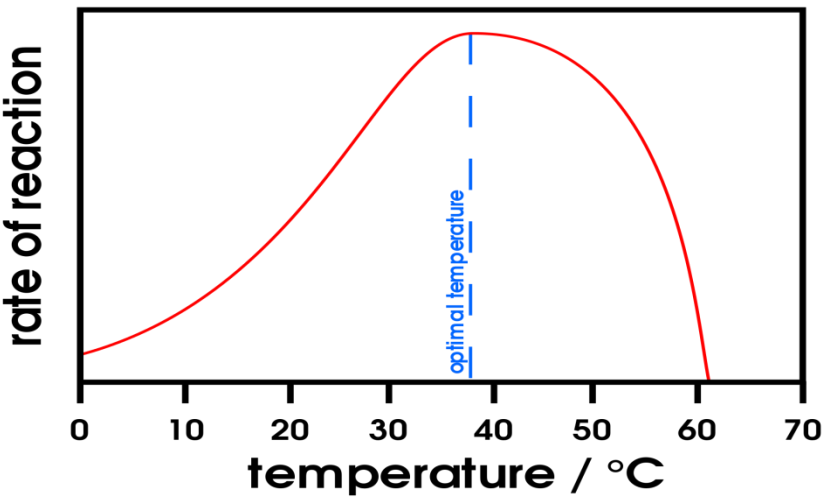


Figure. Effect of temperature on enzymatic reaction

3. Effect of pH

The concentration of H^+ affects reaction velocity in which the enzyme and substrate usually requires specific chemical groups in an ionized or unionized state in order to interact.

Extreme pH can lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid chains.

The pH at which maximum enzyme activity is achieved is different for different enzymes.

For example, pepsin, a digestive enzyme in the stomach, has maximum action at pH 2, where as other enzymes, designed to work at neutral pH, are denatured by such an acidic environment like carbonic anhydrase, and others need alkaline medium like alkaline phosphatase and trypsin.

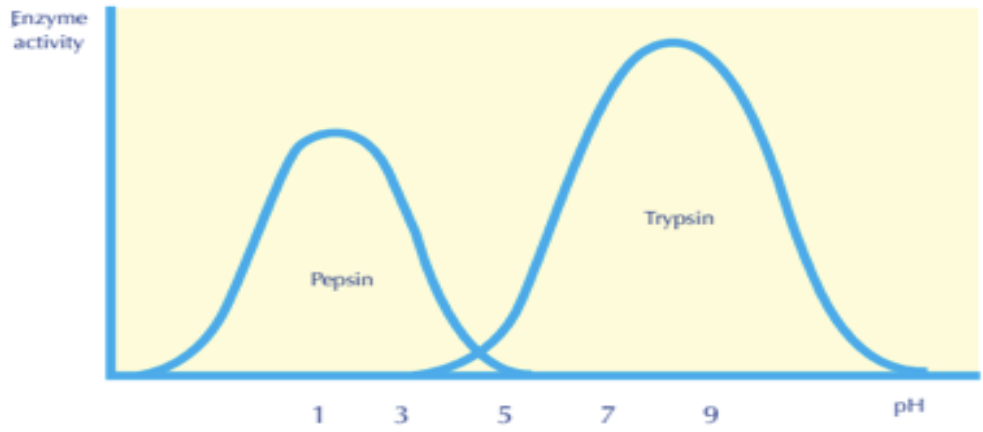


Figure. Effect of pH on enzymatic reaction

4. Concentration of substrate

At fixed enzyme concentration, pH and temperature, the activity of enzymes is influenced by increase in substrate concentration.

An increase in the substrate concentration increases the enzyme activity till a maximum is reached. Further increase in substrate concentration does not increase rate of reaction.

This condition shows that as concentration of substrate is increased, the substrate molecule combine with all available enzyme molecules at their active site till not more active sites are available (the active sites become saturated). At this state the enzymes obtained it maximum rate (V max).

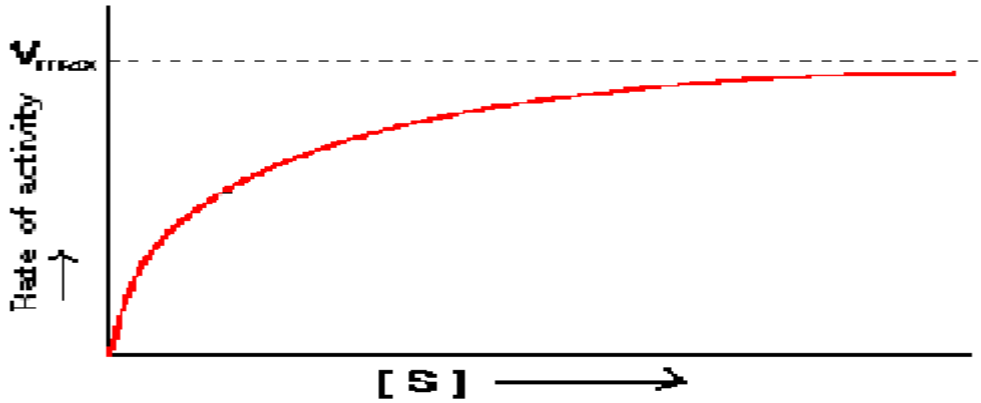


Figure. Effect of Concentration of substrate on enzyme activity

The characteristic shape of the substrate saturation curve for an enzyme can be expressed mathematically by the Michaelis Menten equation:

$$V = \frac{V\max[S]}{Km + [S]}$$

Where: V= Velocity at a given concentration of substrate (initial reaction velocity)

Vmax = Maximal velocity possible with excess of substrate

[S] = concentration of the substrate at velocity V

Km = michaelis-constant of the enzyme for particular substrate.

- Below the relationship between [S] and Km:

a- [S] << Km

$$V = \frac{Vmax\ [S]}{Km} \quad \text{--------- } V\alpha\ [S]$$

b- [S] = Km

$$V = \frac{Vmax\ [S]}{[S] + [S]} = \frac{Vmax\ [S]}{2[S]} = 1/2Vmax$$

c- [S] >> Km

$$V = \frac{Vmax\ [S]}{[S]} \quad \text{-------} \quad V = Vmax$$

When [S] is much less than Km, the velocity of the reaction is roughly proportional to the substrate concentration. The rate of reaction is then said to be first order configuration with respect to substrate. When [S] is much greater than Km, the velocity is constant and equal to Vmax. The rate of reaction is then independent of substrate concentration and said to be zero order with respect to substrate concentration.

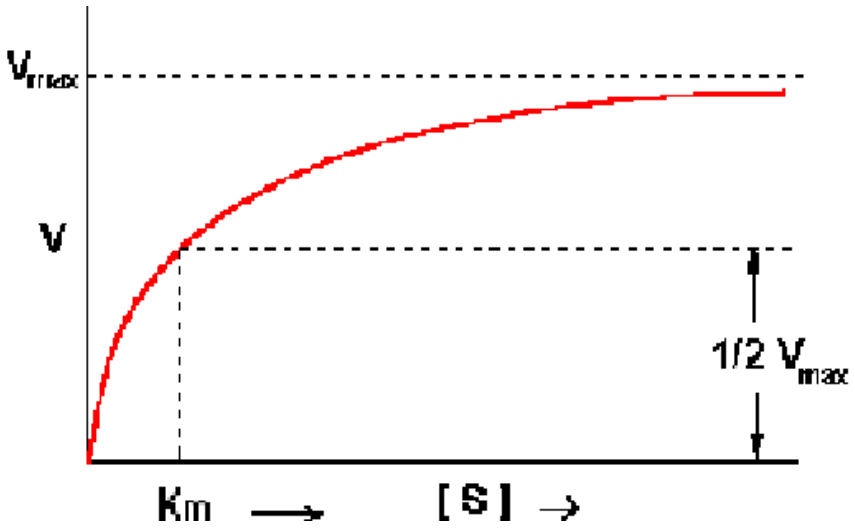


Figure: Relationship between [S] and Km

*** Characteristics of Km**

Km- can defined as the concentration of the substrate at which a reaction velocity equal to ½ Vmax.

Km- is characteristic of an enzyme and a particular substrate, and reflects the affinity of the enzyme for that substrate.

Km- values varies from enzyme to enzyme and used to characterized different enzymes.

Km- values of an enzyme helps to understand the nature and speed of the enzyme catalysis.

High Km value of an enzyme means the catalysis of that enzyme is slow compared to low Km.

Km does not vary with the concentration of enzyme.

*** Enzyme Inhibition**

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor and the process is known as inhibition.

There are two major types of enzyme inhibition, Irreversible and Reversible.

1- Irreversible Inhibition

The type of inhibition that cannot be reversed by increasing substrate concentration or removing the remaining free inhibitor is called Irreversible inhibition Eg. Diisopropyl fluorophosphate (DFP) Inhibits the enzyme acetyl cholinesterase, important in the transmission of nerve impulses. Acetyl cholinesterase catalyzes the hydrolysis of acetylcholine to acetic acid and choline, which acetylcholine is a neurotransmitter substance functioning in certain portions of the nervous system.

- DFP inhibits also trypsin, chymotrypsin, elastase, and phosphglucomutase. Organo-phosphorus compounds like malathion, parathron pesticides-inhibits acetyl cholinesterase by the same way as DFP.

2- Reversible inhibition

This type of inhibition can be Competitive, Non-competitive and uncompetitive.

a- Competitive Inhibition: This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy, therefore, competes with the substrate for that site.

In competitive inhibition the inhibitor and substrate compete for the same active site on the enzyme as a result of similarity in structure. The enzyme substrate complex will be broken dawn to products (E+S $\longrightarrow$ ES $\longrightarrow$ E+P) where as enzyme inhibitor complex; (EI) will not be broken down to products.

A classical example is Malonate that competes with succinate and inhibits the action of succinate dehydrogenase to produce fumarate in the Krebs cycle.

The enzyme can be also inhibited by oxalate and glutarate because of the similarity of these substances with succinate

Eg.2 Allopurinol used for the treatment of Gout

Allopurinol inhibits xanthine oxidase by competing with uric acid precursors for the active site on the enzyme. This competition blocks the conversion of these precursors, and of hypoxanthine and xanthine, to uric acid and result in lower serum urate levels.

Since most clinical drug therapy is based on inhibiting the activity of enzymes.

Effect of competitive inhibitors

The effect of a competitive inhibitor is reversed by increasing [s]. at a sufficiently high substrate concentration, the reaction velocity reaches the Vmax. observed in the absence of inhibitor.

Also a competitive inhibitor increases the apparent Km for a given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve ½ Vmax.

b- Non-Competitive Inhibition

In non-competitive inhibition the inhibitor binds at different site rather than the substrate-binding site. When the inhibitor binds at this site there will be a change in conformation of the enzyme molecules, which leads to the reversible inactivation of the catalytic site.

Non-competitive inhibitors bind reversibly either to the free-enzyme or the ES complex to form the inactive complexes EI and ESI (Enzyme substrate Inhibitor).

The most important non-competitive inhibitors are naturally occurring metabolic intermediates that can combine reversibly with specific sites on certain regulatory enzymes, that changes the activity of their catalytic sites.

An Example: is the inhibition of threonine dehydratase by isoleucine.

*Such type of enzyme is called **Allosteric Enzyme**, which has a specific sites or allosteric site other than the substrate-binding site.

Non-Competitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, non-competitive inhibitors decrease the Vmax of the reaction. Also Non-competitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the

enzyme shows the same Km in the presence or absence of the non-competitive inhibitor.

c- Uncompetitive Inhibition

Uncompetitive Inhibitor binds only to ES complex at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor-binding site available. Inhibition cannot be reversed by substrate.

In this case apparent Vmax. and Km decreased.

*** Regulation of enzyme activity**

There are several means by which the activity of a particular enzyme is specifically regulated.

1. Irreversible covalent activation / zymogen activation

Some enzymes are secreted in an inactive form called Proenzymes or zymogens. At the site of action specific peptide bonds are hydrolysed either enzymatically or by pH changes to convert it into active form, e.g. Pepsinogen to pepsin, Trypsinogen to trypsin, plasminogen to plasmin. After hydrolysis when it is activated, it cannot be reconverted into proenzyme form.

2. Reversible Covalent Modification

By addition of or removal of phosphate or adenylate, certain enzymes are reversibly activated and inactivated as per the requirement. Protein kinase of muscle phosphorylate phosphorylase kinase, glycogen synthetase by making use of ATP.

3. Allosteric Modulation

In addition to simple enzymes that interact only with substrates and inhibitors, there is a class of enzymes that bind small, physiologically important molecules and modulate activity in ways other than those described above. These are known as **allosteric enzymes**; the small regulatory molecules to which they bind are known as **effectors**.

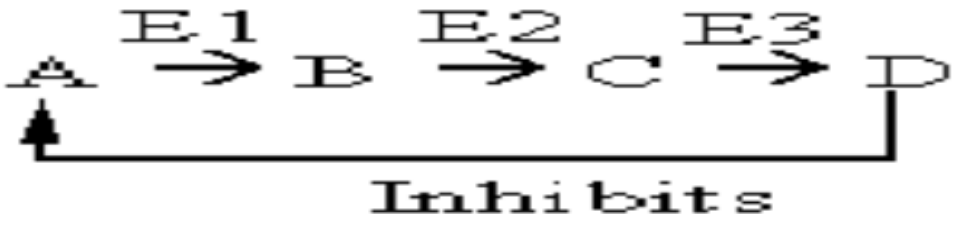
Allosteric effectors bring about catalytic modification by binding to the enzyme at distinct allosteric sites, and causing conformational changes that are transmitted through the bulk of the protein to the catalytically active site.

The hallmark of effectors is that when they bind to enzymes, they alter the catalytic properties of an enzyme's active site. Those that increase catalytic activity are known as positive effectors. Effectors that reduce or inhibit catalytic activity are negative effectors.

There are two ways that enzymatic activity can be altered by effectors: the Vmax can be increased or decreased, or the Km can be raised or lowered.

4. Feedback inhibition

In allosteric regulation in which end products inhibit the activity of the enzyme is called" feedback inhibition".



A high conc. D typically inhibits conversion of A to B.

This involves not simple backing up of intermediates but the activity of D to bind to and inhibit E1. D thus acts as negative allosteric affector or feedback inhibitor of E1.

The kinetics of feedback inhibition can be competitive or mixed. It is the commonest way of regulation of a biosynthetic pathway.

ENZYMES IN CLINICAL DIAGNOSIS

Plasma enzymes can be classified into two major groups

1. Those, relatively, small group of enzymes secreted into the plasma by certain organs (i.e. enzymes those have function in plasma) For example: - the liver secretes zymogens of the enzymes involved in blood coagulation.
2. Those large enzyme species released from cells during normal cell turnover. These enzymes are normally intracellular and have no physiologic function in the plasma. In healthy individuals the levels of these enzymes are fairly constant and represent steady state in which the rate of release from cells into the plasma is balanced by an equal rate or removal from the plasma.

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues.

The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus, the degree of elevation of a particular enzyme activity in plasma is often useful in evaluating the diagnosis and prognosis for the patient.

1. Lipase:

It is an enzyme catalyzing the hydrolysis of fats. It is secreted by tongue, pancreas and Liver.

The plasma lipase level may be low in liver disease, vitamin A deficiency, some malignancies, and diabetes mellitus. It may be elevated in acute pancreatitis and pancreatic carcinoma.

2. α- Amylase

α- amylase is the enzyme concerned with the breakdown of dietary starch and glycogen to maltose. It is present in pancreatic juice and saliva as well as in liver and muscles. The enzyme is excreted in the Urine. The main use of amylase estimations is in the diagnosis of acute pancreatitis.

The plasma amylase level may be low in liver disease and increased in high intestinal obstruction, mumps, acute pancreatitis and diabetes.

3. Acid Phosphatase (ACP)

Acid phosphatases catalyzing the hydrolysis of various phosphate esters at acidic pH is found in the prostate, liver, red cells, platelets and bone. It may be elevated in metastatic prostatic carcinoma.

4. Transaminases

Two transaminases are of clinical interest.

1. Aspartate Transaminase, AST (Glutamate oxaloacetate transaminase, GOT)

catalyzes the transfer of the amino group of aspartic acid to α-ketoglutarate forming glutamate and oxaloacetate.

AST or GOT is widely distributed, with high concentration, in the heart, liver, skeletal muscle, kidney and erythrocytes, and damage to any of these tissues may cause raised levels.

2. Alanine transaminase, ALT (Glutamate pyruvate transaminase, GPT)

Transfer the amino group of alanine to α- ketoglutarate, forming glutamate and pyruvate. It is present in high concentration in liver and to a lesser extent in skeletal muscle, kidney and heart.

- Serum levels of glutamate- pyruvate transaminase (SGPT) and Glutamate-oxaloacetate- transaminase (SGOT) are useful in the diagnosis of liver parenchymal damage and myocardial damage respectively. In liver damage, both enzymes are increased, but SGPT increases more. In myocardial infarction SGOT is increased with little or no increase in SGPT.

5. Lactate Dehydrogenase (LDH)

It catalyzes the reversible interconversion of lactate and pyruvate. It is widely distributed with high concentrations in the heart, skeletal muscle, liver, kidney, brain and erythrocytes.

The enzyme is increased in plasma in myocardial infarction, acute leukemia, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes is more useful in clinical diagnosis to differentiate hepatic disease and myocardial infarction.

6. Creatine kinase (CK) or creatine phosphokinase (CPK)

CK (CPK) is found in heart muscle, brain and skeletal muscle. Measurement of serum creatine phosphokinase activity is of value in the diagnosis of disorders affecting skeletal and cardiac muscle. The level of CPK in plasma highly increased in myocardial infarction.

Dr. Salim.J.Kh.

Endocrine System

Second Lecture

Hormones affect the following important functions:

The various functions performed by hormones may, in general, be discussed under following heads :

1. **Regulatory or homeostatic function**: The hormones have regulatory effects on the composition of the body fluids, the rate of gaseous exchange and the activity of the vascular system and the central nervous system (CNS). **Homeostasis** can be defined as the tendency to maintain uniformity or stability in the internal environment of the organism and to maintain the normal composition of the body fluids. In other words, homoeostasis is the maintenance of a constant internal environment in the face of changes in the external environment.

2. **Permissive function**: Not only does each endocrine gland affect a number of processes, but these glands also affect the functioning of one another. Thus certain hormones require the presence (or 'permission') of another hormone for the expression of their activity. This helps in maintaining a perfect hormonal balance. Derangements of this balance, either clinical or experimental, lead to a variety of metabolic disorders.

3. **Integrative function**: The integrative function of the hormones is reflected in the fact that they support the role of nervous system. However, the integrative properties of the endocrine system are slow and steady whereas those of the nervous system are rapid. This close tie between the two systems has led to the emergence of a new discipline of science called *neuroendocrinology.*

Classification according to function

The major hormones can be divided into 6 general types, based on their role in regulating homeostasis:

1-Master regulators control overall body processes . Most of these hormones come from the pituitary gland, or their production is controlled by the pituitary gland. e.g. thyroid hormones regulate processes like metabolic rate, which is critical for all cells and systems.

2- Several hormones manage energy balance & metabolism. These include insulin & glucagon , cortisol and epinephrine.

3- Several hormones work as a group to control ion balance e.g. PTH and calcitonin that control calcium balance, and aldosterone that controls sodium balance .

4- Hormones controlling growth , development, and reproduction are another group e.g. GH, LH, FSH, estrogen, progesterone & testosterone.

5- A major group of hormones that regulate routine body cycles, like wakefulness, hunger, variations in blood pressure, body composition, seasonal cell turnover , etc. These hormones like melatonin, thyroid stimulating hormone (TSH) and prolactin.

6- The last functional group are hormones that regulate responses to stress like epinephrine & cortisol.

Classification according to structure

Most commonly, hormones are categorized into four structural groups, with members of each group having many properties in common:

1- Peptides and proteins: like LH, FSH, prolactin, ACTH, GH, ADH, and oytocin.

2- Amino acid derivatives: tyrosine give thyroid hormones (T3 and T4) and Catecholamines (epinephrine and norepinephrine).

☼ Tryptophan is the precursor to serotonin and melatonin.

3- Cholesterol Derivatives: examples include the sex steroids such as testosterone and adrenal steroids such as cortisol.

4. **Fatty Acid Derivatives – Eicosanoids**: the principal groups of hormones of this class are prostaglandins and prostacyclins.

Hormone receptor is defines as a molecule or complex of molecules, in or on a cell, that binds its hormone with great selectivity and in so doing is changed in such a manner that a characteristic response or group of responses is initiated.

Characteristics of Receptors: hormone receptors are proteins or glycoproteins that are able to function as follows:

1-They distinguish their hormone from other molecules that may have very similar structures.

2-They bind to the hormone (sometimes called a ligand) even when its concentration is exceedingly low (10^{-8}–10^{-12}M).

3. They undergo a conformational change when bound to the hormone.

4. They catalyze biochemical events to produce a biochemical change.

#Two important terms are used to refer to molecules that bind to the

hormone-binding sites of receptors:

► **Agonists** are molecules that bind the receptor and induce all the post-receptor events that lead to a biologic effect. In other words, they act like the "normal" hormone, although perhaps more or less potently.

► **Antagonists** are molecules that bind the receptor and block binding of the agonist, but fail to trigger intracellular signaling events. Hormone antagonists are widely used as drugs.

Modulation of Hormone Levels

Hormone concentration is influenced by many factors:

a-Rate of hormone synthesis.

b-Release from the endocrine organ.

c- Transport in the circulation.

d- Efficiency of delivery into target cell.

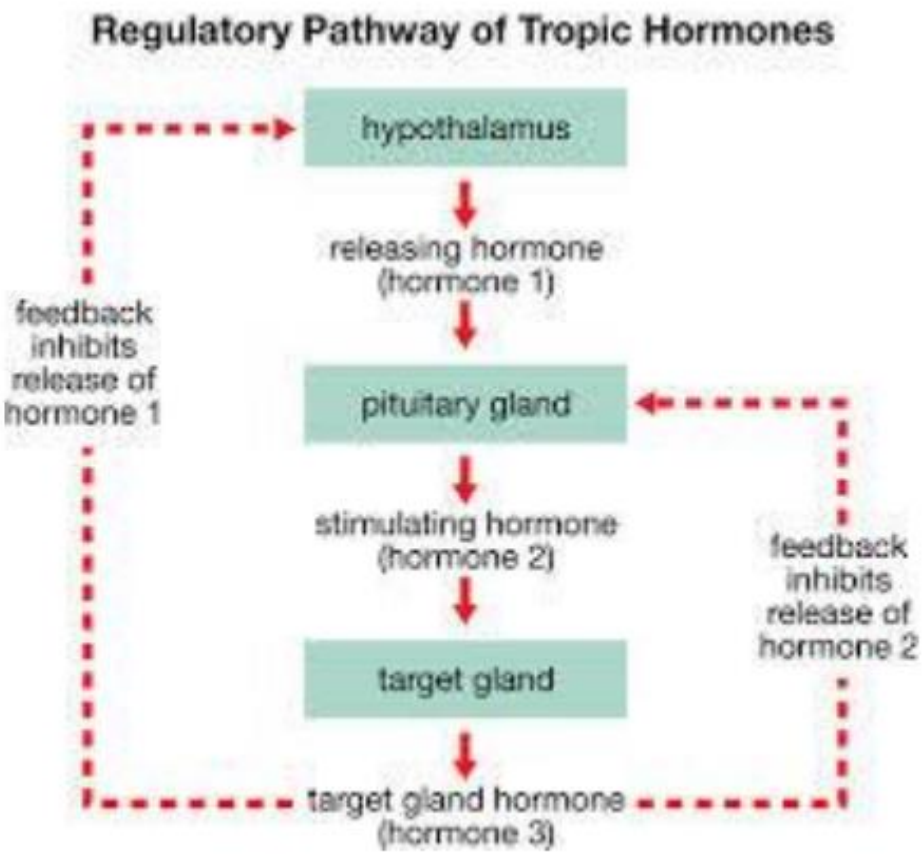
Regulation of Hormone Production

Hormones are produced by endocrine organs in response to:

1-Diverse signals , including other hormones.

2- innervations of the endocrine organ .

3- Environmental signals (including diet).



The hypothalamus-pituitary-target gland system

Hormones half-life: Most hormones are destroyed rapidly after secretion and have a half-life in blood of less than 10 minutes. The half-life of a hormone in blood is defined as that period of time needed for its concentration to be reduced by half and depends on its rate of degradation and on the rapidity with which it can escape from the circulation and equilibrate with fluids in extravascular compartments.

This process is sometimes called the metabolic clearance rate. Some hormones, e.g., epinephrine, have half-lives measured in seconds; others, e.g., thyroid hormones, have half-lives of the order of days.

Pharmacology

Many hormones and their analogues are used as medication. The most commonly prescribed hormones are estrogens and progestagens (as methods of hormonal contraception), thyroxine (as levothyroxine, for hypothyroidism) and steroids (for autoimmune diseases and several respiratory disorders). Insulin is used by many diabetics. Local preparations for use in otolaryngology often contain pharmacologic equivalents of adrenaline, while steroid and vitamin D creams are used extensively in dermatological practice.

A "pharmacologic dose" of a hormone is a medical usage referring to an amount of a hormone far greater than naturally occurs in a healthy body. The effects of pharmacologic doses of hormones may be different from responses to naturally-occurring amounts and may be therapeutically useful. An example is the ability of pharmacologic doses of glucocorticoid to suppress inflammation.

Endocrine System and Hormones

Endocrinology is the study of hormones, their receptors and the intracellular signaling pathways. In addition to the classical endocrine organs, many other cells in the body secrete hormones. Myocytes in the atria of the heart and scattered epithelial cells in the stomach and small intestine are examples of what is sometimes called the "diffuse" endocrine system.

The general function of the endocrine system is to integrate body systems, in conjunction with the nervous system, this by regulating cellular and organ function throughout life and maintaining homeostasis.

The endocrine system is one of the two coordinating and integrating systems of the body. It acts through chemical messengers - hormones – carried in the circulation.

Two systems control all physiologic processes:

☼The **nervous system** exerts point-to-point control through nerves, similar to sending messages by conventional telephone. Nervous control is electrical in nature and fast.

☼The **endocrine system** broadcasts its hormonal messages to essentially all cells by secretion into blood and extracellular fluid. Like a radio broadcast, it requires a receiver to get the message - in the case of endocrine messages, cells must bear a receptor for the hormone being broadcast in order to respond.

There are four types of chemical messengers:

1) Autocrine / Paracrine (prostaglandins / histamine)

• Local chemical messengers.

• Exert effect on neighboring cells (paracrine) or on same cell (autocrine).

2) Neurotransmitters (epinephrine and norepinephrine)

• Short-range chemical messengers.

• Diffuse across narrow space (synapse) to act on adjoining target cell (another neuron, a muscle, or a gland).

3) Neurohormones (oxytocin and antidiuretic hormones)

• Hormones released into blood by neurosecretory neurons.

• Distributed through blood to distant target cells.

4) Hormones (like insulin, parathyroid hormone, etc)

• Long-range messengers.

• Secreted into blood by endocrine glands in response to appropriate signal and act on distant target cells.

Nervous and Endocrine Systems

Property	Nervous System	Endocrine System
Structure	Wired system of neurons	Wireless system of glands
Chemical Messenger	Neurotransmitter	Hormones
Target site	Very close	Far away
Distance of Action	Across synaptic cleft	Carried by blood
Speed of Response	milliseconds	mins to hours
Duration of Action	milliseconds	mins to days

Important definitions:

A. **Endocrine gland**: a gland that secretes hormones directly into the bloodstream; a ductless gland.

B. **Exocrine gland** : a gland that secretes substances into ducts which then leave the body (i.e. sweat/sebaceous glands) or into an internal space or lumen (i.e. digestive glands). Exocrine glands are not part of the endocrine system.

C. **Hormone**: a very powerful substance secreted by an endocrine gland into the bloodstream, that affects the function of another cell or "target cell".

Like all molecules, hormones are synthesized, exist in a biologically active state for a time, and then degrade or are destroyed. Having an appreciation for the "half-life" and mode of elimination of a hormone aids in understanding its role in physiology and is critical when using hormones as drugs.

General characteristics of hormones:

1. needed in very small amounts;

2. produce long-lasting effects in the cells they target;

3. regulate metabolic processes (maintain homeostasis);

4. are regulated by negative-feedback mechanisms;

5. may be steroid (produced from cholesterol = fat-soluble) or non-steroid (water-soluble).

Below is a list of the main glands, some of the hormones they produce and what effects they have on the body.

Hypothalamus: an area in the base of the brain that links the brain to the hormonal system.

- major hormones — anti-diuretic hormone (ADH), oxytocin, dopamine, corticotrophin releasing hormone (CRH), thyrotrophin releasing hormone (TRH), gonadotrophin releasing hormone (GnRH), growth hormone releasing hormone (GHRH) and somatostatin.
- influences — they hypothalamus links the hormonal and nervous systems. Its hormones keep the body stable. They influence sleep rhythms, alertness, appetite, body weight, thirst, blood pressure, heart rate, sex drive, learning, memory, mood and how the body responds to being sick.

Pituitary gland: a kidney bean-shaped gland in the base of the brain.

- major hormones — luteinising hormone (LH), follicle-stimulating hormone (FSH), prolactin, growth hormone, thyroid stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH).
- influences — the pituitary gland helps control other glands and makes hormones that control blood pressure, blood sugar levels, response to stress, menstruation, sperm production, bone growth, muscle mass, contractions during childbirth, making breastmilk and bonding between mother and baby.

Other glands

Pineal gland: a small gland near the center of the brain.

- major hormones — melatonin
- influences — sleep cycle

Thyroid gland: a small gland in the front of the neck, wrapping around the windpipe.

- major hormones — tri-iodothyronine (T3), thyroxine (T4), calcitonin
- influences — metabolism, bone growth, energy levels, body temperature, how the cells use oxygen, heart rate, blood flow, calcium levels, vitamin metabolism, brain development in babies and children, and reproduction

Parathyroid glands: four small glands in the neck behind the thyroid gland.

- major hormones — parathyroid hormone (PTH)

- influences — regulating calcium levels in the blood

Adrenal glands: 2 glands that sit above the kidneys on each side of the body.

- major hormones — adrenaline, cortisol, aldosterone, DHEA, testosterone
- influences — stress response, blood pressure, salt and water control, blood sugar levels, energy, development of sex organs, heart rate, attention, inflammation, development of the fetus.

Pancreas: a long gland behind the stomach, under the liver.

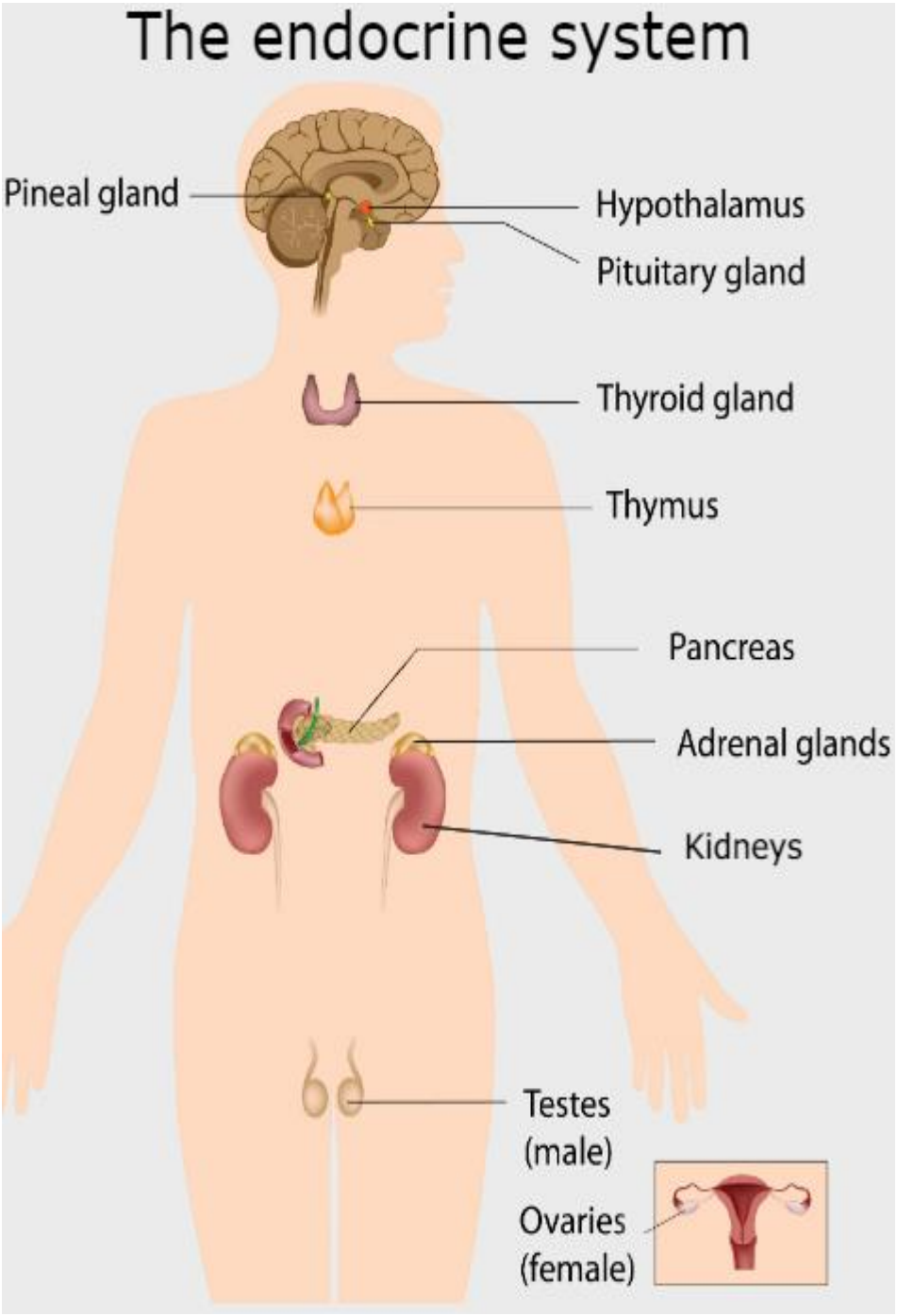
- major hormones — insulin, glucagon, somatostatin, vasoactive intestinal peptide (VIP)
- influences — blood sugar control.

Ovaries (females only): 2 glands found on each side of the uterus in the pelvis.

- major hormones — oestrogen, progesterone, testosterone, anti-mullerian hormone (AMH), Inhibin A and Inhibin B
- influences — female characteristics, storing and releasing eggs.

Testes (males only): 2 glands in the scrotum, behind the penis.

- major hormones — testosterone, anti-mullerian hormone (AMH), estradiol, inhibin B
- influences — male characteristics, sperm production.


The endocrine system
Pineal gland
Hypothalamus
Pituitary gland
Thyroid gland
Thymus
Pancreas
Adrenal glands
Kidneys
Testes
(male)
Ovaries
(female)

" Lipid Metabolism "

* Lipid Metabolism:

Lipid metabolism is the synthesis and degradation of lipids in cells, involving the breakdown and storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes. The energy yield from a gram of fatty acids is approximately 9 kcal, compared to 4 kcal/g for proteins and carbohydrates. The body's store of fat is constantly broken down to balance the body's energy needs with the food available. Groups of specific enzymes help the body break down and process fats. Lipid metabolism is in a constant state of dynamic equilibrium. This means that some lipids are constantly being oxidized to meet the body's metabolic needs, whereas others are being synthesized and stored.

The majority of lipids found in the human body from ingesting food are triglycerides and cholesterol. Other types of lipids found in the body are fatty acids.

Mobilization of Fatty Acids from Adipocytes

When the energy supply from diet is limited, the body responds to this deficiency through hormonal signals transmitted to the adipose tissue by release of glucagon, epinephrine, or adrenocorticotropic hormone.

The hormones bind to the plasma membranes of adipocyte cells and stimulate synthesis of cyclic adenosine monophosphate (cAMP). The cAMP activates a protein kinase that phosphorylates and in turn activates hormone-sensitive triacylglycerol lipases.

These lipases hydrolyze the triacylglycerols at position 1 or 3 to produce diacylglycerols (DAG) and fatty acid, which is the rate limiting step

in the hydrolysis. The diacylglycerol lipases hydrolyze the DAG to monoacylglycerols (MAG) and a fatty acid. Finally MAG lipases hydrolyze MAG to fatty acid and glycerol.

The free fatty acids (FFA) produced by lipolysis move through the plasma membranes of the adipose cells and endothelial cells of blood capillaries by simple diffusion and bind to albumin in the blood plasma, which are transported to peripheral tissues. The glycerol produced is taken up by liver, phosphorylated and oxidized to dihydroxyacetone phosphate, which is isomerised to glyceraldehydes-3-phosphate, an intermediate of both glycolysis and gluconeogenesis.

Therefore, the glycerol is either converted to glucose (gluconeogenesis) or to pyruvate (glycolysis).

Fatty Acid Oxidation:

Although fatty acids are both oxidized to acetyl-Coenzyme A (acetyl-CoA) and synthesized from acetyl-CoA, fatty acid oxidation is not the simple reverse of fatty acid biosynthesis but an entirely different process taking place in a separate compartment of the cell. The separation of fatty acid oxidation in mitochondria from biosynthesis in the cytosol allows each process to be individually controlled and integrated with tissue requirements. The successive oxidative removal of two carbons in the form of acetyl-CoA beginning from the carboxyl end is called β-oxidation.

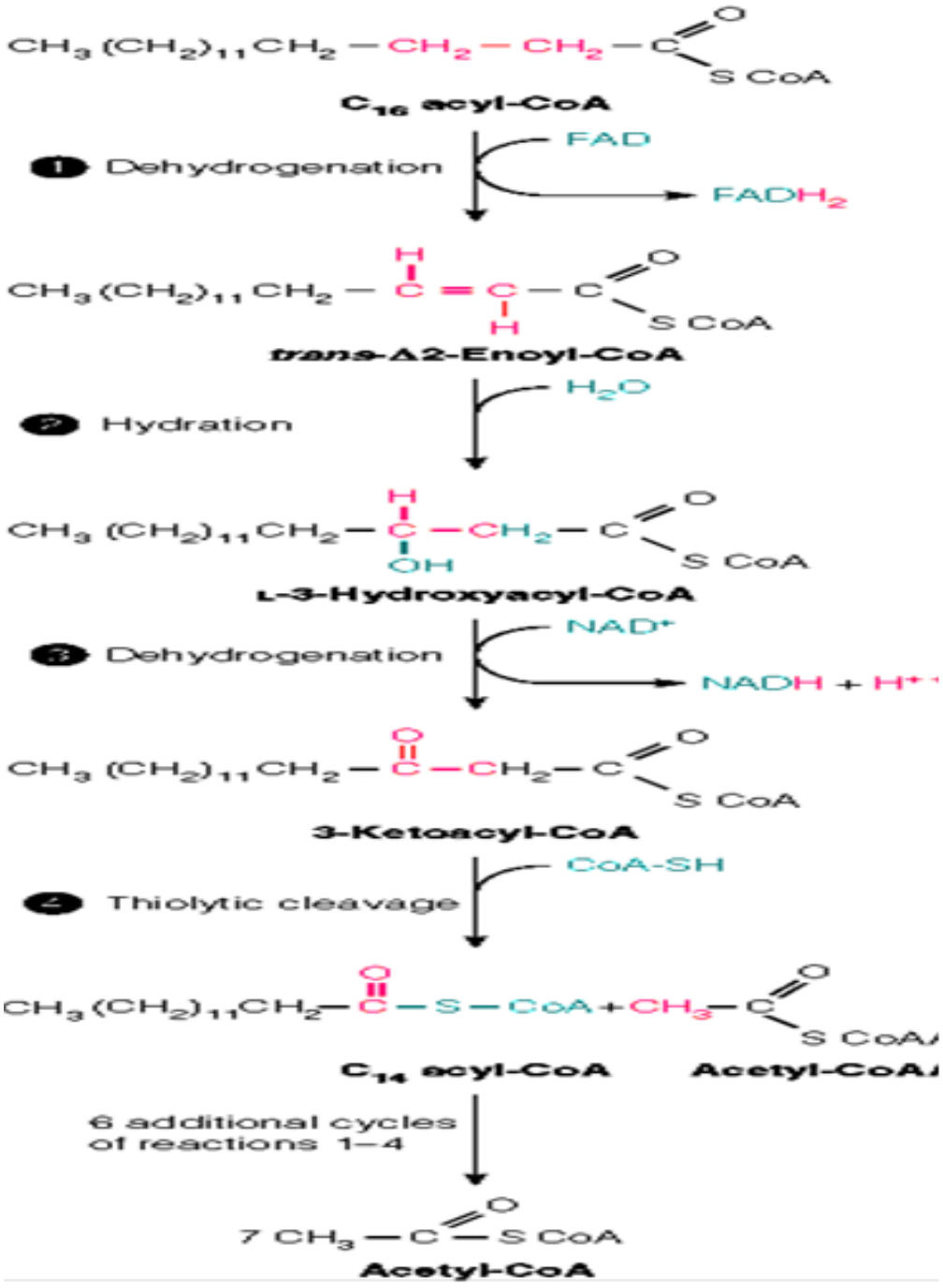
Each step in fatty acid oxidation involves acyl-CoA derivatives catalyzed by separate enzymes, utilizes NAD+ and FAD as coenzymes, and generates ATP. It is an aerobic process, requiring the presence of oxygen. Overall activation of fatty acid requires hydrolysis of two phosphodiester bonds.

1. Acyl CoA dehydrogenase converts acyl CoA to acyl trans enoyl CoA
2. Hydratase converts it to 3-hydroxy acyl CoA.

3. Hydroxy acyl CoA dehydrogenase converts it to 3keto acyl CoA.

4. It is further converted to acyl CoA and acetyl CoA.by Thiolase.

The cycle is repeated 7 times for palmitic acid for complete oxidation.



(β-oxidation of fatty acid)

Complete oxidation of fatty acid can be divided into two stages.

A. Formation of acetyl CoA.

B. Oxidation of acetyl CoA to CO2, water via TCA cycle.

Palmitoyl CoA + 7FAD + 7 NAD +7CoA = 8 Acetyl CoA+7FADH2 +7 NADH2.

Energetics of palmitate oxidation:

Acetyl CoA release energy through TCA cycle.

7 FADH2 → 7 x 2 = 14 ATPs

7NADH2 → 7 x 3 = 21 ATPs

8 Acetyl CoA → 8 x 12 = 96 ATPs

Total ATP produced from one molecule of palmitic acid is 131. Two ATPs (Two energy rich bonds) are utilized, during activation of fatty acid. Therefore total gain of ATPs is 129.

The fates of acetyl-CoA formed by b-oxidation of fatty acids are:

1. Oxidation to CO_2 and H_2O by citric acid cycle.
2. Synthesis of lipids like cholesterol, fatty acids and other steroids.
3. Formation of ketone bodies in the liver.

Regulation of Oxidation of Fatty Acids

• Hormones regulate lipolysis, in adipose tissue more free fatty acids are available for the β- oxidation.

• Insulin inhibits lipolysis.

• Increased concentration of acetyl CoA inhibits Thiolase.

• When the animal is well fed by carbohydrate, fatty acid oxidation is lowered.

Ketone Bodies

When the level of acetyl CoA from β-oxidation increases in excess of that required for entry into the citric acid cycle, It undergoes ketogenesis in the mitochondria of liver (ketone body synthesis).

The three compounds, acetoacetate, β-hydroxybutyrate, and acetone are collectively known as ketone bodies. The synthesis of ketone bodies takes place during severe starvation or severe diabetes mellitus. During such conditions, the body totally depends on the metabolism of stored triacylglycerols to fulfill its energy demand.

In the synthesis, two molecules of acetyl CoA condense together to form acetoacetyl CoA. The acetoacetate, when its concentration is very high in blood is spontaneously decarboxylated to acetone. Acteoacetate can be converted to β-hydroxy butyrate by a dehydrogenase enzyme. It is a reversible reaction.

Causes of Ketosis

1. Prolonged starvation, depletion of carbohydrate stores results in increased fatty acid oxidation and ketosis.
2. Lactating mothers develop ketosis, if the carbohydrate demands are not met with.
3. Diabetic patients with uncontrolled blood glucose, invariably suffer from ketosis, ketoacidosis. Ketosis usually associated with sustained high levels of free fatty acids in blood.

Dr.Salim.J.Kh.

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" Lipid Metabolism "

* Lipid Metabolism:

Lipid metabolism is the synthesis and degradation of lipids in cells, involving the breakdown and storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes. The energy yield from a gram of fatty acids is approximately 9 kcal, compared to 4 kcal/g for proteins and carbohydrates. The body's store of fat is constantly broken down to balance the body's energy needs with the food available. Groups of specific enzymes help the body break down and process fats. Lipid metabolism is in a constant state of dynamic equilibrium. This means that some lipids are constantly being oxidized to meet the body's metabolic needs, whereas others are being synthesized and stored.

The majority of lipids found in the human body from ingesting food are triglycerides and cholesterol. Other types of lipids found in the body are fatty acids.

Mobilization of Fatty Acids from Adipocytes

When the energy supply from diet is limited, the body responds to this deficiency through hormonal signals transmitted to the adipose tissue by release of glucagon, epinephrine, or adrenocorticotropic hormone.

The hormones bind to the plasma membranes of adipocyte cells and stimulate synthesis of cyclic adenosine monophosphate (cAMP). The cAMP activates a protein kinase that phosphorylates and in turn activates hormone-sensitive triacylglycerol lipases.

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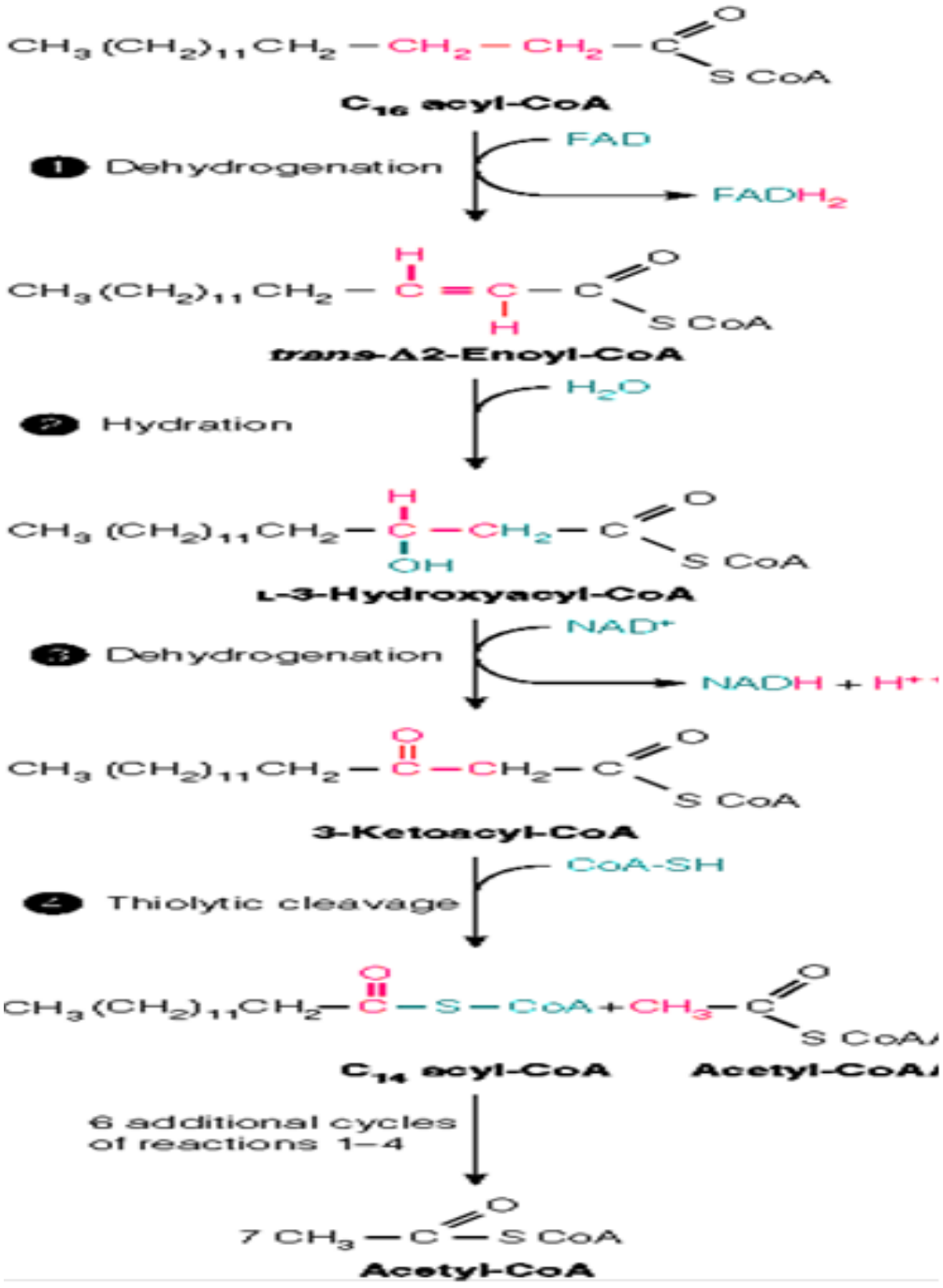

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" Lipids And Lipoproteins "

* Lipids:

Are heterogeneous group of compounds related to the fatty acids. Lipids are biological molecules that are insoluble in aqueous solutions and soluble in organic solvents(ether, chloroform, and benzene), therefore, physical properties reflect the hydrophobic nature of their structures.

-Lipid functions:

1- They serve as structural components of biological membranes.

2- They provide energy reserves, predominantly in the form of triacylglycerides.

3- Both lipids and lipid derivatives serve as hormones.

4- Interactions with vitamins, assist in the regulation of biological processes.

- Classification of lipids:

1- Simple lipids: is ester of fatty acids(F.A) with alcohol include:

a- Fats: ester of fatty acids with trihydric alcohol(glycerol).

- Ester of F.A.(saturated) with glycerol called fats (sold).
- Ester of F.A.(unsaturated) with glycerol called oils (liquid).

They are also known as glycerides (triglycerides). Each molecule of glycerol bind to three molecules of fatty acids (may be the same or different F.A.).

b- Waxes: ester of F.A. with higher molecular weight monohydric alcohol (e.g.: insect secretions, protective coating on animal furs and leaves, Beeswax).

2- Complex lipids : ester of fatty acid -containing groups in addition to fatty acid and alcohol , include:

a- Phospholipids: ester of fatty acid containing phosphoric acid . They frequently have nitrogen containing base. If alcohol is glycerol called

glycerophospholipid, but if alcohol is sphingosine is called sphingophospholipids.

b- Glycolipid: lipid containing fatty acid, sphingosine and carbohydrate e.g.: glycosphingolipid.

c- Other complex lipids like sulfolipids, aminolipids, and lipoproteins may be placed in this group.

3- Precursor and derived lipids : these include fatty acids , glycerol, steroids, fatty aldehydes, ketone bodies, cholesterol and glycerides.

- Fatty acids(F.A.):

Are aliphatic carboxylic acids mostly obtained from the hydrolysis of natural fats and oils. Chemically fatty acids have a general structure of : $CH_3(CH_2)_nCOOH$

Fatty acids that occur in natural fats usually contain an even number of carbon atoms, because they are synthesized from 2 carbon units and are straight-chain derivatives. The chain may be saturated (containing no double bonds), or unsaturated (containing one or more double bonds).

* F.A. have two major roles in the body:

1- As the components of more complex membrane lipids.

2- As the major components of stored fat in the form of triacylglycerols.

The numbering of carbons in fatty acids begins with the carbon of the carboxylate group. At physiological pH, the carboxyl group is readily ionized, rendering a negative charge onto fatty acids in bodily fluids, therefore F.A. are weak acids.

Saturated and unsaturated fatty acids

Saturated Fats	Unsaturated Fats
Contains a single bond.	Contains at least one double bond.
Not to be consumed more than 10 percent of total calories per day.	Not to be consumed more than 30 percent of total calories per day.
Excessive consumption leads to heart diseases.	Good for consumption, but excessive may increase cholesterol.
Increases low- density lipoproteins (LDL), which is called as bad cholesterol.	Increases High-density lipoprotein (HDL), which is commonly known as good cholesterol and also reduce low-density lipoproteins (LDL).
Foods sources of saturated fats are whole milk, butter, cheese, meat, etc.	Foods sources of unsaturated fats are avocado, sunflower oil, soybean oil, fish oil, etc.
High melting point.	Low melting point.
Solid state in room temperature.	Liquid state in room temperature.

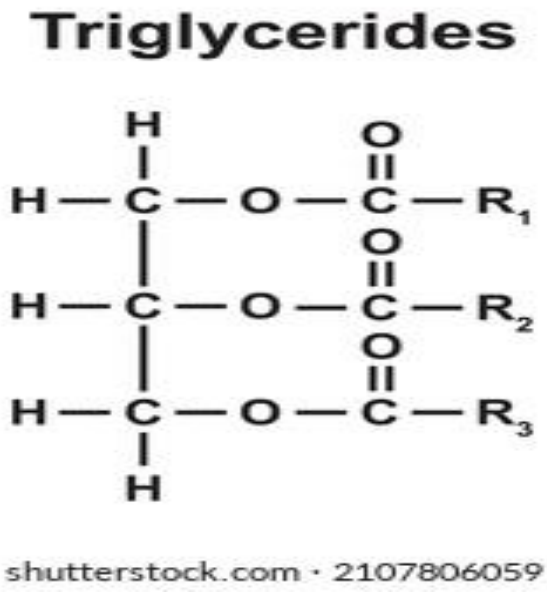
Table of major fatty acids found in plasma

Group	Name	Carbone-chain length	Source
Monounsaturated	Palmitoleic	C16	Plant oil
	Oleic	C18	Olive oil
Polyunsaturated	Linoleic	C18	Plant oil
	Linolenic	C18	Plant oil
	Arachidonic	C20	Plant oil
	Eicosapentaenoic	C20	Fish oil
Saturated	Myristic	C14	Coconut oil
	Palmitic	C16	Animal/plant oil
	Stearic	C18	Animal/plant oil

Triglycerides

Triglycerides are a type of fat that circulates in your blood. Your body makes triglycerides or gets them from the foods you eat. Your body needs some triglycerides for good health. However, high triglycerides in your blood can raise your risk of heart disease and stroke, including obesity and metabolic syndrome — a cluster of conditions that includes too much fat around the waist, high blood pressure, high triglycerides, high blood sugar and abnormal cholesterol levels.

The general structure of a triglyceride molecule is a glycerol unit attached to three fatty acids. Triglycerides are formed through a condensation reaction between a glycerol molecule and the fatty acids. Triglycerides will differ based on the different fatty acids attached to the glycerol.



Cholesterol

Cholesterol is a waxy, fat-like substance that's found in all the cells in your body. Your body needs some cholesterol to make hormones, vitamin D, and substances that help you digest foods. Your body makes all the cholesterol it needs. Cholesterol is also found in foods from animal sources, such as egg yolks, meat, and cheese.

If you have too much cholesterol in your blood, it can combine with other substances in the blood to form plaque. Plaque sticks to the walls of your arteries. This buildup of plaque is known as atherosclerosis. It can lead to coronary artery disease, where your coronary arteries become narrow or even blocked.

Lipoproteins

High-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) are lipoproteins. They are a combination of fat (lipid) and protein. The lipids need to be attached to the proteins so they can move through the blood. Different types of lipoproteins have different purposes:

- HDL : It is sometimes called "good" cholesterol because it carries cholesterol from other parts of your body back to your liver. Your liver then removes the cholesterol from your body.
- LDL : It is sometimes called "bad" cholesterol because a high LDL level leads to the buildup of plaque in your arteries.
- VLDL : Some people also call VLDL a "bad" cholesterol because it too contributes to the buildup of plaque in your arteries. But VLDL and LDL are different; VLDL mainly carries triglycerides and LDL mainly carries cholesterol.

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" Nucleic Acids "

Nucleic acids:

Nucleic acids are large biomolecules that play essential roles in all cells and viruses. A major function of nucleic acids involves the storage and expression of genomic information. Its naturally occurring chemical compound that is capable of being broken down to yield phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines).

The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

Basic structure of nucleic acids:

Nucleic acids are polynucleotides—that is, long chainlike molecules composed of a series of nearly identical building blocks called nucleotides. Each nucleotide consists of a nitrogen-containing aromatic base attached to a pentose (five-carbon) sugar, which is in turn attached to a phosphate group.

Each nucleic acid contains four of five possible nitrogen-containing bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). A and G are categorized as purines, and C, T, and U are collectively called pyrimidines.

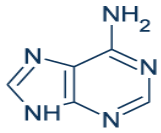
All nucleic acids contain the bases A, C, and G; T, however, is found only in DNA, while U is found in RNA. The pentose sugar in DNA (2′-deoxyribose) differs from the sugar in RNA (ribose) by the absence of a hydroxyl group (—OH) on the 2′ carbon of the sugar ring. Without an

attached phosphate group, the sugar attached to one of the bases is known as a nucleoside.

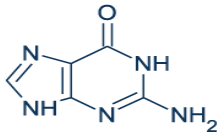
The phosphate group connects successive sugar residues by bridging the 5′-hydroxyl group on one sugar to the 3′-hydroxyl group of the next sugar in the chain. These nucleoside linkages are called phosphodiester bonds and are the same in RNA and DNA.

Purines are larger than pyrimidines because they have a two-ring structure while pyrimidines only have a single ring.

Purines

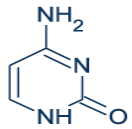


Adenine

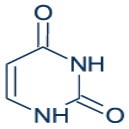


Guanine

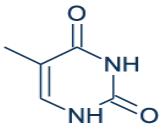
Pyrimidines



Cytosine



Uracil



Thymine

Purines	Pyrimidines
Purine is a heterocyclic aromatic organic compound composed of a pyrimidine ring fused with imidazole ring.	Pyrimidine is a heterocyclic aromatic organic compound that is composed of carbon and hydrogen.
It comprises adenine and guanine as nucleobases.	It comprises cytosine, thymine, and uracil as nucleobases
It consists of two hydrogen-carbon rings and four nitrogen atoms	It consists of one hydrogen-carbon ring and two nitrogen atoms
The melting point of purine is 214 °C	The melting point of pyrimidine is 20-22 °C

Deoxyribonucleic acid (DNA)

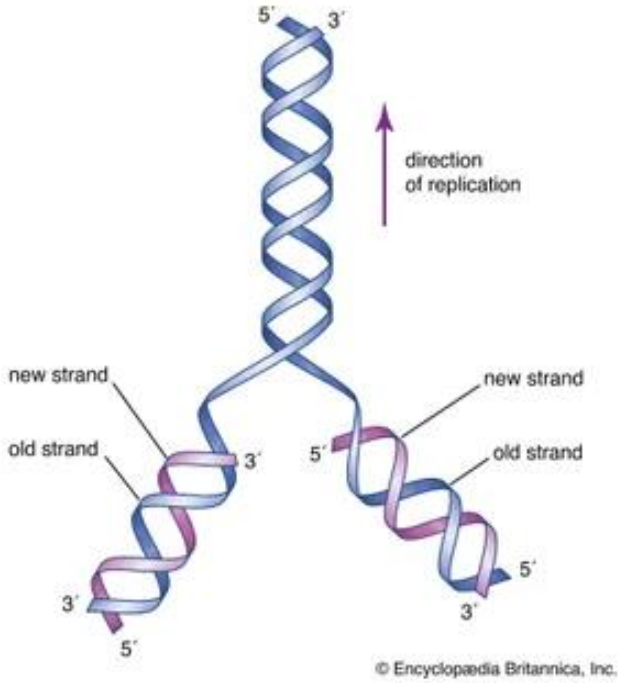
DNA is a polymer of the four nucleotides A, C, G, and T, which are joined through a backbone of alternating phosphate and deoxyribose sugar residues. These nitrogen-containing bases occur in complementary pairs as determined by their ability to form hydrogen bonds between them. A always pairs with T through two hydrogen bonds, and G always pairs with C through three hydrogen bonds. This structure, along with the molecule's chemical stability, makes DNA the ideal genetic material. The bonding between complementary bases also provides a mechanism for the replication of DNA and the transmission of genetic information.

Chemical structure

In 1953 James D. Watson and Francis H.C. Crick proposed a three-dimensional structure for DNA based on low-resolution X-ray crystallographic data which is, in naturally occurring DNA, the amount of T equals the amount of A and the amount of G equals the amount of C. Watson and Crick, who shared a Nobel Prize in 1962 for their efforts, postulated that two strands of polynucleotides coil around each other, forming a double helix.

The two strands, though identical, run in opposite directions as determined by the orientation of the 5′ to 3′ phosphodiester bond. The sugar-phosphate chains run along the outside of the helix, and the bases lie on the inside, where they are linked to complementary bases on the other strand through hydrogen bonds.

Human DNA, consists of 23 pairs of linear chromosomes containing three billion base pairs. In all cells, DNA does not exist free in solution but rather as a protein-coated complex called chromatin. Chromatin contains proteins that control gene expression and determine the characteristic shapes of chromosomes.



Ribonucleic acid (RNA)

RNA is a single-stranded nucleic acid polymer of the four nucleotides A, C, G, and U joined through a backbone of alternating phosphate and ribose sugar residues. It is the first intermediate in converting the information from DNA into proteins essential for the working of a cell. RNA is made by copying the base sequence of a section of double-stranded DNA, called a gene, into a piece of single-stranded nucleic acid. This process, called transcription, is catalyzed by an enzyme called RNA polymerase. There are three types of RNA [Messenger RNA (mRNA), Transfer RNA (tRNA), and Ribosomal RNA (rRNA)].

Chemical structure

Whereas DNA provides the genetic information for the cell and is inherently quite stable, RNA has many roles and is much more reactive chemically. RNA is sensitive to oxidizing agents such as periodate that lead to opening of the 3′-terminal ribose ring. The 2′-hydroxyl group on the ribose ring is a major cause of instability in RNA, because the presence

of alkali leads to rapid cleavage of the phosphodiester bond linking ribose and phosphate groups. In general, this instability is not a significant problem for the cell, because RNA is constantly being synthesized and degraded.

BASIS FOR COMPARISON	mRNA	tRNA	rRNA
Meaning	mRNA or messenger RNA is the connection between gene and protein, and it is the result of the transcribed gene by RNA polymerase.	tRNA or transfer RNA is a cloverleaf shaped RNA molecule and provides specific amino acids to the ribosomes.	rRNA or ribosomal RNA is used for the formation of the ribosomes.
Role	mRNA carries genetic information from the nucleus to ribosomes for the synthesis of proteins.	tRNA carries specific amino acids to the ribosomes to assist the protein biosynthesis.	rRNA these provide the structural framework for the formation of ribosomes.
Synthesized in	Nucleus.	Cytoplasm.	Ribosome.
Size	In mammals, the size of the molecules is around 400 to 12, 000 nucleotides (nt).	The size of the molecule of tRNA is 76 to 90 nucleotides (nt).	The size of the molecule of rRNA may vary from the 30S, 40S, 50S and 60S.
Shape	mRNA is linear in shape.	tRNA is a cloverleaf shape.	rRNA is a sphere shape (complex structure).

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Protein Metabolism

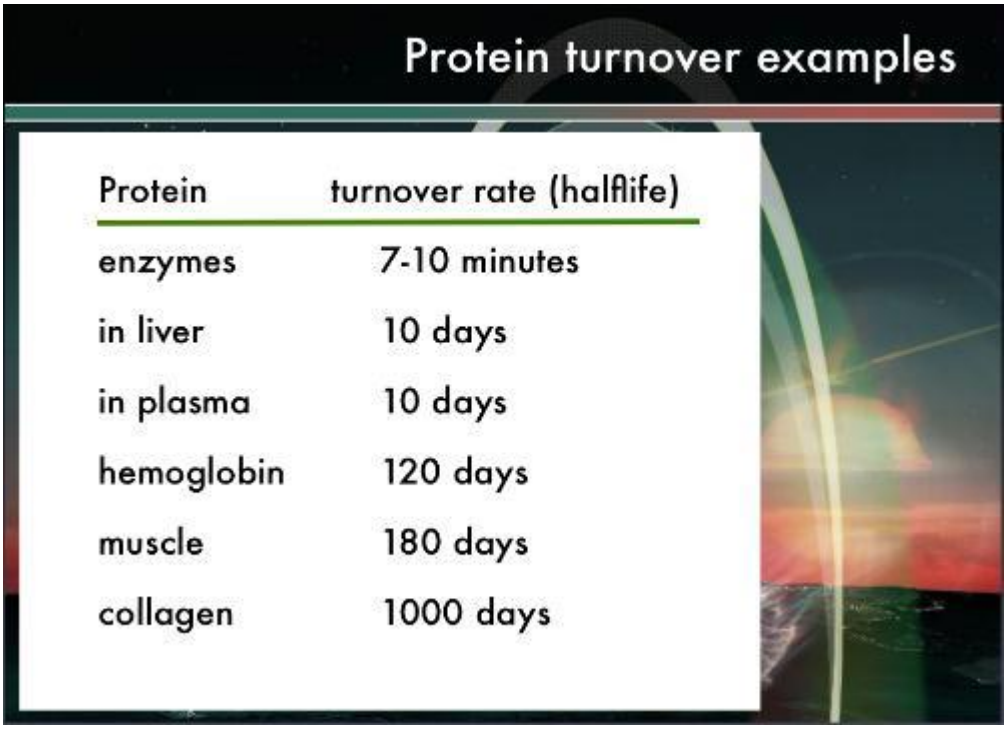
Most of the foods and drinks people ingest are complex materials that the body must break down into simpler substances. This process may involve several steps. The simpler substances are then used as building blocks, which are assembled into the materials the body needs to sustain life. This complicated process of breaking down and converting the substances ingested is called **metabolism**.

The metabolic pathways fall into two categories: (1) **Anabolic pathways** are those involved in the synthesis of compounds. Protein synthesis is such a pathway, as is the synthesis of fuel reserves of triacylglycerol and glycogen.(2) **Catabolic pathways** are involved in the breakdown of larger molecules, commonly involving oxidative reactions.

Metabolism is carried out by chemical substances called enzymes, which are made by the body. If a genetic abnormality affects the function of an enzyme or causes it to be deficient or missing altogether, various disorders can occur.

Protein metabolism is no less important than carbohydrate and lipid metabolism. **(1)** Proteins make up the structural tissue for muscles and tendons, **(2)** transport oxygen as hemoglobin, **(3)** catalyze all biochemical reactions as enzymes, and **(4)** regulate reactions as hormones. Our bodies must be able to synthesize the many proteins, amino acids, and other non-protein nitrogen containing compounds needed for growth, replacement, and repair. Proteins in excess are used to supply energy or build reserves of glucose, glycogen, or lipids.

About 75% of all amino acids are used for the production of protein. Amino acids can come from the protein we eat or from degraded proteins in the body. This degradation is a continuous process as proteins in body are constantly being replace (protein turnover).



Amino acids serve as a source of nitrogen for other compounds in the body:

1- Nitrogen bases of DNA and RNA.

2- Heme and similar structures in myoglobin, hemoglobin, cytochromes, enzymes…..

3- Acetylcholine and other neurotransmetters.

4- Hormones and phospholipids.

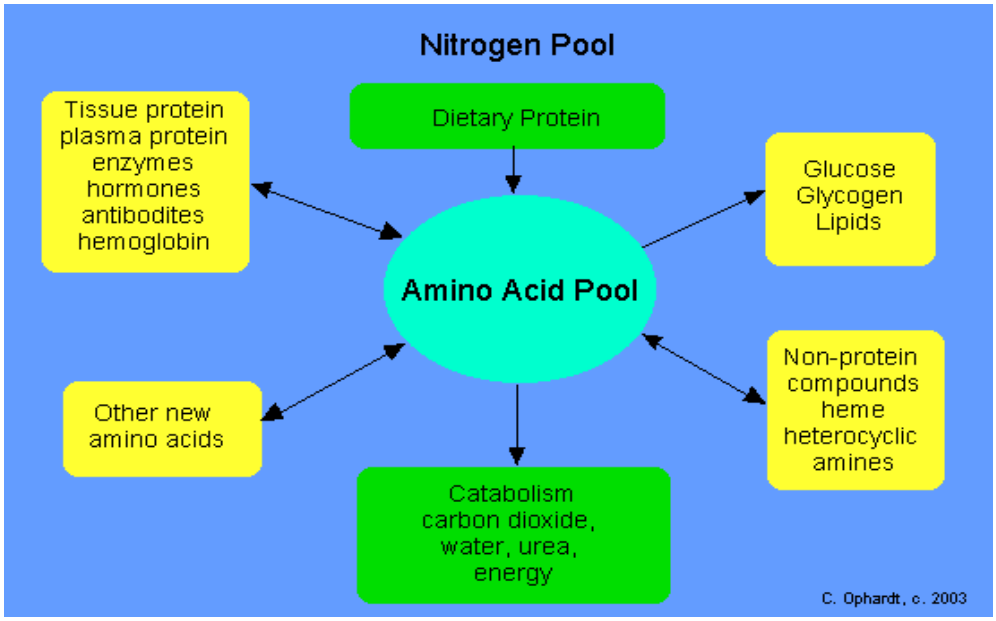
* **Nitrogen Cycle**:

Nitrogen is recycled just as carbon and oxygen are recycled in nature. Various microorganisms have the appropriate enzymes (**nitrogenase**) to convert elemental nitrogen from the air into ammonia. Green plants use the ammonia or nitrate as raw materials for the synthesis of amino acids and proteins. Animals and humans in turn use the plants to supply nitrogen to make amino acids and proteins. We humans are not as versatile as plants since we are unable to synthesize eight amino acids which must be included in the diet. Finally, the nitrogen cycle is completed when plant and animal residues are decayed by microorganisms back to nitrogen gas for the air.

The "nitrogen or amino acid pool" is a grand mixture of amino acids available in the cell derived from dietary sources or the degradation of protein. Since proteins and amino acids are not stored in the body, there is a constant turnover of protein. Some protein is constantly being synthesized while other protein is being degraded. For example, liver and plasma proteins have a half-life of 10 days or more, while enzymes and hormones may be recycled in minutes or hours.

Each day, some of the amino acids are catabolized producing energy and ammonia. The ammonia is converted to urea and excreted from the body and represents a drain on the nitrogen pool.

A nitrogen balance is achieved by a healthy person when the dietary intake is balanced by the excretion of urea wastes. If nitrogen excretion is greater than the nitrogen content of the diet, the person is said to be in negative nitrogen balance. This is usually interpreted as an indication of tissue destruction. If the nitrogen excretion is less than the content of the diet, a positive nitrogen balance indicates the formation of protein.



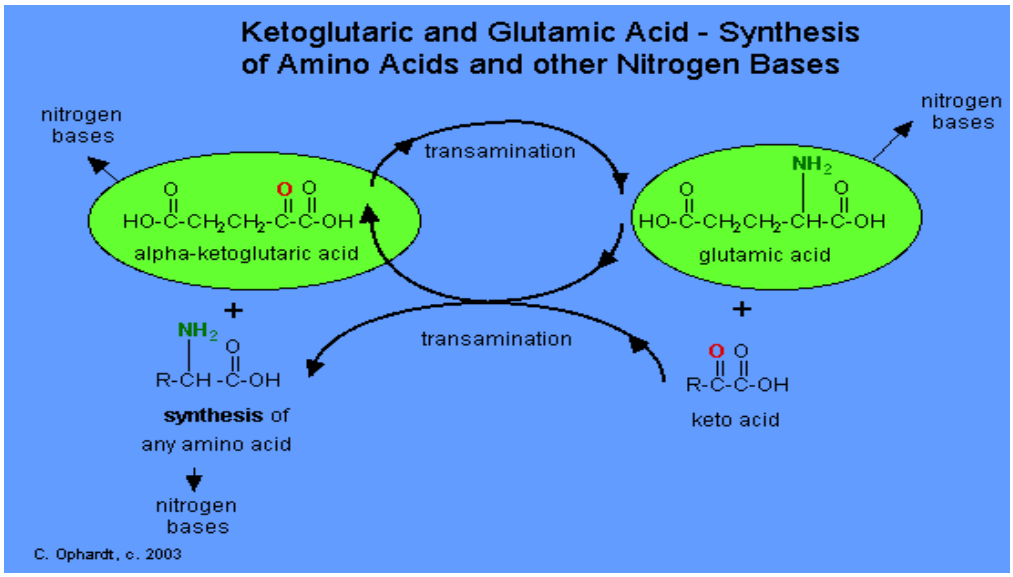
*** Protein biosynthesis:**

Protein synthesis is the process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multi-step process, beginning with amino acid synthesis and transcription which are then used for translation.

* **Amino Acid Synthesis**:

Amino acids are the monomers which are polymerized to produce proteins. Amino acid synthesis is the set of biochemical processes (metabolic pathways) which build the amino acids from carbon sources like glucose. Not all amino acids may be synthesized by every organism, for example adult humans have to obtain 8 of the 20 amino acids from their diet.

For the synthesis of amino acids, the alpha-ketoglutaric acid first uses transamination of a different amino acid to make glutamic acid, which then reacts with a keto acid to make a new amino acid. In effect, the interconversion of alpha-ketoglutaric acid and glutamic acid lies at the very heart of nitrogen metabolism, therefore, glutamate is a key intermediate in amino acid metabolism. These molecules serve as the "collection and receiving agent" for nitrogen. The subsequent fate of the amino group is in new amino acids, any nitrogen bases, or any other nitrogen containing compounds. Carbon skeletons come from intermediates of glycolysis, pentose phosphate pathway(PPP), or citric acid cycle.



*** Transcription:**

Transcription is the process by which an mRNA template, encoding the sequence of the protein in the form of a trinucleotide code, is transcribed from the genome to provide a template for translation. Transcription copies the template from one strand of the DNA double helix, called the template strand. Transcription can be divided into 3 stages: Initiation, Elongation and Termination, each regulated by a large number of proteins such as transcription factors and coactivators that ensure the correct gene is transcribed in response to appropriate signals.

*** Translation:**

The synthesis of proteins is known as translation. Translation occurs in the cytoplasm where the ribosomes are located. Ribosomes are made of a small and large subunit which surrounds the mRNA. In translation, messenger RNA (mRNA) is decoded to produce a specific polypeptide according to the rules specified by the genetic code. This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein. Translation is necessarily preceded by transcription. Translation proceeds in four phases: activation, initiation, elongation and termination (all describing the growth of the amino acid chain, or polypeptide that is the product of translation).

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* Catabolism of amino acids:

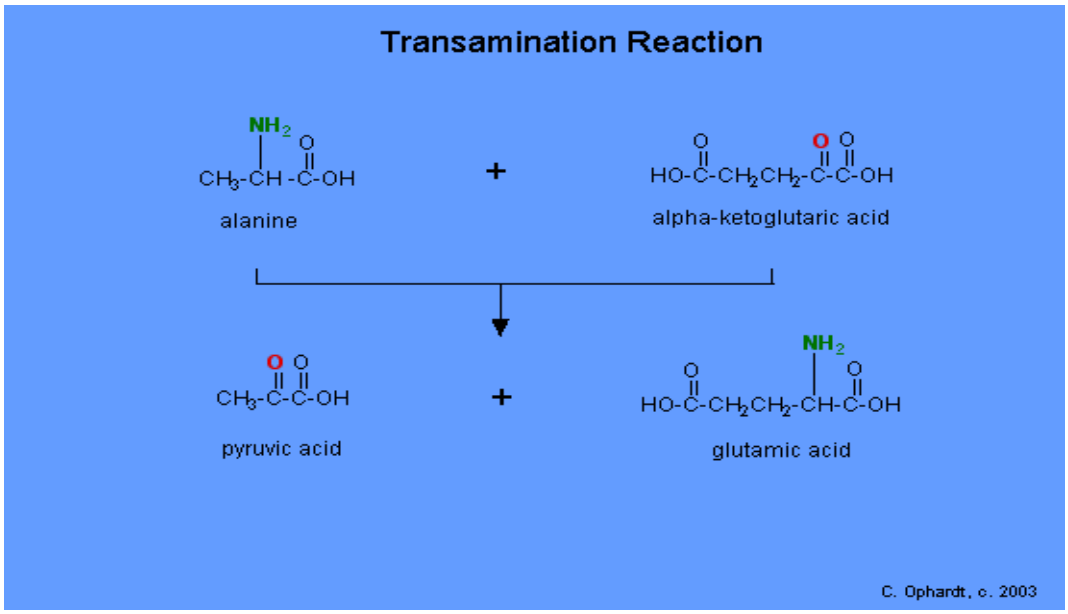
Amino acids cannot be stored by the body, if there is an excess of amino acids or a lack of other energy sources, the body will use them for energy production. Unlike fats and carbohydrates, amino acids require the removal of amine group, it must then be disposed of as it is toxic to the body.

Removal of α-amino group require two step process:

1- Transamination Reaction:

Transamination as the name implies, refers to the transfer of an amine group from one molecule to another. This reaction is catalyzed by a family of enzymes called transaminases. Actually, the transamination reaction results in the exchange of an amine group on one acid with a ketone group on another acid. The most usual and major keto acid involved with transamination reactions

is alpha-ketoglutaric acid, an intermediate in the citric acid cycle. A specific example is the transamination of alanine to make pyruvic acid and glutamic acid.

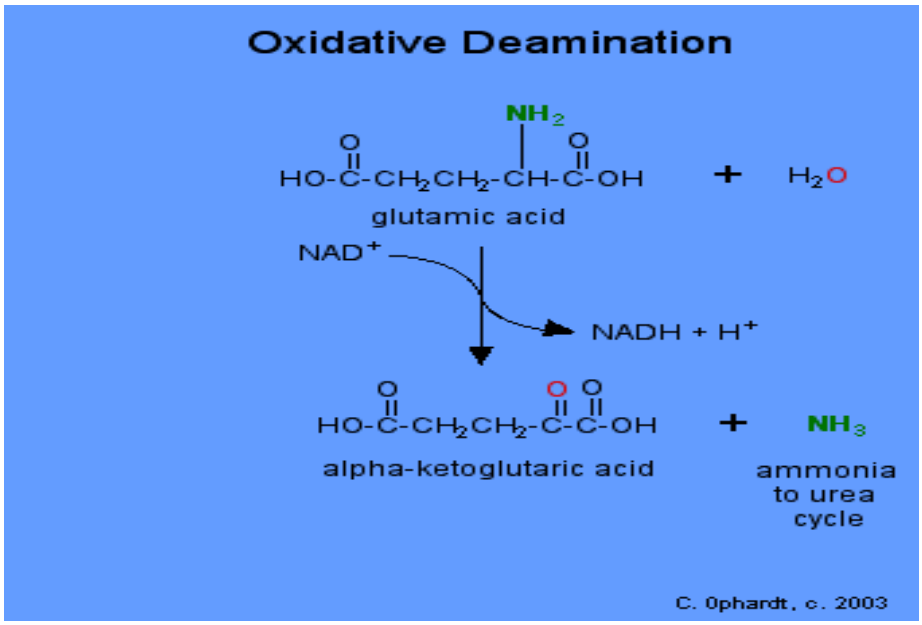


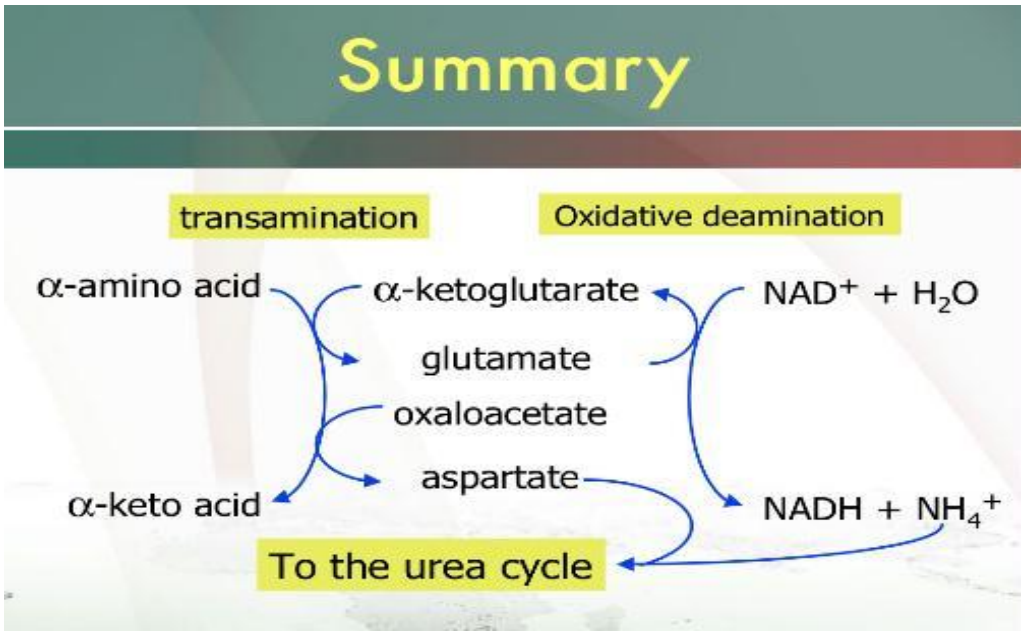
All of the amino acids can be converted through a variety of reactions and transamination into a keto acid which is a part of or feeds into the citric acid cycle.

*** Oxidative Deamination Reaction:**

Deamination is also an oxidative reaction that occurs under aerobic conditions in all tissues but especially the liver. During oxidative deamination, an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as ammonia and the amine functional group is replaced by the ketone group.

Oxidative deamination occurs primarily on glutamic acid because glutamic acid was the end product of many transamination reactions.



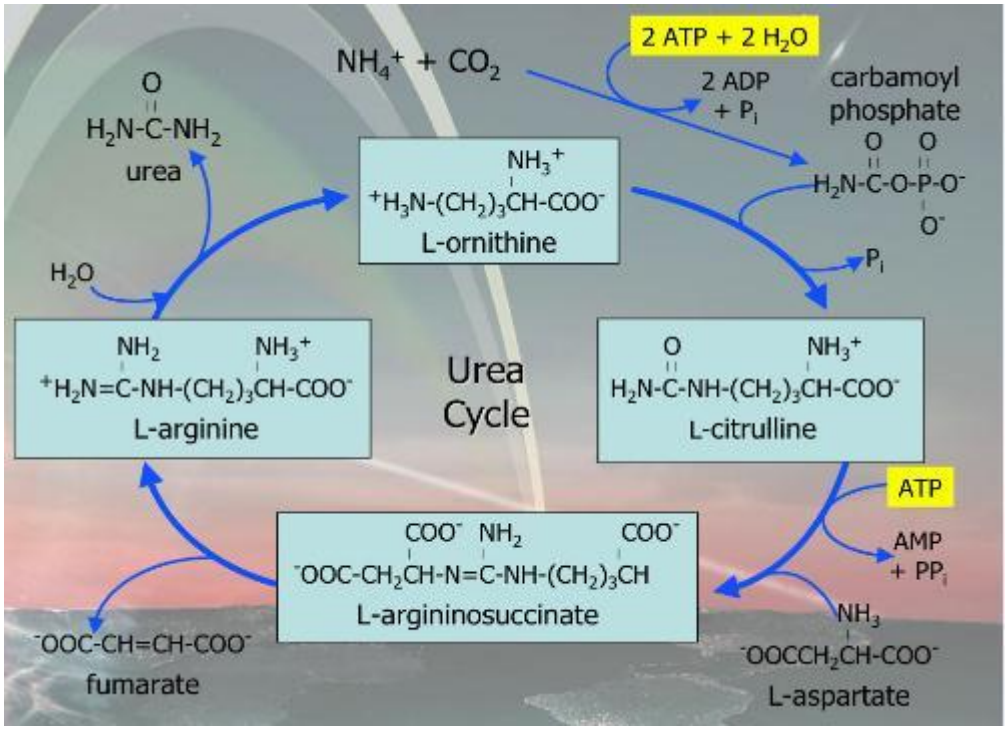


*** <u>Urea Cycle</u> :**

Urea is the major end product of nitrogen metabolism in humans and mammals. Ammonia, the product of oxidative deamination reactions, is toxic in even small amounts and must be removed from the body. The urea cycle or the ornithine cycle describes the conversion reactions of ammonia into urea. Since these reactions occur in the liver, the urea is then transported to the kidneys where it is excreted.

Ammonia + carbon dioxide + 3ATP ---> urea + water + 3 ADP

Urea is routinely measured in the blood as: Blood Urea Nitrogen (BUN). BUN levels may be elevated (a condition called uremia) in both acute and chronic renal (kidney) failure. Various diseases damage the kidney and cause faulty urine formation and excretion. Congestive heart failure leads to a low blood pressure and consequent reduced filtration rates through the kidneys, therefore, BUN may be elevated. Urinary tract obstructions can also lead to an increased BUN. In severe cases, hemodialysis is used to remove the soluble urea and other waste products from the blood. Waste products diffuse through the dialyzing membrane because their concentration is lower in the dialyzing solution. Ions, such as Na+ and Cl- which are to remain in the blood, are maintained at the same concentration in the dialyzing solution - no net diffusion occurs.



High ammonia levels are toxic to humans. A complete block of any step in the urea cycle is fatal since there is no known alternative pathway for the synthesis of urea. Inherited disorders from defective enzymes may cause a partial block in some of the reactions and results in hyperammonemia which can lead to mental retardation. Extensive ammonia accumulation leads to extensive liver damage and death.

*** Disorders of Amino Acid Metabolism:**

*** Phenylketonuria:** Phenylketonuria (PKU) is a disorder that causes a buildup of the amino acid phenylalanine, which is an essential amino acid that cannot be synthesized in the body but is present in food. Excess phenylalanine is normally converted to tyrosine, another amino acid, and eliminated from the body. Without the enzyme that converts it to tyrosine, phenylalanine builds up in the blood and is toxic to the brain, causing mental retardation.

Symptoms include seizures, nausea and vomiting, an eczema-like rash, lighter skin and hair than their family members, aggressive or self-injurious behavior, hyperactivity, and sometimes psychiatric symptoms. Untreated children often give off a "mousy" body and urine odor as a result of a by-product of phenylalanine (phenylacetic acid) in their urine and sweat.

To prevent mental retardation, phenylalanine intake must be restricted, beginning in the first few weeks of life. Because all natural sources of protein contain too much phenylalanine for children with PKU, affected children cannot have meat, milk, or other common foods that contain protein. Instead, they must eat a variety of phenylalanine-free processed foods, which are low-protein natural foods, such as fruits, vegetables.

*** Maple Syrup Urine Disease:** Children with maple syrup urine disease are unable to metabolize certain amino acids. By-products of these amino acids build up, causing neurologic changes, including seizures and mental retardation. These by-products also cause body fluids, such as urine and sweat, to smell like maple syrup.

Infants with severe disease are treated with dialysis. Some children with mild disease benefit from injections of the vitamin B_1 (thiamin). After the disease has been brought under control, children must always consume a special artificial diet that is low in the particular amino acids that are affected by the missing enzyme.

*** Homocystinuria:** Children with homocystinuria are unable to metabolize the amino acid homocysteine, which, along with certain toxic by-products, builds up to cause a variety of symptoms. Symptoms may be mild or severe, depending on the particular enzyme defect.

Infants with this disorder are normal at birth. The first symptoms, including dislocation of the lens of the eye, causing severely decreased vision, usually begin after 3 years of age. Most children have skeletal abnormalities, including osteoporosis; the child is usually tall and thin with a curved spine, elongated limbs, and long, spiderlike fingers. Psychiatric and behavioral disorders and mental retardation are common. Homocystinuria makes the blood more likely to spontaneously clot, resulting in strokes, high blood pressure, and many other serious problems.

In a few states, children are screened for homocystinuria at birth with a blood test. The diagnosis is confirmed by a test measuring enzyme function in liver or skin cells. Some children with homocystinuria improve when given vitamin B_6 (pyridoxine) or vitamin B_{12} (cobalamin).

* **Tyrosinemia:** Children with tyrosinemia are unable to completely metabolize the amino acid tyrosine. By-products of this amino acid build up, causing a variety of symptoms. In some states, the disorder is detected on the newborn screening tests.

There are two main types of tyrosinemia: I and II. Type I tyrosinemia is most common in children of French-Canadian or Scandinavian descent. Children with this disorder typically become ill sometime within the first year of life with dysfunction of the liver, kidneys, and nerves, resulting in irritability, rickets, or even liver failure and death. Restriction of tyrosine in the diet is of little help. An experimental drug, which blocks production of toxic metabolites, may help children with type I tyrosinemia. Often, children with type I tyrosinemia require a liver transplant.

Type II tyrosinemia is less common. Affected children sometimes have mental retardation and frequently develop sores on the skin and eyes. Unlike type I tyrosinemia, restriction of tyrosine in the diet can prevent problems from developing.

" Vitamins "

Vitamins are organic nutrients (molecules), that are required in small quantities for a variety of biochemical functions,(the most prominent function is as cofactors for enzymatic reactions), also they are essential for the normal processes of metabolism, including growth and maintenance of health.

The distinguishing feature of the vitamins is that they generally cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet, (except, the body is able to produce part or even all of its requirements for some of the vitamins, Example: Vitamin D from cholesterol and niacin from Tryptophan).

The vitamins are classified into two major groups:

1- Water-soluble vitamins: (thiamin (B_1) , riboflavin (B_2) , niacin(B_3) , pantothenic acid (B_5) , pyridoxal (pyridoxine, pyridoxamine (B_6) , biotin , cobalamin (B_{12}) , folic acid and ascorbic acid).

2- Fat-soluble vitamins: (vitamin A , vitamin D , vitamin E ,vitamin K).

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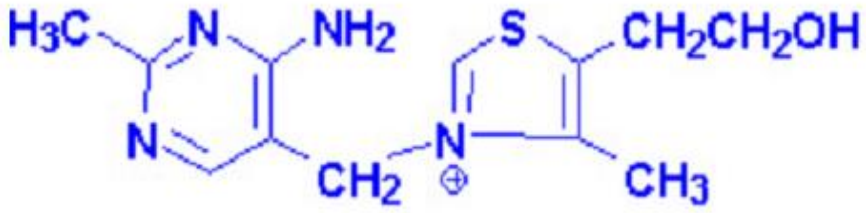
* Water-soluble vitamins: it's a polar hydrophilic molecules and, therefore, are soluble in water.

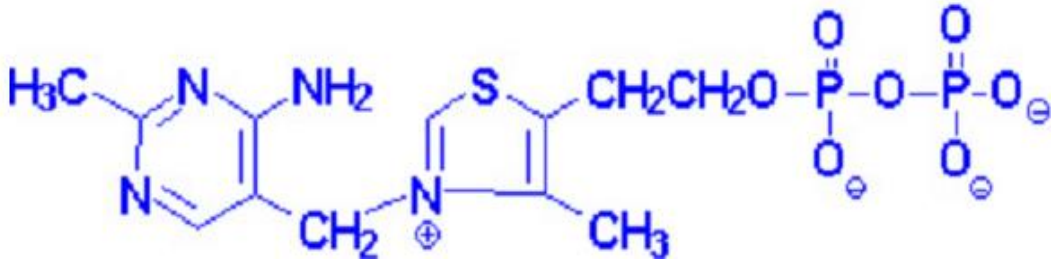
Because of their water solubility, excesses of these vitamins are excreted in urine and so rarely accumulate in toxic concentrations, for this reason their storage is limited.

1-Thiamin (B_1) :

Thiamin is also known as vitamin B_1, is derived from a substituted pyrimidine and a thiazole which are coupled by a methylene bridge.



Thiamin is rapidly converted to its active form, thiamin pyrophosphate (TPP), in the brain and liver by specific enzyme, thiamin diphosphotrans-ferase.



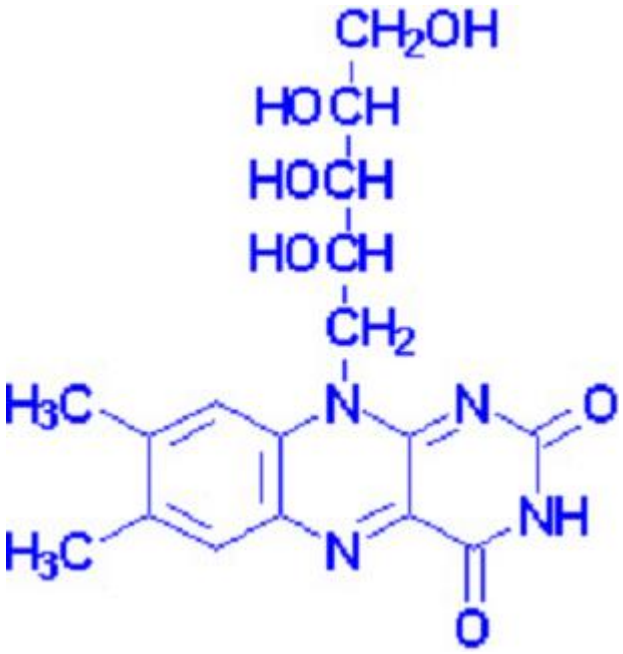
TPP is necessary as a cofactor for the pyruvate and α-ketoglutarate dehydrogenase catalyzed reactions like in pentose phosphate pathway, therefore, a deficiency in thiamin intake leads to a severely reduced capacity of cells to generate energy as a result of its role in these reactions.

The dietary requirement for thiamin is proportional to the caloric intake of the diet and ranges from 1.0-1.5 mg/day for normal adults. If the carbohydrate content of the diet is excessive, then an excess thiamin intake will be required.

The severe thiamin deficiency disease known as beriberi is the result of a diet that is carbohydrate rich and thiamin deficient, in such individuals TPP dependent reactions are prevented, leading to accumulation of substrates like Pyruvate, Pentose sugars etc .

Thiamin is present in almost all plant and animal tissues commonly used as foods like seeds, nuts, wheat, & lean meat.

2- Riboflavin (B_2) :



Riboflavin is also known as vitamin B_2, is the precursor for the coenzyme, flavin mononucleotide(FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins.

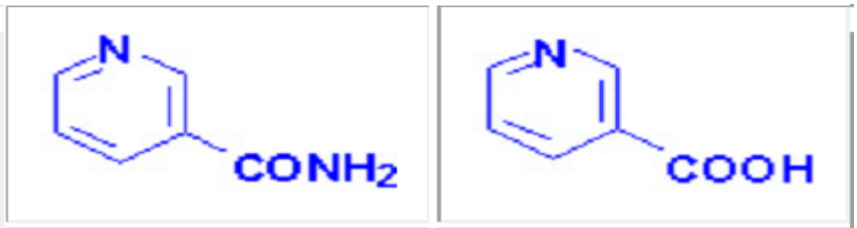
Riboflavin deficiencies are rare due to the presence of adequate amounts of the vitamin in eggs, milk, meat, and cereals.

Requirement's about 1.5-2.5mg for adults, infants 0.6mg, children 1.0-1.8mg.

Deficiency: Lack of riboflavin in the diet causes a generally non fatal syndrome of inflammation of the corner of mouth (angular stomatitis), painful glossitis of tongue (Purple) and Scaly dermatitis.

Riboflavin decomposes when exposed to visible light, this characteristic can lead to riboflavin deficiencies in newborns treated for hyperbilirubinemia by phototherapy.

3-Niacin (B_3) :



Nicotinamide	Nicotinic Acid

Niacin (nicotinic acid and nicotinamide) is also known as vitamin B_3. Both nicotinic acid and nicotinamide can serve as dietary sources of vit.B_3.

Niacin is required for the synthesis of the active forms of vit.B_3, which are nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both NAD^+ and $NADP^+$ function as cofactors for numerous dehydrogenases, e.g.: lactate and malate dehydrogenases.

Niacin is not a true vitamin, since it can be derived from the amino acid tryptophan.

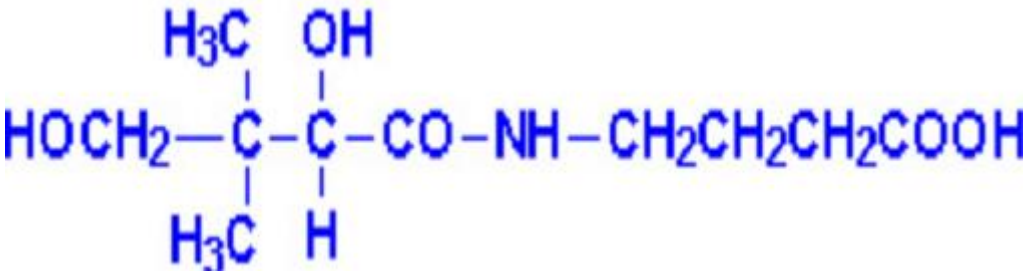
The ability to utilize tryptophan for niacin synthesis is inefficient, due to (60) mg of tryptophan are required to synthesize (1) mg of niacin, also, synthesis of niacin from tryptophan requires vitamins B_1, B_2, and B_6 which may be limited in the diet.

The daily requirement for niacin is 17-21mg for adults, infants 6mg.The requirement increases with increased intake of calories, illness, severe injury ,infection ,burns, high corn (maize) diet, pregnancy and lactation.

Lack of niacin causes of the deficiency syndrome which called "pellagra" in peoples depend on corn as diet, a disease involving gastrointestinal tract (GIT) and central nervous system (CNS).

The major action of nicotinic acid is reduction the fatty acid mobilization from adipose tissue, therefore, it used in treatment of hypercholesterolemia.

4- Pantothenic acid (B_5) :



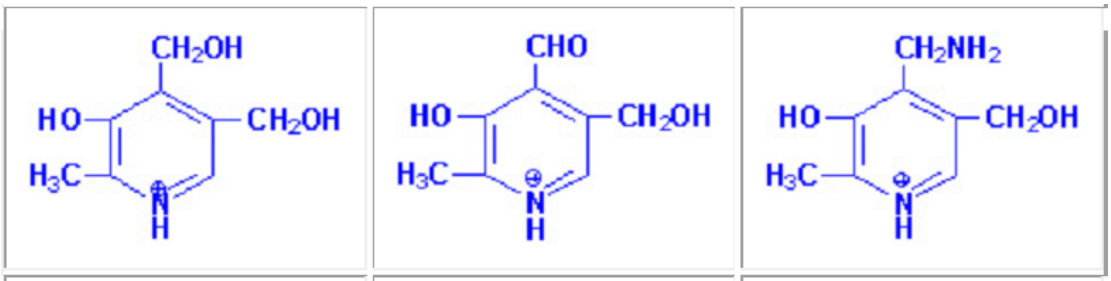
Pantothenic acid is also known as vitamin B_5. Its formed from β-alanine and pantoic acid. Pantothenate is required for synthesis of coenzyme A (CoA) and is a component of the acyl carrier protein(ACP), which is (ACP) used in fatty acid synthesis.

Pantothenate is, therefore, required for the metabolism of carbohydrate and all fats and proteins. At least (70) enzyme have been identified as requiring CoA or ACP derivatives for their function.

Daily requirements about 4-7mg and deficiency is rare due to its widespread distribution in whole grain cereals, legumes and meat.

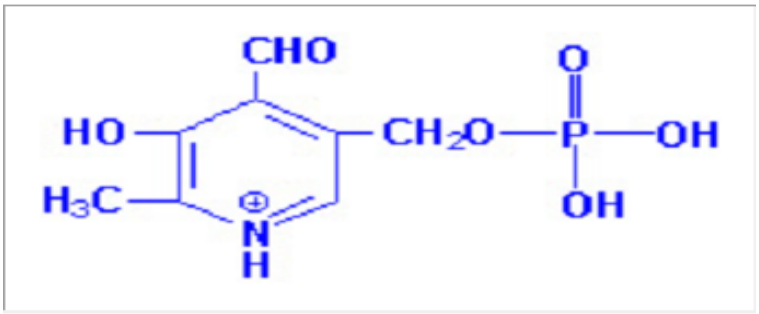
The burning foot syndrome in prisoners which is associated with reduced capacity for acetylation is ascribed to pantothenic acid deficiency.

5- Vitamin B_6 :



Pyridoxine	Pyridoxal	Pyridoxamine

Pyridoxal, pyridoxine, and pyridoxamine are collectively known as vitamin B_6. All three compounds are efficiently converted to the biologically active form of vit.B_6, pyridoxal phosphate, this conversion is catalyzed by the ATP requiring enzyme, pyridoxal kinase.



Pyridoxal Phosphate

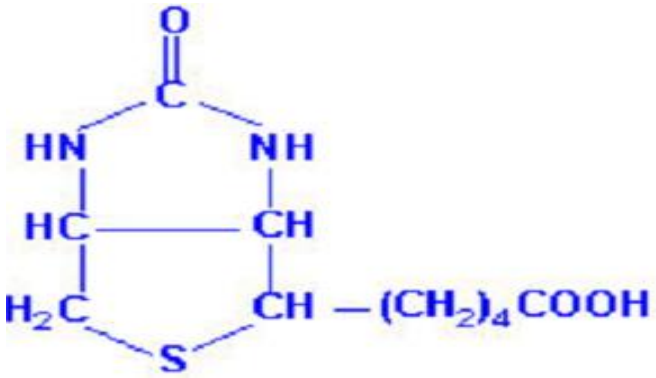
Pyridoxal phosphate functions as a cofactor in enzymes involved in transamination reactions required for the synthesis and catabolism of the amino acids as well as in glycogenolysis as a cofactor for glycogen phosphorylase.

The requirement for vitamin B6 in the diet is proportional to the level of protein consumption ranging from 1.4-2.0 mg/day for a normal adult.

During pregnancy and lactation the requirement for vit.B_6 increases approximately 0.6 mg/day.

Deficiencies of vit.B_6 are rare and usually are related to an overall deficiency of all the B-complex vitamins and if happened may lead to hypochromic microcytic anemia since it is required for heme synthesis .

6- Biotin :



It is the cofactor required for enzymes that are involved in carboxylation reactions (e.g.: acetyl-CoA carboxylase and pyruvate carboxylase).

Biotin is found in numerous foods and also is synthesized by intestinal bacteria and as such deficiencies of the vitamin are rare. Deficiencies are generally seen after long antibiotic therapies which deplete the intestinal fauna, also deficiency may happen after excessive consumption of raw eggs, due to the egg white protein (avidin) prevent intestinal absorption of the biotin.

Daily requirements about 100-200µg/day. Requirement increase in pregnancy and lactation. Patients on oral antibiotics for a long period of time require more of this vitamin.

7- Cobalamin :

It is more commonly known as vit.B_{12}. It is composed of a complex tetrapyrol ring structure (corrin ring) and a cobalt ion in the center.

There are two reactions in the body that require this vitamin as a cofactor :

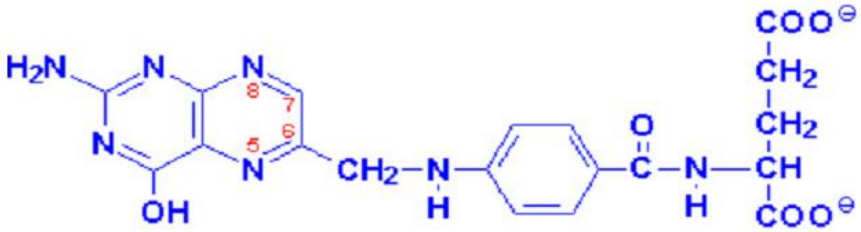
1- During the catabolism of fatty acids, and the amino acids valine, isoleucine and threonine, the enzyme required is methylmalonyl-CoA mutase.

2- During the conversion of homocysteine to methionine and is catalyzed by methionine synthase.

Deficiencies of vit.B_{12} is rare due to the liver can store it up to six years, but if happened, deficiencies lead to pernicious anemia (due to impaired DNA synthesis) and also lead to neurological complications (due to progressive demyelination of nerve cells).

Daily requirements about 3mg, and its synthesized in small quantities by microorganisms.

8- Folic acid :



Folic acid is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (PABA). Folic acid is obtained primarily from yeasts and leafy vegetables as well as animal liver.

The active form of folic acid is Tetra hydro folate (THF). Animals cannot synthesize PABA, thus, requiring folate intake in the diet. Folate deficiency is rare, but if happened, leads to impairment of DNA synthesis and this lead to abnormally large erythrocytes (macrocytic anemia).

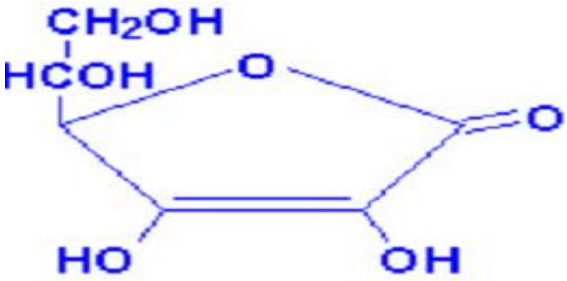
Daily requirements about 100µg (during Lactation & pregnancy are 500 - 800µ g/day).

Deficiency: The causes of folate deficiency are inadequate intake, impaired absorption, increased demand during pregnancy, lactation and impaired metabolism that leads to megaloblastic anemia. In this condition

production of erythrocytes slows down, macrocytic erythrocytes with fragile membrane are formed.

Inadequate folate levels during the early stages of pregnancy increases the risk of neural tube defects (a type of birth defect) and spontaneous abortions.

9- Ascorbic acid :



Ascorbic acid is more commonly known as vitamin C. Ascorbic acid is derived from glucose via the uronic acid pathway.

The active form of vitamin C is ascorbate itself. The main functions of ascorbate in: collagen biosynthesis, degradation of tyrosine, absorption of iron, steroidogenesis, adrenaline synthesis, bile acid formation, bone mineral metabolism and as potent antioxidant.

Deficiency of vit.C leads to the disease Scurvy due to the role of the vitamin in modification of collagen.

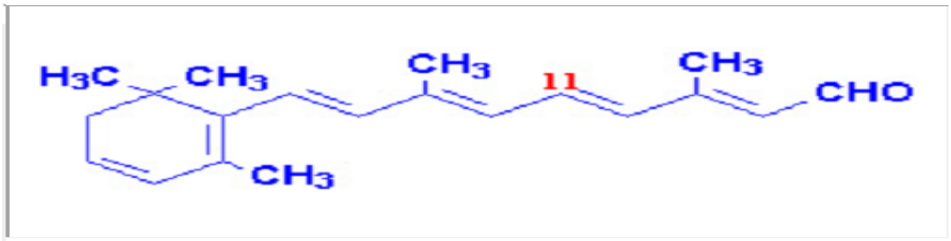
WBC's are rich in vit C and plays an important role in immunity.

Source: citrus fruits, potato, tomato & green vegetables, and the daily requirements about 60mg.

* Fat-soluble vitamins :

Ample reserves of fat soluble vitamins are stored in the tissues as they are not readily absorbed from the food. With the exception of Vit. K, they do not serve as coenzymes. Indeed Vit D act more like hormone.

1-Vitamin A:



All-*trans*-retinal

Vitamin A consists of three biologically active molecules, retinol, retinal and retinoic acid. Each of these compounds are derived from the plant precursor molecule, β-carotene.

Functions: β-carotene has an antioxidant role and prevents the development of diseases in which the action of free radicals is implicated .

It plays a protective role against cancer and cardiovascular disease, as the normal proliferation of epithelial cell growth and differentiation depends on retinoids.

Vitamin A is necessary for vision, and deficiency cause night blindness due to the visual pigment, rhodopsin is found in the rod-cells of the retina and is formed by the binding of retinal to the apoprotein opsin.

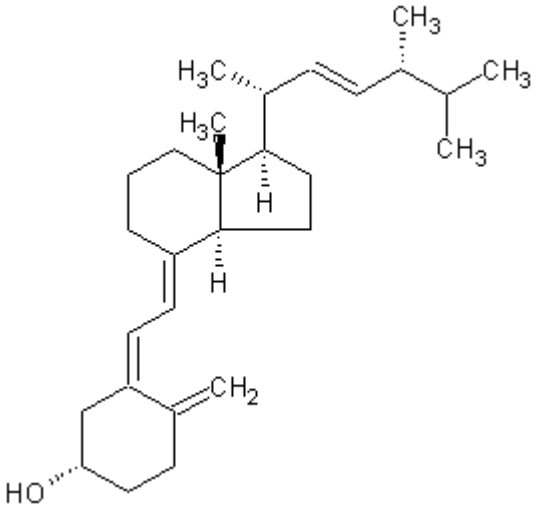
Source: a rich source is liver, but leafy vegetables and some fruits provide the largest amount of β-carotene. Liver, egg yolk, butter and milk are good sources of β-carotene.

Vitamin A is stored in the liver and deficiency of the vitamin occurs only after prolonged lack of dietary intake. The earliest symptoms of vitamin A deficiency are night blindness. Severe Vit A deficiency leads to progressive keratinization of the cornea and possibly permanent blindness.

The deficiency causes dryness and roughness of skin developing keratosis of hair follicles. Bone growth is markedly impaired.

Excessive intake of vitamin A (hypervitaminosis), in humans cause head ache, nausea, vomiting and dizziness. This might be related to increased spinal fluid pressure.

2-Vitamin D :



Vitamin D is the only vitamin that is usually not required in the diet, for this reason it is rather classified as a hormone since under conditions of inadequate exposure to sunlight that dietary intake is required.

Vitamin D is a steroid hormone that functions to regulate specific gene expression following interaction with its intracellular receptor.

The biologically active form of the hormone is 1,25-dihydroxy vitamin D_3 (1,25-$(OH)_2$ D_3), also termed calcitriol.

Function: Calcitriol functions in concert with parathyroid hormone(PTH) and calcitonin to regulate serum calcium and phosphorous levels.

- Intestine: This vitamin promotes absorption of calcium, phosphates.

- Kidney: Reabsorption of calcium, phosphate are enhanced.

- Bone: It promotes synthesis of osteocalcin which is needed for bone mineralization. It also promotes bone collagen synthesis.

Sources: Fish oils, egg yolk are naturally rich sources of Vit D.

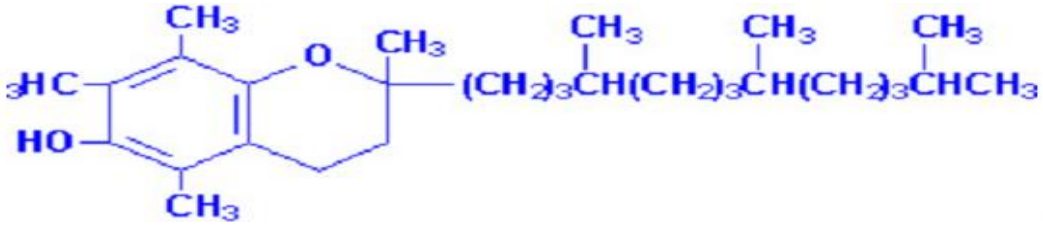
Deficiency: usually deficiency of Vit D are due to insufficient exposure to sunlight, inadequate dietary intake, gastrointestinal disorder, obstructive jaundice and Partial gastrectomy.

The main symptom of vitamin D deficiency in children is rickets and in adults is osteomalacia.

Vit D toxicity :

Excess Vit. D level enhances calcium absorption leading to hypercalcemia and metastatic calcium deposits. There is a tendency to develop kidney stones from the hypercalciuria, secondary to hypercalcemia.

3- Vitamin E :



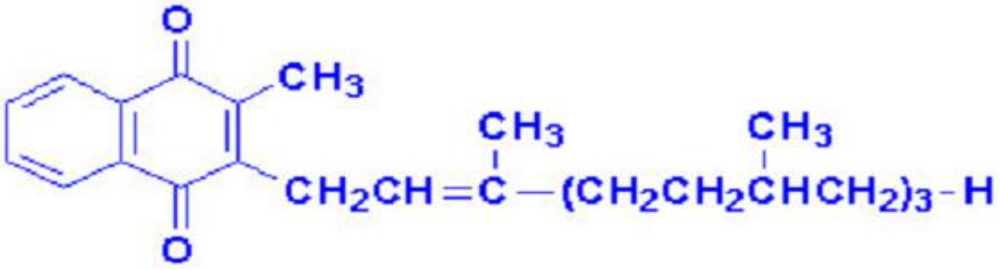
Vitamin E is a mixture of several related compounds known as tocopherols. The α-tocopherol molecule is the most potent of the tocopherols.

The major function of vitamin E is to act as a natural antioxidant, therefore, it's important for preventing peroxidation of polyunsaturated membrane fatty acids, this protective phenomenon is very much evident in the prevention of hemolysis of RBCs by H_2O_2.

Source: The reachest source is vegetable oils and nuts.

The major symptom of vitamin E deficiency in humans is an increase in red blood cell fragility. Neurological disorders have been associated with vitamin E deficiencies.

4- Vitamin K :



The major function of the K vitamin is in the maintenance of normal levels of the blood clotting proteins.

Deficiency of the vitamin in adults is rare, but the long time antibiotic treatment can lead to deficiency in adults. The intestine of newborn infants don't contain bacteria, therefore , deficiency is possible if lacking from the early diet. The primary symptom of a deficiency in infants is a hemorrhagic syndrome.

Dr.Salim.J.Kh.

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