Biosynthesis of Food Constituents: Vitamins. 2. Water-Soluble Vitamins: Part 2 – a Review

Jan VELÍŠEK and Karel CEJPEK

Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

Velíšek J., Cejpek K. (2007): **Biosynthesis of food constituents: Vitamins. 2. Water-soluble vitamins: Part 2 – a Review**. Czech J. Food Sci., **25**: 101–118.

This review article gives a survey of the biosynthetic pathways that lead to water-soluble vitamins in microorganisms, plants and some animals. The biosynthetic pathways leading to some the B-group vitamins (biotin, folacin, cobalamins) and to vitamin C are described in detail using reaction schemes and mechanisms with enzymes involved and detailed explanations based on chemical principles and mechanisms.

Keywords: biosynthesis; B-group vitamins; biotin; folates; folic acid; cobalamins; vitamin B_{12}; vitamin C; L-ascorbic acid; D-erythro-ascorbic acid

The majority of water-soluble vitamins (thiamin, riboflavin, pantothenic acid, vitamin B_6) and their active forms were already reviewed (Velíšek & Cejpek 2007).

6 BIOTIN

The biologically active (3*aS*,4*S*,6*aR*)-isomer of biotin or [3*aS*-(3*a*α,4β,6*a*α)]hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid, also known as vitamin H (and formerly as bios II, factor X, coenzyme R, or vitamin B_8), is required by all living cells. It occurs as a prosthetic group of a variety of enzymes known as carboxylases, transcarboxylases, and decarboxylases that catalyse the transfer of carbon dioxide and thus play a significant role in the biosynthesis of fatty acids saccharides and in catabolism of branched-chain amino acids.

Plants, fungi, and the majority of microorganisms synthesise biotin and the pathway of its biosynthesis seems to be conserved throughout the evolution (Ploux 2000). This common pathway starts from pimelic acid (Figure 20), which is first transformed

Partly supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6046137305.

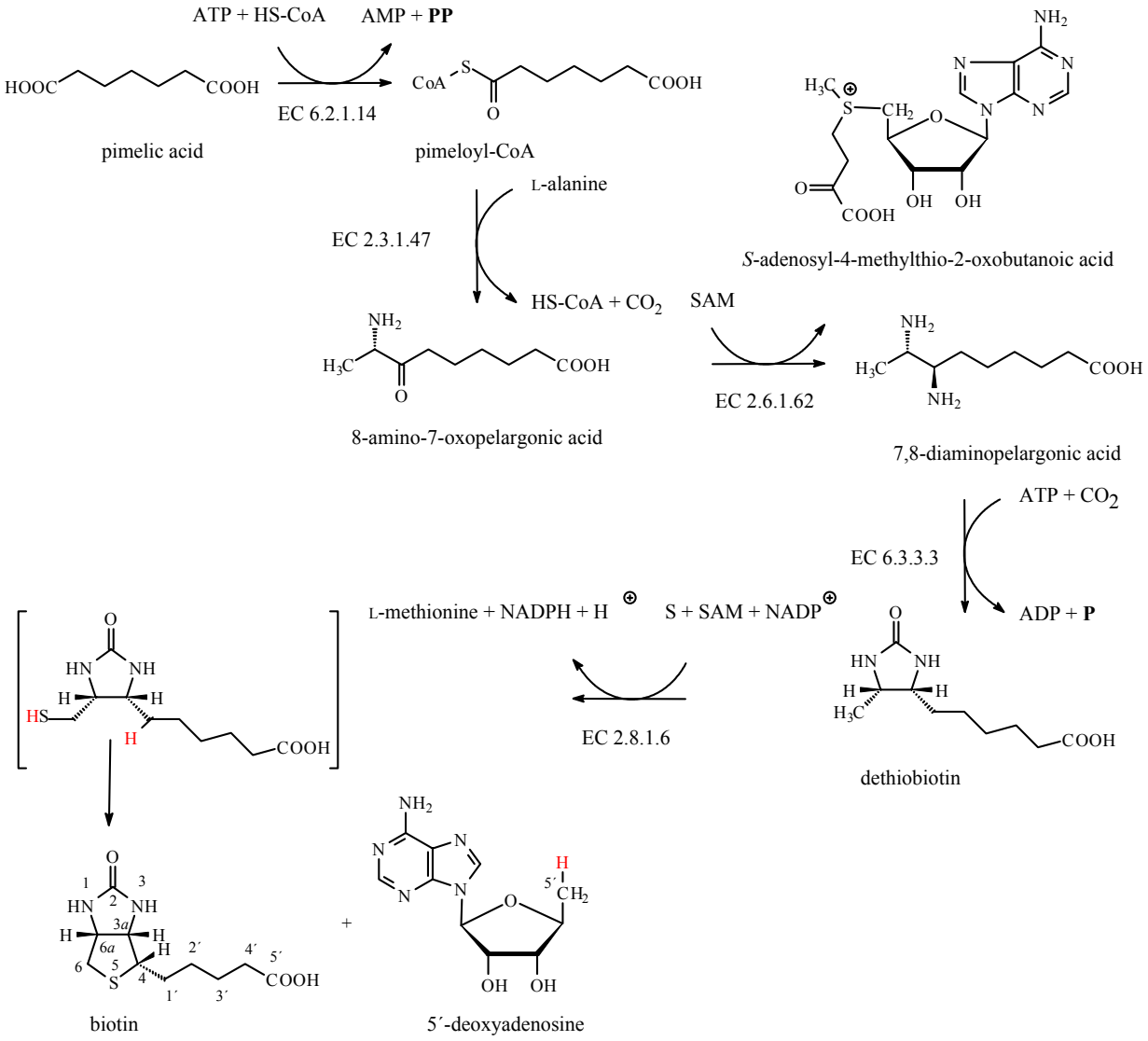

Figure 20

to the active form, pimeloyl-CoA 1)[8], by the action of pimeloyl-CoA synthetase (6-carboxyhexanoyl-CoA ligase, EC 6.2.1.14).

Under the catalysis by 8-amino-7-oxopelargonate synthase (or 8-amino-7-oxononanonate synthase, EC 2.3.1.47), pimeloyl-CoA reacts with L-alanine and yields 8-amino-7-oxopelargonic acid (8-amino-7-oxononanoic acid), which is further transformed to 7,8-diaminopelargonic acid (7,8-diaminononanoic acid) by the action of 7,8-diaminopelargonate transaminase (EC 2.6.1.62). Dethiobiotin synthase (EC 6.3.3.3) then catalyses the formation of the imidazole part of dethiobiotin. The final unprecedented reaction in biochemistry, the transformation of dethiobiotin to biotin is catalysed by biotin synthase (EC 2.8.1.6)[9]. The biotin synthase Fe-S cluster functions as the immediate sulfur donor for the biotin formation. This

[8]For example, *Bacillus subtilis* requires free pimelic acid, but pimeloyl-CoA rather than free pimelic acid is required by *Escherichia coli*.

[9]This complex enzyme system comprises biotin synthase, L-cystein, dithioerythritol, Fe^{2+} ion, *S*-adenosyl-L-methionine (SAM), and a reducing agent (flavodoxin, flavodoxin reductase, and $NADP^+$ or photoreduced deazaflavin). When biotin synthase is reduced, the cluster dimerises to give a $[4Fe\text{-}4S]^+$ cluster, which is the catalytically important species (Begley *et al.* 1999). Biotin is formed in substoichiometric amounts. Approximately three moles of SAM are required per mole of biotin produced.

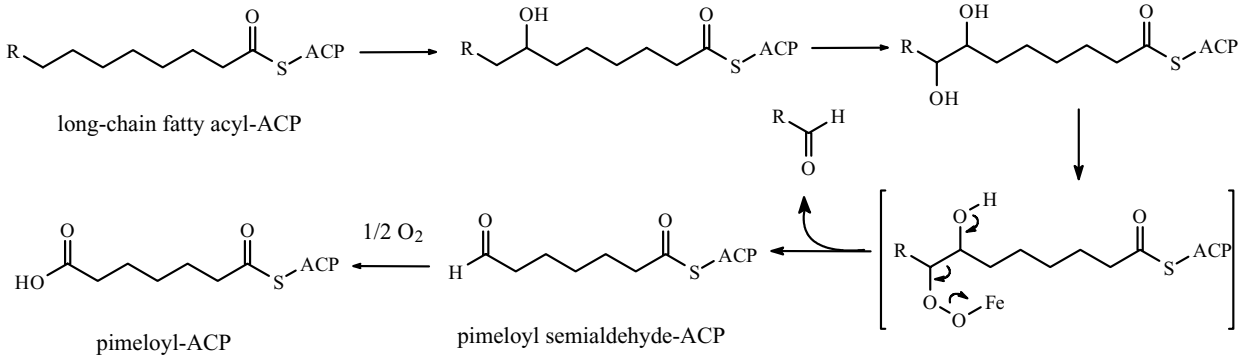

Figure 21

reaction requires *S*-adenosyl-L-methionine (SAM) which is reductively cleaved by the reduced Fe-S cluster of biotin synthase to yield the adenosyl radical. This radical then abstracts hydrogen from the CH_3 group of dethiobiotin and the resulting radical probably reacts with one of the sulfides of the Fe-S cluster to yield the intermediate thiol, and the abstraction of hydrogen from the first CH_2 group in the side-chain then yields biotin. The SAM derived products are L-methionine and 5'-deoxyadenosine (Begley *et al.* 1999).

Pimelic acid formation has been proposed to proceed by oxidative cleavage of ACP-bound long-chain fatty acid (Stok & De Voss 2000). The reaction proceeds via two sequential hydroxylation reactions to produce a diol intermediate. A third oxidative reaction then produces either peroxide or a peroxyl radical which subsequently decomposes with C-C bond cleavage to yield two carbonyl compounds, one of which is a pimeloyl semialdehyde derivative of ACP. The conversion of the pimeloyl semialdehyde derivative to pimeloyl-ACP may proceed either via autoxidation, cytochrome P_{450} catalysed oxidation[10], or both (Figure 21). The acyl chain can be released as a free pimelic acid e.g. by acyl-ACP hydrolase (acyl-ACP thioesterase, EC 3.1.2.14), analogously to the fatty acids formation (Velíšek & Cejpek 2006b).

7 FOLACIN

Folacin is a common name for the biologically active derivatives of folic (pteroyglutamic) acid (formerly also known as vitamin B_9, vitamin B_c or vitamin M) (Friedrich 1988; Dewick 2002). Folic acid, is a so called conjugate of 4-aminobenzoic acid and L-glutamic acid, i.e. 4-[(pteridin-6-yl-methyl)amino]benzoic acid attached to one or more L-glutamic acid residues (IUPAC). Generally, three to eight glutamic acid residues occur in natural folates. The active constituent of folates is (6*S*)-5,6,7,8-tetrahydrofolic acid, i.e. (6*S*)-5,6,7,8-tetrahydropteroylglutamic acid (H_4PteGlu).

The activity of tetrahydrofolic acid is analogous to that of cobalamins as it functions as a carrier of one-carbon functional groups which may be in the form of methyl, methylene, methenyl, or formyl groups. The vitamin is a coenzyme that play a vital role in the metabolism of amino acids, purine, and pyrimidine nucleotides.

Many microorganisms and plants synthesise 7,8-dihydrofolic (7,8-dihydropteroylglutamic) acid (7,8-H_2PteGlu), the precursor of folic acid coenzymes, *de novo*. Mammals, however, are dependent for their nutrition on the basic molecule, and can only carry out two step reduction of folic acid to tetrahydrofolic acid via dihydrofolic acid and the biosynthesis of dihydrofolic acid from folic acid. Mammals can also add or remove additional glutamyl residues.

7.1 7,8-Dihydrofolic acid

The biosynthesis of 7,8-dihydrofolic acid in microorganisms and plants starts from guanosine 5'-triphosphate (GTP), the common precursor in the biosynthetic pathway to dihydrofolic acid and to riboflavin (Friedrich 1988; KEGG) (Fig-

[10]These proteins contain a cysteine-ligated heme with which they activate molecular oxygen, producing a molecule of water and an equivalent of an Fe(V) oxo species that is responsible for the vast array of oxidative reactions that they perform. The activation of oxygen requires two electrons, which are ultimately derived from NADH or NADPH and are usually supplied by cytochrome P_{450} reductase (EC 1.6.2.4) in eucaryotes and an iron-sulfur redoxin (EC 1.8.4.8) in procaryotes.

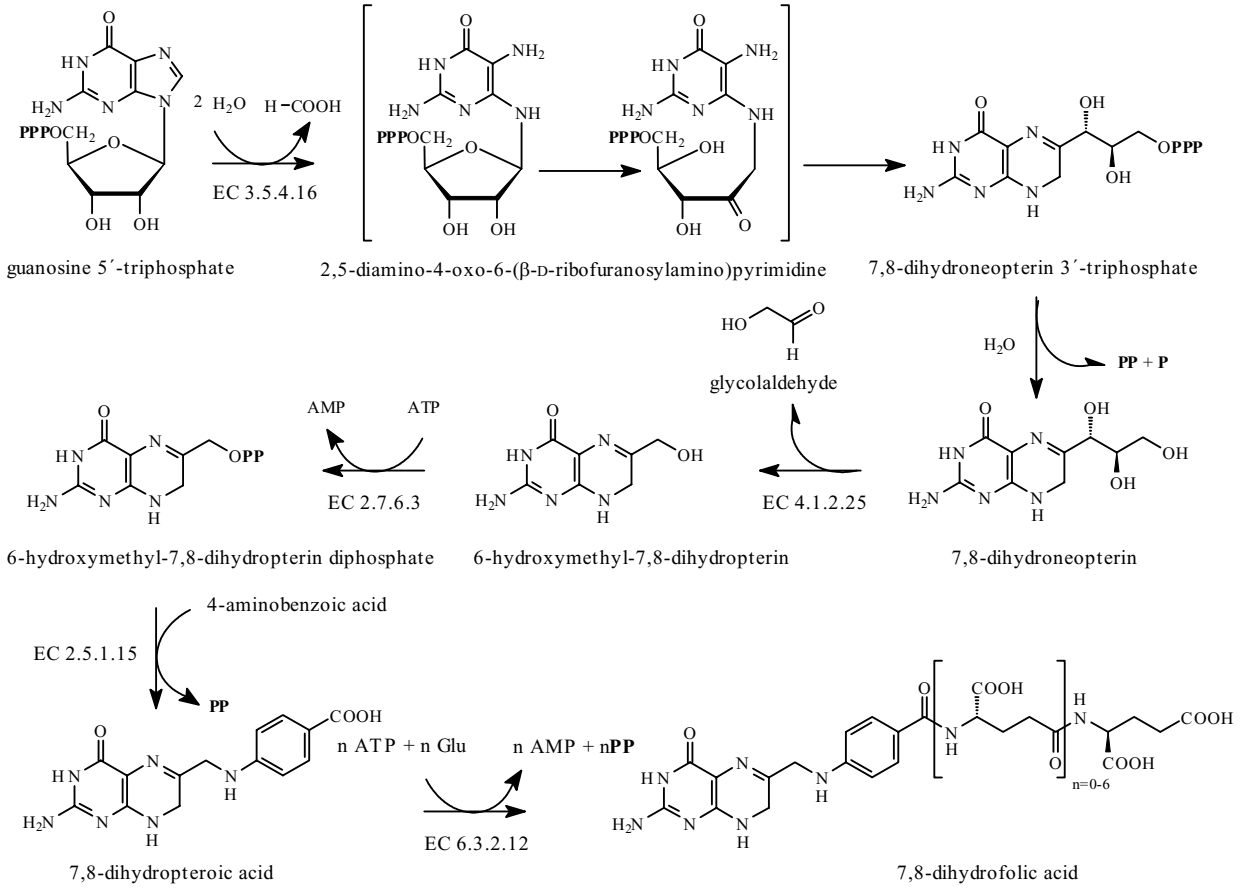

Figure 22

ure 22). The cleavage of the imidazole ring of GTP and the elimination of the C-8 carbon as formic acid is catalysed by GTP cyclohydrolase I (EC 3.5.4.16) and yields 2,5-diamino-4-oxo-6-(ribofuranosylamino)pyrimidine as the first intermediate. The elimination reaction is followed by the Amadori rearrangement of thus formed *N*-substituted β-D-ribofuranosylamine to yield the corresponding Amadori product, *N*-substituted 1-amino-1-deoxy-D-ribulose), and then followed by ring closure of the pyrazine ring to form 7,8-dihydroneopterin 3'-triphosphate (the common precursor for tetrahydrofolic acid, biopterin, and many other natural pterins). Its dephosphorylation by not yet characterised enzyme(s) yields 7,8-dihydroneopterin. The chain shortening of the side-chain of 7,8-dihydroneopterin (elimination of glycolaldehyde by a retroaldolisation reaction), catalysed by dihydroneopterin aldolase, EC 4.1.2.25) to form 6-hydroxymethyl-7,8-dihydroneopterin, is followed by a reaction with ATP to form the corresponding diphosphate (2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase, EC 2.7.6.3). 6-Hydroxymethyl-7,8-dihydroneopterin diphosphate then reacts, under catalysis of dihydropteroate synthase (EC 2.5.15), with 4-aminobenzoic acid to form 7,8-dihydropteroic acid. The formation of 7,8-dihydrofolic acid from 7,8-dihydropteroic acid and L-glutamic acid, with participation of ATP, is catalysed by dihydrofolate synthase (EC 6.3.2.12).

4-Aminobenzoic acid, forming part of the structure of 7,8-dihydrofolic acid, is produced from chorismic acid (Figure 23). Chorismic acid is first aminated to give 4-amino-4-deoxychorismic acid (aminodeoxychorismate synthase/glutamine aminotransferase), which eliminates pyruvic acid (aminodeoxychorismate lyase, EC 4.1.3.38) to yield 4-aminobenzoic acid.

7.2 Coenzyme forms of folic acid

The biosynthesis of the coenzyme forms of folic acid consists of several steps, the reduction of dihydrofolic acid to tetrahydrofolic acid (Figure 24) catalysed by dihydrofolate reductase (EC 1.5.1.3), the incorporation and interconversion of C_1 units of various kinds,

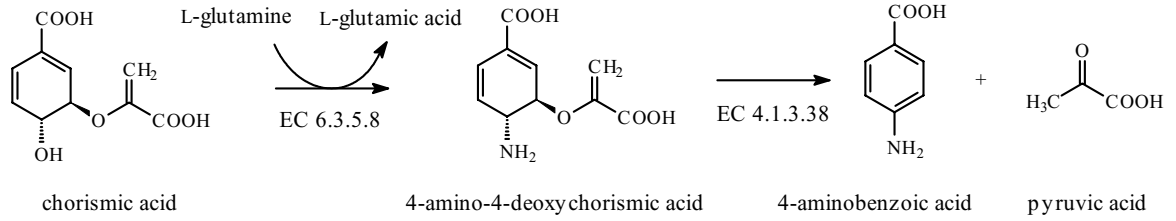

Figure 23

and the synthesis of the polyglutamyl chain. These reactions are catalysed by a number of enzymes, which have been studied in detail (KEGG).

8 COBALAMINS

The term vitamin B_{12} is used in two different ways. In a broader sense, it refers to a group of cobalt-containing organometallic compounds (the covalent C-Co bond with cobalt(III) ion is the only carbon-metal bond known in biochemistry). These compounds contain 5,6-dimethylbenzimidazole and are known as cobalamins. The most important cobalamins are cyanocobalamin (vitamin B_{12}), the intermediate forms of the cofactors, i.e. aquacobalamin (also known as vitamin B_{12a}), and hydroxocobalamin (vitamin B_{12b}, aquacobalamin is the conjugate acid of hydroxocobalamin), and the two metabolically active coenzyme forms of vitamin B_{12}, methylcobalamin (methylvitamin B_{12}) and 5'-deoxy-5'-adenosylcobalamin (often called 5'-deoxyadenosylcobalamin or adenosylvitamin B_{12}). In a more specific way, the term vitamin B_{12} is used to refer to only one of these forms, cyanocobalamin, which is the principal vitamin B_{12} form used for foods and in nutritional supplements (IUPAC).

Cobalamins belong to a group of compounds called corrinoids that are based upon the skeleton of corrin (Figure 25). The corrin nucleus contains four partially reduced pyrrole rings (A, B, C, and D) joined into a macrocyclic-ring system by links between their α position. Three of these links are formed by one-carbon unit (methine bridge) and the other by a direct Cα-Cα bond between rings A and D. This highly substituted macrocycle is adorned with seven peripheral methyl groups. Attached to the corrin ring are seven amide chains, named *a-g*. On the side α axial of the pseudo-planar framework is a distinct nucleotide loop, containing a 2,3-dimethylimidazole that coordinates cobalt in 1-position and continues as a nucleotide-like moiety from the 3-position, i.e. with a ribose ring, a phosphate and eventually an *N*-propyl propanamide linkage back to the corrin ring at the *f*-chain. The β axial ligand of the corrin ring can be a variety of neutral species and anions. The species of known biochemical occurrence include 5'-deoxy-5'-adenosylcobalamin (R = 5'-de-

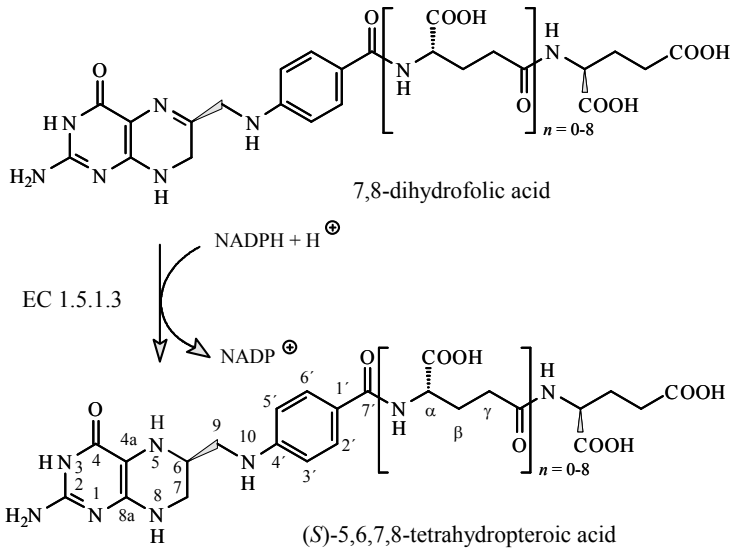

Figure 24

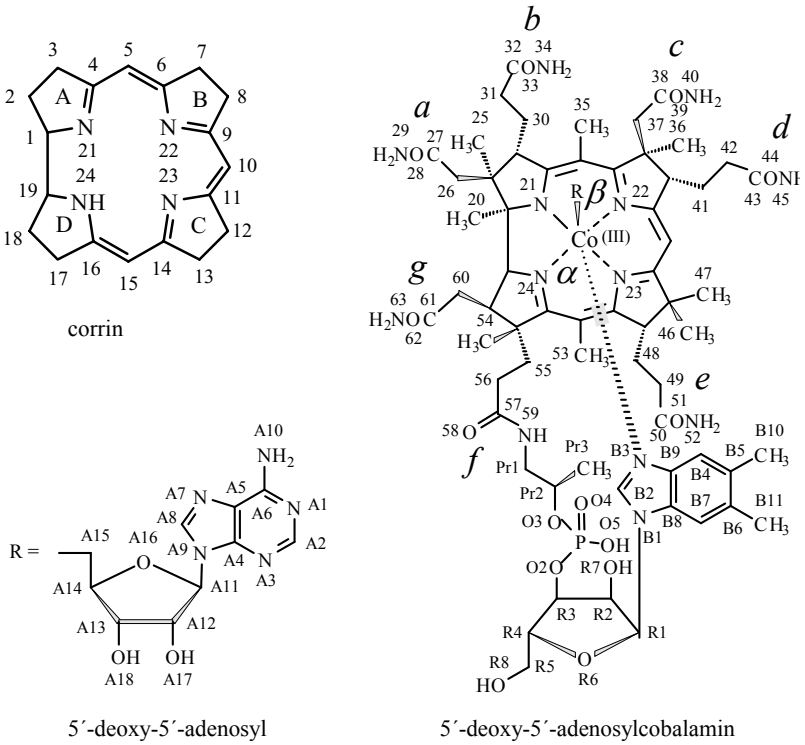

Figure 25

oxy-5'-adenosyl), methylcobalamin (R = CH_3), aquacobalamin (R = H_2O), cyanocobalamin (R = CN-), with the ligands coordinated to cobalt (Sirovatka *et al.* 2000; Jensen & Mikkelsen 2001; Marques *et al.* 2001).

There is a close structural relationship between corrinoids and porphyrins (primarily represented by chlorophylls and heme pigments). However, one of the most prominent features of corrinoids is that the macrocycle has undergone a unique extrusion of one of the ring carbons (C-20) during the process of the ring contraction. The numbering of corrinoids is thus the same as that of porphyrin nucleus, number 20 being omitted to preserve the identity (Figure 25).

In spite of their apparently similar structures, the B12 coenzymes function as cofactors for two quite different types of enzymatic reactions (Jensen & Mikkelsen 2001; Marques *et al.* 2001; Marsh & Drennan 2001). Methylcobalamin is used in certain transmethylation reactions, including methylation of homocysteine to form methionine and in the pathway for fixing carbon dioxide in certain anaerobic acetogenic microorganisms. All methylcobalamin-dependent enzymes transfer a methyl cation by heterolytic cleavage of the cobalt-carbon bond, giving the Co(I) intermediate. The 5'-deoxy-5'-adenosylcobalamin-dependent enzymes, on the other hand, generate a substrate radical and the Co(II) square-pyramidal intermediate, induced by homolytic cleavage of the Co-C bond (the so-called activation of the coenzyme, which is the first step in the catalytic cycle). The cofactor is involved in the enzymatic catalysis of

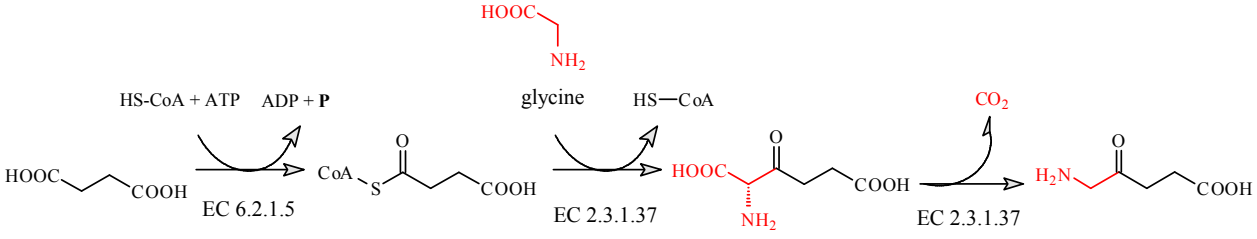

Figure 26

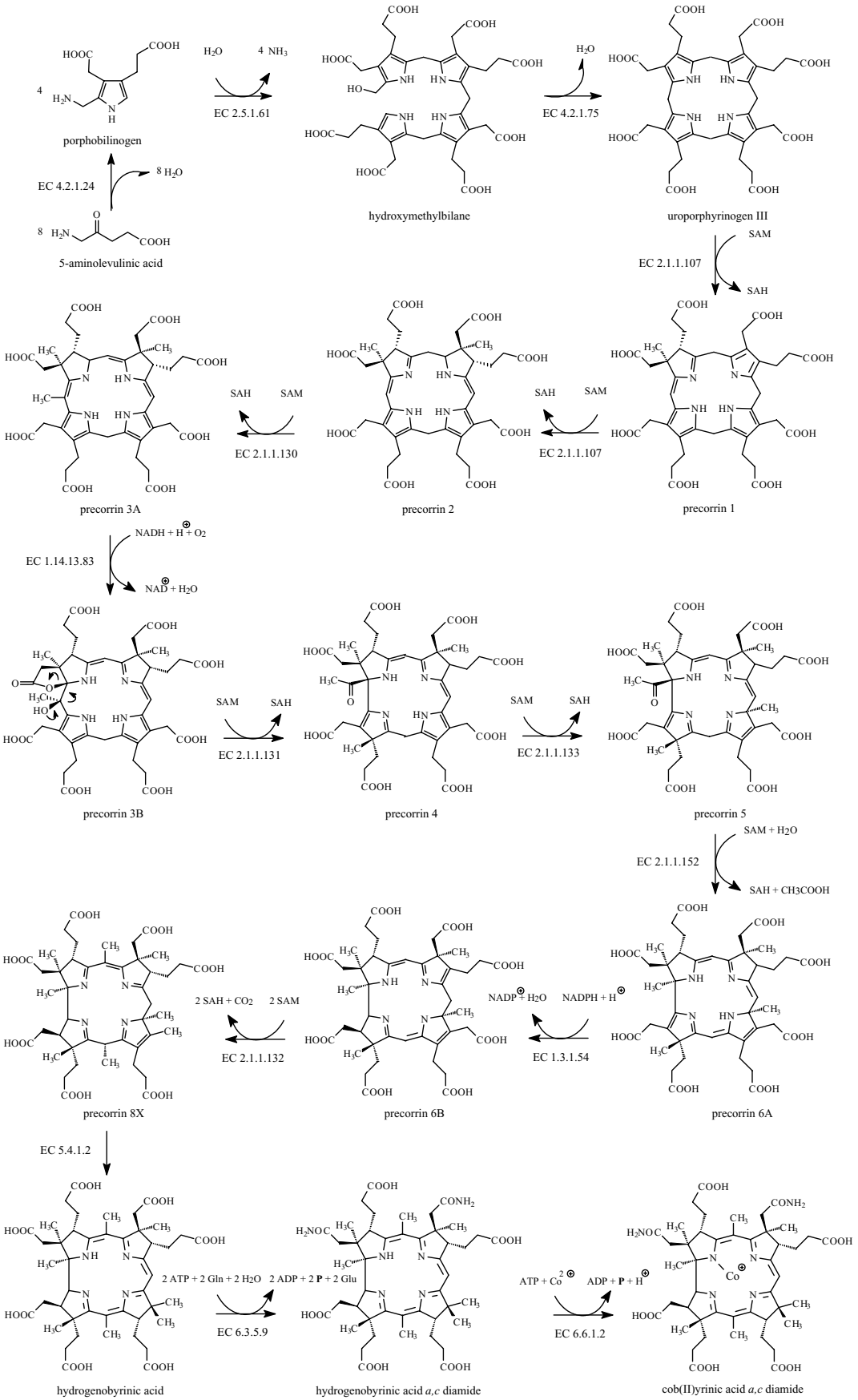
porphobilinogen
5-aminolevulinic acid
hydroxymethylbilane
uroporphyrinogen III
precorrin 3A
precorrin 2
precorrin 1
precorrin 3B
precorrin 4
precorrin 5
precorrin 8X
precorrin 6B
precorrin 6A
hydrogenobyrinic acid
hydrogenobyrinic acid a,c diamide
cob(II)yrinic acid a,c diamide
EC 2.5.1.61
EC 4.2.1.75
EC 4.2.1.24
EC 2.1.1.107
EC 2.1.1.130
EC 1.14.13.83
EC 2.1.1.131
EC 2.1.1.133
EC 2.1.1.152
EC 2.1.1.132
EC 1.3.1.54
EC 5.4.1.2
EC 6.3.5.9
EC 6.6.1.2

Figure 27

a variety of chemically difficult intramolecular 1,2-rearrangements catalysed by 5'-deoxy-5'-adenosylcobalamin-dependent isomerases and in the reduction of ribonucleotides to deoxyribonucleotides in some organisms.

Vitamin B_{12} is synthesised by many bacteria, some cyanobacteria and even some yeasts. The ultimate source of cobalamin for mammalian metabolism is microbial synthesis. The cobalamins from the microbial flora in the gastrointestinal system of herbivorous animals are absorbed by the host and stored in their tissues. From there, cobalamin is passed on to other animals in the food chain.

The biosynthesis of cobalamin by bacteria proceeds either aerobically or anaerobically (IUPAC; KEGG; Roessner *et al.* 2001). The starting compound of both pathways is 5-aminolevulinic (5-amino-4-oxopentanoic) acid derived from the glycine metabolism. The condensation of glycine with succinyl-CoA is catalysed by 5-aminolevulinate synthetase (EC 2.3.1.37) and yields L-2-amino-3-oxoadipic (L-2-amino-3-oxohexanedioic) acid, which eliminates carbon dioxide to produce 5-aminolevulinic acid. In the presence of Mg^{2+} and ATP, the biosynthesis of succinyl-CoA from succinic acid is catalysed by succinate-CoA ligase (ADP-forming) (EC 6.2.1.5) also known as succinate thiokinase (Figure 26).

The first three steps of both pathways (aerobic and anaerobic) follow those of porphyrin biosynthesis (Figure 27). These steps start by the condensation of two 5-aminolevulinic acids to form porphobilinogen (aminolevulinic acid dehydratase, also known as porphobilinogen synthase, EC 4.2.1.24). This reaction is followed by the conversion of four porphobilinogens to yield hydroxymethylbilane (hydroxymethylbilane synthase, EC 2.5.1.61)[11]. In the presence of a second enzyme, uroporphyrinogen III synthase (EC 4.2.1.75), which is often called cosynthase, hydroxymethylbilane is cyclised under the ring D inversion to form uroporphyrinogen III (urogen III), the common intermediate in the biosyn-

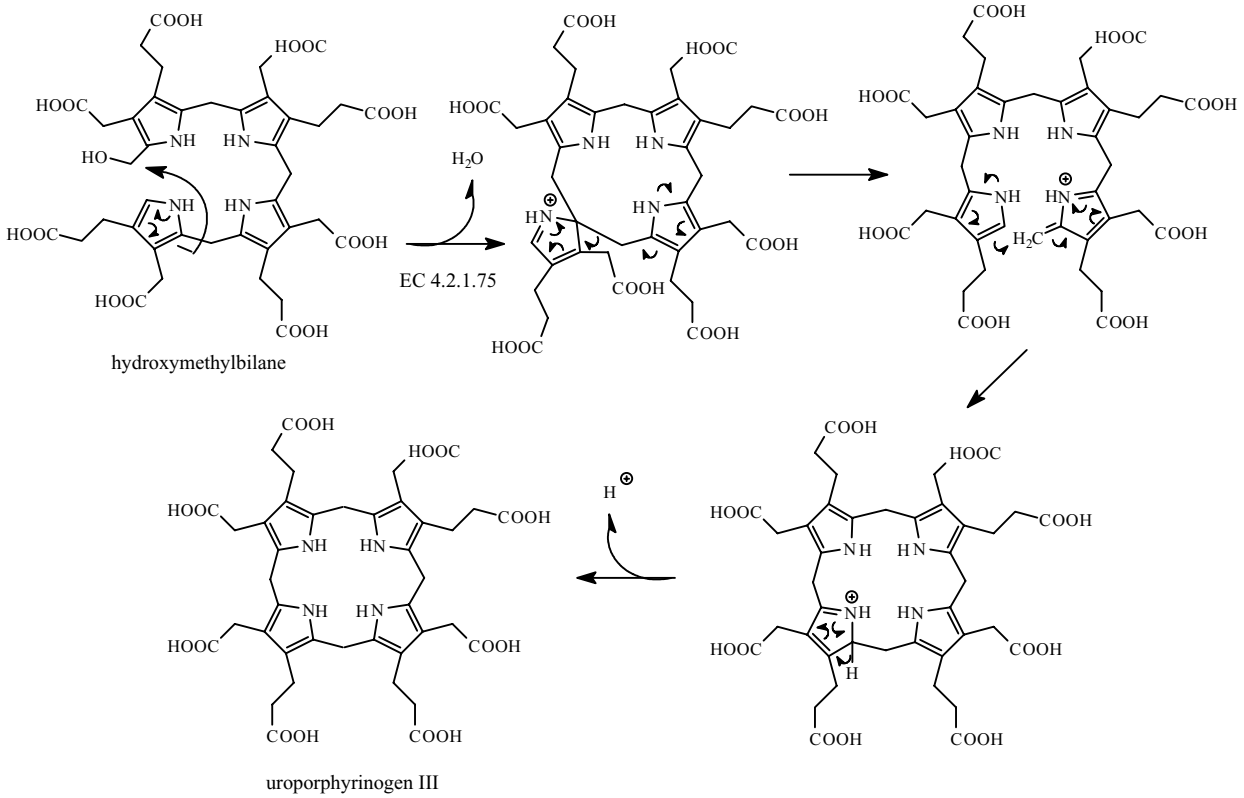

Figure 28

[11]The enzyme contains at its active site a unique dipyrrolomethane cofactor on which the tetrapyrrole is built. The enzyme works by stepwise addition of pyrrolylmethyl groups until a hexapyrrole is present at the active centre. The terminal tetrapyrrole is then hydrolysed to yield the product, leaving a cysteine-bound dipyrrole on which the assembly continues.

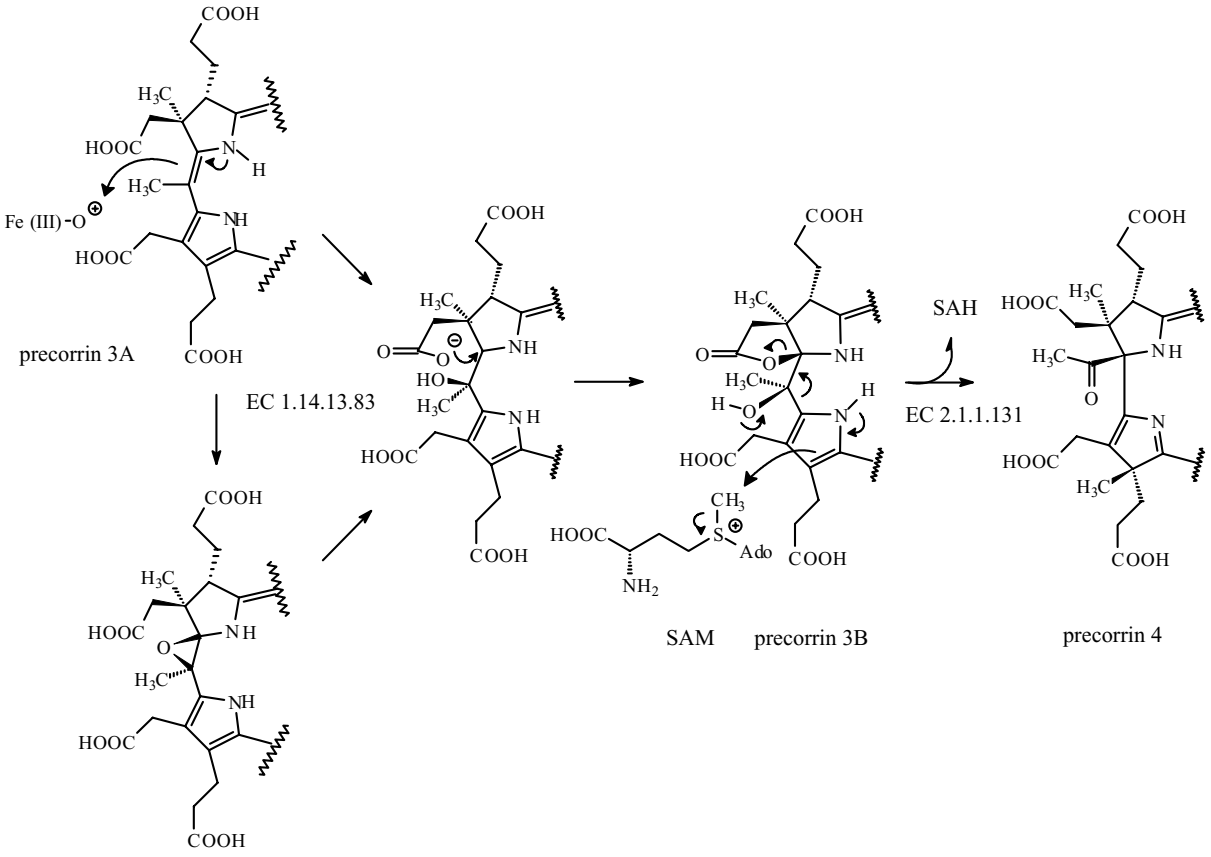

Figure 29

thesis of the corrinoids and the porphyrins (Figure 28). If uroporphyrinogen III synthase is absent, hydroxymethylbilane cyclises spontaneously. The next step is catalysed by uroporphyrin III methyltransferase (EC 2.1.1.107). The enzyme catalyses two sequential methylation reactions, the first forming precorrin 1 by methylation of C-2 carbon, which is further methylated at C-7 to form precorrin 2. Up to this point the aerobic and anaerobic pathways are virtually undistinguishable.

In the aerobic cobalamin biosynthesis (e.g. by *Pseudomonas denitrificans*), the next product formed by methylation of precorrin 2 at C-20 is precorrin 3A (precorrin 2 C-20-methyltransferase, EC 2.1.1.130) (Figure 27). The subsequent reaction (an oxygen atom from oxygen is incorporated into the macrocycle at C-20 as a hydroxyl group and a γ-lactone is formed; this hydroxylation spring-loads the mechanism for the contraction of the macrocycle by installing a masked pinacol) is carried out by precorrin 3B synthase (EC 1.14.13.83), yielding precorrin 3B (precorrin 3 hydroxylactone) as the product. The mechanism of hydroxylation at C-20 of precorrin 3A with the subsequent formation of a γ-lactone between C-1 and the acetate of ring A is explained by either the direct insertion of oxygen from an Fe(III)-O^+ species or by the formation of a 1,20-epoxide, which, for steric reasons, requires ring opening through the participation of the nitrogen electron pair rather than by a direct attack by the carboxylate of the C-2 acetate side chain (Figure 29). The formation of precorrin 3B is followed by three methylation reactions which introduce methyl groups at C-17 (precorrin 3B C-17-methyltransferase, EC 2.1.1.131, Figures 27 and 29)[12], C-11 (precorrin 4 C-11-methyltransferase, EC 2.1.1.133), and C-1 (precorrin 6A synthase, deacetylating, EC 2.1.1.152) of the macrocycle followed by acetic acid extrusion, giving rise to precorrin 4, precorrin 5 and pre-

[12]The addition of the fourth methyl group at C-17 to afford precorrin 4, catalysed by precorrin 3B C17-methyltransferase (EC 2.1.1.131), triggers the ring contraction, and is accompanied by the formation of a methyl ketone pendant from C-1 that has been derived by a pinacol-type rearrangement.

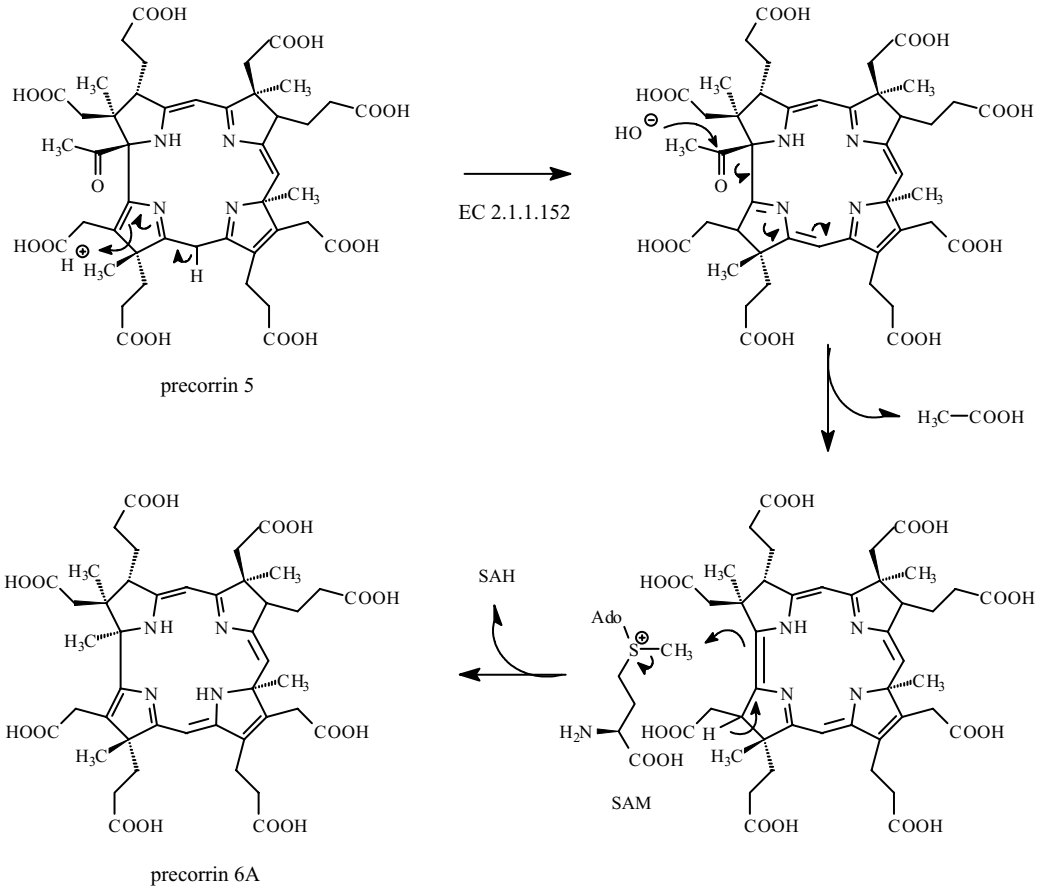

Figure 30

corrin 6A, respectively (Figure 30)[13]. Precorrin A reductase (EC 1.3.1.54) then catalyses the formation of precorrin 6B (dihydroprecorrin 6) from precorrin 6A (C-18/C-19 reduction) (Figure 31). This reaction is followed by the formation of precorrin 8 also known as precorrin 8X (C-5 and C-15 methylation and decarboxylation) using precorrin 6Y C-5,-15-methyltransferase (decarboxylating) (EC 2.1.1.132). A rearrangement of the C-11 methyl group (to become the C-12 *re*-methyl group found in vitamin B_{12}) then yields hydrogenobyrinic acid (precorrin 8X methylmutase, EC 5.4.1.2). The next step in the aerobic biosynthesis of cobalamin, *a*,*c*-amidation, generates hydrogenobyrinic acid *a,c* diamide (hydrogenobyrinic acid *a,c* diamide synthase, EC 6.3.5.9), the substrate required by cobaltochelatase (EC 6.6.1.2), which adds cobalt (Co^{2+} ion) to the macrocycle yielding cob(II)yrinic acid *a,c* diamide.

The first difference between the biosynthesis of vitamin B_{12} in aerobic and anaerobic conditions is that the anaerobes (e.g. *Salmonella typhimurium* and *Propionibacterium shermanii*) synthesise, in an NAD^+-dependent dehydrogenation, sirohydrochlorin (the precursor of the pigment siroheme in methanogenic bacteria formed under the catalysis of sirohydrochlorin ferrochelatase, EC 4.99.1.4) from precorrin 2 instead of precorrin 3 (precorrin 2 dehydrogenase, EC 1.3.1.76), and then the cobalt atom is inserted at this stage (sirohydrochlorin cobaltochelatase, EC 4.99.1.3) to yield cobalt precorrin 2 (Co-precorrin 2 or Co-sirohydrochlorin) (Figure 32). Methylation at C-20 of Co-precorrin 2 yields Co-precorrin 3 (precorrin 2 C-20-methyltransferase, EC 2.11.130). Methylation of Co-precorrin 3 at C-17, followed by the ring contraction and δ-lactone formation (precorrin 3B C-17-methyltrans-

[13]Precorrin 6A synthase (EC 2.1.1.152) is a bifunctional enzyme that catalyses the removal of the methyl ketone moiety of a tautomer of precorrin 5 (deacetylating enzyme). Deacetylation provides an intermediate that then undergoes C-1 methylation to afford a tautomer of precorrin 6A, which finally isomerises to precorrin 6A.

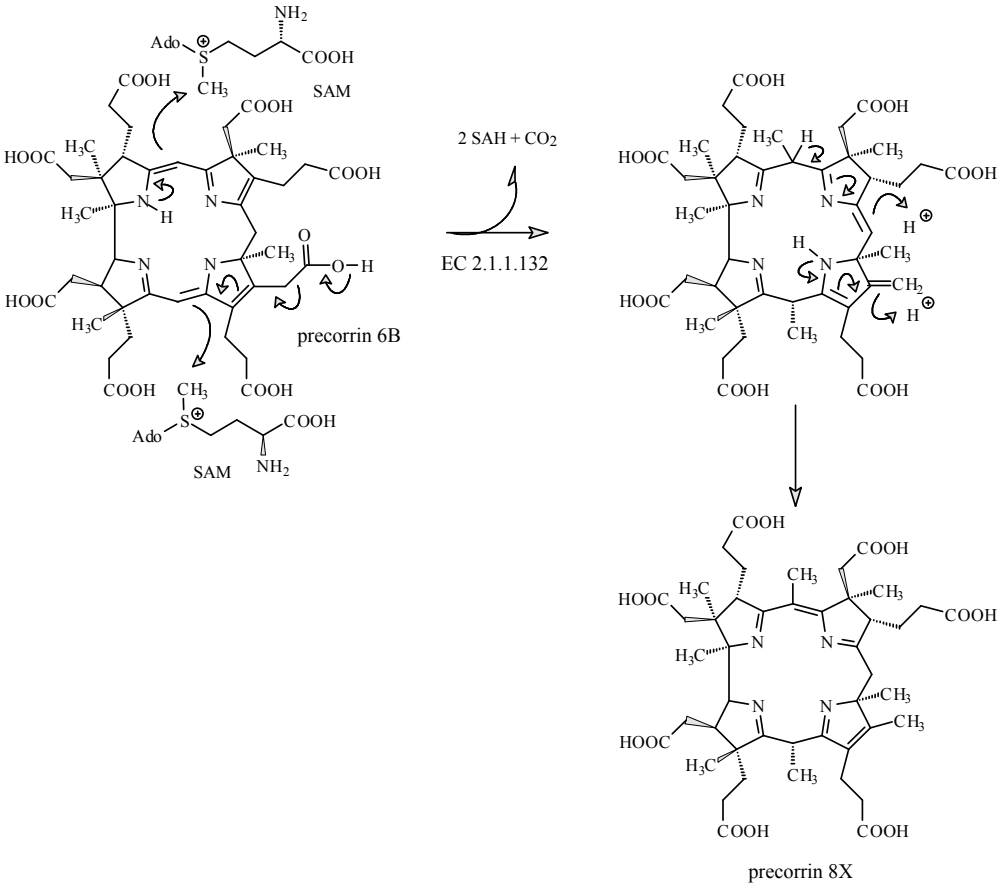

Figure 31

ferase, EC 2.1.1.131) yields Co-precorrin 4 which is methylated at C-11 (precorrin 4 C-11-methyltransferase, EC 2.1.1.133) to form Co-precorrin 5. Methylation at C-1 of Co-precorrin 5, followed by acetaldehyde extrusion (methyltransferase, EC 2.1.1.-), yields Co-precorrin 6A (Figure 33), which is reduced at C-18/C-19 (precorrin 6A reductase, EC 1.3.1.54) to Co-precorrin 6B, and this reaction is followed by C-5 and C-15 methylation and decarboxylation (precorrin 6Y C-5, -15-methyltransferase, EC 2.1.1.132) to yield Co-precorrin 8X. The C-11 methyl rearrangement (precorrin 8X methylmutase, EC 5.4.1.2) produces cobyrinic acid, which is finally amidated by an amide synthase (EC 6.3.1.-) to produce cob(II)yrinic acid *a,c*-diamide.

Cob(II)yrinic acid *a,c* diamide is the product from which the two pathways converge so that the next steps are the same in both aerobes and anaerobes (Figure 34). This compound is first reduced to a Co^{1+} complex, cob(I)yrinic acid *a,c* diamide, under the action of cob(II)yrinic acid *a,c* diamide reductase (EC 1.16.8.1). The reduction is followed by adenosylation of cob(I)yrinic acid *a,c* diamide catalysed by cob(I)yrinic acid *a,c* diamide 5'-deoxy-5'-adenosyltransferase (EC 2.5.1.17), which yields adenosylcobyrinic acid *a,c*-diamide containing Co(III). 5'-Deoxy-5'-adenosylcobyric acid synthase (EC 6.3.5.10) then catalyses the four-step amidation sequence from cobyrinic acid *a,c*-diamide to 5'-deoxy-5'-adenosylcobyrinic acid hexaamide, known as 5'-deoxy-5'-adenosylcobyric acid, via the formation of cobyrinic acid triamide, tetraamide, and pentaamide intermediates. The same enzyme also catalyses the attachment of (*R*)-1-aminopropan-2-ol to 5'-deoxy-5'-adenosylcobyric acid. The substrate for this reaction, (*R*)-1-aminopropan-2-yl phosphate, is produced by threonine phosphate decarboxylase (EC 4.1.1.81). The enzyme that converts L-threonine to *O*-phospho-L-threonine (L-threonine phosphate) is not yet characterised. Adenosylcobinamide kinase (EC 2.7.1.156), adenosylcobinamide phosphate guanylyltransferase (EC 2.7.7.62), and cobalamin synthase (EC 2.7.8.26) catalyse reactions in the nucleotide loop assembly pathway, which convert 5'-deoxy-5'-adenosylcobinamide into 5'-deoxy-5'-adenosylcobalamin, vitamin B_{12} prosthetic group.

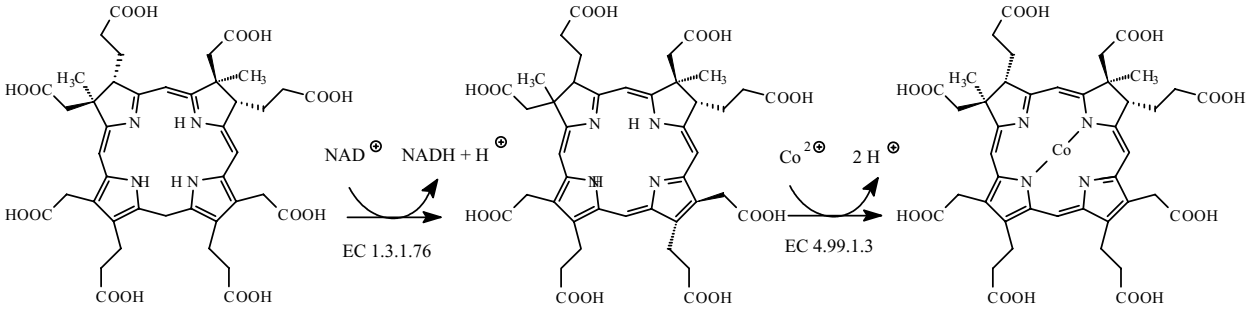

Figure 32

Nicotinate mononucleotide-dimethylimidazole phosphoribosyltransferase (EC 2.4.2.21) and α-ribazole phosphatase (EC 3.1.3.73) are involved in 5,6-dimethylbenzimidazole activation whereby 5,6-dimethylbenzimidazole is converted to its riboside, α-ribazole, *N*1-(α-D-ribofuranosyl)-5,6-dimethylbenzimidazole (Figure 35). The second branch of the nucleotide loop assembly pathway is the cobinamide activation branch where 5'-deoxy-5'-adenosylcobinamide or 5'-deoxy-5'-adenosylcobinamide phosphate is converted to the activated intermediate 5'-deoxy-5'-adenosylcobinamide-GDP. The final step in 5'-deoxy-5'-adenosylcobalamin biosynthesis is the condensation of adenosylcobinamide-GDP with α-ribazole to yield 5'-deoxy-5'-adenosylcobalamin[14].

9 VITAMIN C

Vitamin C is the collective term that comprises L-ascorbic acid, the product of its one-electron oxidation, L-ascorbyl radical or L-monodehydroascorbic acid or L-semidehydroascorbic acid, and the product of its two-electron oxidation, L-dehydroascorbic acid (Velíšek 2002). Plant-related functional roles involving L-ascorbic acid are largely related to its redox properties. It has a role(s) in photosynthesis and plant stress through the control of active oxygen, in cell wall growth and development, and possibly also in cell division. Chain cleavage of L-ascorbic acid by hydrogen peroxide may have a major role in plant metabolism as it results in specific end products such as L-threonic acid, L-tartaric acid,

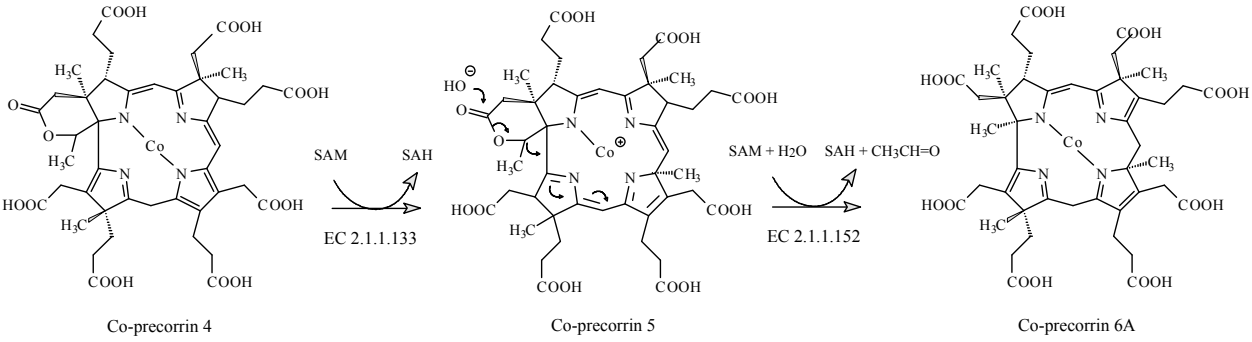

Figure 33

[14]In the corrinoid adenosylation pathway, reduction of Co(III) to Co(II) in aquacob(III)alamin (vitamin B_{12a}) proceeds by a one-electron transfer. This can be carried out by an NADH-linked flavoprotein aquacobalamin reductase (EC 1.16.1.3) or non-enzymatically in the presence of dihydroflavin nucleotides, to yield vitamin B_{12r}. In the next reaction, Co(II) is reduced to Co(I) in a second single-electron transfer by cob(II)alamin reductase (EC 1.16.1.4), and the cob(I)alamin (vitamin B_{12s}) conducts a nucleophilic attack on the adenosyl moiety of ATP to leave the cobalt atom in a Co(III) state and yield vitamin B_{12} coenzyme. The reaction is catalysed by cob(I)yrinic acid *a,c*-diamide adenosyltransferase (EC 2.5.1.17). A flavoprotein cyanocobalamin reductase (EC 1.16.1.6) catalyses the formation of cob(I)alamin from cyanocob(III)alamin (vitamin B_{12}). The formation of methylcobalamin (methylvitamin B_{12}) probably occurs in the process of the complex methyltransferase reaction catalysed by N^5-methyltetrahydrofolate homocysteine methyltransferase (EC 2.1.1.13).

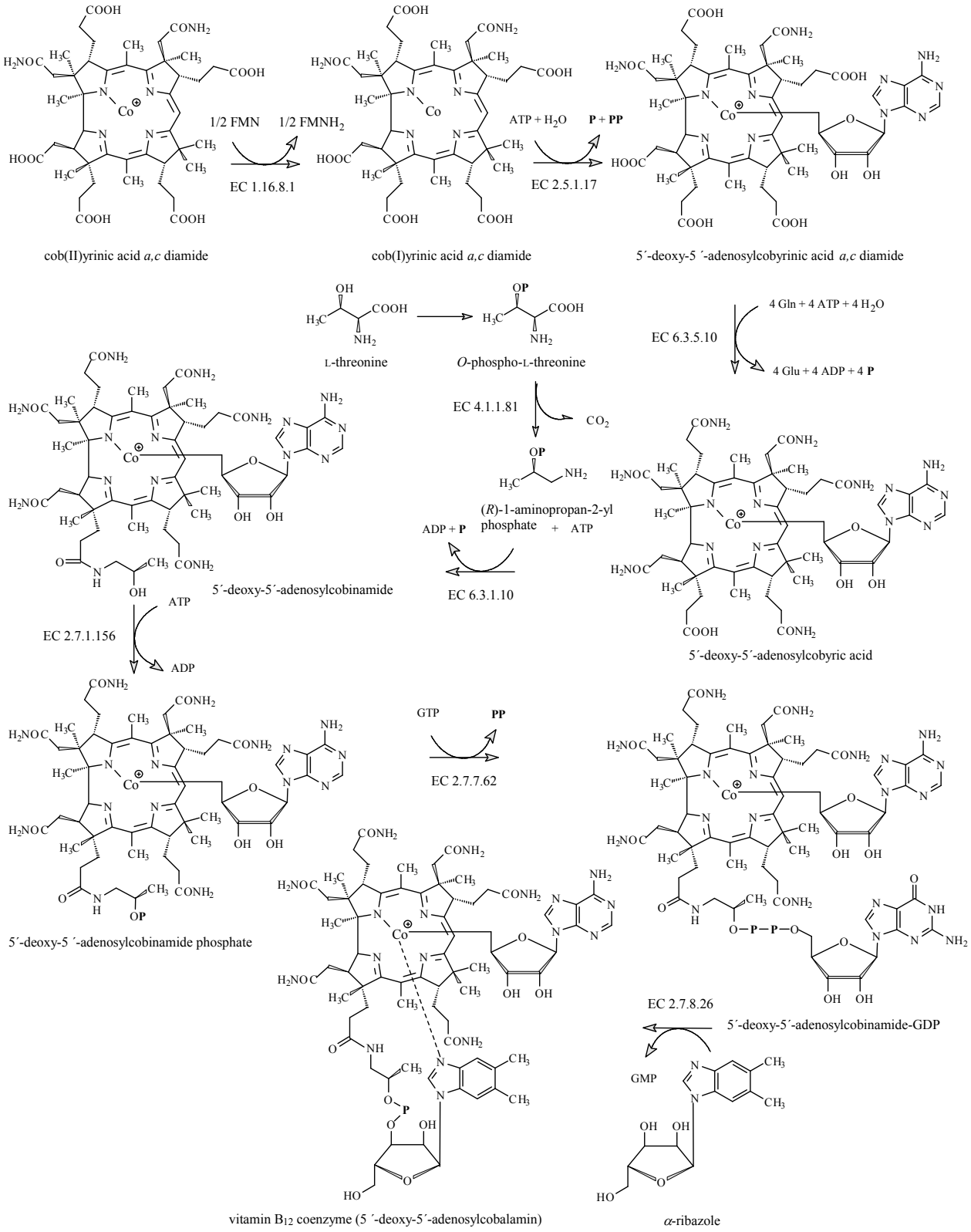
1/2 FMN
1/2 FMNH2
EC 1.16.8.1
ATP + H2O
P + PP
EC 2.5.1.17
cob(II)yrinic acid a,c diamide
cob(I)yrinic acid a,c diamide
5´-deoxy-5 ´-adenosylcobyrinic acid a,c diamide
L-threonine
O-phospho-L-threonine
4 Gln + 4 ATP + 4 H2O
EC 6.3.5.10
4 Glu + 4 ADP + 4 P
EC 4.1.1.81
CO2
(R)-1-aminopropan-2-yl phosphate + ATP
ADP + P
EC 6.3.1.10
5´-deoxy-5´-adenosylcobinamide
5´-deoxy-5´-adenosylcobyric acid
ATP
EC 2.7.1.156
ADP
GTP
PP
EC 2.7.7.62
5´-deoxy-5 ´-adenosylcobinamide phosphate
5´-deoxy-5´-adenosylcobinamide-GDP
EC 2.7.8.26
GMP
vitamin B12 coenzyme (5 ´-deoxy-5´-adenosylcobalamin)
α-ribazole

Figure 34

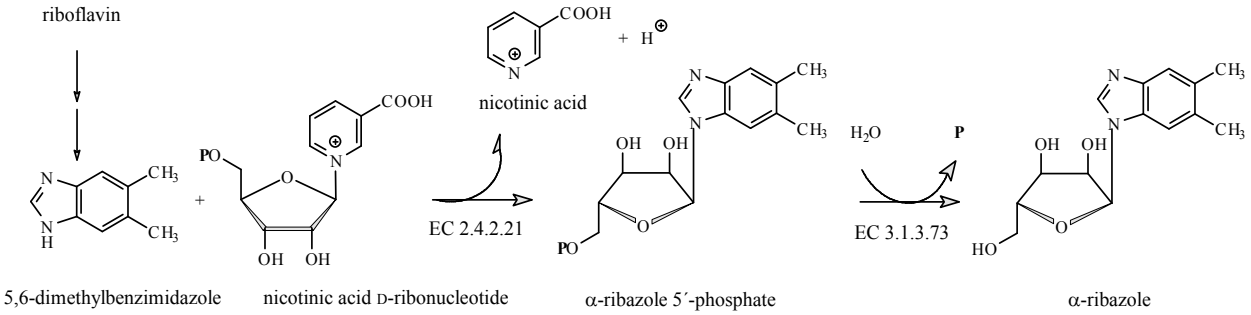

Figure 35

L-glyceric acid, oxalic acid, and CO_2, or is recycled through triose and hexose phosphates. In animals, L-ascorbic acid is essential for the formation of collagen, the principal structural protein in skin, bone, tendons, and ligaments, being a cofactor in the hydroxylation of L-proline to (*E*)-4-hydroxy-L-proline and of L-lysine to 5-hydroxy-L-lysine (Dewick 2002; Velíšek & Cejpek 2006a). Ascorbic acid is also associated with the hydroxylation of L-tyrosine in the pathway to catecholamines (Velíšek *et al.* 2006), in the biosynthesis of homogentisic acid, and the precursor of tocopherols and plastoquinones. It is best known as an antioxidant that can detoxify reactive products of oxygen metabolism, e.g. superoxide radical, hydrogen peroxide, and singlet oxygen (Velíšek 2002).

The biosynthesis of L-ascorbic acid occurs by different pathways in plants and animals. It is biosynthesised in all chlorophyll-containing plants. Among animals, this ability is absent only in some of the more primitive forms (i.e. in the insects and invertebrates), most fish and a few avian and mammalian species including humans. The ability to synthesise ascorbic acid begins in the amphibians, and is located in the kidneys in this class, as well as in reptiles, more primitive orders of birds, and egg-laying mammals. Many marsupials use both their kidneys and livers for this synthesis. The higher orders of birds and most mammals synthesise ascorbic acid in the liver. The most recent orders of birds, as well as several mammals (among others guinea pigs, bats, and some primates including humans), have lost their ability to synthesise ascorbic acid and must ingest exogenous ascorbic acid in their food.

9.1 L-Ascorbic acid

9.1.1 Biosynthesis in animals

In animals, L-ascorbic acid is formed from the active form of D-glucose (Smirnoff 2001), UDP-D-glucose (UDP-D-Glc), as a part of the UDP-glucose interconversion pathway.[15] UDP-D-Glc is oxidised at C-6 by UDP-D-glucose dehydrogenase (EC 1.1.1.22), forming irreversibly UDP-D-glucuronic acid (UDP-D-GlcA) (Figure 36). UDP-D-GlcA is then transformed to UDP-D-glucuronic acid 1-phosphate (UDP-D-GlcA1P) which is hydrolysed releasing D-GlcA or D-glucurono-3,6-lactone. The intermediates and enzymes, which could include (pyro)phosphorylases and (pyro)phosphatases (e.g. UDP-glucuronic acid pyrophosphatase and glucuronic acid 1-phosphate phosphatase), involved in these steps, do not appear to have been identified. There is apparently no information on these enzymes. Non-specific lysosomal acid phosphatases could account for some of the activity. Reduction at C-1 of D-GlcA by D-glucuronate reductase (EC 3.1.1.19) or of D-glucurono-3,6-lactone by D-glucuronolactone reductase (EC 3.1.1.20) then yields L-gulonic acid (L-GulA)[16] or L-gulono-1,4-lactone (L-*xylo*-hexulono-1,4-lactone). Moreover,

[15]The starting point of the biosynthetic pathway can be considered D-fructose 6-phosphate (D-Fru6P), which reversibly isomerises to D-glucose 6-phosphate (D-Glc6P) by phosphoglucose isomerase (EC 5.3.1.9). Transformation of D-Glc6P to D-glucose 1-phosphate (D-Glc1P) is a reaction catalysed by phosphoglucomutase (EC 5.4.2.2). UDP-D-glucose (UDP-D-Glc) is formed from D-Glc1P and uridine 5'-triphosphate (UTP) in a reaction catalysed by UDP-D-glucose pyrophosphorylase (EC 2.7.7.9). UDP-D-Glc can also arise from UDP-D-Gal via an epimerisation reaction involving UDP-D-glucose 4-epimerase (EC 5.1.3.2) (Velíšek & Cejpek 2005).

[16]The enzyme EC 1.1.1.19 also reduces D-galacturonic acid. May be identical with EC 1.1.1.2. The enzyme EC 3.1.1.25 is specific for 1,4-lactones with 4-8 carbon atoms (requires Ca^{2+}). The enzyme EC 1.1.3.8 is a flavoprotein (FAD).

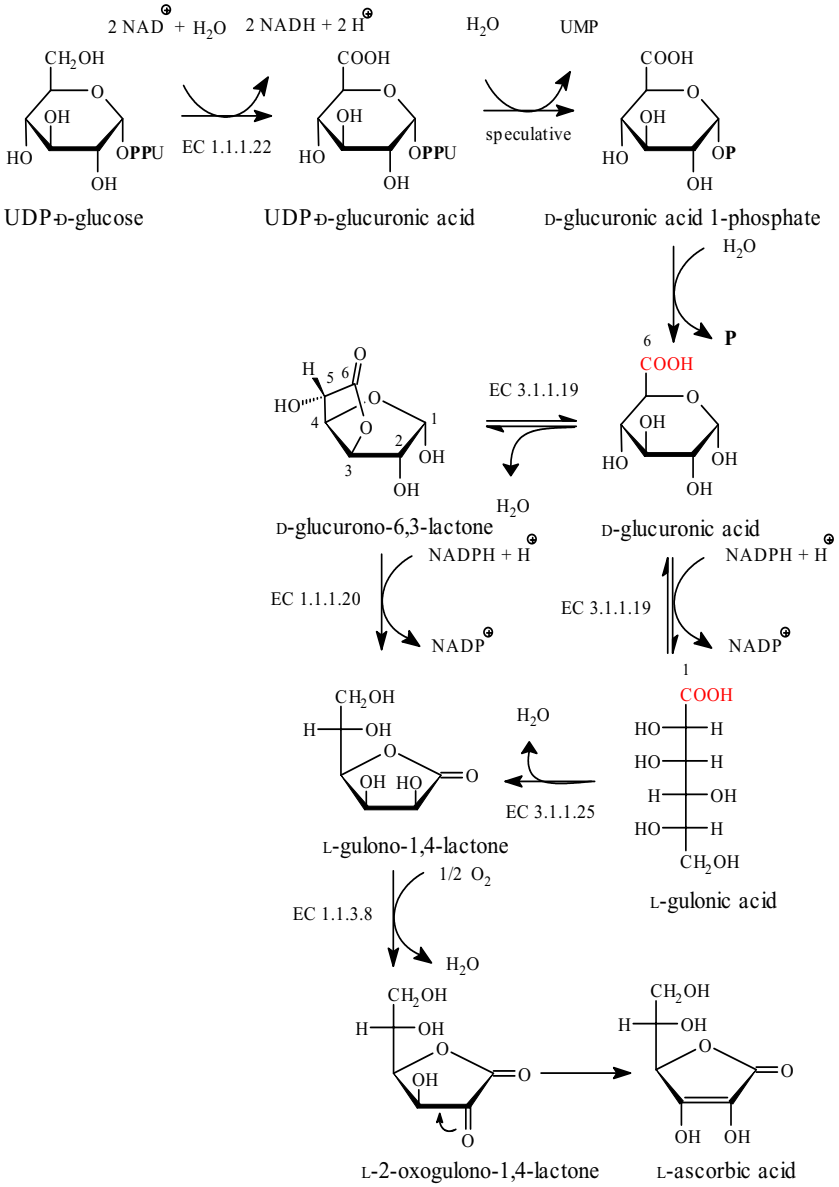

Figure 36

L-GulA can be transformed to L-gulono-1,4-lactone by the action of 1,4-lactonase (EC 3.1.1.25). L-Gulono-1,4-lactone is oxidised at C-2 to L-2-oxogulono-1,4-lactone (L-*xylo*-hex-2-ulosono-1,4-lactone) by L-gulonolactone oxidase (EC 1.1.3.8), and spontaneously isomerises to L-ascorbic acid. During the course of these transformations, an apparent inversion of configuration occurs so that the C-1 of D-Glc is incorporated into the C-6 of L-ascorbic acid molecule (inversion of the hexose chain).

9.1.2 Biosynthesis in plants

Surprisingly, the biosynthetic pathway of L-ascorbic acid in plants was not elucidated until relatively recently (Wheeler *et al.* 1998). Plants form L-ascorbic acid from GDP-D-mannose (GDP-D-Man).[17] The proposed reaction sequence (Figure 37) involves the oxidation of the C-4 hydroxyl group to a carbonyl group, water elimination, the readdition of water from the opposite face of the double bond, and

[17]The starting point of the biosynthetic pathway in plant can be considered to be D-Fru6P which reversibly isomerises to D-mannose 6-phosphate (D-Man6P) (under the catalysis by phosphomannose isomerase, EC 5.4.2.8) and D-Man6P to D-mannose 1-phosphate (D-Man1P) (phosphomannomutase, EC 5.4.2.8). D-Man1P then reacts with guanosine 5'-triphosphate (GTP) to yield GDP-D-mannose (GDP-mannose pyrophosphorylase, EC 2.7.7.22) (Velíšek & Cejpek 2005).

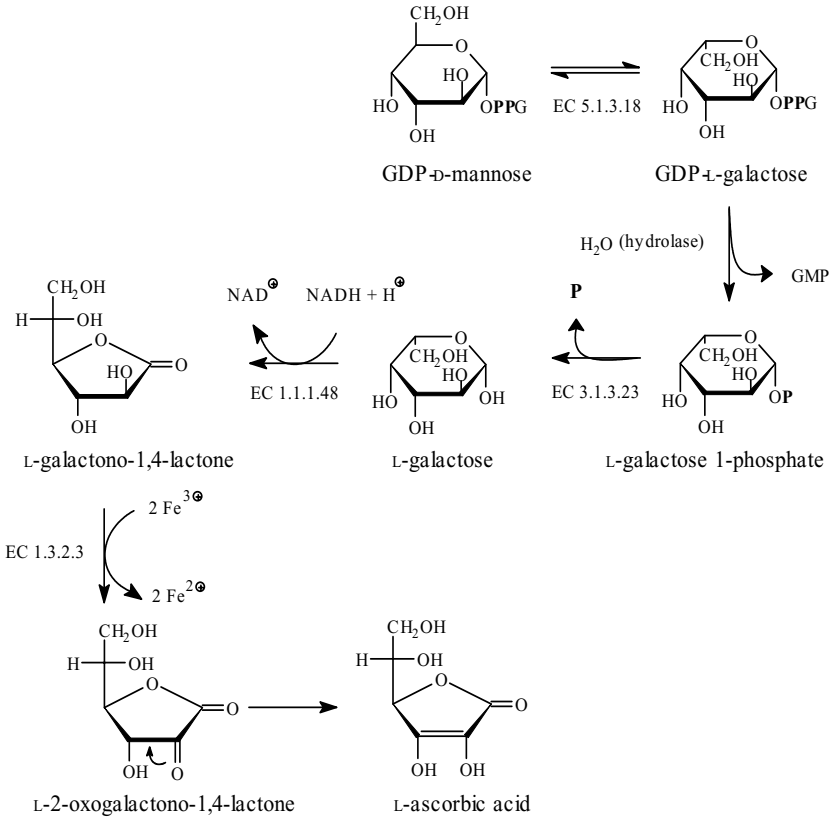

Figure 37

the reduction of the C-4 carbonyl group, also from the opposite face, yielding GDP-L-galactose (GDP-L-Gal). This reaction is catalysed by GDP-mannose-3,5-isomerase (EC 5.1.3.18). In the next step, GDP-L-Gal is probably transformed to GDP-L-galactose 1-phosphate (L-Gal1P). It is not yet known if L-Gal1P is an intermediate. It is shown although this is speculative. L-Gal1P is then hydrolysed to L-Gal by an unknown enzyme (probably by sugar-phosphatase, EC 3.1.3.23), and L-Gal is oxidised to L-galactono-1,4-lactone under the action of L-galactose dehydrogenase (EC 1.1.1.48). The final oxidation of L-galactono-1,4-lactone at C-2 to L-2-oxogalactono-1,4-lactone (L-*lyxo*-hex-2-ulosono-1,4-lactone) is catalysed by L-galactono-1,4-lactone dehydrogenase (EC 1.3.2.3) using ferricytochrome-c as an electron acceptor. This lactone then spontaneously isomerises to L-ascorbic acid (Wheeler *et al.* 1998; Davey *et al.* 1999; Loewus 1999; Smirnoff 2001).

It seems that there exist some other alternative pathways, revealing a more complex picture of L-ascorbic acid biosynthesis. For example, GDP-L-gulose (GDP-L-Gul) and *myo*-inositol have been suggested to be another intermediates in L-ascorbic acid biosynthesis, indicating that part of the animal pathway may also be operating in plants (Valpuesta & Botella 2004).

9.2 L-Ascorbic acid analogues

Microorganisms (including *Saccharomyces cerevisiae* yeasts) and higher fungi do not posses the ability to synthesise L-ascorbic acid from D-aldoses.[18] Instead, they synthesise D-*erythro*-ascorbic acid (D-*glycero*-pent-2-enono-1,4-lactone), a five carbon analogue of L-ascorbic acid. This is thought to perform antioxidant functions in fungi resembling those performed by L-ascorbic acid in other eukaryotes. Both compounds have similar

[18]However, the synthesis of L-ascorbic acid in e.g. *Saccharomyces cerevisiae* can be induced by supply of unphysiological substrates such as L-galactose and various 1,4-lactones, which are converted into L-ascorbic acid via the enzymes acting in the D-*erythro*-ascorbic acid biosynthesis.

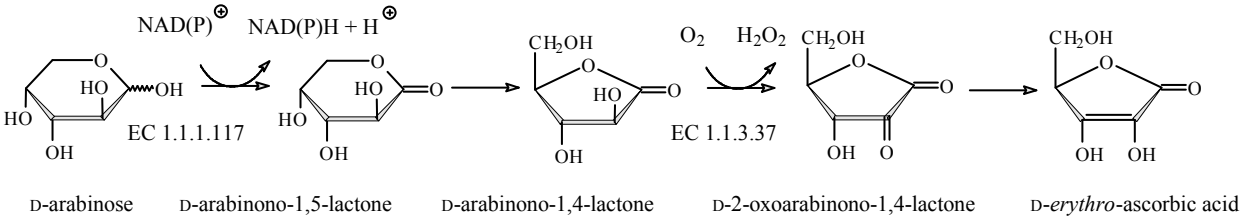

Figure 38

redox properties and are substrates for L-ascorbate oxidase (EC 1.10.3.3).

The pathway of D-*erythro*-ascorbic acid biosynthesis shares many features with the pathway for L-ascorbic acid in plants (Loewus 1999; Hancock *et al*. 2000). The first step, oxidation of D-arabinose (D-Ara), is catalysed by a $NAD(P)^+$-specific D-arabinose dehydrogenase (EC 1.1.1.117), which oxidises D-Ara at C-1 to produce the corresponding aldonolactone, possibly L-arabinono-1,5-lactone, which then spontaneously rearranges to the more stable D-arabinono-1,4-lactone (Figure 38). The final step, the oxidation of D-arabinono-1,4-lactone at C-2, is catalysed by an FAD-containing enzyme D-arabinono-1,4-lactone oxidase (EC 1.1.3.37). Finally, the oxidation product, D-2-oxoarabinono-1,4-lactone (D-*erythro*-pent-2-ulosono-1,4-lactone), spontaneously isomerises to D-*erythro*-ascorbic acid.

Apart from D-*erythro*-ascorbic acid, other fungal ascorbic acid analogues and their glycosides currently known include 6-deoxy-L-ascorbic acid (a potential source is L-fucose), 6-deoxy-5-*O*-(α-D-xylopyranosyl)-L-ascorbic acid, 6-deoxy-5-*O*-(α-D-glucopyranosyl)-L-ascorbic acid, 5-*O*-(α-D-xylopyranosyl)-D-*erythro*-ascorbic acid, 5-*O*-(α-D-glucopyranosyl)-D-*erythro*-ascorbic acid, isolated from eatable mushrooms (*Basidiomycetes*), and 5-*O*-(α-D-galactopyranosyl)-D-*erythro*-ascorbic acid isolated from *Sclerotinia sclerotiorum* (*Ascomyces*). Glycosidation (occurring at C-5) of ascorbic acid analogues appears to be a common feature in fungi. In the presence of H_2O_2 and under alkaline conditions, D-*erythro*-ascorbic acid is cleaved between C-2 and C-3 to yield oxalic acid and D-glyceric acid (Loewus 1999; Hancock *et al*. 2000).

EC (Enzyme Commission) numbers and some common abbreviation

EC (Enzyme Commission) numbers, assigned by IUPAC-IUBMB, were taken from KEGG. In many structures, the unionised forms are depicted to simplify the structures, to eliminate the need for counter-ions, and to avoid the mechanistic confusion.

ACP – acyl carrier protein
Ado – adenosine
Ara – arabinose
ADP – adenosine 5'-diphosphate
AMP – adenosine 5'-monophosphate
ATP – adenosine 5'-triphosphate
CoA – coenzyme A as a part of a thioester
DNA – deoxyribonucleic acid
FAD – flavine adenine dinucleotide
FMN – flavin mononucleotide
Fru – fructose
H_2PteGlu – 7,8-dihydrofolic acid
H_4PteGlu – (6*S*)-5,6,7,8-tetrahydropteroylglutamic acid
Gal – galactose
GDP – guanosine-5'-diphosphate
Glc – glucose
GlcA – glucuronic acid
Gln – L-glutamine
Glu – L-glutamic acid
GMP – guanosine-5'-monophosphate
GTP – guanosine-5'-triphosphate
Gul – gulose
GulA – gulonic acid
Man – mannose
NADH – nicotinamide adenine dinucleotide
NADPH – nicotinamide adenine dinucleotide phosphate
P – phosphoric acid
PP – diphosphoric acid
PPP – triphosphoric acid
SAH – *S*-adenosyl-L-homocysteine (AdoHcy)
SAM – *S*-adenosyl-L-methionine (AdoMet)
UDP – uridine-5'-diphosphate
UMP – uridine-5'-monophosphate
UTP – uridine-5'-triphosphate

References

Begley T.P., Xi J., Kinsland C., Taylor S., McLafferty F. (1999): The enzymology of sulfur activation during thiamin and biotin biosynthesis. Current Opinion in Chemical Biology, **3**: 623–629.

Davey M.W., Gilot C., Persiau G., Østergaard J., Han Y., Bauw G.C., Van Montagu M.C. (1999): Ascorbate biosynthesis in *Arabidopsis* cell suspension culture. Plant Physiology, **121**: 535–543.

Dewick P.M. (2002): Medicinal Natural Products. A Biosynthetic Approach. 2nd Ed. Wiley, New York.

Friedrich W. (1988): Vitamins. Walter de Gruyter, Berlin.

Hancock R.D., Galpin J.R., Viola R. (2000): Biosynthesis of L-ascorbic acid (vitamin C) by *Saccharomyces cerevisiae*. FEMS Microbiology Letters, **186**: 245–250.

Jensen K.P., Mikkelsen K.V. (2001): Semi-empirical studies of cobalamins, corrin models, and cobaloximes. The nucleotide loop does not strain the corrin ring in cobalamins. Inorganica Chimica Acta, **323**: 5–15.

Loewus F.A. (1999): Biosynthesis and metabolism of ascorbic acid in plants and analogs of ascorbic acid in fungi. Phytochemistry, **52**: 193–210.

Marsh E.N.G., Drennan C.L. (2001): Adenosylcobalamin-dependent isomerases. New insights into structure and mechanism. Current Opinion in Chemical Biology, **5**: 499–505.

Marques H.M., Ngoma B., Egan T.J., Brown K.L. (2001): Parameters for the AMBER force field for the molecular mechanics modelling of the cobalt corrinoids. Journal of Molecular Structure, **561**: 71–91.

Ploux O. (2000): Biotin. In: De Leenheer A.P., Lambert W.E., Van Bocxlaer J.F. (eds): Modern Chromatographic Analysis of Vitamins. 3rd Ed. Marcel Dekker, New York: 479–509.

Roessner C.A., Santander P.J., Scott A.I. (2001): Multiple biosynthetic pathways for vitamin B_{12}: Variations on a central theme. Vitamins and Hormones, **61**: 267–297.

Sirovatka J.M., Rappé A.K., Finke R.G. (2000): Molecular mechanics studies of coenzyme B12 complexes with constrained Co-N (axial base) bond lengths: introduction of the universal force field (UFF) to coenzyme B12 chemistry and its use to probe the plausibility of an axil-base-induced, ground-state corrin butterfly conformational steric effect. Inorganica Chimica Acta, **300–302**: 545–555.

Smirnoff N. (2001): L-Ascorbic acid biosynthesis. Vitamins and Hormones, **61**: 241–266.

Stok E., De Voss J.J. (2000): Expression, purification, and characterisation of Biol: a carbon-carbon bond cleaving cytochrome P450 involved in biotin biosynthesis in *Bacillus subtilis*. Archives of Biochemistry and Biophysics, **384**: 351–360.

Valpuesta V., Botella M.A. (2004): Biosynthesis of L-ascorbic acid in plants: new pathway for an old antioxidant. Trends in Plant Science, **9**: 573–577.

Velíšek J. (2002): Chemie potravin. Ossis, Tábor.

Velíšek J., Cejpek K. (2005): Biosynthesis of food constituents: Saccharides. 1. Monosaccharides, oligosaccharides, and related compounds – a review. Czech Journal of Food Sciences, **23**: 129–144.

Velíšek J., Cejpek K. (2006a): Biosynthesis of food constituents: Amino acids. 3. Modified proteinogenic amino acids – a review. Czech Journal of Food Sciences, **24**: 59–61.

Velíšek J., Cejpek K. (2006b): Biosynthesis of food constituents: Lipids. 1. Fatty acids and their derivatives – a review. Czech Journal of Food Sciences, **24**: 193–216.

Velíšek J., Cejpek K. (2007): Biosynthesis of food constituents:Vitamins. 2. Water-soluble vitamins: Part 1 – a review. Czech Journal of Food Sciences, **25**: 49–64.

Velíšek J., Kubec R., Cejpek K. (2006): Biosynthesis of food constituents: Amino acids. 4. Non-protein amino acids – a review. Czech Journal of Food Sciences, **24**: 93–109.

Wheeler G.L., Jones M.A., Smirnoff N. (1998): The biosynthetic pathway of vitamin C in higher plants. Nature, **393**: 365–369.

IUPAC (International Union of Pure and Applied Chemistry). Available from http://www.chem.qmul.ac.uk/iupac/.

KEGG, Genome Net, Bioinformatics Centre, Institute for Chemical Research, Kyoto University. Available from http://www.genome.ad.jp/.

Received for publication February 20, 2006
Accepted after corrections October 30, 2006

Corresponding author:

Prof. Ing. Jan Velíšek, DrSc., Vysoká škola chemicko-technologická v Praze, Fakulta potravinářské a biochemické technologie, Ústav chemie a analýzy potravin, Technická 5, 166 28 Praha 6, Česká republika
tel.: + 420 220 443 177, fax: + 420 233 339 990, e-mail: jan.velisek@vscht.cz