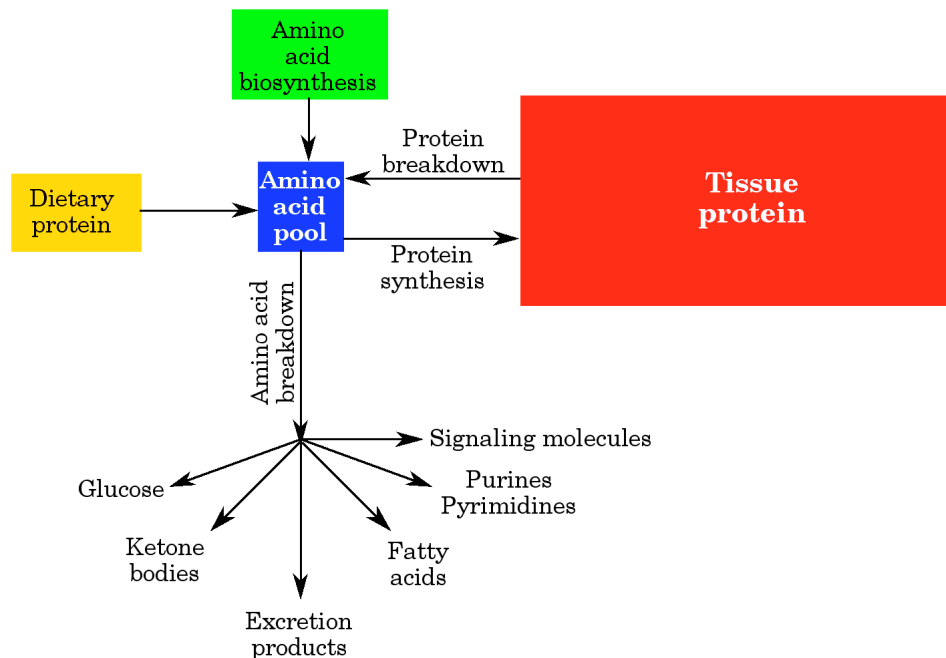


Introduction to amino acid metabolism

Overview

The body has a small pool of free amino acids. The pool is dynamic, and is constantly being used as a source of substrate for various reactions, and is constantly being replenished. Free amino acids are **not stored**, except as part of larger molecules (*i.e.* proteins).

Amino acids can be used for a variety of functions. The primary function of amino acids is to act as the monomer unit in protein synthesis. Amino acids can be also used as substrates for biosynthetic reactions; the nucleotide bases, heme, and a number of hormones and neurotransmitters are derived from amino acids. Finally, the carbon skeleton of all of the amino acids broken down for energy.



Nitrogen metabolism

Unlike glucose or fatty acids, amino acids contain nitrogen. Biologically relevant inorganic nitrogen molecules include dinitrogen (N_2), nitrogen oxides (including NO_2^- and NO_3^-), and ammonium (NH_4^+)⁴.

Ammonium is the most useful form of inorganic nitrogen in most organisms, especially in animals. Unfortunately ammonium is toxic to animals. The reasons for this toxicity are incompletely understood, but most symptoms involve altered brain function, suggesting that the central nervous system is vulnerable to ammonium-induced damage. Ammonium therefore must be handled carefully, and physiological nitrogen metabolism must take this into account.

⁴ Ammonium is the protonated form of ammonia (NH_3); ammonium is the major species in aqueous solution.

Ammonium can come from several sources depending on the organism: 1) organic nitrogen: nitrogen attached to organic molecules that can be metabolized; 2) free ammonium; 3) nitrogen oxides (especially nitrate); and 4) dinitrogen.

Nitrogen fixation

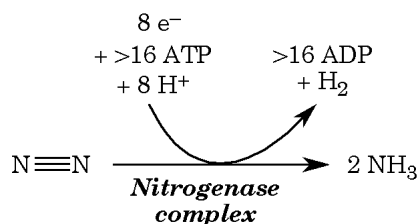
N_2 is inaccessible to most organisms, because of the strong bond between the nitrogen atoms. Although the conversion of N_2 to NH_3 has a ΔG° of about -33 kJ/mol, the activation energy barrier for the reduction of N_2 is very large.

A few bacteria are capable of reducing dinitrogen to ammonia. These are called nitrogen-fixing bacteria; some are free living, but many are symbiotes of plants, especially legumes such as soybeans, peas and alfalfa.

The nitrogen fixation reaction requires specialized proteins, the products of the *nif* genes, which code for nitrogenase and its accessory proteins. Nitrogenase requires iron, sulfur, and molybdenum as cofactors. Nitrogenase is rapidly denatured by oxygen, and therefore requires an oxygen-free environment.

Legumes have leghemoglobin, a monomeric globin with high oxygen affinity. It functions to protect the bacteria from free oxygen, by transferring oxygen only to the bacterial cytochrome *c* oxidase. Free-living bacteria either live in anaerobic environments, or use uncoupling agents to increase their rate of oxygen reduction to protect their nitrogenase complex.

The nitrogen fixation reaction is expensive: at least 16 ATP are required to overcome the energy barrier in dinitrogen. The actual energy requirements are usually higher than the minimum stoichiometry shown below due to wasteful side reactions. This means that organisms capable of fixing nitrogen have considerable energy requirements. Legumes use $\sim 20\%$ of their ATP production to supply energy for their symbiotic bacteria.



The nitrogen fixation process requires electrons. In free living cyanobacteria, the electrons are derived from a photosynthetic electron transport chain. The symbiotic nitrogen-fixing bacteria of legumes are present in root nodules; because they are not exposed to sunlight, these bacteria must use electrons from metabolic sources (such as the pyruvate dehydrogenase reaction) to drive nitrogen reduction.

Humans can only perform N_2 reduction using technological assistance. The Haber process, invented shortly before World War I, uses high pressures of hydrogen gas (200 atmospheres) and temperatures (700 K) to achieve what the bacteria manage at ambient temperature and pressure.

Nitrogen assimilation

Plants can use either ammonium or nitrogen oxides (especially nitrate) as sources of usable nitrogen. Nitrate is formed by microorganisms that can use ammonium as an energy source, and is thus the lowest energy form of nitrogen. On the other hand, nitrate and other nitrogen oxides are major components in explosives, which is why fertilizer can be dangerous. The explosive potential of the common fertilizer NH_4NO_3 when combined with readily available carbon compounds such as diesel oil has led to some limits on the sale of this material.

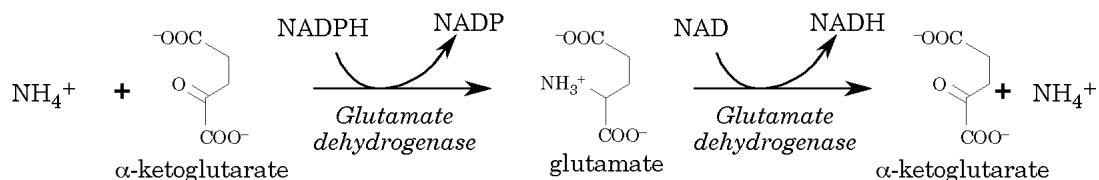
Nitrate reduction requires electrons, derived from photosynthesis, to produce ammonium. The reduction of nitrate must be followed by **ammonium fixation**, the process of attaching ammonium ions to carbon compounds. The reactions used for this purpose are discussed below.

Unlike plants, animals use organic nitrogen derived from their diet for essentially all of their nitrogen requirements. Animals require nitrogen in reduced form and release most nitrogen in reduced form; in general, animals cannot reduce nitrogen oxides, and do not excrete these compounds.

Most organisms have three major reactions that incorporate inorganic nitrogen into organic compounds. These reactions are catalyzed by glutamate dehydrogenase, glutamine synthetase, and one isozyme of carbamoyl phosphate synthase (this last enzyme we will discuss later, during the discussion of the urea cycle).

In addition, one pathway for glycine synthesis uses inorganic ammonium; under most conditions, this reaction is a relatively minor ammonium fixation reaction. Finally, some microorganisms can fix ammonium using asparagine synthetase, although higher organisms use glutamine as the ammonium donor for this reaction.

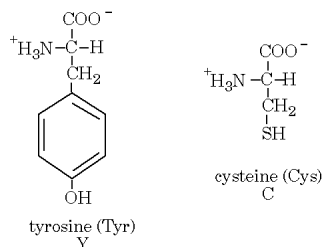
Glutamate dehydrogenase uses reducing equivalents from NADPH to bind ammonium to α -ketoglutarate. It can also catalyze the reverse reaction, releasing α -ketoglutarate and ammonium; in doing so, however, it usually uses NAD and produces NADH. The ammonium release reaction is a key step in the catabolism of many amino acids.



Glutamate dehydrogenase has a high K_m for ammonium. Because ammonium is toxic to animals, ammonium concentration is normally maintained at too low a level to allow glutamate dehydrogenase to synthesize significant amounts of glutamate. Instead, another reaction, catalyzed by glutamine synthetase, is more important for ammonium fixation in most species. In many plants, glutamine synthetase is the sole ammonium fixation enzyme. **Glutamine synthetase** uses ATP as the source of energy for the reaction.

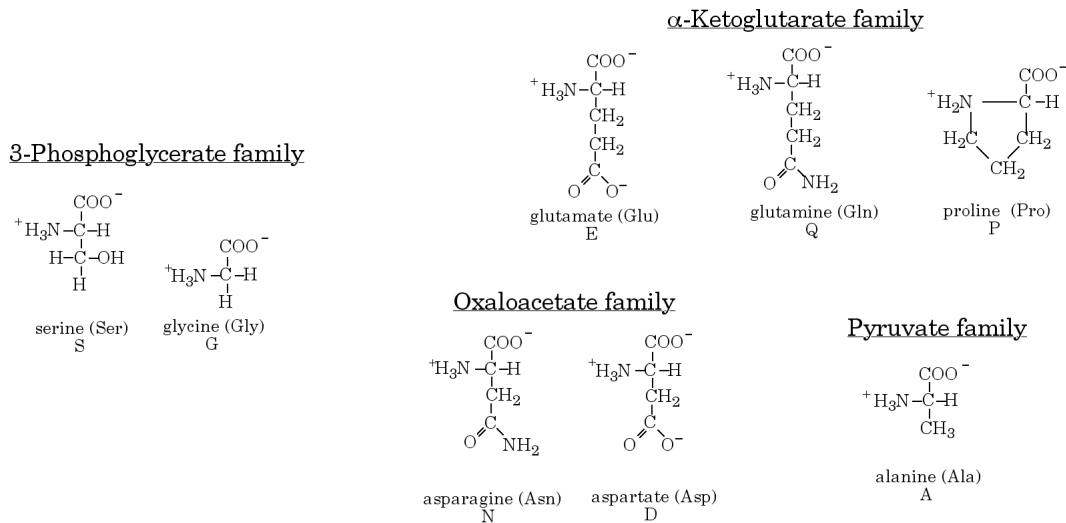
Arginine is normally considered to be an essential amino acid. Although humans and most animals can synthesize arginine, synthesis rates are typically lower than requirements, especially during growth and development.

Two other amino acids, cysteine and tyrosine are typically considered to be non-essential amino acids. This is not really accurate, because each of these can only be synthesized from an essential amino acid. Cysteine is synthesized from methionine, and tyrosine is synthesized from phenylalanine. If the essential amino acid precursor is not available, these two amino acids are also unavailable.



Examination of the amino acids shown above reveals that animals are incapable of synthesizing the aromatic amino acids, the hydrophobic amino acids larger than alanine, and the basic amino acids.

“Non-essential” amino acids are also **required** in order to make proteins. However, most organisms can synthesize these compounds, and therefore do not require dietary sources of these amino acids. In general, the synthesis pathways for the essential amino acids are complex, and involve a large number of reactions. Non-essential amino acids are at least as important as essential amino acids. In fact, they are so important that animals have retained the enzyme pathways necessary to synthesize these compounds. “Nutritionally non-essential” is therefore a better term.



The “non-essential” amino acids can all be synthesized from compounds that have other uses. The precursors for the non-essential amino acids are intermediates in major pathways.

Animals can thus synthesize the acidic amino acids, the amide containing amino acids, glycine and proline, and serine.

Transamination

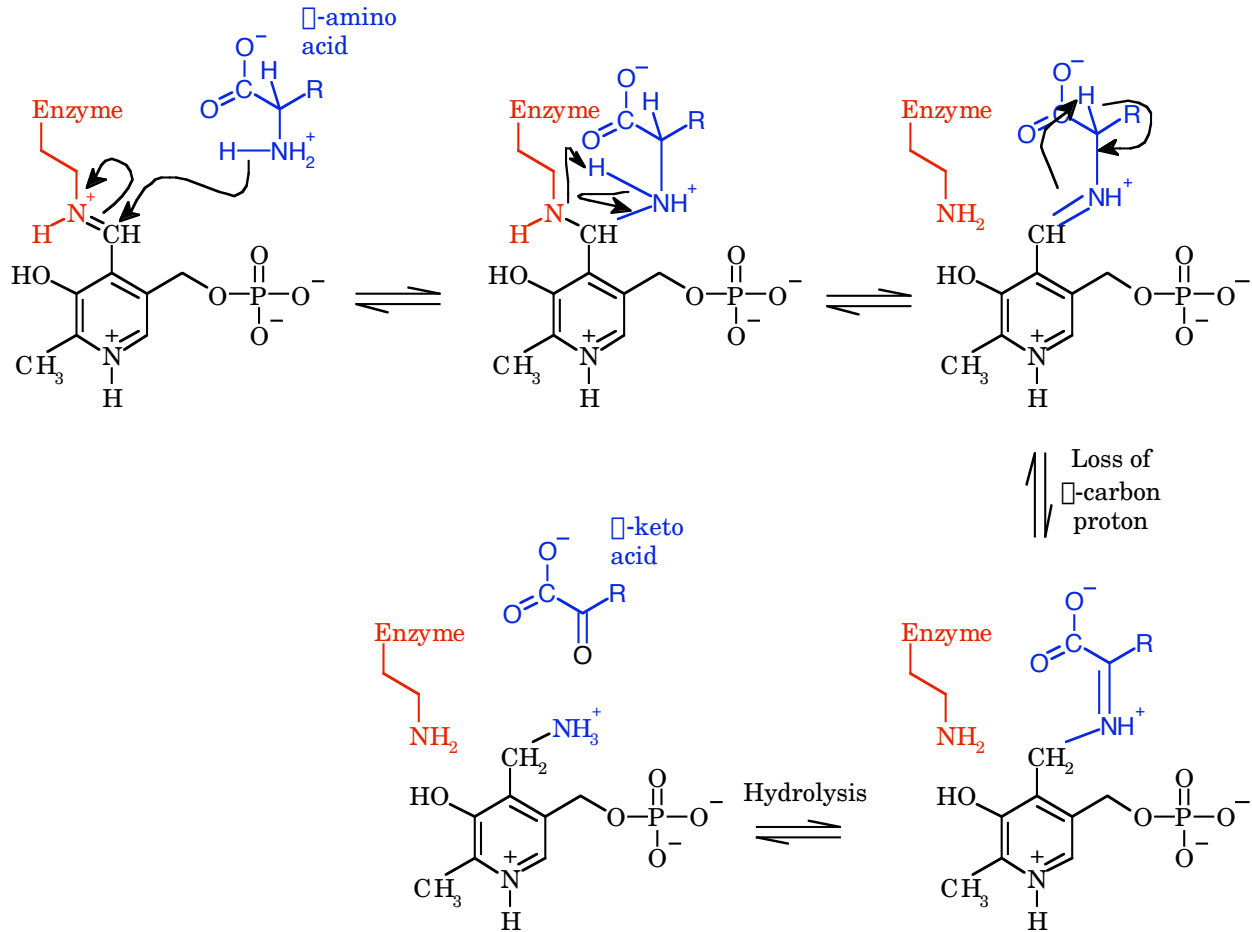
In animals, addition of free ammonium to α -ketoacids or to α -amino acids is limited to α -ketoglutarate, glutamate, and glycine. All other amino acids receive their nitrogen by transfer of organic nitrogen from one amino acid to another.

In amino acid metabolism, the most common nitrogen donor is glutamate, and the most common acceptor is α -ketoglutarate. This is logical, since glutamate is a direct link (via glutamate dehydrogenase) to inorganic ammonium. In some cases, however, alanine and pyruvate or aspartate and oxaloacetate are used instead of glutamate and α -ketoglutarate. Note that in each case, the pairs of compounds listed are the amino acids and their corresponding α -ketoacid.

Transamination is an **exchange** reaction. In transamination reactions, no free ammonium is released at any stage of the reaction; the reaction begins and ends with a ketoacid and an amino acid. The class name for the enzymes catalyzing these reactions is **aminotransferase** (usually with a specific name referring to the substrate specificity, such as glutamate-aspartate aminotransferase. (Note: the older literature referred to these enzymes as transaminases; aminotransferase is the currently accepted term.)

Aminotransferases are pyridoxal phosphate-dependent enzymes. The mechanism involves the formation of a Schiff base between the pyridoxal group and the amine function. This then rearranges, and then releases the keto acid, with the nitrogen attached to the pyridoxamine. The enzyme can then donate the amine to a different ketoacid. Aminotransferases catalyze reversible reactions, with overall ΔG° near zero.

A slightly abbreviated mechanism for a generic aminotransferase reaction is shown below. It illustrates two important points: 1) the critical role played by pyridoxal phosphate in altering the chemistry at the α -carbon, a role this cofactor plays in a large number of different reactions involving amino acids, and 2) the reason these reactions are reversible and require both an ammonium donor and acceptor. An α -ketoacid binding an aminotransferase that contains pyridoxamine will be converted to an amino acid. An amino acid that binds an aminotransferase with pyridoxal will become an α -ketoacid.



Aminotransferases are critical to both the synthesis and breakdown of amino acids.

Nutritionally non-essential Amino acid synthesis pathways in Animals

Ammonium dependent reactions

Glutamate is synthesized from α -ketoglutarate, the TCA cycle intermediate, by any of a large number of aminotransferase isozymes. It is also synthesized from free ammonium by glutamate dehydrogenase.

Glutamine is synthesized from glutamate by glutamine synthetase. These reactions, which were discussed above, are the *primary* ammonium fixing reactions in most species.

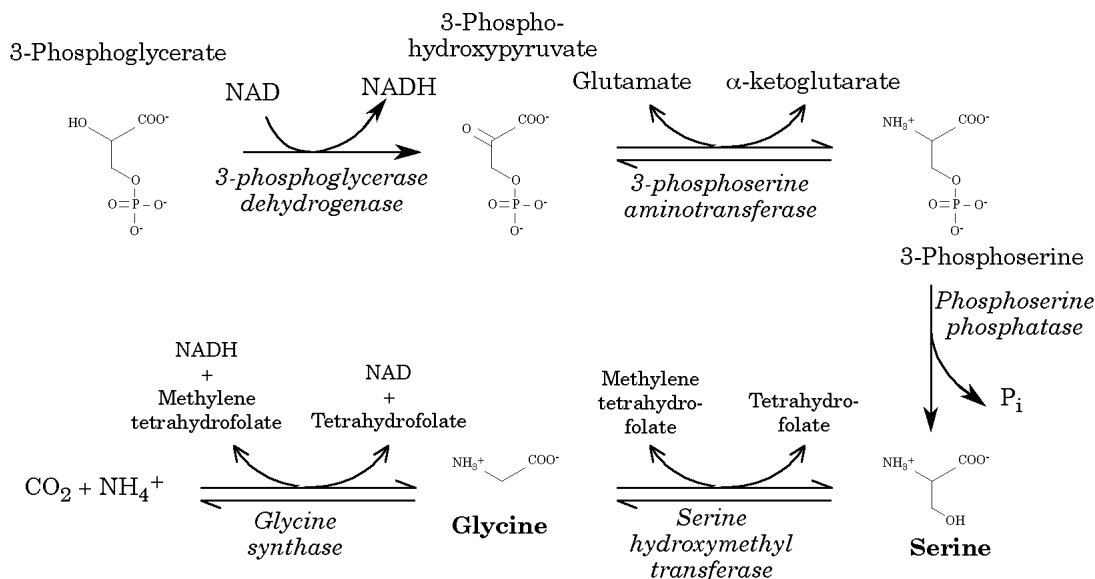
Ammonium transfer reactions

Alanine is synthesized from pyruvate and **aspartate** is synthesized from oxaloacetate by aminotransferases. The most common ammonium donor is glutamate, although other amino acids are used under some conditions.

always contains the only amino form of the compound.

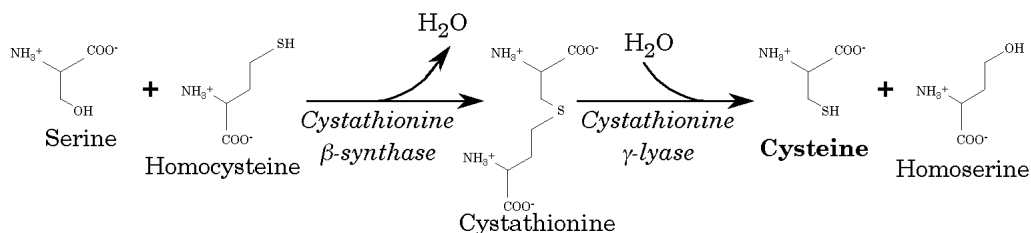
More complex pathways

Serine is synthesized from 3-phosphoglycerate, the glycolytic intermediate, by a short pathway. The first step is the oxidation of the 3-phosphoglycerate hydroxyl to a ketone, forming an α -ketoacid. This α -ketoacid is then converted to an α -amino acid in an aminotransferase reaction, followed by removal of the phosphate from the serine hydroxyl by **phosphoserine phosphatase**. Serine can also be synthesized from glycine by **serine hydroxymethyltransferase**.



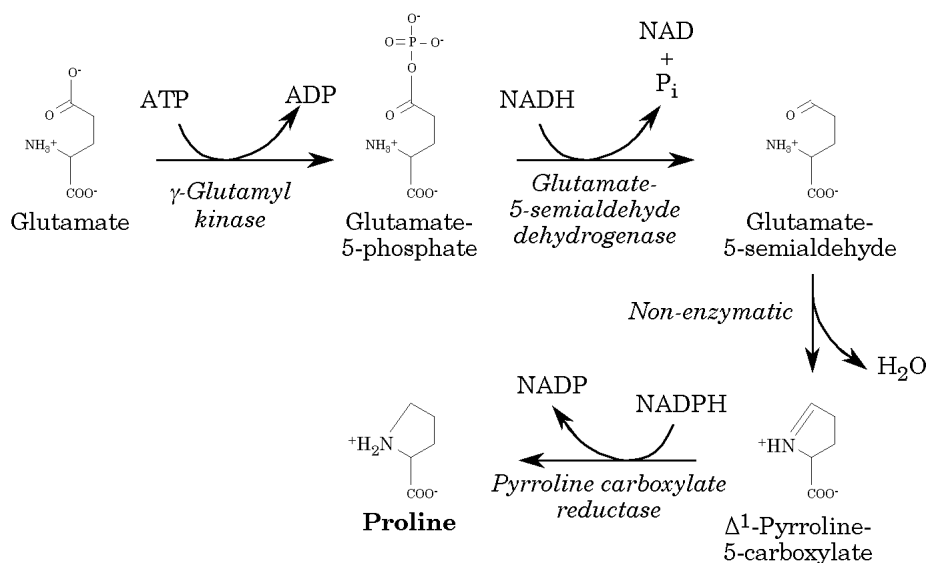
Glycine is synthesized from several precursors. The two major pathways for glycine production are shown above. In one pathway, the α -carbon of serine is transferred to tetrahydrofolate. The resulting methylene tetrahydrofolate can then be used to synthesize another molecule of glycine from ammonium and carbon dioxide. Net fixation of ammonium is limited by availability of methylene tetrahydrofolate. Methylene tetrahydrofolate is also used for other biosynthetic reactions; the interconversion of serine and glycine acts as a method for generating methylene tetrahydrofolate when necessary, or consuming excess methylene tetrahydrofolate when this compound is no longer necessary. The interconversion of glycine and serine are also involved in some of their breakdown pathways.

Cysteine synthesis requires serine and homocysteine. Homocysteine is a product of reactions involving methionine that will be discussed later.



In plants and microorganisms, cysteine is also synthesized from serine, either by direct addition of inorganic sulfur, or more commonly by addition of inorganic sulfur to activated serine. These pathways do not occur in humans.

Proline is synthesized from glutamate by a several step pathway. The first step is the phosphorylation of the glutamate side-chain carboxylate group. This is followed by a reaction similar to the glyceraldehyde-3-phosphate dehydrogenase reaction, which releases free inorganic phosphate, and glutamate with the side-chain carboxylate reduced to an aldehyde. The aldehyde then undergoes a non-enzymatic formation of a Schiff base linkage to the α -amino group. The double bond must then be reduced to a single bond by **pyrroline carboxylate reductase** to form proline.



Summary

Amino acids are used for protein synthesis, for energy, and as precursors for a variety of biosynthetic reactions.

Atmospheric nitrogen cannot be incorporated into organic molecules. In order to become useful, the atmospheric nitrogen must be fixed by conversion to ammonia in the energetically expensive nitrogenase reaction. Only a small number of microorganisms are capable of fixing nitrogen.

Once ammonium has been produced, it must be converted to organic nitrogen in one of a small number of reactions that all organisms use. This organic nitrogen is then used to synthesize amino acids and other nitrogen containing biological compounds.

Even if supplied with ammonium, a carbon source, and energy, animals are only capable of synthesizing eight amino acids in sufficient quantities to support growth (and one, arginine, in sufficient quantities to support life, but not growth). The remaining amino acids must be derived from the diet (either directly, or, in the case of tyrosine and cysteine, indirectly in the form of other amino acids that can be used as precursors).

The nutritionally non-essential amino acids can be synthesized from glycolytic or TCA cycle intermediates.