M1 Nitrogen fixation and assimilation

Key Notes

The nitrogen cycle

The nitrogen cycle is the movement of nitrogen through the food chain from simple inorganic compounds, mainly ammonia, to complex organic compounds.

Nitrogen fixation

Nitrogen fixation is the conversion of N_2 gas into ammonia, a process carried out by some soil bacteria, cyanobacteria and the symbiotic bacteria *Rhizobium* that invade the root nodules of leguminous plants. This process is carried out by the nitrogenase complex, which consists of a reductase and an iron–molybdenum-containing nitrogenase. At least 16 ATP molecules are hydrolyzed to form two molecules of ammonia. Leghemoglobin is used to protect the nitrogenase in the *Rhizobium* from inactivation by O_2.

Nitrogen assimilation

Ammonia is assimilated by all organisms into organic nitrogen-containing compounds (amino acids, nucleotides, etc.) by the action of glutamate dehydrogenase (to form glutamate) and glutamine synthetase (to form glutamine).

Related topics

Myoglobin and hemoglobin (B4)
Citric acid cycle (L1)
Electron transport and oxidative phosphorylation (L2)
Photosynthesis (L3)
Amino acid metabolism (M2)
Hemes and chlorophylls (M4)

The nitrogen cycle

The nitrogen cycle refers to the movement of nitrogen through the food chain of living organisms (*Fig. 1*). This complex cycle involves bacteria, plants and animals. All organisms can convert **ammonia** (NH_3) to organic nitrogen compounds, that is compounds containing C–N bonds. However, only a few microorganisms can synthesize ammonia from nitrogen gas (N_2). Although N_2 gas makes up about 80% of the earth's atmosphere, it is a chemically unreactive compound. The first stage in the nitrogen cycle is the reduction of N_2 gas to ammonia, a process called **nitrogen fixation**. Ammonia can also be obtained by reduction of nitrate ion (NO_3^-) that is present in the soil. **Nitrate reduction** can be carried out by most plants and microorganisms. The ammonia resulting from these two processes can then be assimilated by all organisms. Within the biosphere there is a balance between total inorganic and total organic forms of nitrogen. The conversion of organic to inorganic nitrogen comes about through catabolism, denitrification and decay (*Fig. 1*).

Nitrogen fixation

The process of converting atmospheric N_2 gas into ammonia (nitrogen fixation) is carried out by only a few microorganisms, termed **diazatrophs**. These are

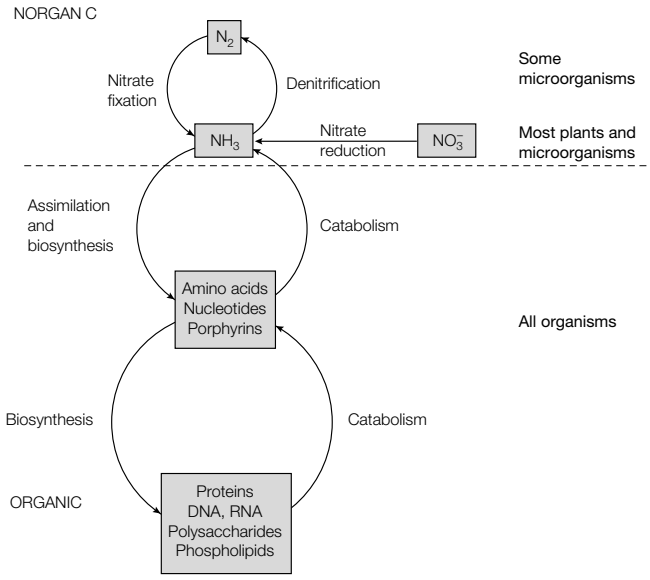

Fig. 1. The interrelationships between inorganic and organic nitrogen metabolism.

some free-living soil bacteria such as *Klebsiella* and *Azotobacter*, cyanobacteria (blue–green algae), and the **symbiotic bacteria** (mainly *Rhizobium*). The symbiotic *Rhizobium* bacteria invade the roots of **leguminous green plants** (plants belonging to the pea family, e.g. beans, clover, alfalfa) and form **root nodules** where nitrogen fixation takes place. The amount of N_2 fixed by these diazatrophic microorganisms has been estimated to be in the order of 10^{11} kg per year, about 60% of the earth's newly fixed nitrogen. Lightning and ultraviolet radiation fix another 15%, with the remainder coming from industrial processes.

The chemical unreactivity of the N≡N bond is clearly seen when one considers the industrial process of nitrogen fixation. This process, devised by Fritz Haber in 1910 and still used today in fertilizer factories, involves the reduction of N_2 in the presence of H_2 gas over an iron catalyst at a temperature of 500°C and a pressure of 300 atmospheres.

$$N_2 + 3\,H_2 \rightleftharpoons 2\,NH_3$$

Nitrogenase complex

Biological nitrogen fixation is carried out by the **nitrogenase complex** which consists of two proteins: a **reductase**, which provides electrons with high reducing power, and a **nitrogenase**, which uses these electrons to reduce N_2 to NH_3 (*Fig.* 2). The reductase is a 64 kDa dimer of identical subunits that contains one iron–sulfur cluster and two ATP binding sites. The nitrogenase is a larger protein of 220 kDa that consists of two α- and two β-subunits ($\alpha_2\beta_2$) and contains an iron–molybdenum complex. The transfer of electrons from the reductase to the nitrogenase protein is coupled to the hydrolysis of ATP by the reductase.

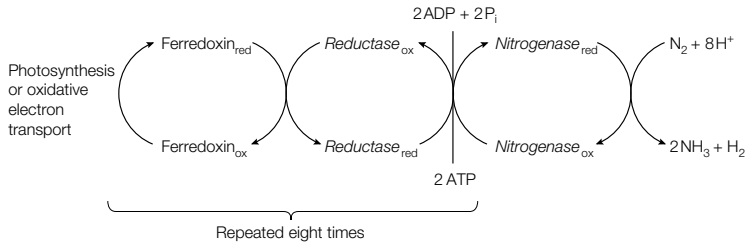

Fig. 2. The flow of electrons in the nitrogenase-catalyzed reduction of N_2.

Although the reduction of N_2 to NH_3 is only a six-electron process:

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

the reductase is imperfect and H_2 is also formed. Thus two additional electrons are also required:

$$N_2 + 8\,e^- + 8\,H^+ \rightarrow 2\,NH_3 + H_2$$

The eight high-potential electrons come from reduced **ferredoxin** that is produced either in chloroplasts by the action of photosystem I or in oxidative electron transport (*Fig. 2*) (see Topics L2 and L3). The overall reaction of biological nitrogen fixation:

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

highlights that it is energetically very costly, with at least 16 ATP molecules being hydrolyzed.

Leghemoglobin

The nitrogenase complex is extremely sensitive to inactivation by O_2, so the enzyme must be protected from this reactive substance. In the root nodules of leguminous plants, protection is afforded by the symbiotic synthesis of **leghemoglobin**. The globin part of this monomeric oxygen-binding protein (see Topic B4) is synthesized by the plant, whereas the **heme** group (see Topic M4) is synthesized by the *Rhizobium*. The leghemoglobin has a very high affinity for O_2, so maintaining a low enough concentration to protect the nitrogenase.

Nitrogen assimilation

The next step in the nitrogen cycle is the assimilation of inorganic nitrogen, in the form of ammonia, into organic nitrogen-containing compounds. All organisms assimilate ammonia via two main reactions catalyzed by **glutamate dehydrogenase** and **glutamine synthetase** giving rise to the amino acids glutamate (Glu) and glutamine (Gln), respectively. The amino nitrogen in Glu and the amide nitrogen in Gln are then used in further biosynthetic reactions to give rise to other compounds.

Glutamate dehydrogenase

Glutamate dehydrogenase catalyzes the reductive amination of the citric acid cycle intermediate α-ketoglutarate (*Fig. 3a*) (see Topic L1). Although the reaction is reversible, the reductant used in the biosynthetic reaction is NADPH. This enzyme is also involved in the catabolism of amino acids (see Topic M2).

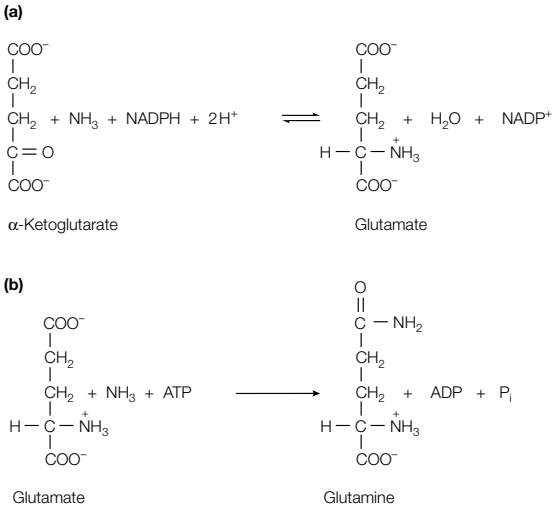

Fig. 3. Assimilation of ammonia by (a) glutamate dehydrogenase and (b) glutamine synthetase.

Glutamine synthetase

Glutamine synthetase catalyzes the incorporation of ammonia into glutamine, deriving energy from the hydrolysis of ATP (*Fig. 3b*). This enzyme is named a **synthetase**, rather than a synthase, because the reaction couples bond formation with the hydrolysis of ATP. In contrast, a **synthase** does not require ATP.

M2 Amino acid metabolism

Key Notes

Biosynthesis of amino acids

Some organisms can synthesize all of the 20 standard amino acids, others cannot. Nonessential amino acids are those that can be synthesized, essential amino acids have to be taken in the diet. The 20 standard amino acids can be grouped into six biosynthetic families depending on the metabolic intermediate from which their carbon skeleton is derived.

Amino acid degradation

Amino acids are degraded by the removal of the α-amino group and the conversion of the resulting carbon skeleton into one or more metabolic intermediates. Amino acids are termed glucogenic if their carbon skeletons can give rise to the net synthesis of glucose, and ketogenic if they can give rise to ketone bodies. Some amino acids give rise to more than one intermediate and these lead to the synthesis of glucose as well as ketone bodies. Thus these amino acids are both glucogenic and ketogenic.

Transamination

The α-amino groups are removed from amino acids by a process called transamination. The acceptor for this reaction is usually the α-keto acid, α-ketoglutarate, which results in the formation of glutamate and the corresponding α-keto acid. The coenzyme of all transaminases is pyridoxal phosphate which is derived from vitamin B_6 and which is transiently converted during transamination into pyridoxamine phosphate.

Oxidative deamination of glutamate

The glutamate produced by transamination is oxidatively deaminated by glutamate dehydrogenase to produce ammonia. This enzyme is unusual in being able to use either NAD^+ or $NADP^+$, and is subject to allosteric regulation. GTP and ATP are allosteric inhibitors, whereas GDP and ADP are allosteric activators.

Amino acid oxidases

Small amounts of amino acids are degraded by L- and D-amino acid oxidases that utilize flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzyme, respectively.

Metabolism of phenylalanine

Phenylalanine is first converted to tyrosine by the monooxygenase phenylalanine hydroxylase; a reaction involving the coenzyme tetrahydrobiopterin. The tyrosine is then converted first by transamination and then by a dioxygenase reaction to homogentisate, which in turn is further metabolized to fumarate and acetoacetate.

Inborn errors of metabolism

Inborn errors of metabolism are inherited metabolic disorders caused by the absence of an enzyme in a metabolic pathway. Alkaptonuria is caused by the lack of homogentisate oxidase and is harmless, whereas phenylketonuria, which is due to a lack of phenylalanine hydroxylase, can cause severe mental retardation.

Related topics

- Amino acids (B1)
- Introduction to enzymes (C1)
- Regulation of enzyme activity (C5)
- Glycolysis (J3)
- Gluconeogenesis (J4)
- Pentose phosphate pathway (J5)
- Fatty acid breakdown (K2)
- Fatty acid synthesis (K3)
- Citric acid cycle (L1)
- Electron transport and oxidative phosphorylation (L2)
- Nitrogen fixation and assimilation (M1)
- The urea cycle (M3)

Biosynthesis of amino acids

Plants and microorganisms can synthesize all of the 20 standard amino acids. Mammals, however, cannot synthesize all 20 and must obtain some of them in their diet. Those amino acids that are supplied in the diet are referred to as **essential**, whereas the remainder that can be synthesized by the organism are termed **nonessential**. This designation refers to the needs of an organism under a particular set of conditions. In humans the nonessential amino acids are Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, Pro, Ser and Tyr, while the essential ones are His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val (see Topic B1 for the structures of the 20 standard amino acids). The pathways for the biosynthesis of amino acids are diverse and often vary from one organism to another. However, they all have an important feature in common: their carbon skeletons come from key intermediates in central metabolic pathways (glycolysis, Topic J3; the citric acid cycle, Topic L1; or the pentose phosphate pathway, Topic J5) (*Fig. 1*). The amino acids can be grouped together into six biosynthetic pathways depending on the intermediate from which they are derived (*Fig. 1*). The primary amino group usually comes from transamination of glutamate.

Amino acid degradation

As there is no store for excess amino acids, and as proteins are constantly being turned over, amino acids have to be continually degraded. The α-amino group is removed first and the resulting carbon skeleton is converted into one or more major metabolic intermediates and used as metabolic fuel. The carbon skeletons of the 20 standard amino acids are funneled into only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate (*Fig. 2*). Amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate are termed **glucogenic** as they can give rise to the net synthesis of glucose. This is because the citric acid cycle intermediates and pyruvate can be converted into phosphoenolpyruvate and then into glucose via gluconeogenesis (see Topics J4 and L1). In contrast, amino acids that are degraded to acetyl CoA or acetoacetyl CoA are termed **ketogenic** because they give rise to ketone bodies (see Topic K2); the acetyl CoA or acetoacetyl CoA can also be used to synthesize lipids (see Topic K3). Of the standard set of 20 amino acids, only Leu and Lys are solely ketogenic. Ile, Phe, Trp and Tyr are both ketogenic and glucogenic as some of their carbon atoms end up in acetyl CoA or acetoacetyl CoA, whereas others end up in precursors of glucose. The remaining 14 amino acids are classified as solely glucogenic.

Transamination

Prior to the metabolism of their carbon skeletons into a major metabolic intermediate, the α-amino group of the amino acid has first to be removed by a process known as **transamination**. In this process the α-amino group of most amino

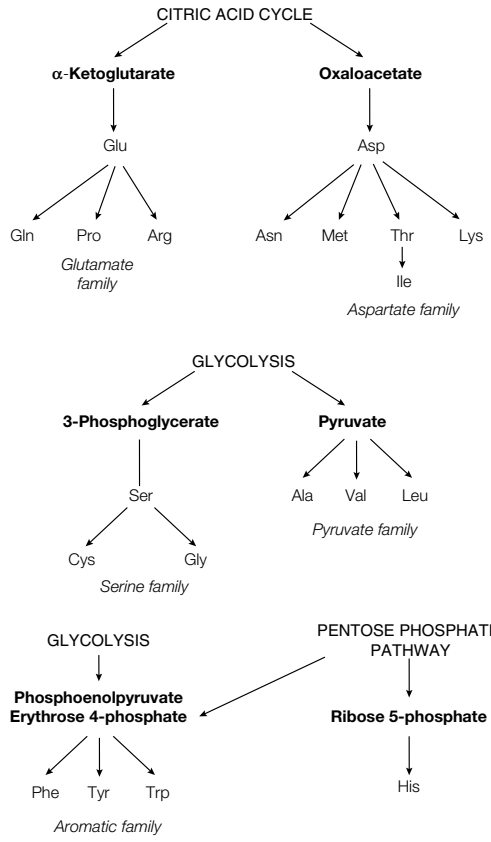

Fig. 1. Biosynthetic families of amino acids.

acids is transferred to **α-ketoglutarate** to form **glutamate** and the corresponding α-keto acid:

$$\alpha\text{-amino acid} + \alpha\text{-ketoglutarate} \rightleftharpoons \alpha\text{-keto acid} + \text{glutamate}$$

The enzymes that catalyze these reactions are called **transaminases** (aminotransferases) and in mammals are found predominantly in the liver. For example, aspartate transaminase catalyzes the transfer of the amino group of aspartate to α-ketoglutarate (*Fig. 3a*), while alanine transaminase catalyzes the transfer of the amino group of alanine to α-ketoglutarate (*Fig. 3b*).

Pyridoxal phosphate

The coenzyme (or prosthetic group) of all transaminases is **pyridoxal phosphate**, which is derived from pyridoxine (**vitamin B_6**), and which is transiently converted into **pyridoxamine phosphate** during transamination (*Fig. 4*). In the absence of substrate, the aldehyde group of pyridoxal phosphate forms a covalent **Schiff base linkage** (imine bond) with the amino group in the side-chain of a specific lysine residue in the active site of the enzyme. On addition of

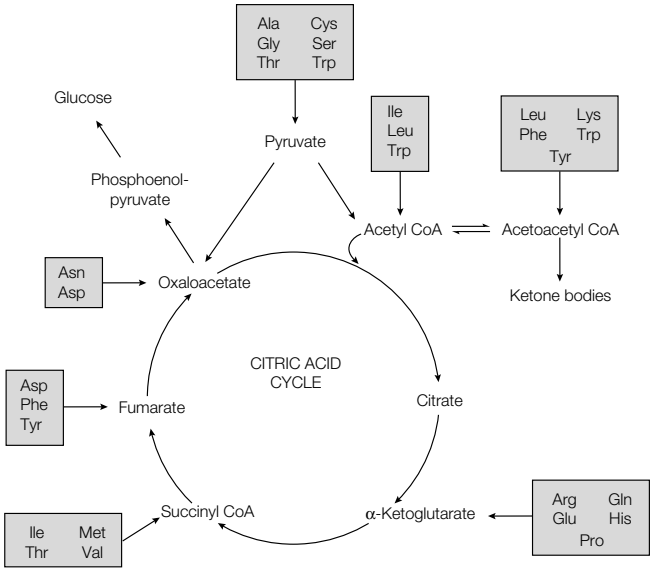

Fig. 2. Fates of the amino acid carbon skeletons.

substrate, the α-amino group of the incoming amino acid displaces the amino group of the active site lysine and a new Schiff base linkage is formed with the amino acid substrate. The resulting amino acid–pyridoxal phosphate–Schiff base that is formed remains tightly bound to the enzyme by multiple noncovalent interactions.

The amino acid is then hydrolyzed to form an α-keto acid and pyridoxamine

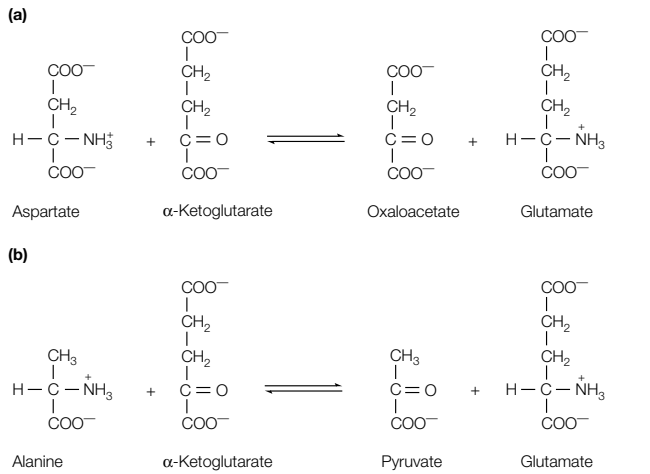

Fig. 3. Reactions catalyzed by (a) aspartate transaminase and (b) alanine transaminase.

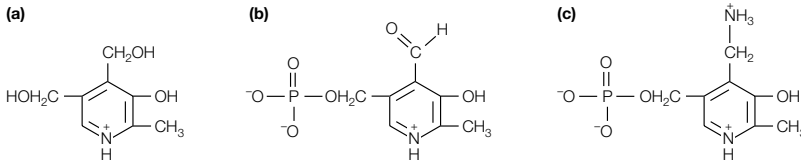

Fig. 4. Structures of (a) pyridoxine (vitamin B_6), (b) pyridoxal phosphate and (c) pyridoxamine phosphate.

phosphate, the α-amino group having been temporarily transferred from the amino acid substrate on to pyridoxal phosphate (*Fig. 5*). These steps constitute one half of the overall transamination reaction. The second half occurs by a reversal of the above reactions with a second α-keto acid reacting with the pyridoxamine phosphate to yield a second amino acid and regenerate the enzyme–pyridoxal phosphate complex (*Fig. 5*).

The reactions catalyzed by transaminases are **anergonic** as they do not require an input of metabolic energy. They are also freely reversible, the direction of the reaction being determined by the relative concentrations of the amino acid–keto acid pairs. Pyridoxal phosphate is not just used as the coenzyme in transamination reactions, but is also the coenzyme for several other reactions

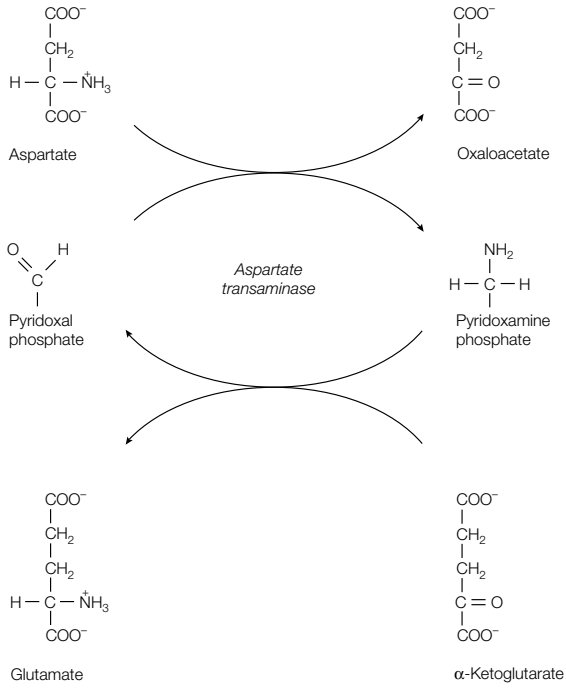

Fig. 5. The overall reaction of transamination.

involving amino acids including decarboxylations, deaminations, racemizations and aldol cleavages.

Oxidative deamination of glutamate

The α-amino groups that have been funneled into glutamate from the other amino acids are then converted into **ammonia** by the action of **glutamate dehydrogenase** (*Fig. 6*). This enzyme is unusual in being able to utilize either NAD^+ or $NADP^+$ (see Topic C1). In the biosynthesis of glutamate, the $NADP^+$ form of the coenzyme is used (see Topic M1), whereas NAD^+ is used in its degradation. Glutamate dehydrogenase consists of six identical subunits and is subject to **allosteric regulation** (see Topic C5 for a detailed description of allosteric regulation). GTP and ATP are **allosteric inhibitors**, whereas GDP and ADP are **allosteric activators**. Hence, when the energy charge of the cell is low (i.e. there is more ADP and GDP than their triphosphate forms) glutamate dehydrogenase is activated and the oxidation of amino acids increases. The resulting carbon skeletons are then utilized as metabolic fuel, feeding into the citric acid cycle (see Topic L1) and ultimately giving rise to energy through oxidative phosphorylation (see Topic L2).

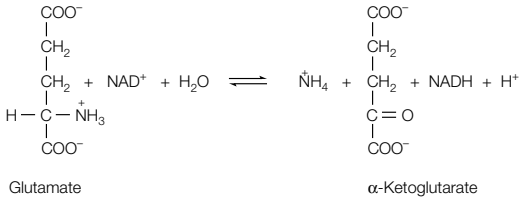

Fig. 6. Oxidative deamination of glutamate by glutamate dehydrogenase.

Amino acid oxidases

The major route for the deamination of amino acids is transamination followed by the oxidative deamination of glutamate. However, a minor route also exists that involves direct oxidation of the amino acid by **L-amino acid oxidase**. This enzyme utilizes **flavin mononucleotide (FMN)** as its coenzyme (see Topic C1), with the resulting $FMNH_2$ being reoxidized by molecular O_2, a process that also generates the toxic H_2O_2 (*Fig. 7*). The H_2O_2 is rendered harmless by the action of **catalase**. Kidney and liver are also rich in the **FAD**-containing **D-amino acid oxidase**. However, the function of this enzyme in animals is unclear, since the

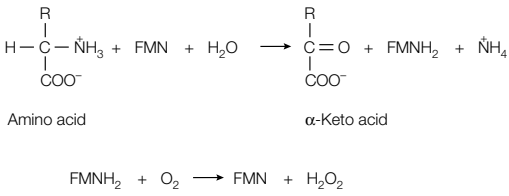

Fig. 7. Action of L-amino acid oxidase.

D-isomers of amino acids are rare in nature, only occurring in bacterial cell walls (see Topic A1) and certain antiobiotics.

Metabolism of phenylalanine

The metabolism of phenylalanine will now be considered in some detail, as two inborn errors of metabolism are known that affect this pathway. **Phenylalanine** is first hydroxylated by **phenylalanine hydroxylase** to form another aromatic amino acid **tyrosine** (*Fig. 8*). The coenzyme for this reaction is the reductant **tetrahydrobiopterin** which is oxidized to **dihydrobiopterin**. Phenylalanine hydroxylase is classified as a **monooxygenase** as one of the atoms of O_2 appears in the product and the other in H_2O. The tyrosine is then transaminated to *p*-hydroxyphenylpyruvate, which is in turn converted into **homogentisate** by *p*-hydroxyphenylpyruvate hydroxylase. This hydroxylase is an example of a **dioxygenase**, as both atoms of O_2 become incorporated into the product (*Fig. 8*). The homogentisate is then cleaved by homogentisate oxidase, another dioxygenase, before fumarate and acetoacetate are produced in a final reaction. The fumarate can feed into the citric acid cycle (see Topic L1), whereas acetoacetate can be used to form ketone bodies (see Topic K2). Thus phenylalanine and tyrosine are each both glucogenic and ketogenic.

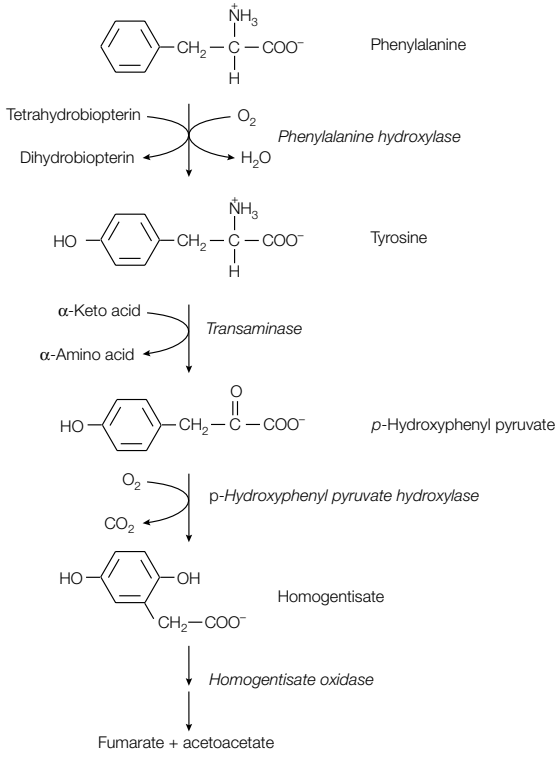

Fig. 8. The metabolism of phenylalanine.

Inborn errors of metabolism

Two inborn errors of metabolism are known that affect phenylalanine metabolism. These are **inherited metabolic disorders** caused by the absence of one of the enzymes in the pathway. One of these disorders, **alkaptonuria**, is caused by the absence of **homogentisate oxidase**. This results in the accumulation of homogentisate that is subsequently excreted in the urine, and which oxidizes to a black color on standing. This defect is harmless, in contrast with the other disorder, **phenylketonuria**. In phenylketonuria there is a block in the hydroxylation of phenylalanine to tyrosine caused by an absence or deficiency of **phenylalanine hydroxylase** or, more rarely, of its tetrahydrobiopterin coenzyme. The result is a 20-fold increase in the levels of phenylalanine in the blood with its subsequent **transamination** to phenylpyruvate. If untreated, severe mental retardation occurs, with a life expectancy of on average 20 years. With an incidence of 1:20 000 this condition is now screened for at birth, with treatment being to restrict the intake of phenylalanine (by putting the individual on a **low phenylalanine diet**) and thus minimize the need to metabolize the excess. However, enough phenylalanine must be provided to meet the needs for growth and replacement.

M3 THE UREA CYCLE

Key Notes

Ammonia excretion

Excess nitrogen is excreted as ammonia. Ammonotelic organisms excrete ammonia directly, uricotelic organisms excrete it as uric acid, and ureotelic organisms excrete it as urea.

The urea cycle

In the urea cycle ammonia is first combined with CO_2 to form carbamoyl phosphate. This then combines with ornithine to form citrulline. Citrulline then condenses with aspartate, the source of the second nitrogen atom in urea, to form argininosuccinate. This compound is in turn split to arginine and fumarate, and the arginine then splits to form urea and regenerate ornithine The first two reactions take place in the mitochondria of liver cells, the remaining three in the cytosol.

Link to the citric acid cycle

The fumarate produced in the urea cycle can enter directly into the citric acid cycle and be converted into oxaloacetate. Oxaloacetate can then be either transaminated to aspartate which feeds back into the urea cycle, or be converted into citrate, pyruvate or glucose.

Hyperammonemia

Hyperammonemia is an increase in the levels of ammonia in the blood caused by a defect in an enzyme of the urea cycle. The excess ammonia is channeled into glutamate and glutamine with a deleterious effect on brain function.

Formation of creatine phosphate

The urea cycle intermediate arginine can be condensed with glycine to form guanidinoacetate, which in turn is methylated by the methyl donor *S*-adenosyl methionine to creatine. The creatine is then phosphorylated to form creatine phosphate, a high-energy store found in muscle.

The activated methyl cycle

S-Adenosyl methionine is the major methyl donor in biological reactions. It is regenerated via the intermediates *S*-adenosyl homocysteine, homocysteine and methionine in the activated methyl cycle.

Uric acid

Uric acid, the major nitrogenous waste product of uricotelic organisms, is also formed in other organisms from the breakdown of purine bases. Gout is caused by the deposition of excess uric acid crystals in the joints.

Related topics

- Cytoskeleton and molecular motors (A3)
- Thermodynamics (C2)
- DNA structure (F1)
- RNA structure (G1)
- Gluconeogenesis (J4)
- Glycogen metabolism (J6)
- Triacylglycerols (K4)
- The citric acid cycle (L1)
- Amino acid metabolism (M2)

Ammonia excretion

There is no store for nitrogen-containing compounds as there is for carbohydrate (glycogen) or lipids (triacylglycerol) (see Topics J6 and K4). Thus nitrogen

ingested in excess of what is required by the organism has to be excreted. The excess nitrogen is first converted into **ammonia** and is then excreted from living organisms in one of three ways. Many aquatic animals simply excrete the ammonia itself directly into the surrounding water. Birds and terrestrial reptiles excrete the ammonia in the form of **uric acid**, while most terrestrial vertebrates convert the ammonia into **urea** before excretion. These three classes of organisms are called: **ammonotelic**, **uricotelic** and **ureotelic**, respectively.

The urea cycle

Urea is synthesized in the liver by the **urea cycle**. It is then secreted into the bloodstream and taken up by the kidneys for excretion in the urine. The urea cycle was the first cyclic metabolic pathway to be discovered by Hans Krebs and Kurt Henseleit in 1932, 5 years before Krebs discovered the citric acid cycle (see Topic L1). The overall reaction of the pathway is:

$$NH_4^+ + HCO_3^- + H_2O + 3\ ATP + aspartate \rightarrow$$
$$urea + 2\ ADP + AMP + 2\ P_i + PP_i + fumarate$$

One of the nitrogen atoms of urea comes from ammonia, the other is transferred from the amino acid aspartate, while the carbon atom comes from CO_2. **Ornithine**, an amino acid that is not in the standard set of 20 amino acids and is not found in proteins, is the carrier of these nitrogen and carbon atoms. Five enzymatic reactions are involved in the urea cycle (*Fig. 1*), the first two of which take place in mitochondria, the other three in the cytosol:

1. **Carbamoyl phosphate synthetase**, which is technically not a member of the urea cycle, catalyzes the condensation and activation of ammonia (from the oxidative deamination of glutamate by glutamate dehydrogenase; Topic M2) and CO_2 (in the form of bicarbonate, HCO_3^-) to form **carbamoyl phosphate**. The hydrolysis of two ATP molecules makes this reaction essentially irreversible.
2. The second reaction also occurs in the mitochondria and involves the transfer of the carbamoyl group from carbamoyl phosphate to **ornithine** by **ornithine transcarbamoylase**. This reaction forms another nonstandard amino acid **citrulline** which then has to be transported out of the mitochondrion into the cytosol where the remaining reactions of the cycle take place.
3. The citrulline is then condensed with aspartate, the source of the second nitrogen atom in urea, by the enzyme **argininosuccinate synthetase** to form **argininosuccinate**. This reaction is driven by the hydrolysis of ATP to AMP and PP_i, with subsequent hydrolysis of the pyrophosphate. Thus both of the high-energy bonds in ATP are ultimately cleaved.
4. **Argininosuccinase** then removes the carbon skeleton of aspartate from argininosuccinate in the form of **fumarate**, leaving the nitrogen atom on the other product **arginine**. As the urea cycle also produces arginine, this amino acid is classified as nonessential in ureotelic organisms. Arginine is the immediate precursor of urea.
5. The **urea** is then formed from arginine by the action of **arginase** with the regeneration of ornithine. The ornithine is then transported back into the mitochondrion ready to be combined with another molecule of carbamoyl phosphate.

Link to the citric acid cycle

The synthesis of fumarate by argininosuccinase links the urea cycle to the citric acid cycle (*Fig. 2*). Fumarate is an intermediate of this latter cycle which is then hydrated to malate, which in turn is oxidized to **oxaloacetate** (see Topic L1).

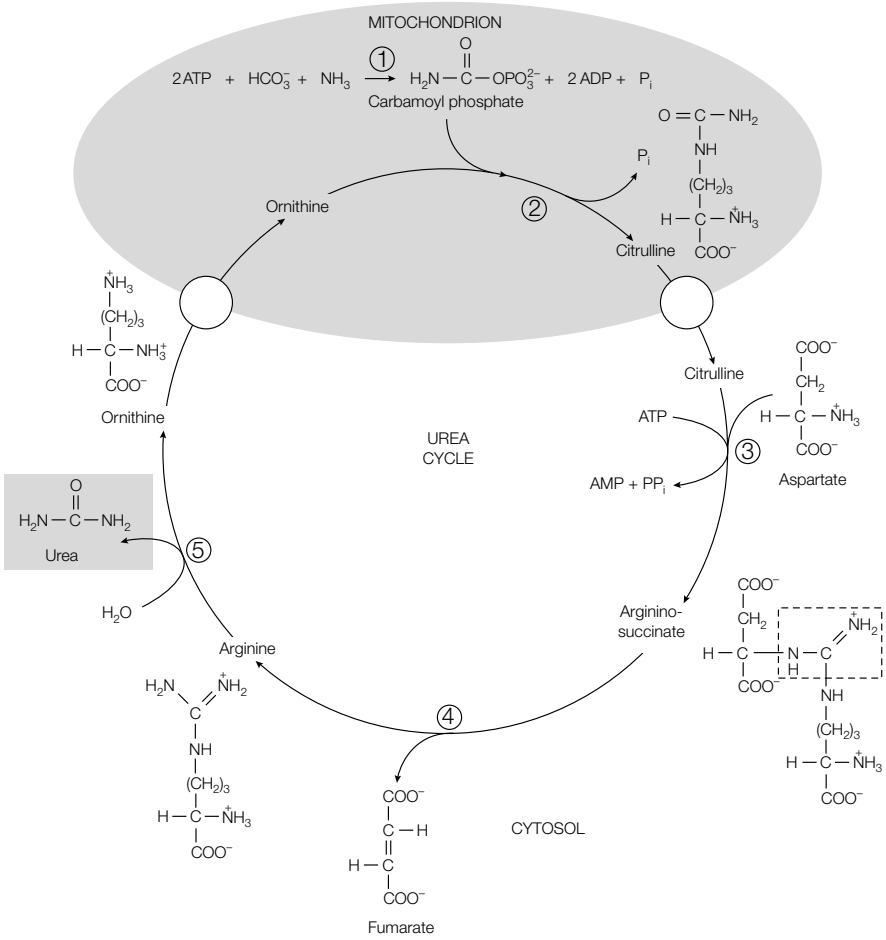

Fig. 1. The urea cycle. The enzymes involved in this cycle are: (1) carbamoyl phosphate synthetase; (2) ornithine transcarbamoylase; (3) argininosuccinate synthetase; (4) arginosuccinase; and (5) arginase.

Oxaloacetate has several possible fates:

- **transamination** to aspartate (see Topic M2) which can then feed back into the urea cycle;
- condensation with acetyl CoA to form citrate which then continues on round the citric acid cycle (see Topic L1);
- conversion into glucose via gluconeogenesis (see Topic J4);
- conversion into pyruvate.

Hyperammonemia Why do organisms need to detoxify ammonia in the first place? The answer to this question is obvious when one considers what happens if there is a block in

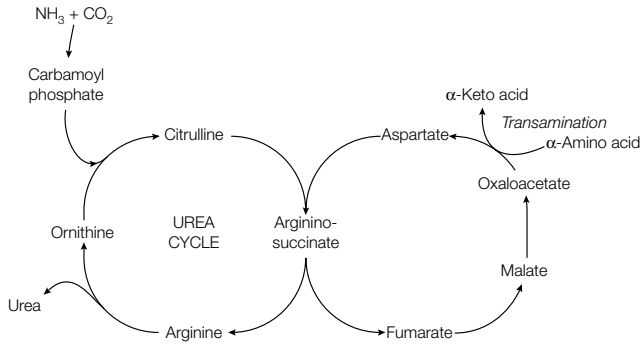

Fig. 2. The urea cycle and the citric acid cycle are linked by fumarate and the transamination of oxaloacetate to aspartate.

the urea cycle due to a defective enzyme. A block in any of the urea cycle enzymes leads to an increase in the amount of ammonia in the blood, so-called **hyperammonemia**. The most common cause of such a block is a genetic defect that becomes apparent soon after birth, when the afflicted baby becomes lethargic and vomits periodically. If left untreated, coma and irreversible brain damage will follow. The reasons for this are not entirely clear but may be because the excess ammonia leads to the increased formation of **glutamate** and **glutamine** (*Fig. 3*) (see Topic M1). These reactions result via depletion of the citric acid cycle intermediate α-ketoglutarate which may then compromise energy production, especially in the brain. It also leads to an increase in the acidic amino acids glutamate and glutamine which may directly cause damage to the brain.

Formation of creatine phosphate

The urea cycle is also the starting point for the synthesis of another important metabolite **creatine phosphate**. This **phosphagen** provides a reservoir of high-energy phosphate in muscle cells (see Topic A3) as the energy released upon its hydrolysis is greater than that released upon the hydrolysis of ATP (ΔG for creatine phosphate hydrolysis = –10.3 kcal mol^{-1} compared with –7.3 kcal mol^{-1} for ATP hydrolysis) (see Topic C2). The first step in the formation of creatine phosphate is the condensation of arginine and glycine to form guanidinoacetate (*Fig. 4*). Ornithine is released in this reaction and can then be re-utilized by the urea cycle. The guanidinoacetate is then methylated by the methyl group donor ***S*-adenosyl methionine** to form creatine, which is in turn phosphorylated by **creatine kinase** to form creatine phosphate (*Fig. 4*).

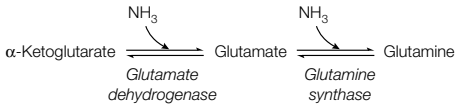

Fig. 3. Excess ammonia leads to the formation of glutamate and glutamine.

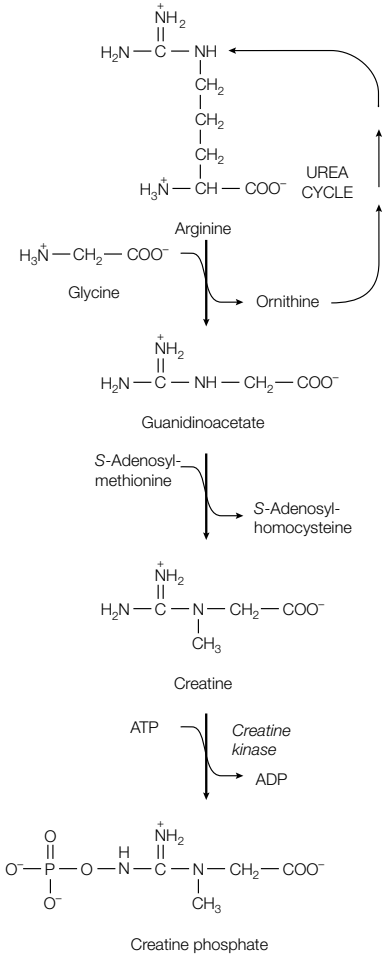

Fig. 4. Formation of creatine phosphate.

The activated methyl cycle

***S*-Adenosyl methionine** serves as a donor of **methyl groups** in numerous biological reactions [e.g. in the formation of creatine phosphate (see above) and in the synthesis of nucleic acids]. It is formed through the action of the activated methyl cycle (*Fig. 5*). During donation of its methyl group to another compound, *S*-adenosyl methionine is converted into *S*-adenosyl homocysteine. To regenerate *S*-adenosyl methionine, the adenosyl group is removed from the *S*-adenosyl homocysteine to form homocysteine. This is then methylated by the enzyme homocysteine methyltransferase, one of only two **vitamin B_{12}**-containing enzymes found in eukaryotes, to form **methionine**. The resulting methionine is then activated to *S*-adenosyl methionine with the release of all three of the phosphates from ATP.

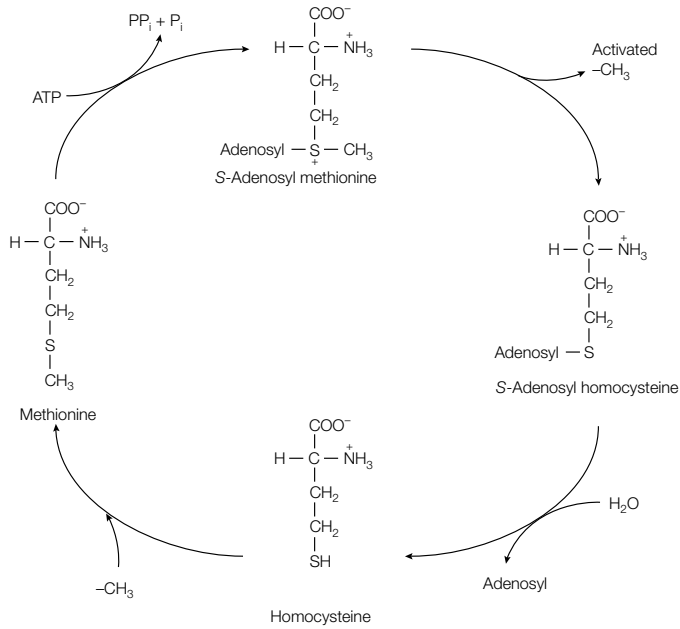

Fig. 5. The activated methyl cycle.

Uric acid

Uric acid (*Fig. 6*) is the main nitrogenous waste product of uricotelic organisms (reptiles, birds and insects), but is also formed in ureotelic organisms from the breakdown of the **purine bases** from DNA and RNA (see Topics F1 and G1). Some individuals have a high serum level of sodium urate (the predominant form of uric acid at neutral pH) which can lead to crystals of this compound being deposited in the joints and kidneys, a condition known as **gout**, a type of arthritis characterized by extremely painful joints.

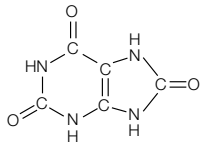

Fig. 6. Uric acid.

M4 HEMES AND CHLOROPHYLLS

Key Notes

Tetrapyrroles

The tetrapyrroles are a family of pigments based on a common chemical structure that includes the hemes and chlorophylls. Hemes are cyclic tetrapyrroles that contain iron and are commonly found as the prosthetic group of hemoglobin, myoglobin and the cytochromes. The chlorophylls are modified tetrapyrroles containing magnesium that occur as light-harvesting and reaction center pigments of photosynthesis in plants, algae and photosynthetic bacteria.

Biosynthesis of hemes and chlorophylls

The starting point for heme and chlorophyll synthesis is aminolaevulinic acid (ALA) which is made in animals from glycine and succinyl CoA by the enzyme ALA synthase. This pyridoxal phosphate-requiring enzyme is feedback-regulated by heme. Two molecules of ALA then condense to form porphobilinogen in a reaction catalyzed by ALA dehydratase. Porphobilinogen deaminase catalyzes the condensation of four porphobilinogens to form a linear tetrapyrrole. This compound then cyclizes to form uroporphyrinogen III, the precursor of hemes, chlorophylls and vitamin B12. Further modifications take place to form protoporphyrin IX. The biosynthetic pathway then branches, and either iron is inserted to form heme, or magnesium is inserted to begin a series of conversions to form chlorophyll.

Heme degradation

Heme is broken down by heme oxygenase to the linear tetrapyrrole biliverdin. This green pigment is then converted to the red-orange bilirubin by biliverdin reductase. The lipophilic bilirubin is carried in the blood bound to serum albumin, and is then converted into a more water-soluble compound in the liver by conjugation to glucuronic acid. The resulting bilirubin diglucuronide is secreted into the bile, and finally excreted in the feces. Jaundice is due to a build up of insoluble bilirubin in the skin and whites of the eyes. In higher plants heme is broken down to the phycobiliprotein phytochrome which is involved in coordinating light responses, while in algae it is metabolized to the light-harvesting pigments phycocyanin and phycoerythrin.

Related topics

- Myoglobin and hemoglobin (B4)
- Regulation of enzyme activity (C5)
- Citric acid cycle (L1)
- Electron transport and oxidative phosphorylation (L2)
- Photosynthesis (L3)
- Amino acid metabolism (M2)

Tetrapyrroles

The red **heme** and green **chlorophyll** pigments, so important in the energy-producing mechanisms of respiration and photosynthesis, are both members of the family of pigments called **tetrapyrroles**. They share similar structures (*Fig. 1*), and have some common steps in their synthesis and degradation. The basic

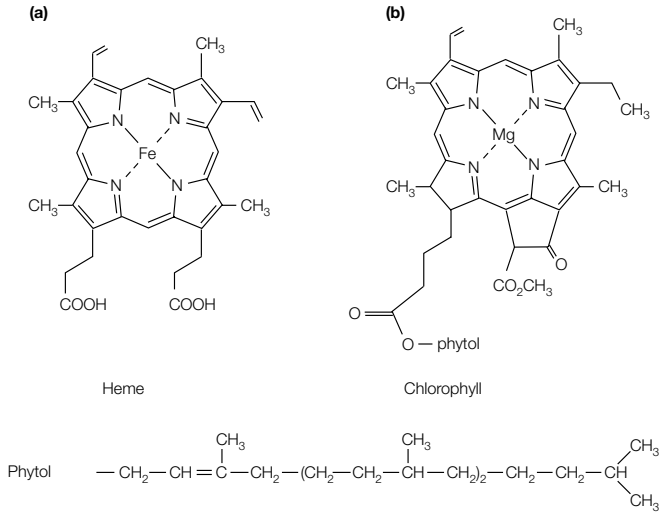

Fig. 1. Structure of (a) heme and (b) chlorophyll.

structure of a tetrapyrrole is four pyrrole rings surrounding a central metal atom.

Hemes (*Fig. 1a*) are a diverse group of tetrapyrrole pigments, being present as the prosthetic group of both the **globins** (hemoglobin and myoglobin; Topic B4) and the **cytochromes** (including those involved in respiratory and photosynthetic electron transport; Topic L2 and L3) and the cytochrome P450s that are used in detoxification reactions. Some enzymes, including the catalases and peroxidases, contain heme. In all these hemoproteins the function of the heme is either to bind and release a ligand to its central **iron atom**, or for the iron atom to undergo a change in oxidation state, releasing or accepting an electron for participation in a redox reaction.

The chlorophylls are also a diverse family of pigments, existing in different forms in photosynthetic bacteria, algae and higher plants. They share a common function in all of these organisms to act as light-harvesting and reaction center pigments in **photosynthesis** (see Topic L3). This function is achieved by a number of modifications to the basic tetrapyrrole structure. These include: the insertion of **magnesium** as the central metal ion, the addition of a fifth ring to the tetrapyrrole structure, loss of a double bond from one or more of the pyrrole rings, and binding of one specific side-chain to a long fat-like molecule called **phytol** (*Fig. 1b*). These changes give chlorophylls and bacteriochlorophylls a number of useful properties. For example, chlorophylls are membrane bound, absorb light at longer wavelengths than heme, and are able to respond to excitation by light. In this way, chlorophylls can accept and release light energy and drive photosynthetic electron transport (see Topic L3).

Biosynthesis of hemes and chlorophylls

In animals, fungi and some bacteria, the first step in tetrapyrrole synthesis is the condensation of the amino acid **glycine** with **succinyl CoA** (an intermediate of the citric acid cycle; Topic L1) to form **aminolaevulinic acid** (ALA). This reaction is catalyzed by the enzyme **ALA synthase** (*Fig. 2a*) which requires the coenzyme **pyridoxal phosphate** (see Topic M2) and is located in the mitochondria of eukaryotes. This committed step in the pathway is subject to regulation. The synthesis of ALA synthase is feedback-inhibited by heme (see Topic C5). In plants, algae and many bacteria there is an alternative route for ALA synthesis that involves the conversion of the intact five-carbon skeleton of glutamate in a series of three steps to yield ALA. In all organisms, two molecules of ALA then condense to form **porphobilinogen** in a reaction catalyzed by **ALA dehydratase** (also called **porphobilinogen synthase**) (*Fig. 2a*). Inhibition of this enzyme by lead is one of the major manifestations of acute **lead poisoning**.

Four porphobilinogens then condense head-to-tail in a reaction catalyzed by **porphobilinogen deaminase** to form a **linear tetrapyrrole** (*Fig. 2b*). This enzyme-bound linear tetrapyrrole then cyclizes to form **uroporphyrinogen III**, which has an asymmetric arrangement of side-chains (*Fig. 2b*). Uroporphyrinogen III is the common precursor of all hemes and chlorophylls, as well as of **vitamin B_{12}**. The pathway continues with a number of modifications to groups attached to the outside of the ring structure, finally forming **protoporphyrin IX** (*Fig. 2b*). At this point either iron or magnesium is inserted into the central cavity, committing the porphyrin to either heme or chlorophyll synthesis, respectively. From here further modifications occur, and finally the specialized porphyrin prosthetic groups are attached to their respective **apoproteins** (the form of the protein consisting of just the polypeptide chain) to form the biologically functional **holoprotein**.

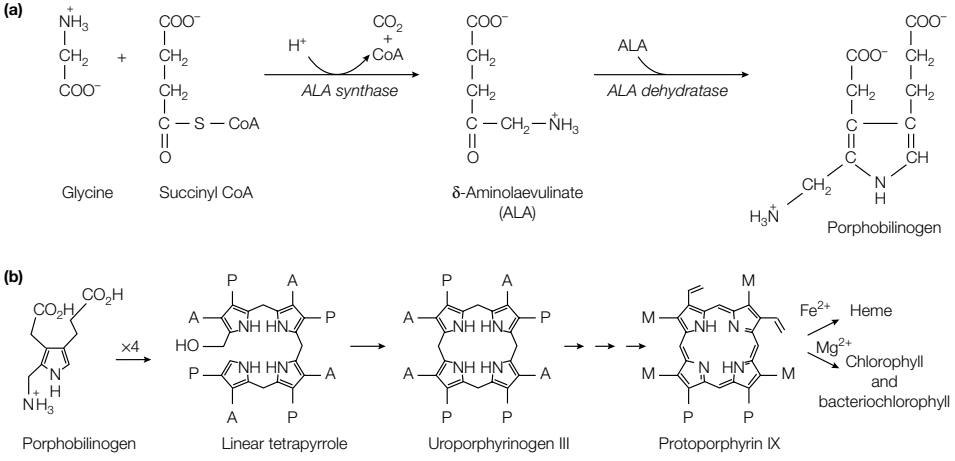

Fig. 2. Pathway of the synthesis of heme and chlorophyll. (a) Synthesis of porphobilinogen from glycine and succinyl CoA; (b) synthesis of protoporphyrin IX from porphobilinogen. A = CH_2COOH, M = CH_3, P = CH_2CH_2COOH.

Heme biosynthesis takes place primarily in immature erythrocytes (85% of the body's heme groups), with the remainder occurring in the liver. Several genetic defects in heme biosynthesis have been identified that give rise to the disorders called **porphyrias**.

Heme degradation

Bile pigments exist in both the plant and animal kingdoms, and are formed by breakdown of the cyclic tetrapyrrole structure of heme. In animals this pathway is an excretory system by which the heme from the hemoglobin of aging red blood cells, and other hemoproteins, is removed from the body. In the plant kingdom, however, heme is broken down to form bile pigments which have major roles to play in coordinating light responses in higher plants (the phycobiliprotein **phytochrome**), and in light harvesting in algae (the phycobiliproteins **phycocyanin** and **phycoerythrin**).

In all organisms, the degradation of heme begins with a reaction carried out by a single common enzyme. This enzyme, **heme oxygenase**, is present mainly in the spleen and liver of vertebrates, and carries out the oxidative ring opening of heme to produce the green bile pigment **biliverdin**, a linear tetrapyrrole (*Fig. 3*). Heme oxygenase is a member of the **cytochrome P450** family of enzymes, and requires NADPH and O_2. In birds, reptiles and amphibians this water-soluble pigment is the final product of heme degradation and is excreted directly. In mammals, however, a further conversion to the red-orange **bilirubin** takes place; a reaction catalyzed by **biliverdin reductase** (*Fig. 3*). The changing color of a bruise is a visible indicator of these degradative reactions. The bilirubin, like other lipophilic molecules such as free fatty acids, is then transported in the blood bound to **serum albumin**. In the liver, its water solubility is

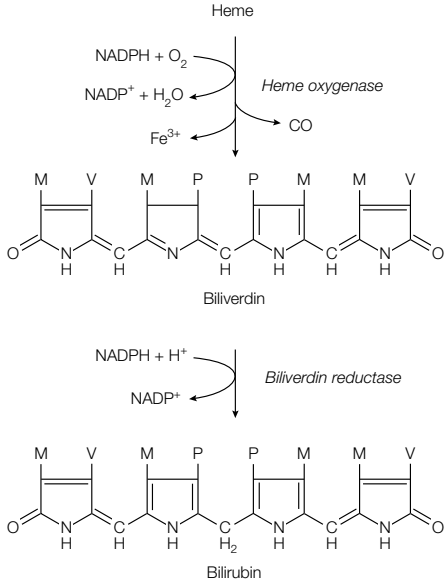

Fig. 3. Degradation of heme to the bile pigments biliverdin and bilirubin. M = methyl (CH_3), V = vinyl ($CH = CH_2$), P = propionyl ($CH_2CH_2CH_2OH$).

increased by conjugation to two molecules of **glucuronic acid**, a sugar residue that differs from glucose in having a COO^- group at C-6 rather than a CH_2OH group. The resulting **bilirubin diglucuronide** is secreted into the bile and then into the intestine, where it is further metabolized by bacterial enzymes and finally excreted in the feces.

When the blood contains excessive amounts of the insoluble bilirubin, it is deposited in the skin and the whites of the eyes, resulting in a yellow discoloration. This condition, called **jaundice**, is indicative either of impaired liver function, obstruction of the bile duct, or excessive breakdown of erythrocytes.