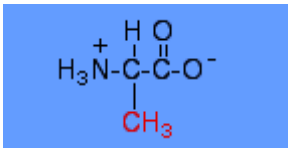
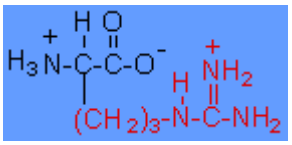
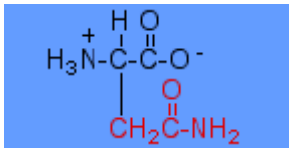
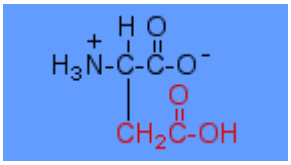
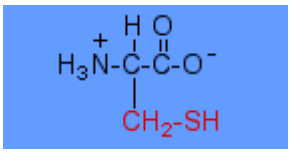
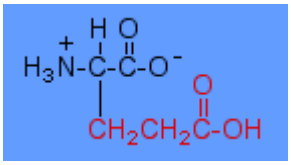
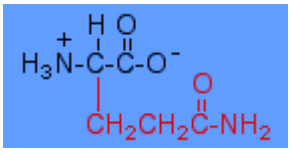
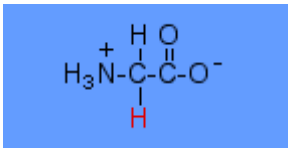
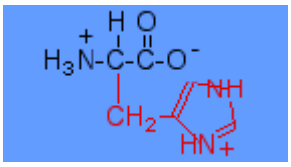
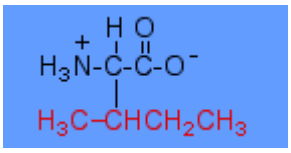
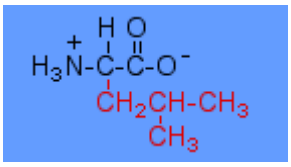
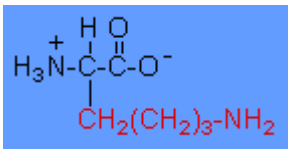
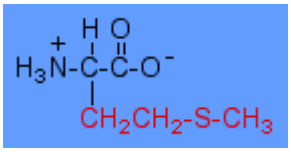
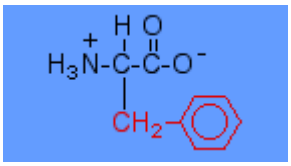
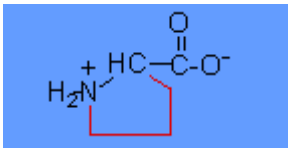
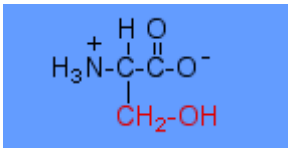
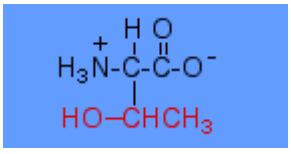
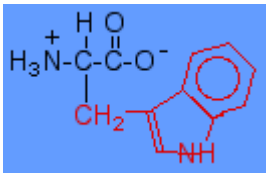
Structures of Amino Acids				
R = any number carbons in a hydrocarbon chain *CHIME plug-in required to view these images.				
Amino Acid Name	**Abrev.**	**Abrev.**	**Structure of R group (red)**	**Comments**
Alanine	**ala**	A		Neutral Non-polar
Arginine	**arg**	R		Basic Polar
Asparagine	**asn**	N		Neutral Polar
Aspartic Acid	**asp**	D		Acidic Polar
Cysteine	**cys**	C		Neutral Slightly Polar
Glutamic Acid	**glu**	E		Acidic Polar
Glutamine	**gln**	Q		Neutral Polar
Glycine	**gly**	G		Neutral Non-polar

Histidine	**his**	H		Basic Polar
Isoleucine	**ile**	I		Neutral Non-polar
Leucine	**leu**	L		Neutral Non-polar
Lysine	**lys**	K		Basic Polar
Methionine	**met**	M		Neutral Non-polar
Phenyl- alanine	**phe**	F		Neutral Non-polar
Proline	**pro**	P		Neutral Non-polar
Serine	**ser**	S		Neutral Polar
Threonine	**thr**	T		Neutral Polar
Trypto- phan	**trp**	W		Neutral Slightly polar

Nitrogen Fixation

Before discussing the pathways for amino acid synthesis, we'll see how nitrogen sources are obtained through the process of *nitrogen fixation*

Once a usable source of nitrogen has been obtained, it is fed into amino acid production through a couple of key reactions involving *glutamate* and *glutamine*

The coenzyme *pyridoxal phosphate* (PLP) plays an important role in transferring the primary amines to new amino acids and helps to establish the correct stereochemistry at the chiral carbon in a *transamination* reaction

Next, we'll consider the starting points for all 20 of the amino acids, which consist of six molecules from the core pathways in metabolism, including *glycolysis*, the *pentose phosphate pathway* and the *citric acid cycle*

In many animals (including humans) not all amino acids can be synthesized and so we'll consider how some of them must be obtained in the diet as *essential amino acids*

Nitrogen Fixation

The primary elements in biomolecules are Carbon, Nitrogen, Oxygen and Hydrogen (C, N, O, H). Carbon, oxygen and hydrogen can be obtained from atmospheric carbon dioxide (CO_2) and water (H_2O) through the process of *photosynthesis*, which occurs in many plants and microorganisms.

The remaining primary element nitrogen can obtained from nitrogen gas (N_2) in the atmosphere by the process of *nitrogen fixation*

Nitrogen fixation involves the conversion of the highly stable N_2 into a biologically-usable form, ammonium (NH_4^+)

Higher plants and animals are ultimately dependent upon these prokaryotic sources for nitrogen. Some of these bacterial organisms exist in a symbiotic relationship with plants, as in the case of nodules found in the root structures of clover and legumes

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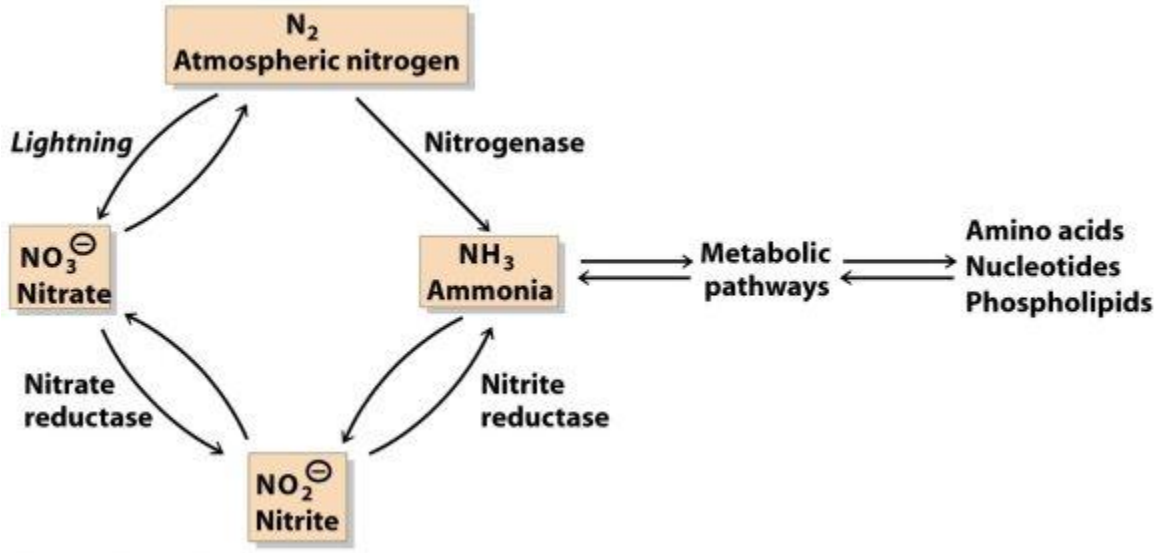
Nitrogen is often a limiting factor in biological growth. In human agriculture, fertilizers are commonly required to provide a source of nitrogen for large-scale production. In addition to natural sources of nitrogen, the *Haber process* is an industrial method for fixing nitrogen at very high temperatures and pressures

Biological fixation of nitrogen is done by the enzyme complex *nitrogenase*, which is highly-conserved across different species. The process is energetically very expensive, requiring 16 molecules of ATP per N_2 molecule to be fixed. The process is also quite slow, with just a few molecules processed each second per complex

In spite of this, large quantities of nitrogen are fixed by *diazotrophic* (nitrogen-fixing) microorganisms, with estimates of 10 kg produced per year. Lightning and UV radiation are estimated to fix another 15% and industrial processes account for the remaining 25%

The Nitrogen Cycle



The flow of nitrogen between the atmosphere, nitrates, nitrites and ammonia is called the *nitrogen cycle*. Most of the flow is between ammonia and nitrate. Ammonia from decaying organisms is oxidized by soil bacteria to nitrate in a process called *nitrification*

Most green plants and some microorganisms have nitrate and nitrite reductases that can catalyze the reverse conversion of nitrogen oxides to ammonia. The fixation of nitrogen from the atmosphere into ammonia is catalyzed by the enzyme *nitrogenase*

Nitrogenase

The N-N triple bond has a high energy and is hard to break. In spite of this, it's energetically favorable to convert N_2 and H_2 to NH_3. However, the intermediate transition states have an even higher energy, so ATP hydrolysis is used to stabilize the transition states and lower the intermediate energy barriers

The nitrogenase complex that catalyzes this process consists of two proteins, a *reductase* that receives electrons with high reducing power, and the *nitrogenase*, which is the site at which N_2 binds and the triple bond is broken:

The Nitrogen Fixation Reaction

The basic reaction for nitrogen fixation is the reduction of one molecule of N_2 by 6 electrons into two molecules of ammonia:

$N_2 + 6\ e^- + 6\ H^+ \rightarrow 2\ NH_3$

In the industrial Haber process mentioned previously, this can be accomplished with an iron catalyst at about 500° C and a pressure of 300 atmospheres

By contrast, the biological reaction can occur at standard room temperature and pressure. This is done by the investment of a large amount of ATP. In addition, a molecule of H_2 is generated as a byproduct, so that 8 electrons are needed on the input side:

$$N_2 + 8\ e^- + 8\ H^+ + 16\ ATP + 16\ H_2O \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i$$

These electrons are fed into the nitrogenase complex from a suitable donor such as ferrodoxin. They are then transferred to the actual reaction center by the *reductase* component

The reductase part of the complex is called the *Fe protein* because it is composed of a dimer of two polypeptide chains linked by a 4Fe-4S cluster

The Fe protein also contains the binding sites for ATP. The hydrolysis of ATP triggers a conformational change that moves the reductase component closer to the nitrogenase component, allowing the electron transfer to take place

Amino Acid Metabolism

Introduction

All tissues have some capability for synthesis of the non-essential amino acids, amino acid remodeling, and conversion of non-amino acid carbon skeletons into amino acids and other derivatives that contain nitrogen. However, the liver is the major site of nitrogen metabolism in the body. In times of dietary surplus, the potentially toxic nitrogen of amino acids is eliminated via transaminations, deamination, and urea formation; the carbon skeletons are generally conserved as carbohydrate, via gluconeogenesis, or as fatty acid via fatty acid synthesis pathways. In this respect amino acids fall into three categories: glucogenic, ketogenic, or glucogenic and ketogenic. Glucogenic amino acids are those that give rise to a net production of pyruvate or TCA cycle intermediates, such as a-ketoglutarate or oxaloacetate, all of which are precursors to glucose via gluconeogenesis. All amino acids except lysine and leucine are at least partly glucogenic. Lysine and leucine are the only amino acids that are solely ketogenic, giving rise only to acetylCoA or acetoacetylCoA, neither of which can bring about net glucose production.

A small group of amino acids comprised of isoleucine, phenylalanine, threonine, tryptophan, and tyrosine give rise to both glucose and fatty acid precursors and are thus characterized as being glucogenic and ketogenic. Finally, it should be recognized that amino acids have a third possible fate. During times of starvation the reduced carbon skeleton is used for energy production, with the result that it is oxidized to CO2 and H2O.

Essential vs. Nonessential Amino Acids

Nonessential

Alanine
Asparagine
Aspartate
Cysteine
Glutamate
Glutamine
Glycine
Proline
Serine
Tyrosine

Essential

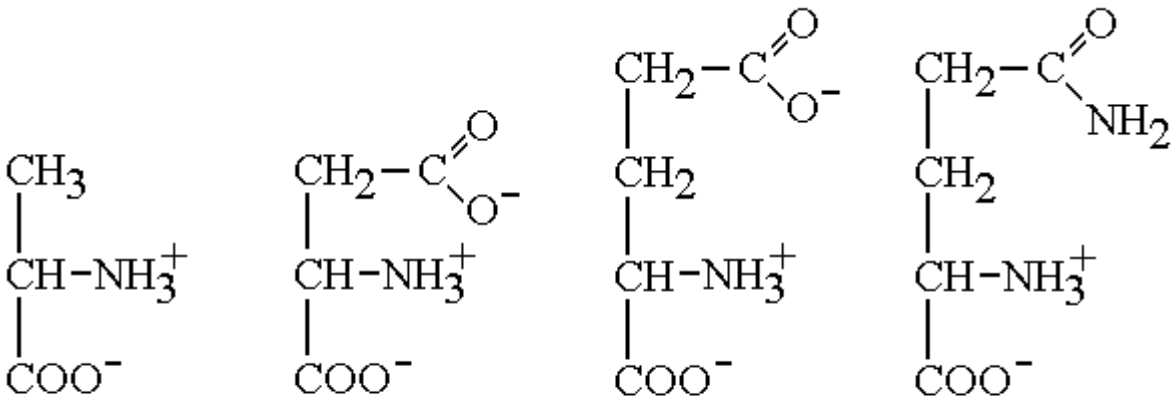
Arginine*
Histidine
Isoleucine
Leucine
Lysine
Methionine*
Phenylalanine*
Threonine
Tyrptophan
Valine

*The amino acids arginine, methionine and phenylalanine are considered essential for reasons not directly related to lack of synthesis. Arginine is synthesized by mammalian cells but at a rate that is insufficient to meet the growth needs of the body and the majority that is synthesized is cleaved to form urea. Methionine is required in large amounts to produce cysteine if the latter amino acid is not adequately supplied in the diet. Similarly, phenyalanine is needed in large amounts to form tyrosine if the latter is not adequately supplied in the diet. :rabbi:

Central role of glutamate

Four of the amino acids: glutamate, aspartate, alanine and glutamine are present in cells at much higher concentrations than the other 16. All four have major metabolic functions in

addition to their roles in proteins, but glutamate occupies the prime position.

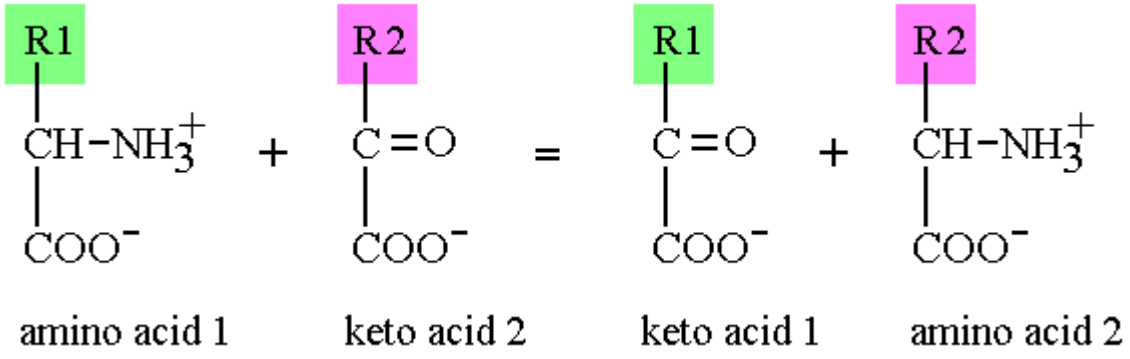


Glutamate and aspartate function as excitatory neurotransmitters in the central nervous system, and glutamate is partly responsible for the flavour of food. (It is the mono sodium glutamate listed on processed food labels.) However, glutamate also occupies a special position in amino acid breakdown, and most of the nitrogen from dietary protein is ultimately excreted from the body via the glutamate pool.

Glutamate is special because it is chemically related to 2-oxoglutarate (= alpha keto glutatarate) which is a key intermediate in the citric acid (Krebs) cycle. Glutamate can be reversibly converted into oxoglutarate by transaminases or by glutamate dehydrogenase. In addition, glutamate can be reversibly converted into glutamine, an important nitrogen carrier, and the most common free amino acid in human blood plasma.

Transamination reactions

Most common amino acids can be converted into the corresponding keto acid by transamination. This reaction swops the amino group from one amino acid to a different keto acid, thereby generating a new pairing of amino acid and keto acid.
here is no overall loss or gain of nitrogen from the system

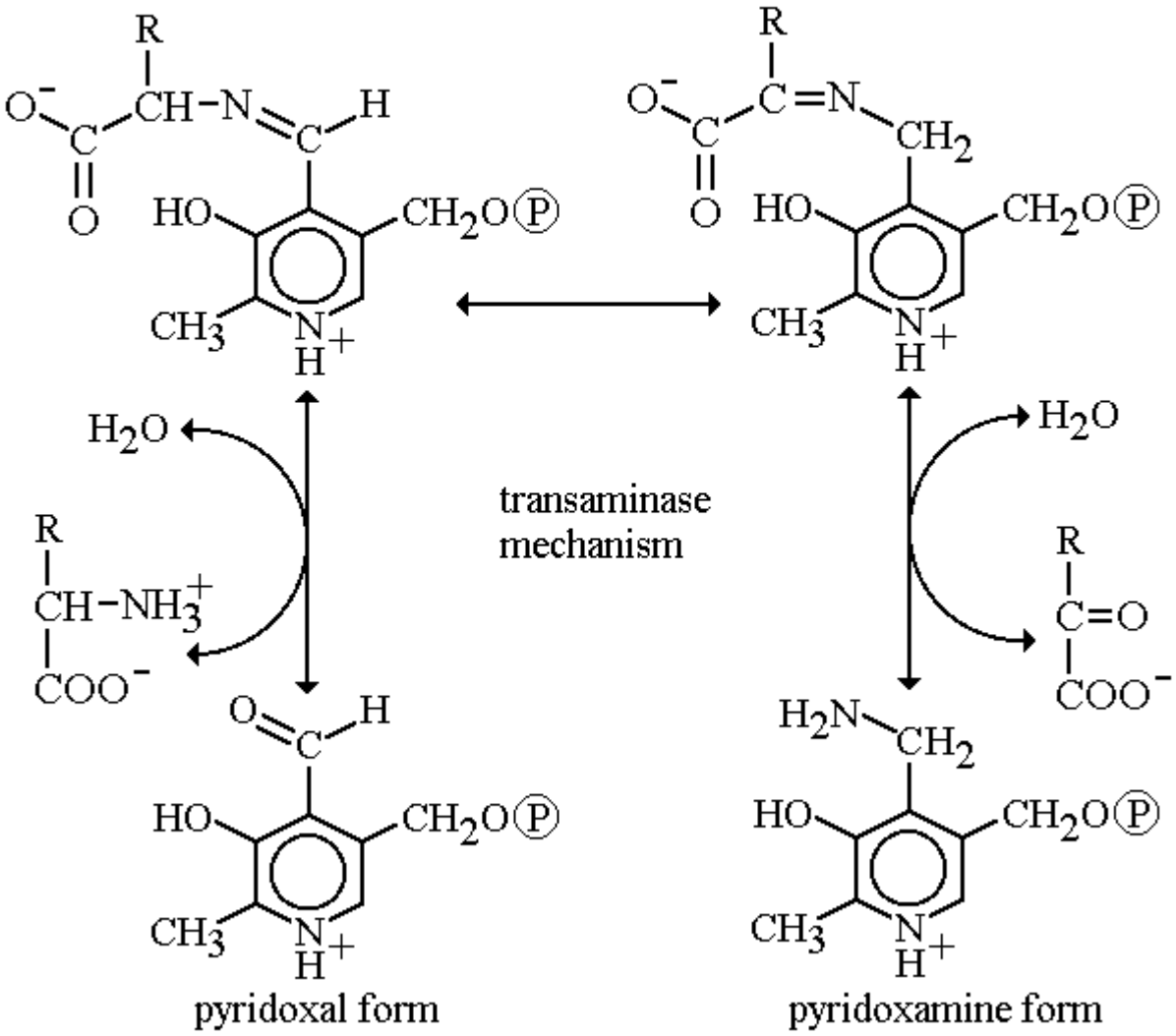


Transamination reactions are readily reversible, and the equilibrium constant is close to 1.
One of the two pairs is almost invariably glutamate and its corresponding keto acid

oxoglutarate, although there are a few exceptions to this rule. All transaminases require pyridoxal phosphate (derived from vitamin b6) as a cofactor.

The substrates bind to the active centre one at a time, and the function of the pyridoxal phosphate is to act as a temporary store of amino groups until the next substrate comes along. In the process the pyridoxal phosphate is converted into pyridoxamine phosphate, and then back again. Enzymologists call this a "ping pong

mechanism, and it leads to a characteristic pattern in the reaction kinetics.

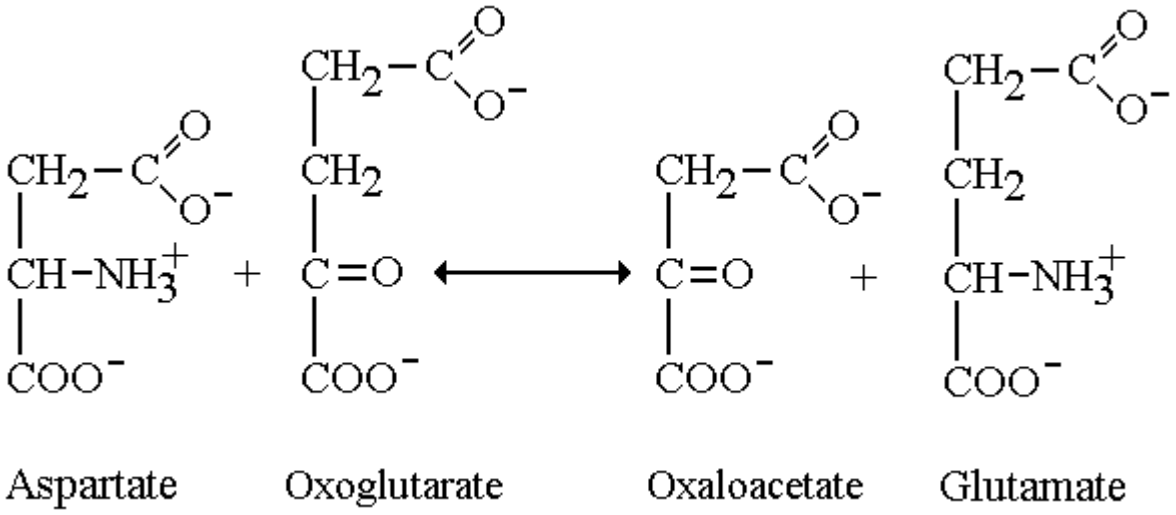


The condensation between the alpha amino group and the aromatic aldehyde to form a "Schiff base" makes the alpha carbon atom chemically reactive, so the isomerisation of the Schiff base takes place very easily. In practice the pyridoxal form of the coenzyme condenses with the amino group of a lysine residue in the enzyme protein when no amino acid is bound, and the free aldehyde form of the coenzyme has only a transitory existence. Many of the enyzmes

that metabolise amino acids require pyridoxal phosphate as a cofactor. Unexpectedly, this compound also serves in a different manner in the active centre of glycogen phosphorylase.

Glutamate:oxaloacetate transaminase [GOT]

This enzyme is also known as aspartate aminotransferase and is one of the most active enzymes in the cell. It exists in mitochondrial and cytosolic variants, and the detailed iso-enzyme pattern is tissue-specific. It escapes in large amounts from dead or dying tissues and enters the bloodstream, so GOT is often measured in blood samples for medical diagnostic purposes.

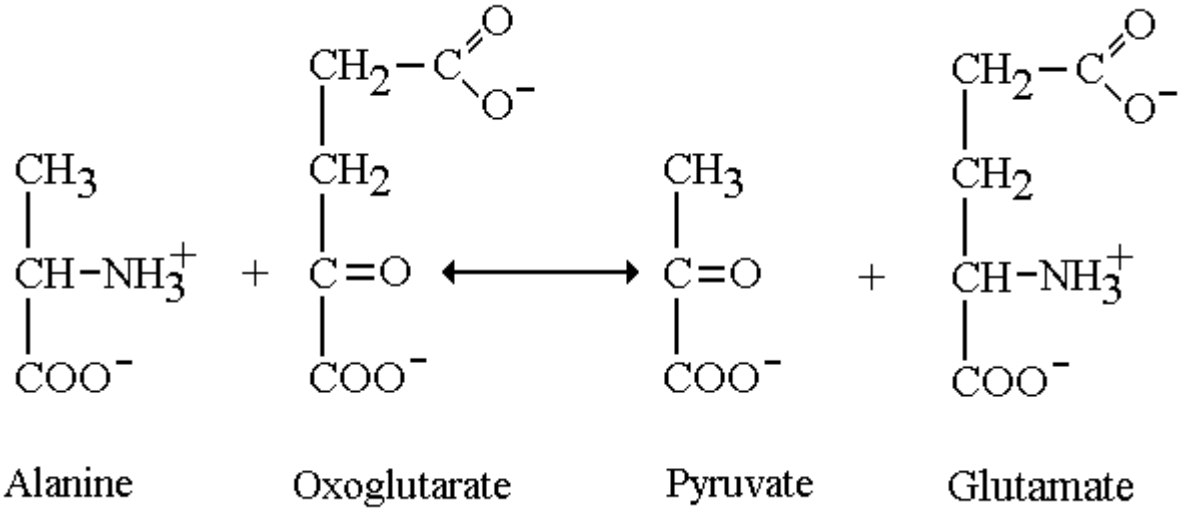


The metabolic importance of this enzyme is that it brings about a free exchange of amino groups between glutamate (which is the most common amino acid) and aspartate which is a second major amino acid pool. Glutamate and aspartate are each required for separate but essential steps in the urea cycle, which is responsible for ammonia detoxication and nitrogen excretion. The free movement of nitrogen between the glutamate and aspartate pools is an important balancing process that is vital for normal cellular metabolism.

Glutamate:pyruvate transaminase [GPT]

This very active enzyme is also known as alanine aminotransferase and exists in mitochondrial and cytosolic variants. The detailed iso-enzyme pattern is tissue-specific. It escapes in large amounts from dead or dying tissues and GPT may be measured in blood

samples for medical diagnostic purposes.

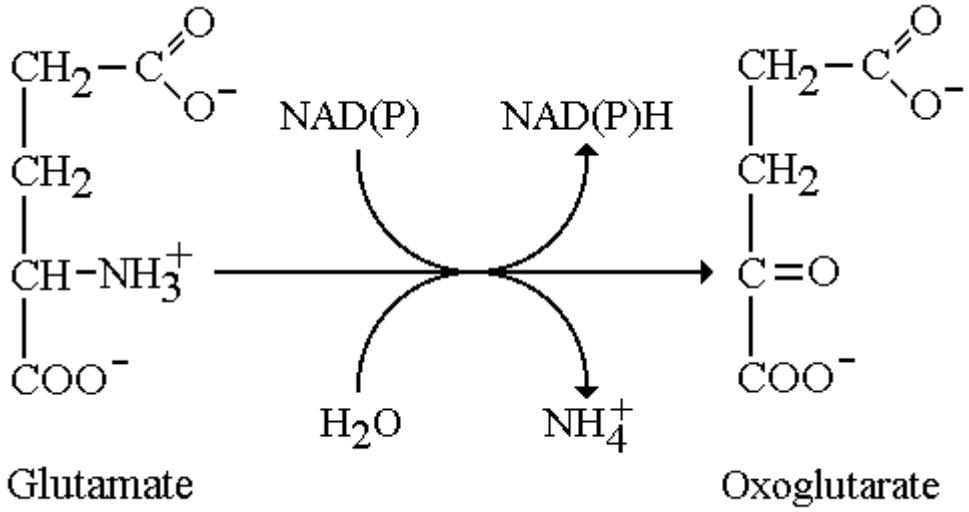


Alanine is the principal amino acid released from muscle tissue during starvation. It is an important substrate for hepatic gluconeogenesis, and alanine transamination is required for the proper maintenance of fasting blood glucose concentrations.

Glutamate dehydrogenase [GluDH]

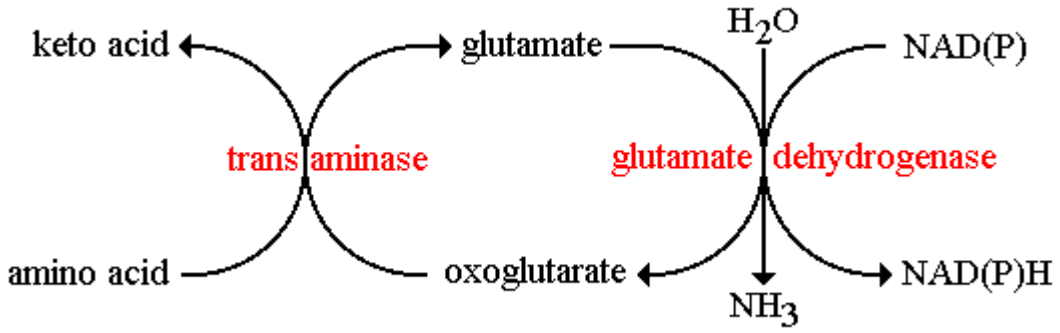
This enzyme is the first committed step on the final common pathway for mammalian nitrogen excretion, leading eventually to urea. A few of the amino acids have specific deamination pathways, but about 75% of ingested protein nitrogen follows the glutamate route.

Glutamate dehydrogenase in mammals is almost entirely confined to the liver mitochondrial matrix space, where it accounts for a significant proportion of the total protein. In contrast to the transamination reactions which merely swop amino groups from one compound to another, GluDH catalyses a net loss of nitrogen from the amino acid pool. The process is therefore termed "oxidative deamination". It is the only common dehydrogenase which is non-specific for NAD or NADP, and this may be important for its overall regulation.



Trans-deamination

Most transaminases share a common substrate and product (glutamate and oxoglutarate) with glutamate dehydrogenase, and this permits a combined nitrogen excretion pathway for individual amino acids that is commonly described as "trans-deamination".



This process underlines the central role of glutamate in the overall control of nitrogen metabolism.

Urea cycle

Ammonium ions are in equilibrium with about 1% free ammonia at physiological pH. Ammonium salts are toxic compounds, causing vomiting, convulsions and ultimately coma and death when the blood concentration exceeds approximately 0.25mM. It is not entirely clear why this should be so: it may be that ammonium ions mimic potassium ions, but gain access as uncharged ammonia to areas from which they should be excluded. Alternatively, they may favour the synthesis of excessive amounts of glutamate and glutamine which have excitatory effects on neural tissues.

It is therefore necessary to have an efficient means to remove ammonia from the body. Water-living species commonly excrete free ammonia through their gills [ammonotelism], but this easy option is not available to land dwellers which produce a variety of less toxic nitrogenous end products. Urea synthesis and excretion [ureotelism] first evolved in lungfish and primitive amphibia about 400 million years ago. The process is replicated today when ammonotelic tadpoles leave the water and metamorphose into ureotelic frogs. Urea is also used in humans, and in all placental mammals, which start to express the urea cycle genes around the time of birth. Urea is very soluble, but still requires appreciable quantities of water for its removal via the kidneys. This imposes a minimum daily water requirement and limits the range of environments that these species can exploit.

Urea is not the only possible solution to the problem: spiders excrete guanine, which packs no less than 5 surplus nitrogen atoms into a single small molecule, while reptiles and birds excrete mainly uric acid [uricotelism]. Uric acid is an extremely insoluble purine compound that readily forms supersaturated solutions. This has been turned to advantage in uricotelic species, which can survive in extremely arid environments. They regurgitate concentrated urine, supersaturated with uric acid, from the cloaca into the hindgut where the uric acid crystalises and the residual water is resorbed. The uric acid forms the fine pasty mass of white crystals that is familiar to us in bird droppings.

Uricotelism is also an advantage to animals that lay shelled eggs, which of necessity have a zero water intake. The uric acid crystalises within the allantois, part of which eventually becomes incorporated into the lower gut as the embryo develops. In humans the insolubility

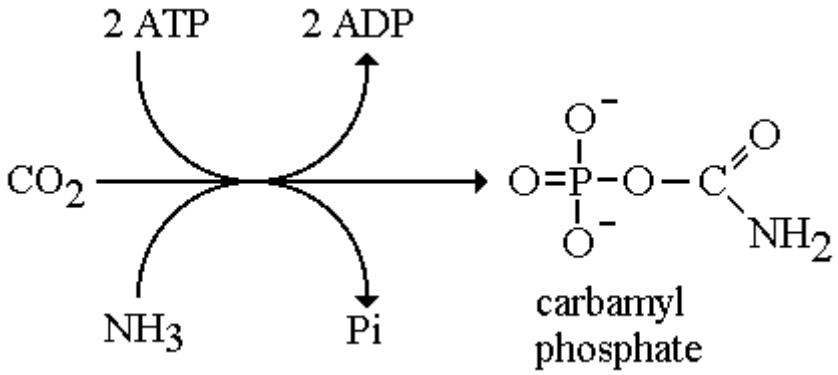
of uric acid is a considerable nuisance, since it gives rise to the extremely painful deposits of small crystals [called "tophi"] within the joints of patients suffering from gout.

Urea is synthesised via the urea cycle, which is confined to mammalian liver. Individual enzymes from the urea cycle are present in other tissues, and may be important for arginine biosynthesis, but the complete cycle does not occur. Extra-hepatic tissues export their surplus nitrogen to the liver by other routes, principally as the amino acids alanine and glutamine. In addition, the cleavage of arginine by nitric oxide synthetase generates citrulline, which is a urea cycle intermediate. Citrulline is recycled to arginine, and in tissues which use the nitric oxide signalling system the relevant urea cycle enzymes have sufficient activity to maintain cellular arginine supplies.

The urea cycle takes place partly in the cytosol and partly in the mitochondria, and the individual reactions are as follows.

carbamyl phosphate synthetase 1 [CPS1]

This mitochondrial enzyme converts the ammonia produced by glutamate dehydrogenase into carbamyl phosphate (=carbamoyl phosphate) which is an unstable high energy compound. It is the mixed acid anhydride of carbamic acid and phosphoric acid, and requires two molecules of ATP to drive its synthesis.

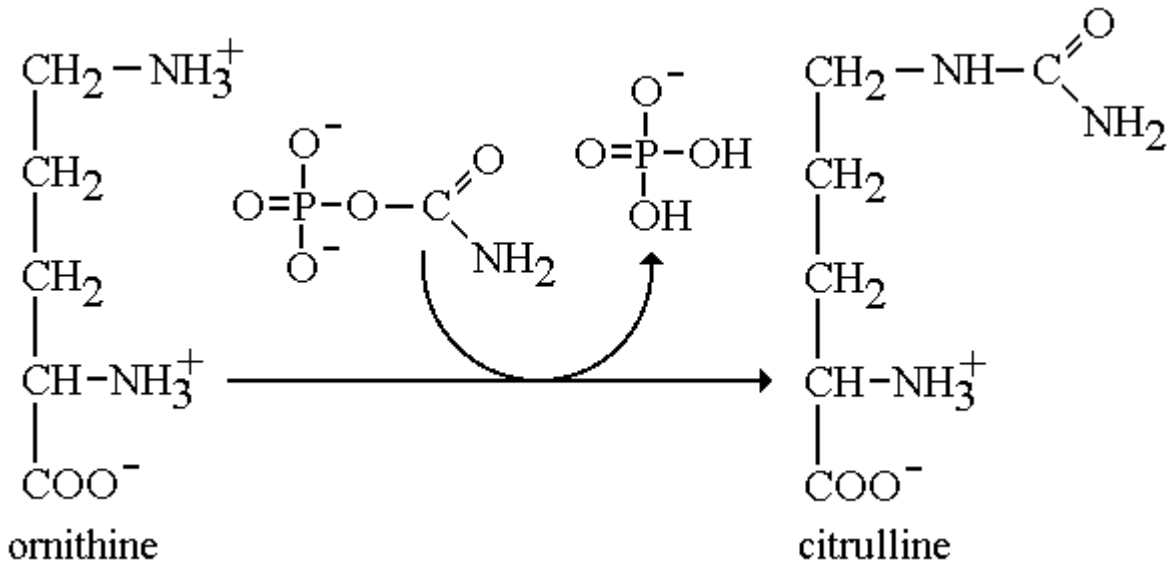


CPS1 is strongly activated by N-acetyl glutamate, which controls the overall rate of urea production. This bizarre method of regulation is not fully understood: N-acetyl glutamate is an intermediate in the bacterial synthesis of ornithine, but this feature has been lost from mammals and only the regulatory system has survived. There is a futile cycle catalysed by the enzymes N-acetylglutamate synthetase and N-acetylglutamate hydrolase. This is plainly important for the control of nitrogen metabolism, but we do not yet know how it works.

CPS1 deficiency results in hyperammonemia. The neonatal cases are usually lethal, but there is also a less severe, delayed-onset form. Ammonia-dependent CPS1 is present only in the liver mitochondrial matrix space. It should be distinguished from a second *cytosolic* glutamine-dependent carbamyl phosphate synthetase [CPS2] which is found in all tissues and is involved in pyrimidine biosynthesis. Carbamyl phosphate synthesis is a major burden for liver mitochondria. This enzyme accounts for about 20% of the total protein in the matrix space. Glutamate dehydrogenase is also present in very large amounts.

ornithine transcarbamylase [OTCase]

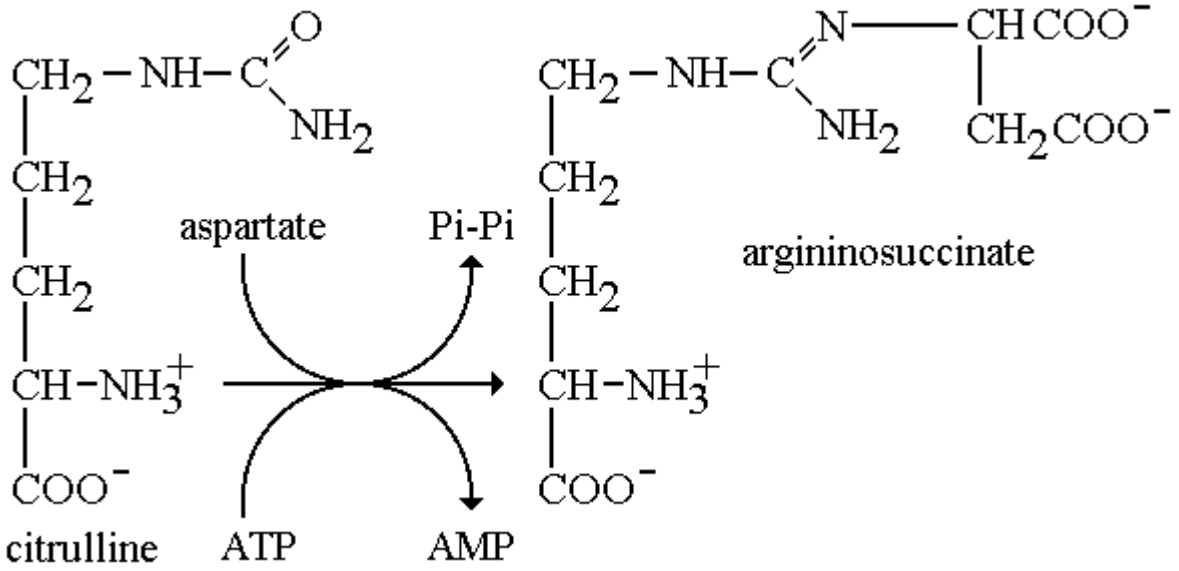
The next reaction also takes place in the liver mitochondrial matrix space, where ornithine is converted into citrulline.



This enzyme has no regulatory significance. However, the OTCase gene is on the X chromosome and an inherited deficiency is observed in males, with an incidence of about 1 in 80,000 people. This is the most common of the inherited urea cycle defects. Patients show all the symptoms of hyperammonemia, and in addition may excrete abnormal amounts of orotate, since the unused carbamyl phosphate escapes into the cytosol and enters the pyrimidine biosynthetic pathway.

arginino-succinate synthetase

Once in the cytosol, citrulline condenses with aspartate and the reaction is driven by ATP. In this way aspartate contributes the second nitrogen atom to urea, the firsthaving come from glutamate.

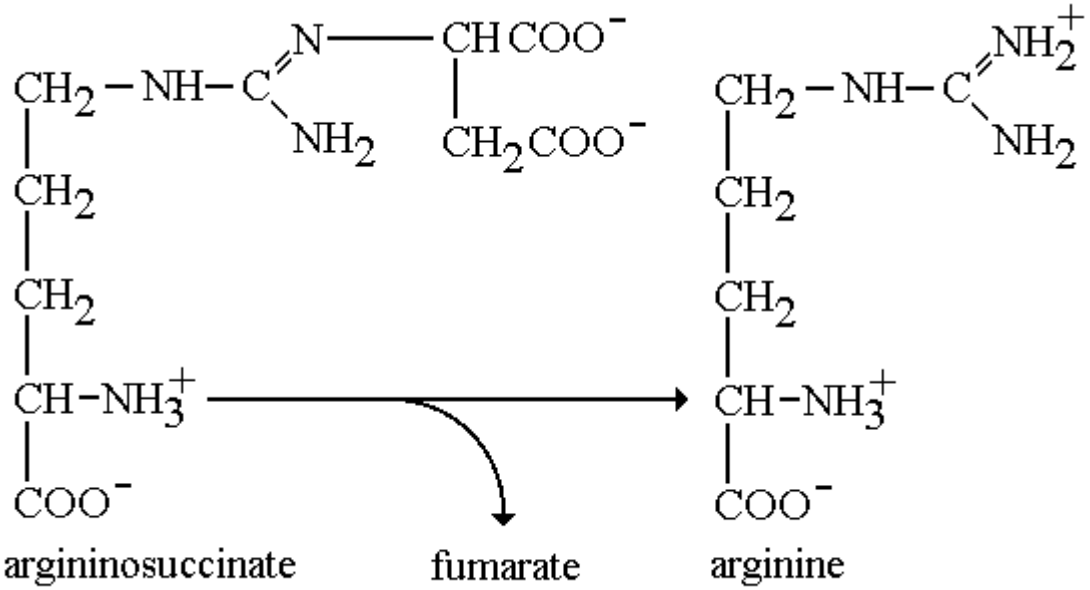


Production of arginino-succinate is an energetically expensive process, since the ATP is split to AMP and pyrophosphate. The pyrophosphate is then cleaved to inorganic phosphate using

pyrophosphatase, so the overall reaction costs two equivalents of high energy phosphate per mole.

arginino-succinate lyase

Elimination of fumarate from arginino-succinate then yields arginine.

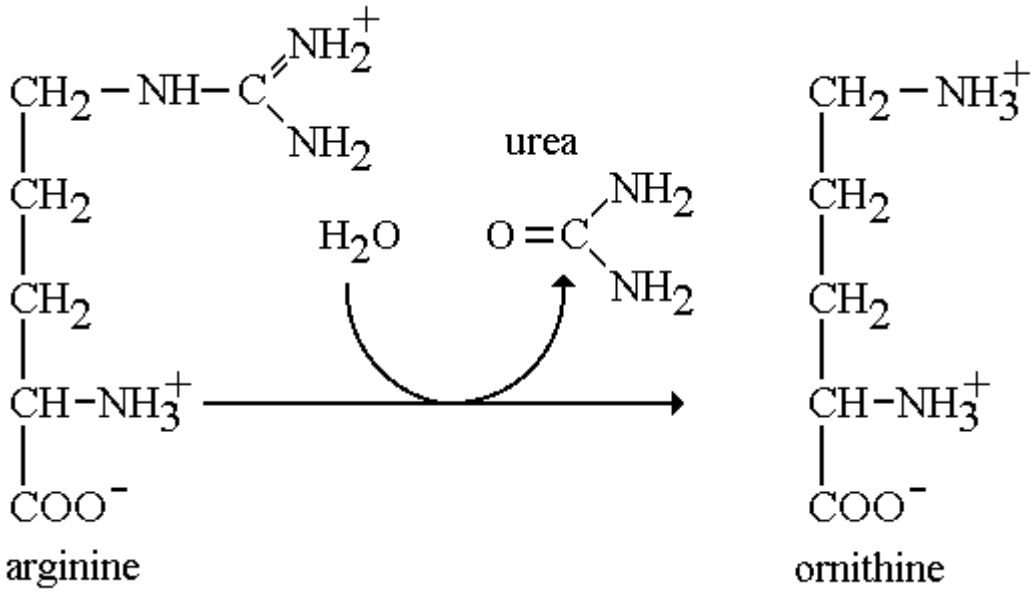


. Fumarate is not transported by mitochondria, so this requires the presence of cytosolic **fumarase** to form malate.

$$\text{fumarate} + H_2O = \text{malate}$$

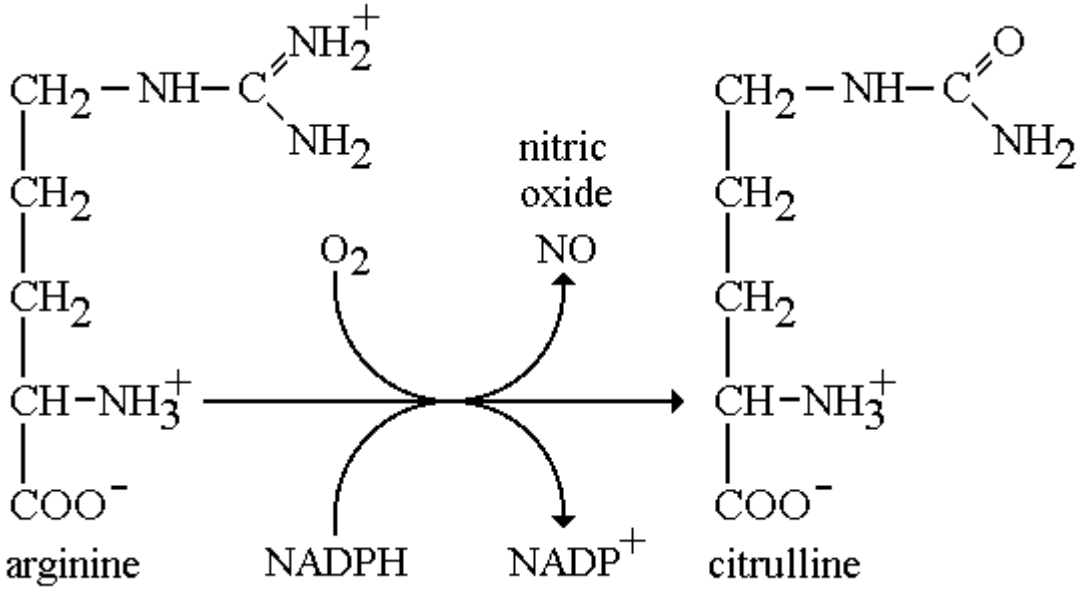
The reaction is readily reversible, and the equilibrium slightly favours malate. The cytosolic and mitochondrial fumarase isoenzymes are extremely similar and derived from the same gene through alternative mRNA splicing reactions. Cleavage of arginine by **arginase** to

produce urea regenerates ornithine, which is then available for another round of the cycle.



Nitric Oxide

In addition to its metabolic functions in the urea cycle, arginine is also the immediate precursor for nitric oxide [NO], an important signalling molecule involved in the local regulation of blood flow. Nitric oxide synthase uses oxygen and NADPH, and the other products are citrulline and NADP.



The citrulline is reconverted to arginine using two of the urea cycle enzymes, although the full urea cycle does not take place outside the liver.

Essential and non-essential amino acids

Humans can degrade all the amino acids that are commonly found in proteins, but we have very limited synthetic capacity. However, the initial transamination step in most of the amino

acid breakdown pathways is freely reversible. If the corresponding keto acids are produced during normal metabolism, then it may be possible to use surplus nitrogen from other sources to make good a dietary deficiency in some of these "non-essential" amino acids, providing that the total nitrogen intake is sufficient.

In addition, a few amino acids are degraded to form other amino acids (for example, phenylalanine is metabolised initially to tyrosine) so that tyrosine is essential on a minimal diet, but becomes non-essential if sufficient phenylalanine is eaten. Tyrosine is therefore described as a "conditionally essential" amino acid.

Relatively few keto acids and amino acids can be produced from alternative sources, so about half of the amino acids are essential in the diet. [See table below.]

Glycogenic and ketogenic amino acids

The carbon skeletons from the majority of amino acids are degraded to Krebs cycle intermediates after removal of the amino group by transamination. This means that they can give rise to blood glucose via the gluconeogenic pathway. They are termed 'glycogenic' amino acids, because it was observed many years ago that they made diabetic glycosuria worse. In contrast to this 'ketogenic' amino acids exacerbated diabetic ketoacidosis, and these amino acids are degraded to compounds such as acetoacetate and acetyl-CoA. 'Mixed' amino acids are degraded to both Krebs cycle acids and to acetyl-CoA. The situation is summarised in the following table:

amino acid	made from	degraded to	glyco / keto	Comments
alanine	pyruvate	pyruvate	glycogenic	large amount in cells
arginine	glutamate	glutamate	glycogenic	strongly basic, urea cycle
asparagine	aspartate	aspartate	glycogenic	Glycoproteins
aspartate	oxaloacetate	oxaloacetate	glycogenic	acidic, large amount in cells
cysteine	(methionine)*	pyruvate	glycogenic	-SH group
glutamate	oxoglutarate	oxoglutarate	glycogenic	acidic, very large amount in cells
glutamine	glutamate	glutamate	glycogenic	large amount in cells
glycine	serine	one-carbon pool***	glycogenic	no side chain, collagen
histidine	essential	glutamate	glycogenic	weak base
isoleucine	essential	acetyl-CoA + propionyl-CoA	mixed	branched side-chain
leucine	essential	acetyl-CoA	ketogenic	branched side-chain
lysine	essential	not known	mixed	long side chain, basic
methionine	essential	propionyl-CoA	glycogenic	contains sulphur, methyl donor
phenylalanine	essential	tyrosine	mixed	aromatic, phenylketonuria
proline	glutamate	glutamate	glycogenic	imino acid
serine	phosphoglycerate	pyruvate	glycogenic	-OH group

threonine	essential	disputed****	glycogenic	-OH group
tryptophan	essential	not known	mixed	Aromatic
tyrosine	(phenylalanine)**	fumarate + acetoacetate	mixed	aromatic, phenolic
valine	essential	propionyl-CoA	glycogenic	branched side-chain

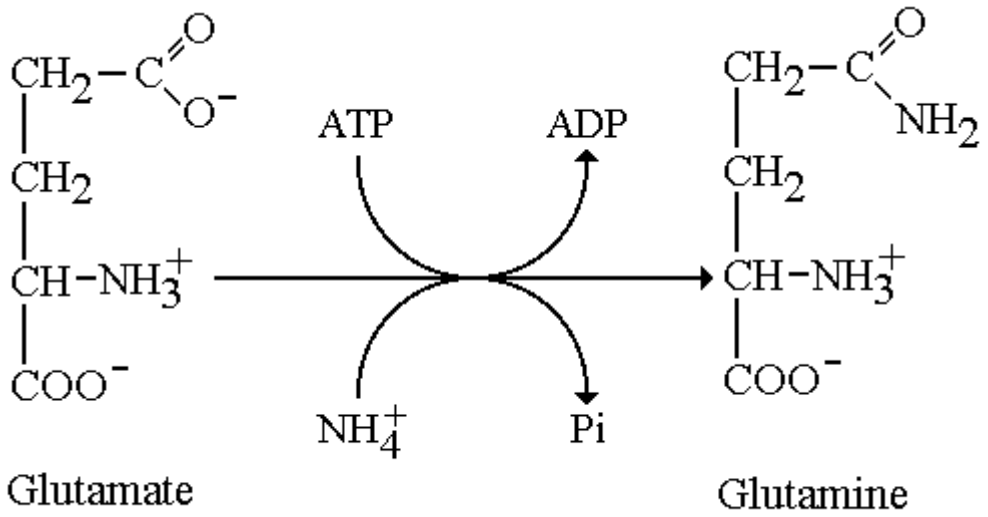
Notes:

* Cysteine is conditionally essential - it can be formed from methionine
** Tyrosine is conditionally essential - it can be formed from phenylalanine
*** Multiple pathways for glycine degradation - beware of species differences
**** Multiple pathways for threonine degradation - beware of species differences

Glutamine metabolism

In view of the toxicity of free ammonia and ammonium salts, cells require a non-toxic source of nitrogen for use in nitrogen transport and biosynthetic reactions. This need is satisfied by glutamine, which is the most common free amino acid in human blood plasma.

Glutamine is readily synthesised from glutamate and ammonium ions by the enzyme glutamine synthetase. This enzyme is present in liver and in many other body tissues. works efficiently at non-toxic ammonium concentrations. The required energy comes from ATP:



Glutamine supplies most of the nitrogen required for purine and pyrimidine biosynthesis, and for the manufacture of amino sugars. When necessary it can be degraded back to glutamate by the enzyme glutaminase:

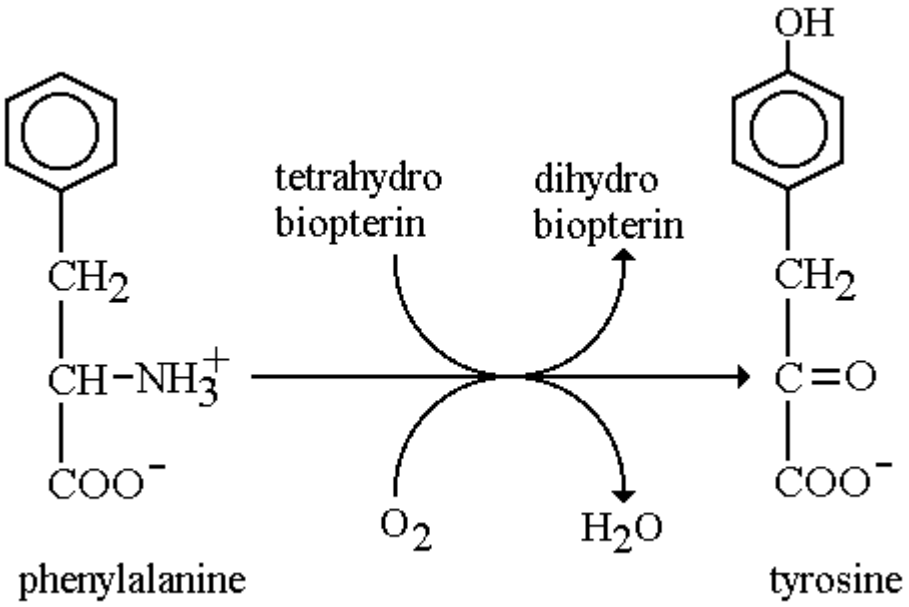
$$\text{glutamine} + H_2O = \text{glutamate} + NH_4^+$$

Glutaminase is activated by inorganic phosphate. It is obvious that glutamine synthetase and glutaminase constitute a potential futile cycle, and the arrangment must be delicately regulated to avoid wasteful hydrolysis of ATP. *The two enzymes are commonly present in different cells,* and this seems to be particularly important in the liver and in the central nervous system. In liver, the urea cycle enzymes, glutamate:pyruvate transaminase and

glutaminase are concentrated in the periportal cells, whereas glutamine synthetase is concentrated in the perivenous cells near the hepatic veins. In addition to the numerous biosynthetic uses for glutamine, kidney tubules can use ammonia derived from glutamine to control the urinary pH, and avoid large cation losses under acidotic conditions. In neural tissues, formation of glutamine is thought to terminate the action of glutamate, an excitatory neurotransmitter. Glutamine is a particularly important fuel for the cells lining the gut, and has been used experimentally in the treatment of gastrointestinal disease. Large amounts of glutamine and alanine are released from muscle cells during starvation, and these are important substrates for hepatic gluconeogenesis under fasting conditions.

Phenylketonuria

Phenylalanine is normally metabolised by conversion to tyrosine. The enzyme responsible for this conversion is phenylalanine hydroxylase, a mixed function oxygenase with a tetrahydrobiopterin cofactor:



Half of the oxygen molecule re-appears in the tyrosine -OH group and the other half is reduced to water. The "dihydrobiopterin" in the above reaction is an isomer of the folic acid compounds involved in one-carbon metabolism. It is recycled back to tetrahydrobiopterin using NADH:

"dihydrobiopterin" + NADH = tetrahydrobiopterin + NAD