Biosynthesis of Food Constituents: Amino Acids: 2. The Alanine-Valine-Leucine, Serine-Cysteine-Glycine, and Aromatic and Heterocyclic Amino Acids Groups – a Review

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Abstract

Velíšek J., Cejpek K. (2006): **Biosynthesis of food constituents: Amino acids: 2. The alanine-valine-leucine, serine-cysteine-glycine, and aromatic and heterocyclic amino acids groups – a review**. Czech J. Food Sci., **24**: 45–58.

This review article gives a survey of principal pathways that lead to the biosynthesis of the proteinogenic amino acids of the alanine-valine-leucine group starting with pyruvic acid from the glycolytic pathway and serine-cysteine-glycine group starting with 3-phospho-D-glyceric acid from the glycolytic pathway. A survey is further given to the aromatic and heterocyclic amino acids (phenylalanine, tyrosine, tryptophan, histidine) starting with 3-phosphoenolpyruvic acid from the glycolytic pathway and D-erythrose 4-phosphate, an intermediate in the pentose phosphate cycle and Calvin cycle.

Keywords: biosynthesis; amino acids; alanine; valine; leucine; serine; glycine; cysteine; phenylalanine; tyrosine; tryptophan; histidine

1 THE ALANINE, VALINE, AND LEUCINE GROUP

1.1 Alanine

L-Alanine and the essential amino acids L-valine and L-leucine have a common precursor, i.e. pyruvic acid from the glycolytic pathway (Figure 1).

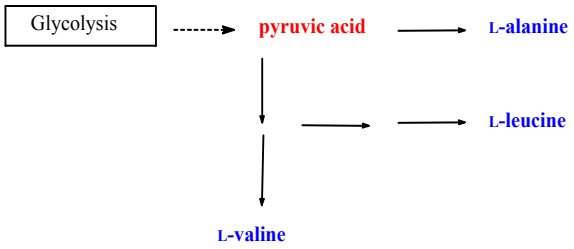

Figure 1

Alanine is formed from pyruvic acid in a simple transamination reaction (Figure 2) catalysed by alanine transaminase (EC 2.6.1.2) (Sengbusch). The transamination reactions are catalysed by transaminases dependent on the coenzyme pyridoxal 5'-phosphate (PLP).

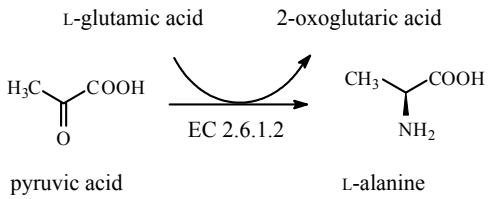

Figure 2

Partly supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project MSM 6046137305.

1.2 Valine

Valine is synthesised in a set of four reactions, and an extension of the valine pathway results in leucine synthesis (Figure 3) (IUBMB 2003). The first three enzymes involved in the biosynthesis of valine and leucine, i.e. acetolactate synthase (EC 2.2.1.6), ketol-acid reductoisomerase (EC 1.1.1.86), and dihydroxyacid-dehydratase (EC 4.2.1.9), also catalyse analogous reactions leading to the biosynthesis of isoleucine. 3-Methyl-2-oxobutanoic acid is the common precursor of both amino acids, valine and leucine. This carboxylic acid then yields valine by the action of the branched-chain-amino-acid transaminase (EC 2.6.1.42).

1.3 Leucine

Leucine is synthesised from 3-methyl-2-oxobutanoic acid in six steps (Figure 3) (IUBMB 2003). The first step, catalysed by 2-isopropylmalate synthase (EC 2.3.3.13, requires K^+ ions), is the condensation of 3-methyl-2-oxobutanoic acid with acetyl-CoA to (*S*)-2-isopropylmalic acid. The next two steps are catalysed by 3-isopropylmalate dehydratase (EC 4.2.1.33). 2-Isopropylmaleic acid arises from 2-isopropylmalic acid by elimination of water and addition of water to 2-isopropylmaleic acid yields (2*R*,3*S*)-3-isopropylmalic acid. The enzyme also hydrates the product back to (*S*)-2-isopropylmalic acid, thus bringing about the interconversion between the two isomers. In the next step, 3-isopropylmalic acid is oxidised to (*S*)-2-isopropyl-3-oxosuccinic acid by the NAD^+-dependent 3-isopropylmalate dehydrogenase (EC 1.1.1.85). The product decarboxylates spontaneously to 4-methyl-2-oxopentanoic acid (4-oxoisocapronic acid), which is, in the last step, transformed to leucine by either branched-chain-amino-acid transaminase (EC 2.6.1.42) or more specific leucine transaminase (EC 2.6.1.6) that does not act on valine or isoleucine.

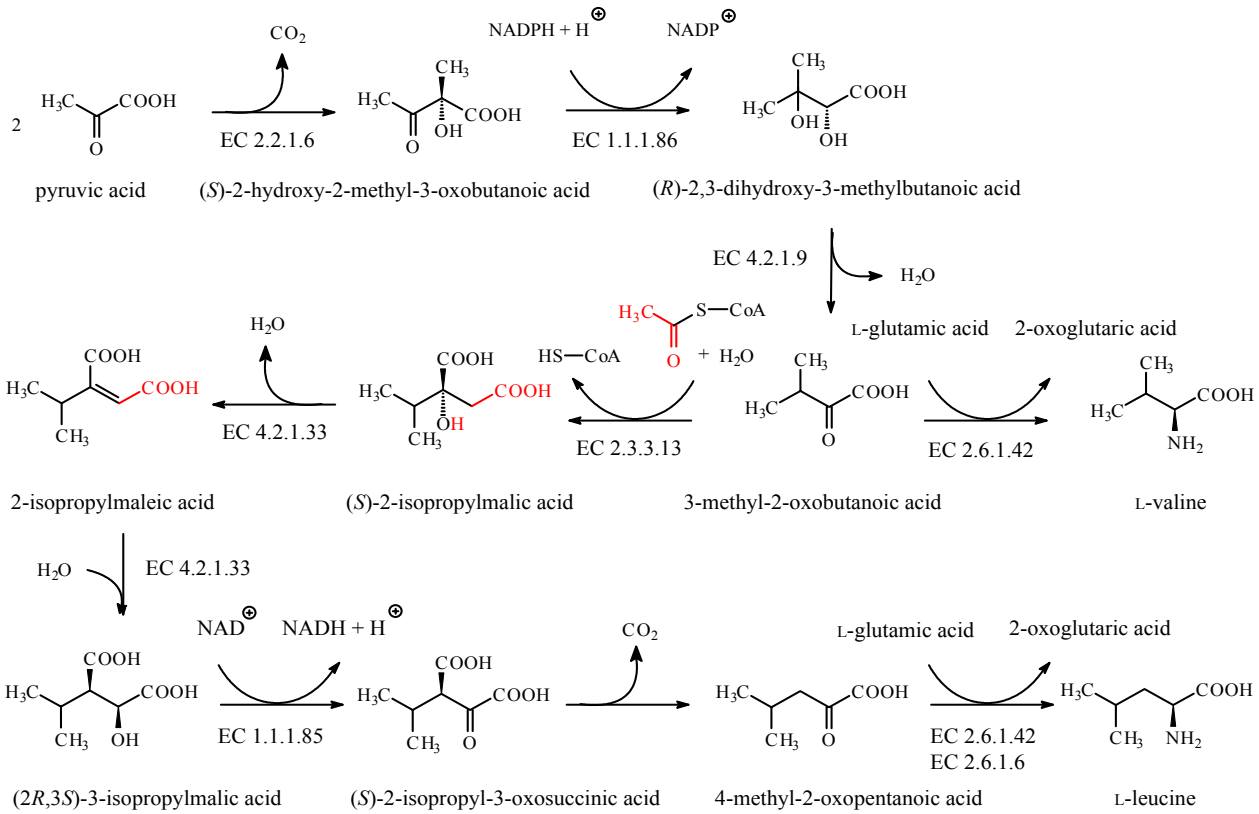

Figure 3

2 THE SERINE, GLYCINE, AND CYSTEINE GROUP

2.1 Serine

L-Serine is generated in a three-step reaction from 3-phospho-D-glyceric acid, formed in the glycolytic pathway, and becomes the precursor of glycine and L-cysteine (Figure 4).

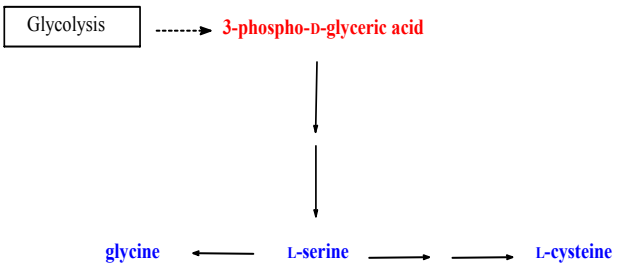

Figure 4

The first step of serine biosynthesis is the oxidation of 3-phospho-D-glyceric acid at C-2 to 3-phosphopyruvic acid catalysed by 3-phosphoglycerate dehydrogenase (EC 1.1.1.95). This phosphorylated oxo-analogue of serine is transaminated by PLP-dependent phosphoserine transaminase (EC 2.6.1.52) in the second step yielding 3-phospho-L-serine. Finally, in the third step, phosphoserine is hydrolysed by phosphoserine phosfatase (EC 3.1.3.3) to serine (Figure 5) (Sengbusch).

2.2 Glycine

The major pathway of glycine biosynthesis is the direct transformation of L-serine, which is catalysed by PLP protein, i.e. serine hydroxymethyl transferase (EC 2.1.2.1). The hydroxymethyl group of the serine side chain is split off and this C1 unit is accepted by tetrahydrofolic acid (H_4PteGlu, R = benzoyl glutamic acid residue) under the formation of 5,10-methylene-tetrahydrofolic acid (5,10-methylene-H_4PteGlu) (Figure 6) (Sengbusch).

2.3 Cysteine

Cysteine directly or indirectly provides sulfide for structural, regulatory, or catalytic purposes in peptides, proteins, and numerous low molecular weight compounds such as methionine, glutathione, biotin, and thiamine. Biosynthesis of the cysteine -SH group needs sulfur and thus provides an exclusive entry of reduced sulfur into cellular metabolism. Plants take up sulfur as inorganic sulfate ions. After its uptake by roots, sulfate is distributed into different organs and, to be assimilated, it has to be reduced in a process called assimilatory sulfate reduction performed in chloroplasts. The first step in the sulfate assimilation pathway is catalysed by ATP sulfurylase (EC 2.7.7.4). This enzyme activates sulfate via an ATP-dependent reaction that leads to the formation of adenylyl-

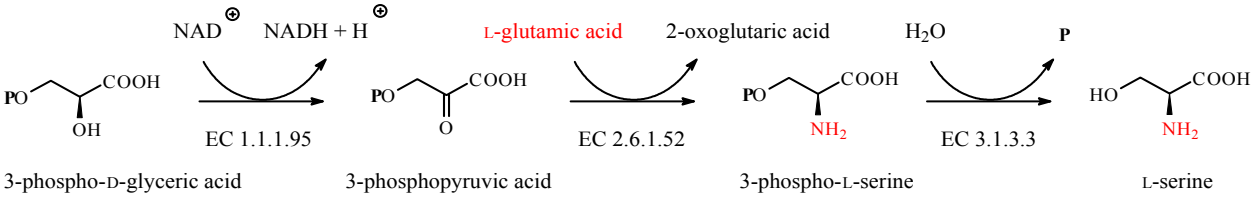

Figure 5

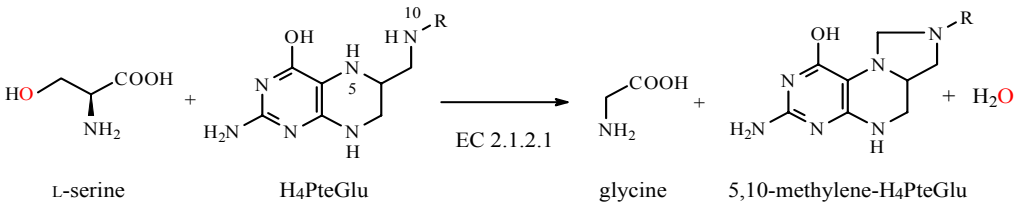

Figure 6

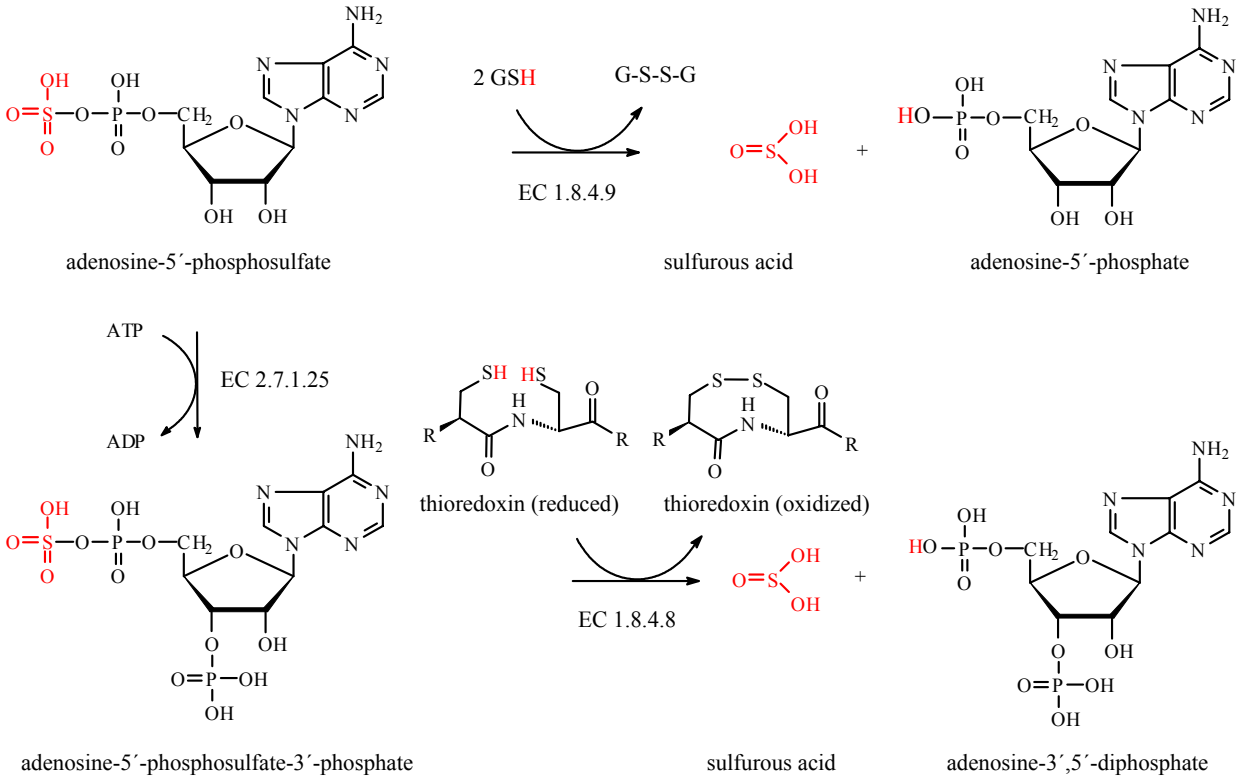

Figure 7

sulfate (adenosine-5'-phosphosulfuric acid, APS) and inorganic diphosforic acid (**PP**).

To accomplish the incorporation of sulphur into biomolecules, specifically amino acids, sulphate in APS is transformed into sulphite and this into sulphide. This process may occur through two different pathways, depending on the organism. One of them involves phosforylation of APS by adenosine 5'-phosphosulfate kinase (EC 2.7.1.25) using ATP to produce 3'-phosfoadenosine-5'-phosphosulfate (PAPS) and ADP. In the following reaction, PAPS reductase (EC 1.8.4.8) firstly reacts with reduced thioredoxin and then with PAPS to generate free sulphite. The other pathway involves the direct reduction of APS by 5'-adenylylsulfate reductase (EC 1.8.4.9), which uses GSH as an electron source to produce sulphite. In yeasts and many bacteria, sulphite is synthesised via adenosine 5'-phosphosulfate kinase, whereas in plants, green algae, and phototrophic bacteria, sulfate is transformed into sulphite via 5'-adenylylsulfate reductase (Figure 7).

Once sulfate has been reduced to sulphite, the subsequent step is identical in bacteria, fungi, and plants. Sulfite is reduced to sulphide at the expense of oxidising three molecules of NADPH, by sulphite reductase (EC 1.8.7.1)[1].

Depending on the organism, there are two different ways by which sulfide is incorporated into a carbon backbone to produce L-cysteine (Figure 8). First, the physiological *O*-ester substrate *O*-acetylserine is synthesised from L-serine by serine acetyltransferase (EC 2.3.1.30). Sulfide is condensed with *O*-acetylserine by the PLP-dependent enzyme *O*-acetylserine (thiol)-lyase (also called *O*-acetylserine sulfhydrylase, EC 2.5.1.47) to form cysteine directly. Because the substrate *O*-acetylserine is derived from the carbon and nitrogen assimilatory pathways, the *O*-acetylserine (thiol)-lyase links the sulfur and nitrogen assimilatory pathways together.

O-acetylserine sulphhydrylase (EC 2.5.1.47) also catalyses the condensation of sulfide with *O*-acetylhomoserine to form L-homocysteine. *O*-Acetylhomoserine is synthesised from L-homoserine by homoserine *O*-acetyltransferase (EC 2.3.1.31). Then homocysteine is transformed into cysteine by *trans*-sulfuration, i.e. L-homocysteine asso-

[1]Sulphite reductase contains a special acidic heme group called siroheme and catalyses the reduction of sulfite using electrons donated by ferredoxin.

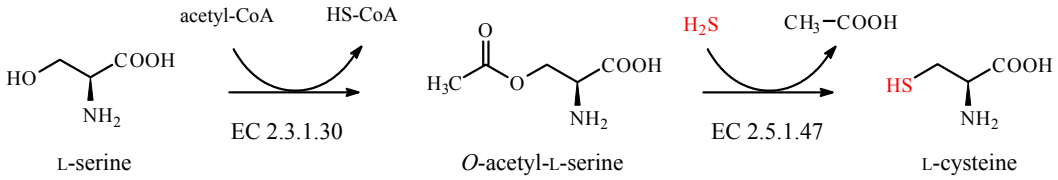

Figure 8

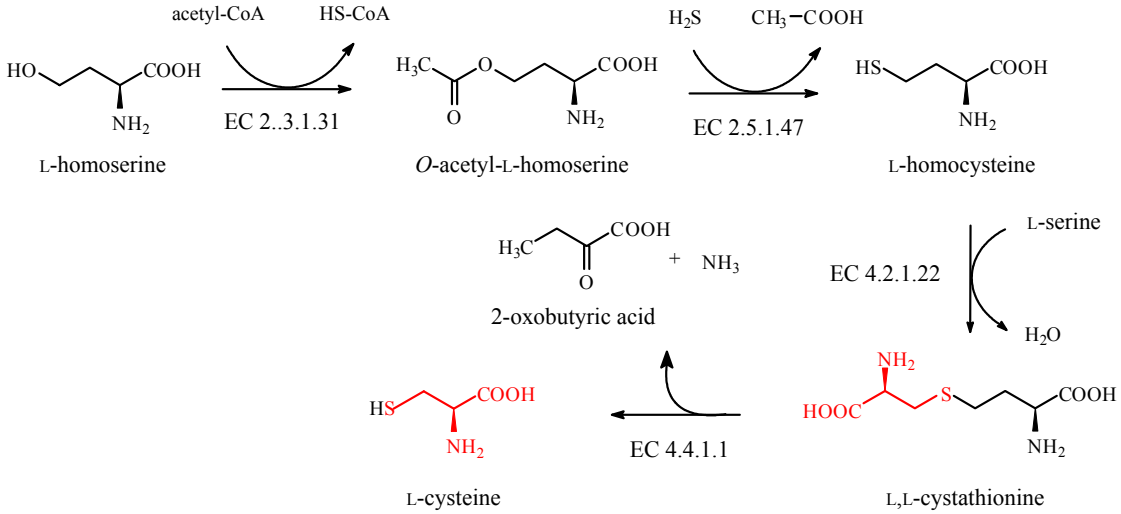

Figure 9

ciates with serine to form L,L-cystathionine by action of cystathionine β-synthase (EC 4.3.1.22). Cystathionine in turn dissociates into cysteine, 2-oxobutyric acid, and ammonia by cystathionine γ-lyase (EC 4.4.1.1) (Figure 9) (Jost *et al.* 2000). Cysteine may also be transformed into homocysteine by reverse *trans*-sulphuration catalysed by cystathionine γ-synthase (EC 2.5.1.48) and cystathionine β-lyase (EC 4.4.1.8).

The serine acetyltransferase pathway is used by plants and by enteric bacteria. Fungi use different cysteine biosynthetic pathway depending on the species. In plants, cysteine can also be produced by the degradation of methionine.

3 THE AROMATIC AND HETEROCYCLIC AMINO ACIDS GROUP

3.1 Phenylalanine and tyrosine

Biosynthetic pathways of the aromatic and heterocyclic amino acids family is schematically shown in Figure 10. The shikimic acid pathway provides a route to the aromatic amino acids L-phenylalanine and L-tyrosine and to the heterocyclic amino acid L-tryptophan (IUBMB 2001). Shikimic acid[2], a central intermediate in this pathway, is formed by a sequence of reactions from 3-phosphoenolpyruvic acid (from glycolysis) and D-erythrose 4-phosphate (from the pentose phosphate cycle and the Calvin cycle). Both shikimic acid and the subsequent chorismic acid are important intermediates. The latter is the starting compound for three different pathways that lead to the end products phenylalanine, tyrosine, and tryptophan. An activated form of D-ribose 5-phosphate (5-phospho-α-D-ribose 1-diphosphate), is another intermediate of tryptophan biosynthesis. It has also a key position in the biosynthesis of L-histidine. The biosynthetic pathway of histidine is unusual in that histidine is produced from a purine. Animals employ none of these pathways and, accordingly, these amino acids feature among those essential amino acids for man that have to be obtained in the diet.

[2]Shikimic acid has been isolated from plants of *Illicium anisatum* L., syn. *I. religiosum* Sieb. et Zucc., Japanese shikimi.

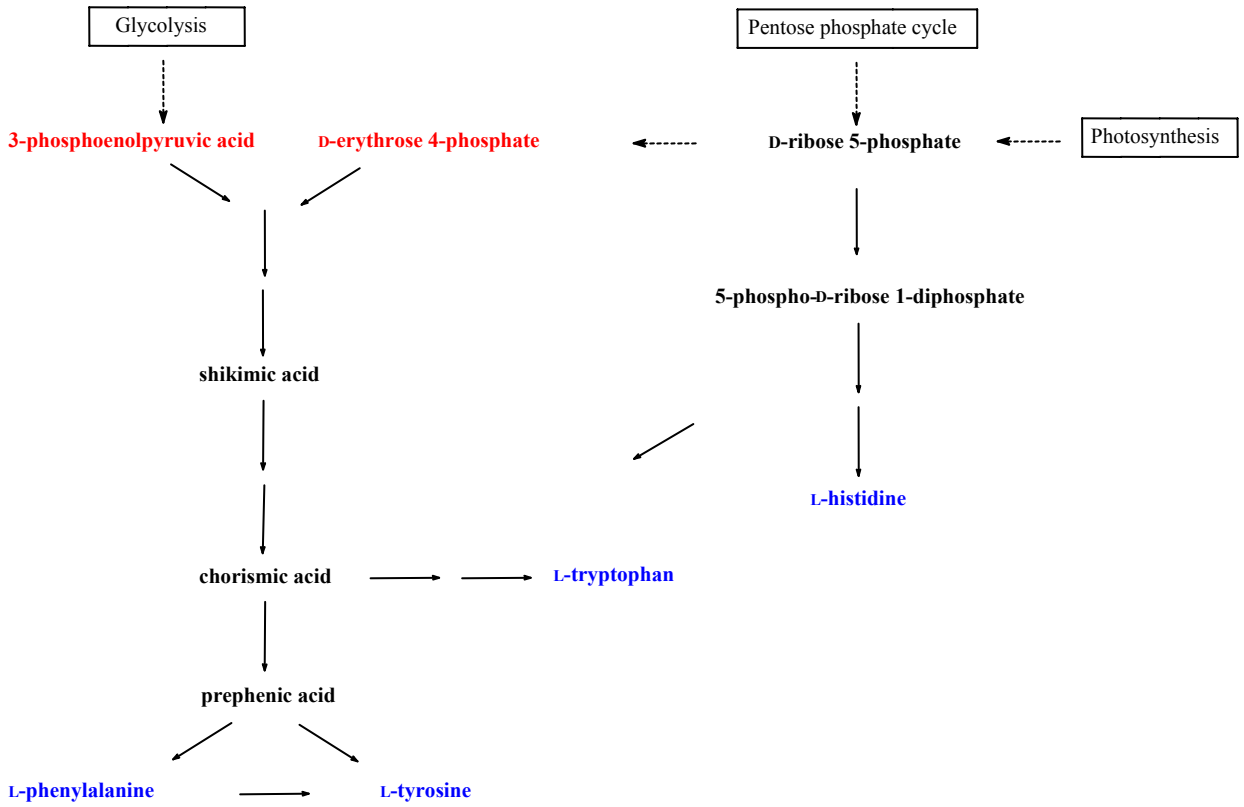

Figure 10

The shikimic acid pathway begins with an aldol-type condensation of phosphoenolpyruvic acid with D-erythrose 4-phosphate to give the seven-carbon 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (3-deoxy-7-phospho-D-*arabino*-heptulosonic acid, Figure 11). This reaction catalysed by 3-deoxy-7-phosphoheptulonate synthase (EC 2.5.1.54) is mechanistically a complex reaction (Figure 12) (IUBMB 2002). Elimination of phosphoric acid from 3-deoxy-7-phospho-D-*arabino*-heptulosonic acid followed by intramolecular aldol reaction generates the first alicyclic intermediate 3-dehydroquinic acid. The elimination of phosphoric acid by 3-dehydroquinate synthase (EC 4.2.3.4) actually follows a NAD^+-dependent oxidation (NAD^+ is bound as a prosthetic group) of the central secondary hydroxyl group, which is then re-formed in a NADH-dependent reduction reaction on the intermediate carbonyl compound prior to the aldol reaction occurring (Figure 13) (Bender *et al.* 1989; Gourley *et al.* 1999; IUBMB 2001).

Reduction of 3-dehydroquinic acid with either quinate dehydrogenase (EC 1.1.1.24) or quinate/shikimate dehydrogenase (EC 1.1.1.282) yields quinic acid, a fairly common acid frequently occurring in foods in the free and esterified form[3]. 3-Dehydroshikimic acid is formed from 3-dehydroquinic acid by dehydration catalysed by 3-dehydroquinate dehydratase (EC 4.2.1.10) (Figure 14) (IUBMB 2001). Reduction of 3-dehydroshikimic acid by $NADP^+$-dependent shikimate 3-dehydrogenase (EC 1.1.1.25) or quinate/shikimate dehydrogenase (EC 1.1.1.282) yields shikimic acid. This second shikimate dehydrogenase enzyme differs from shikimate 3-dehydrogenase (EC 1.1.1.25) in that it can use both quinate and shikimate as substrate and either NAD^+ or $NADP^+$ as acceptor.

An important branch point compound in the shikimate pathway is chorismic acid. Chorismic acid forms from shikimic acid, which is first phosphorylated by shikimate kinase (EC 2.7.1.71) and yields 3-phosphoshikimic acid. 3-Phosphoshikimic acid reacts, under catalysis of 3-phosphoshikimate 1-carboxyvinyltransferase (EC 2.5.1.19), with further molecule of phosphoenolpyruvic acid, which is incorporated as an enol ether side-chain in

[3]Reduction of 3-dehydroquinic acid in bacteria can be achieved also by a quinoprotein, NAD(P)-independent quinate-dehydrogenase (pyrroloquinoline-quinone) (EC 1.1.99.25).

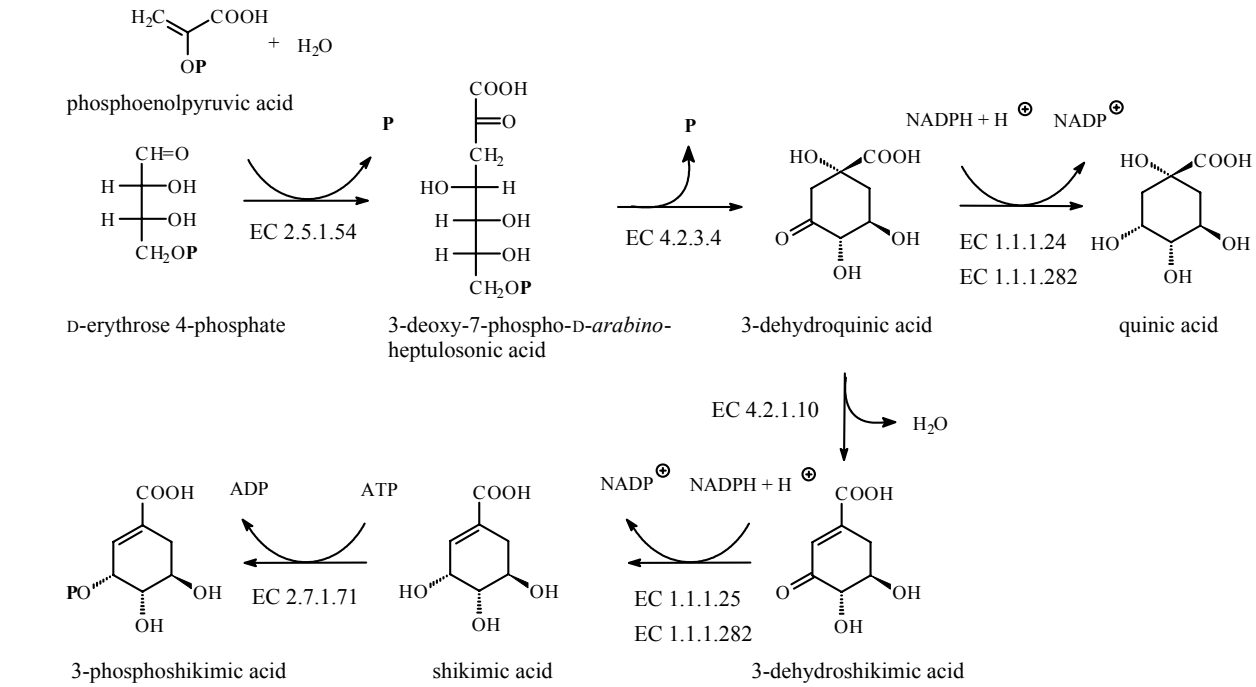

Figure 11

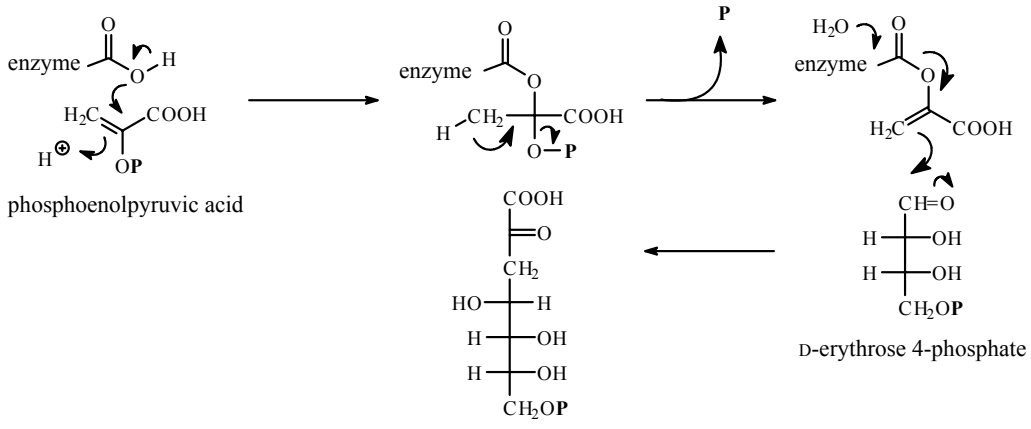

Figure 12

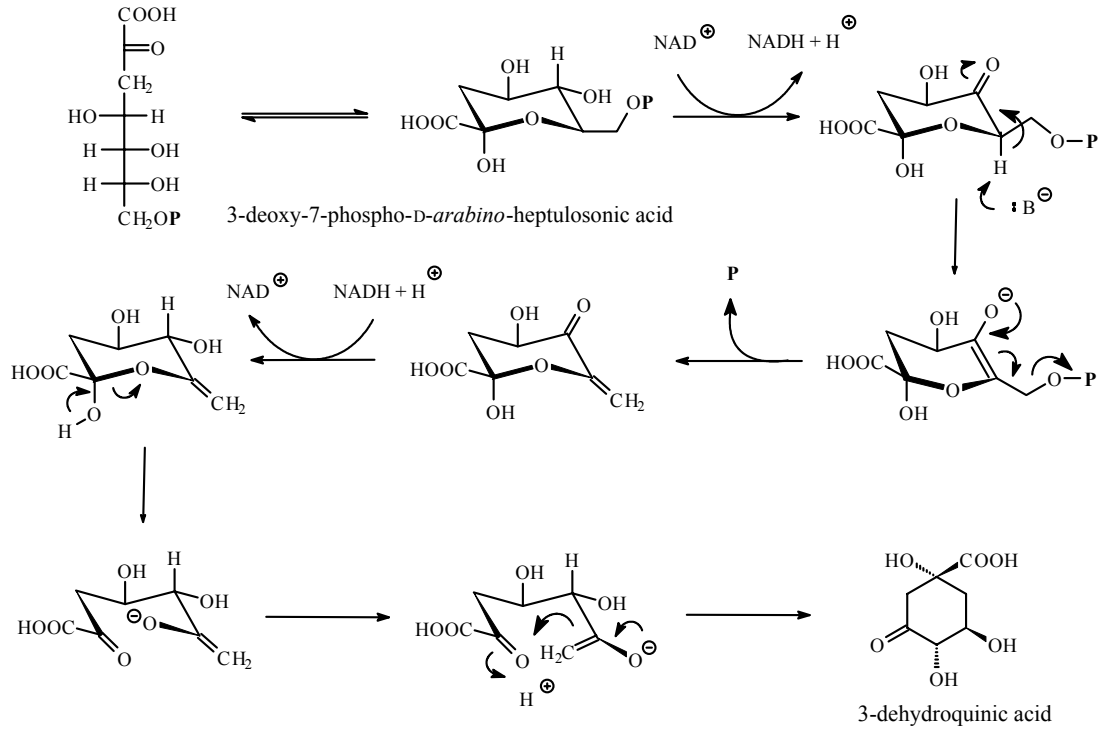

Figure 13

5-(1-carboxyvinyl)-3-phosphoshikimic acid (Figure 15). The transformation of 5-(1-carboxyvinyl)-3-phosphoshikimic acid to chorismic acid involves a 1,4-elimination of phosphoric acid by chorismate synthase (EC 4.2.3.5) (Figure 16) (Floss *et al.* 1972; Bornemann *et al.* 1996, 2000; Jakeman *et al.* 1998; Lewis *et al.* 1999; Osborne *et al.* 2000; IUBMB 2001).

The biologically unique conversion of chorismic acid to prephenic acid by chorismate mutase (EC 5.4.99.5) is formally a Claisen rearrangement reaction (Figures 17 and 18). Decarboxylation and loss of hydroxyl group from prephenic acid (prephenate dehydratase, EC 4.2.1.51) yields phenylpyruvic acid, and the PLP-dependent transamination catalysed by aromatic aminotransferase

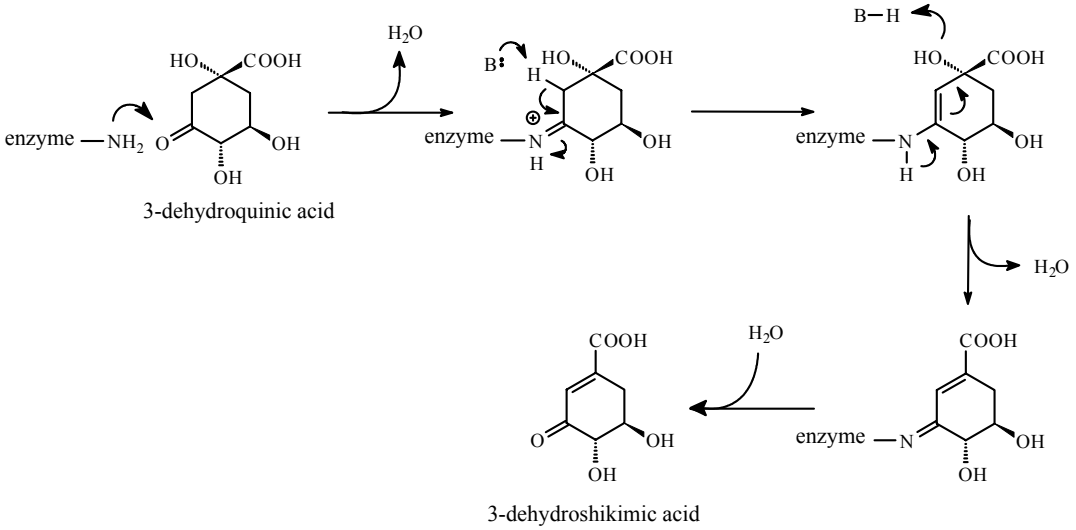

Figure 14

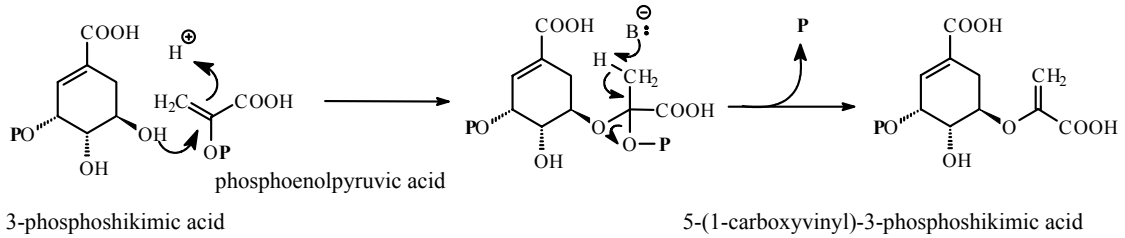

Figure 15

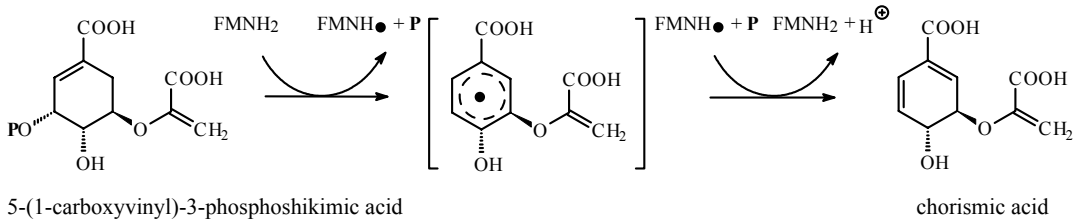

Figure 16

(EC 2.6.1.57) forms phenylalanine. In the presence of a NAD^+-dependent dehydrogenase (prephenate dehydrogenase, EC 1.3.1.12), or a $NADP^+$-dependent prephenate dehydrogenase ($NADP^+$) (EC 1.3.1.13) decarboxylation occurs with retention of the hydroxyl function. Transamination of the resultant 4-hydroxyphenylpyruvic acid by aromatic-amino-acid transaminase (EC 2.6.1.57)

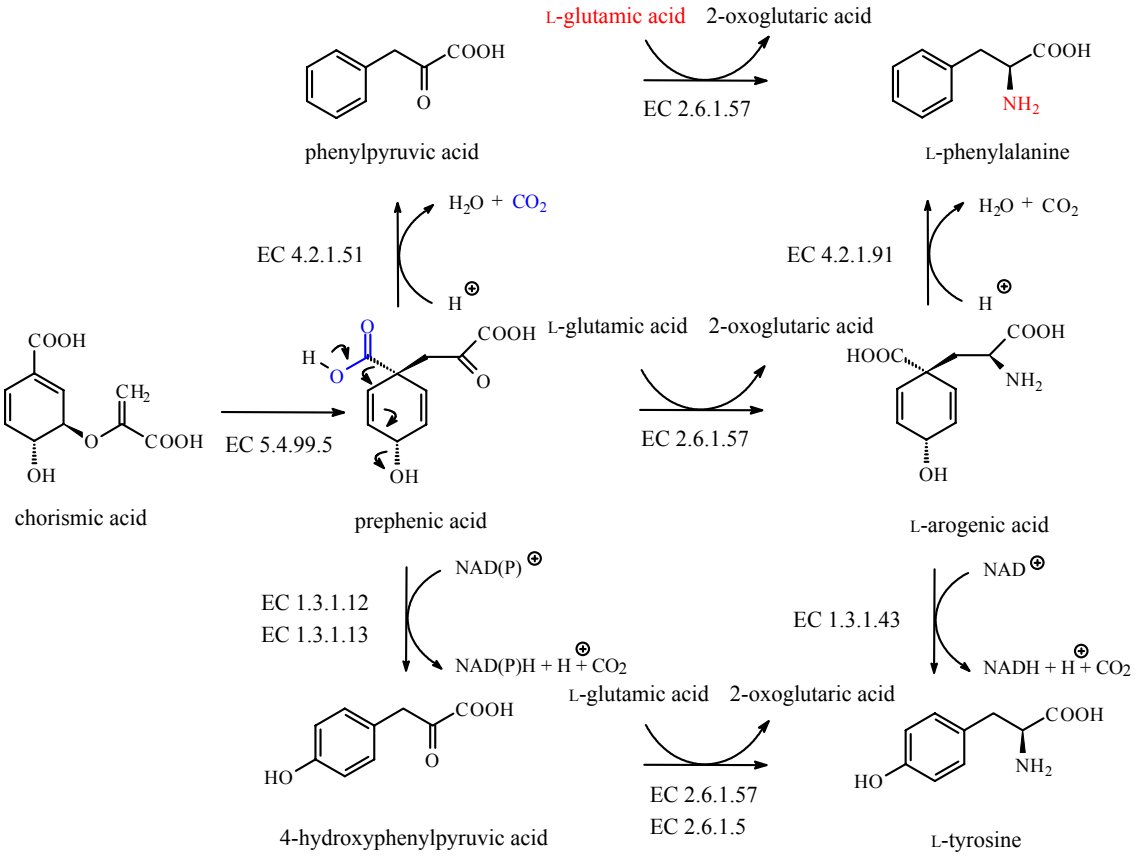

Figure 17

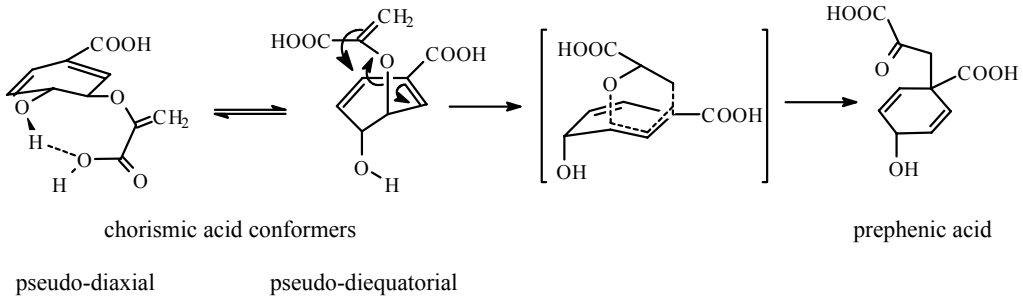

Figure 18

or tyrosine transaminase (EC 2.6.1.5) subsequently gives tyrosine (Clark *et al.* 1990; IUBMB 2001; Martí *et al.* 2003).

L-Arogenic acid is the result of transamination of prephenic acid occurring prior to the decarboxylation, and can be transformed into both phenylalanine and tyrosine depending on the absence or presence of a suitable enzyme activity, e.g. activity of arogenate dehydratase (EC 4.2.1.91) and arogenate dehydrogenase (EC 1.3.1.43), respectively (IUBMB 2003). In animals, which lack this pathway, direct hydroxylation of phenylalanine to tyrosine (and of tyrosine to DOPA) may be achieved. This reaction is catalysed by (6*R*)-5,6,7,8-tetrahydro-L-biopterin (L-*erythro*-5,6,7,8-tetrahydrobiopterin, BH_4)-dependent hydroxylase enzyme (phenylalanine hydroxylase, EC 1.14.16.1)[4], the hydroxyl oxygen being derived from molecular oxygen. The second oxygen atom is reduced to water (Figure 19). The rationalised reaction mechanism is given in Figure 20 (Carr *et al.* 1995; IUBMB 2001).

3.2 Tryptophan

Tryptophan is derived from chorismic acid via anthranilic (2-aminobenzoic) acid in a reaction catalysed by anthranilate synthase (EC 4.1.3.27) (Figure 21). In some organisms this enzyme is a part of a multifunctional protein having more other enzymatic activities for the biosynthesis of tryptophan, i.e. anthranilate phosphoribosyltransferase (EC 2.4.2.18), phosphoribosylanthranilate isomerase (EC 5.3.1.24), indole-3-glycerol-phosphate synthase (EC 4.1.1.48), and tryptophan synthase (EC 4.2.1.20) activity (IUBMB 2001).

Chorismic acid is first aminated at C-2 by L-glutamine to give 2-amino-2-deoxyisochorismic acid,

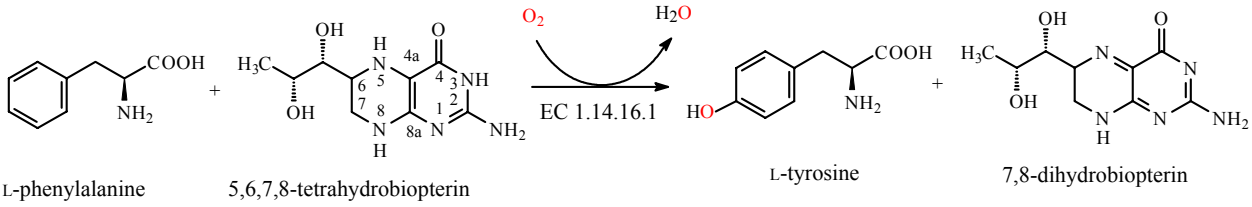

Figure 19

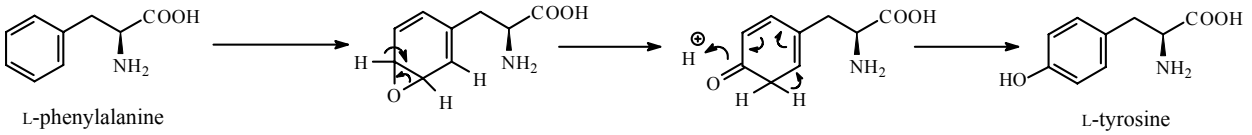

Figure 20

[4]The product of tetrahydrobiopterin reduction is 4a-hydroxytetrahydrobiopterin. It can dehydrate 6,7-dihydrobiopterin, both spontaneously and by the action of 4a-hydroxytetrahydropterin dehydratase (EC 4.2.1.96). The 6,7-dihydrobiopterin can be enzymatically reduced back to tetrahydrobiopterin by 6,7-dihydropteridine reductase (EC 1.5.1.34), or spontaneously rearranges to the more stable 7,8-dihydrobiopterin.

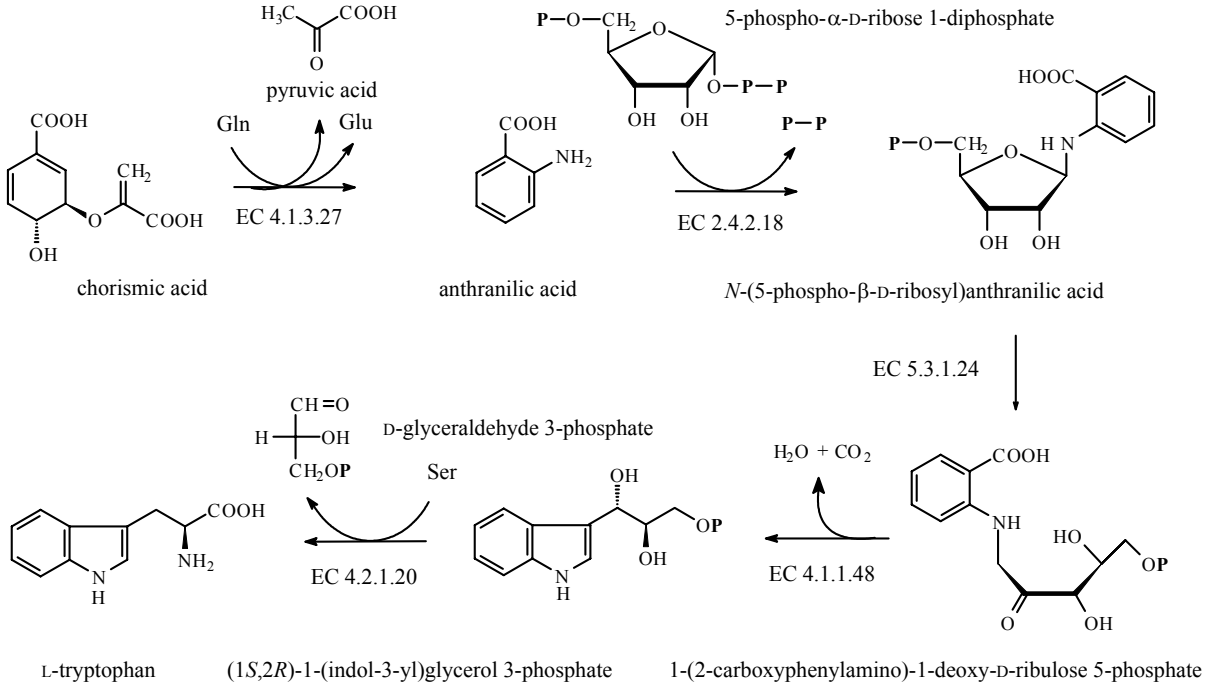

Figure 21

which yields anthranilic acid by elimination of the C-4 hydroxyl as water and C-3 substituent as pyruvic acid (Figure 22). The transformation involves an S_N2'-type of reaction, incoming ammonia, generated from glutamine, acts as a nucleophile attacking the diene system.

5-Phospho-α-D-ribose 1-diphosphate is the activated form of ribose. It forms from either AMP in a reaction catalysed by adeninephosphoribosyl transferase (EC 2.4.2.7) or from D-ribose 5-phosphate (forming in pentose phosphate pathway) and ATP by ribose-phosphate diphosphokinase

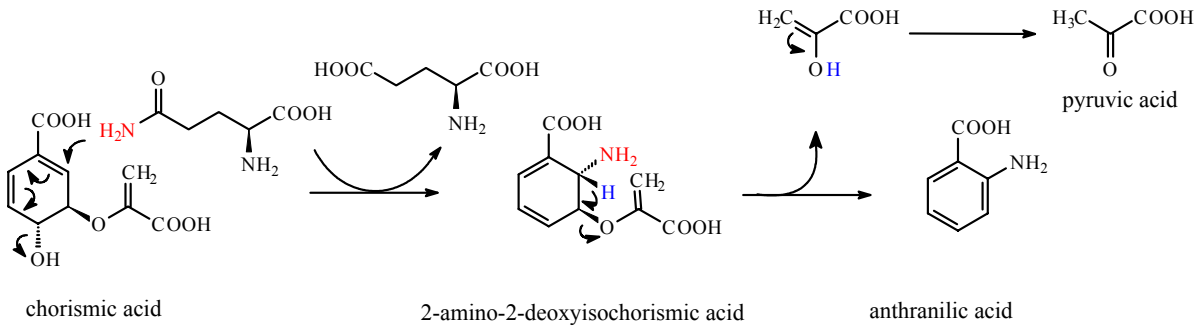

Figure 22

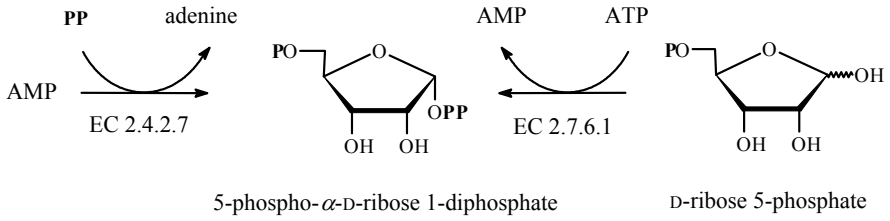

Figure 23

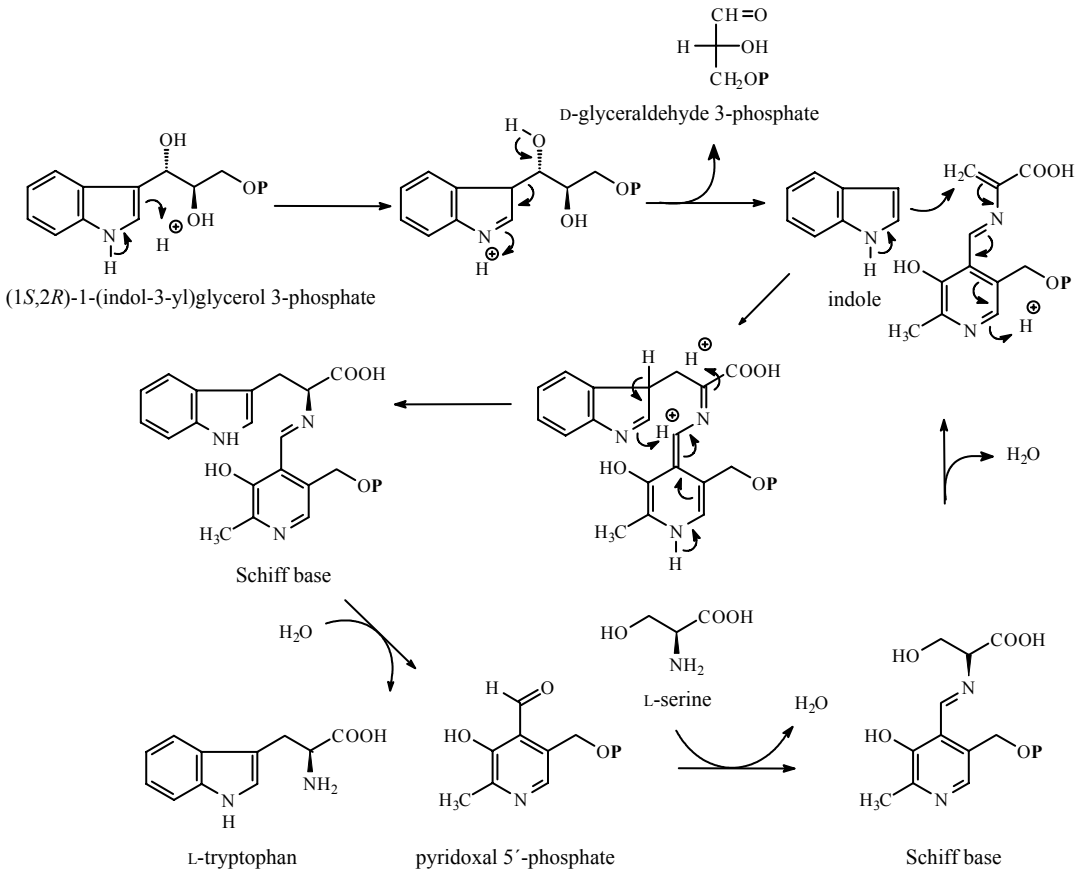

Figure 24

(EC 2.7.6.1) (Figure 23). It has also a key position in the biosynthesis of histidine and purine nucleotides (IUBMB 2003).

The tryptophan synthase multienzyme complex (EC 4.2.1.20) catalyses the final step of tryptophan biosynthesis in its two subunits. In the first enzyme subunit, (1*S*,2*R*)-1-(indol-3-yl)glycerol 3-phosphate splits off D-glyceraldehyde 3-phosphate and yields indole. Indole moves to the second enzyme subunit where it reacts with the dehydrated Schiff base of L-serine yielding the tryptophan-PLP Schiff base, which is then hydrolysed to the parent compounds, tryptophan and PLP (Figure 24).

3.3 Histidine

The biosynthetic pathway of L-histidine is unusual in that five of its carbon atoms are produced from 5-phospho-α-D-ribose 1-diphosphate, the key intermediate of tryptophan biosynthesis. One carbon atom comes from ATP (Figure 25). The early stages of histidine biosynthesis involve condensation of 5-phospho-α-D-ribose 1-diphosphate with ATP, followed by elimination of diphosphoric acid, which is catalysed by ATP phosphoribosyltransferase (EC 2.4.2.17). Elimination of diphosphoric acid from this product, N^1-5'-phosphoribosyl-ATP, is catalysed by phosphoribosyl-ATP diphosphatase (EC 3.6.1.31) and yields *N*'-5'-phosphoribosyl-AMP. The pyrimidine ring of this compound opens by phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19) under the formation of *N*'-5'-phosphoribosylformimino-5-amino-imidazole-4-carboxamide ribonucleotide. Then 1-(5-phosphoribosyl)-5-[(phosphoribosyl amino)methylene amino]imidazole-4-carboxamide isomerase (EC 5.3.1.16) splits the ribose attached at N^1 to form 5-amino-4-carboxamide ribonucleotide and the intermediate fragment, which reacts with ammonia from glutamine to form 3-(imidazol-4-yl)-1-glycerol phosphate. The reaction catalysed by imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19) converts this compound

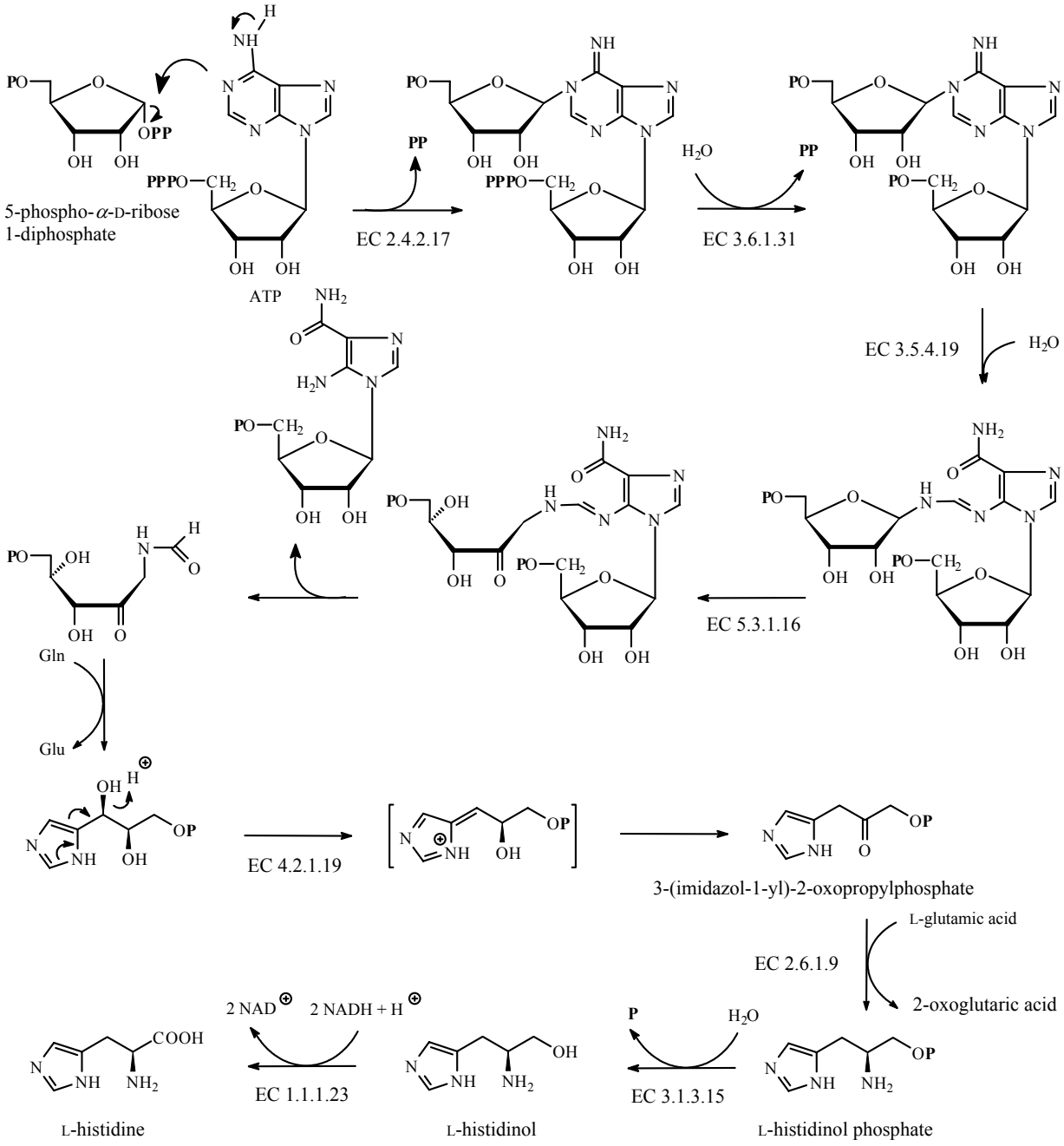

Figure 25

to 3-(imidazol-4-yl)-2-oxopropyl phosphate, which yields, in a transamination reaction catalysed by a PLP protein histidinol-phosphate transaminase (EC 2.6.1.9), L-histidinol phosphate. Histidinol phosphate is hydrolysed to L-histidinol by histidinol-phosphatase (EC 3.1.3.15) and histidinol is finally oxidised to L-histidine by the NAD-dependent histidinol dehydrogenase (EC 1.1.1.23) (IUBMB 2001).

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 ionized, 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 – denosine 5'-diphosphate
AMP – adenosine 5'-monophosphate
APS – adenosine-5'-phosphosulfuric acid
ATP – adenosine 5'-triphosphate
CoA – coenzyme A as a part of a thioester
FMN – flavin mononucleotide
GSH – glutathione (reduced)
GSSG – glutathione (oxidised)
NADH – nicotinamide adenine dinucleotide
NADPH – nicotinamide adenine dinucleotide phosphate
P – phosphoric acid
PAPS – 3'-phosphoadenosine-5'-phosphosulfate
PLP – pyridoxal 5'-phosphate
PP – diphosphoric acid

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