Biosynthesis of Food Constituents: Amino Acids: 1. The Glutamic Acid and Aspartic Acid Groups – a Review

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Abstract

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This review article gives a survey of principal pathways that lead to the biosynthesis of the proteinogenic amino acids of the glutamic acid group (glutamic acid, glutamine, proline, arginine) and aspartic acid group (aspartic acid, asparagine, threonine, methionine, lysine, isoleucine) starting with oxaloacetic acid from the citric acid cycle. There is an extensive use of reaction schemes, sequences, and mechanisms with the enzymes involved and detailed explanations using sound chemical principles and mechanisms.

Keywords: biosynthesis; amino acids; glutamic acid; glutamine; proline; arginine; aspartic acid; asparagine; threonine; methionine; lysine; isoleucine

Commonly, 20 L-amino acids encoded by DNA represent the building blocks of animal, plant, and microbial proteins, and a limited number of amino acids participate in the biosynthesis of certain shikimate metabolites and particularly in the formation of alkaloids. The basic amino acids encountered in proteins are called proteinogenic amino acids. The biosynthesis of some of these amino acids proceeds by ribosomal processes only in microorganisms and plants while the ability to synthesise them is lacking in animals including human beings. These amino acids have to be obtained in the diet (or produced by hydrolysis of body proteins) since they are required for normal good health; they are referred to as essential amino acids. The essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The rest of the encoded amino acids are referred to as non-essential amino acids (alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, tyrosine). Arginine and histidine are classified as essential, sometimes as semi-essential amino acids, as their amounts synthesised in the body is not sufficient for normal growth of children. Although it is itself non-essential, cysteine (sometimes classified as conditionally essential amino acid) can partly replace methionione which is an essential amino acid. Similarly, tyrosine can partly replace phenylalanine.

1 THE GLUTAMIC ACID GROUP

1.1 Glutamic acid and glutamine

Free ammonium ions are toxic to living cells and are rapidly incorporated into organic compounds. One of such transformations is the reaction of ammonia with 2-oxoglutaric acid from the citric acid cycle to produce L-glutamic acid. This reac-

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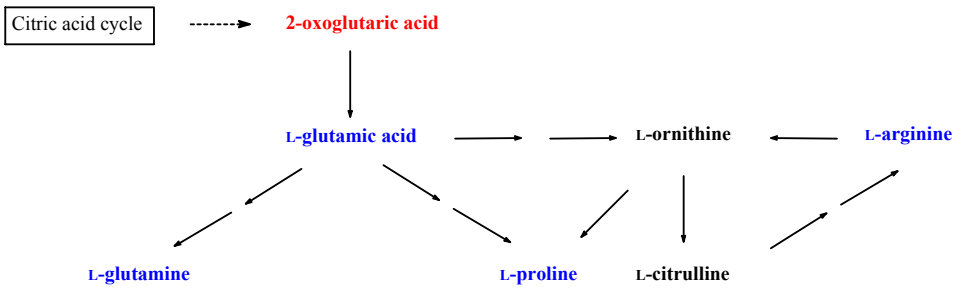

Figure 1

tion is known as reductive amination. Glutamic acid is, accordingly, the first amino acid generated. Glutamic acid itself can bind a further ammonium ion to form L-glutamine, the second amino acid. Glutamic acid then serves as the precursor of L-proline, L-ornithine, L-citrulline, and L-arginine. The biosynthetic pathway of the glutamic acid amino acid family is schematically shown in Figure 1 (Sengbusch).

The formation of glutamic acid (as well as the reverse rection) is catalysed by glutamate dehydrogenase. Nicotinamide adenine dinucleotide (NADH)-dependent glutamate dehydrogenase (EC 1.4.1.2) or the enzyme using nicotinamide adenine dinucleotide phosphate (NADPH) as the coenzyme (EC 1.4.1.4) is employed as hydrogen donor by microorganisms (e.g. *Saccharomyces cerevisiae*). In plants[1] is the reaction mainly dependent on the coenzyme NAD(P)H (EC 1.4.1.3) (Figure 2).

L-Glutamine arises as a product of amidation of glutamic acid that binds a further ammonium ion (Sengbusch). This amidation of glutamic acid is catalysed by glutamine synthetase (EC 6.3.1.2) and proceeds through the intermediate L-glutam-5-yl phosphate-ATP which splits off ADP and phosphoric acid (Figure 3).

Very important for many amino acid syntheses is the group of enzymes called transaminases. They are able to transfer an amino group (mostly that of glutamine) to a 2-oxo acid according to the following equation:

$$(\text{amino acid})_1 + (\text{2-oxo acid})_2 = (\text{2-oxo acid})_1 + (\text{amino acid})_2$$

Thus, the glutamine amino group is transferred to 2-oxoglutaric acid (Figure 4) producing glutamic acid. The reaction is catalysed by glutamate synthases having either NADPH (EC 1.4.1.13) or NADH (EC 1.4.1.14) as cofactor.

The transamination reaction catalysed by transaminases is dependent on the coenzyme pyridoxal 5'-phosphate (PLP). The reaction starts with the addition of the unprotonised amino group of an amino acid to the electron deficient carbon of

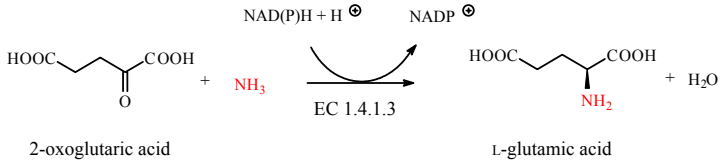

Figure 2

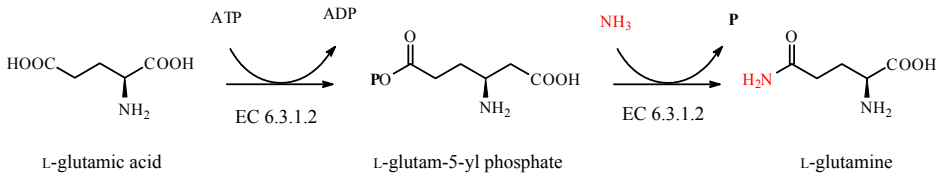

Figure 3

[1]Plants take up nitrogen as nitrates and in smaller amounts also as ammonium ions. A few species, mainly leguminose plants, live in symbiosis with nitrogen-fixing bacteria that are able to reduce atmospheric nitrogen. Microorganisms mainly require ammonium ions as the nitrogen source, some bacteria and moulds are able to use nitrates similarly to plants. Plants reduce nitrates in two steps. In the first step, nitrate is reduced to nitrite by nitrate reductase (NADH) (EC 1.7.1.1). In the second step, nitrite is reduced to ammonia by ferredoxin-nitrite reductase (EC 1.7.7.1).

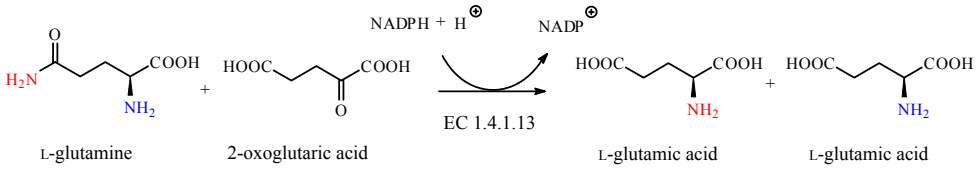

Figure 4

the polarised carbonyl group of PLP under the formation of a monotopic carbinolamine. The carbinolamine dehydration then leads to an aldimine (Schiff base). The α-hydrogen atom of the original amino acid in the aldimine is now much more acidic and can be readily eliminated. Isomerisation of the resulting intermediate leads to a ketimine (an imine of PLP with an 2-oxo acid). Hydrolysis of this ketimine generates pyridoxamine 5'-phosphate and the 2-oxo acid (Figure 5). The amino group of pyridoxamine 5'-phosphate is then transferred to another 2-oxo acid molecule and PLP is regenerated (Dewick 2002).

1.2 Proline

L-Proline is generated from L-glutamic acid by a ring formation (IUBMB 2001). The reaction is facilitated by a preliminary phosphorylation of glutamic acid by γ-glutamyl kinase (EC 2.7.2.11) that leads to an unstable intermediate L-glutam-5-yl phosphate under consumption of one molecule of ATP. The reduction of the phosphorylated γ-carboxyl to a carbonyl group is catalysed by the NAD(P)H dependent glutamate semialdehyde dehydrogenase (EC 1.2.1.41). Glutamic acid 5-semialdehyde formed yields spontaneously a cyclic Schiff base (*S*)-1-pyrroline-5-carboxylic acid, i.e. (*S*)-3,4-dihydro-2*H*-pyrrole-2-carboxylic acid. Its reduction to proline is achieved by pyrroline-5-carboxylate reductase (EC 1.5.1.2) and the hydrogen needed is supplied by NAD(P)H. An alternative pathway of proline biosynthesis is a cyclisation of L-ornithine catalysed by ornithine cyclodeamidase (EC 4.3.1.12) (Figure 6).

1.3 Arginine

The biosynthesis of the essential amino acid L-arginine in microorganisms and plants starts from L-glutamic acid (IUBMB 2001). It proceeds via L-ornithine in several intermediate steps and needs the reduction of the γ-carboxyl group to carbonyl group under the participation of ATP similarly to proline biosynthesis. To prevent the spontaneous cyclisation of the semialdehyde, the first step is acetylation of glutamic acid with acetyl-CoA catalysed by amino-acid *N*-acetyltransferase (EC 2.3.1.1). The carboxyl group of the intermediate *N*-acetyl-L-glutam-5-yl phosphate thus formed is then reduced to a carbonyl group by NADPH-dependent *N*-acetyl-γ-glutamyl-phosphate reductase

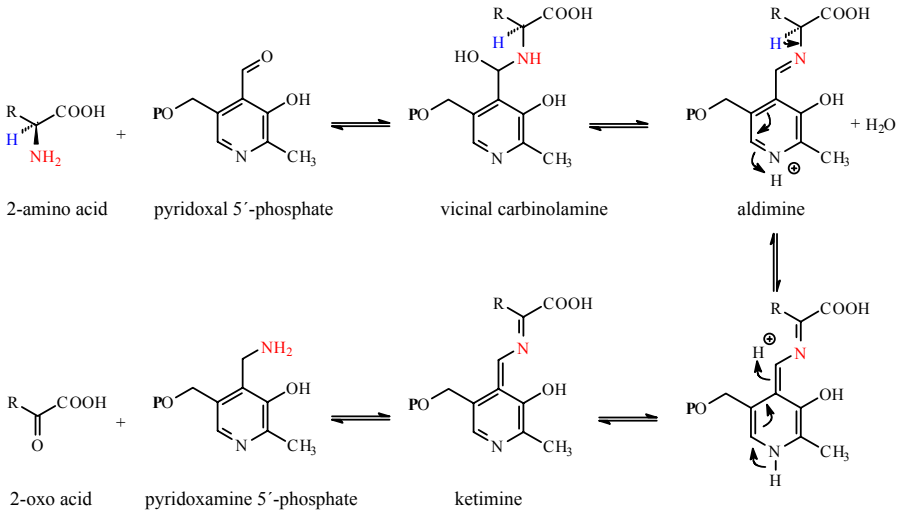

Figure 5

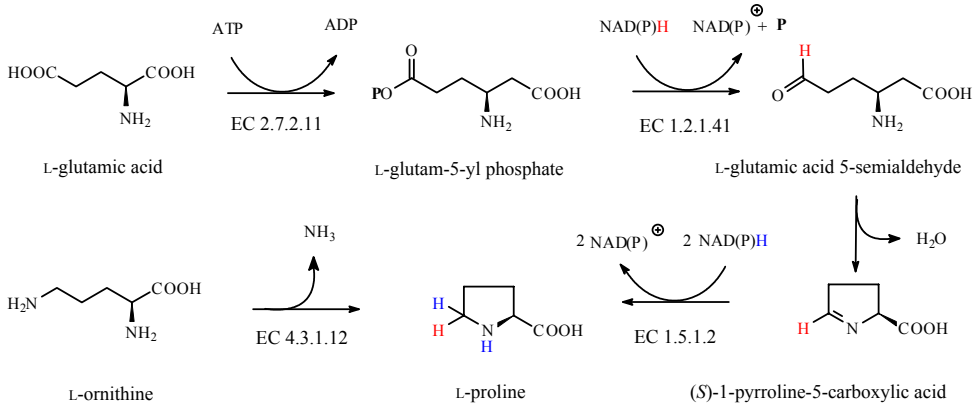

Figure 6

(EC 1.2.1.38) and the next intermediate *N*-acetyl-L-glutamic acid 5-semialdehyde is transformed to *N*-acetyl-L-ornithine in a transamination reaction catalysed by a PLP protein N^2-acetylornithine 5-transaminase (EC 2.6.1.11). Finally, hydrolysis of *N*-acetyl-L-ornithine to L-ornithine is catalysed by acetylornithine deacetylase (EC 3.5.1.16) (Figure 7).

Ornithine functions as an acceptor of carbamoyl phosphate in the urea (ornithine) cycle. At the same time, a phosphoric acid is split off and the resulting next intermediate is L-citrulline. Both intermediates belong to the group of amino acids that is common in cells but is not used in the synthesis of proteins. The reaction is catalysed by ornithine carbamoyl transferase (EC 2.1.3.3). The next reaction leading from citrulline to L-argininosuccinic acid is catalysed by argininosuccinate synthase (EC 6.3.4.5). Oxygen of the ureido group of citrulline is activated with ATP via the intermediate citrullyl-AMP; AMP is replaced by amino group of aspartic acid forming L-argininosuccinic acid. Argininosuccinate lyase (EC 4.3.2.1) then splits this intermediate to arginine and fumaric acid (Figure 8). The release of urea from arginine is catalysed by arginase (EC 3.5.3.1) and generates ornithine.

2 THE ASPARTIC ACID GROUP

2.1 Aspartic acid and asparagine

L-Aspartic acid is generated by transamination of oxaloacetic acid (oxosuccinic acid) from the citric acid cycle. From aspartic acid branches a pathway to L-homoserine, an intermediate that is the starting point of the biosyntheses of L-threonine and L-methionine. The biosynthesis of methionine requires the sulphur transfer from L-cysteine. From aspartic acid then lead pathways to L-lysine and the branched amino acid L-isoleucine. Biosyntheses of both these amino acid require pyruvic acid from the glycolytic pathway (Azevedo *et al.*

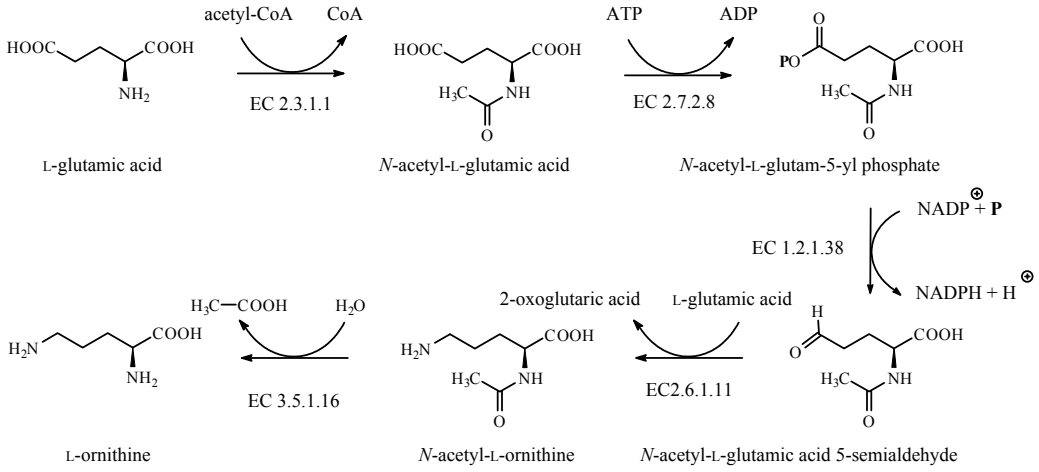

Figure 7

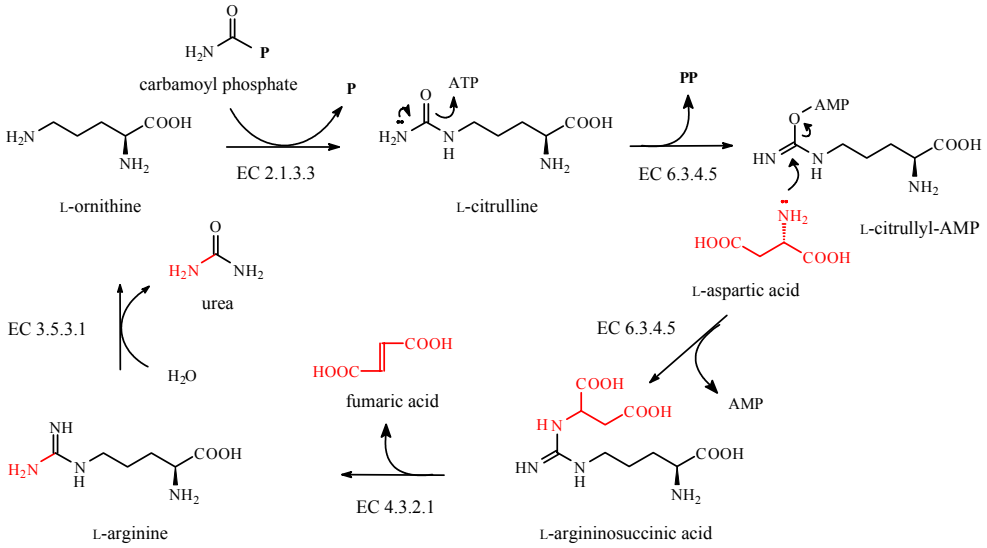

Figure 8

1997; Sengbusch). The biosynthetic pathway of the aspartic acid amino acid family is schematically shown in Figure 9.

The transamination of oxaloacetic acid to L-aspartic acid is a reaction catalysed by aspartate transaminase (EC 2.6.1.1), an enzyme dependent on the coenzyme PLP (Figure 10).

L-Asparagine forms from aspartic acid by the addition of a further amino group. This may be achieved by the fixation of an ammonium ion

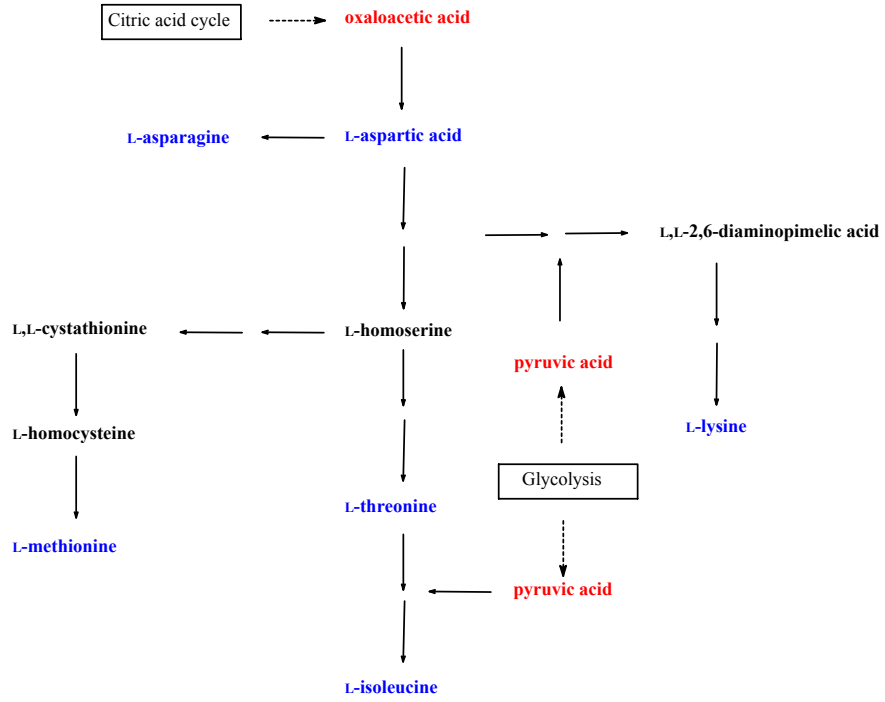

Figure 9

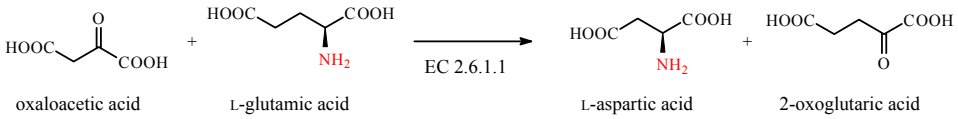

Figure 10

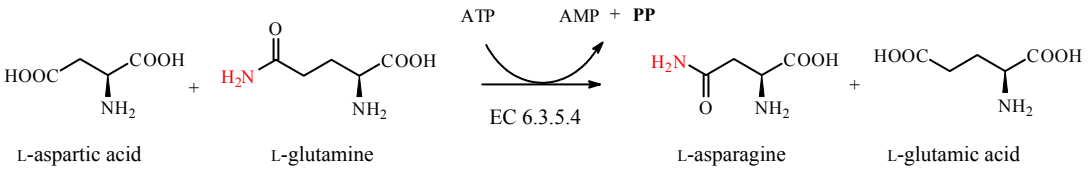

Figure 11

(analogous to the synthesis of glutamine). This reaction (in some microorganisms) is catalysed by ammonia-dependent asparagine synthetases, e.g. asparagine synthetase (AMP forming) (EC 6.3.1.1) or asparagine synthetase (ADP forming) (EC 6.3.1.4). On the other hand, asparagine may also form by the transfer of one amino group from glutamine to aspartic acid. In plants and animals, however, all the asparagine synthases so far characterised belong to the glutamine-dependent class (Gálvez-Valdivieso 2005). This amidation is catalysed by asparagine synthetase (EC 6.3.5.4) and the required energy supplied by ATP that yields AMP and diphosphoric acid (Figure 11).

2.2 Threonine

The biosynthesis of the essential amino acid threonine in microorganisms and plants consists of five steps and consumes two molecules of ATP, one NAD(P)H + H^+, and one NADH + H^+ (Figure 12). Aspartate kinase (EC 2.7.2.4) is the first enzyme of the pathway catalysing the conversion of aspartic acid into L-aspart-4-yl phosphate (*β*-aspartyl phosphate). L-Aspart-4-yl phosphate is reduced to L-aspartic acid 4-semialdehyde (*β*-aspartic acid semialdehyde) by aspartate-semialdehyde dehydrogenase (EC 1.2.1.11). Homoserine dehydrogenase (EC 1.1.1.3) catalyses the conversion of L-aspartic acid 4-semialdehyde to L-homoserine in the presence of coenzyme NADH or NADPH (in plants). Homoserine kinase (EC 2.7.1.39) catalyses the reaction common to threonine, isoleucine, and methionine synthesis, in which homoserine is converted in the presence of ATP to yield *O*-phospho-L-homoserine (4-phospho-L-homoserine) and ADP. The last step involves the irreversible hydrolysis of *O*-phosphohomoserine into threonine, which is catalysed by the enzyme threonine synthase (EC 4.2.3.1) (Azevedo *et al.* 1997; IUBMB 2001).

2.3 Methionine

The biosynthesis of the essential amino acid L-methionine starts with L-homoserine and involves the transfer of sulphur from the C_3 skeleton of L-cysteine to the C_4 skeleton of L-homoserine (Figure 13).

This process termed *trans*-sulphuration is closely related to the biosynthesis of cysteine from serine and sulphide. Plants from most of the major phylogenetic divisions were only observed to utilise *O*-phospho-L-homoserine (some bacteria use *O*-succinyl-L-homoserine, yeasts and fungi utilise *O*-acetyl-L-homoserine). (Azevedo *et al.* 1997; Hesse & Hoefgen 2003).

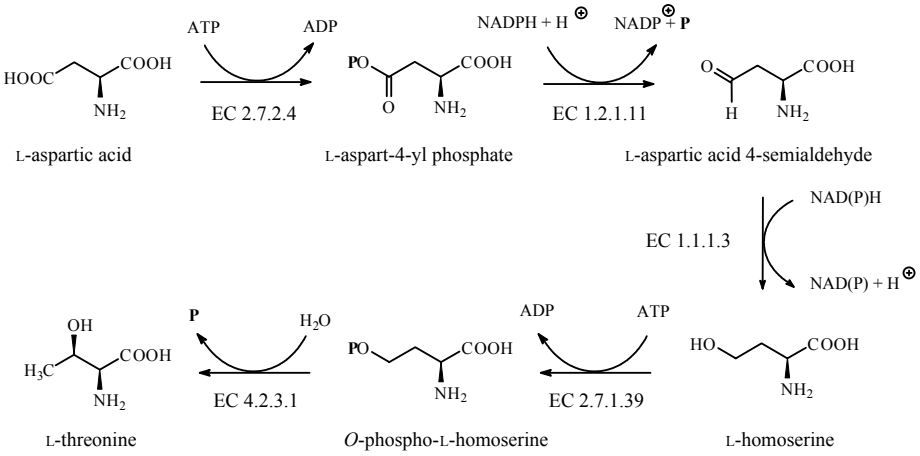

Figure 12

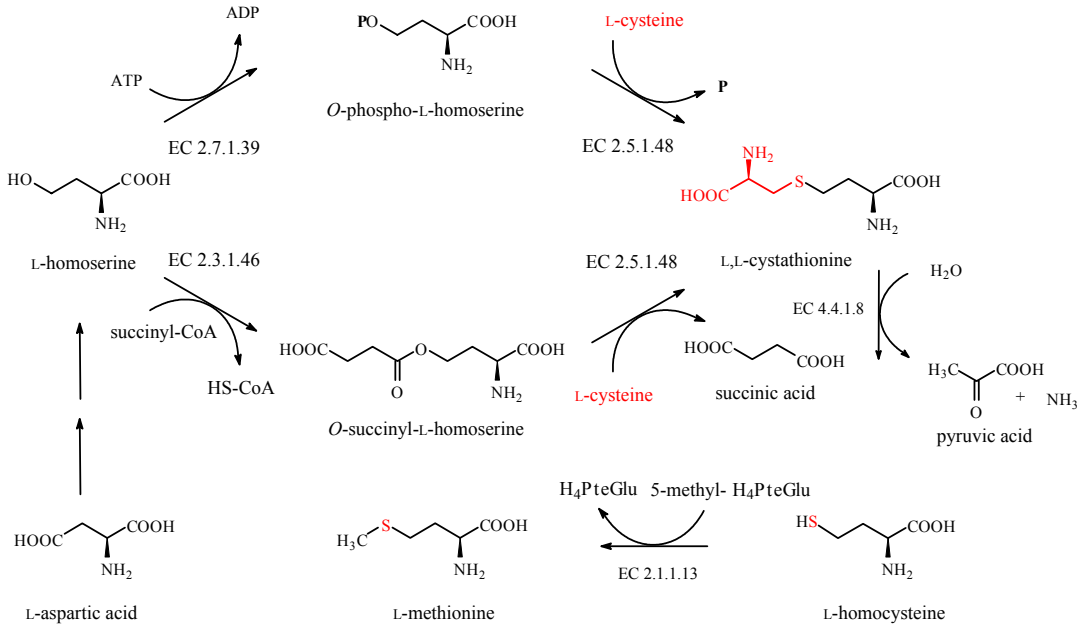

Figure 13

Homoserine is transformed to *O*-phosphohomoserine in a reaction catalysed by homoserine kinase (EC 2.7.1.39) which serves as the starting compound for methionine biogenesis in plants. In microorganisms, the formation of *O*-succinylhomoserine is catalysed by homoserine *O*-succinyltransferase (EC 2.3.1.46). Cystathionine *γ*-lyase (EC 2.5.1.48), the first enzyme unique to methionine biosynthesis, catalyses the synthesis of L,L-cystathionine from cysteine and either *O*-phosphohomoserine or *O*-succinylhomoserine. Cystathionine-*β*-lyase (EC 4.4.1.8) catalyses the *β*-cleavage of cystathionine to yield homocysteine, pyruvic acid and ammonia. The final reaction of methionine biosynthesis, the methylation of homocysteine, is catalysed by homocysteine methyltransferase (EC 2.1.1.13). The methyl group is derived from N^5-methyltetrahydropteroylglutamic acid (5-methyl-H_4PteGlu) in a cobalamin dependent reaction. It appears that the triglutamic acid form, 5-methyl-H_4PteGlu$_3$, is the methyl donor for methionine biosynthesis in plants whereas bacteria use the monoglutamic acid form. The function of this enzyme is both *de novo* synthesis of methionine and the regeneration of *S*-adenosyl-L-methionine (SAM) from *S*-adenosyl-L-homocysteine (SAH) after methylation reactions.

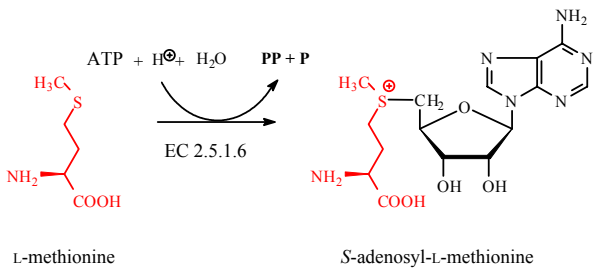

Figure 14

Eventually, about 20% of methionine in plants is incorporated into proteins and 80% is converted to SAM, the end product of the methionine biosynthetic pathway. The biosynthesis of SAM from methionine and ATP is catalysed by methionine adenosyltransferase (EC 2.5.1.6) (Dewick 2002) (Figure 14).

2.4 Lysine

The enzyme dihydrodipicolinate synthase (EC 4.2.1.52) is the first enzyme of the essential amino acid L-lysine biosynthesis branch of the aspartic acid metabolic pathway (Azevedo *et al.* 1997; Born & Blanchard 1999; IUBMB 2001). Dihydrodipicolinate synthase catalyses the aldolisation of L-aspartic acid 4-semialdehyde and pyruvic acid from the glycolytic pathway. The product of this aldolisation reaction spontaneously forms an intermediate cyclic Schiff base under the elimination of water. Its dehydration yields (*R*)-2,3-dihydropyridine-2,6-dicarboxylic acid (2,3-dihydrodipicolinic acid). Dihydrodipicolinate reductase (EC 1.3.1.26) catalyses the NAD(P)H-dependent reduction of 2,3-dihydrodipicolinic acid

to (*R*)-2,3,4,5-tetrahydropyridine-2,6-dicarboxylic acid (2,3,4,5-tetrahydrodipicolinic acid). The tetrahydropyridine ring then opens and the released amino group is succinylated by 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate *N*-succinyltransferase (EC 2.3.1.117) yielding (*R*)-2-succinylamino-6-oxoheptanedioic acid (*N*-succinyl-L-2-amino-6-oxopimelic acid). Transamination of this oxo acid, catalysed by the PLP-dependent succinyldiaminopimelate transaminase (EC 2.6.1.17), forms *N*-succinyl-L,L-2,6-diaminoheptanedioic acid (*N*-succinyl-L,L-2,6-diaminopimelic acid). Succinyl-diaminopimelate desuccinylase (EC 3.5.1.18) splits off succinic acid from *N*-succinyl-L,L-2,6-diaminopimelic acid yielding L,L-2,6-diaminoheptanedioic acid (L,L-2,6-diaminopimelic acid). Diaminopimelate epimerase (EC 5.1.1.7) catalyses isomerisation of L,L-2,6-diaminopimelic acid to *meso*-2,6-diaminoheptanedioic acid (*meso*-2,6-diaminopimelic acid). The last step in lysine biosynthesis involves the PLP-dependent decarboxylation of *meso*-2,6-diaminopimelic acid to lysine, which is catalysed by the enzyme diaminopimelate decarboxylase (EC 4.1.1.20) (Figure 15).

2.5 Isoleucine

The first step of the essential amino acid L-isoleucine biosynthesis, catalysed by threonine ammonia-lyase (EC 4.3.1.19) yields 2-oxobutyric (2-oxobutanoic) acid and ammonia (Figure 16) (IUBMB 2003). This transformation of threonine to 2-oxobutanoic acid probably involves the initial elimination of water, followed by isomerisation and hydrolysis of the product with C = N bond breakage (Figure 17).

The next step, the formation of (*S*)-2-ethyl-2-hydroxy-3-oxobutanoic acid (also known as 2-aceto-2-hydroxybutanoic acid), is catalysed by acetolactate synthase (EC 2.2.1.6). This enzyme catalyses

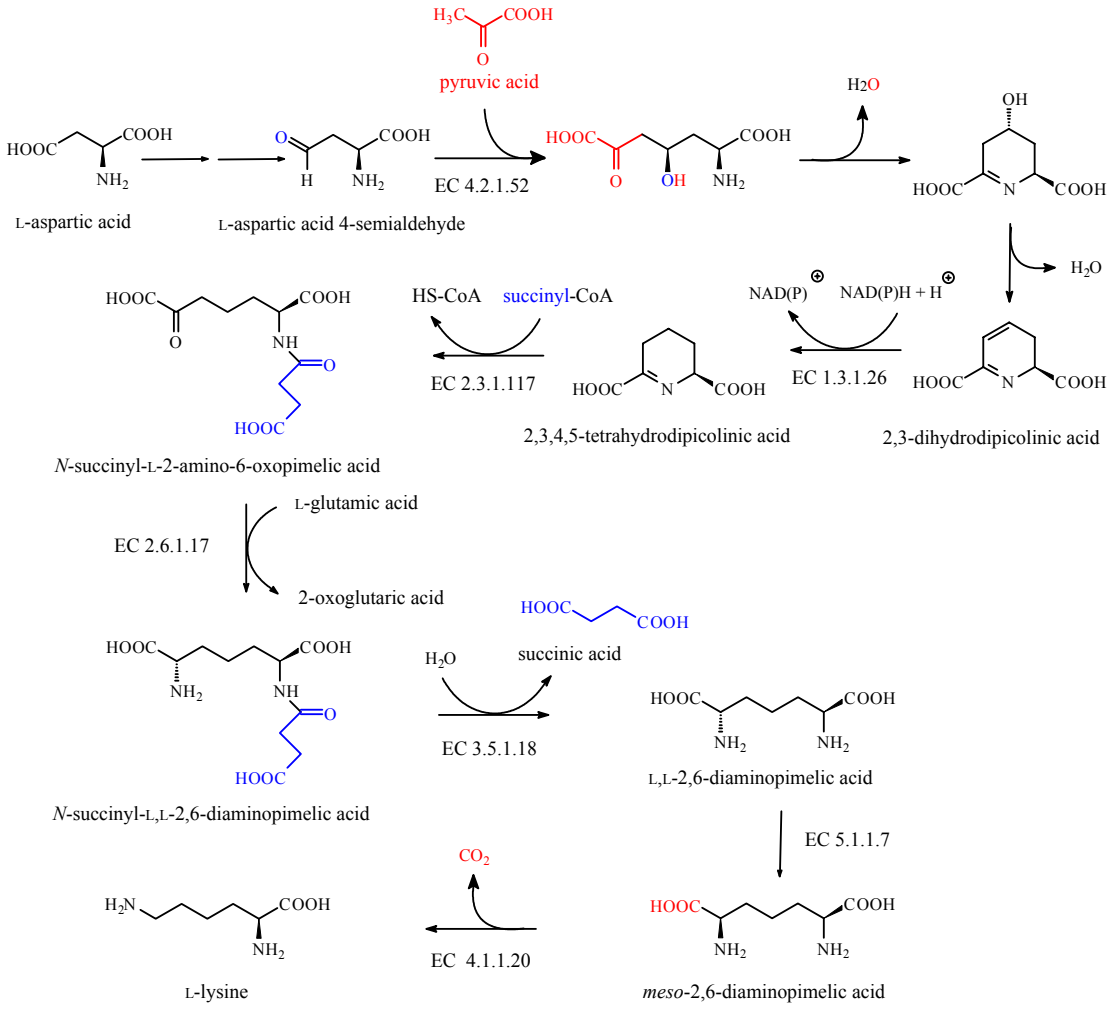

Figure 15

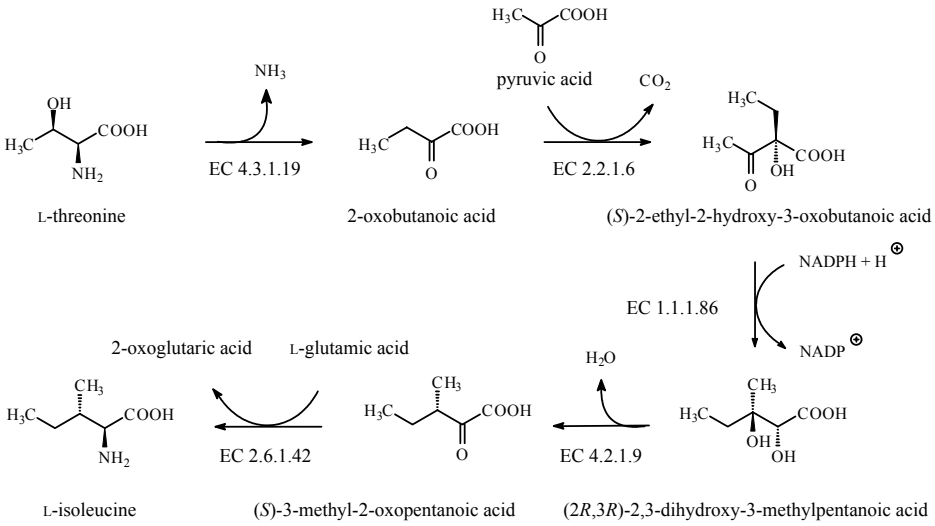

Figure 16

the condensation of 2-oxobutanoic acid and pyruvic acid and requires divalent metal ions, FAD, and thiamine diphosphate. Thiamine diphosphate mediated decarboxylation of pyruvic acid produces one acetaldehyde equivalent bound in the form of enamine which reacts as a nucleophile in the addition reaction with 2-oxobutanoic acid. The subsequent release from the thiamine diphosphate carrier generates (*S*)-2-ethyl-2-hydroxy-3-oxobutanoic acid (Figure 18).

Ketol-acid reductoisomerase (EC 1.1.1.86) catalyses an unusual reaction in the biosynthesis of isoleucine in which (*S*)-2-ethyl-2-hydroxy-3-oxobutanoic acid (acetohydroxy acid) is converted to (*R*)-3-hydroxy-3-methyl-2-oxopentanoic acid (methylhydroxyketol acid) in an alkyl migration (the alkyl migration is highly specific for Mg^{2+}), which is followed by the NADPH-dependent reduction in the presence of a divalent metal ion (such as Mg^{2+}, Mn^{2+} or Co^{2+}) to yield (2*R*,3*R*)-2,3-dihydroxy-3-methylpentanoic acid (dihydroxy acid) (Figure 19). Dihydroxy-acid dehydratase (EC 4.2.1.9) catalyses the next step in isoleucine biosynthesis which involves the dehydration and tautomerisation of 2,3-dihydroxy-3-methylpentanoic acid to (*S*)-3-methyl-2-oxopentanoic acid. 3-Methyl-2-oxopentanoic acid is finally converted to isoleucine in a transamination reaction catalysed by branched-chain-amino-acid transaminase (EC 2.6.1.42) (Azevedo *et al.* 1997; Lee *et al.* 2005).

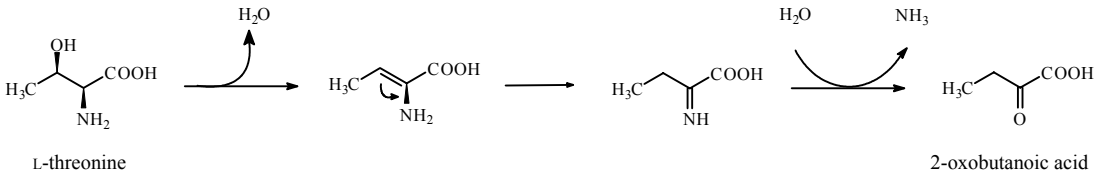

Figure 17

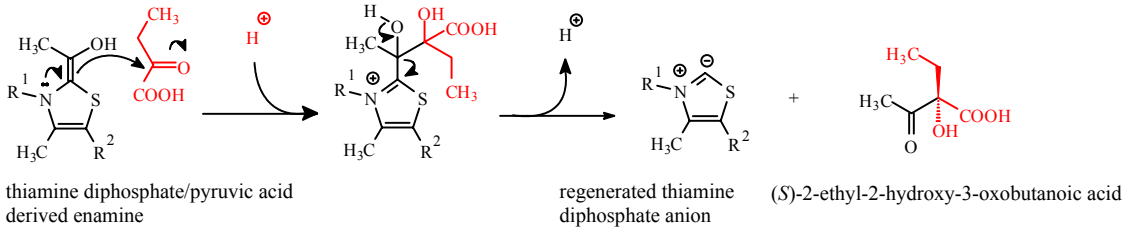

Figure 18

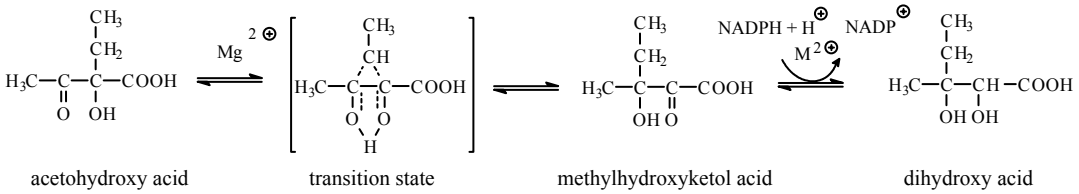

Figure 19

EC (Enzyme Commission) numbers and some common abbreviations

EC (Enzyme Commission) numbers, assigned by IUPAC-IUBMB, were taken from KEGG: Kyoto Encyclopedia of Genes and Genomes, http://www.biologie.uni-hamburg.de. In many structures, the abbreviation **P** is used to represent the phosphate group and **PP** the diphosphate group. At physiological pH, these and some other groups will be ionised, but in pictures the unionised forms are depicted to simplify the structures, to eliminate the need for counter-ions, and to avoid the mechanistic confusion.

ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
CoA	coenzyme A as part of a thioester
FAD	flavine adenine dinucleotide
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
P	phosphoric acid
PLP	pyridoxal 5'-phosphate
PP	diphosphoric acid
SAH	*S*-adenosyl-L-homocysteine
SAM	*S*-adenosyl-L-methionine

References

Azevedo R.A., Arruda P., Turner W.L., Lea P.J. (1997): The biosynthesis and metabolism of the aspartate derived amino acids in higher plants. Phytochemistry, **46**: 395–419.

Born T.L., Blanchard J.S. (1999): Structure/function studies on enzymes in the diaminopimelate pathway of bacterial cell wall biosynthesis. Current Opinion in Chemical Biology, **3**: 607–613.

Dewick P.M. (2002): Medicinal natural products. A biosynthetic approach. 2nd Ed. Wiley, New York.

Gálvez-Valdivieso G., Osuna D., Maldonado J.M., Pineda M., Aquilar M. (2005): Purification of a functional asparagines synthetase (PVAS2) from common bean (*Phaseolus vulgaris*), a protein predominantly found in root tissues. Plant Science, **168**: 89–95.

Hesse H., Hoefgen R. (2003): Molecular aspects of methionine biosynthesis. Trends in Plant Science, **8**: 259–262.

Lee Y.T., Ta H.T., Duggleby R.G. (2005): Cyclopropane-1,1-dicarboxylate is a slow, tight-binding inhibitor of rice ketol-acid reductoisomerase. Plant Science, **168**: 1035–1040.

IUBMB (2001): http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/.

IUBMB (2003): http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/.

Sengbusch P.: Botany Online –The Internet Hypertextbook. http://www.biolobie.uni-hamburg.de/b_online/e00/default.htm.

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