Sulfur, Phosphorus, and Iron Metabolism in Plants

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12.1 Sulfur Metabolism

Sulfur is an essential element for plant growth. Though it constitutes only 0.1% of the dry weight of a plant, its requirement for the plant is crucial. It is a constituent of sulfur-containing amino acids, cysteine, and methionine which are integral to the protein structure. Cysteine residues are responsible for holding proteins in proper conformation because of disulfide linkages (-S-S-) between two –SH containing amino acids. Iron-sulfur (4Fe-4S) clusters present in various proteins are engaged in electron transport reactions. Sulfur is also a constituent of a number of molecules such as lipoic acid, thiamin, biotin, ACP, and coenzyme A, which are required as cofactors by various enzymes. Sulfur-containing lipids, sulfoquinovosyldiacylglycerol, are structural constituents of thylakoids. Various secondary metabolites produced from cysteine and methionine have diverse roles in plants. Many molecules synthesized by the plants in response to abiotic and biotic stress contain sulfur. These include **phytoalexins,** thioredoxin, **alliins, glucosinolates**, etc. Alliins are found in onion and garlic, while glucosinolates are found in members of family Brassicaceae and are responsible for their flavor and smell. In some plants, elemental sulfur is deposited which functions as a potent fungicide (Fig. 12.1).

Sulfur is available to plants in oxidized form as sulfate ions from soil and/or as a pollutant from air in the form of SO_2. Plants absorb sulfate through their roots as well as through the stomatal chambers in the leaves. Once absorbed by roots, sulfate is transported to plastids where it is metabolized or translocated to leaves where sulfur metabolism is significant and occurs in chloroplasts. Extra sulfate ions may be stored in vacuoles. Metabolism of sulfur includes reduction of sulfate ions to sulfide before its incorporation into cysteine, which can further be converted to methionine. Various other sulfur-containing compounds are synthesized from cysteine (Fig. 12.2).

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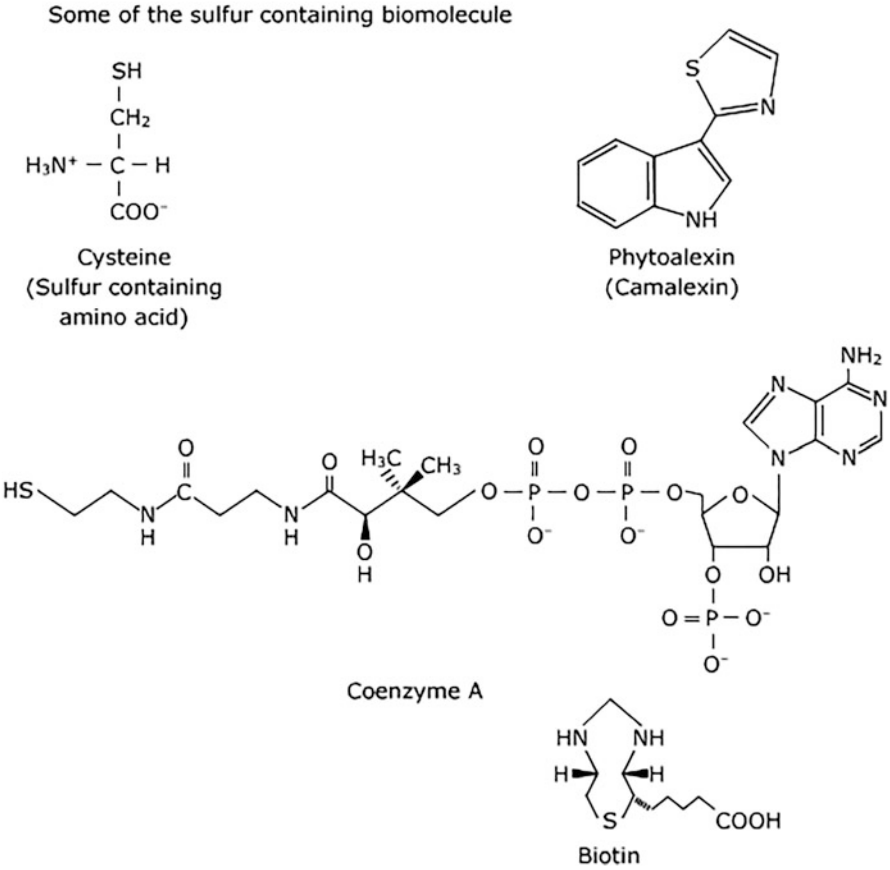


Fig. 12.1 Some of the sulfur-containing biomolecules

12.1.1 Biogeochemical Cycle of Sulfur

In nature, sulfur is present both in oxidized and reduced forms. Interconversion of these forms constitutes the **biogeochemical cycle**. Oxidized forms of sulfur include sulfates present in the soil, which are absorbed by the plants. Once sulfates gain entry into the plants, they are reduced to sulfides which are metabolized further or lead to cysteine biosynthesis. Sulfides also act as precursor for the biosynthesis of other biomolecules. Some bacteria also reduce SO_4^{2-}. Reduced form of sulfur is oxidized during animal metabolism or by some of the chemoautotrophs, which use reduced sulfur either as H_2S, sulfur, or SO_3^- as the terminal electron acceptors in place of oxygen. Since animals, including human, are not able to reduce sulfur, it needs to be provided in their diet. Since plants are the major source for providing reduced sulfur to animals, studying sulfur metabolism in plants is significant. Plants also absorb

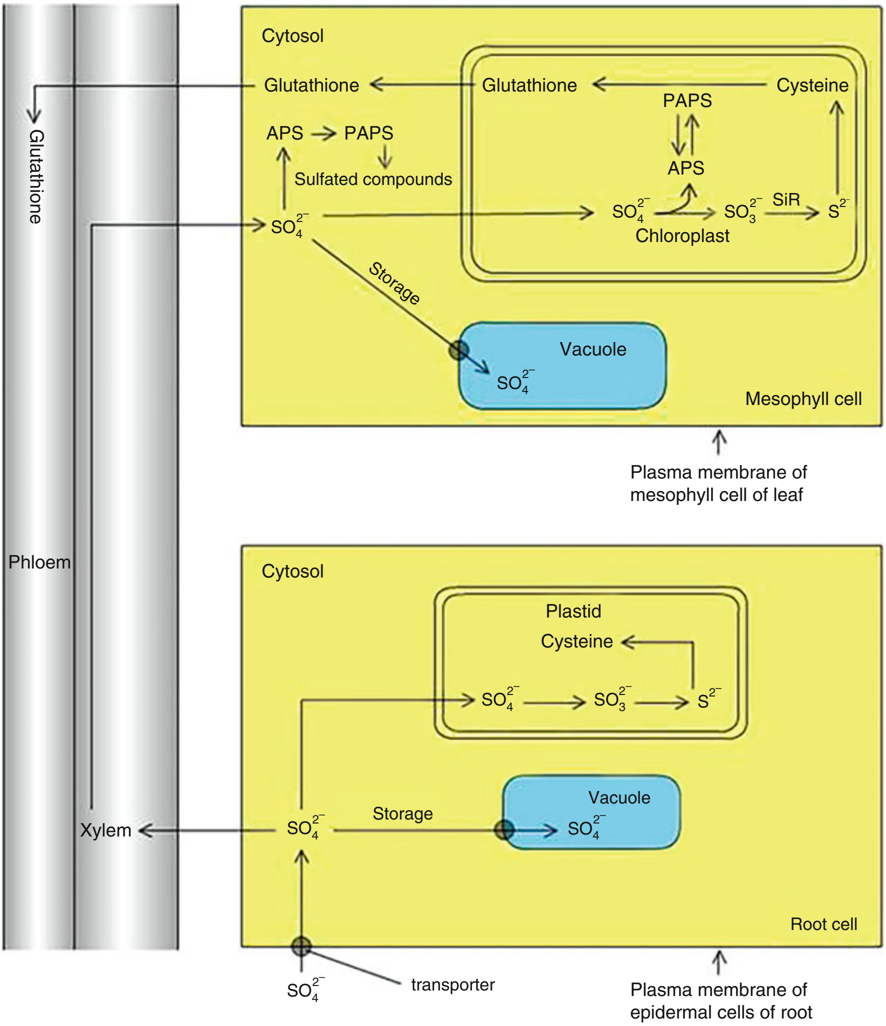


Fig. 12.2 Overview of sulfur metabolism

sulfur in the form of SO_2 from the air where it accumulates because of the volcanic activity or due to burning of fossil fuels. The major source of sulfur in plants is through soil in the form of sulfate fertilizers. Phytoplanktons also produce **dimethylsulfoniopropionate (DMSP)** which functions as an osmoprotectant and a cryoprotectant. After DMSP is released from algae, it is converted to **dimethyl sufide (DMS)**, which is a volatile sulfur-containing compound released into the atmosphere. DMS is oxidized to **DMSO (dimethyl sulfoxide)**, sulfite, and sulfate (Fig. 12.3).

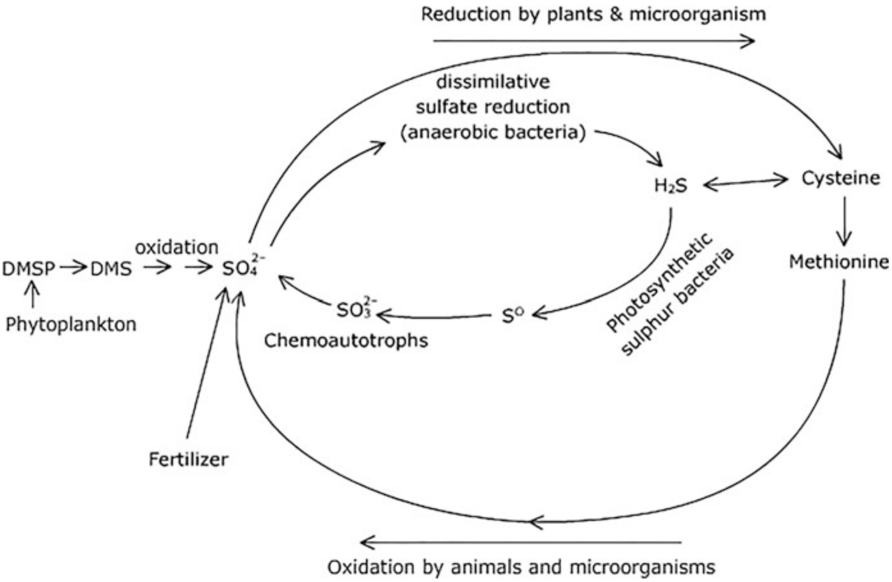


Fig. 12.3 Biogeochemical cycle of sulfur. Plants and microorganisms reduce sulfate to sulfide which then assimilated to cysteine. Animals and some microorganisms utilize H_2S or cysteine/ methionine through aerobic metabolism. *DMS* dimethyl sulfide, *DMSP* dimethylsulfoniopropionate

12.1.2 Uptake of Sulfur

Plants absorb sulfur from the soil in the form of sulfates with the help of sulfate transporters. These transporters are present in root epidermis, cortical cells, vascular systems, vacuoles, and also in the membrane of plastids and chloroplasts in the mesophyll cells. Sulfate being principally metabolized in chloroplasts needs to be translocated to mesophyll cells through xylem stream after being absorbed through the roots. Extra sulfur is stored in the vacuoles of both root and mesophyll cells. Studies with *Arabidopsis* have indicated the involvement of multigene family for encoding sulfate transporters. As many as 14 genes have been reported to encode for the transporters required for sulfate transport. This indicates the significance of the sulfate uptake. Different transporters are expressed in various compartments of the plant cell involved in accumulation of sulfates from micromolar to millimolar concentration. These transporters belong to different groups which include high-affinity sulfate transporters (operate in the range of 1–7 μM range) and low-affinity transporters (operate in the range of 100 μM to 1.2 mM sulfate). In plasma membrane of root epidermal cells, high-affinity SO_4^{2-} cotransporters are also present which are H^+/SO_4^{2-} symporters. These transporters accumulate sulfate against electrochemical gradient and are powered by ATPase-mediated proton pumps in the plasma membrane. Movement of $3H^+$ occurs in response to proton motive force (PMF), which is coupled with the transport of one SO_4^{2-}. SULTR-2 has been shown to be the major sulfate transporter in *Arabidopsis* and, it is located in plasma

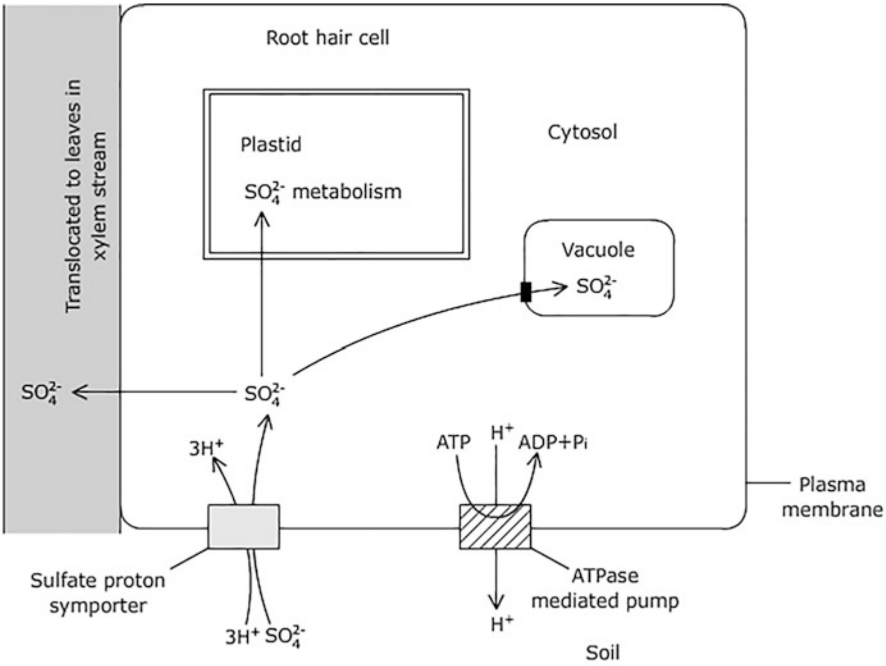


Fig. 12.4 Sulfate uptake by root cells

membrane of root epidermis. It consists of 500–700 amino acids having 10–12 predicted transmembrane helices. Low-affinity transporters are found to function in the vascular system of plants. These are involved in translocation of sulfate within the plant. Unlike plasma membrane-localized sulfate transporters, electrochemical gradient is thought to drive diffusion of sulfate into vacuoles through sulfate-specific channels present on the tonoplast membranes. However, in vacuoles, there seems to be an involvement of uniporters. Involvement of transporters similar to those of plasma membrane might be possible in plastids (Fig. 12.4).

12.1.3 Sulfate Metabolism

Almost 80–85% sulfate accumulated in roots is reduced and incorporated into cysteine, mainly in chloroplasts. Translocation of sulfate to leaves occurs through xylem. Similar to nitrate reduction, sulfate is also reduced in two steps. First step involves reduction of sulfate (SO_4^{2-}) to sulfite (SO_3^{2-}), while sulfite is reduced to sulfide (S^{2-}) before being incorporated into cysteine which is metabolized further.

$$SO_4^{2-} \rightarrow SO_3^{2-} \rightarrow S^{2-} \rightarrow \text{Cysteine}$$

Reduction of sulfate to sulfite requires two electrons transfer provided by reduced glutathione. Being relatively inert molecule, sulfate requires activation before being reduced to sulfite. Sulfate is activated by reacting with ATP, resulting in formation of **adenosine 5′-phosphosulfur** (APS). Reaction is catalyzed by the enzyme **ATP sulfurylase**. APS contains a phosphoric acid-sulfuric acid anhydride bond which makes $SO_4{}^{2-}$ reduction possible. Free energy of the reaction ($\Delta G^{0\prime}$) is estimated to be +41.8 $kJ.mol^{-1}$.

$$SO_4^{2-} + Mg.ATP \rightarrow Mg.PP_i + APS$$

Forward reaction is possible with removal of the products of the reaction. Pyrophosphatase hydrolyzes PP_i, while APS is at branch point and can be further metabolized by either of the two enzymes APS kinase or APS reductase (Fig. 12.5). APS sulfurylase is a homotetramer consisting of 52–54 kDa polypeptides. In leaves, it exists in two isoforms. Enzyme found in the leaf plastids represent about 90% of the total enzyme, while the one found in the cytosol is the minor component. At least three genes encoding APS sulfurylase have been identified in *Arabidopsis*. These are APS1, APS3, and APS4, while the cytosolic isoform is encoded by the fourth gene-APS2. The enzyme binds to $Mg.ATP^{2-}$ and $SO_4{}^{2-}$ sequentially. Demand of the end products might regulate activity of the enzyme since expression of the genes encoding the enzyme is downregulated by the addition of reduced glutathione.

Adenosine 5′-phosphosulfur (APS) is a high-energy compound due to the presence of high-energy phosphoric acid-sulfuric acid anhydride bond. It is because of this high-energy bond that it facilitates further metabolism of $SO_4{}^{2-}$ to $SO_3{}^{2-}$. There are two alternative pathways for APS. Sulfur present in APS in the form of $SO_4{}^{2-}$ is either reduced to $SO_3{}^{2-}$ by the enzyme **APS reductase**, or APS is converted to 3′-phosphoadenosine 5′-phosphosulfate (PAPS) through the activity of **APS kinase**. Although PAPS acts as a sulfur donor in plants, however in some organisms such as cyanobacteria, PAPS can be reduced to sulfite or may also provide precursor for cysteine biosynthesis. Reaction catalyzed by APS kinase is as follows:

$$APS + ATP \rightarrow PAPS + ADP$$

Three reactions are involved in $SO_4{}^{2-}$ reduction. First APS is reduced to sulfite, catalyzed by APS reductase. The reaction occurs as follows:

$$APS + 2glutathione_{red} \rightarrow SO_3^{2-} + 2glutathione_{ox} + AMP + 2H^+$$

$SO_3{}^{2-}$ is reduced to sulfide (S^{2-}) which is followed by formation of cysteine. Reduction of APS to sulfite by APS reductase is unique in plants and occurs in plastids. **Glutathione** functions as a reductant for this reaction. Multiple isoforms of APS reductase are expressed in plants in response to environmental signals such as the availability of reduced sulfur. Three genes in *Arabidopsis* encode for APS

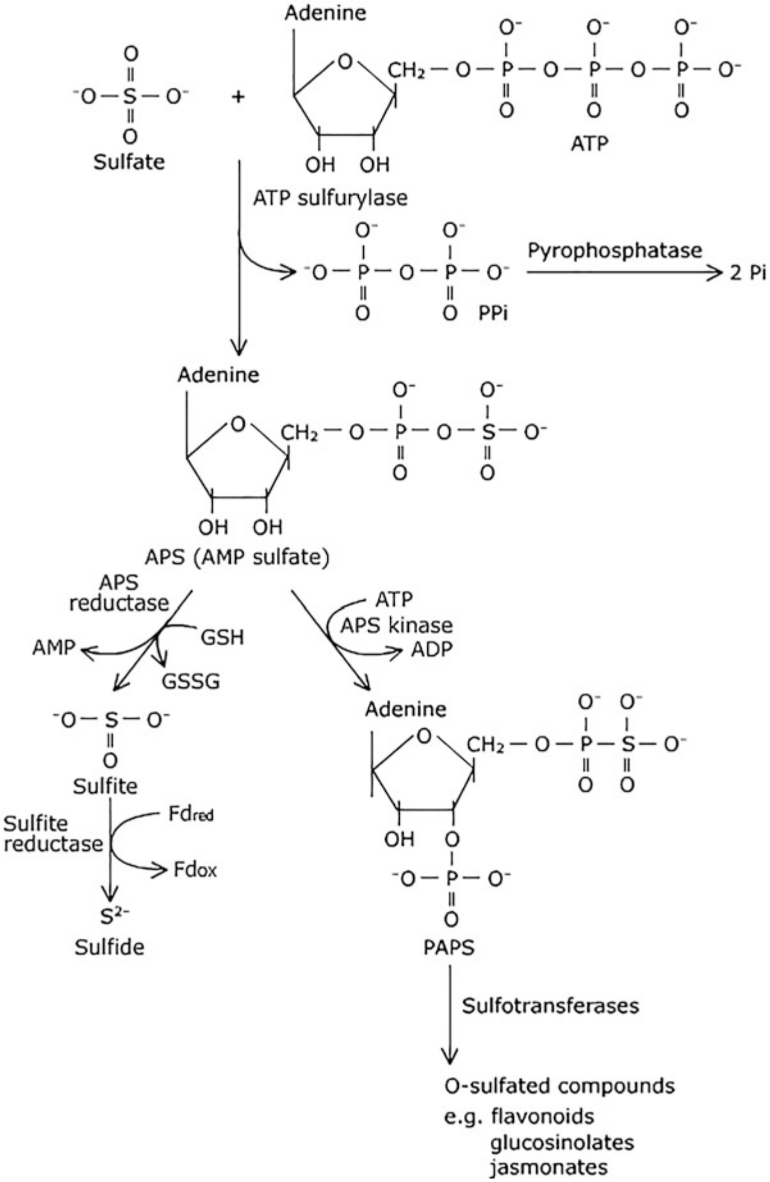


Fig. 12.5 Reduction of sulfate to sulfide and synthesis of some of the sulfur-containing compounds. *APS* adenosine-5′-phosphosulfates, *PAPS* 3′-phosphoadenosine-5′-phosphosulfate

reductase. In the presence of reduced sulfur, expression of the gene encoding this enzyme is downregulated, resulting in the accumulation of SO_4^{2-}. However, overexpression of the gene also results in accumulation of reduced sulfur which may cause cellular damage. The enzyme is synthesized in cytosol by cytosolic

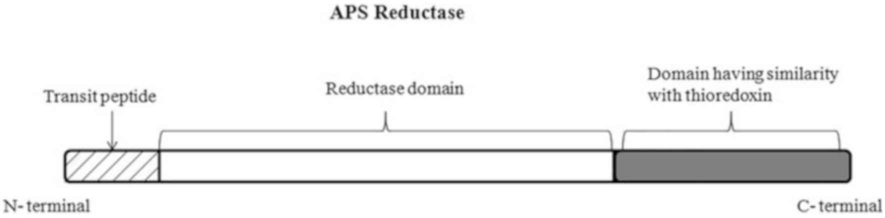


Fig. 12.6 Structure of APS reductase

ribosomes, and the presence of transit peptide at the N-terminus of the polypeptide facilitates its movement into the plastid. The mature enzyme is a homodimer consisting of 43 kDa monomeric subunits. It is characterized by the presence of a C-terminal redox domain and a N-terminal reductase domain (Fig. 12.6). Reaction between the redox region of the enzyme and glutathione is the source of electrons used for reduction of $SO_4{}^{2-}$ to $SO_3{}^{2-}$. Enzyme contains 4Fe-4S cluster at the N-terminal-localized reductase region. $SO_4{}^{2-}$ binds to this region with simultaneous release of AMP and its reduction to $SO_3{}^{2-}$. Probably electrons are provided by the reductase with simultaneous release of $SO_3{}^{2-}$. Reduced glutathione provides two electrons for reduction necessary for the recovery of active enzyme.

Next step in sulfur metabolism is reduction of sulfite to sulfide. Sulfite, which is produced as a result of $SO_4{}^{2-}$ reduction, is very reactive and extremely toxic for the plants, so it has to be immediately metabolized. Reduction of $SO_3{}^{2-}$ is catalyzed by the enzyme sulfite reductase (SiR) and requires transfer of 6 electrons.

$$SO_3^{2-} + 6\text{ferredoxin}_{\text{red}} \rightarrow \quad S^{2-} + \quad \text{ferredoxin}_{\text{ox}}$$

Since accumulation of $SO_3{}^{2-}$is very toxic, level of sulfite reductase in the tissue is half saturated at 10^{-6} mole/L of sulfite. Thus, the enzyme is able to reduce efficiently any amount of sulfite which has been produced due to reduction of $SO_4{}^{2-}$. In chloroplasts, reduced ferredoxin is produced from NADPH during noncyclic electron transport during light reaction of photosynthesis. However, in the cytosol or chloroplasts, ferredoxin is reduced from NADPH, which is produced during oxidative pentose monophosphate pathway. In plants, SiR is a monomeric hemeprotein of 65-kDa molecular mass. Both SiR and NiR (nitrite reductase) primarily function in plastids. Similar to NiR, SiR also catalyzes transfer of 6 electrons facilitated by a Fe_4-S_4 center and a siroheme (Fig. 12.7). A similarity exists in their tertiary structures and mechanism of action as well. SiR isolated and purified from spinach is capable of reducing nitrite, though with lower efficiency. Both enzymes show about 20% similarity in their amino acids composition, and probably a primitive bifunctional reductase might have been a common ancestor for both the enzymes.

Detoxification of sulfite also occurs due to its oxidation by **sulfite oxidase** which protects plants against surplus $SO_3{}^{2-}$. Though this enzyme has been reported earlier

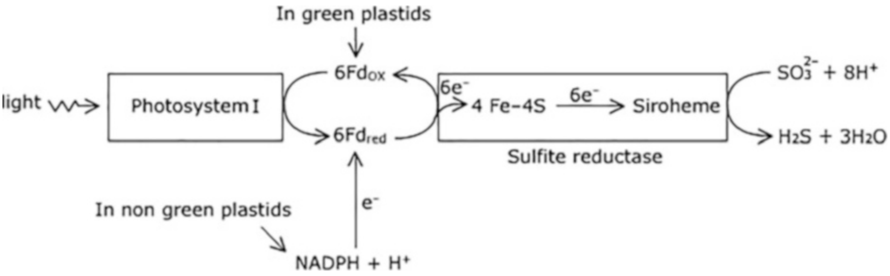


Fig. 12.7 Sulfite reductase: sulfite is reduced to sulfide in chloroplasts. Reduced ferredoxin produced during light reactions is used as the reductant

both in animals and microorganisms, its role in plants is not very clear. Reports about sulfite oxidase in plants are comparatively recent. Reaction catalyzed by this enzyme is as follows:

$$SO_3^{2-} + H_2O + O_2 \rightarrow SO_4^{2-} + H_2O_2$$

Conversion of SO_3^{2-} back to SO_4^{2-} is **futile cycle** and is prevented by compartmentalization. Both pathways including reduction of SO_4^{2-} to SO_3^{2-} and oxidation of SO_3^{2-} to SO_4^{2-} occur in different compartments of the cell. Sulfite reduction occurs in plastids, while oxidation of sulfite occurs in peroxisomes where H_2O_2 produced can be degraded. Sulfite oxidase belongs to family of molybdenum-containing enzymes.

Final step in reductive sulfate assimilation is biosynthesis of cysteine from sulfide (S^{2-}). Precursors required are O-acetylserine (OAS) and sulfide (S^{2-}). Sulfide exists as bisulfide (HS) at physiological pH. O-acetylserine is produced from serine and acetyl-CoA. Acetylation of serine is catalyzed by **serine-acetyl-CoA transferase (SAT)**.

$$\text{Serine} + \text{acetyl} - \text{CoA} \rightarrow \text{O} - \text{acetylserine} + \text{CoA}$$

Formation of acety-CoA from acetate and CoA requires ATP, and the reaction is catalyzed by the enzyme **acetyl-CoA synthetase** present in chloroplasts. Pyrophosphate released in the process is hydrolyzed by pyrophosphatase. Activation of serine to O-acetylserine costs two ATPs in chloroplasts. SAT is a dimer of two trimers. Each trimer consists of 29-kDa monomers. There are six active sites in the hexamer, and a histidine residue is present in each active site which can bind to hydroxyl group of serine and activate it to bind with the acetyl group from acetyl-CoA. Three isoforms of SAT have been isolated which differ in regulation of their activity, especially in their sensitivity to inhibition by cysteine accumulation. The enzyme is indispensable for plant growth, and the mutants for this enzyme results in growth

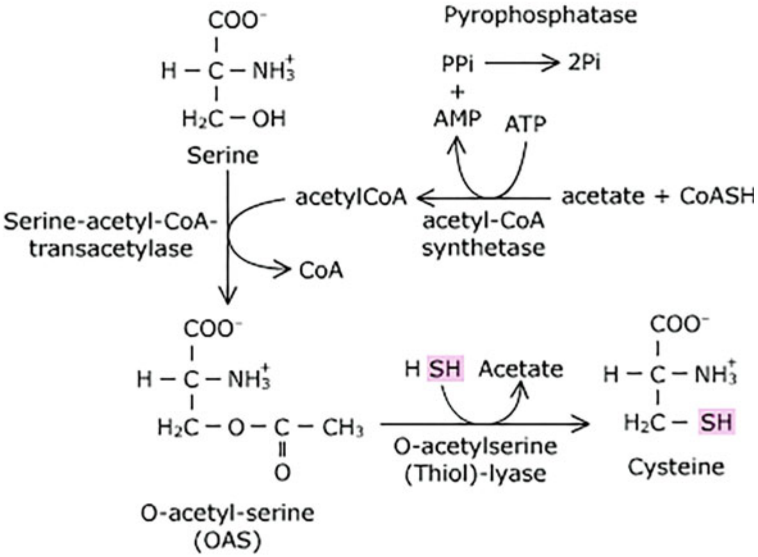


Fig. 12.8 Cysteine biosynthesis from serine using the hydrogen sulfide produced as a result of sulfite reduction

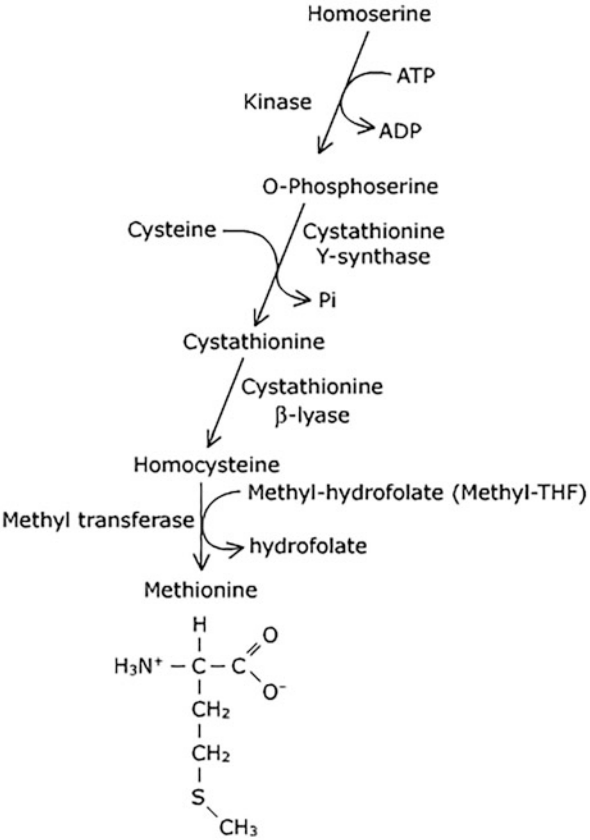
retardation. Subsequent fixation of H_2S in the form of cysteine from O-acetylserine requires the enzyme **O-acetylserine (thiol) lyase**. The enzyme requires pyridoxal phosphate as the prosthetic group. It has high affinity both for H_2S and O-acetylserine. Ester linkage of O-acetylserine is cleaved, and SH group from H_2S is incorporated with simultaneous release of acetyl unit and cysteine biosynthesis (Fig. 12.8). Cysteine biosynthesis primarily occurs in cytosol. O-acetylserine is synthesized in mitochondria, and sulfide production occurs in the plastids. OAS and OAS (thiol) lyase form a complex called as **cysteine synthase**. Active complex consists of a hexamer of OAS and two dimers of OAS (thiol) lyase. Protein-protein interaction is responsible for regulation of activity of the enzyme complex in response to concentration of OAS and sulfide. OAS may act as the signal for fine-tuning of the pathway involved.

12.1.4 Cysteine Metabolism

Cysteine is an important sulfur-containing amino acid since it readily undergoes dithiol (-SH-HS-) and disulfide (-S-S-) interconversion similar to glutathione molecule. It plays an important role in protein structure if the polypeptide contains two cysteine residues. Protein folding is promoted if two cysteine residues are covalently linked to each other by disulfide (-S-S-) bonds under oxidative cellular conditions, while reducing conditions will promote unfolding due to reduction of disulfide bonds to sulfhydryl (-SH) groups. Cysteine is also a precursor of various biomolecules, such as methionine (another sulfur-containing amino acid), glutathione, glucosinolates, etc. Besides being structural component of proteins, methionine has many other important roles in plant metabolism. It is the methyl group donor for lignin biosynthesis. And its derivative, S-adenosyl methionine, is the precursor for ethylene biosynthesis. Besides crop plants, many legumes also have low methionine content. So, enriching the plant dietary sources with these sulfur-containing amino acids is one of the goals of the biotechnologists. Methionine is synthesized from cysteine. Precursor for methionine biosynthesis is homoserine which is produced as a result of reduction of aspartic acid. Phosphorylation of homoserine results in the production of O-phosphohomoserine. The phosphate group is displaced by sulfhydryl group of cysteine, resulting in the formation of cystathionine, followed by cleavage of alanine group to yield homocysteine. Cofactor methyl-tetrahydrofolate of the enzyme methyl transferase is responsible for methylation of sulfhydryl group of homocysteine resulting in the formation of methionine (Fig. 12.9).

Glutathione (GSH) is a tripeptide which plays an important role as cellular redox buffer. It acts as an antioxidant since it provides protection to cellular constituents against reactive oxygen species (ROS) together with ascorbate. It also provides protection against toxicity by heavy metals since it is required for formation of **phytochelatins** (Box 12.1), herbicides, and **xenobiotic** by forming complexes with them. It consists of amino acid residues glutamate, cysteine, and glycine in which γ-carboxylic group of glutamate is linked to amino group of cysteine which forms peptide bond with glycine. When in oxidized state, thiol groups of cysteine amino acids present in two glutathione molecules are linked together to form disulfide (-S-S-) bond, while under reduced conditions, these exist as thiol groups (-SH). Conversion of oxidized glutathione to reduced form is catalyzed by a flavoprotein, **glutathione reductase** which requires NADPH (Fig. 12.10). Oxidized form of glutathione is present in micromolar concentrations, predominantly in endoplasmic reticulum and Golgi apparatus, while reduced form is present in millimolar concentrations. Glutathione biosynthesis requires two enzymes and three amino acid precursors. **γ-glutamyl-cysteine synthetase** (the enzyme found in plastids) catalyzes an ATP requiring reaction by forming an amide linkage between γ-carboxylic group of glutamic acid and amino group of cysteine. A second peptide bond is formed between carboxylic group of cysteine and amino group of glycine. This bond formation requires another molecule of ATP and is catalyzed by the enzyme **glutathione synthetase** (Fig. 12.11). Isoforms of the enzyme are found both in plastids and cytoplasm. Glutathione also acts as the reservoir for organic sulfur, and cysteine is released free when required by the plant.

Fig. 12.9 Biosynthesis of methionine from cysteine



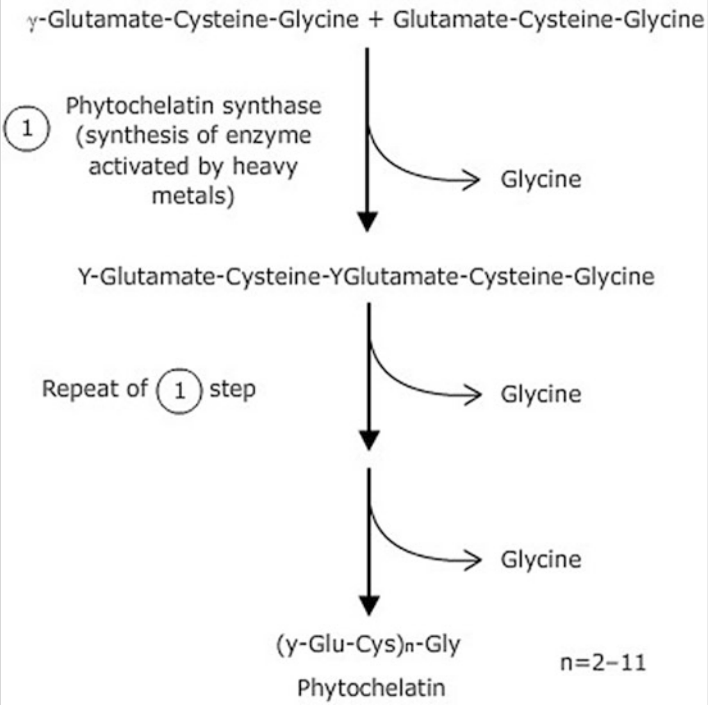
Box 12.1: Glutathione as a Precursor of Phytochelatins

Phytochelatins are oligomers of glutathione produced by the enzyme phytochelatin synthase, a transpeptidase which transfers free amino group of glutamate component of glutathione to carboxyl group of cysteine of another glutathione molecule. Reaction is accompanied by release of glycine and is repeated resulting in the formation of a chain of up to 11 repeats of dipeptide (Glu-Cys). Synthesis of enzyme phytochelatin synthase is triggered in

(continued)

Box 12.1 (continued)

response to heavy metals, such as Zn^+, Cd^+, Pb^+, Hg^+, etc. which is then responsible for de novo synthesis of phytochelatins. Heavy metals form tight complexes with the thiol group of cysteines of phytochelatin, which are then transported across tonoplast to the vacuoles. Transport of the phytochelatin-heavy metal complex requires ATP. Once inside the vacuoles, the complex breaks down releasing heavy metal which gets accumulated there. This property of phytochelatins has been exploited by certain plants effectively for phytoremediation. Since glutathione is the precursor for the biosynthesis of phytochelatins, glutathione level in plant falls down in response to heavy metals.



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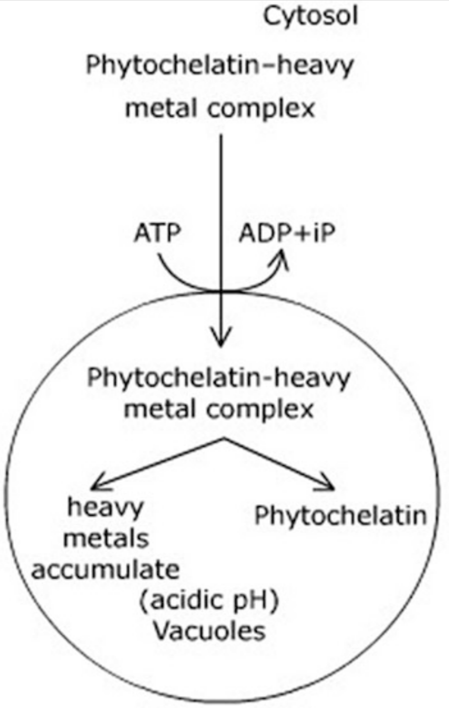
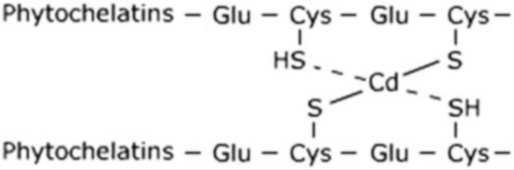




Heavy metals form tight complexes with thiol groups of cysteine of phytochelatins

Transport of phytochelatin-heavy metal complex into the vacuoles

12.1.5 Sulfated Compounds

Besides being used for sulfate reduction to sulfite, APS is also metabolized by alternate pathway. APS is phosphorylated by APS kinase resulting in production of *PAPS* (**adenosine-3′-phosphate-5′-phosphosulfate)** which is sulfuryl group

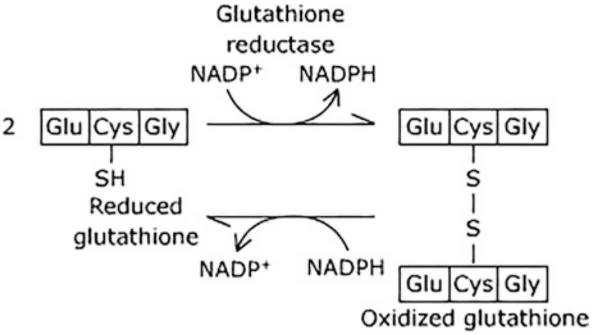


Fig. 12.10 Glutathione: interconversion of reduced and oxidized form

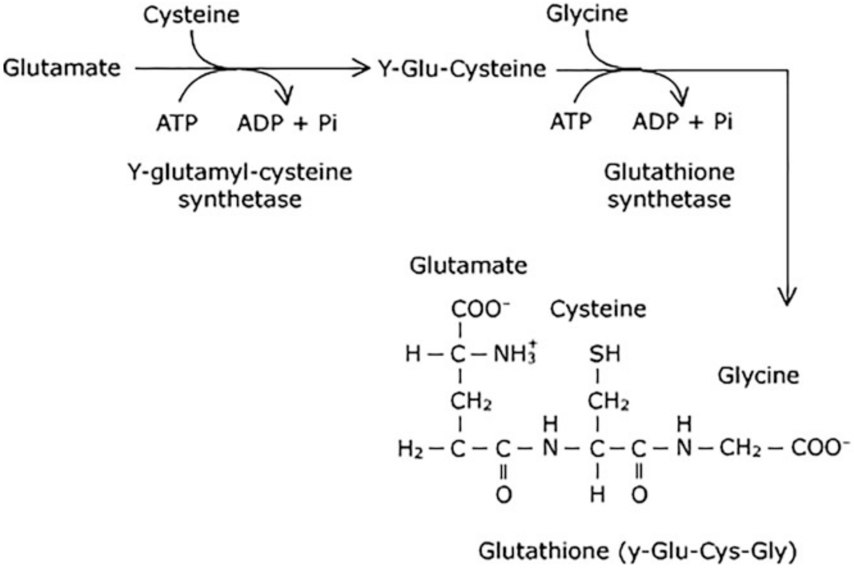
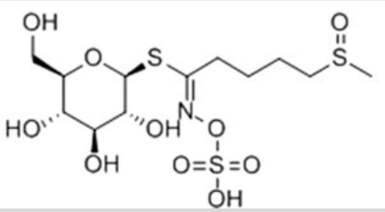


Fig. 12.11 Glutathione biosynthesis

donor for synthesis of various sulfated compounds required by plants for defense against abiotic and biotic stress, such as **glucosinolates** (Box 12.2) and sulfated derivatives of jasmonic acid, etc. (Fig. 12.12). Besides, another large group includes sulfated compounds which are medicinally important, such as alliin (Box 12.3). Some of the sulfated compounds are also involved in seismonastic movements in *Mimosa pudica.* Small sulfated peptides such as **phytosulfokines** are used as regulators of plant growth. APS kinase is present both in plastids and cytosol. Since many of the sulfated compounds produced from PAPS are required for stress responses, synthesis of APS kinase is believed to be regulated in response to stress.

Box 12.2: Glucosinolates

Glucosinolates are natural compounds present in the members of family Brassicaceae such as in mustard, cabbage, or horseradish. These are derived either from cysteine or from compounds of cysteine biosynthetic pathway. Pungency of these plants is due to the presence of glucosinolates. These are responsible for providing protection to plants against herbivores. On being hydrolyzed by the enzyme myrosinase, glucosinolates produce isothiocyanates which are toxic to insect herbivores since these bind with the digestive enzymes of the insects. Some of the derivatives of isothiocyanates are volatile, because of which insects avoid these plants by detecting their odor. These volatile compounds are responsible for the characteristic odor of *Brassica* sp. Glucosinolates and myrosinase are stored in separate compartments which get mixed when the tissue is damaged. Isothiocyanates are detoxified by glutathione in human liver. Glucoraphanin is the major type of glucosinolate found in broccoli. Sulforaphane is the hydrolysis product of glucoraphanin which may protect humans against certain forms of cancers.



Structure of glucoraphanin

Reactions requiring PAPS as the sulfate group donor and compound with hydroxyl group as the acceptor molecule are catalyzed by the enzymes sulfotransferases (SOT). Multiple sulfotransferases have been isolated which are required for the structural diversity of the biological molecules with hydroxyl groups, which act as the acceptor of sulfate groups from PAPS. Cysteine is the key amino acid whose biosynthesis is influenced by carbon, nitrogen, and sulfate metabolism. In plants exposed to nitrogen starvation, sulfate transporters as well as APS reductase are downregulated. Sulfate starvation leads to reduced nitrate uptake as well as reduction in nitrate reductase activity. In a similar way, under reduced CO_2 assimilation, both sulfur and nitrogen metabolisms are reduced. Under stress situations, growth regulators such as salicylate and ethylene upregulate APS reductase, which is the key enzyme in sulfur metabolism. Under biotic stress, genes involved in sulfate assimilation and glucosinolate biosynthesis are also upregulated by the jasmonates.

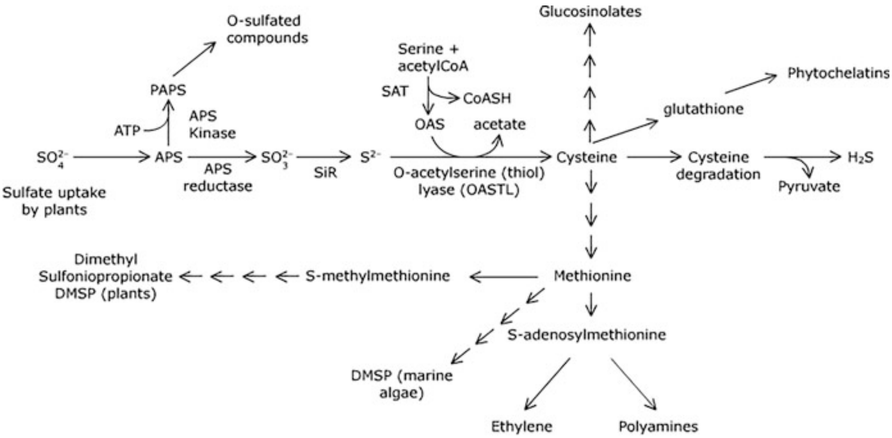
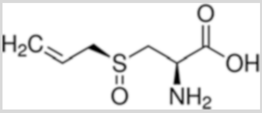


Fig. 12.12 Cysteine metabolism

Box 12.3: Alliin

Alliin is a sulfoxide which is a derivative of amino acid cysteine. It is generally found in *Allium* species which includes onion and garlic. Plants store alliin in the inactive form as conjugated with sugar molecules. When plant cells are injured, an enzyme **alliinase** is released from vacuoles which results in the removal of the sugar moiety. An odoriferous compound is released which has antibacterial properties. In onion, alliin is further decomposed to produce propane thiol-S oxide which triggers tears in the eyes. Alliins have an important role in protecting plants against herbivory. In cell culture, alliins have been found to be effective in inhibiting cell division and are effective in inducing apoptosis in human cells. So, they may be effective in protection against cancer.



Structure of alliin

12.2 Phosphorus Metabolism

Phosphorus is essential macronutrient which is central to plant metabolism. It is the constituent of nucleic acids as **phosphodiester** linkages join two subsequent nucleotides. Many of the intermediates of glycolysis and Calvin cycle are phosphorylated sugars. Phosphate-containing nucleotides play an important role in almost all the energy transformation reactions. ATP occupies the central role in energy metabolism. Almost 70% of the proteins in a cell need phosphorylation at some point of time. Phosphorylation of proteins is key metabolic requirement as part of post-translational regulation of enzyme activity. These include regulation of nitrate reductase, phosphoenolpyruvate dehydrogenase, and the enzyme involved in starch biosynthesis besides many other enzymes. Protein kinases and phosphatases are responsible for catalyzing the post-translational modifications of the proteins which is also required for cell signaling. Phosphorus is an essential structural component of membranes as phospholipids. Inositol phosphate (produced as a result of hydrolysis of a membrane lipid phosphatidylinositol by phospholipase) is required as the signaling molecule. Thus, phosphorus is constituent of the biomolecules which are indispensable for life.

In this section, we will briefly discuss about the forms in which phosphorus is available to plants, transport of inorganic phosphates from soil to root cells and at the subcellular level, phosphorus metabolism. This will include role of inorganic phosphate as the potential phosphate group donor, role of various phosphorylated compounds in energy transfer reactions, role of ATP as the phosphate group donor and reactions involving transphosphorylation between nucleotides, and role of polyphosphates as the storage molecule as well as its significance in cell signaling and remobilization of phosphorus during phosphorus deficiency and leaf senescence.

12.2.1 Biogeochemical Cycle of Phosphorus

Phosphorus is a macronutrient found in lowest concentration in the soil. Despite being an essential element, its availability in the soil is limited. Unlike nitrogen (N_2), phosphorus is not biologically fixed. In contrast to sulfur which can be taken up by plant in form of SO_2, phosphorus is also not available in gaseous form. However, phosphorus is available in both inorganic and organic forms in the soil. Phosphorus is found in soil solutions in the form of $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}, depending upon the pH of the soil solution. In the pH range of 3–7, it is the $H_2PO_4^-$ which dominates. This form of phosphorus ($H_2PO_4^-$) which is denoted by inorganic phosphate (P_i) is absorbed by the plant. Organic form of phosphorus is present in organic molecules as ester derivatives. It is made available by the action of certain enzymes produced by the plants. Bones and many other structures of dead animals are rich source of phosphorus. Phosphorus derived from organic wastes of animals and plants is recycled by the plants, and through plants, by the animals. Unlike NO_3^- and SO_4^-, phosphates are not reduced, and it is the oxidized form which is incorporated in the biomolecules (Fig. 12.13).

12.2.2 Phosphate Transporters

Phosphorus concentration in soil is very low (0.1 μM–1.0 μM) which is about three times lower than present inside plants. Phosphorus is present in the soil as $H_3PO_4^-$,

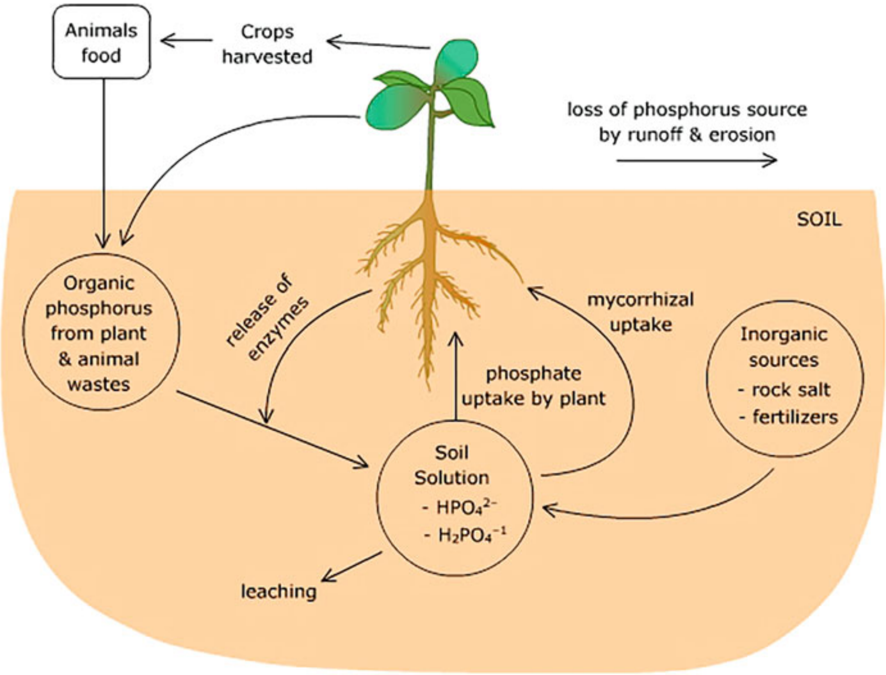


Fig. 12.13 Biogeochemical cycle of phosphorus

$H_2PO_4^-$, HPO_4^-, and PO_4^-. PO_4^- is not available since it forms insoluble salts with different mineral elements, making them unavailable for the plant. $H_2PO_4^-$ is the predominant form of phosphorus available in soil at pH 5–6. Plants absorb phosphorus in the form of $H_2PO_4^-$. There are high-affinity and low-affinity transporters which work in the range of 2.5–12 μM and 50–100 μM, respectively. Low-affinity transporters are constitutive, while high-affinity transporters are expressed in response to phosphorus deficiency. Phosphorus starvation increases its uptake capacity by increasing the high-affinity transporters. Phosphate transporters belong to major facilitator family of transporters known as **MFS transporters.** These transporters consist of single polypeptide chain mediating transport of small molecules in response to electrochemical gradient. These transporters are formed by 12 transmembrane domains and one long hydrophilic domain in the middle. These differ from **ABC transporters** since these do not have any binding site for ATP. However, energy demand is met through ATPase-mediated H^+ pumps. Phosphate transporters are H^+/P_i symporters with 2 or 4 H^+ being transported for each P_i transported. Various categories of P_i transporters are involved in transport of P_i from soil solution to the plant through root systems, while others are involved in subcellular redistribution and long-distance transport. PHT1 transporters are involved in transport of P_i from apoplast/soil solution to the root cell through the plasma membrane. It belongs to multigene family. In *Arabidopsis*, 9 genes encode for

PHT1, while 13, 15, and 8 genes have been found to code for PHT1 in rice, soybean, and tomato, respectively. P_i is mobile and can migrate from one region to another based upon the need of the plant. Another class of transporters includes PHO1, which mediates P_i efflux into xylem for long-distance transport. Other P_i transporters are responsible for P_i transport in various subcellular compartments of the cell as well. Transport of P_i across the inner mitochondrial membrane is mediated by PHT3 proteins, while PHT2 and several members of PHT4 mediate P_i transport across plastid membrane. They have at least eight transmembrane alpha-helices and mediate counter-exchange of P_i with phosphorylated C_3, C_5, or C_6 sugars. These transporters are of four types—triose phosphate translocators (TPT), PEP translocators (PPT), glucose-6-P translocator (GPT), and xylulose-5-P translocators (XPT). Nucleotide triphoshate transporters (NPT) are also present which exchange ATP with ADP. Exchange of ATP^{4-} with ADP^{3-} creates imbalance of the charge, suggesting unidirectional transport of P_i. Transport of nucleoside monophosphate (NMP) in exchange of nucleoside diphosphate (NDP) is facilitated by nucleoside sugar transporters (NSTs) localized in Golgi apparatus. Peroxisomal adenine nucleotide carriers (PNCs) are responsible for counter-exchange of ATP with ADP/AMP. Since vacuole serves as the major reservoir for excess P_i in the cell and acts as buffer for cytosolic P_i, learning about P_i transporters in tonoplast becomes significant. However, proteins mediating P_i transport across tonoplast have not yet been identified. Besides very little is known about P_i uptake by the cells which are not symplastically connected to their neighbors, such as guard cells or the developing embryo.

12.2.3 Role of Phosphorus in Cell Metabolism

Many biomolecules participating in metabolic reactions are charged species since it keeps them water soluble and also prevents them from diffusing across plasma membrane. One of the most common biologically ionic groups is the phosphate group. Almost all Calvin-Benson cycle intermediates are phosphorylated with P_i being released at the time of synthesis of the end products such as sugars and starch. Except starch, most of the end products of photosynthesis are synthesized in cytosol. Triose phosphates need to be transported out of the plastids and P_i, which is released in the cytosol and is cycled back to plastids. This transport is facilitated by antiporters located on the inner envelope of the plastids (Fig. 12.14). Photosynthesis is regulated by the availability of P_i and is inhibited when P_i supply becomes limiting.

Phosphorus is capable of forming five covalent bonds. In the inorganic phosphoryl form, phosphorus is covalently linked to oxygen by three P-O and one P=O bond. Since oxygen is more electronegative than phosphorus, sharing of bond between phosphorus and oxygen is unequal. At physiological pH, the electronegative oxygen in P=O bond of ATP attracts electrons away from phosphorus, resulting in slight electropositive charge accumulation (δ^+) on phosphorus than oxygen, which carries partial negative charge (δ^-). This provides an electrophilic property to phosphorus. As a result, phosphoryl group is transferred from ATP to a

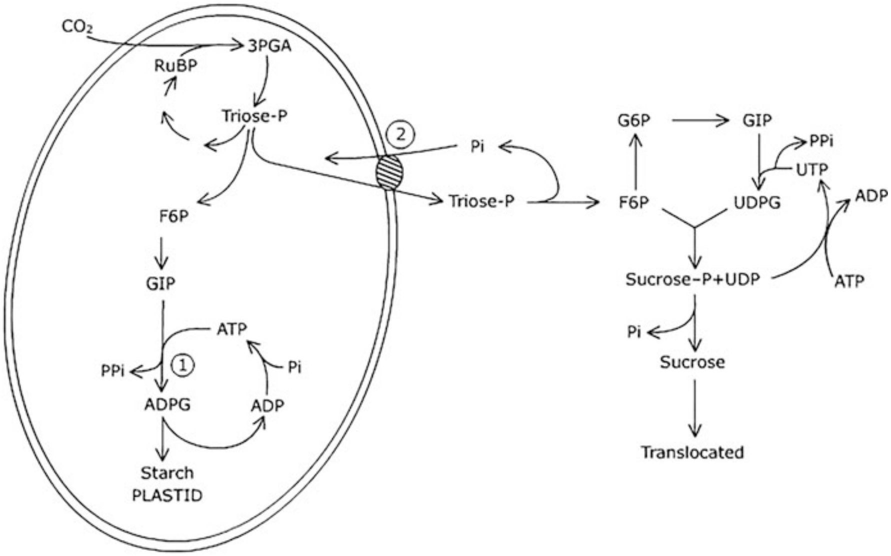


Fig. 12.14 Role of phosphates in carbohydrate metabolism and its regulation. (1) ADP-glucose pyrophosphorylase; (2) triose-P antiporter

nucleophilic group, such as a carboxylic group, forming an ester. Phosphoryl group transfer reactions are important group transfer reactions required for activation of intermediates of the pathway. A good leaving group is central to metabolic reactions. "Leaving group" is a metabolic fragment which departs with unpaired electrons due to **heterolytic bond cleavage**. Generally, a good "leaving group" is attached to activate an intermediate of the metabolic reactions so that it can participate in the reaction. Though intermediates with good leaving groups are fairly stable, their low activation energy requirement in the presence of enzymes makes them suitable for faster reactions. Inorganic orthophosphate groups which include $H_2PO_4^-$ and HPO_4^- (which are commonly referred as inorganic phosphates) are good "leaving groups" which participate in nucleophilic substitution reactions. Nucleophilic substitutions, which include $-PO_3^-$ as the leaving group, occur in many metabolic reactions. Phosphorus plays vital role in plant processes that involve energy transfer. It is a constituent of various biomolecules involved in energy transfer reactions, such as ATP, NTPs, and NADPH.

One important role of phosphorus is in post-translational regulation of activities of various enzymes by phosphorylation and dephosphorylation, thus regulating metabolism. Phosphoryl group is transferred to –OH group of either serine, threonine, or tryptophan residues of the protein. P_i acts as potent inhibitor of starch synthesis by acting as negative modulator of the enzyme ADP-glucose pyrophosphorylase. Another example which is significant in plants is post-translational modification of PEP dehydrogenase. About 70% of all eukaryotic proteins are possibly phosphorylated at some point of time. Protein kinases and "super family of proteins" possibly amount to 4% of the entire genome. O-Phosphorylation of proteins at serine, threonine, or tyrosine residues occurs in the ratio of 75:25:5.

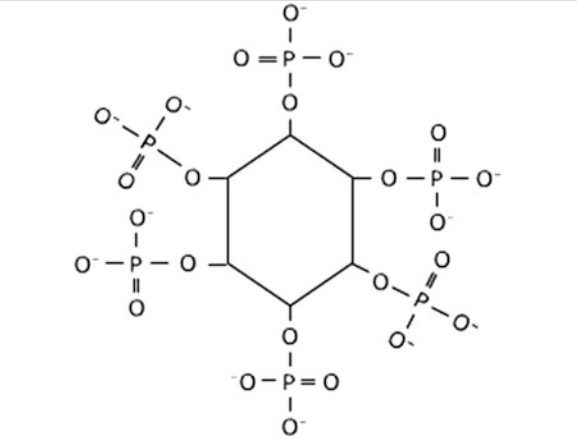
Phosphorylation of proteins as a regulatory mechanism is significant during development as well as in response to stress conditions. Thus, understanding phosphorylation of the proteins is significant. Use of mass-spectrometry has helped in identification of the phosphoproteins. Phosphorus homeostasis includes regulating the cytosolic concentration of P_i. If there is deficiency of phosphorus, it is mobilized from vacuolar storage pools, or genes involved in acquisition or transport of phosphorus are upregulated. When phosphorus is present in excess amounts, it can be stored in vacuoles so as to avoid toxicity. In case of deficiency, mobilization of phosphorus from senescing leaves occurs, which is known as **retrotranslocation**. Many of the senescence-induced genes (because of P_i deficiency) are involved in lipid degradation. This includes phospholipase A, phospholipase D, etc. This results in the production of IP_3 which acts as the signal molecule. There is overlapping of upregulation and downregulation of genes involved in P_i deficiency and senescence which signifies the importance of phosphorus recycling. Major pool of organic phosphorus is present in nucleic acids, phosphorylated proteins, various phosphorylated metabolites, and phospholipids. During senescence, phosphatases, nucleases, and phosphoesterases play important roles in the release of P_i from the organic forms. Transporters are required for uploading P_i into phloem so that it can be supplied to younger parts of the plant. In thylakoids, phospholipids might be replaced by sulfolipids or galactolipids under P_i deficiency. However, phosphorus is irreplaceable in many phosphorus-containing biomolecules.

12.2.4 Mobilization of Phosphorus

In plants phosphorus is mostly present in organic forms as organic esters. In seeds, it is present as phytic acid (inositol hexakisphosphate) (Box 12.4). Phytic acid can bind to various anions resulting in the formation of phytate. At the time of germination, phytase is responsible for release of phosphates from phytates. Numerous enzymes including phosphatases and diesterases present in the plant are responsible for breaking down of the organic forms (prevalent as phosphomonoesters and diesters) thus making phosphorus available to plants. After reserves are exhausted, activity of these enzymes declines. Mobile nature of phosphorus is responsible for maintaining the homeostasis at the time of its deficiency or during leaf senescence. There are phosphatases which differ in their optimal activity at different pH. Many plant phosphatases which have acid optima absorb light at 560 nm wavelength in pure solution. These are known as purple acid phosphatases (PAPs). PAPs play very important role in plant nutrition. PO_4^- is an inhibitor of PAPs. A large pool of phosphorus is in the form rRNA. In developing leaves, rRNA pool increases, while it decreases in the senescing leaves where phosphorus concentration decreases up to 78%. During P_i deficiency and senescence of leaves, genes required for synthesis of PAPs are upregulated. However, mobilization of P_i from senescing leaves remains the major source for P_i which is required by various sinks in the plants, such as young leaves, reproductive structures, and storage tissues. Under P_i deficiency, many genes required for

Box 12.4: Phytase Enzyme

A number of plants store phosphorus in seeds in the form of phytate. Phytic acid is chemically inositol hexakisphosphate which on dissociation in aqueous solutions bind to cations such as Ca, Mg, Fe, or Zn in anionic form (phytate). This can be hydrolyzed by phytase enzyme which is present in microorganisms and plants. However, the presence of this enzyme has not been reported in animals. Bacteria present in ruminant stomachs of Artiodactyla not only release cellulase required for the digestion of cellulose but also phytase. Plant-derived feed for poultry and animals is often supplemented with phytase, which results in making inorganic phosphorus available for growth.



Structure of phytic acid (Inositol hexakisphosphate)

hydrolysis of phospholipids and for biosynthesis of galactolipids and sulfolipids are upregulated. Understanding the recycling and mobilization of phosphorus will help in improving P_i use efficiency of crop plants which will further minimize the usage of P_i fertilizers.

12.3 Iron Metabolism

Iron is an essential element in all living beings. It is required as a component of various proteins and as an activator in various enzyme-catalyzed reactions. It is important in redox reactions of the cell because of its variable valency. Since it is a constituent of heme, it has structural role in all heme proteins, including cytochromes. The cofactor of cytochromes consists of porphyrin ring with iron in the center (Fig. 12.15). Various cytochromes, which absorb different light

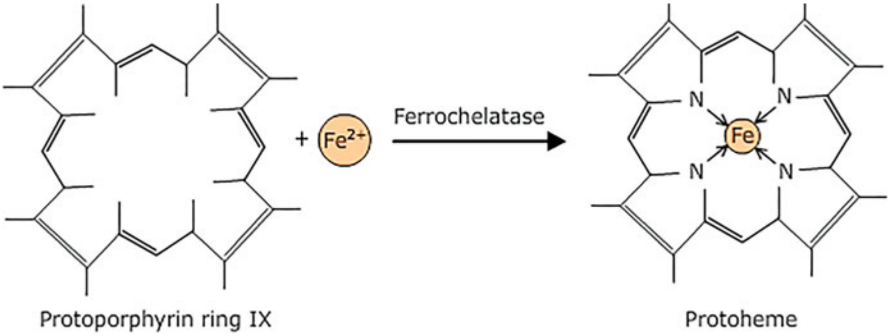
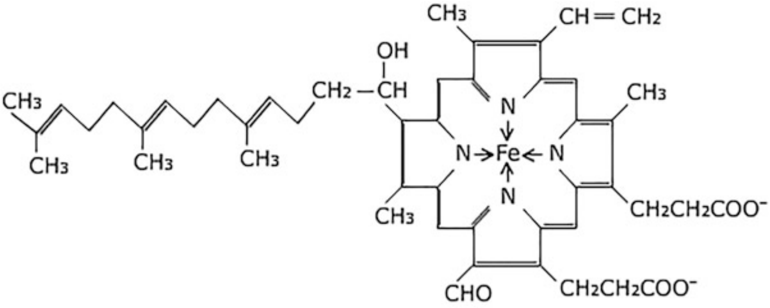
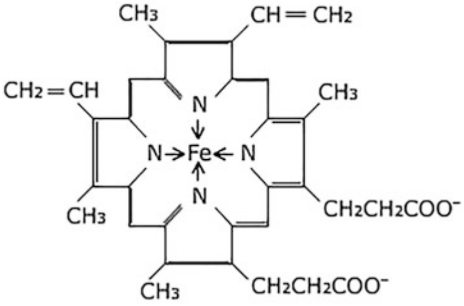


Fig. 12.15 Ferrochelatase catalyzes the terminal step in heme biosynthesis. It catalyzes chelation of ferrous ion into the protoporphyrin IX ring to form protoheme. In plants, heme is synthesized both in mitochondria and chloroplasts

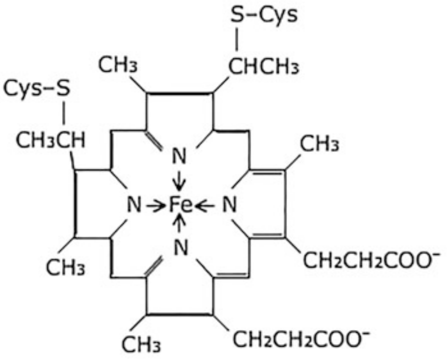
wavelengths, have been identified. These include cytochromes a, b, and c, which differ in the side groups attached to porphyrin ring (Fig. 12.16). The longest wavelength absorbed is near 600 nm in type "a" cytochromes, near 560 nm in type "b," while it is near 550 nm in case of cytochrome "c." Cytochromes are constituents of electron transport chain both in mitochondria and chloroplasts, which differ in their redox potential. Additionally, non-heme Fe also participates in various subcellular redox reactions as **iron-sulfur proteins (ISP)**, in which Fe is present as a complex with sulfur. A coordination between the metabolism of these two nutrients, i.e., Fe and S, is required since most of the metabolically important Fe is covalently bound to S as Fe-S complex. Chelated iron and reduced sulfur are required for the biosynthesis of Fe-S clusters. Fe-S clusters (Fig. 12.17) are component of various proteins involved in electron transport. There are multiple types of Fe-S clusters, the simplest one having one Fe coordinated to four cysteine -SH groups. The complex may also be present as Fe_2-S_2, Fe_3S_3, and Fe_4-S_4. **Rieske iron-sulfur proteins** are Fe_2S_2 type in which one Fe is coordinated with two histidine residues of the apoprotein rather than with -SH of cysteine, while one Fe is coordinated with two -SH groups of cysteine. These are called **Rieske protein** after the name of the scientist John S. Rieske and coworkers who discovered and isolated them in 1964. In each cluster, iron is present either in reduced state (Fe II) or in oxidized state (Fe III). Each center is able to carry only one electron at a time. Rieske proteins are part of the electron transport system with electron reduction potential ranging from −150 to +400 mV. These are components of cytochromes bc_1 complexes in respiratory electron transport chain (ETC) and cytochrome b_6f in ETC of photosynthesis. Ferredoxin type [Fe_2S_2] iron clusters are coordinated with four cysteine residues in a protein. Nitrogenase also has Fe-S containing cofactor, which mediates electron transfer between reduced ferredoxin and nitrogen. Catalase and peroxidase are also iron requiring heme proteins. Iron is also required for chlorophyll biosynthesis. But unlike heme, it is not a structural component of chlorophyll. The rate-



Heme a
(in a-type cytochromes)



Iron protoporphyrin IX
(in b-type cytochromes)



Herne c
(in c-type cytochromes)

Fig. 12.16 Prosthetic group of cytochromes. Heme is a tetrapyrrole that binds ferrous iron (Fe^{2+}) at four coordinated nitrogens in the protophyrin ring system and is incorporated into various apoproteins as a prosthetic group. Protophyrin IX is the immediate precursor for heme biosynthesis. Protophyrin IX complexed with iron to form iron protophyrin IX, i.e., heme of cyt b type of proteins. Heme c is bound covalently through thioester bonds in cyt c type of proteins, while heme a exhibits long isoprenoid tail attached to one of the five-membered rings, which is present in a-type cytochromes

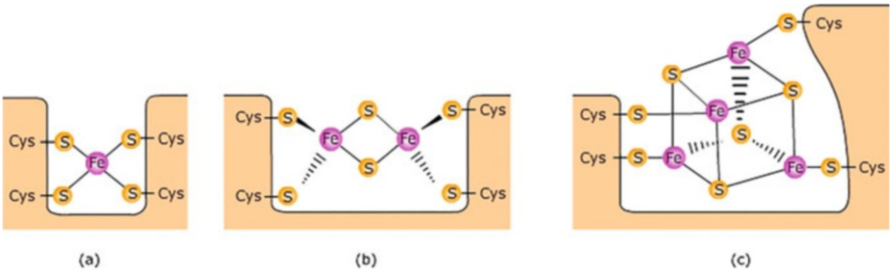


Fig. 12.17 Iron-sulfur clusters (Fe-S) of Fe-S proteins. (**a**) Single Fe is coordinated to four sulfur atoms of cysteine residues of the protein. (**b**) In 2Fe-2S cluster 2 Fe and, in (**c**) 4 Fe are coordinated with 4 Cys-SH groups of the protein. All iron-sulfur proteins are involved in single electron transfer, since single Fe takes part in electron transfer

limiting step in chlorophyll biosynthesis is catalyzed by iron-dependent enzyme glutamyl-tRNA reductase. Iron also has a critical role in DNA replication.

12.3.1 Biogeochemical Cycle of Iron and Iron Uptake by the Plant

In all terrestrial forms of life, including human beings, iron is obtained from the plants, which sequester it from the soil. Thus, iron enters the food chain through the plants. Iron constitutes approximately 3% of the earth's crust. It ranks fourth among all the elements which are available in the earth crust. Though present in high amount, iron is not easily available, and plants exhibit deficiency symptoms. The reason most of the plants show iron deficiency even when iron is present in soils in significant amount is that soluble inorganic forms of iron (Fe^{2+}) which plants are capable of absorbing are present in very low concentrations in the soil, i.e., in the range of 0.01–1 nm. In aerated soils, acidophilic bacteria such as *Acidithiobacillus ferrooxidans* oxidize Fe(II) to Fe(III). These bacteria grow autotrophically on Fe (II) using O_2 as the terminal electron acceptor.

$$2Fe^{2+} + {}^1/_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$$

Oxidation of ferrous ions by bacteria is used both for generating ATP and for reduction of CO_2 to carbohydrates.

$$4Fe^{2+} + HCO_3^- + 10H_2O + h\nu \rightarrow 4Fe(OH)_3 + (CH_2O) + 7H^-$$

Ferric hydroxide is insoluble, and its formation is favored in pH 5–8. However, if pH of the soil is more than 8, iron is present as Fe $(OH)_4$, which is sparingly used thus not available to plants. Aerobic soils have moderate to basic pH, and therefore, iron is unavailable to plants. Iron is present in oxidized state or is complexed with

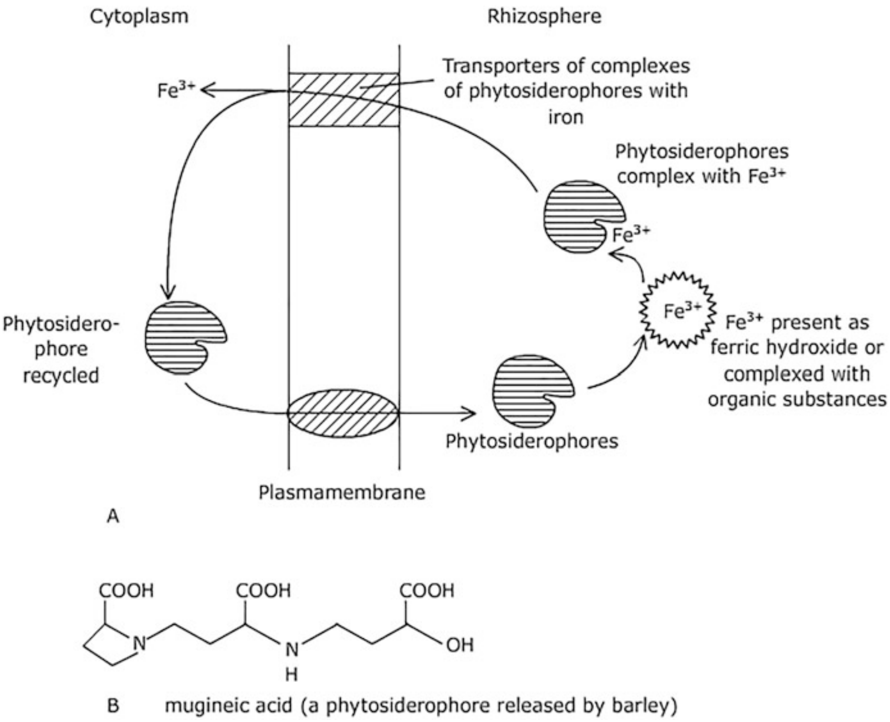


Fig. 12.18 (**a**) Iron uptake as complex with phytosiderophore released by the plant. (**b**) Mugineic acid, an amino acid secreted by some members of the grass family under iron deficiency

organic material in soils having neutral and basic pH. However, in waterlogged soils, iron is available as Fe^{2+}. Plants can absorb reduced form of iron (Fe^{2+}) since transporters for these are present in plasma membrane of the root epidermis. Two strategies are adopted by plants for the uptake of iron. In grasses, plants release organic compounds known as **phytosiderophores (PS)** that efficiently chelate and solubilize ferric ions. The Fe (III)-PS complex is the soluble form of the iron complexes which can be absorbed by high-affinity transporters present in the plasma membrane of root epidermis (Fig. 12.18). Transporters of Fe (III)-PS complexes are integral membrane protein with 12 putative transmembrane domains encoded by the gene **yellow stripe 1 (YS1).** The protein is responsible for proton-coupled symport of Fe (III)-PS complexes. In oat and barley, phytosiderophores have been characterized which consist of non-protein amino acids avenic acid and mugineic acid. These amino acids are synthesized by the plants in response to iron deficiency from methionine. Function of phytosiderophores is less affected by pH. ATPase-mediated H^+ pumps in root epidermis pump out protons altering pH of rhizosphere adjacent to roots to acidic. In acidic pH, Fe^{3+} is released from organic complexes. In

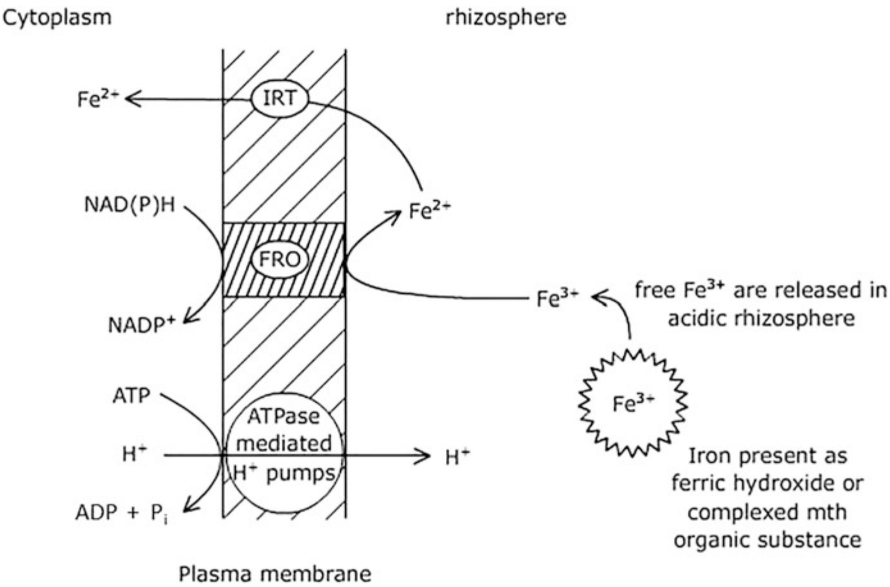


Fig. 12.19 Mechanism of iron uptake in roots of the plant. FRO, ferredoxin reductase/oxidase; IRT, iron-regulated transporter

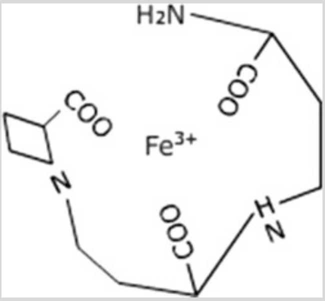
many species of monocots other than grasses, enzymes of **ferredoxin reductase/ oxidase (FRO)** family are synthesized (Fig. 12.19). These are **flavocytochromes** with intramembrane heme moieties and large cytoplasmic loops having binding sites for FAD and NAD(P)H. FROs facilitate electron transfer from NAD(P)H to Fe^{3+} mediated by heme as a result of which Fe^{3+} is reduced to Fe^{2+}. The presence of **iron-regulated transporter (IRT1)** in root epidermis is responsible for the absorption of Fe^{2+} ions. Coordinated role of ATPase-mediated H^+ pumps, FROs and IRT, is required for iron uptake by the monocots and eudicots.

12.3.2 Transport of Iron Within Plant

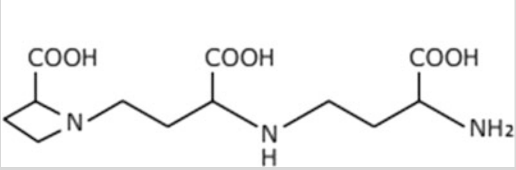
After uptake from the soil through different strategies adopted in different plants, iron is made available in the cytosol of the root epidermal cells complexed with some organic compounds which are released in xylem. The pH of the xylem sap is acidic (5.5). Iron released in the xylem form complex with citrate or some other compounds (in grasses this may be a phytosiderophore) which facilitates iron transport to shoots along with the transpiration stream. In case iron is required to be transported to plant parts with lower transpiration rate, it takes place in phloem (pH 7.2). The iron-chelating compound identified in *Ricinus communis* is a 17-kD protein, which is called **iron transport protein (ITP)**. It binds with Fe (III) form of iron rather than with Fe (II). Another compound **nicotianamine (NA)** forms a stable complex with Fe

(II) rather than Fe (III) at neutral pH (Box 12.5). According to a proposed model, iron must be transported as Fe (III) complexed possibly with IRT. However, at the time of unloading, iron forms a complex with NA, and the Fe:NA complex crosses plasma membrane through yellow stripe-like (YSL) transporters. A reductase may reduce Fe (III) to Fe (II) prior to binding with NA.

Box 12.5: Nicotianamine (NA)



Nicotianamine (NA) is a metal chelator which plays an important role in long-distance transport and homeostasis of Fe in plants. It is an intermediate in the biosynthesis of **phytosiderophores**. NA is synthesized from methionine as a result of single-step condensation of three molecules of S-adenyl-methionine catalyzed by the enzyme NA synthase. NA has three amine and three carboxylic groups in an **azetidine** ring. At cytosolic pH (around 7.2), NA can form chelates with both forms of Fe (Fe^{2+} and Fe^{3+}). NA aminotransferase (NAAT) catalyzes amino group transfer from NA during the biosynthesis of phytosiderophores by graminaceous plants. Activity of NAAT affects both iron homeostasis and its transport. Introduction and overexpression of the NAAT gene in tobacco result in reduction of endogenous NA, which significantly affects iron transport and iron homeostasis, resulting in the production of abnormal phenotype of the plant. It also affects the genes involved in iron uptake machinery of the plants. NA-Fe complexes are transported across membranes, and this process seems to be very significant for intracellular transport of Fe in the plants. NA-Fe transporters are members of oligopeptide transporters (OPT) groups which are found in archaea, fungi, bacteria, and plants.



Nicotianamine (NA)
Chelation of Fe^{3+} with NA

12.3.3 Redistribution of Iron at the Subcellular Level

Long-distance transport of iron as well as intracellular transport requires specialized proteins and small molecular weight chelators because the presence of O_2 iron is insoluble and toxic. For long-distance transport, iron may get chelated with nicotianamine or organic acids. Ferric reductase and transporters may possibly be involved in the transport of iron into the mesophyll cells. However, exact mechanism is not known. Iron metabolism in chloroplasts and mitochondria is particularly important since these organelles are the major sinks for iron. Both organelles may have a prokaryotic type of iron transport system. Iron may be able to cross the outer membrane of both the organelles freely through porins. Mitochondria are the major sinks for iron because it is the major site for biogenesis of Fe-S cluster and for heme biosynthesis. There is a possible role of metalloreductase in iron transport across inner mitochondrial membrane. An iron transporter known as **mitochondrial iron transporter (MIT)** has been identified in rice. Iron-sulfur cluster biogenesis machinery has a very important role in maintaining iron homeostasis. Two proteins play a significant role in iron metabolism of mitochondria. **Frataxin (FH)**, a conserved mitochondrial protein, is a molecular chaperon which regulates biogenesis of Fe-S cluster and heme biosynthesis. Maintaining iron concentration in mitochondria is a challenge since pH of the matrix is alkaline. Role of frataxin and a storage protein **ferretin** is significant in maintaining iron homeostasis. Ferretin is the iron-storage protein which stores excess iron and protects the cellular machinery from ROS production. Role of ferretin as the iron-storage protein complex is more significant in chloroplasts, while frataxin has more significance in mitochondria (Fig. 12.20). Iron in the form of free ions is toxic in mitochondria and chloroplasts since these are the major sites for ROS generation in the cell. The conserved proteins in ferretin, which are oligomerized to form a hollow

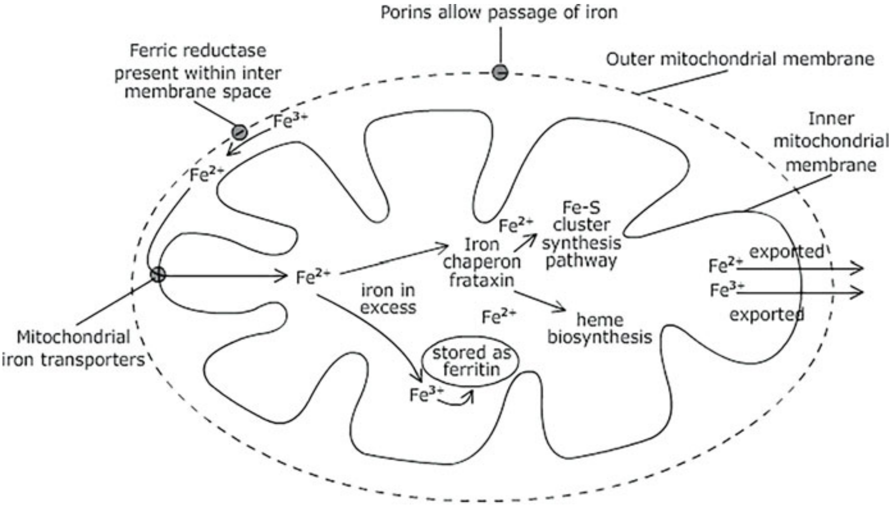


Fig. 12.20 Iron metabolism in mitochondria

sphere, exhibit ferroxidase activity (Fig. 12.21). These oxidize Fe^{2+} to Fe^{3+} which is stored within the core of the protein complex in the form of hydrous ferric oxide along with phosphates. There may be as many as 2000–4000 Fe^{3+} atoms per molecule of ferritin. Mechanism of the release of Fe^{3+} from ferretin is not yet understood. Transport of iron into chloroplasts requires ferric chelate reductase (FRO7). Another cell organelle involved in iron storage is vacuole (Fig. 12.22). Transport of iron into the vacuoles is mediated by **vacuolar iron transporter (VIT 1)**. Once inside the vacuole, iron is chelated with nicotianamine (NA). Release of Fe from chloroplasts and vacuoles is possibly mediated by efflux transporters YSL (yellow stripe-like) and by **NRAMP** family of transporters, respectively. Since iron can catalyze formation of deleterious ROS, it may be used by the host as a defense mechanism against invading pathogens (iron homeostasis). A cell needs to have a sensing and signaling mechanism

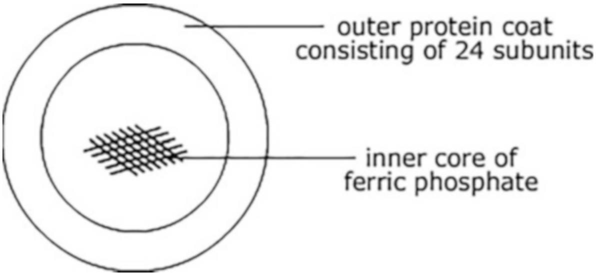


Fig. 12.21 Phytoferritin complex: the sphere complex consists of outer shell composed of 24 ferritin molecules and an inner core consisting of a ferric oxide-phosphate complex

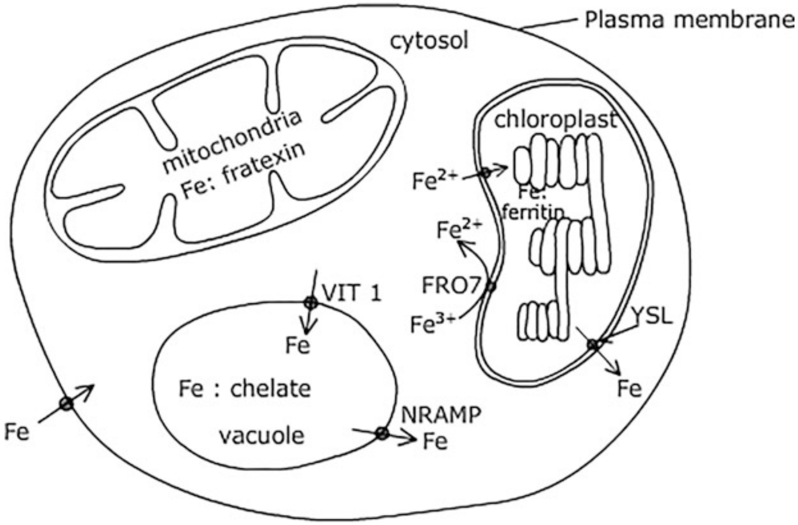


Fig. 12.22 Cell organelles involved in iron metabolism. *VIT* vacuolar iron transporter, *YSL* yellow stripe-like, an efflux transporter, *FRO7* ferric chelate reductase, *NRAMP* family of natural resistance-associated macrophage protein (metal ion transporters, which play a major role in metal iron homeostasis)

by which iron homeostasis can be maintained because both deficiency and excess iron can affect various physiological processes in the cell. Iron homeostasis is required for heme biosynthesis, for assembly of Fe-S proteins as well as incorporation of iron with apoproteins. Under iron deficiency, expression of FIT1 is upregulated, which is an iron-induced transcription factor and might be involved in sensing iron deficiency. There may also be possible involvement of hormonal signaling in sensing iron deficiency.

Summary

- Sulfur is an essential macronutrient which is a structural component of various biomolecules, including amino acids cysteine and methionine, cofactors, coenzymes, lipoic acids, sulfolipids, and many others. In soil, sulfur is available to plants as sulfate ions and as SO_2 in the air present as a pollutant. Sulfate transporters in the root hair epidermis facilitate uptake of sulfate. Sulfate is metabolized in root plastids, and the excess amount is either stored in vacuoles or is transported to leaves via xylem where it is metabolized in chloroplasts.
- Primary route for assimilation of SO_4^- is similar to that of nitrate assimilation. In chloroplasts, ATP sulfurylase catalyzes reaction of SO_4^- with ATP to form adenosine 5′-phosphate sulfate (APS). While attached to APS, sulfate is reduced to SO_3^- using the electrons provided by glutathione and SO_3^-, and AMP is released free. Sulfite reductase catalyzes reduction of SO_3^- to S^{2-} (sulfide) using six electrons from reduced ferredoxin. Sulfide is added to O-acetyl serine (OAS) to form cysteine with simultaneous release of acetate. Cysteine is the precursor for various other sulfur-containing biomolecules like methionine, glutathione, phytochelatins, glucosinolates, etc.
- APS kinase catalyzes conversion of APS to PAPS. This is followed by sulfation reactions resulting in biosynthesis of various other sulfur-containing biomolecules. Since animals, including human, do not have the enzyme for SO_4^- reduction, cysteine biosynthesis does not occur in them, and it needs to be provided in their diet. So, increasing cysteine and methionine content of various crop plants remains to be an important and challenging goal for the biotechnologists.
- Inorganic phosphorus present in soil is derived either from rock salt or is present in soil due to fertilizer application. The organic form of phosphorus present in soil is derived from plant and animal wastes. The inorganic form of phosphorus available to plants is released from the organic forms due to action of various phosphatases released by the plant roots or by the microorganism. Inorganic phosphorus refers to HPO_4^- and $H_2PO_4^-$.
- Phosphorus is a mobile element. During its deficiency, P_i is released from the organic forms in the older leaves or is released during leaf senescence and translocated to younger leaves as well as the reproductive parts of the plant. Phosphorus homeostasis is maintained by increasing P_i supply from vacuoles under P_i deficiency and promoting storage in vacuoles if phosphorus is in excess so as to avoid phosphorus toxicity in cytosol of the cell.

- Phosphorus is required in the cell as the structural component of biomolecules which include phospholipids, nucleic acids, ATP, etc. Various intermediates of Calvin cycle and respiration are phosphorylated. Phosphorylation and dephosphorylation of proteins are essential for regulation of activities of various enzymes. Many of the intermediates of various metabolic pathways including photosynthesis and respiration are phosphorylated because phosphorylation lowers the activation energy requirement during enzyme-catalyzed reactions.
- Various phosphate transporters are present in plasma membrane and in other cellular membranes, which regulate uptake of phosphates from the soil and its distribution within cell and within the plant. At the root hair epidermis, a symporter is responsible for exchange of 2 or 4 H^+ with inorganic phosphate.
- In spite of iron being present as the fourth major element in earth crust, its availability to plants is limited which is mainly influenced by pH of the soil. Plants adopt different strategies to absorb iron from the soil which include release of chelating organic compounds, phytosiderophores, or release of H^+ by the roots, which facilitate release of iron from the organic complex forms, and reducing Fe^{3+} to Fe^{2+} form. This is followed by its absorption through transporters present on the root epidermis.
- Iron is an essential element which is required by all living beings as the structural component of heme proteins as well as Fe-S protein complexes which participate in electron transport reactions besides its requirement as an activator of some of the enzymes.
- Once inside the cytosol, iron is either transported to the shoots or there is intracellular redistribution of Fe within the root cell/mesophyll cell mediated by various transporters which include mitochondrial iron transporters and vacuolar iron transporters or is transported to chloroplasts. Mitochondria and chloroplasts are the major sink for iron since these two organelles house the electron transport chain.
- In case of nonavailability of iron or when excess free iron is present which is toxic, plants show deficiency symptoms. To maintain iron homoeostasis, it is either transported out to the subcellular organelles or is stored as frataxin in mitochondria or ferritin in chloroplasts. Iron is also transported as the complex of nicotianamine.

Multiple-Choice Questions

1. Phytoplanktons produce:
   (a) Dimethyl sulfide
   (b) Sulfur dioxide
   (c) Dimethylsulfoniopropionate
   (d) Dimethyl sulfoxide

2. SO_4^- uptake in root hair cell is facilitated by the plasma membrane localized:
   (a) 3 H^+/SO_4^- symporter
   (b) H^+/SO_4^- symporter
   (c) 3 H^+/SO_4^- antiporter
   (d) H^+/SO_4^- antiporter
3. The enzyme ATP sulfurylase catalyzes synthesis of:
   (a) Cysteine
   (b) Disulfide
   (c) Glutathione
   (d) Adenosine 5′-phosphosulfur
4. Which of the following statements is not correct?
   (a) Reduction of SO_3^- to S^{2-} involves transfer of 6 electrons.
   (b) Sulfite reductase draws electrons from reduced ferredoxin for reduction of SO_3^-.
   (c) Sulfite reductase draws electrons from NADH for reduction of SO_3^-.
   (d) Both nitrite reductase and sulfite reductase are similar since these consist of Fe_4-S_4 and siroheme.
5. Which of the following statements is correct?
   (a) Methionine is the precursor of cysteine.
   (b) Methionine is synthesized from cysteine.
   (c) Cysteine biosynthesis occurs in the plastids.
   (d) Glutathione acts as buffer for the changes in cytosolic pH.
6. Rieske iron-sulfur proteins are:
   (a) Fe_4S_4 types in which the iron atoms are coordinated with four cysteine residues of the apoprotein.
   (b) Fe_2S_2 types in which the iron atoms are coordinated with the cysteine residues of the apoprotein.
   (c) Fe_3S_3 types in which all iron are coordinated with histidine of the apoprotein.
   (d) Fe_2S_2 types in which one iron is coordinated with two histidine residues while another Fe is ordinated with two cysteine residues of the apoprotein.
7. The enzyme, ferredoxin reductase/oxidase (FRO), which is localized in plasma membrane of root hair epidermis facilitates Fe absorption because:
   (a) It is transporter of the iron phytosiderophore complex.
   (b) It facilitates reduction of Fe^{3+} to Fe^{2+} which can pass through the transporters of the membrane.
   (c) It pumps protons out of the cell to make the soil pH acidic which results in release of Fe from the complex with organic compounds.
   (d) It oxidizes Fe^{2+} to Fe^{3+} which makes the iron soluble and it can be absorbed.
8. The iron-storage protein is:
   (a) Ferretin in chloroplasts
   (b) Ferretin in mitochondria
   (c) Frataxin in mitochondria
   (d) Frataxin in chloroplasts

Answers

1.c 2. a 3. d 4. c 5. b 6. d 7. b
8. a

Suggested Further Readings

Jones RL, Helen O, Howard T, Susan W (2013) The molecular life of plants. Wiley-Blackwell, Chichester, pp 477–491

Smith AM, Coupland G, Liam D, Harberd N, Jones J, Martin C, Sablowski R, Amey A (2010) Plant biology. Garland Science/Taylor & Francis Group, New York, pp 284–293

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Long SR, Kahn M, Seefeldt L, Tsay Y, Kopriva S (2015) Nitrogen and sulfur. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. Wiley-Blackwell, Chichester, pp 746–767