Biosynthesis of Amino Acids

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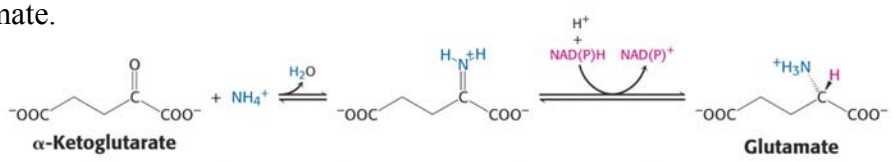
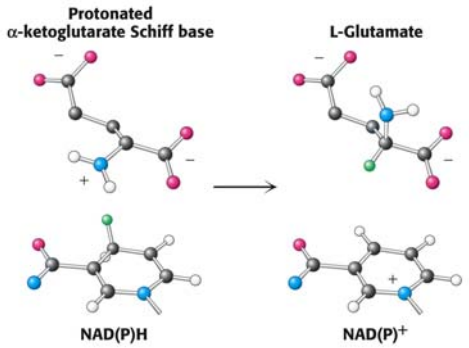
I. Nitrogen Sources

In order to synthesize amino acids, a source of nitrogen is needed. In animals glutamate and glutamine play the pivotal roles. The α–amino group of most of the amino acids comes from the transamination reaction transferring the amino group from glutamate to an α-ketoacid acceptor. Glutamate is synthesized from ammonia and α-ketoglutarate by the action of glutamate dehydrogenase. We have already discussed this enzyme in the degradation of amino acids. It catalyzes the following reaction:

NH_4^+ + α-ketoglutarate + NAD(P)H + H^+ ⟷ Glutamate + $NAD(P)^+$ + H_2O

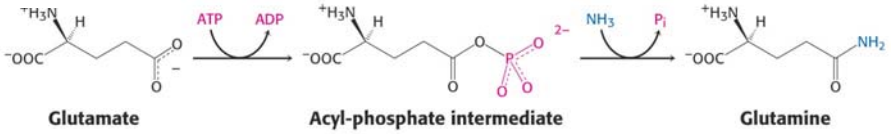
This reaction occurs in two steps first a Schiff base is formed between the ammonia and the ketone of a-ketoglutarate. The Schiff base is reduced by hydride transfer from either NADH or NADPH to form glutamate.





The importance of the hydride transfer is that it establishes the stereochemistry at the α-carbon. Glutamate dehyrogenase binds α-ketoglutarate is such a way that the hydride is transferred to specific face to form only the L-isomer of glutamate.

A second ammonium ion is incorporated into glutamate by the action of glutamine synthetase to form glutamine. This amidation is driven by ATP as we have previously discussed.

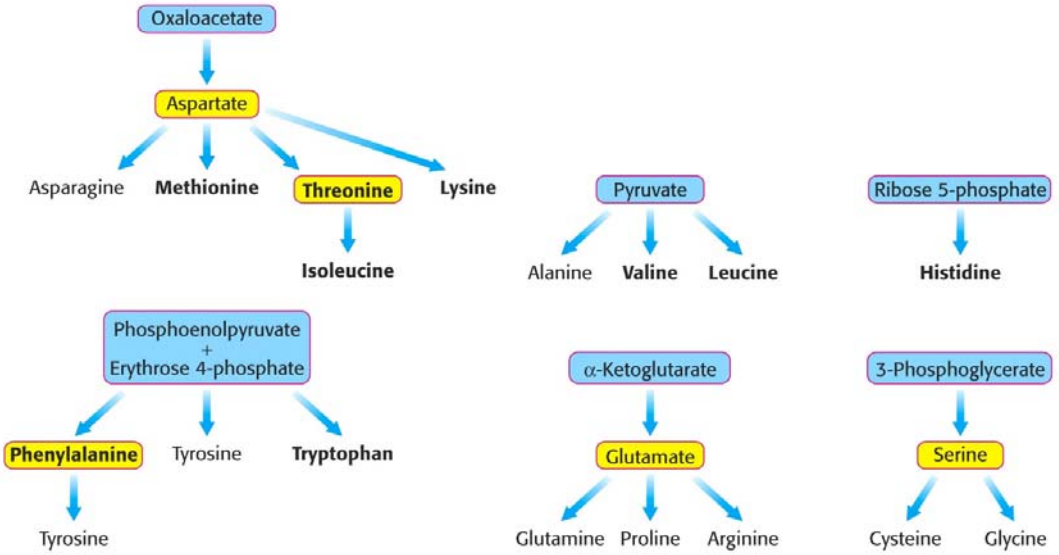


The regulation of glutamine synthetase plays a crucial role in controlling nitrogen metabolism. The dynamic duo of glutamate dehydrogenase and glutamine synthetase are found in all living organisms.

II. Amino Acids are Synthesized From Metabolites of Other Pathways.

Plants and bacteria can synthesize all 20 of the amino acids. Where as we humans cannot synthesize 9 of them. These nine amino acids must come from our diets and are called essential amino acids. The essential amino acids are:
Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine.
The other 11 amino acids are called nonessential amino acids they include:
Alanine, Arginine, Asparagine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Proline, Serine, and Tyrosine.

These nonessential amino acids are synthesize by simple pathways. Whereas the biosynthesis of the essential amino acids are quite complex.

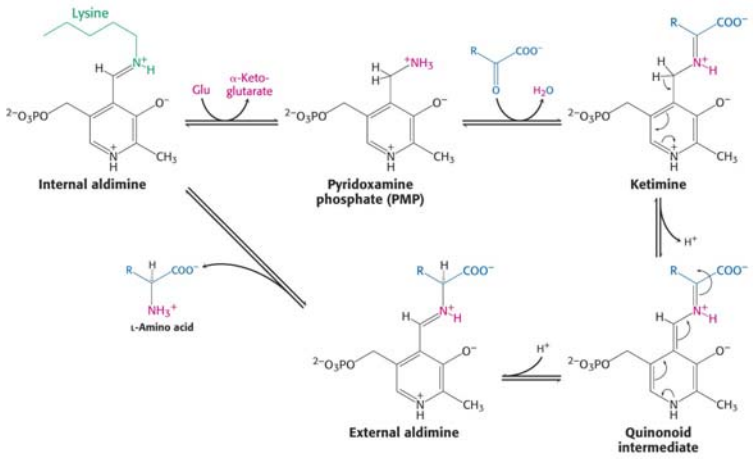


Shown above are the major metabolic precursors of the amino acids. The amino acids that are precursors for other amino acids are shown in yellow. The nine essential amino acids are shown in boldface. The carbon skeletons come from intermediates of glycolysis, the pentose phosphate pathway and the citric acid cycle. On the basis of the starting points the 20 amino acids can be group into 6 categories depending on the precursor: oxaloacetate, PEP, α-ketoglutarate, pyruvate, 3-phosphoglycerate, ribose-5P.

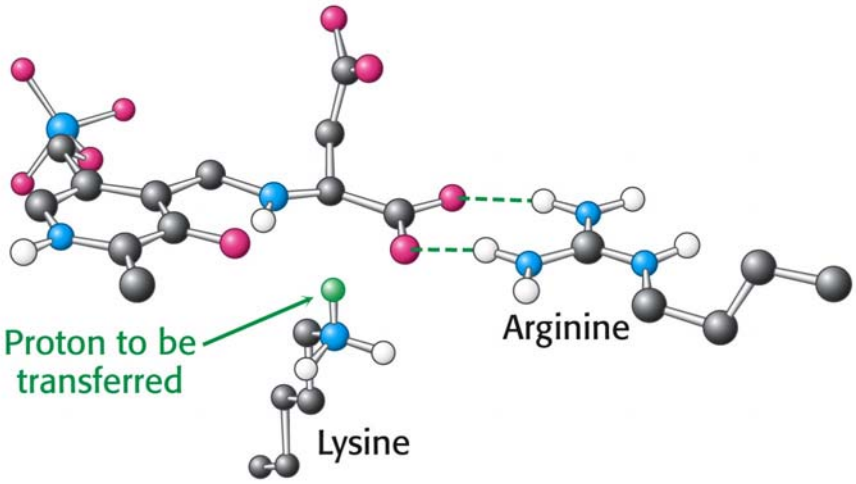
III. Aspartate, Glutamate and Alanine Biosynthesis

Oxaloacetate, pyruvate and α-ketoglutarate are all α-ketoacids which are substrates for transamination reactions that we very familiar with by now. Asparatate aminotransferase transfers an amino group from glutamine to oxaloacetate to form aspartate and α-ketoglutarate. There are a number of transaminase that will transfer an amino group from an amino acid such as aspartate or alanine and transfer it to α-ketoglutarate to form glutamate. Alanine aminotransferase transfers the amino group of glutamine to pyruvate to form alanine and α-ketoglutarate.

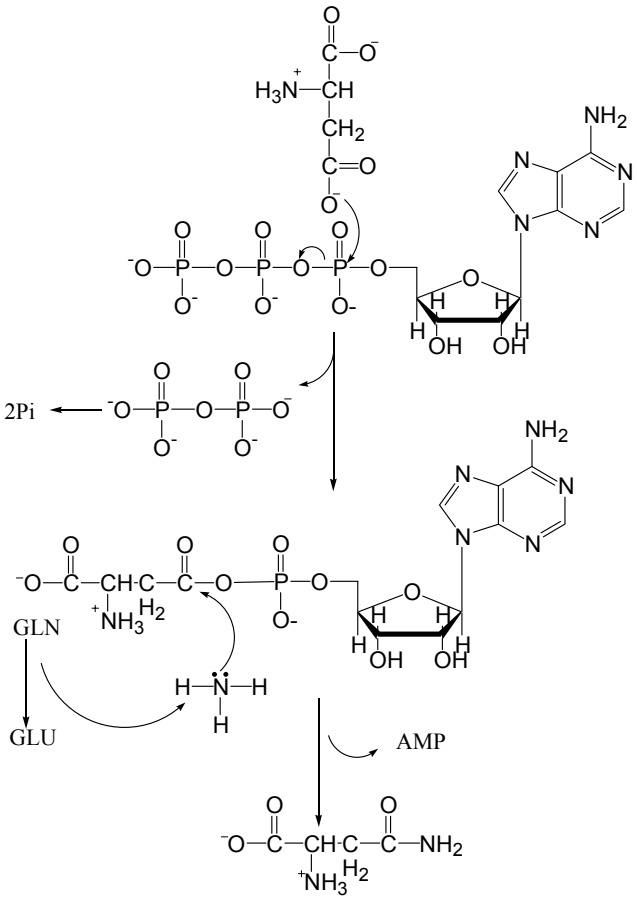
All of these transamination reactions are catalyzed by enzymes that require pyridoxal phosphate as a cofactor. Review the pyridoxal phosphate mechanism. The enzyme reaction begins with the formation of the external aldimine between PLP and the amino group donor displacing the active site lysine residue. The amino group is transferred to PLP to form the pyridixoamine phosphate intermediate releasing the α-ketoacid remnant of the donor. The second half of the reaction is the reverse of the first. The amino group acceptor is another α-ketoacid which forms a Schiff base with the PMP to form a ketimine intermediate. The amino group is transferred to the acceptor to form the amino acid.



The important step in the transamination reaction is the protonation of the α-carbon of the quinonoid intermiate. This protonation determines the stereochemistry at the α-carbon. In the case of aspartate amino transferase (the postor child of transaminases) the chirality of the amino acid is determined by binding interactions with the substrate. Particularly, the interaction of the conserved arginine residue (Arg-386) with the carboxylate group of the substrate. This interaction orients the substrate so that when Lys-268 protonates the α–carbon of the quinonoid intermediate it generates an external aldimine with the L-configuration at the α-carbon.



IV. Asparagine Biosynthesis



Asparagine synthetase catalyzes the formation of asparagine from aspartate.

The first step is to activate the carboxylate group of aspartate by reacting with ATP to form an aspartyl-adenylate intermediate.

The activated carbonyl of aspartyl-adenylate intermediate is attacked by an ammonia nucleophile to produce asparagines.

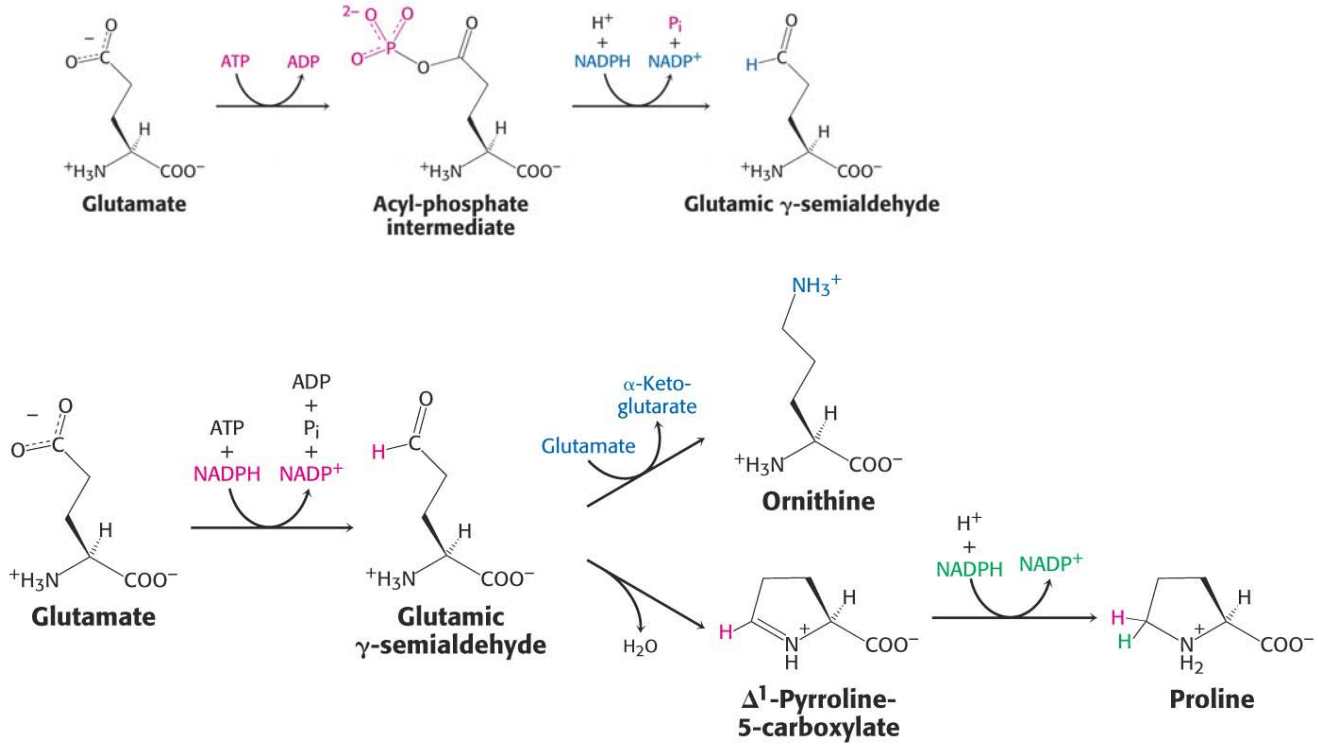
In bacteria ammonia is the nitrogen source for this reaction.

In mammals the nitrogen source for this reaction is derived from the hydolysis of glutamine into glutamate. The ammonia produced in this reaction is channeled to the aspartyl-adenylate intermediate to from asparagine.

The advantage of this pathway of using glutamine as the nitrogen source is that the ammonia is produced by the enzyme and channeled directly to the intermediate. The cells are not exposed to ammonia which is toxic to animals.

V. Proline and Arginine Biosynthesis.

Proline and arginine are both derived from glutamate. The first step is to form glutamate γ-semialdehyde. The γ-carboxylate group of glutamate is activated by phosphorylation with ATP to from a γ-glutamylphosphate intermediate. The mixed anhydride is then reduced with NADPH liberating the phosphate and producing glutamate γ-semialdehyde.



Glutamate γ-semialdehyde will spontaneously cyclize eliminating H_2O and forming pyrroline 5-carboxylate which is then reduced by pyrolline-5-carboxylate reductase to form proline.

Glutamate γ-semialdehyde is also a substrate for ornithine-δ-aminotransferase which will transfer and amino group from glutamate to glutamate γ-semialdehyde to from ornithine and α-ketoglutarate. Ornithine of course is a metabolite of the urea cycle which reacts with carbamoyl phosphate to form citrulline. Citrulline is condensed with aspartate to form argininosuccinate which is then cleaved to form arginine and fumarate.

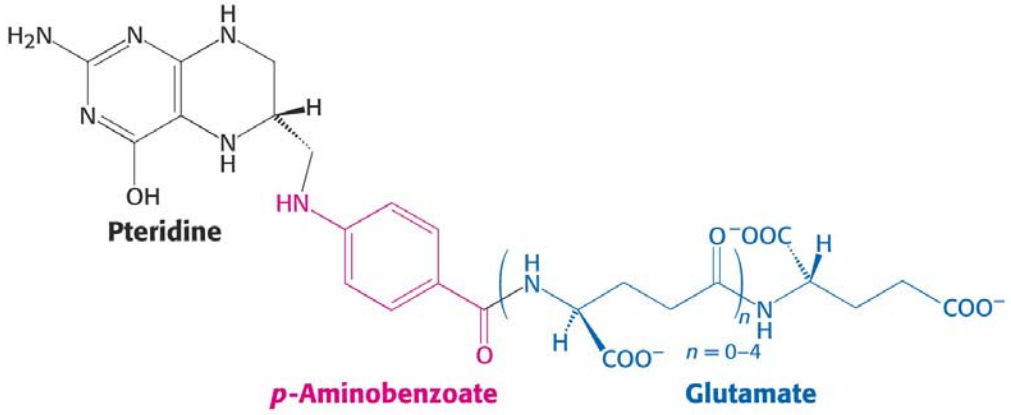
VI. Serine, Cysteine and Glycine Biosynthesis.

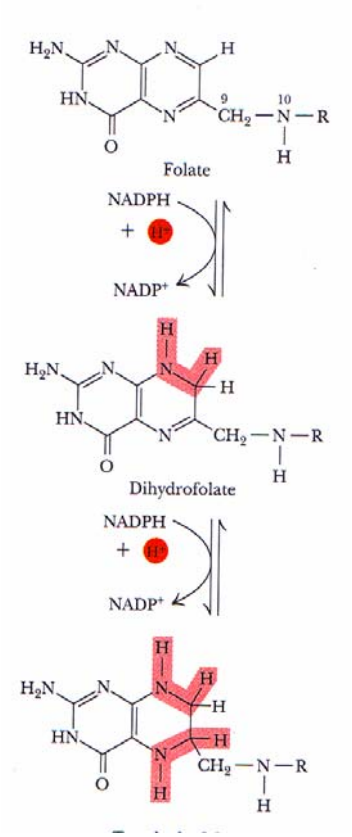
Serine is synthesized from 3-phosphoglycerate. Serine is a precursor for both cysteine and glycine. The first step is the oxidation of 3-phosphoglycerate into 3-phosphohydroxypyruvate. The enzyme that catalyzes the oxidation is 3-phosphoglycerate dehydrogenase. This oxidation generates an α-ketoacid which can now be transaminated by transferring an amino group from glutamate to 3-phosphoglycerate to generate 3-phosphoserine and α-ketoglutarate. The phosphate group is removed by phosphoserine phosphatase to produce serine.

Serine is converted into glycine by serine hydroxymethyltransferase which contains both a pyridoxal phosphate cofactor and a tetrahydrofolate cofactor. It is time to take a closer look as this remarkable cofactor and this remarkable enzyme.

Tetrahydrofolate-Carrier of Activated One-Carbon Units.

Tetrahydrofolate





Tetrahydrofolate

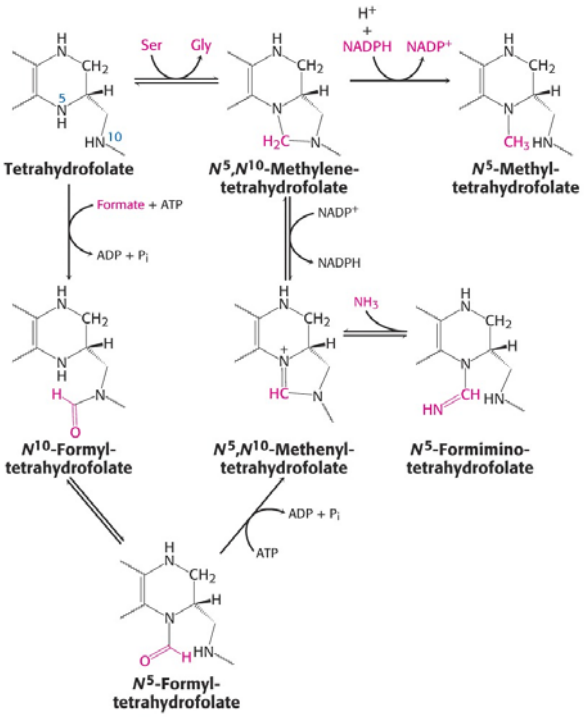
Tetrahydrofolate is a versatile carrier of one carbon units. It consists of 3 groups. A pteridine, an amino benzoate and a chain of one or more glutamate residues. Mammals can synthesize all three components but lack the enzymes that conjugate them together. Microorganisms do contain the necessary conjugating enzymes and can produce folate. We must obtain folate from our diet or from the microorganism that inhabit our intestinal tract. Folic acid is member of the vitamin B complexes and is found in green vegetables, fruits, yeast and liver. Folate is reduced by dihydrofolate reductase to form THF. This is a two step reduction requiring 2 hydride transfers from NADPH.

One carbon units may be bound to THF at the N^5 and N^{10} nitrogens. The carbons carried can exist in three oxidation state. The most reduced state of a THF carried carbon is a methyl group, the intermediate oxidation state carries a methylene group, the most oxidized state carries a formimino group. The next higher oxidation state is the carboxylate which is carried by the biotin cofactor rather than THF.

The one carbon units carried by THF are interconvertible as shown below

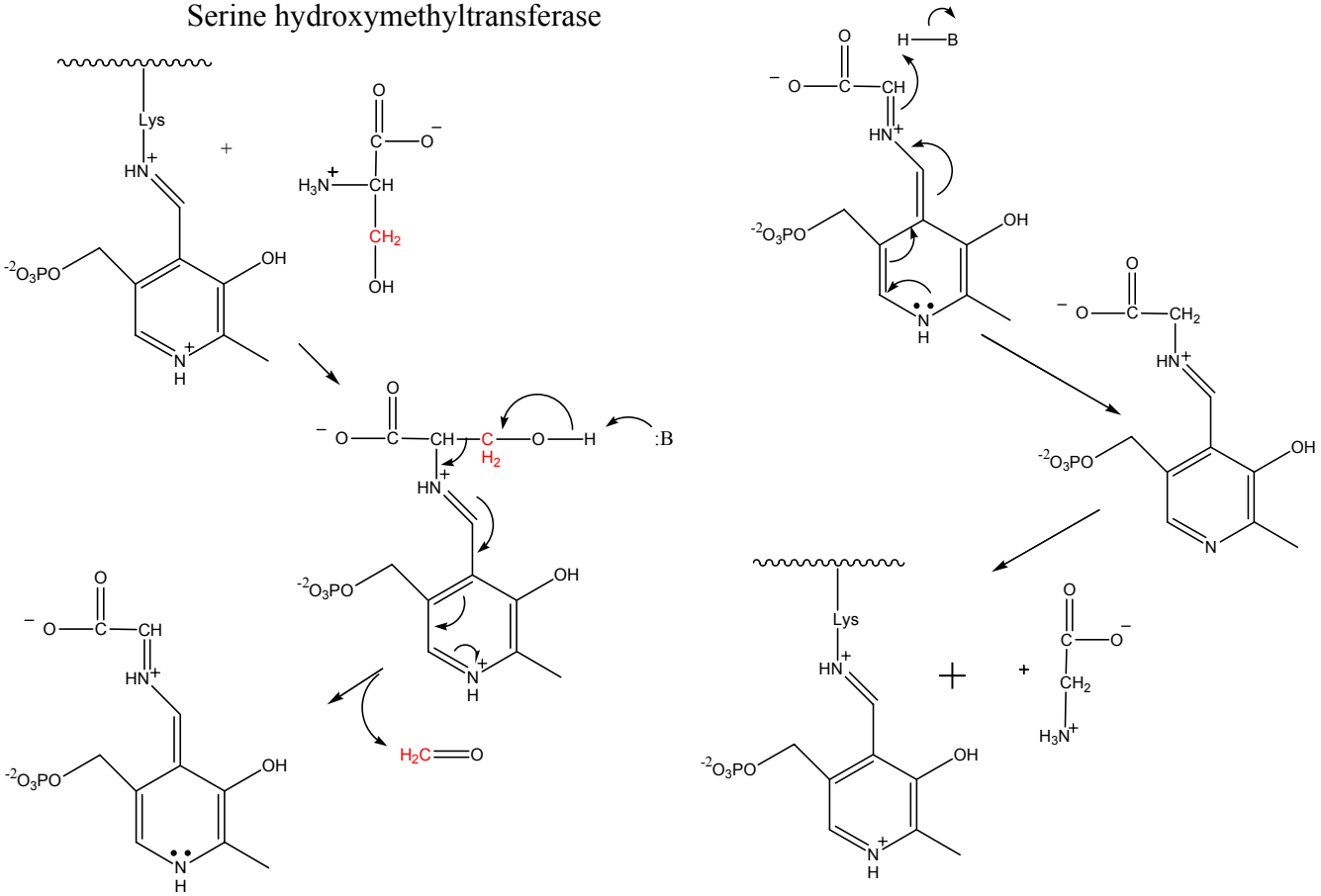
TABLE 24.2 One-carbon groups carried by tetrahydrofolate

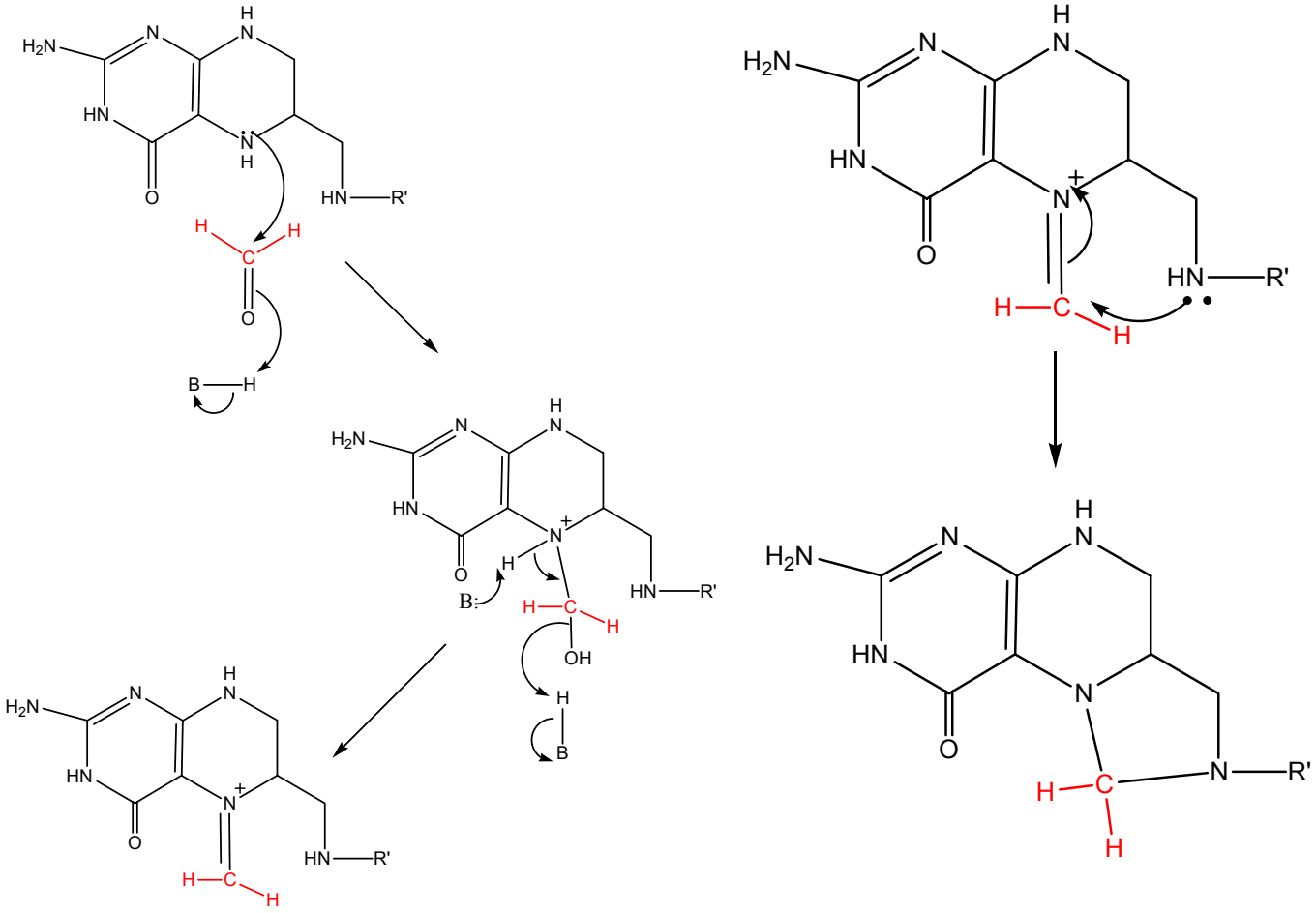
Oxidation state	Group	
Most reduced (= methanol)	$-CH_3$	Methyl
Intermediate (= formaldehyde)	$-CH_2-$	Methylene
Most oxidized (= formic acid)	–CHO	Formyl
	–CHNH	Formimino
	–CH=	Methenyl



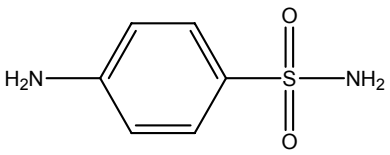
All of these interconvertible tetrahydrofolate derivatives carry activated one carbon units which can be used for a variety of biosyntheses. THF also serves as one carbon unit acceptor in degradative reactions.

Serine hydroxymethyltransferase

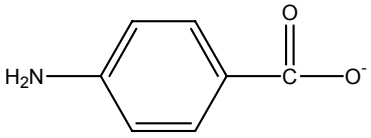




Sulfonamides such as sulfanilamide are analogs of the p-aminobenzoate substituent of folic acid. The are competitive inhibitors of the bacterial conjugating enzyme for the p-aminobenzoate binding site. They block the synthesis of folate producing a deficiency which inactivates all of the enzymes that require a THF cofactor. Since mammals do not possess the conjugating enzymes, sulfonamides are not toxic which is why sulfonamides are such widely used antibiotics.



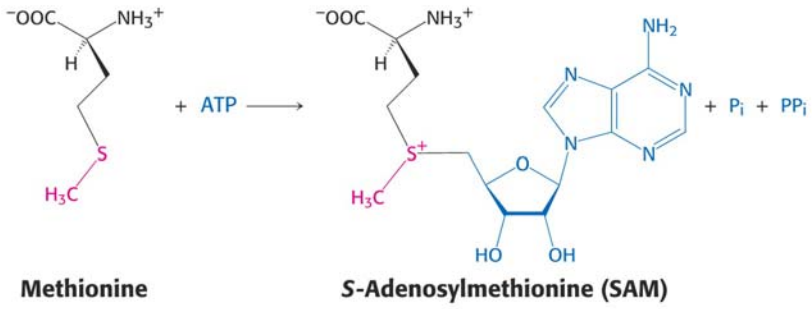
Sulfonamide

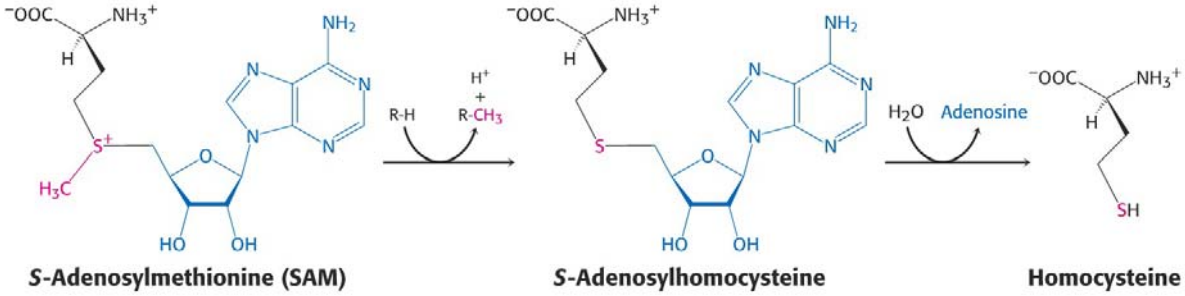


p-Aminobenzoate

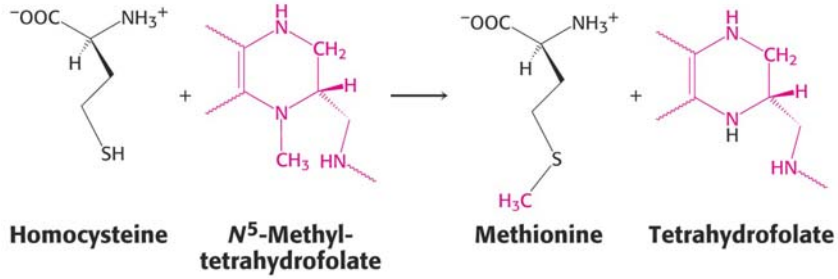
SAM, the Major Donor of Methyl Units

THF can carry a methyl group, but the transfer potential is not very high enough for most biochemical methylations. The activated methyl carrier of choice is S-Adenosylmethionine. SAM is produced by the transfer of an adenosyl group from ATP to methionine. The methyl group of the methionine group is activated by the positive charge on the adjacent sulfur atom. This positive charge makes the methyl transfer more energetic than from N^5-methyl-THF. SAM carries the methyl groups used for the methylation reactions of DNA.

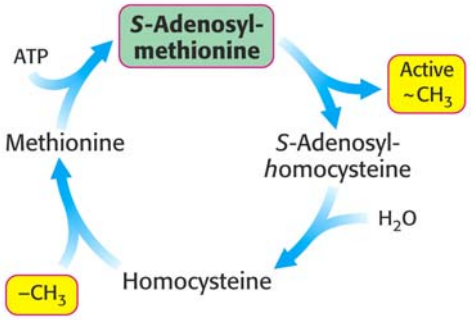




When the methyl group of SAM has been transferred to an acceptor, SAM is converted into S-adenosylhomocysteine which is then hydrolyzed to form homocysteine. Homocysteine can be converted back into methionine by the transfer of a methyl group from N^5-methyltetrahydrofolate in a reaction catalyzed by homocysteine methyltransferase. This enzyme requires cofactor B_{12}.



These reactions constitute the activated methyl cycle shown below.



Cysteine Biosynthesis

Homocysteine is an intermediate in cysteine biosynthesis. Serine and homocysteine are condensed together by crystathionine β-synthase to form cystathionine which is then deaminated and cleaved by cystathioninase. Both of these enzymes require pyridoxal phosphate cofactors.