TABLE 3-1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/symbol	M_r[a]	pK_a values: pK_1 (—OCOOH)	pK_2 ($-NH_3^+$)	pK_R (R group)	pI	Hydropathy index[b]	Oc in
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	
Alanine	Ala A	89	2.34	9.69		6.01	1.8	
Proline	Pro P	115	1.99	10.96		6.48	−1.6[d]	
Valine	Val V	117	2.32	9.62		5.97	4.2	
Leucine	Leu L	131	2.36	9.60		5.98	3.8	
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	
Methionine	Met M	149	2.28	9.21		5.74	1.9	
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	
Cysteine[e]	Cys C	121	1.96	10.28	8.18	5.07	2.5	
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	

[a] M_r values reflect the structures as shown in Figure 3-5. The elements of water (M_r 18) are deleted when the amino acid is incorporated into a polypeptide.

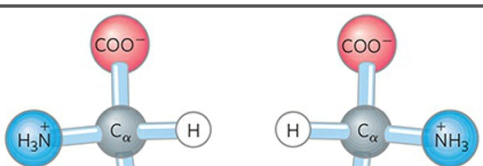
[b] A scale combining hydrophobicity and hydrophilicity of R groups. The values reflect the free energy (ΔG) of transfer of the amino acid side chain from a hydrophobic solvent to water. This transfer is favorable ($\Delta G < 0$; negative value in the index) for charged or polar amino acid side chains, and unfavorable ($\Delta G > 0$; positive value in the index) for amino acids with nonpolar or more hydrophobic side chains. See Chapter 11. Source: J. Kyte and R. F. Doolittle, *J. Mol. Biol.* 157:105, 1982.

[c] Average occurrence in more than 1,150 proteins. Source: R. F. Doolittle, in *Prediction of Protein Structure and the Principles of Protein Conformation* (G. D. Fasman, ed.), p. 599, Plenum Press, 1989.

[d] As originally composed, the hydropathy index takes into account the frequency with which an amino acid residue appears on the surface of a protein. As proline often appears on the surface in β turns, it has a lower score than its chain of methylene groups would suggest.

[e] Cysteine is generally classified as polar despite having a positive hydropathy index. This reflects the ability of the sulfhydryl group to act as a weak acid and to form a weak hydrogen bond with oxygen or nitrogen.

In some cases, such as amino acids with heterocyclic R groups (such as histidine), the Greek lettering system is ambiguous and the numbering convention is therefore used. For branched amino acid side chains, equivalent carbons are given numbers after the Greek letters. Leucine thus has $\delta 1$ and $\delta 2$ carbons (see the structure in Fig. 3-5).



of the paper, the dashed bonds behind it. In projection formulas (c), the horizontal bonds are assumed to project out of the plane of the paper, the vertical bonds behind. However, projection formulas are often used casually and are not always intended to portray a specific stereochemical configuration.

Special nomenclature has been developed to specify the **absolute configuration** of the four substituents of asymmetric carbon atoms. The absolute configurations of simple sugars and amino acids are specified by the **D, L system (Fig. 3-4)**, based on the absolute configuration of the three-carbon sugar glyceraldehyde, a convention proposed by Emil Fischer in 1891. (Fischer knew what groups surrounded the asymmetric carbon of glyceraldehyde but had to guess at their absolute configuration; he guessed right, as was later confirmed by x-ray diffraction analysis.) For all chiral compounds, stereoisomers having a configuration related to that of L-glyceraldehyde are designated L, and stereoisomers related to D-glyceraldehyde are designated D. The functional groups of L-alanine are matched with those of L-glyceraldehyde by aligning those that can be interconverted by simple, one-step chemical reactions. Thus the carboxyl group of L-alanine occupies the same position about the chiral carbon as does the aldehyde group of L-glyceraldehyde, because an aldehyde is readily converted to a carboxyl group via a one-step oxidation. Historically, the similar L and D designations were used for levorotatory (rotating plane-polarized light to the left) and dextrorotatory (rotating light to the right). However, not all L-amino acids are levorotatory, and the convention shown in Figure 3-4 was needed to avoid potential ambiguities about absolute configuration. By Fischer's convention, L and D refer *only* to the absolute configuration of the four substituents around the chiral carbon, not to optical properties of the molecule.

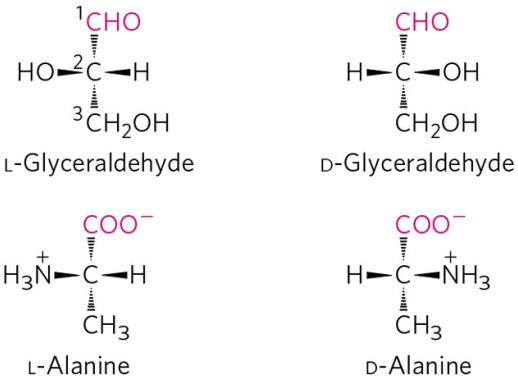


FIGURE 3-4 Steric relationship of the stereoisomers of alanine to the absolute configuration of L- and D-glyceraldehyde. In these perspective formulas, the carbons are lined up vertically, with the chiral atom in the center. The carbons in these molecules are numbered beginning with the terminal aldehyde or carboxyl carbon (red), 1 to 3 from top to bottom as shown. When presented in this way, the R group of the amino acid (in this case the methyl group of alanine) is always below the α carbon. L-Amino acids are those with the α-amino group on the left, and D-amino acids have the α-amino group on the right.

Another system of specifying configuration around a chiral center is the **RS system**, which is used in the systematic nomenclature of organic chemistry and describes more precisely the configuration of molecules with more than one chiral center (p. 19).

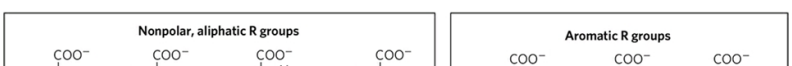
The Amino Acid Residues in Proteins Are L Stereoisomers

Nearly all biological compounds with a chiral center occur naturally in only one stereoisomeric form, either D or L. The amino acid residues in protein molecules are exclusively L stereoisomers. D-Amino acid residues have been found in only a few, generally small peptides, including some peptides of bacterial cell walls and certain peptide antibiotics.

It is remarkable that virtually all amino acid residues in proteins are L stereoisomers. When chiral compounds are formed by ordinary chemical reactions, the result is a racemic mixture of D and L isomers, which are difficult for a chemist to distinguish and separate. But to a living system, D and L isomers are as different as the right hand and the left. The formation of stable, repeating substructures in proteins (Chapter 4) generally requires that their constituent amino acids be of one stereochemical series. Cells are able to specifically synthesize the L isomers of amino acids because the active sites of enzymes are asymmetric, causing the reactions they catalyze to be stereospecific.

Amino Acids Can Be Classified by R Group

Knowledge of the chemical properties of the common amino acids is central to an understanding of biochemistry. The topic can be simplified by grouping the amino acids into five main classes based on the properties of their R groups (Table 3-1), particularly their **polarity**, or tendency to interact with water at biological pH (near pH 7.0). The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble). A few amino acids are somewhat difficult to characterize or do not fit perfectly in any one group, particularly glycine, histidine, and cysteine. Their assignments to particular groupings are the results of considered judgments rather than absolutes.



It is remarkable that virtually all amino acid residues in proteins are L stereoisomers. When chiral compounds are formed by ordinary chemical reactions, the result is a racemic mixture of D and L isomers, which are difficult for a chemist to distinguish and separate. But to a living system, D and L isomers are as different as the right hand and the left. The formation of stable, repeating substructures in proteins (Chapter 4) generally requires that their constituent amino acids be of one stereochemical series. Cells are able to specifically synthesize the L isomers of amino acids because the active sites of enzymes are asymmetric, causing the reactions they catalyze to be stereospecific.

Amino Acids Can Be Classified by R Group

Knowledge of the chemical properties of the common amino acids is central to an understanding of biochemistry. The topic can be simplified by grouping the amino acids into five main classes based on the properties of their R groups (Table 3-1), particularly their **polarity**, or tendency to interact with water at biological pH (near pH 7.0). The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble). A few amino acids are somewhat difficult to characterize or do not fit perfectly in any one group, particularly glycine, histidine, and cysteine. Their assignments to particular groupings are the results of considered judgments rather than absolutes.

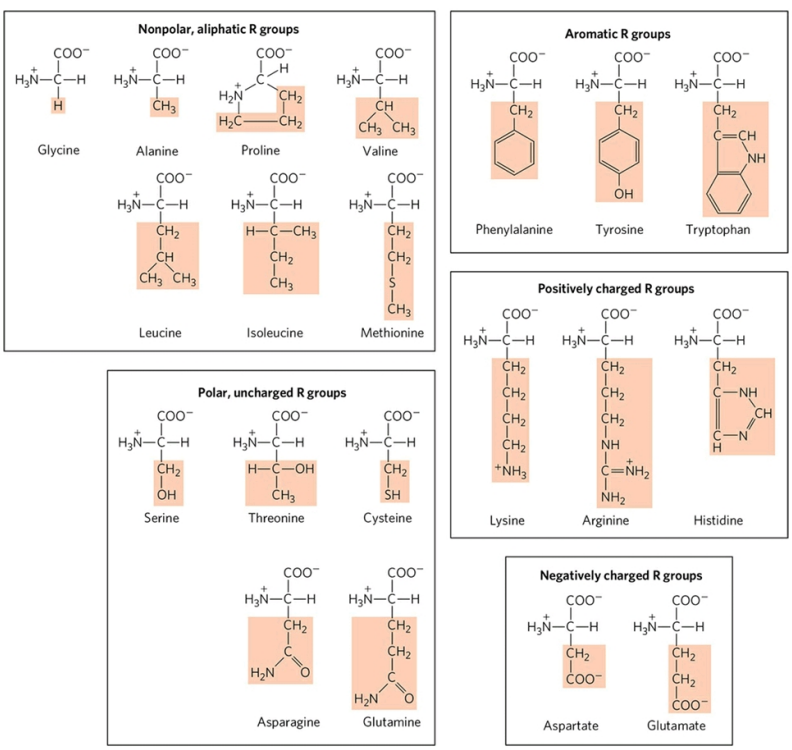


FIGURE 3-5 The 20 common amino acids of proteins. The structural formulas show the state of ionization that would predominate at pH 7.0. The unshaded portions are those common to all the amino acids; the shaded portions are the R groups. Although the R group of histidine is shown uncharged, its pK_a (see Table 3-1) is such that a small but significant fraction of these groups are positively charged at pH 7.0. The protonated form of histidine is shown above the graph in Figure 3-12b.

The structures of the 20 common amino acids are shown in **(Figure 3-5)**, and some of their properties are listed in Table 3-1. Within each class there are gradations of polarity, size, and shape of the R groups.

Nonpolar, Aliphatic R Groups The R groups in this class of amino acids are nonpolar and hydrophobic. The side chains of **alanine**, **valine**, **leucine**, and **isoleucine** tend to cluster together within proteins, stabilizing protein structure through the hydrophobic effect. **Glycine** has the simplest structure. Although it is most easily grouped with the nonpolar amino acids, its very small side chain makes no real contribution to interactions driven by the hydrophobic effect. **Methionine**, one of the two sulfur-containing amino acids, has a slightly nonpolar thioether group in its side chain. **Proline** has an aliphatic side chain with a distinctive cyclic structure. The secondary amino (imino) group of proline residues is held in a rigid conformation that reduces the structural flexibility of polypeptide regions containing proline.

Aromatic R Groups **Phenylalanine**, **tyrosine**, and **tryptophan**, with their aromatic side chains, are relatively nonpolar (hydrophobic). All can contribute to the hydrophobic effect. The hydroxyl group of tyrosine can form hydrogen bonds, and it is an important functional group in some enzymes. Tyrosine and tryptophan are significantly more polar than phenylalanine because of the tyrosine

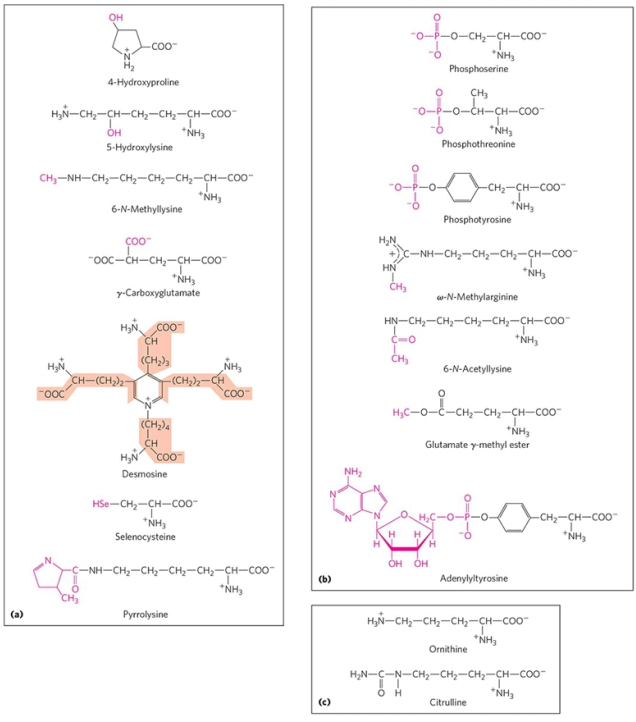


FIGURE 3-8 Uncommon amino acids. (a) Some uncommon amino acids found in proteins. Most are derived from common amino acids. (Note the use of either numbers or Greek letters in the names of these structures to identify the altered carbon atoms.) Extra functional groups added by modification reactions are shown in red. Desmosine is formed from four Lys residues (the carbon backbones are shaded in light red). Selenocysteine and pyrrolysine are exceptions: these amino acids are added during normal protein synthesis through a highly specialized expansion of the standard genetic code described in Chapter 27. Both are found in very small numbers of proteins. **(b)** Reversible amino acid modifications involved in regulation of protein activity. Phosphorylation is the most common type of regulatory modification. **(c)** Ornithine and citrulline, which are not found in proteins, are intermediates in the biosynthesis of arginine and in the urea cycle.

Amino Acids Have Characteristic Titration Curves

Acid-base titration involves the gradual addition or removal of protons (Chapter 2). **Figure 3-10** shows the titration curve of the diprotic form of glycine. The two ionizable groups of glycine, the carboxyl group and the amino group, are titrated with a strong base such as NaOH. The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine. Each of the two stages resembles in shape the titration curve of a monoprotic acid, such as acetic acid (see Fig. 2-17) and can be analyzed in the same way. At very low pH, the predominant ionic species of glycine is the fully protonated form, $^{+}H_3N—CH_2—COOH$. In the first stage of the titration, the —COOH group o

glycine loses its proton. At the midpoint of this stage, equimolar concentrations of the proton-donor ($^{+}H_3N—CH_2—COOH$) and proton-acceptor ($^{+}H_3N—CH_2—COO^{-}$) species are present. As in the titration of any weak acid, a point of inflection is reached at this midpoint where the pH is equal to the pK_a of the protonated group being titrated (see Fig. 2-18). For glycine, the pH at the midpoint is 2.34, thus its —COOH group has a pK_a (labeled pK_1 in Fig. 3-10) of 2.34. (Recall from Chapter 2 that pH and pK_a are simply convenient notations for proton concentration and the equilibrium constant for ionization, respectively. The pK_a is a measure of the tendency of a group to give up a proton, with that tendency decreasing tenfold as the pK_a increases by one unit.) As the titration of glycine proceeds, another important point is reached at pH 5.97. Here there is another point of inflection, at which removal of the first proton is essentially complete and removal of the second has just begun. At this pH glycine is present largely as the dipolar ion (zwitterion) $^{+}H_3N—CH_2—COO^{-}$. We shall return to the significance of this inflection point in the titration curve (labeled pI in Fig. 3-10) shortly.

examine the routes by which α-amino groups are incorporated into glutamate and then into aspartate and ammonia. In Section 26-2, we discuss urea biosynthesis from these precursors.

Most amino acids are deaminated by **transamination,** the transfer of their amino group to an α-keto acid to yield the α-keto acid of the original amino acid and a new amino acid, in reactions catalyzed by **aminotransferases** (alternatively, **transaminases**). The predominant amino group acceptor is α-ketoglutarate, producing glutamate as the new amino acid:

$$\text{Amino acid} + \alpha\text{-ketoglutarate} \rightleftharpoons \alpha\text{-keto acid} + \text{glutamate}$$

Glutamate's amino group, in turn, is transferred to oxaloacetate in a second transamination reaction, yielding aspartate:

$$\text{Glutamate} + \text{oxaloacetate} \rightleftharpoons \alpha\text{-ketoglutarate} + \text{aspartate}$$

Transamination, of course, does not result in any net deamination. Deamination occurs largely through the oxidative deamination of glutamate by **glutamate dehydrogenase (GDH),** yielding ammonia. The reaction requires NAD^+ or $NADP^+$ as an oxidizing agent and regenerates α-ketoglutarate for use in additional transamination reactions:

$$\text{Glutamate} + NAD(P)^+ + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+ + NAD(P)H$$

The mechanisms of transamination and oxidative deamination are the subjects of this section. We also consider other means of amino group removal from specific amino acids.

A. *Transamination*

a. Aminotransferase Reactions Occur in Two Stages

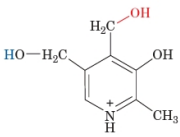
1. The amino group of an amino acid is transferred to the enzyme, producing the corresponding keto acid and the aminated enzyme.

$$\text{Amino acid} + \text{enzyme} \rightleftharpoons \alpha\text{-keto acid} + \text{enzyme—}NH_2$$

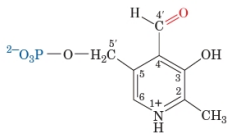
2. The amino group is transferred to the keto acid acceptor (e.g., α-ketoglutarate), forming the amino acid product (e.g., glutamate) and regenerating the enzyme.

$$\alpha\text{-Ketoglutarate} + \text{enzyme—}NH_2 \rightleftharpoons \text{enzyme} + \text{glutamate}$$

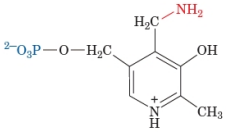
To carry the amino group, aminotransferases require participation of an aldehyde-containing coenzyme, ***pyridoxal-5′-phosphate (PLP),*** *a derivative of* ***pyridoxine (vitamin B_6;*** Fig. 26-1*a,b*). The amino group is accommodated by conversion of this coenzyme to **pyridoxamine-5′-phosphate (PMP;** Fig. 26-1*c*). PLP is covalently attached to the enzyme via a Schiff base (imine) linkage formed by the



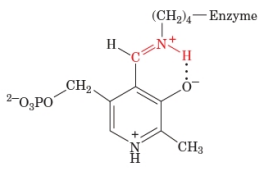
(*a*) **Pyridoxine (vitamin B_6)**



(*b*) **Pyridoxal-5′-phosphate (PLP)**



(*c*) **Pyridoxamine-5′-phosphate (PMP)**



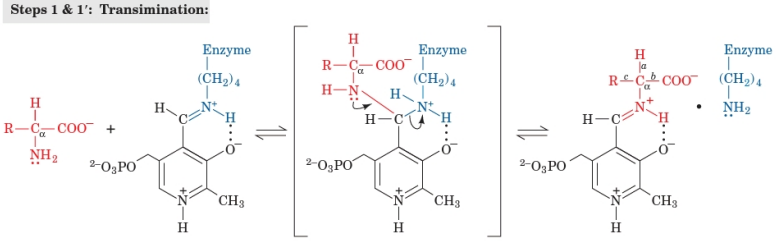
(*d*) **Enzyme–PLP Schiff base**

Figure 26-1 **Forms of pyridoxal-5′-phosphate.** (*a*) Pyridoxine (vitamin B_6). (*b*) Pyridoxal-5′-phosphate (PLP). (*c*) Pyridoxamine-5′-phosphate (PMP). (*d*) The Schiff base that forms between PLP and an enzyme ε-amino group.

condensation of its aldehyde group with the ε-amino group of an enzymatic Lys residue (Fig. 26-1*d*). This Schiff base, which is conjugated to the coenzyme's pyridinium ring, is the focus of the coenzyme's activity.

Esmond Snell, Alexander Braunstein, and David Metzler demonstrated that the aminotransferase reaction occurs via a Ping Pong Bi Bi mechanism whose two stages consist of three steps each (Fig. 26-2):

Steps 1 & 1′: Transimination:



α-Amino acid | **Enzyme–PLP Schiff base** | **Geminal diamine intermediate** | **Amino acid–PLP Schiff base (aldimine)**

Steps 2 & 2′: Tautomerization:

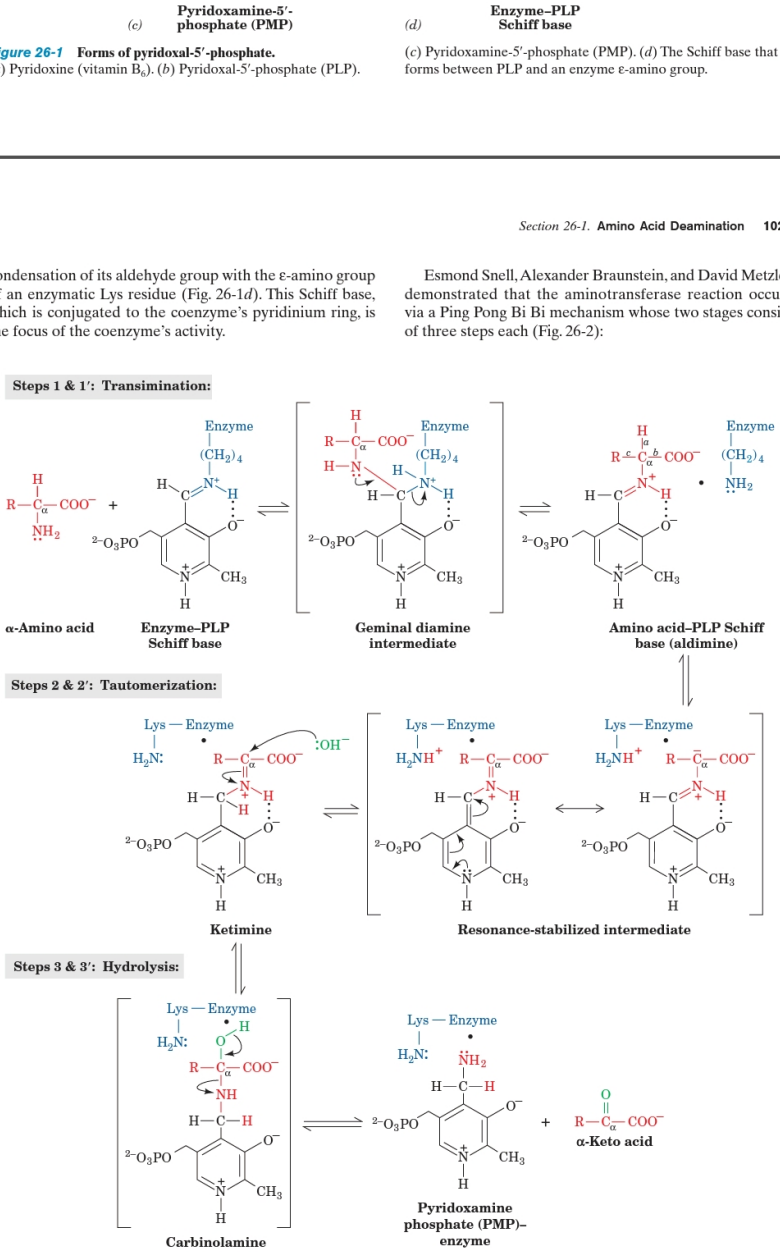


Figure 26-2 **The mechanism of PLP-dependent enzyme-catalyzed transamination.** The first stage of the reaction, in which the α-amino group of an amino acid is transferred to PLP yielding an α-keto acid and PMP, consists of three steps: **(1)** transimination; **(2)** tautomerization, in which the Lys released during the transimination reaction acts as a general acid–base catalyst; and **(3)** hydrolysis. The second stage of the reaction, in which the amino group of PMP is transferred to a different α-keto acid to yield a new α-amino acid and PLP, is essentially the reverse of the first stage: Steps 3′, 2′, and 1′ are, respectively, the reverse of Steps 3, 2, and 1. See the Animated Figures

b. Stage I: Conversion of an Amino Acid to an α-Keto Acid

Step 1. The amino acid's nucleophilic amino group attacks the enzyme–PLP Schiff base carbon atom in a **transimination (trans-Schiffization)** reaction to form an amino acid–PLP Schiff base (aldimine), with concomitant release of the enzyme's Lys amino group. This Lys is then free to act as a general base at the active site.

Step 2. The amino acid–PLP Schiff base tautomerizes to an α-keto acid–PMP Schiff base by the active site Lys–catalyzed removal of the amino acid α hydrogen and protonation of PLP atom C4′ via a resonance-stabilized carbanion intermediate. This resonance stabilization facilitates the cleavage of the C_α—H bond.

delocalized all the way to the coenzyme's protonated pyridinium nitrogen atom; that is, PLP functions as an electron sink. For transamination reactions, this electron-withdrawing capacity facilitates removal of the α proton (*a* bond cleavage) in the tautomerization of the Schiff base. PLP-dependent reactions involving *b* bond cleavage (amino acid decarboxylation) and *c* bond labilization are discussed in Section 26-4B and in Sections 26-3Bb and 26-3G, respectively.

Aminotransferases differ in their specificity for amino acid substrates in the first stage of the transamination reaction, thereby producing the correspondingly different α-keto acid products. Most aminotransferases, however, accept only α-ketoglutarate or (to a lesser extent) oxaloacetate as the α-keto acid substrate in the second stage of the reaction, thereby yielding glutamate or aspartate as

(*c*) Pyridoxamine-5′-phosphate (PMP)

(*d*) Enzyme–PLP Schiff base

Figure 26-1 **Forms of pyridoxal-5′-phosphate.** (*a*) Pyridoxine (vitamin B_6). (*b*) Pyridoxal-5′-phosphate (PLP). (*c*) Pyridoxamine-5′-phosphate (PMP). (*d*) The Schiff base that forms between PLP and an enzyme ε-amino group.

condensation of its aldehyde group with the ε-amino group of an enzymatic Lys residue (Fig. 26-1*d*). This Schiff base, which is conjugated to the coenzyme's pyridinium ring, is the focus of the coenzyme's activity.

Esmond Snell, Alexander Braunstein, and David Metzler demonstrated that the aminotransferase reaction occurs via a Ping Pong Bi Bi mechanism whose two stages consist of three steps each (Fig. 26-2):

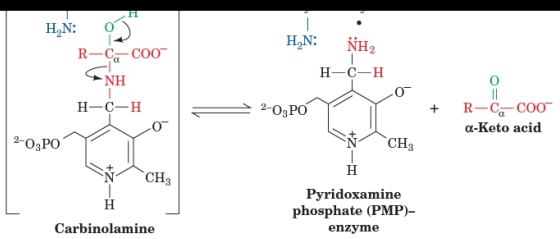


Figure 26-2 **The mechanism of PLP-dependent enzyme-catalyzed transamination.** The first stage of the reaction, in which the α-amino group of an amino acid is transferred to PLP yielding an α-keto acid and PMP, consists of three steps: **(1)** transimination; **(2)** tautomerization, in which the Lys released during the transimination reaction acts as a general acid–base catalyst; and **(3)** hydrolysis. The second stage of the reaction, in which the amino group of PMP is transferred to a different α-keto acid to yield a new α-amino acid and PLP, is essentially the reverse of the first stage: Steps 3′, 2′, and 1′ are, respectively, the reverse of Steps 3, 2, and 1. See the Animated Figures

b. Stage I: Conversion of an Amino Acid to an α-Keto Acid

Step 1. The amino acid's nucleophilic amino group attacks the enzyme–PLP Schiff base carbon atom in a **transimination (trans-Schiffization)** reaction to form an amino acid–PLP Schiff base (aldimine), with concomitant release of the enzyme's Lys amino group. This Lys is then free to act as a general base at the active site.

Step 2. The amino acid–PLP Schiff base tautomerizes to an α-keto acid–PMP Schiff base by the active site Lys–catalyzed removal of the amino acid α hydrogen and protonation of PLP atom C4′ via a resonance-stabilized carbanion intermediate. This resonance stabilization facilitates the cleavage of the C_α—H bond.

Step 3. The α-keto acid–PMP Schiff base is hydrolyzed to PMP and an α-keto acid.

c. Stage II: Conversion of an α-Keto Acid to an Amino Acid

To complete the aminotransferase's catalytic cycle, the coenzyme must be converted from PMP back to the enzyme–PLP Schiff base. This involves the same three steps as above, but in reverse order:

Step 3′. PMP reacts with an α-keto acid to form a Schiff base.

Step 2′. The α-keto acid–PMP Schiff base tautomerizes to form an amino acid–PLP Schiff base.

Step 1′. The ε-amino group of the active site Lys residue attacks the amino acid–PLP Schiff base in a transimination reaction to regenerate the active enzyme–PLP Schiff base, with release of the newly formed amino acid.

The reaction's overall stoichiometry therefore is

Amino acid 1 + α-keto acid 2 $\rightleftharpoons$ α-keto acid 1 + amino acid 2

Examination of the amino acid–PLP Schiff base's structure (Fig. 26-2, Step 1) reveals why this system is called "an electron-pusher's delight." *Cleavage of any of the amino acid C_α atom's three bonds (labeled a, b, and c) produces a resonance-stabilized C_α carbanion whose electrons are delocalized all the way to the coenzyme's protonated pyridinium nitrogen atom; that is, PLP functions as an electron sink.* For transamination reactions, this electron-withdrawing capacity facilitates removal of the α proton (*a* bond cleavage) in the tautomerization of the Schiff base. PLP-dependent reactions involving *b* bond cleavage (amino acid decarboxylation) and *c* bond labilization are discussed in Section 26-4B and in Sections 26-3Bb and 26-3G, respectively.

Aminotransferases differ in their specificity for amino acid substrates in the first stage of the transamination reaction, thereby producing the correspondingly different α-keto acid products. Most aminotransferases, however, accept only α-ketoglutarate or (to a lesser extent) oxaloacetate as the α-keto acid substrate in the second stage of the reaction, thereby yielding glutamate or aspartate as their only amino acid products. *The amino groups of most amino acids are consequently funneled into the formation of glutamate or aspartate, which are themselves interconverted by* ***glutamate–aspartate aminotransferase:***

Glutamate + oxaloacetate $\rightleftharpoons$ α-ketoglutarate + aspartate

Oxidative deamination of glutamate (Section 26-1B) yields ammonia and regenerates α-ketoglutarate for another round of transamination reactions. Ammonia and aspartate are the two amino group donors in the synthesis of urea.

d. The Glucose–Alanine Cycle Transports Nitrogen to the Liver

An important exception to the foregoing is a group of muscle aminotransferases that accept pyruvate as their α-keto acid substrate. The product amino acid, alanine, is released into the bloodstream and transported to the liver, where it undergoes transamination to yield pyruvate for use in gluconeogenesis (Section 23-1A). The resulting glucose is returned to the muscles, where it is glycolytically degraded to pyruvate. This is the **glucose–alanine cycle** (Fig. 26-3). The amino group ends up in either ammonium ion or aspartate for urea biosynthesis. Evidently, the glucose–alanine cycle functions to transport nitrogen from muscle to liver.

During starvation the glucose formed in the liver by this route is also used by the other peripheral tissues, breaking the

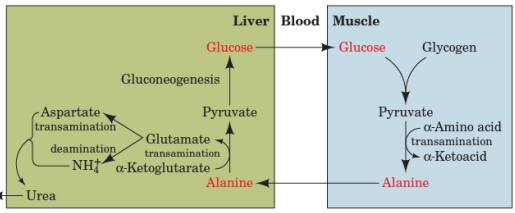


Figure 26-3 **The glucose–alanine cycle.** See the Animated Figures

cycle. Under these conditions both the amino group and the pyruvate originate from muscle protein degradation, provid-

monomer of the bovine GDH–glutamate–NADH–GTP complex (Fig. 26-5*b*) with that of the 96% identical human

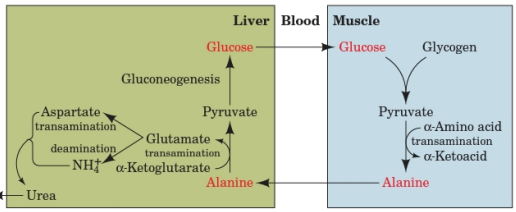


Figure 26-3 **The glucose–alanine cycle.** See the Animated Figures

cycle. Under these conditions both the amino group and the pyruvate originate from muscle protein degradation, providing a pathway yielding glucose for other tissue use (recall that muscle is not a gluconeogenic tissue; Section 23-1).

Nitrogen is also transported to the liver in the form of glutamine, synthesized from glutamate and ammonia in a reaction catalyzed by **glutamine synthetase** (Section 26-5Ab). The ammonia is released for urea synthesis in liver mitochondria or for excretion in the kidney through the action of **glutaminase** (Section 26-3D).

B. *Oxidative Deamination: Glutamate Dehydrogenase*

Glutamate is oxidatively deaminated in the mitochondrial matrix by glutamate dehydrogenase (GDH), the only known enzyme that, in at least some organisms, can accept either NAD^+ or $NADP^+$ as its redox coenzyme. Oxidation is thought to occur with transfer of a hydride ion from glutamate's C_α to $NAD(P)^+$, thereby forming α-iminoglutarate, which is hydrolyzed to α-ketoglutarate and ammonia (Fig. 26-4). GDH is allosterically inhibited by GTP, NADH, and nonpolar compounds such as palmitoyl-CoA and steroid hormones. It is activated by ADP, NAD^+, and leucine (the most abundant amino acid in proteins; Table 4-1).

a. The X-Ray Structures of GDH Reveal its Allosteric Mechanism

The X-ray structures of homohexameric GDH from bovine and human liver mitochondria, determined by Thomas Smith, reveal that each monomer has three domains, a substrate domain, a coenzyme domain, and an antenna domain. The protein, which has D_3 symmetry, can be considered to be a dimer of trimers, with the antenna domains of each trimer wrapping around each other about the 3-fold axis (Fig. 26-5*a*). Structural comparison of a 501-residue

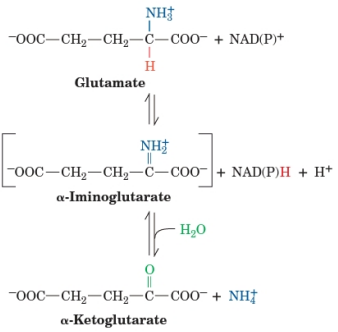


Figure 26-4 **The oxidative deamination of glutamate by glutamate dehydrogenase.** This reaction involves the intermediate formation of α-iminoglutarate.

monomer of the bovine GDH–glutamate–NADH–GTP complex (Fig. 26-5*b*) with that of the 96% identical human **apoenzyme** (no active site or regulatory ligands bound; Fig. 26-5*c*) reveals that, on binding ligands, the coenzyme binding domain rotates about the so-called pivot helix so as to close the cleft between the coenzyme and substrate domains. Simultaneously, the antenna domain twists in a way that unwinds one turn of the antenna helix that is connected to the pivot helix. Although the closed form is required for catalysis, the open form favors the association and dissociation of substrates and products. In the open state, Arg 463 (human numbering) in the center of the pivot helix interacts with the activator ADP (whose binding site in the bovine complex is occupied by the ADP moiety of an NADH; Fig. 26-5*b*), whereas in the closed state, the side chain of His 454 hydrogen-bonds to the γ-phosphate of the inhibitor GTP. The GTP binding site is distorted and blocked in the open state so that GTP binding favors the closed form of the enzyme. This results in tight binding of substrates and products and hence inhibition of the enzyme. ADP binding favors the open form, allowing product dissociation, and therefore activates the enzyme. Allosteric interactions appear to be communicated between subunits through the interactions of the antenna domains. In fact, bacterial GDHs, which lack allosteric regulation, differ from mammalian GDHs mainly by the absence of antenna domains.

b. Hyperinsulinism/Hyperammonemia (HI/HA) Is Caused by Uncontrolled GDH Activity

Charles Stanley has reported a new form of congenital hyperinsulinism that is characterized by hypoglycemia and **hyperammonemia** (**HI/HA;** hyperammonemia is elevated levels of ammonia in the blood) and has shown that it is caused by mutations in GDH at the N-terminal end of its pivot helix in the GTP binding site or in the antenna domain near its joint with the pivot helix. The mutant enzymes have reduced sensitivity to GTP inhibition but retain their ability to be activated by ADP. The GDH mutants S448P, H454Y, and R463A, which were respectively designed to affect the antenna region, the GTP binding site, and the ADP binding site (Fig. 26-5*b*), all have decreased sensitivity to GTP inhibition (Fig. 26-6), with H454Y and S448P, which were previously known to be associated with HI/HA, conferring the most resistance to GTP inhibition. The hypoglycemia and hyperammonemia in HI/HA patients arises from the increased activity of the GDH mutants in the breakdown direction, producing increased amounts of α-ketoglutarate and NH_3. The increased levels of α-ketoglutarate stimulate the citric acid cycle and oxidative phosphorylation, which has been shown to lead to increased insulin secretion and hypoglycemia, thereby producing the symptoms of the disease. The NH_4^+ produced is usually converted to urea (Section 26-2) but can also be exported to the bloodstream.

If this scenario for the cause of HI/HA is correct, it requires a reassessment of the role of GDH in ammonia homeostasis. The equilibrium position of the GDH reaction greatly favors the synthesis of Glu ($\Delta G'^{\circ} \approx 30$ kJ · mol^{-1} for the reaction as written in Fig. 26-4), but studies of cellular

Figure 26-5 **X-ray structures of glutamate dehydrogenase (GDH).** (*a*) Bovine GDH in complex with glutamate, NADH, and GTP. The homohexameric enzyme, which has D_3 symmetry, is viewed along one of its 2-fold axes with its 3-fold axis vertical. Each of its subunits is differently colored. The bound substrates and ligands are shown in space-filling form with glutamate orange, the substrate NADH pink, the NADH bound at the ADP effector site brown, and the GTP effector gray. (*b*) One subunit of the

substrate and product concentrations suggested that the enzyme functions close to equilibrium ($\Delta G \approx 0$) *in vivo*. It was therefore widely accepted that increases in $[NH_3]$, high levels of which are toxic, would cause GDH to act in reverse, removing NH_3 and hence preventing its buildup to toxic levels. However, since HI/HA patients have increased GDH activity yet have higher levels of NH_3 than normal, this accepted role of GDH cannot be correct. Indeed, if GDH functioned close to equilibrium, changes in its activity resulting from allosteric interactions would not result in significant flux changes.

C. *Other Deamination Mechanisms*

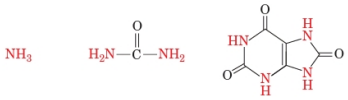
Two nonspecific amino acid oxidases, **L-amino acid oxidase** and **D-amino acid oxidase,** catalyze the oxidation of L- and D-amino acids, utilizing FAD as their redox coenzyme [rather than $NAD(P)^+$]. The resulting $FADH_2$ is reoxidized by O_2.

$$\text{Amino acid} + \text{FAD} + H_2O \longrightarrow \alpha\text{-keto acid} + NH_3 + FADH_2$$
$$FADH_2 + O_2 \longrightarrow FAD + H_2O_2$$

D-Amino acid oxidase occurs mainly in kidney. Its function is an enigma since D-amino acids are associated mostly with bacterial cell walls (Section 11-3Ba). A few amino acids, such as serine and histidine, are deaminated nonoxidatively (Sections 26-3B and 26-3D).

2 THE UREA CYCLE

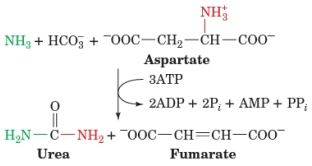
Living organisms excrete the excess nitrogen resulting from the metabolic breakdown of amino acids in one of three ways. Many aquatic animals simply excrete ammonia. Where water is less plentiful, however, processes have evolved that convert ammonia to less toxic waste products that therefore require less water for excretion. One such product is urea, which is excreted by most terrestrial vertebrates; another is **uric acid,** which is excreted by birds and terrestrial reptiles:



Ammonia **Urea** **Uric acid**

Accordingly, living organisms are classified as being either **ammonotelic** (ammonia excreting), **ureotelic** (urea excreting), or **uricotelic** (uric acid excreting). Some animals can shift from ammonotelism to ureotelism or uricotelism if their water supply becomes restricted. Here we focus our attention on urea formation. Uric acid biosynthesis is discussed in Section 28-4A.

Urea is synthesized in the liver by the enzymes of the ***urea cycle.*** It is then secreted into the bloodstream and sequestered by the kidneys for excretion in the urine. The urea cycle was elucidated in outline in 1932 by Hans Krebs and Kurt Henseleit (the first known metabolic cycle; Krebs did not elucidate the citric acid cycle until 1937). Its individual reactions were later described in detail by Sarah Ratner and Philip Cohen. The overall urea cycle reaction is



Thus, the two urea nitrogen atoms are contributed by NH_3 and aspartate, whereas the carbon atom comes from HCO_3^-. Five enzymatic reactions are involved in the urea cycle, two of which are mitochondrial and three cytosolic (Fig. 26-7). In this section, we examine the mechanisms of these reactions and their regulation.

A. *Carbamoyl Phosphate Synthetase: Acquisition of the First Urea Nitrogen Atom*

Carbamoyl phosphate synthetase (CPS) is technically not a urea cycle enzyme. It catalyzes the condensation and activation of NH_3 and HCO_3^- to form **carbamoyl phosphate,** the first of the cycle's two nitrogen-containing substrates, with the concomitant hydrolysis of two ATPs. Eukaryotes have two forms of CPS:

1. Mitochondrial **CPS I** uses NH_3 as its nitrogen donor and participates in urea biosynthesis.

2. Cytosolic **CPS II** uses glutamine as its nitrogen donor and is involved in pyrimidine biosynthesis (Section 28-2A). The reaction catalyzed by CPS I involves three steps (Fig. 26-8):

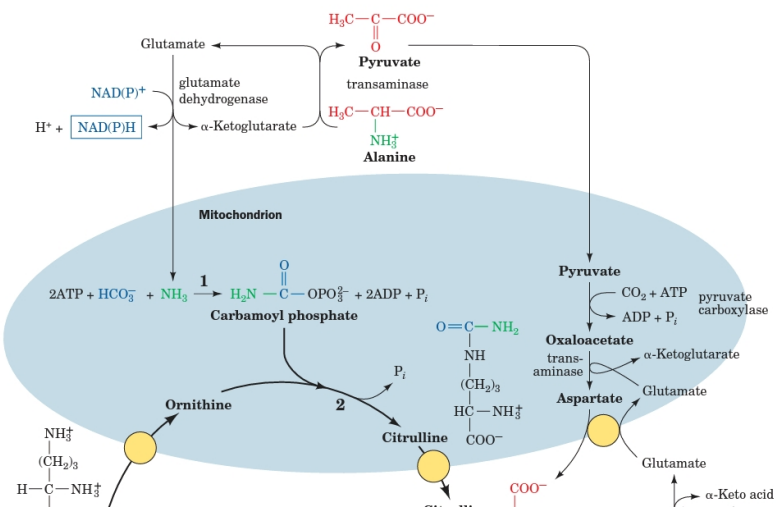
1. Activation of HCO_3^- by ATP to form **carboxyphosphate** and ADP.

2. Nucleophilic attack of NH_3 on carboxyphosphate, displacing the phosphate to form **carbamate** and P_i.

3. Phosphorylation of carbamate by the second ATP to form carbamoyl phosphate and ADP.

The reaction is essentially irreversible and is the rate-limiting step of the urea cycle. CPS I is subject to allosteric activation by ***N*-acetylglutamate** as is discussed in Section 26-2F.

E. coli contains only one type of CPS, which is homologous to both CPS I and CPS II. The enzyme is a heterodimer but when allosterically activated by ornithine (a urea cycle intemediate), it forms a tetramer of heterodimers, $(\alpha\beta)_4$. Its small subunit (382 residues) functions to hydrolyze glutamine and deliver the resulting NH_3 to its large subunit (1073 residues). However, if the enzyme's **glutaminase (glutamine**



their water supply becomes restricted. Here we focus our attention on urea formation. Uric acid biosynthesis is discussed in Section 28-4A.

Urea is synthesized in the liver by the enzymes of the ***urea cycle.*** It is then secreted into the bloodstream and but when allosterically activated by ornithine (a urea cycle intemediate), it forms a tetramer of heterodimers, $(\alpha\beta)_4$. Its small subunit (382 residues) functions to hydrolyze glutamine and deliver the resulting NH_3 to its large subunit (1073 residues). However, if the enzyme's **glutaminase (glutamine**

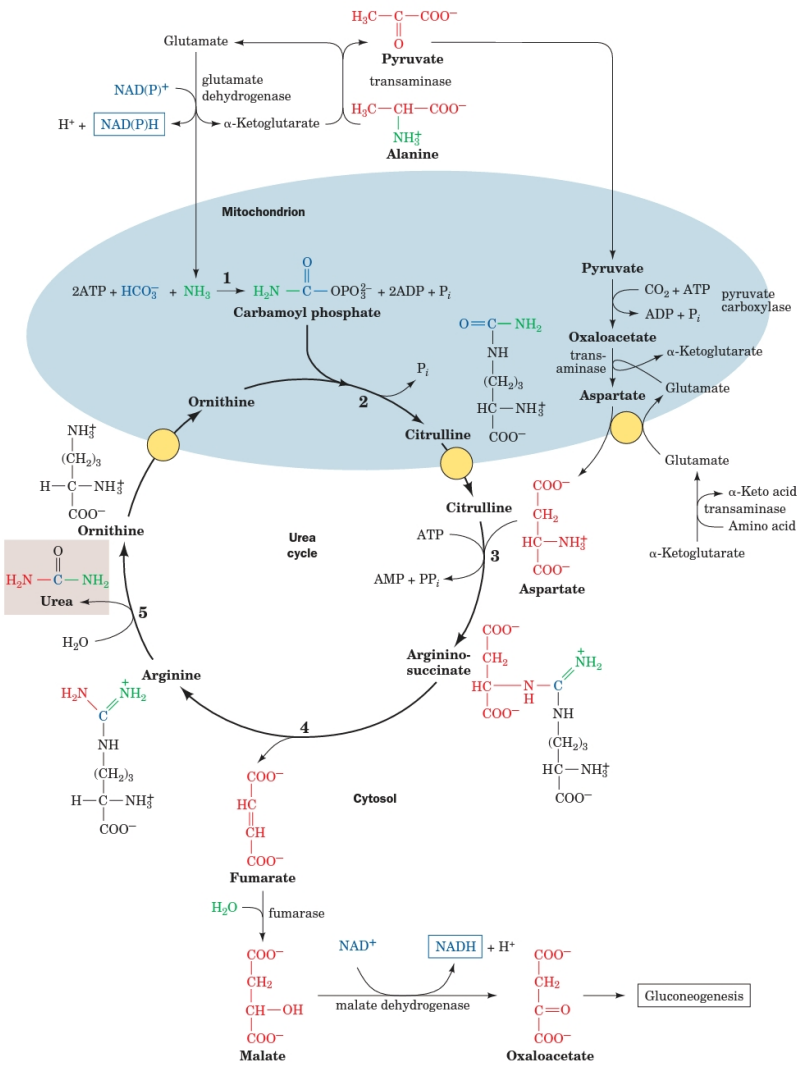


Figure 26-7 *(Opposite)* **The urea cycle.** Its five enzymes are **(1)** carbamoyl phosphate synthetase, **(2)** ornithine transcarbamoylase, **(3)** argininosuccinate synthetase, **(4)** argininosuccinase, and **(5)** arginase. The reactions occur in part in the mitochondrion and in part in the cytosol with ornithine and citrulline being transported across the mitochondrial membrane by specific transport systems (*yellow circles*). One of the urea amino groups (*green*) originates as the NH_3 product of the glutamate dehydrogenase reaction (*top*). The other amino group (*red*) is obtained from aspartate through the transfer of an amino acid to oxaloacetate via transamination (*right*). The fumarate product of the argininosuccinase reaction is converted to oxaloacetate for entry into gluconeogenesis via the same reactions that occur in the citric acid cycle but take place in the cytosol (*bottom*). The ATP utilized in Reactions 1 and 3 of the cycle can be regenerated by oxidative phosphorylation from the superimposable halves that have 40% sequence identity. The N-terminal half contains the carboxyphosphate synthetic component and an oligomerization domain while the C-terminal half contains the carbamoyl phosphate synthetic component and an allosteric binding domain.

a. *E. coli* CPS Contains an Extraordinarily Long Tunnel

The X-ray structure of *E. coli* CPS in complex with Mn^{2+}, ADP, P_i, and ornithine, determined by Hazel Holden and Ivan Rayment, reveals that the active site for synthesis of the carboxyphosphate intermediate is ~45 Å away from the ammonia synthesis site and also ~35 Å away from the carbamoyl phosphate synthesis active site. Astonishingly, the three sites are connected by a narrow 96-Å-long molec-

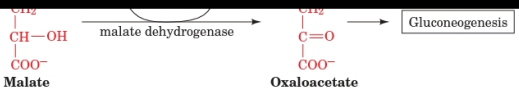


Figure 26-7 *(Opposite)* **The urea cycle.** Its five enzymes are **(1)** carbamoyl phosphate synthetase, **(2)** ornithine transcarbamoylase, **(3)** argininosuccinate synthetase, **(4)** argininosuccinase, and **(5)** arginase. The reactions occur in part in the mitochondrion and in part in the cytosol with ornithine and citrulline being transported across the mitochondrial membrane by specific transport systems (*yellow circles*). One of the urea amino groups (*green*) originates as the NH_3 product of the glutamate dehydrogenase reaction (*top*). The other amino group (*red*) is obtained from aspartate through the transfer of an amino acid to oxaloacetate via transamination (*right*). The fumarate product of the argininosuccinase reaction is converted to oxaloacetate for entry into gluconeogenesis via the same reactions that occur in the citric acid cycle but take place in the cytosol (*bottom*). The ATP utilized in Reactions 1 and 3 of the cycle can be regenerated by oxidative phosphorylation from the NAD(P)H produced in the glutamate dehydrogenase (*top*) and malate dehydrogenase (*bottom*) reactions. **See the Animated Figures**

amidotransferase) activity is eliminated (e.g., by site-directed mutagenesis), the large subunit can still produce carbamoyl phosphate if NH_3 is supplied in high enough concentration. The large subunit is composed of two nearly superimposable halves that have 40% sequence identity. The N-terminal half contains the carboxyphosphate synthetic component and an oligomerization domain while the C-terminal half contains the carbamoyl phosphate synthetic component and an allosteric binding domain.

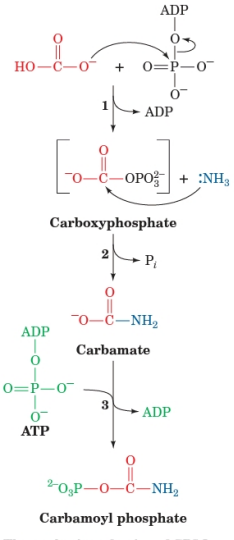


Figure 26-8 **The mechanism of action of CPS I.** **(1)** Activation of HCO_3^- by phosphorylation forms the intermediate, carboxyphosphate; **(2)** nucleophilic attack on carboxyphosphate by NH_3 forms the reaction's second intermediate, carbamate; and **(3)** phosphorylation of carbamate by ATP yields the reaction product carbamoyl phosphate.

a. *E. coli* CPS Contains an Extraordinarily Long Tunnel

The X-ray structure of *E. coli* CPS in complex with Mn^{2+}, ADP, P_i, and ornithine, determined by Hazel Holden and Ivan Rayment, reveals that the active site for synthesis of the carboxyphosphate intermediate is ~45 Å away from the ammonia synthesis site and also ~35 Å away from the carbamoyl phosphate synthesis active site. Astonishingly, the three sites are connected by a narrow 96-Å-long molecular tunnel that runs nearly the length of the elongated protein molecule (Fig. 26-9). It therefore appears that CPS guides its intermediate products from the active site in which they are formed to that in which they are utilized. This phenomenon, in which the intermediate of two reactions is directly transferred from one enzyme active site to

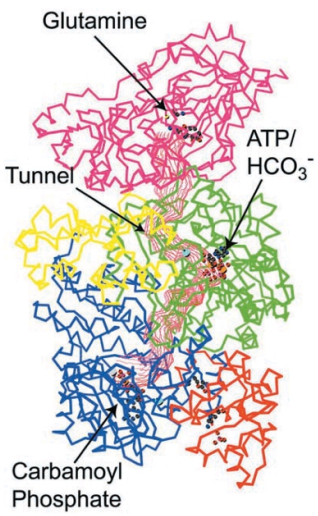


Figure 26-9 **X-ray structure of *E. coli* carbamoyl phosphate synthetase (CPS).** The protein is represented by its C_α backbone. The small subunit (*magenta*) contains the glutamine binding site where NH_3 is produced or bound. The large subunit consists of the carboxyphosphate domain (*green*), the oligomerization domain (*yellow*), the carbamoyl phosphate domain (*blue*), and the allosteric binding domain (*orange*). The 96-Å-long tunnel connecting the three active sites is outlined in red. [Courtesy of Hazel Holden and Ivan Rayment, University of Wisconsin. PDBid 1JDB.]

another, is called **channeling** (the term "tunneling" is reserved for certain quantum mechanical phenomena).

Channeling increases the rate of a metabolic pathway by preventing the loss of its intermediate products as well as protecting the intermediate from degradation. NH_3 must travel ~45 Å down the CPS tunnel to react with carboxyphosphate to form the next intermediate, carbamate. The carbamate, in turn, must travel an additional ~35 Å to the site where it is phosphorylated by ATP to form the final product carbamoyl phosphate. The NH_3 transfer tunnel is lined with polar groups capable of forming hydrogen bonds with NH_3, whereas the tunnel through which carbamate travels is lined with backbone atoms and lacks charged groups

citrullyl–AMP intermediate, which is subsequently displaced by the aspartate amino group. Support for the existence of the citrullyl–AMP intermediate comes from experiments using ^{18}O-labeled citrulline (* in Fig. 26-10). The label was isolated in the AMP produced by the reaction, demonstrating that at some stage of the reaction, AMP and citrulline are linked covalently through the ureido oxygen atom.

D. *Argininosuccinase*

With formation of argininosuccinate, all of the urea molecule components have been assembled. However, the amino group donated by aspartate is still attached to the