Amino acids

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Organic compounds possessing one or more basic amino groups and one or more acidic carboxyl groups. Of the more than 80 amino acids which have been found in living organisms, about 20 serve as the building blocks for the proteins.

All the amino acids of proteins, and most of the others which occur naturally, are α-amino acids, meaning that an amino group ($—NH_2$) and a carboxyl group (—COOH) are attached to the same carbon atom. This carbon (the α carbon, being adjacent to the carboxyl group) also carries a hydrogen atom; its fourth valence is satisfied by any of a wide variety of substituent groups, represented by the letter R in **Fig. 1**.

In the simplest amino acid, glycine, R is a hydrogen atom. In all other amino acids, R is an organic radical; for example, in alanine it is a methyl group ($—CH_3$), while in glutamic acid it is an aliphatic chain terminating in a second carboxyl group ($—CH_2$ —CH—COOH). Chemically, the amino acids can be considered as falling roughly into nine categories based on the nature of R (see **table**).

Occurrence of conjugated amino acids

Amino acids occur in living tissues principally in the conjugated form. Most conjugated amino acids are peptides, in which the amino group of one amino acid is linked to the carboxyl group of another. This type of linkage is known as a peptide bond; a molecule of water is split out when a peptide bond is formed, and a molecule of

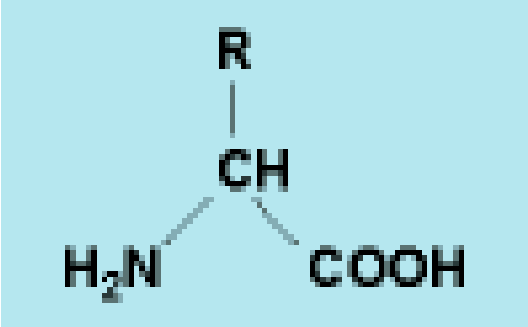


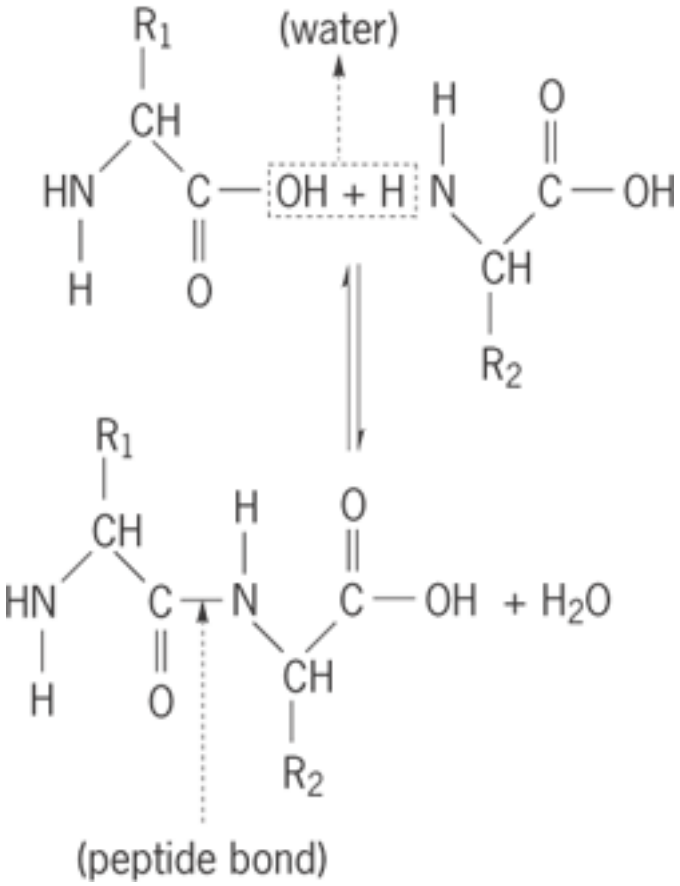
Fig. 1 **Structural formula for an amino acid.**

Amino acids of proteins, grouped according to the nature of R

Amino acids*	R
Glycine	Hydrogen
Alanine, valine, leucine, isoleucine	Unsubstituted aliphatic chain
Serine, threonine	Aliphatic chain bearing a hydroxyl group
Aspartic acid, glutamic acid	Aliphatic chain terminating in an acidic carboxyl group
Asparagine, glutamine	Aliphatic chain terminating in an amide group
Arginine, lysine	Aliphatic chain terminating in a basic amino group
Cysteine, cystine, methionine	Sulfur-containing aliphatic chain
Phenylalanine, tyrosine	Terminates in an aromatic ring
Tryptophan, proline, histidine	Terminates in a heterocyclic ring

* See articles on the individual amino acids listed in the table.

water must be added when a peptide bond is broken, as shown in the reaction below.



Since each amino acid possesses both an amino group and a carboxyl group, the acids are capable of linking together to form chains of various lengths, called polypeptides. Proteins are polypeptides ranging in size from about 50 to many thousand amino acid residues. The process by which peptides are formed from free amino acids actually cannot be as simple as pictured in the equation, for a considerable amount of energy is required. This process is discussed later in this article.

Although most of the conjugated amino acids in nature are proteins, numerous smaller conjugates occur naturally, many with important biological activity. The line between large peptides and small proteins is difficult to draw, with insulin (molecular weight = 7000; 50 amino acids) usually being considered a small protein and adrenocorticotropic hormone (molecular weight = 5000; 39 amino acids) being considered a large peptide. In addition to their role as hormones, peptides often occur in coenzymes (such as folic acid and glutathione), bacterial capsules (the polyglutamic acid capsule which contributes to the pathogenicity of *Bacillus anthracis*), fungal toxins (the tomato wilt toxin of *Fusarium* and the toxins of the poisonous mushroom *Amanita phalloides*), and antibiotics (chloramphenicol, penicillin, bacitracin, and polymixins). Elucidation of the structure of bacterial cell walls has shown that they are composed in part of a series of cross-linked peptides, the

cross-linking providing the wall with a large part of its rigidity. The action of penicillin in inhibiting this cross-linking reaction accounts for its antibiotic activity. Finally, a considerable part of the phospholipid fraction of any organism contains serine linked by phosphoester bond to glycerol phosphate. *See also:* ANTIBIOTIC; COENZYME; TOXIN.

Occurrence of free amino acids

Free amino acids are found in living cells, as well as the body fluids of higher animals, in amounts which vary according to the tissue and to the amino acid. The amino acids which play key roles in the incorporation and transfer of ammonia, such as glutamic acid, aspartic acid, and their amides, are often present in relatively high amounts, but the concentrations of the other amino acids of proteins are extremely low, ranging from a fraction of a milligram to several milligrams per 100 g wet weight of tissue. In view of the fact that amino acid and protein synthesis go on constantly in most of these tissues, the presence of free amino acids in only trace amounts points to the existence of extraordinarily efficient regulation mechanisms. Each amino acid is ordinarily synthesized at precisely the rate needed for protein synthesis. The regulation mechanism has been found most often to be one of feedback control; each amino acid acts as an inhibitor of its own biosynthesis. If any amino acid is formed in excess of that required for protein synthesis, the biosynthesis of that amino acid is slowed down until the excess has been used.

In addition to the amino acids of protein, a variety of other free amino acids occurs naturally. Some of these are metabolic products of the amino acids of proteins; for example, γ-aminobutyric acid occurs as the decarboxylation product of glutamic acid. Others, such as homoserine and ornithine, are biosynthetic precursors of the amino acids of protein. However, the origin and role of many unusual free amino acids is not yet known.

General properties

The amino acids are characterized physically by the following: (1) the pK_1, or the dissociation constant of the various titratable groups; (2) the isoelectric point, or pH at which a dipolar ion does not migrate in an electric field; (3) the optical rotation, or the rotation imparted to a beam of plane-polarized light (frequently the D line of the sodium spectrum) passing through 1 decimeter of a solution of 100 grams in 100 milliliters; and (4) solubility. *See also:* IONIC EQUILIBRIUM; ISOELECTRIC POINT; OPTICAL ACTIVITY.

At ordinary temperatures, the amino acids are white crystalline solids; when heated to high temperatures, they decompose rather than melt. They are stable in aqueous solution, and with few exceptions can be heated as high as 120°C (248°F) for short periods without decomposition, even in acid or alkaline solution. Thus, the hydrolysis of proteins can be carried out under such conditions with the complete recovery of most of the constituent free amino acids. The exceptions are as follows: Acid hydrolysis of protein destroys most of the tryptophan and some of the serine and threonine, oxidizes cysteine to cystine, and deamidates glutamine and asparagine; alkaline hydrolysis destroys serine, threonine, cystine, cysteine, and arginine, and also causes deamidations.

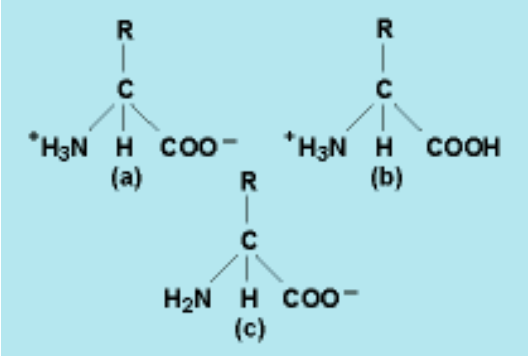


Fig. 2 **Ionic states of an amino acid. (*a*) Zwitterion. (*b*) Cation. (*c*) Anion.**

Enantiomorphs. Since all of the amino acids except glycine possess a center of asymmetry at the α carbon atom, they can exist in either of two optically active, mirror-image forms or enantiomorphs. All of the common amino acids of proteins appear to have the same configuration about the α carbon; this configuration is symbolized by the prefix L-. The opposite, generally unnatural, form is given the prefix D-. Some amino acids, such as isoleucine, threonine, and hydroxyproline, have a second center of asymmetry and can exist in four stereoisomeric forms. The prefix allo- is used to indicate one of the two alternative configurations at the second asymmetric center; thus, isoleucine, for example, can exist in the L, L-allo, D, and D-allo forms. *See also:* STEREOCHEMISTRY.

Unlike chemical syntheses, which lead to mixtures of D and L forms, biosynthetic processes invariably produce optically active amino acids. For most amino acids, only the L isomer occurs naturally; but in a few cases, the D isomer is found also. For example, the cell walls of certain bacteria contain D-alanine and D-glutamic acids, and the D isomers of phenylalanine, leucine, serine, and valine occur in some antibiotic peptides.

Ionic state. Another important general property of all amino acids is their ionic state (**Fig. 2**). The basic amino group can bind a proton from solution and become a cation; the acidic carboxyl group can release a proton into solution and become an anion. At the isoelectric point (the pH at which the molecule has no net charge), amino acids exist as dipolar ions or zwitterions; while in strong acid solution, the carboxyl group exists in the undissociated form, and the molecule becomes a cation. If such an acidic solution is titrated with strong alkali, two dissociations of protons are observed. The carboxyl group, having the weakest affinity for its proton, dissociates at a fairly low pH; its pK (the pH at which half of the molecules are dissociated) in most cases is close to 2.0. As more alkali is added, the proton on the amino group begins to dissociate; pK values for this dissociation are generally found close to 9.5. When sufficient alkali has been added to pull off all the dissociable protons, the amino acid exists as an anion.

Since the amino acids are ions, they can be prepared as their salts. For example, the titration of an amino acid solution with hydrochloric acid (HCl) leads to formation of the amino acid hydrochloride, while titration with sodium hydroxide (NaOH) forms the sodium salt (**Fig. 3**).

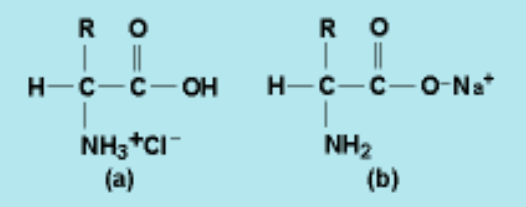


Fig. 3 **Salts of amino acids. (*a*) Amino acid hydrochloride. (*b*) Sodium amino acid.**

When the R radical contains an ionizable group, the amino acid will have correspondingly more ionic forms. Those amino acids whose radicals contain carboxyl groups (aspartic and glutamic acids) are known as the acidic amino acids, since a solution of the zwitterion will be strongly acidic. Similarly, histidine, lysine, and arginine are known as the basic amino acids, and the rest as the neutral amino acids. (It is important to note that a solution of the zwitterion of a neutral amino acid will in fact be slightly acidic.)

The salts are, in general, more soluble in water or alcohol than the corresponding zwitterions.

Isolation and determination

Since most amino acids occur in conjugated form, their isolation usually requires their prior release in free form by acid or alkaline hydrolysis. Hydrolysates of proteins or other polypeptides, or crude extracts of plants, animal, or microbial materials, serve as the starting point for the isolation in pure form of single amino acids. Prior to the application of chromatography in the early 1940s, the isolation of amino acid depended on slight differences in the solubilities of amino acid salts in various solvents and at different pH values. For example, the isolation of aspartic acid was accomplished by adding an excess of calcium hydroxide to an aqueous solution of amino acids and then precipitating the calcium aspartate with alcohol.

Such methods, although used successfully to isolate each of the common amino acids of protein, are difficult as well as tedious, and require relatively large amounts of starting material. Chromatography, on the other hand, is simple, rapid, and capable of isolating amino acids even when they are present in microgram quantities. Thus chromatography has been the method of choice for amino acid isolation ever since its first application by A. J. P. Martin and R. L. M. Synge in 1941. *See also:* CHROMATOGRAPHY.

Chromatography is carried out by using either cylindrical glass tubes (columns) packed with a porous solid or by using sheets of filter paper. In the former method, the column is packed with any of a variety of substances, such as starch, powdered cellulose, or cation-exchange resin, and is saturated with the chosen solvent. A solution of amino acids is allowed to percolate into the top of the column, and then solvent is forced through the column at a controlled rate. The solvent is allowed to flow out through an opening at the bottom of the column, and the eluate is caught in a series of test tubes.

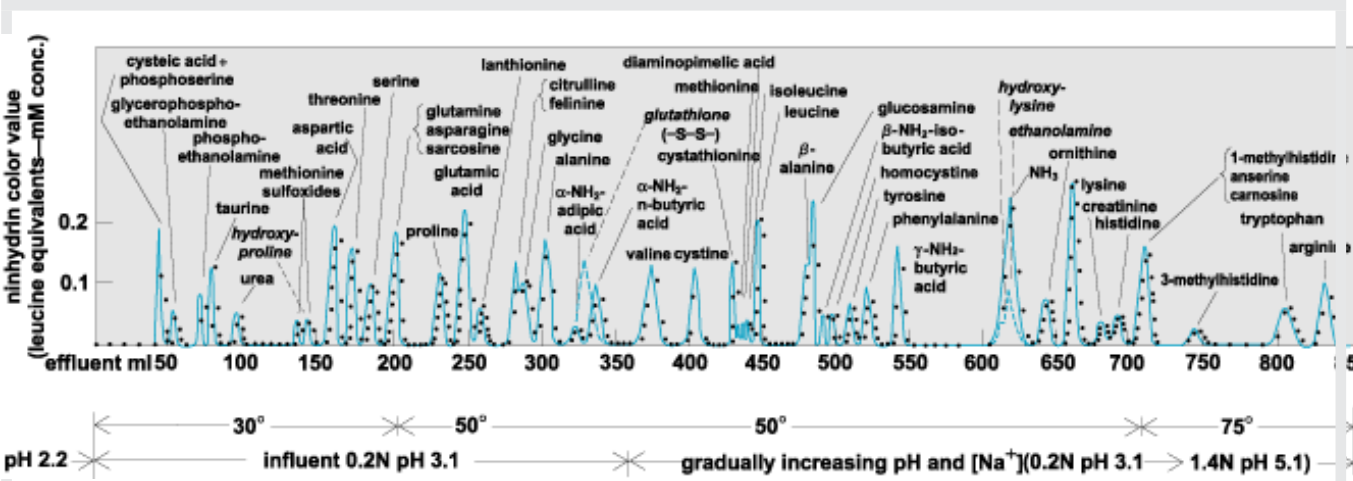


Fig. 4 **Typical protein fractionation into amino acids shown in a cation-exchange resin column chromatogram.** (*After S. Moore and W. H. Stein, J. Biol. Chem., 211:893–906, 1954*)

A given amino acid will have been eluted from the column at a time depending on its own characteristic rate of movement, and will have been caught in one or a few tubes; a different amino acid will have been eluted into a separate set of tubes. To detect their presence, as well as to determine their exact quantity, a substance which reacts with amino acids to give a visible color is then added to each tube. The best such reagent is ninhydrin, which reacts with amino acids to produce carbon dioxide, ammonia, and aldehyde, and forms a purple compound with the liberated ammonia. The amount of color which develops under standard conditions can then be measured in a photoelectric colorimeter, and the precise amount of amino acid determined by comparison with a standard curve based on reactions with known quantities. **Figure 4** shows a typical separation by passing water adjusted to different pH values and temperatures through a column of cation-exchange resin, collecting fractions, and determining the amount of amino acid in each with ninhydrin.

The column method described above is capable of giving the most precise quantitative data, and can handle relatively large quantities of amino acids. For the analysis of mixtures containing only a few micrograms of each amino acid, however, paper chromatography is the most simple and rapid procedure, and can also be made quantitative. In this procedure, the mixture of amino acids is applied as a drop of solution to a spot close to one corner of a sheet of paper. The sheet is then placed in a vapor-tight chamber with one edge of the paper dipping into a chosen, water-saturated organic solvent. The solvent flows through the paper by capillarity, water becoming bound to the paper and the organic solvent flowing past it. The amino acids travel through the paper as discrete spots, exactly as described above for their travel through a column. When the solvent has traveled to the opposite edge of the paper, the sheet is removed, dried, and sprayed with a reagent such as ninhydrin. The position of each amino acid is then revealed by a colored spot which appears when the sheet is heated.

If the solvent used brings two or more amino acids to the same position, two-dimensional chromatography is employed. The dried sheet is not sprayed, but is rotated 90° and is placed with the edge along which the amino

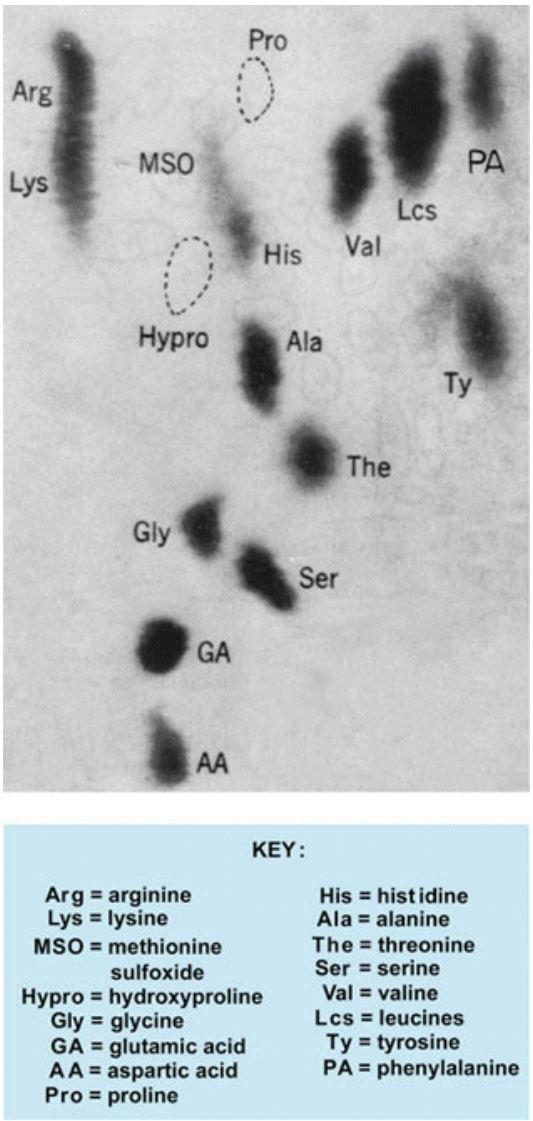


Fig. 5 **Two-dimensional chromatogram of a protein; the solvents were phenol and lutidine. (*From R. J. Block, E. L. Durrum, and G. Zweig, A Manual of Paper Chromatography and Paper Electrophoresis, 2d ed., Academic Press, 1958*)**

acids are located in a second solvent. This solvent flows through the paper at right angles to the direction taken by the first solvent, and if correctly chosen, will separate those amino acids which stayed together in the first solvent. The sheet is then dried and sprayed to locate the amino acids, and then the spots can be cut out with scissors and eluted with water in separate tubes for colorimetric determination. A two-dimensional chromatogram of the amino acids of a protein is shown in **Fig. 5**.

The determination of total α-amino acids in extracts of natural materials can be carried out by using the ninhydrin method, described above, or the Van Slyke method, which measures the amount of nitrogen gas given off on

treatment with nitrous acid. Specific determinations of a given amino acid were previously carried by means of sensitive microbiological assays which measured the growth of a microorganism dependent on the amino acid as a function of the concentration of the unknown material. Nowadays this rather difficult and cumbersome method has been largely replaced by ion-exchange chromatography.

There are relatively specific color reactions for many amino acids; while still used to some extent as quantitative assays, these now find most widespread use as qualitative tests for the presence of the amino acids.

Amino Acid Metabolism

Although amino acids and some other charged molecules can enter a cell passively by means of simple diffusion, there are present in all cells so far examined special systems for concentrating such small molecules inside the cell. These systems, called permeases, are localized in the cell membrane. They are protein complexes, probably containing at least two parts, which utilize metabolic energy to transport small molecules against the concentration gradient. Bacterial amino acid permeases are capable of achieving a concentration inside a cell 1000 times greater than that outside the cell.

The criteria for a permease, or active transport system, are (1) it must require energy, (2) it must be relatively specific, and (3) it must concentrate the transported substance against a gradient. Most permeases obey the classical Michaelis-Henry enzyme kinetics. Portions of the system which concentrates valine, isoleucine, and leucine in *Escherichia coli* have been purified; thus the mechanism of action of this permease may soon be clear.

Biosynthesis

Since amino acids, as precursors of proteins, are essential to all organisms, all cells must be able to synthesize those they cannot obtain from their environment. The selective advantage of being able rapidly to shift from endogenous to exogenous sources of these compounds has led to the evolution in bacteria and many other organisms of very complex and precise methods of adjusting the rate of synthesis to the available level of the compound. These regulatory mechanisms can be divided according to whether they require a short or a long time to take effect.

The immediately effective control is that of feedback inhibition. As Figs. 9–Fig. 14 show, the biosynthesis of amino acids is relatively complicated and usually requires at least three enzymatic steps. In most cases so far examined, the amino acid end product of the biosynthetic pathway inhibits the first enzyme to catalyze a reaction specific to the biosynthesis of that amino acid. This inhibition is extremely specific; the enzymes involved have special sites for binding the inhibitor. This inhibition functions to shut off the pathway in the presence of transient high levels of the product, thus saving both carbon and energy for other biosynthetic reactions. When the level of the product decreases, the pathway begins to function once more.

The one exception to this general rule is the growth factor requirement which results from the presence in the environment of a metabolic inhibitor. For example, a certain strain of bacterium is very sensitive to inhibition by valine, and this inhibition can be overcome by isoleucine. In the presence of valine, then, this strain requires isoleucine for growth. There are many such antagonisms between amino acids, with the result that organisms which require several amino acids must receive them in balanced amounts; any one in excess may prove inhibitory.

If a microorganism is grown for several generations in the presence of an amino acid, the levels of the enzymes of the biosynthetic pathway decrease considerably. This phenomenon is called enzyme repression, and it comes about because the synthesis of the enzymes is decreased in the presence of the end product. However, the enzyme already present in the cell is stable; therefore several generations are required before it is diluted to its lowest level by being apportioned among daughter cells at each division. (The level of such enzymes never reaches zero.) If at any time during this process the amino acid ceases to be available to the microorganism, synthesis of the enzyme immediately begins and continues until the proper intracellular concentration of the amino acid is reached. At this point the biosynthesis of the enzyme slows down and an equilibrium is reached such that the level of the enzyme remains constant until there is another alteration in the exogenous level of the amino acid. In contrast to feedback inhibition, which requires only milliseconds to act, repression and derepression require from one-half to several generations to reach a new equilibrium.

The actual metabolic pathways by which amino acids are synthesized are presented in diagrammatic form in Figs. 9–Fig. 14. These pathways generally are found to be the same in all living cells investigated, whether microbial or animal. Biosynthetic mechanisms thus appear to have developed soon after the origin of life and to have remained unchanged through the divergent evolution of modern organisms. The major exception is lysine, which is formed from aspartic acid via diaminopimelic acid in bacteria, but from α-ketoglutaric acid in the fungi. Indeed, the occurrence of diaminopimelic acid as a precursor of lysine, or as a constituent of proteins, or both, is a major taxonomic property of the bacteria and the related blue-green algae.

Formation and transfer of amino groups

The biosynthetic pathway diagrams reveal only one quantitatively important reaction by which organic nitrogen enters the amino groups of amino acids: the reductive amination of α-ketoglutaric acid to glutamic acid by the enzyme glutamic acid dehydrogenase. All other amino acids are formed either by transamination (transfer of an amino group, ultimately from glutamic acid) or by a modification of an existing amino acid. An example of the former is the formation of valine by transfer of the amino group from glutamic acid to α-ketoisovaleric acid; an example of the latter is the reduction and cyclization of glutamic acid to form proline.

Two other direct conversions of inorganic nitrogen to amino acid nitrogen are known: the reductive amination of pyruvic acid to alanine and the addition of ammonia to fumaric acid to form aspartic acid. However, there is no evidence that either of these reactions is quantitatively important in amino nitrogen formation. In any case,

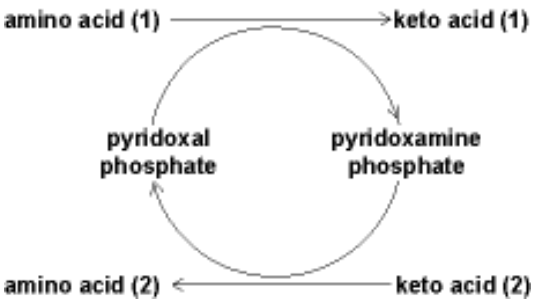


Fig. 6 Graphic representation of transamination mechanism by true transaminase.

ammonia is the only form of inorganic nitrogen which has been clearly shown to enter organic compounds directly; nitrate (NO_3^-), nitrite (NO_2^-), and nitrogen gas (N_2) are probably reduced to free intracellular ammonia before being converted to organic form in those plants and microorganisms which can use them as a nutritional source of nitrogen.

The principal mechanism of amino group transfer, transamination, is extremely important in many phases of nitrogen metabolism. Although many transamination reactions are known (all or most naturally occurring amino compounds probably participate in transamination in one tissue or another), the actual number of transaminases involved is uncertain. The few transaminases which have been highly purified all catalyze amino group exchange between more than just one pair of amino acids.

A true transaminase uses pyridoxal phosphate or pyridoxamine phosphate as coenzyme; the amino group is transferred to the former, which then gives it up to the keto acid acceptor (**Fig. 6**). A different mechanism of amino group transfer occurs in the biosyntheses of arginine and of adenylic acid; here, aspartic acid is added to a keto group to form a stable intermediate; a second enzyme then cleaves the intermediate to fumaric acid plus the new amino compound (see Fig. 10, showing the pathway of arginine biosynthesis).

One other important route by which ammonia enters organic compounds is by way of the amide group of glutamine. This group is formed by the direct addition of ammonia to glutamic acid, the necessary energy coming from the breakdown of adenosine triphosphate (ATP), first to adenosine diphosphate (ADP), then to inorganic phosphate. Once formed, amide nitrogen can be transferred to suitable acceptors to form precursors of the purine and histidine rings, as well as to hexose 6-phosphate to form glucosamine 6-phosphate.

Asparagine is another important amide, but the mechanism of asparagine formation is still in some doubt, and the only known product of the asparagine amide group is free ammonia. Glutamine is also readily deamidated to ammonia; both glutamine and asparagine serve as important storage forms of ammonia in higher plants and animals, as well as being constituent amino acids of proteins.

Fig. 7 General features of degradative pathways for amino acids.

Degradation

Most organisms are capable of degrading some amino acids, and metabolic pathways leading to degradation to CO_2 and H_2O are known for each of the common amino acids. These pathways are detailed in the articles on the individual amino acids. There are, however, general features (**Fig. 7**) characteristic of degradative pathways which are discussed below.

The first step in the degradation of all amino acids, with the exception of tyrosine and phenylalanine, is the labilization of one of the four groups on the α-carbon atom. The labilization always involves an enzyme containing pyridoxal phosphate as a cofactor, except in the case of oxidative deamination where a flavoprotein is

involved. Pyridoxal phosphate acts by forming a Schiff's base (shown as **1** in Fig. 7) with the amino acid, as shown in Fig. 7. By a rearrangement of electrons and protons (a tautomerization) the double bond moves to the position shown in **2**, with a concomitant alteration in the electron distribution in the pyridine ring of pyridoxal phosphate and the loss of the hydrogen on the α-carbon to the solvent. Intermediate **2** may now be hydrolyzed to yield the α-keto acid (**3**) corresponding to the amino acid, or it may return to intermediate **1**. In the latter case there is often a racemization, since **2** is a symmetric compound and the reversal of the tautomerization is not always carried out in an asymmetric manner.

If, instead of the hydrogen atom, the carboxyl group is labilized by donating its electrons to form the new double bond, intermediate **2** *a* is formed. A reversal of the tautomerization cannot regenerate an amino acid in this case; the reaction is a decarboxylation and the product is an amine. Similarly, the organic radical R may be lost (**2** *b*) whereupon the reversal of the tautomerization yields glycine. The last reaction is possible only when the radical is substituted with a hydroxyl group on the β-carbon (adjacent to the α-carbon). A final variation on the basic reactions of pyridoxal phosphate takes place when the R group contains an electronegative substituent on the β-carbon (**4**). In this case it is possible to expel the substituent, leading to an allyl amino acid, intermediate **5**, analogous to **1**. Intermediate **5** is now hydrolyzed to yield the extremely unstable intermediate **6** which rearranges to give the keto acid, ammonia, and X^- from the amino acid.

Transamination. Transamination is accomplished by hydrolysis of intermediate **2** to yield the keto acid and pyridoxamine phosphate. The latter compound reacts with another keto acid, yielding pyridoxal phosphate and the new amino acid. Transaminase enzymes are relatively nonspecific, reacting with groups of amino acids with similar R groups (for example, the aromatic amino acids or the branched-chain amino acids). The equilibrium constant of a transaminase reaction is usually very close to 1, reflecting the similarity in the free energies of formation of keto acids from amino acids. Transamination is much commoner than deamination in nature, since a deamination without a subsequent transfer to the amino group to another keto acid renders the coenzyme inactive. Most transaminase reactions involve aspartate or glutamate as one partner, with alanine also being rather common.

Decarboxylation. Decarboxylations are carried out by rather specific enzymes, and their products are often biologically active substances. Histamine, tryptamine (serotonin), and dopamine are all examples of decarboxylation products which are very active in animal tissues.

β Elimination. These reactions result in the loss of an electronegative group, such as OH^- or SH^-, from the carbon adjacent to the α carbon. Usually the net result is a deamination as well, since α,β unsaturated amino acids rapidly tautomerize to α,β saturated imino acids, which hydrolyze to yield the corresponding keto acid. Sometimes, however, there is addition to the α,β double bond of the pyridoxal phosphate–amino acid complex, so that the net result is a β substitution. This type of reaction is important in the synthesis of tryptophan.

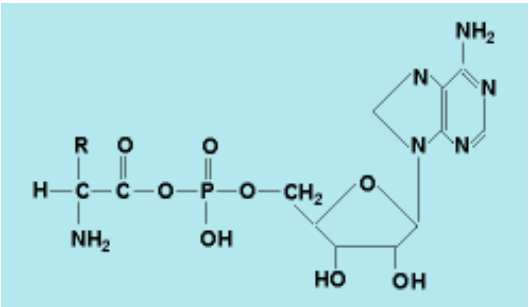


Fig. 8 **Structural formula for amino-acyl adenylate.**

β Cleavage. Instead of the α hydrogen or the carboxyl group, the β carbon can be eliminated, giving rise to glycine. These reactions only occur when the β substituent is electronegative, as in serine or threonine.

Conjugation

Until 1956 the mechanism of protein synthesis was totally unknown. Since then most of the details have been elucidated. A brief outline of the steps involved follows. The brevity of the outline precludes mention of many of the details.

The structure of a protein is determined by the arrangement of bases in the portion of the deoxyribonucleic acid (DNA) making up the gene for that protein. This sequence of DNA is transcribed into a complementary molecule of ribonucleic acid (RNA). The specific RNA, called messenger RNA (mRNA), binds to ribosomes, which are complex subcellular particles composed of a different sort of RNA, ribosomal RNA (rRNA), and protein. The ribosomes serve as the sites where conjugation of the amino acids takes place.

The amino acids are activated by reaction with a molecule of ATP to form an amino-acyl adenylate (**Fig. 8**) that remains temporarily bound to the enzyme which catalyzes the activation, an amino-acyl-tRNA synthetase (tRNA indicates transfer RNA). The amino-acyl moiety is then transferred to a specific molecule of RNA, that is, to tRNA. The linkage of the amino acid to the tRNA takes place by esterification to the 2′-hydroxyl of the ribose moiety of the 3′-terminal end. The tRNA molecule has a complex secondary structure which exposes a sequence of three bases in the interior of the molecule. These three bases are called the anticodon. Their nature and order are specific for the amino acid involved. The amino-acyl-tRNA synthetase contributes the specificity which ensures, with very high certainty, that the amino acid is attached to a tRNA with the proper anticodon.

The amino-acyl-tRNA interacts with the ribosome messenger complex when there is a sequence of three bases (the codon) on the messenger complementary, in the Watson-Crick sense, to the anticodon. The tRNA is aligned on the ribosome in such a way that the nascent protein chain, held by a C-terminal amino acid still linked to its tRNA, is brought into proximity with the free α-amino group of the amino acid. A series of enzymes effects

formation of the peptide bond, and the nascent protein chain has been elongated by one amino acid. The chain is now held to the ribosome by the tRNA of the latest residue added, and it is ready to accept the amino acid specified by the next codon. Thus the ribosome progresses along the mRNA, "reading" it, until all the amino acids specified by the original DNA gene have been added. It then reaches a codon which signifies termination of the chain, and a soluble protein is released.

The nascent protein chain begins in bacteria with a special methionyl-tRNA complex, in which the amino group of methionine is blocked with a formyl group. This is the first amino-acyl-tRNA to bind to the ribosome, and apparently all genes begin with the codon specific for this tRNA. In the first peptide bond formed, the *N*-formyl-methionyl-tRNA plays the part of the nascent protein chain.

For smaller peptides, such as glutathione and the mucopeptide of bacterial cell walls, the conjugation process is catalyzed by specific enzymes and involves different intermediates. Necessarily this means separate enzymes for each such peptide, in contrast to protein synthesis where the same machinery (except for the mRNA) serves for all chains of whatever sequence.

Amino Acids in Nutrition

The nutritional requirement for the amino acids of protein can vary from zero, in the case of an organism which synthesizes them all, to the complete list, in the case of an organism in which all the biosynthetic pathways are blocked. There are 8 or 10 amino acids required by certain mammals; most plants synthesize all of their amino acids, while microorganisms vary from types which synthesize all, to others (such as certain lactic acid bacteria) which require as many as 18 different amino acids. *See also:* AMINO ACID METABOLISM; NUTRITION.

It seems likely that, when life originated, amino acids were taken from the rich organic medium which the oceans then offered, and biosynthetic abilities evolved only slowly as the supply of exogenous materials became depleted. A stage must have eventually been reached, however, at which all the amino acids were being synthesized metabolically, and none was required nutritionally. As evolution progressed, food chains developed, and some forms of life became adapted to obtain many of their organic nutrients at the expense of other living forms, either directly or indirectly. In these dependent types, mutations had occurred, causing the loss of specific biosynthetic enzymes and hence the gain of nutritional requirements. It is easy to duplicate this process in the laboratory: A microorganism with full biosynthetic ability can be induced to undergo random mutations, and selective methods can then be used to isolate mutants requiring amino acids, vitamins, or other normal metabolites. In every case, it is found that a given mutation deprives the cell of a single biosynthetic enzyme, blocking the reaction which that enzyme catalyzes and thus the entire pathway of which that reaction is a part. *See also:* PREBIOTIC ORGANIC SYNTHESIS.

In summary, the nutrition of many organisms must include the provision of growth factors, which are defined as organic compounds which the organism requires for its growth but which it cannot synthesize. Growth factor

requirements reflect the heritable loss of biosynthetic enzymes, as the result of gene mutations. Amino acids are typical growth factors for many organisms.

Graphic Presentation of Amino Acid Biosynthesis

The amino acids are grouped into families on the basis of their common biosynthetic origins (Figs. 9-13). Lysine is shown in two families, because its biosynthesis in bacteria differs from that in fungi. Intermediates which are hypothetical are shown in brackets. The notation −2H or +2H refers to the removal or addition of two electrons and two hydrogen ions with the aid of either diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN), both of which are coenzymes of hydrogen transfer. Symbols used are ~Ac, coenzyme A–bound acetate; PRRP, phosphoribosyl pyrophosphate; CAP, carbamyl phosphate; and ATP, adenosine triphosphate. An arrow between two compounds in the diagrams does not necessarily imply a single enzymatic reaction. In many cases, the arrow represents a sequence of reactions for which the intermediates are unknown.

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Aromatic family

This family is composed of phenylalanine, tyrosine, tryptophan, and two other important metabolites, *p*-aminobenzoic acid and *p*-hydroxybenzoic acid. The initial precursors for the biosynthesis of these amino acids, phosphoenol pyruvate and D-erythrose 4-phosphate, are metabolites of glucose catabolism.

Although the intermediates shown in **Fig. 9** have all been isolated and identified, not all of the enzymes (particularly those involved in chorismic acid metabolism) have been studied. It is probable that *p*-hydroxybenzoic acid and prephenic acid are synthesized directly from chorismic acid by a single enzymatic reaction, and *p*-aminobenzoic acid may also be a direct metabolite. It is certain that other unidentified intermediates exist in the pathway between chorismic acid and anthranilic acid.

Two different enzymes for the conversion of chorismic acid to prephenic acid have been demonstrated in microorganisms. One of these enzymes is controlled by the pool size of tyrosine, while the other is controlled by phenylalanine.

The glycerol phosphate side chain of indoleglycerol phosphate derived from phosphoribosyl pyrophosphate can be exchanged directly for serine without the formation of free indole as an intermediate. In the absence of serine, the enzyme liberates free indole from indoleglycerol phosphate, and the same enzyme will condense indole with serine to form tryptophan.

There is some evidence for the existence of an anthranilic acid–tryptophan cycle in microorganisms. Formylkynurenine produced by the action of tryptophan pyrrolase on tryptophan can regenerate anthranilic acid by the combined action of kynureninase and kynurenine formamidase.

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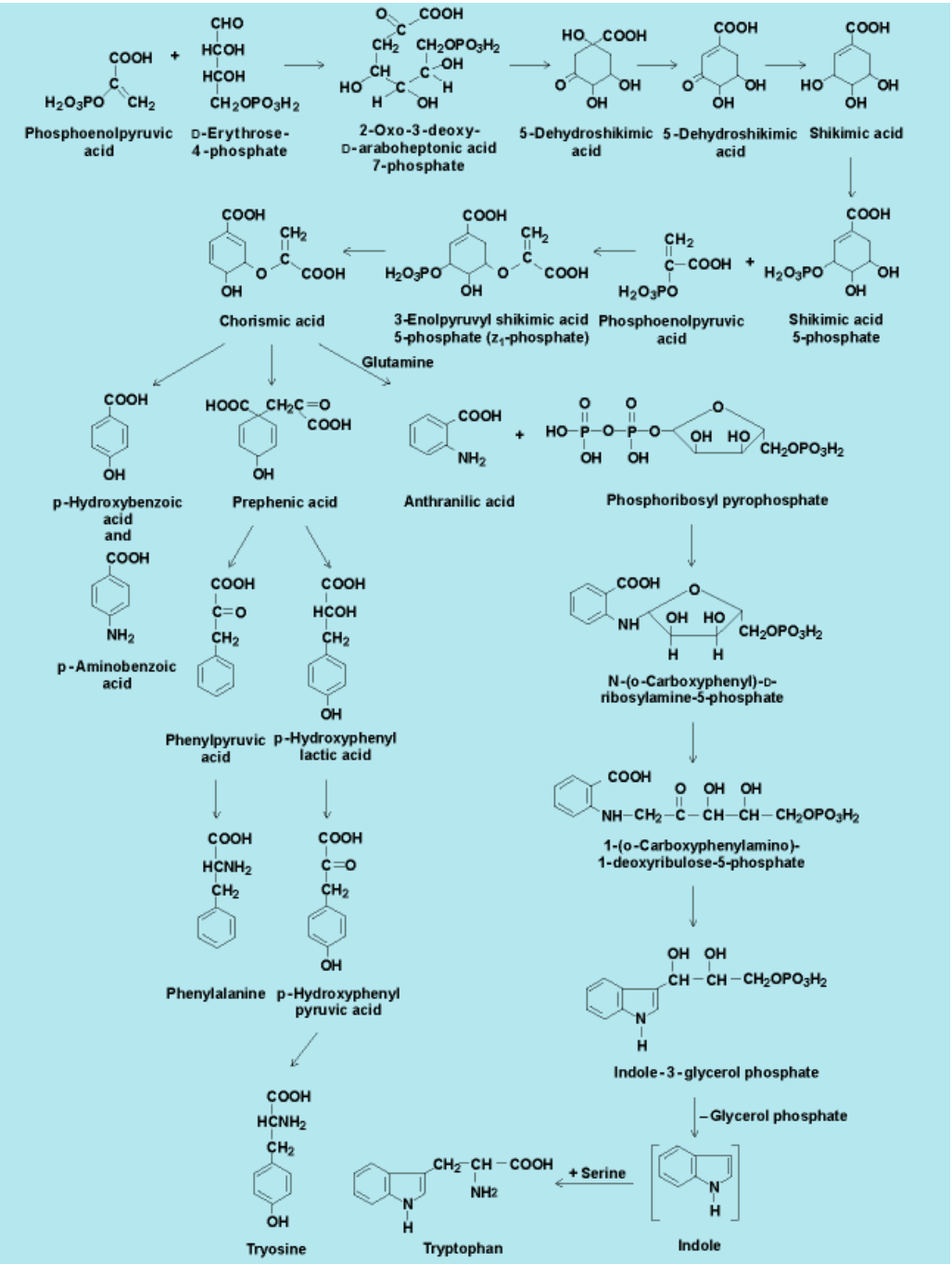

Fig. 9 **Metabolic pathways for the aromatic family of amino acids.**

α-Ketoglutaric acid family

This family is composed of glutamic acid, proline, lysine, and arginine. The numbered items refer to **Fig. 10**.

Fig. 10 **Metabolic pathways for the α-ketoglutaric family of amino acids.**

1. In yeast and other fungi, lysine is formed from α-ketoglutaric acid plus a C_2 fragment derivable from acetate. Lysine is formed by a different pathway in bacteria (see following section on aspartic acid).

2. Presumably by transamination.

3. This reductive amination is the main source of organic nitrogen for most microorganisms.

4. The cyclization takes place spontaneously.

5. The acetylation of glutamic acid prevents cyclization at the next step and permits the eventual formation of ornithine. This mechanism has been demonstrated in *Escherichia coli*, but does not take place in the fungus *Neurospora*; the fungus appears able to form ornithine via the nonacetylated intermediates.

6. Transamination.

7. Carbamyl phosphate (CAP).

Aspartic acid family

This family is composed of aspartic acid, lysine, threonine, methionine, and isoleucine. The numbered items refer to **Fig. 11**.

1. Aspartate arises principally by the transamination of oxaloacetate. In plants and in some microorganisms, it is formed by the direct amination of fumaric acid.

2. The compound formed by the transmination at carbon 6 and the subsequent desuccinylation is L,L-diaminopimelic acid. For the decarboxylase to function, the compound must be racemized to form *meso*-diaminopimelic acid.

3. In bacteria, and presumably in blue-green algae, lysine is formed by decarboxylation of diaminopimelic acid. In fungi and in higher animals, lysine is formed by a different route as seen in the α-ketoglutaric acid family (Fig. 10).

4. A series of reactions probably involves transfer of an active formaldehyde group from serine, followed by reduction.

5. Intramolecular rearrangement and reduction in α-aceto-α-hydroxybutyric acid take place in one step. The same enzyme catalyzes the analogous step in valine biosynthesis (Fig. 13).

6. Transamination from glutamic acid. The same transaminase functions for the keto acids of both isoleucine and valine.

Serine family

The serine family is composed of serine, glycine, cysteine, and tryptophan. The numbered items refer to **Fig. 12**.

Fig. 11 **Metabolic pathways for the aspartic acid family of amino acids.**

1. Transamination from alanine. There is equal evidence for a second pathway in which dephosphorylation precedes transamination.

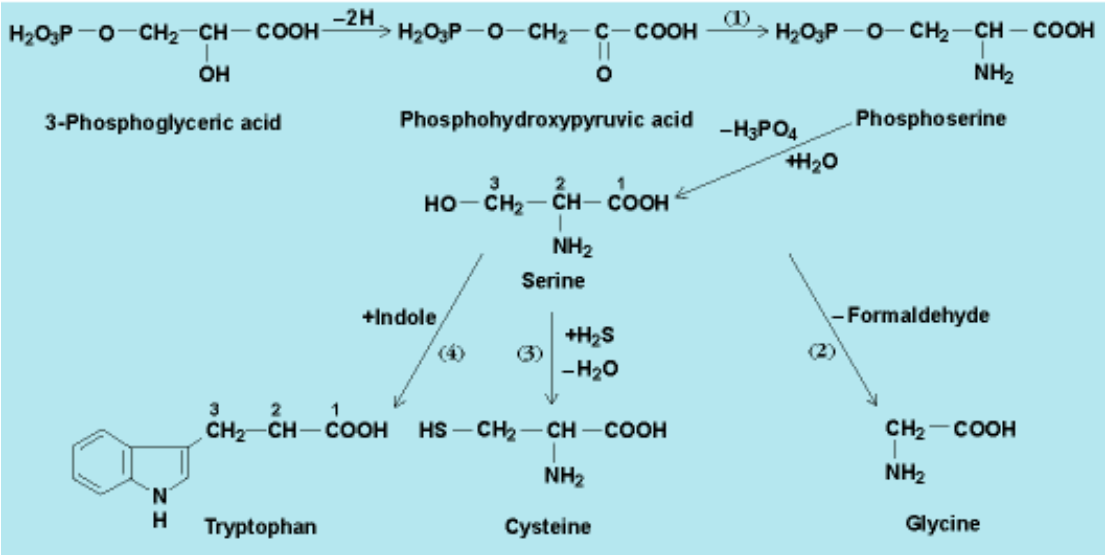

Fig. 12 **Metabolic pathways for the serine family of amino acids.**

2. The terminal group of serine is transferred to tetrahydrofolic acid (THFA) to form *N*(10)-hydroxymethyl-THFA. In this form, it can be transferred at various levels of oxidation for biosynthesis of compounds methionine, purine, and thymine.

3. This reaction, inferred to occur in microorganisms, is yet to be directly demonstrated. In animal tissues, serine receives the sulfhydryl group by transsulfuration from homocysteine, which is formed in animal tissues from dietary methionine.

4. See the aromatic family for considerations of this reaction.

Pyruvic acid family

This family is composed of valine, leucine, and alanine. The numbered items refer to **Fig. 13**.

1. Intramolecular rearrangement and reduction take place in one step.

2. Generally by transamination from glutamate, although cases of direct reductive amination with ammonia have been cited.

3. A single enzyme, dihydroxy acid dehydrase, catalyzes the dehydration of both the isoleucine and valine dihydroxy acid precursors.

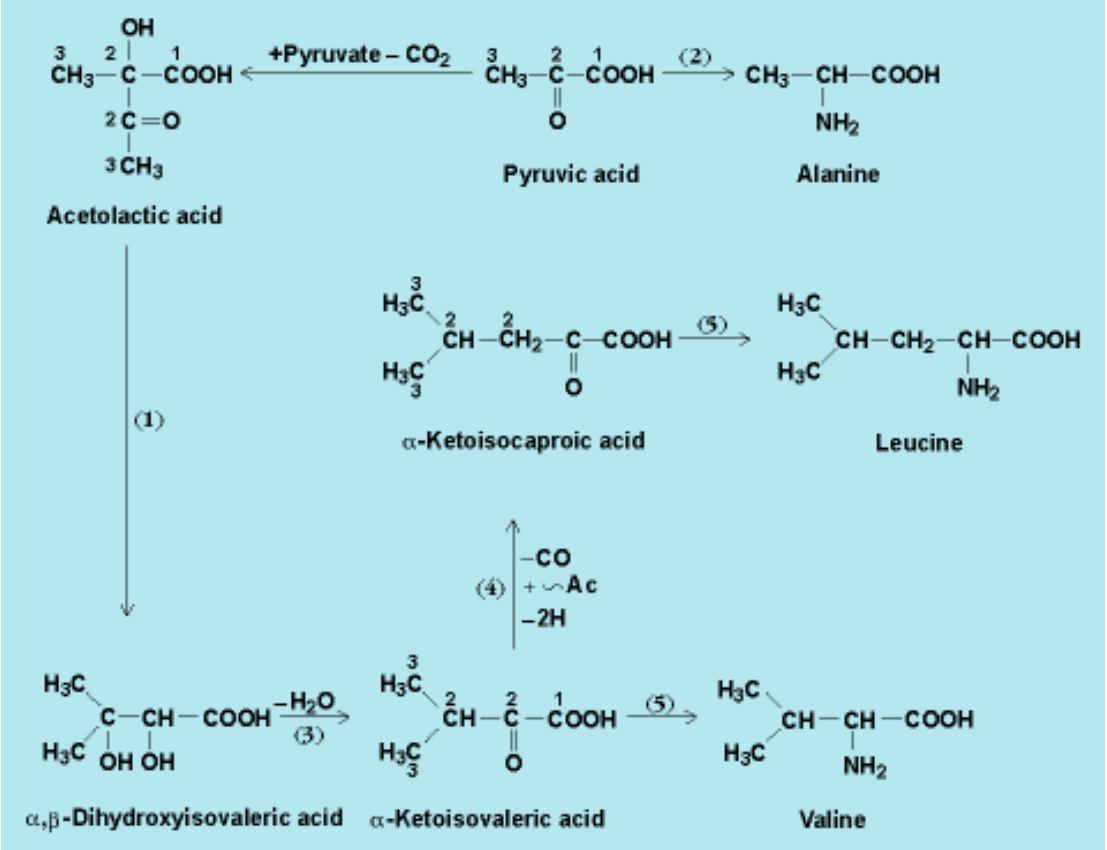

Fig. 13 Metabolic pathways for the pyruvic acid family of amino acids.

4. The complex series of reactions involved in the formation of α-ketoisocaproic acid is exactly analogous to the steps in the citric acid cycle leading to formation of α-ketoglutarate, with the keto acid taking the place of oxaloacetate.

5. Transamination from glutamate. The valine transaminase also functions in isoleucine biosynthesis.

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Histidine biosynthesis

The pathway of histidine biosynthesis shown in **Fig. 14** is known to occur in the mold *Neurospora* and in coliform bacteria. It should be noted that the imidazole ring of histidine is formed by the pathway and is not derived from the five-membered ring of adenosine triphosphate (ATP).

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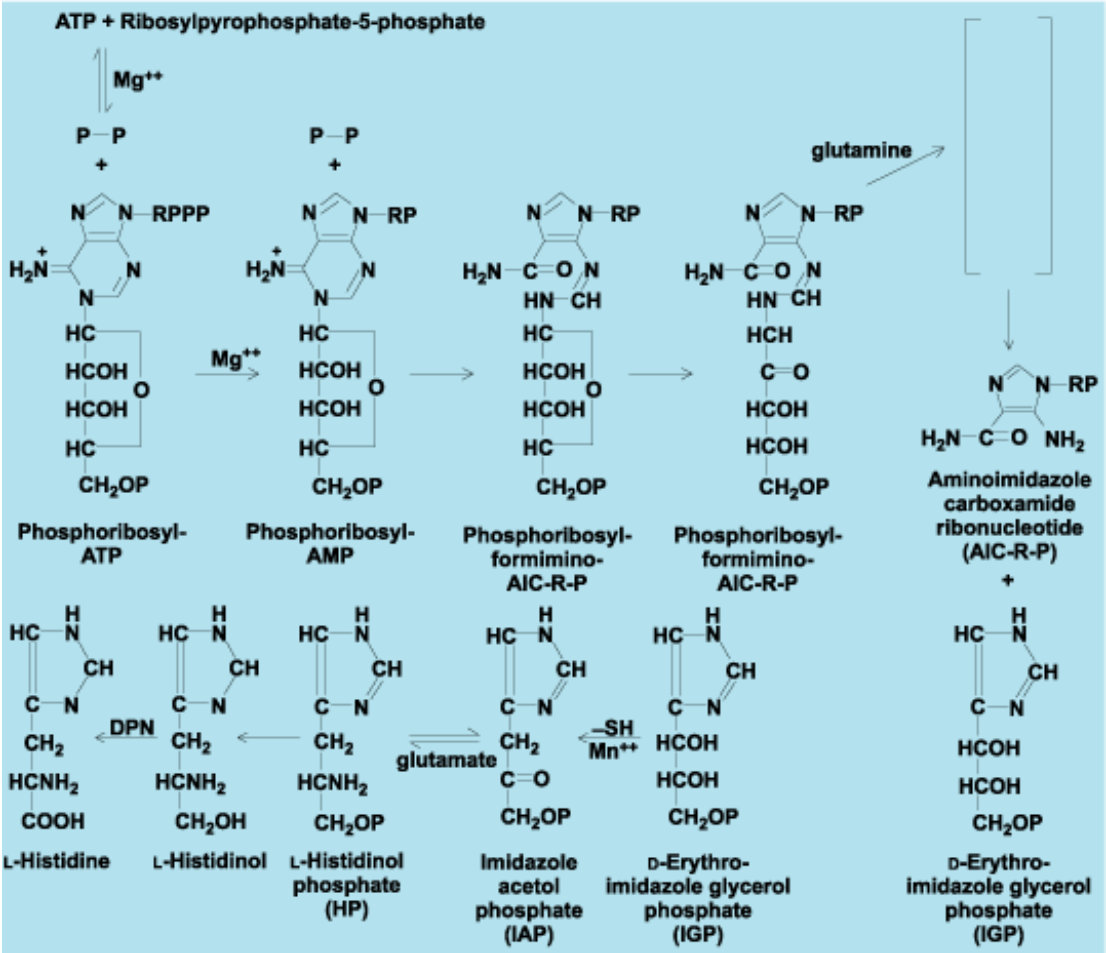

Fig. 14 Metabolic pathways in histidine biosynthesis.

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