

CHAPTER 1

Structure, Distribution, Biosynthesis, Catabolism and Function

1.1. Introduction

Porphyryns are a large class of natural or synthetic pigments having a substituted aromatic macrocyclic ring consisting of four pyrrole residues linked together by four methine bridging groups¹ (Fig. 1.1). They are deeply coloured (red or purple), fluorescent compounds with an intense and characteristic absorbance band between 390–425 nm (the Soret band or B band) and two to four much weaker bands (Q bands) between 480–700 nm. The Soret band, with extinction coefficient of 150,000 or more, is often used for the sensitive spectrophotometric detection of porphyryns following separation by high-performance liquid chromatography (HPLC). Porphyryns also have a characteristic fluorescence spectrum. Using an excitation wavelength of 400–405 nm and an emission wavelength of around 600 nm, a much higher intrinsic sensitivity of detection than absorption spectrophotometry can be achieved. A fluorescence detector is therefore preferred for the sensitive detection of porphyryns in chromatographic analysis.

Porphyryns are widely distributed in nature. They occur as coloured pigments in the downs of young birds and in higher concentrations in feathers of birds such as Turacos² (as the copper complex of uroporphyrin III), owls, and bustards.³ The eggshells of birds may also contain porphyryns and/or bile pigments,^{4,5} usually protoporphyrin IX and biliverdin IX α . The calcareous shells⁶ and pearls⁷ of shellfishes often contain porphyryns and the shells of some deep-sea bivalves are found to contain high concentrations of pink or red

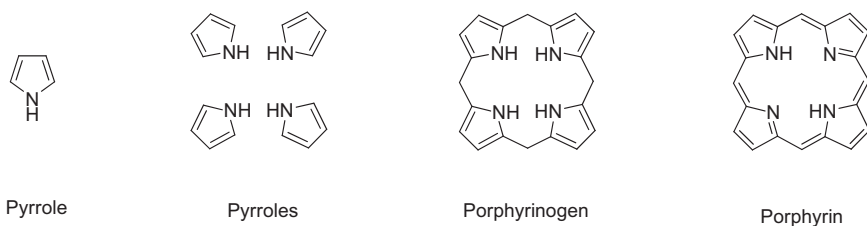


Figure 1.1. Structures of pyrrole, porphyrinogen and unsubstituted porphyrin macrocycle.

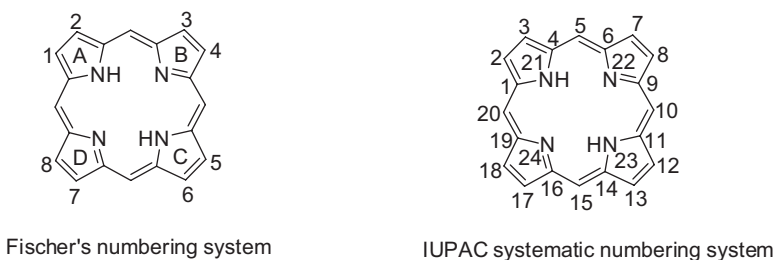


Figure 1.2. The numbering of unsubstituted porphyrin macrocycle.

fluorescent porphyrin deposits, mainly uroporphyrin I. Petroporphyrins⁸ in coal, oil or shale are formed from dead plants and other photosynthetic organisms by diagenesis deep in the earth over millions of years. It has even been suggested that porphyrin is an ideal biomarker in the search for extraterrestrial life⁹ because of its presence in virtually all living organisms on Earth.

The main physiological significance of porphyrins lies in the pathways of haem^{10,11} and chlorophyll biosynthesis,¹²⁻¹⁴ of which they can be considered as intermediary metabolites or oxidised by-products.

1.2. Nomenclature

In the conventional Fischer system^{15,16} of nomenclature, the peripheral positions of the macrocyclic ring are numbered from 1 to 8 (Fig. 1.2). The four pyrrole rings are labelled A, B, C and D and the

four methine bridges (the *meso*-positions) are designated α , β , γ and δ . Trivial names are given to porphyrins of biological and clinical importance and are commonly used.¹ They are also used in this book (Table 1.1), unless otherwise stated. In the systematic IUPAC nomenclature^{17,18} all atoms, including the nitrogen atoms, are numbered. IUPAC system of naming allows a more precise description of a substituent on a carbon or nitrogen atom of the porphyrin macrocycle. Table 1.1 shows the trivial names and structures of some naturally occurring porphyrins.

1.3. Biosynthesis of Porphyrins, Haem and Chlorophyll

The first step (Fig. 1.3) is the condensation of glycine with succinyl coenzyme A (CoA), a derivative of succinic acid, to form 5-aminolaevulinic acid (ALA or 5-ALA). The reaction is catalysed by the enzyme 5-aminolaevulinic acid synthase (ALA-S) in the matrix compartment of the mitochondrion. This pathway, called Shemin pathway,^{19,20} occurs in animals. In plants, the C₅ pathway in which ALA is formed from glutamate occurs.²¹

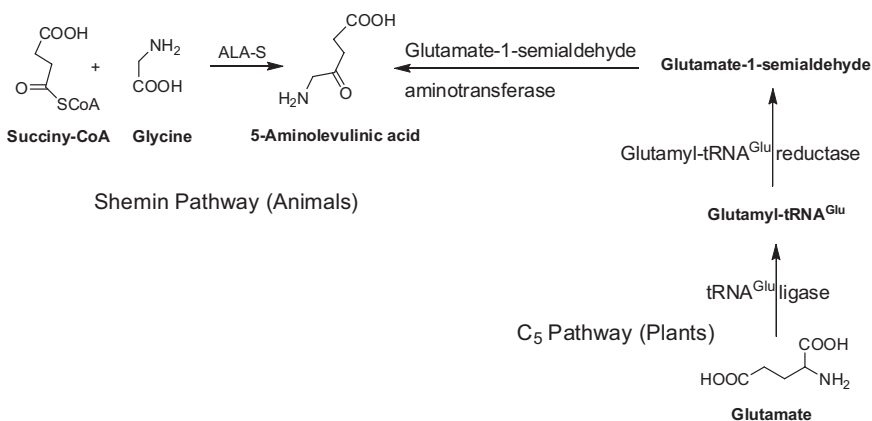
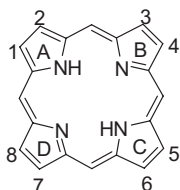


Figure 1.3. The biosynthesis of 5-aminolaevulinic acid (ALA) from glycine and succinyl-CoA (Shemin pathway) and from glutamate (C₅) pathway. ALA-S = 5-aminolaevulinic acid synthase.

Table 1.1. Trivial Names and Structures of some Naturally Occurring Porphyrins.**SIDE-CHAIN SUBSTITUENTS**

Porphyrin	1	2	3	4	5	6	7	8
Uroporphyrin I	Ac	Pr	Ac	Pr	Ac	Pr	Ac	Pr
Uroporphyrin III	Ac	Pr	Ac	Pr	Ac	Pr	Pr	Ac
Heptacarboxylic acid porphyrin I	Ac	Pr	Ac	Pr	Ac	Pr	Me	Pr
Heptacarboxylic acid porphyrin III (7d)	Ac	Pr	Ac	Pr	Ac	Pr	Pr	Me
Hexacarboxylic acid porphyrin I (6lab)	Me	Pr	Me	Pr	Ac	Pr	Ac	Pr
Hexacarboxylic acid porphyrin I (6lac)	Me	Pr	Ac	Pr	Me	Pr	Ac	Pr
Hexacarboxylic acid porphyrin III (6ad)	Me	Pr	Ac	Pr	Ac	Pr	Pr	Me
Pentacarboxylic acid porphyrin I	Ac	Pr	Me	Pr	Me	Pr	Me	Pr
Pentacarboxylic acid porphyrin III (5abd)	Me	Pr	Me	Pr	Ac	Pr	Pr	Me
Pentacarboxylic acid porphyrin III (5abc)	Me	Pr	Me	Pr	Me	Pr	Pr	Ac
Pentacarboxylic acid porphyrin III (5acd)	Me	Pr	Ac	Pr	Me	Pr	Pr	Me
Pentacarboxylic acid porphyrin III (5bcd)	Ac	Pr	Me	Pr	Me	Pr	Pr	Me
Coproporphyrin I	Me	Pr	Me	Pr	Me	Pr	Me	Pr
Coproporphyrin III	Me	Pr	Me	Pr	Me	Pr	Pr	Me
Isocoproporphyrin	Me	Et	Me	Pr	Ac	Pr	Pr	Me
Tricarboxylic acid porphyrin	Me	V	Me	Pr	Me	Pr	Pr	Me
Protoporphyrin IX	Me	V	Me	V	Me	Pr	Pr	Me
Mesoporphyrin IX	Me	Et	Me	Et	Me	Pr	Pr	Me
Deuteroporphyrin IX	Me	H	Me	H	Me	Pr	Pr	Me

Side-chain abbreviations: Me = methyl, Et = ethyl, Ac = CH_2COOH , Pr = $\text{CH}_2\text{CH}_2\text{COOH}$, V = vinyl. The letters a,b,c and d denote the positions of the methyl group on rings A, B, C and D, respectively.

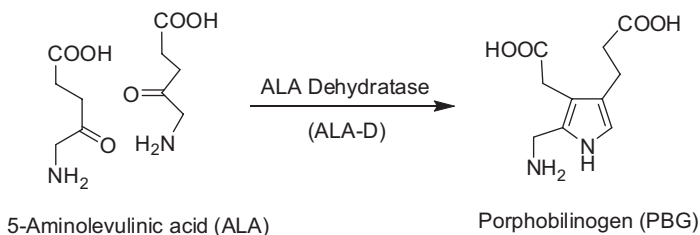


Figure 1.4. The biosynthesis of PBG from two molecules of ALA.

Two molecules of ALA are then condensed with each other (Fig. 1.4) in the soluble part of the cytoplasm to form the monopyrrolic precursor, porphobilinogen (PBG). This reaction is catalysed by the enzyme 5-aminolaevulinic acid dehydratase (ALA-D) or porphobilinogen synthase (PBG-S).²²

In the next step, four molecules of PBG condense together in a head-to-tail fashion (Fig. 1.5) to yield the symmetrical, linear tetrapyrrole, hydroxymethylbilane (HMB).^{23,24} This reaction is catalysed by porphobilinogen deaminase (PBG-D), also known as hydroxymethylbilane synthase (HMB-S).^{25,26}

HMB is rearranged and cyclised to yield the asymmetrical uroporphyrinogen III (Fig. 1.5). The reaction is catalysed by uroporphyrinogen III synthase (Urogen III-S).^{27,28} In the absence of Urogen III-S the unstable HMB, with a half-life of less than 5 minutes at neutral pH, is cyclised spontaneously to the symmetrical and physiologically unimportant uroporphyrinogen I (Fig. 1.5). Uroporphyrinogen III is the common precursor²⁴ in the biosynthesis of haem, sirohaem, the cofactor of sulphite and nitrite reductase, and vitamin B₁₂ (Fig. 1.6).

The type isomers I and III denote the arrangements of the four acetic acid groups and four propionic acid groups around the macrocyclic periphery (positions 1–8) of the porphyrins (Table 1.1). There are four possible arrangements and Fischer called these isomers type I, II, III and IV isomers.

At this stage of haem biosynthesis, and also in the next three steps, the intermediates are porphyrinogens or hexahydroporphyrins.

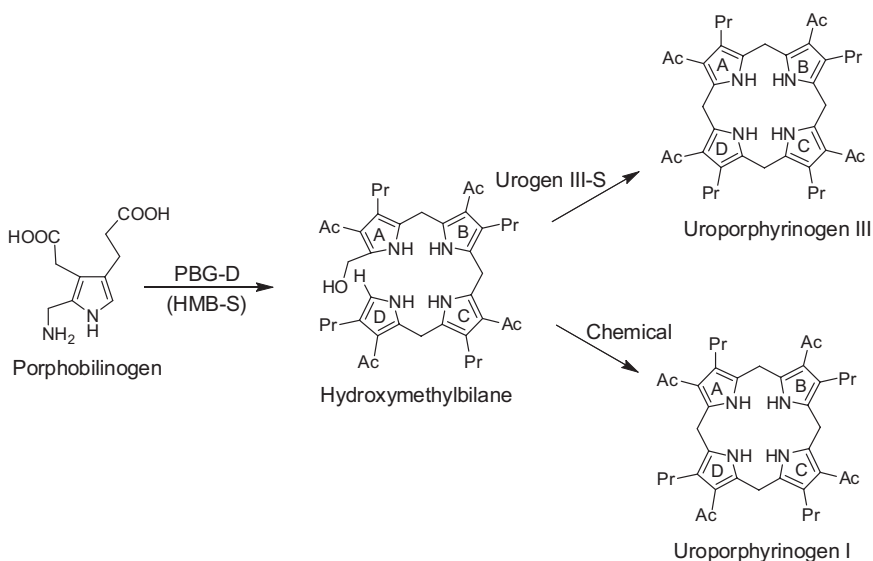


Figure 1.5. The biosynthesis of hydroxymethylbilane and uroporphyrinogen III. PBG-D = porphobilinogen deaminase; HMB-S = hydroxymethylbilane synthase; Urogen III-S = uroporphyrinogen III synthase; Ac = CH_2COOH ; Pr = $\text{CH}_2\text{CH}_2\text{COOH}$.

They are colourless, non-fluorescent intermediates in which the pyrrole rings are joined together by methylene rather than methine bridges (Fig. 1.1). The porphyrins are oxidative by-products at these stages and cannot be metabolised themselves.

The four acetic acid groups of uroporphyrinogen III are sequentially decarboxylated to methyl groups in a step-wise fashion, starting from ring D through rings A, B and C, to give coproporphyrinogen III (Fig. 1.6).²⁹ The reaction is catalysed by the cytoplasmic enzyme uroporphyrinogen decarboxylase (Urogen-D).³⁰ Although the clockwise decarboxylation pathway from ring D through rings A, B and C is preferred,³¹ random decarboxylation has also been observed leading to a complex mixture of isomeric, hepta-, hexa- and penta-carboxylic acid porphyrinogen intermediates.^{32,33} There are four possible type III hepta-, six type III hexa- and four type III penta-carboxylic acid porphyrinogens.

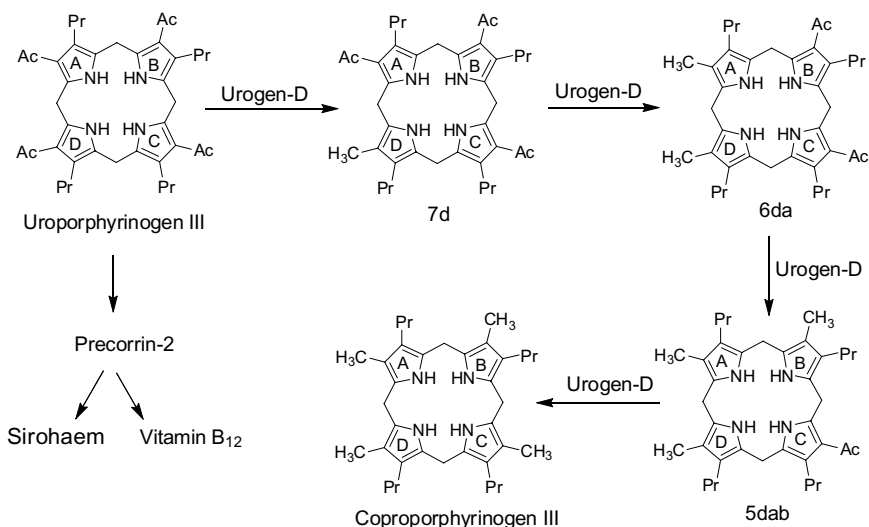


Figure 1.6. The biosynthesis of coproporphyrinogen III by sequential decarboxylation of the four side-chain acetic acid groups of uroporphyrinogen III. Uroporphyrinogen I is similarly decarboxylated to coproporphyrinogen I. Urogen-D = uroporphyrinogen decarboxylase. The letters a, b, c, and d denote the position on which the acetic acid group on ring A, B, C, and D, respectively, has been decarboxylated to a methyl group.

Urogen-D is not specific for uroporphyrinogen III and the symmetrical uroporphyrinogen I is similarly decarboxylated to coproporphyrinogen I.

Coproporphyrinogen III is taken up into the mitochondrion where the remaining steps of haem biosynthesis take place. Coproporphyrinogen III is converted into protoporphyrinogen IX, via 2-vinyl-4,6,7-tricarboxylic acid porphyrinogen, by oxidative decarboxylation of the two propionic acid groups on rings A and B to vinyl groups (Fig. 1.7).^{10,11} The reaction is catalysed by coproporphyrinogen oxidase (Coprogen-O). This enzyme is highly specific for the type III isomer and will not decarboxylate coproporphyrinogen I which will not be further metabolised.

Protoporphyrinogen IX is then oxidised to protoporphyrin IX by protoporphyrinogen oxidase (Protogen-O)^{10,11} and finally haem is

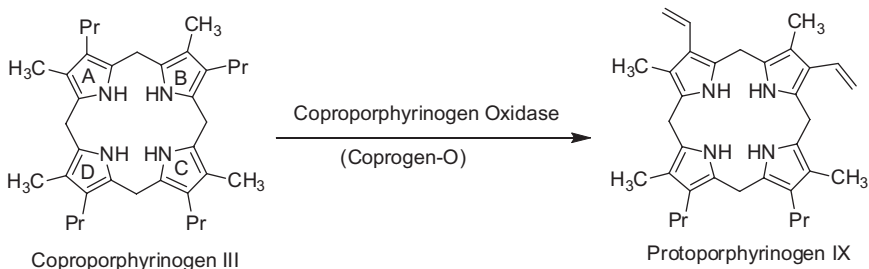


Figure 1.7. The biosynthesis of protoporphyrin IX from coproporphyrinogen III.

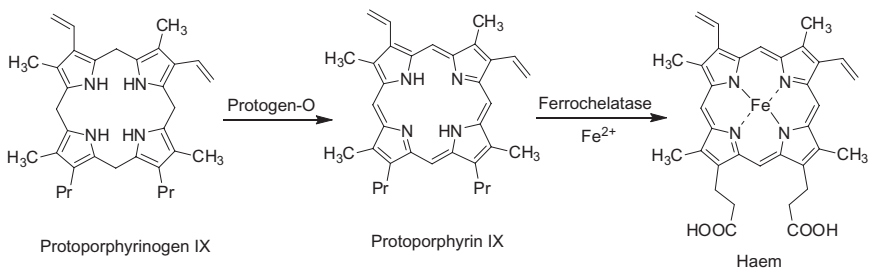


Figure 1.8. The biosynthesis of protoporphyrin IX and haem. Protophen-O = protoporphyrinogen oxidase.

produced by insertion of ferrous iron into protoporphyrin IX (Fig. 1.8), a step catalysed by the last enzyme of haem biosynthesis, ferrochelatase (FECH).^{10,11} Note that protoporphyrin IX is the only porphyrin formed in the pathway.

An outline of the pathway of haem biosynthesis from glycine to haem is shown in Fig. 1.9. The first enzyme of the pathway, ALA-S, plays a key role in the regulation of haem biosynthesis. Haem, the end product, exercises a negative feedback control on its synthesis,³⁴ by modulating the amount of ALA-S.

Protoporphyrin IX is also a precursor in chlorophyll biosynthesis¹²⁻¹⁴ via the formation of Mg-protoporphyrin IX followed by monomethyl esterification, methylation, vinyl group reduction and

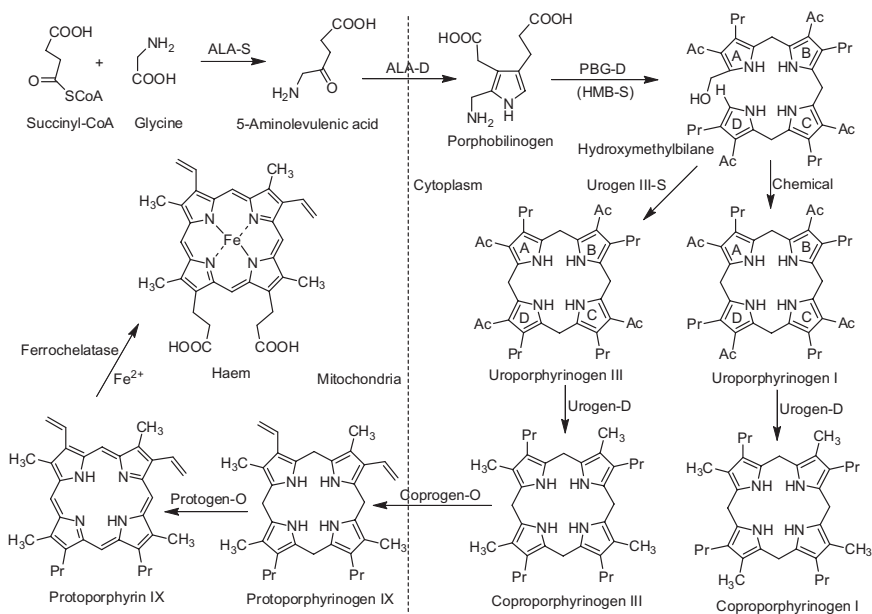


Figure 1.9. The haem biosynthetic pathway.

formation of a fifth ring (ring E) to give protochlorophyllide. In the presence of light protochlorophyllide is reduced to chlorophyll *a*, which is esterified by phytyl diphosphate to form chlorophyll *a* (Fig. 1.10).

1.4. Biosynthesis of Bilins in Animals and Plants

1.4.1. Degradation of haem to bile pigments

Bilins is the general term for open-chain tetrapyrroles^{17,18} derived from names given to bile pigments, the haem degradation products excreted in animal bile. In humans the conversion of haem derived from haemoglobin of effete red cells at the end of their life span to the green bile pigment biliverdin IX α occurs in the reticulo-endothelial system by a series of reactions which are catalysed by the microsomal haem oxygenase system.³⁵⁻³⁷ A small proportion comes from the turnover of other haemoproteins, e.g. cytochrome P450s,

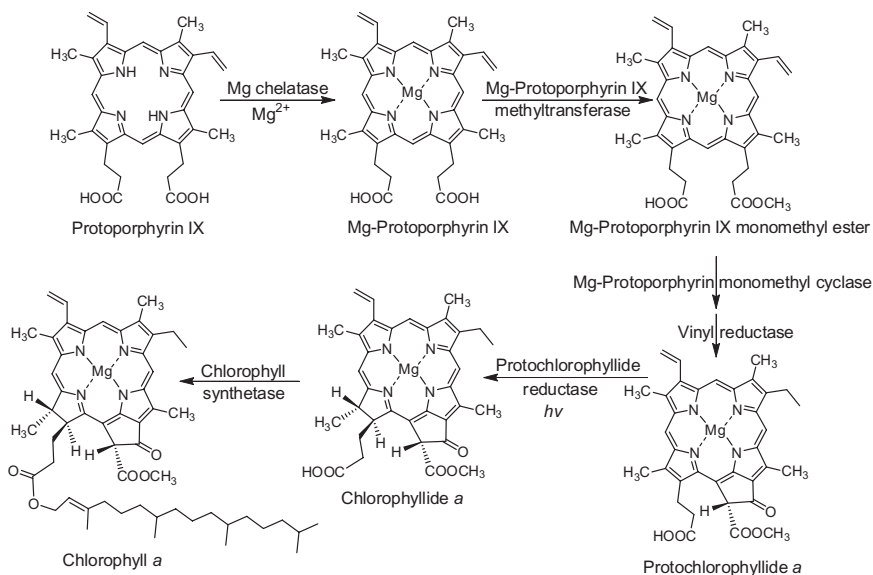


Figure 1.10. The biosynthesis of chlorophyll a from protoporphyrin IX.

immature erythroid cells and free haem which turnover at a faster rate. Biliverdin IX α is reduced at the C-10 position to bilirubin IX α , the yellow bile pigment (Fig. 1.11). The reaction is catalysed by the cytosolic enzyme NADPH-dependent biliverdin reductase.^{38,39}

Bilirubin forms extensive intra-molecular hydrogen bonds which give it a strongly hydrophobic property.^{40–42} It is insoluble in aqueous solution at physiological pH and is transported in blood tightly bound to albumin.⁴³ Bilirubin is excreted in the bile into the intestine mainly as the polar bilirubin mono- and di-glucuronide conjugates after esterification of the C-8 and/or C-12 propionic acid side-chains with uridine diphosphate glucuronic acid in the liver. The reaction is catalysed by the microsomal enzyme bilirubin UDP-glucuronosyl transferase (UGT1A1).^{44,45} Xylose and glucose conjugates have also been detected in small quantities. In the intestine de-conjugation and sequential hydrogenation by intestinal flora results in a series of chromogens which on hydrogenation give a variety of faecal bile pigments with varying degree of double bond conjugation and colour⁴⁶

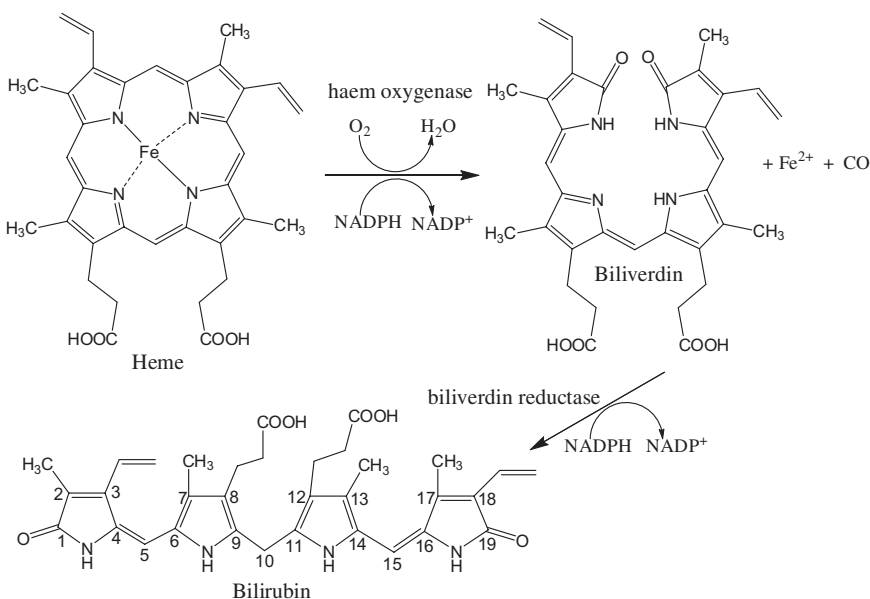


Figure 1.11. The degradation of haem by haem oxygenase system.

ranging from the green-blue biliverdins, violet biliviolins, yellow bilirubins, orange urobilins to the colourless urobilinogens. The structures of these bile pigments are shown in Fig. 1.12.

1.4.2. Biosynthesis of bilins in plants, algae and cyanobacteria

Haem oxygenase is present not only in animals but also in plants, algae and cyanobacteria.⁴⁷⁻⁴⁹ The same pathway of enzyme reaction that converts protohaem to biliverdin IX α is observed for all these organisms. From the same universal precursor, plants, algae and cyanobacteria convert biliverdin IX α into phycobilins,^{47,48} the open-chain tetrapyrroles, which include phytochromobilin, phycoerythrobilin, phycocyanobilin, phycobiliviolin and phycourobilin. These, together with chlorophyll *a* and β -carotene, give the immense variety of colouration seen in algae and cyanobacteria, from blue-green, purple, red and orange to yellow.⁵⁰

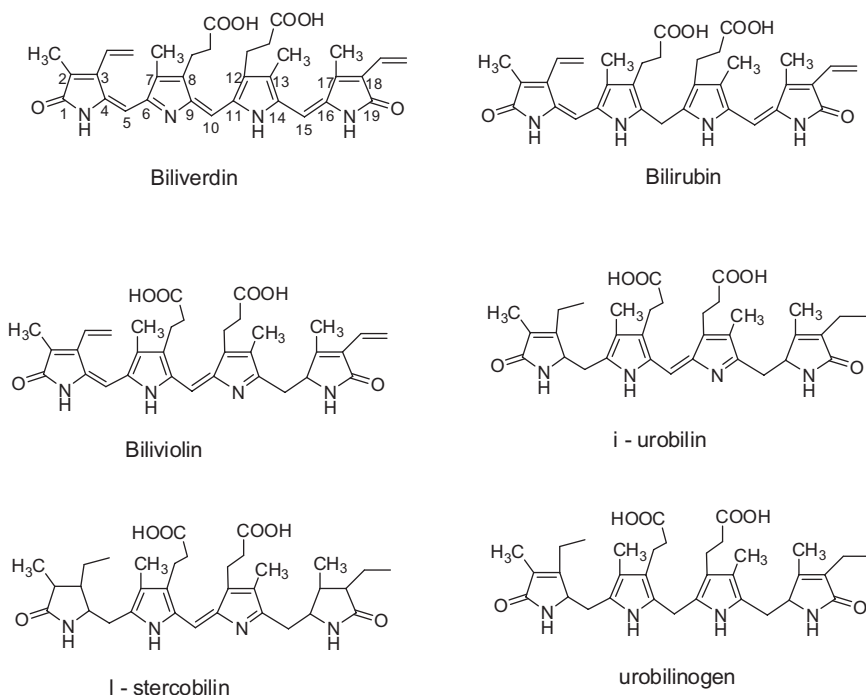


Figure 1.12. Structures of bile pigments.

In higher plants biliverdin IX α is converted to 3(*Z*)-phytychromobilin by the plastid-localised enzyme phytychromobilin synthase (P Φ B synthase) which is a bilin 2,3-reductase (Fig. 1.13). This gives the ethylinene group on ring A essential for covalent linkage to apophytochrome, which occurred after isomerisation of 3(*Z*)-phytychromobilin to 3(*E*)-phytychromobilin catalysed by a bilin 3¹,3² *cis-trans* isomerase.^{51,52}

In red algae biliverdin IX α is first reduced to 15,16-dihydrobiliverdin IX α catalysed by a bilin 15,16-reductase.⁵³ This is then followed by reduction at the 2,3-positions, catalysed by a bilin 2,3-reductase, to give 3(*Z*)-phycoerythrobilin (Fig. 1.13). It is also believed that the 3(*Z*)-isomer is isomerised to 3(*E*)-phycoerythrobilin catalysed by a bilin 3¹,3²-isomerase.^{53,54} It has been shown that both the (*Z*)- and (*E*)-isomers are eventually converted into phycocyanobilins.^{47,48,53}

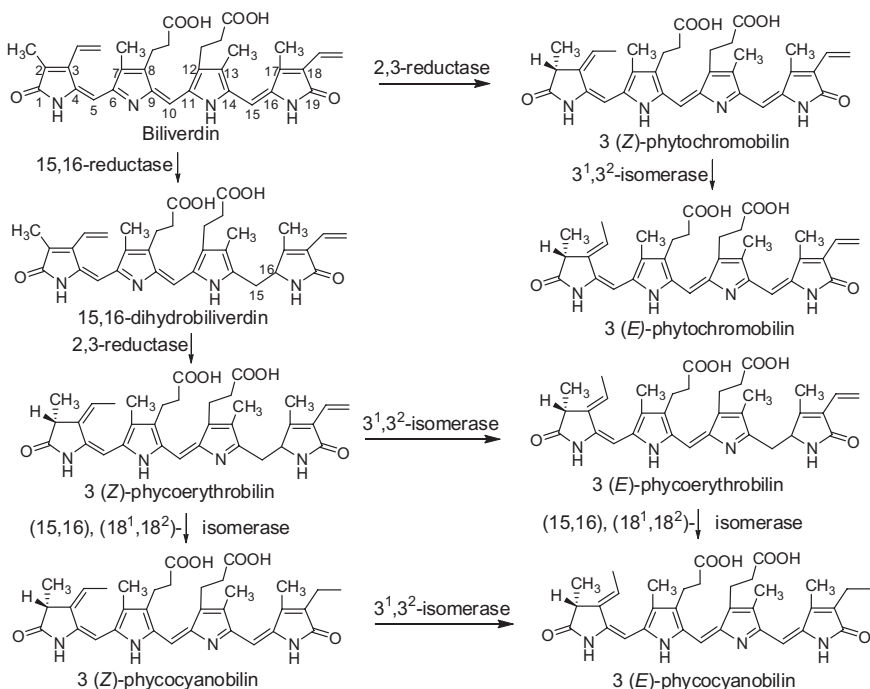


Figure 1.13. Biosynthetic pathways of bilins in plants, algae and cyanobacteria.

In cyanobacteria, only the 3(Z)-phycocyanobilin isomer is produced⁵⁵ and the green algae *Mesotanium caldarium* is reported to synthesise 3(Z)-phycocyanobilin directly from 3(Z)-phytochromobilin⁵⁶ (Fig. 1.13).

1.4.3. Degradation of chlorophyll in senescent higher plants

The degradation of chlorophylls to colourless nonfluorescent catabolites (NCCs)^{57–59} is an integral part of leaf senescence and fruit ripening processes. The breakdown pathway begins with de-phytylation of chlorophyll *a* by chlorophyllase (chlase), followed

by the removal of Mg by a Mg-dechelating substance or Mg-dechelataase to give phaeophobide *a*. Oxidative ring opening then takes place, catalysed by phaeophobide *a* oxygenase (PAO), with the conversion of phaeophobide *a* into the open-chain tetrapyrrole red chlorophyll catabolite (RCC). It has been suggested that chlorophyll *b* is reduced to chlorophyll *a* before entering the pathway through PAO.⁶⁰ RCC is then reduced to a primary fluorescent chlorophyll catabolite (pFCC), catalysed by RCC reductase. Modification of several peripheral side-chains of pFCC occurred and the modified FCCs are transported to the vacuoles⁶¹ where, under weakly acidic conditions, they undergo rapid, stereospecific isomerisation to give ubiquitous NCCs (Fig. 1.14). The type of peripheral side-chain modifications within different NCCs are species specific.^{62,63}

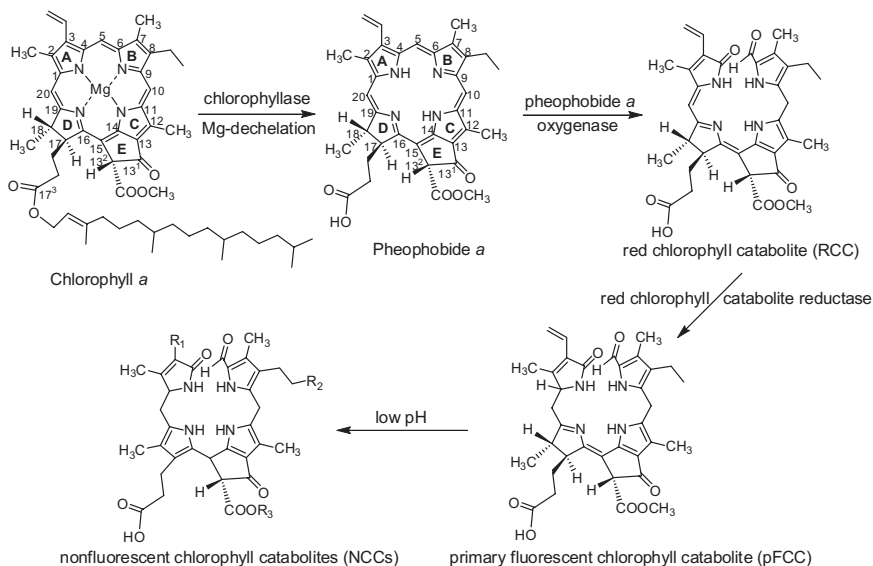


Figure 1.14. The pathway of chlorophyll breakdown in higher plants. Sites of peripheral modifications as present in different NCCs are indicated ($R_1 - R_3$). $R_1 = \text{CH}_2\text{CH}_3$; $R_2 = \text{H, OH or O-glucosyl}$; $R_3 = \text{H or CH}_3$.

1.5. Functions of Porphyrins and Other Tetrapyrroles

The macrocyclic tetrapyrrole structure is ideal for the insertion of metal atoms to form metallo-complexes which are the prosthetic groups in the formation of metalloproteins and metalloenzymes where many essential biochemical processes and bioenergetic reactions of life take place. They are nature's most important catalysts. Protoporphyrin IX complexes with iron to form the oxygen transport metalloprotein haemoglobin which uses reversible oxygen coordination to iron II for transport of oxygen to organs throughout the body. Myoglobin, found in large amounts in skeletal and cardiac muscles, stores oxygen for use when needed and transports oxygen by diffusion. Other haem containing proteins include the cytochromes, peroxidases, reductases and catalase, which carry out a wide range of important oxidation and reduction reactions vital for all living cells. Sirohaem is the cofactor of sulphite and nitrite reductases. Chlorophylls are magnesium tetrapyrrole complexes which capture and convert absorbed sunlight into usable energy in photosynthesis. Vitamin B₁₂ or cyanocobalamin, a cofactor in methyltransferases, is a cobalt tetrapyrrole complex. Factor F₄₃₀ is involved in methane formation in certain bacteria, and is a nickel tetrapyrrole complex. Uroporphyrinogen III is the common intermediate to all these cellular tetrapyrrole metal complexes.

Bile pigments, especially bilirubin, possess significant antioxidant⁶⁴⁻⁶⁶ and anti-mutagenic properties.⁶⁷ They are potent free radical scavengers and have been shown to inhibit the mutagenic effects of oxidants and aromatic mutagens such as poly aromatic hydrocarbons and heterocyclic amines.

Bilirubin has been hypothesised to have a circadian regulation role in humans.⁶⁸ The albumin-bound bilirubin resembles phytochromes, which set the biological clock in plants.

In higher plants, phytochromobilin, the open-chain tetrapyrrole, is the chromophore of phytochrome which functions as a light-sensing pigment or photoreceptor in plant development.^{52,69-71} It has the ability to photo-interconvert between red and far-red light absorbing forms by sensing the ambient light conditions. Phytochrome-like

molecules have also been identified in algae,⁷² ferns and mosses⁷³ and cyanobacteria.⁷⁴

Phycobiliproteins⁵⁰ are a homologous family of phycobilin-protein complexes present in cyanobacteria,⁵⁰ red algae,⁵⁰ cryptomonads,⁷⁵ and some species of prochlorophytes.⁷⁶ They are the light-harvesting antennae of these organisms⁵⁰ with the open-chain tetrapyrrole chromophores covalently linked to protein molecules via cysteine residues.

Phycobiliproteins, especially phycocyanin, the blue, light-harvesting pigment in cyanobacteria, rhodophytes and cryptophytes, are water soluble antioxidants with strong fluorescent properties. Phycocyanin has been investigated for potential applications in the food, cosmetic and biotechnology industries, and in diagnostic medicine because of these useful properties.⁷⁷

Porphyrins and related compounds are excellent photosensitisers⁷⁸⁻⁸⁰ used in photodynamic therapy (PDT) of diseases, including cancer,^{81,82} dermatological conditions^{83,84} and wet age-related macular degeneration (AMD).^{85,86} PDT comprises exogenous administration of a light-absorbing compound (photosensitiser) which can accumulate in a target tissue. Light of wavelength matching its absorption characteristics is directed at the target tissue to photoactivate the sensitiser. This generates free radicals, especially singlet oxygen, at a rate that overwhelms tissue defence and causes cell death.

PDT has been investigated as a new anti-microbial strategy^{87,88} for treating localised infections caused by MRSA and for modulating wound healing. Anti-microbial PDT has also been suggested as a possible method for eliminating pathogenic oral bacteria within the oral cavity.⁸⁸

Porphyrin photosensitisers have been used for photocatalytic patterning.⁸⁹ Porphyrins are excited to generate radical species that photocatalytically oxidise, and thereby pattern, chemistries in the local vicinity. The technique, suitable for a wide variety of substrates including proteins and cells, has potential applications in biological and medical sciences.

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