Chapter

12

Assimilation of Mineral Nutrients

HIGHER PLANTS ARE AUTOTROPHIC ORGANISMS that can synthesize their organic molecular components out of inorganic nutrients obtained from their surroundings. For many mineral nutrients, this process involves absorption from the soil by the roots (see Chapter 5) and incorporation into the organic compounds that are essential for growth and development. This incorporation of mineral nutrients into organic substances such as pigments, enzyme cofactors, lipids, nucleic acids, and amino acids is termed **nutrient assimilation**.

Assimilation of some nutrients—particularly nitrogen and sulfur—requires a complex series of biochemical reactions that are among the most energy-requiring reactions in living organisms:

- In nitrate (NO_3^-) assimilation, the nitrogen in NO_3^- is converted to a higher-energy form in nitrite (NO_2^-), then to a yet higher-energy form in ammonium (NH_4^+), and finally into the amide nitrogen of glutamine. This process consumes the equivalent of 12 ATPs per nitrogen (Bloom et al. 1992).
- Plants such as legumes form symbiotic relationships with nitrogen-fixing bacteria to convert molecular nitrogen (N_2) into ammonia (NH_3). Ammonia (NH_3) is the first stable product of natural fixation; at physiological pH, however, ammonia is protonated to form the ammonium ion (NH_4^+). The process of biological nitrogen fixation, together with the subsequent assimilation of NH_3 into an amino acid, consumes about 16 ATPs per nitrogen (Pate and Layzell 1990; Vande Broek and Vanderleyden 1995).
- The assimilation of sulfate (SO_4^{2-}) into the amino acid cysteine via the two pathways found in plants consumes about 14 ATPs (Hell 1997).

For some perspective on the enormous energies involved, consider that if these reactions run rapidly in reverse—say, from NH_4NO_3 (ammonium nitrate) to N_2—they become explosive, liberating vast amounts of energy as motion, heat, and light. Nearly all explosives are based on the rapid oxidation of nitrogen or sulfur compounds.

Assimilation of other nutrients, especially the macronutrient and micronutrient cations (see Chapter 5), involves the formation of complexes with organic compounds. For example, Mg^{2+} associates with chlorophyll pigments, Ca^{2+} associates with pectates within the cell wall, and Mo^{6+} associates with enzymes such as nitrate reductase and nitrogenase. These complexes are highly stable, and removal of the nutrient from the complex may result in total loss of function.

This chapter outlines the primary reactions through which the major nutrients (nitrogen, sulfur, phosphate, cations, and oxygen) are assimilated. We emphasize the physiological implications of the required energy expenditures and introduce the topic of symbiotic nitrogen fixation.

NITROGEN IN THE ENVIRONMENT

Many biochemical compounds present in plant cells contain nitrogen (see Chapter 5). For example, nitrogen is found in the nucleoside phosphates and amino acids that form the building blocks of nucleic acids and proteins, respectively. Only the elements oxygen, carbon, and hydrogen are more abundant in plants than nitrogen. Most natural and agricultural ecosystems show dramatic gains in productivity after fertilization with inorganic nitrogen, attesting to the importance of this element.

In this section we will discuss the biogeochemical cycle of nitrogen, the crucial role of nitrogen fixation in the conversion of molecular nitrogen into ammonium and nitrate, and the fate of nitrate and ammonium in plant tissues.

Nitrogen Passes through Several Forms in a Biogeochemical Cycle

Nitrogen is present in many forms in the biosphere. The atmosphere contains vast quantities (about 78% by volume) of molecular nitrogen (N_2) (see Chapter 9). For the most part, this large reservoir of nitrogen is not directly available to living organisms. Acquisition of nitrogen from the atmosphere requires the breaking of an exceptionally stable triple covalent bond between two nitrogen atoms ($N{\equiv}N$) to produce ammonia (NH_3) or nitrate (NO_3^-). These reactions, known as **nitrogen fixation,** can be accomplished by both industrial and natural processes.

Under elevated temperature (about 200°C) and high pressure (about 200 atmospheres), N_2 combines with hydrogen to form ammonia. The extreme conditions are required to overcome the high activation energy of the reaction. This nitrogen fixation reaction, called the *Haber–Bosch process*, is a starting point for the manufacture of many industrial and agricultural products. Worldwide industrial production of nitrogen fertilizers amounts to more than 80×10^{12} g yr^{-1} (FAOSTAT 2001).

Natural processes fix about 190×10^{12} g yr^{-1} of nitrogen (Table 12.1) through the following processes (Schlesinger 1997):

- *Lightning*. Lightning is responsible for about 8% of the nitrogen fixed. Lightning converts water vapor and

TABLE 12.1
The major processes of the biogeochemical nitrogen cycle

Process	Definition	Rate (10^{12} g yr^{-1})[a]
Industrial fixation	Industrial conversion of molecular nitrogen to ammonia	80
Atmospheric fixation	Lightning and photochemical conversion of molecular nitrogen to nitrate	19
Biological fixation	Prokaryotic conversion of molecular nitrogen to ammonia	170
Plant acquisition	Plant absorption and assimilation of ammonium or nitrate	1200
Immobilization	Microbial absorption and assimilation of ammonium or nitrate	N/C
Ammonification	Bacterial and fungal catabolism of soil organic matter to ammonium	N/C
Nitrification	Bacterial (*Nitrosomonas* sp.) oxidation of ammonium to nitrite and subsequent bacterial (*Nitrobacter* sp.) oxidation of nitrite to nitrate	N/C
Mineralization	Bacterial and fungal catabolism of soil organic matter to mineral nitrogen through ammonification or nitrification	N/C
Volatilization	Physical loss of gaseous ammonia to the atmosphere	100
Ammonium fixation	Physical embedding of ammonium into soil particles	10
Denitrification	Bacterial conversion of nitrate to nitrous oxide and molecular nitrogen	210
Nitrate leaching	Physical flow of nitrate dissolved in groundwater out of the topsoil and eventually into the oceans	36

Note: Terrestrial organisms, the soil, and the oceans contain about 5.2×10^{15} g, 95×10^{15} g, and 6.5×10^{15} g, respectively, of organic nitrogen that is active in the cycle. Assuming that the amount of atmospheric N_2 remains constant (inputs = outputs), the *mean residence time* (the average time that a nitrogen molecule remains in organic forms) is about 370 years [(pool size)/(fixation input) = $(5.2 \times 10^{15}$ g + 95×10^{15} g)/$(80 \times 10^{12}$ g yr^{-1} + 19×10^{12} g yr^{-1} + 170×10^{12} g $yr^{-1})$] (Schlesinger 1997).

[a]N/C, not calculated.

oxygen into highly reactive hydroxyl free radicals, free hydrogen atoms, and free oxygen atoms that attack molecular nitrogen (N_2) to form nitric acid (HNO_3). This nitric acid subsequently falls to Earth with rain.

- *Photochemical reactions*. Approximately 2% of the nitrogen fixed derives from photochemical reactions between gaseous nitric oxide (NO) and ozone (O_3) that produce nitric acid (HNO_3).
- *Biological nitrogen fixation*. The remaining 90% results from biological nitrogen fixation, in which bacteria or blue-green algae (cyanobacteria) fix N_2 into ammonium (NH_4^+).

From an agricultural standpoint, biological nitrogen fixation is critical because industrial production of nitrogen fertilizers seldom meets agricultural demand (FAOSTAT 2001).

Once fixed in ammonium or nitrate, nitrogen enters a biogeochemical cycle and passes through several organic or inorganic forms before it eventually returns to molecular nitrogen (Figure 12.1; see also Table 12.1). The ammonium (NH_4^+) and nitrate (NO_3^-) ions that are generated through fixation or released through decomposition of soil organic matter become the object of intense competition among plants and microorganisms. To remain competitive, plants have developed mechanisms for scavenging these ions from the soil solution as quickly as possible (see Chapter 5). Under the elevated soil concentrations that occur after fertilization, the absorption of ammonium and nitrate by the roots may exceed the capacity of a plant to assimilate these ions, leading to their accumulation within the plant's tissues.

Stored Ammonium or Nitrate Can Be Toxic

Plants can store high levels of nitrate, or they can translocate it from tissue to tissue without deleterious effect. However, if livestock and humans consume plant material that is high in nitrate, they may suffer methemoglobinemia, a disease in which the liver reduces nitrate to nitrite, which combines with hemoglobin and renders the hemoglobin unable to bind oxygen. Humans and other animals may also convert nitrate into nitrosamines, which are potent carcinogens. Some countries limit the nitrate content in plant materials sold for human consumption.

In contrast to nitrate, high levels of ammonium are toxic to both plants and animals. Ammonium dissipates transmembrane proton gradients (Figure 12.2) that are required for both photosynthetic and respiratory electron transport (see Chapters 7 and 11) and for sequestering metabolites in

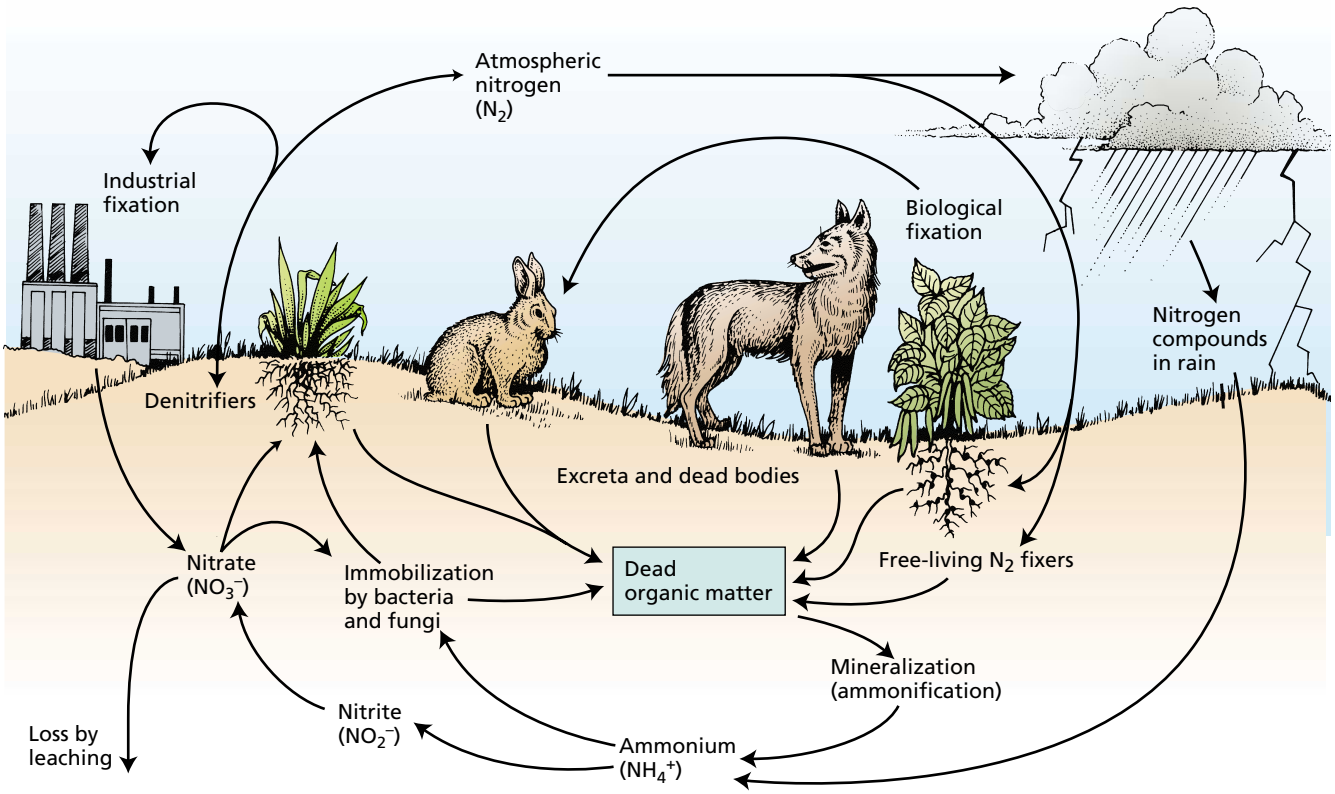


FIGURE 12.1 Nitrogen cycles through the atmosphere as it changes from a gaseous form to reduced ions before being incorporated into organic compounds in living organisms. Some of the steps involved in the nitrogen cycle are shown.

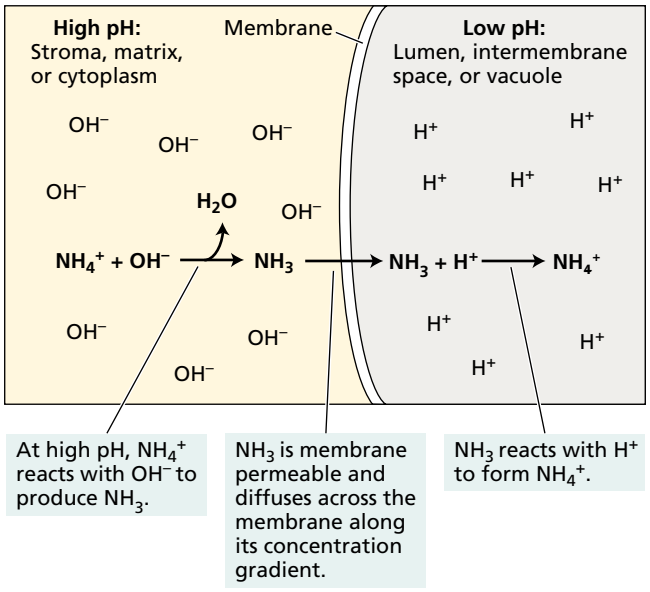


FIGURE 12.2 NH_4^+ toxicity can dissipate pH gradients. The left side represents the stroma, matrix, or cytoplasm, where the pH is high; the right side represents the lumen, intermembrane space, or vacuole, where the pH is low; and the membrane represents the thylakoid, inner mitochondrial, or tonoplast membrane for a chloroplast, mitochondrion, or root cell, respectively. The net result of the reaction shown is that both the OH^- concentration on the left side and the H^+ concentration on the right side have been diminished; that is, the pH gradient has been dissipated. (After Bloom 1997.)

the vacuole (see Chapter 6). Because high levels of ammonium are dangerous, animals have developed a strong aversion to its smell. The active ingredient in smelling salts, a medicinal vapor released under the nose to revive a person who has fainted, is ammonium carbonate. Plants assimilate ammonium near the site of absorption or generation and rapidly store any excess in their vacuoles, thus avoiding toxic effects on membranes and the cytosol.

In the next section we will discuss the process by which the nitrate absorbed by the roots via an H^+–NO_3^- symporter (see Chapter 6 for a discussion of symport) is assimilated into organic compounds, and the enzymatic processes mediating the reduction of nitrate first into nitrite and then into ammonium.

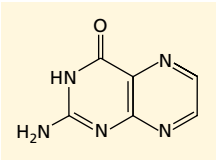
NITRATE ASSIMILATION

Plants assimilate most of the nitrate absorbed by their roots into organic nitrogen compounds. The first step of this process is the reduction of nitrate to nitrite in the cytosol (Oaks 1994). The enzyme **nitrate reductase** catalyzes this reaction:

$$NO_3^- + NAD(P)H + H^+ + 2\ e^- \rightarrow NO_2^- + NAD(P)^+ + H_2O \qquad (12.1)$$

where NAD(P)H indicates NADH or NADPH. The most common form of nitrate reductase uses only NADH as an electron donor; another form of the enzyme that is found predominantly in nongreen tissues such as roots can use either NADH or NADPH (Warner and Kleinhofs 1992).

The nitrate reductases of higher plants are composed of two identical subunits, each containing three prosthetic groups: FAD (flavin adenine dinucleotide), heme, and a molybdenum complexed to an organic molecule called a *pterin* (Mendel and Stallmeyer 1995; Campbell 1999).



A pterin (fully oxidized)

Nitrate reductase is the main molybdenum-containing protein in vegetative tissues, and one symptom of molybdenum deficiency is the accumulation of nitrate that results from diminished nitrate reductase activity.

Comparison of the amino acid sequences for nitrate reductase from several species with those of other well-characterized proteins that bind FAD, heme, or molybdenum has led to the three-domain model for nitrate reductase shown in Figure 12.3. The FAD-binding domain accepts two electrons from NADH or NADPH. The electrons then pass through the heme domain to the molybdenum complex, where they are transferred to nitrate.

Nitrate, Light, and Carbohydrates Regulate Nitrate Reductase

Nitrate, light, and carbohydrates influence nitrate reductase at the transcription and translation levels (Sivasankar and Oaks 1996). In barley seedlings, nitrate reductase mRNA was detected approximately 40 minutes after addition of nitrate, and maximum levels were attained within 3 hours (Figure 12.4). In contrast to the rapid mRNA accumulation,

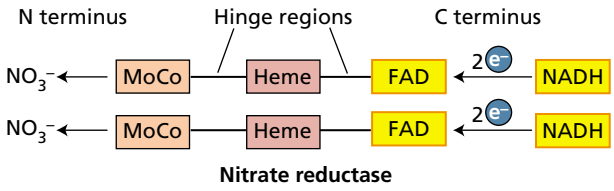


FIGURE 12.3 A model of the nitrate reductase dimer, illustrating the three binding domains whose polypeptide sequences are similar in eukaryotes: molybdenum complex (MoCo), heme, and FAD. The NADH binds at the FAD-binding region of each subunit and initiates a two-electron transfer from the carboxyl (C) terminus, through each of the electron transfer components, to the amino (N) terminus. Nitrate is reduced at the molybdenum complex near the amino terminus. The polypeptide sequences of the hinge regions are highly variable among species.

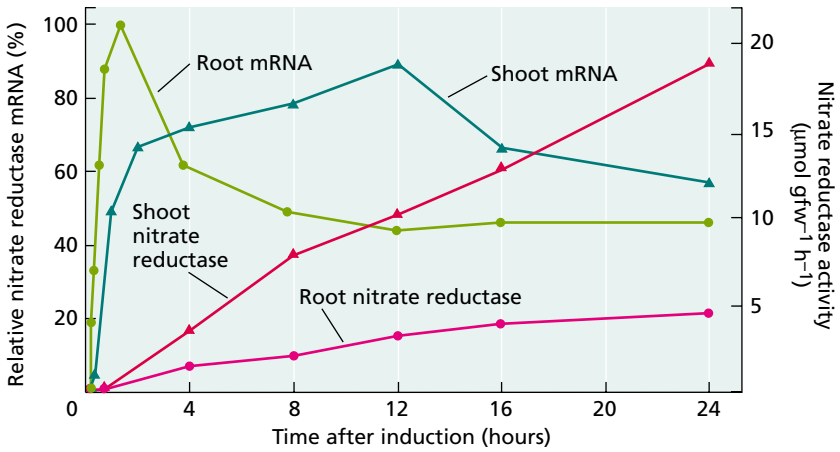


FIGURE 12.4 Stimulation of nitrate reductase activity follows the induction of nitrate reductase mRNA in shoots and roots of barley; gfw, grams fresh weight. (From Kleinhofs et al. 1989.)

there was a gradual linear increase in nitrate reductase activity, reflecting the slower synthesis of the protein.

In addition, the protein is subject to posttranslational modulation (involving a reversible phosphorylation) that is analogous to the regulation of sucrose phosphate synthase (see Chapters 8 and 10). Light, carbohydrate levels, and other environmental factors stimulate a protein phosphatase that dephosphorylates several serine residues on the nitrate reductase protein and thereby activates the enzyme.

Operating in the reverse direction, darkness and Mg^{2+} stimulate a protein kinase that phosphorylates the same serine residues, which then interact with a 14-3-3 inhibitor protein, and thereby inactivate nitrate reductase (Kaiser et al. 1999). *Regulation of nitrate reductase activity through phosphorylation and dephosphorylation provides more rapid control than can be achieved through synthesis or degradation of the enzyme (minutes versus hours).*

Nitrite Reductase Converts Nitrite to Ammonium

Nitrite (NO_2^-) is a highly reactive, potentially toxic ion. Plant cells immediately transport the nitrite generated by nitrate reduction (see Equation 12.1) from the cytosol into chloroplasts in leaves and plastids in roots. In these organelles, the enzyme nitrite reductase reduces nitrite to ammonium according to the following overall reaction:

$$NO_2^- + 6\,Fd_{red} + 8\,H^+ + 6\,e^- \rightarrow NH_4^+ + 6\,Fd_{ox} + 2\,H_2O \quad (12.2)$$

where Fd is ferredoxin, and the subscripts *red* and *ox* stand for *reduced* and *oxidized*, respectively. Reduced ferredoxin derives from photosynthetic electron transport in the chloroplasts (see Chapter 7) and from NADPH generated by the oxidative pentose phosphate pathway in nongreen tissues (see Chapter 11).

Chloroplasts and root plastids contain different forms of the enzyme, but both forms consist of a single polypeptide containing two prosthetic groups: an iron–sulfur cluster (Fe_4S_4) and a specialized heme (Siegel and Wilkerson 1989). These groups acting together bind nitrite and reduce it directly to ammonium, without accumulation of nitrogen compounds of intermediate redox states. The electron flow through ferredoxin (Fe_4S_4) and heme can be represented as in Figure 12.5.

Nitrite reductase is encoded in the nucleus and synthesized in the cytoplasm with an N-terminal transit peptide that targets it to the plastids (Wray 1993). Whereas NO_3^- and light induce the transcription of nitrite reductase mRNA, the end products of the process—asparagine and glutamine—repress this induction.

Plants Can Assimilate Nitrate in Both Roots and Shoots

In many plants, when the roots receive small amounts of nitrate, nitrate is reduced primarily in the roots. As the supply of nitrate increases, a greater proportion of the

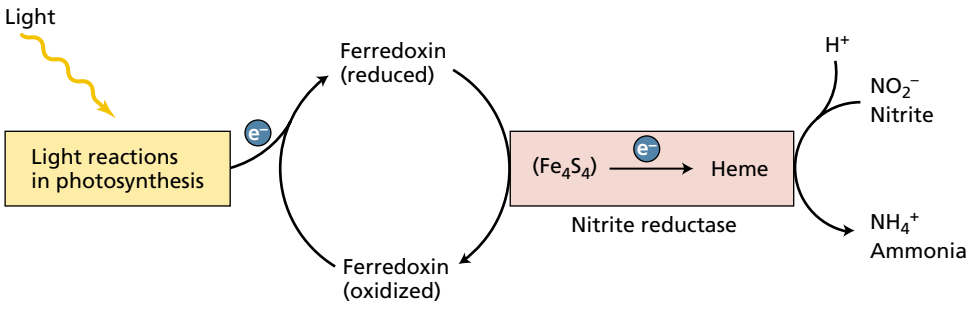


FIGURE 12.5 Model for coupling of photosynthetic electron flow, via ferredoxin, to the reduction of nitrite by nitrite reductase. The enzyme contains two prosthetic groups, Fe_4S_4 and heme, which participate in the reduction of nitrite to ammonium.

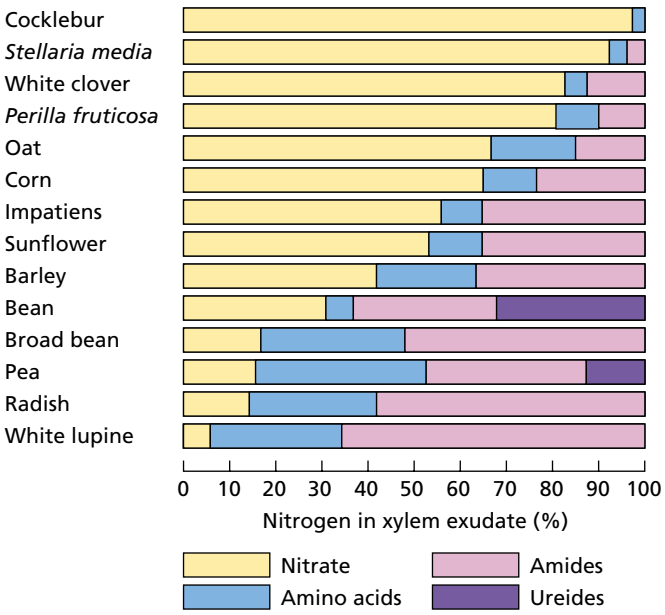


FIGURE 12.6 Relative amounts of nitrate and other nitrogen compounds in the xylem exudate of various plant species. The plants were grown with their roots exposed to nitrate solutions, and xylem sap was collected by severing of the stem. Note the presence of ureides, specialized nitrogen compounds, in bean and pea (which will be discussed later in the text). (After Pate 1983.)

absorbed nitrate is translocated to the shoot and assimilated there (Marschner 1995). Even under similar conditions of nitrate supply, the balance between root and shoot nitrate metabolism—as indicated by the proportion of nitrate reductase activity in each of the two organs or by the relative concentrations of nitrate and reduced nitrogen in the xylem sap—varies from species to species.

In plants such as the cocklebur (*Xanthium strumarium*), nitrate metabolism is restricted to the shoot; in other plants, such as white lupine (*Lupinus albus*), most nitrate is metabolized in the roots (Figure 12.6). Generally, species native to temperate regions rely more heavily on nitrate assimilation by the roots than do species of tropical or subtropical origins.

AMMONIUM ASSIMILATION

Plant cells avoid ammonium toxicity by rapidly converting the ammonium generated from nitrate assimilation or photorespiration (see Chapter 8) into amino acids. The primary pathway for this conversion involves the sequential actions of glutamine synthetase and glutamate synthase (Lea et al. 1992). In this section we will discuss the enzymatic processes that mediate the assimilation of ammonium into essential amino acids, and the role of amides in the regulation of nitrogen and carbon metabolism.

Conversion of Ammonium to Amino Acids Requires Two Enzymes

Glutamine synthetase (**GS**) combines ammonium with glutamate to form glutamine (Figure 12.7A):

$$\text{Glutamate} + NH_4^+ + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i \qquad (12.3)$$

This reaction requires the hydrolysis of one ATP and involves a divalent cation such as Mg^{2+}, Mn^{2+}, or Co^{2+} as a cofactor. Plants contain two classes of GS, one in the cytosol and the other in root plastids or shoot chloroplasts. The cytosolic forms are expressed in germinating seeds or in the vascular bundles of roots and shoots and produce glutamine for intracellular nitrogen transport. The GS in root plastids generates amide nitrogen for local consumption; the GS in shoot chloroplasts reassimilates photorespiratory NH_4^+ (Lam et al. 1996). Light and carbohydrate levels alter the expression of the plastid forms of the enzyme, but they have little effect on the cytosolic forms.

Elevated plastid levels of glutamine stimulate the activity of **glutamate synthase** (also known as *glutamine:2-oxoglutarate aminotransferase*, or **GOGAT**). This enzyme transfers the amide group of glutamine to 2-oxoglutarate, yielding two molecules of glutamate (see Figure 12.7A). Plants contain two types of GOGAT: One accepts electrons from NADH; the other accepts electrons from ferredoxin (Fd):

$$\text{Glutamine} + \text{2-oxoglutarate} + \text{NADH} + H^+ \rightarrow 2\ \text{glutamate} + NAD^+ \qquad (12.4)$$

$$\text{Glutamine} + \text{2-oxoglutarate} + Fd_{red} \rightarrow 2\ \text{glutamate} + Fd_{ox} \qquad (12.5)$$

The NADH type of the enzyme (NADH-GOGAT) is located in plastids of nonphotosynthetic tissues such as roots or vascular bundles of developing leaves. In roots, NADH-GOGAT is involved in the assimilation of NH_4^+ absorbed from the rhizosphere (the soil near the surface of the roots); in vascular bundles of developing leaves, NADH-GOGAT assimilates glutamine translocated from roots or senescing leaves.

The ferredoxin-dependent type of glutamate synthase (Fd-GOGAT) is found in chloroplasts and serves in photorespiratory nitrogen metabolism. Both the amount of protein and its activity increase with light levels. Roots, particularly those under nitrate nutrition, have Fd-GOGAT in plastids. Fd-GOGAT in the roots presumably functions to incorporate the glutamine generated during nitrate assimilation.

Ammonium Can Be Assimilated via an Alternative Pathway

Glutamate dehydrogenase (**GDH**) catalyzes a reversible reaction that synthesizes or deaminates glutamate (Figure 12.7B):

$$\text{2-Oxoglutarate} + NH_4^+ + NAD(P)H \leftrightarrow \text{glutamate} + H_2O + NAD(P)^+ \qquad (12.6)$$

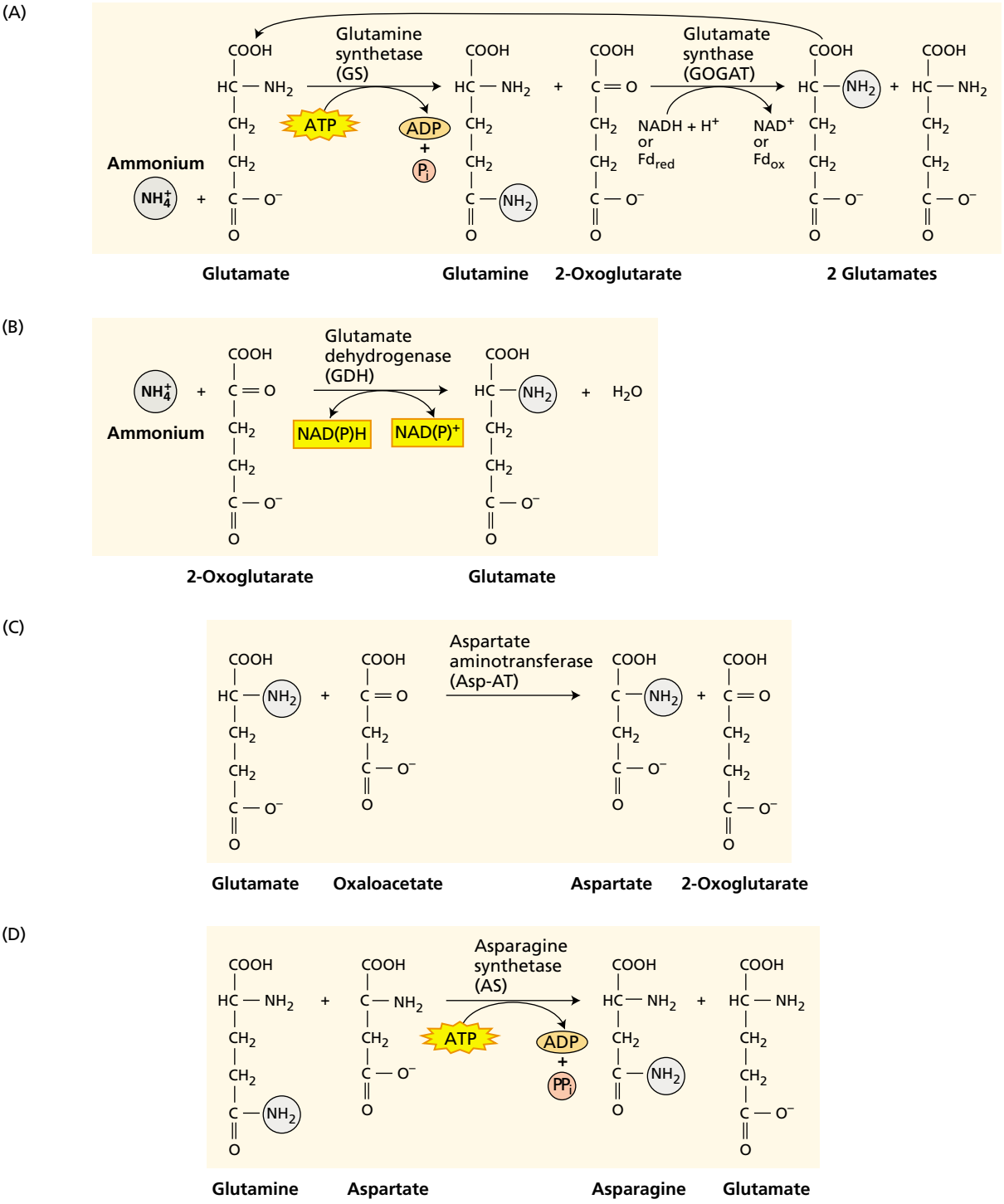


FIGURE 12.7 Structure and pathways of compounds involved in ammonium metabolism. Ammonium can be assimilated by one of several processes. (A) The GS-GOGAT pathway that forms glutamine and glutamate. A reduced cofactor is required for the reaction: ferredoxin in green leaves and NADH in nonphotosynthetic tissue. (B) The GDH pathway that forms glutamate using NADH or NADPH as a reductant. (C) Transfer of the amino group from glutamate to oxaloacetate to form aspartate (catalyzed by aspartate aminotransferase). (D) Synthesis of asparagine by transfer of an amino acid group from glutamine to aspartate (catalyzed by asparagine synthesis).

An NADH-dependent form of GDH is found in mitochondria, and an NADPH-dependent form is localized in the chloroplasts of photosynthetic organs. Although both forms are relatively abundant, they cannot substitute for the GS–GOGAT pathway for assimilation of ammonium, and their primary function is to deaminate glutamate (see Figure 12.7B).

Transamination Reactions Transfer Nitrogen

Once assimilated into glutamine and glutamate, nitrogen is incorporated into other amino acids via transamination reactions. The enzymes that catalyze these reactions are known as aminotransferases. An example is **aspartate aminotransferase** (**Asp-AT**), which catalyzes the following reaction (Figure 12.7C):

$$\text{Glutamate} + \text{oxaloacetate} \rightarrow \text{aspartate} + \text{2-oxoglutarate} \qquad (12.7)$$

in which the amino group of glutamate is transferred to the carboxyl atom of aspartate. Aspartate is an amino acid that participates in the malate–aspartate shuttle to transfer reducing equivalents from the mitochondrion and chloroplast into the cytosol (see Chapter 11) and in the transport of carbon from mesophyll to bundle sheath for C_4 carbon fixation (see Chapter 8). All transamination reactions require pyridoxal phosphate (vitamin B_6) as a cofactor.

Aminotransferases are found in the cytoplasm, chloroplasts, mitochondria, glyoxysomes, and peroxisomes. The aminotransferases localized in the chloroplasts may have a significant role in amino acid biosynthesis because plant leaves or isolated chloroplasts exposed to radioactively labeled carbon dioxide rapidly incorporate the label into glutamate, aspartate, alanine, serine, and glycine.

Asparagine and Glutamine Link Carbon and Nitrogen Metabolism

Asparagine, isolated from asparagus as early as 1806, was the first amide to be identified (Lam et al. 1996). It serves not only as a protein precursor, but as a key compound for nitrogen transport and storage because of its stability and high nitrogen-to-carbon ratio (2 N to 4 C for asparagine, versus 2 N to 5 C for glutamine or 1 N to 5 C for glutamate).

The major pathway for asparagine synthesis involves the transfer of the amide nitrogen from glutamine to asparagine (Figure 12.7D):

$$\text{Glutamine} + \text{aspartate} + \text{ATP} \rightarrow \text{asparagine} + \text{glutamate} + \text{AMP} + \text{PP}_i \qquad (12.8)$$

Asparagine synthetase (**AS**), the enzyme that catalyzes this reaction, is found in the cytosol of leaves and roots and in nitrogen-fixing nodules (see the next section). In maize roots, particularly those under potentially toxic levels of ammonia, ammonium may replace glutamine as the source of the amide group (Sivasankar and Oaks 1996).

High levels of light and carbohydrate—conditions that stimulate plastid GS and Fd-GOGAT—inhibit the expression of genes coding for AS and the activity of the enzyme. The opposing regulation of these competing pathways helps balance the metabolism of carbon and nitrogen in plants (Lam et al. 1996). Conditions of ample energy (i.e., high levels of light and carbohydrates) stimulate GS and GOGAT, inhibit AS, and thus favor nitrogen assimilation into glutamine and glutamate, compounds that are rich in carbon and participate in the synthesis of new plant materials.

By contrast, energy-limited conditions inhibit GS and GOGAT, stimulate AS, and thus favor nitrogen assimilation into asparagine, a compound that is rich in nitrogen and sufficiently stable for long-distance transport or long-term storage.

BIOLOGICAL NITROGEN FIXATION

Biological nitrogen fixation accounts for most of the fixation of atmospheric N_2 into ammonium, thus representing the key entry point of molecular nitrogen into the biogeochemical cycle of nitrogen (see Figure 12.1). In this section we will describe the properties of the nitrogenase enzymes that fix nitrogen, the symbiotic relations between nitrogen-fixing organisms and higher plants, the specialized structures that form in roots when infected by nitrogen-fixing bacteria, and the genetic and signaling interactions that regulate nitrogen fixation by symbiotic prokaryotes and their hosts.

Free-Living and Symbiotic Bacteria Fix Nitrogen

Some bacteria, as stated earlier, can convert atmospheric nitrogen into ammonium (Table 12.2). Most of these nitrogen-fixing prokaryotes are free-living in the soil. A few form symbiotic associations with higher plants in which the prokaryote directly provides the host plant with fixed nitrogen in exchange for other nutrients and carbohydrates (top portion of Table 12.2). Such symbioses occur in nodules that form on the roots of the plant and contain the nitrogen-fixing bacteria.

The most common type of symbiosis occurs between members of the plant family Leguminosae and soil bacteria of the genera *Azorhizobium*, *Bradyrhizobium*, *Photorhizobium*, *Rhizobium*, and *Sinorhizobium* (collectively called **rhizobia**; Table 12.3 and Figure 12.8). Another common type of symbiosis occurs between several woody plant species, such as alder trees, and soil bacteria of the genus *Frankia*. Still other types involve the South American herb *Gunnera* and the tiny water fern *Azolla*, which form associations with the cyanobacteria *Nostoc* and *Anabaena*, respectively (see Table 12.2 and Figure 12.9).

Nitrogen Fixation Requires Anaerobic Conditions

Because oxygen irreversibly inactivates the **nitrogenase** enzymes involved in nitrogen fixation, nitrogen must be fixed under anaerobic conditions. Thus each of the nitro-

TABLE 12.2
Examples of organisms that can carry out nitrogen fixation

Symbiotic nitrogen fixation	
Host plant	**N-fixing symbionts**
Leguminous: legumes, *Parasponia*	*Azorhizobium*, *Bradyrhizobium*, *Photorhizobium*, ` *Rhizobium*, *Sinorhizobium*
Actinorhizal: alder (tree), *Ceanothus* (shrub), *Casuarina* (tree), *Datisca* (shrub)	*Frankia*
Gunnera	*Nostoc*
Azolla (water fern)	*Anabaena*
Sugarcane	*Acetobacter*
Free-living nitrogen fixation	
Type	**N-fixing genera**
Cyanobacteria (blue-green algae)	*Anabaena*, *Calothrix*, *Nostoc*
Other bacteria	
Aerobic	*Azospirillum*, *Azotobacter*, *Beijerinckia*, *Derxia*
Facultative	*Bacillus*, *Klebsiella*
Anaerobic	
Nonphotosynthetic	*Clostridium*, *Methanococcus* (archaebacterium)
Photosynthetic	*Chromatium*, *Rhodospirillum*

gen-fixing organisms listed in Table 12.2 either functions under natural anaerobic conditions or can create an internal anaerobic environment in the presence of oxygen.

In cyanobacteria, anaerobic conditions are created in specialized cells called *heterocysts* (see Figure 12.9). Heterocysts are thick-walled cells that differentiate when filamentous cyanobacteria are deprived of NH_4^+. These cells lack photosystem II, the oxygen-producing photosystem of chloroplasts (see Chapter 7), so they do not generate oxygen (Burris 1976). Heterocysts appear to represent an adaptation for nitrogen fixation, in that they are widespread among aerobic cyanobacteria that fix nitrogen.

Cyanobacteria that lack heterocysts can fix nitrogen only under anaerobic conditions such as those that occur in flooded fields. In Asian countries, nitrogen-fixing cyanobacteria of both the heterocyst and nonheterocyst types are a major means for maintaining an adequate nitrogen supply in the soil of rice fields. These microorganisms fix nitrogen when the fields are flooded and die as the fields dry, releasing the fixed nitrogen to the soil. Another important

TABLE 12.3
Associations between host plants and rhizobia

Plant host	Rhizobial symbiont
Parasponia (a nonlegume, formerly called *Trema*)	*Bradyrhizobium* spp.
Soybean (*Glycine max*)	*Bradyrhizobium japonicum* (slow-growing type); *Sinorhizobium fredii* (fast-growing type)
Alfalfa (*Medicago sativa*)	*Sinorhizobium meliloti*
Sesbania (aquatic)	*Azorhizobium* (forms both root and stem nodules; the stems have adventitious roots)
Bean (*Phaseolus*)	*Rhizobium leguminosarum* bv. *phaseoli*; *Rhizobium tropicii*; *Rhizobium etli*
Clover (*Trifolium*)	*Rhizobium leguminosarum* bv. *trifolii*
Pea (*Pisum sativum*)	*Rhizobium leguminosarum* bv. *viciae*
Aeschenomene (aquatic)	*Photorhizobium* (photosynthetically active rhizobia that form stem nodules, probably associated with adventitious roots)

FIGURE 12.8 Root nodules on soybean. The nodules are a result of infection by *Rhizobium japonicum*. (© Wally Eberhart/Visuals Unlimited.)

source of available nitrogen in flooded rice fields is the water fern *Azolla*, which associates with the cyanobacterium *Anabaena*. The *Azolla–Anabaena* association can fix as much as 0.5 kg of atmospheric nitrogen per hectare per day, a rate of fertilization that is sufficient to attain moderate rice yields.

Free-living bacteria that are capable of fixing nitrogen are aerobic, facultative, or anaerobic (see Table 12.2, bottom):

- *Aerobic* nitrogen-fixing bacteria such as *Azotobacter* are thought to maintain reduced oxygen conditions (microaerobic conditions) through their high levels of respiration (Burris 1976). Others, such as *Gloeothece*, evolve O_2 photosynthetically during the day and fix nitrogen during the night.
- *Facultative* organisms, which are able to grow under both aerobic and anaerobic conditions, generally fix nitrogen only under anaerobic conditions.
- For *anaerobic* nitrogen-fixing bacteria, oxygen does not pose a problem, because it is absent in their habitat. These anaerobic organisms can be either photosynthetic (e.g., *Rhodospirillum*), or nonphotosynthetic (e.g., *Clostridium*).

Symbiotic Nitrogen Fixation Occurs in Specialized Structures

Symbiotic nitrogen-fixing prokaryotes dwell within **nodules**, the special organs of the plant host that enclose the nitrogen-fixing bacteria (see Figure 12.8). In the case of *Gunnera*, these organs are existing stem glands that develop independently of the symbiont. In the case of legumes and actinorhizal plants, the nitrogen-fixing bacteria induce the plant to form root nodules.

Grasses can also develop symbiotic relationships with nitrogen-fixing organisms, but in these associations root nodules are not produced. Instead, the nitrogen-fixing bacteria seem to colonize plant tissues or anchor to the root surfaces, mainly around the elongation zone and the root hairs (Reis et al. 2000). For example, the nitrogen-fixing

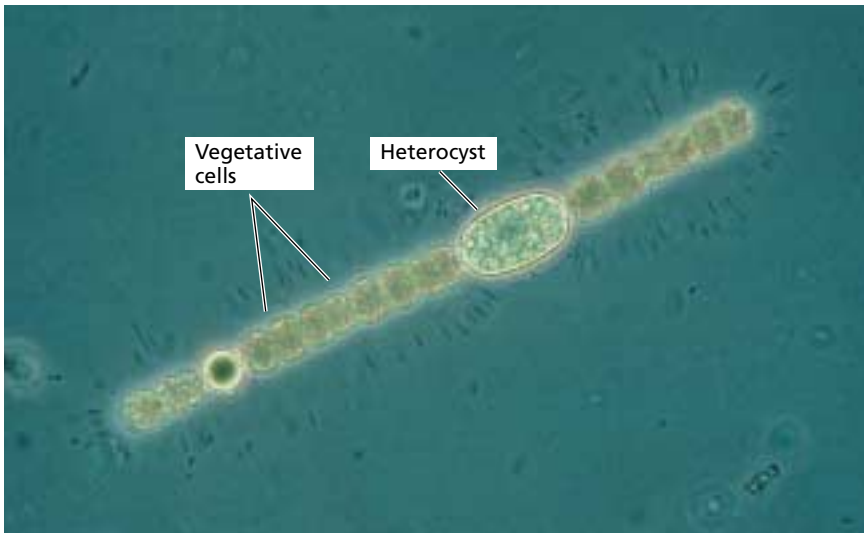


FIGURE 12.9 A heterocyst in a filament of the nitrogen-fixing cyanobacterium *Anabaena*. The thick-walled heterocysts, interspaced among vegetative cells, have an anaerobic inner environment that allows cyanobacteria to fix nitrogen in aerobic conditions. (© Paul W. Johnson/Biological Photo Service.)

bacterium *Acetobacter diazotrophicus* lives in the apoplast of stem tissues in sugarcane and may provide its host with sufficient nitrogen to grant independence from nitrogen fertilization (Dong et al. 1994). The potential for applying *Azospirillum* to corn and other grains has been explored, but *Azospirillum* seems to fix little nitrogen when associated with plants (Vande Broek and Vanderleyden 1995).

Legumes and actinorhizal plants regulate gas permeability in their nodules, maintaining a level of oxygen within the nodule that can support respiration but is sufficiently low to avoid inactivation of the nitrogenase (Kuzma et al. 1993). Gas permeability increases in the light and decreases under drought or upon exposure to nitrate. The mechanism for regulating gas permeability is not yet known.

Nodules contain an oxygen-binding heme protein called **leghemoglobin**. Leghemoglobin is present in the cytoplasm of infected nodule cells at high concentrations (700 μ*M* in soybean nodules) and gives the nodules a pink color. The host plant produces the globin portion of leghemoglobin in response to infection by the bacteria (Marschner 1995); the bacterial symbiont produces the heme portion. Leghemoglobin has a high affinity for oxygen (a K_m of about 0.01 μ*M*), about ten times higher than the β chain of human hemoglobin.

Although leghemoglobin was once thought to provide a buffer for nodule oxygen, recent studies indicate that it stores only enough oxygen to support nodule respiration for a few seconds (Denison and Harter 1995). Its function is to help transport oxygen to the respiring symbiotic bacterial cells in a manner analogous to hemoglobin transporting oxygen to respiring tissues in animals (Ludwig and de Vries 1986).

Establishing Symbiosis Requires an Exchange of Signals

The symbiosis between legumes and rhizobia is not obligatory. Legume seedlings germinate without any association with rhizobia, and they may remain unassociated throughout their life cycle. Rhizobia also occur as free-living organisms in the soil. Under nitrogen-limited conditions, however, the symbionts seek out one another through an elaborate exchange of signals. This signaling, the subsequent infection process, and the development of nitrogen-fixing nodules involve specific genes in both the host and the symbionts.

Plant genes specific to nodules are called **nodulin** (*Nod*) genes; rhizobial genes that participate in nodule formation are called **nodulation** (*nod*) genes (Heidstra and Bisseling 1996). The *nod* genes are classified as common *nod* genes or host-specific *nod* genes. The common *nod* genes—*nodA*, *nodB*, and *nodC*—are found in all rhizobial strains; the host-specific *nod* genes—such as *nodP*, *nodQ*, and *nodH*; or *nodF*, *nodE*, and *nodL*—differ among rhizobial species and determine the host range. Only one of the *nod* genes, the regulatory *nodD*, is constitutively expressed, and as we will explain in detail, its protein product (NodD) regulates the transcription of the other *nod* genes.

The first stage in the formation of the symbiotic relationship between the nitrogen-fixing bacteria and their host is migration of the bacteria toward the roots of the host plant. This migration is a chemotactic response mediated by chemical attractants, especially (iso)flavonoids and betaines, secreted by the roots. These attractants activate the rhizobial NodD protein, which then induces transcription of the other *nod* genes (Phillips and Kapulnik 1995). The promoter region of all *nod* operons, except that of *nodD*, contains a highly conserved sequence called the *nod* box. Binding of the activated NodD to the *nod* box induces transcription of the other *nod* genes.

Nod Factors Produced by Bacteria Act as Signals for Symbiosis

The *nod* genes activated by NodD code for nodulation proteins, most of which are involved in the biosynthesis of Nod factors. **Nod factors** are lipochitin oligosaccharide signal molecules, all of which have a chitin β-1→4-linked *N*-acetyl-D-glucosamine backbone (varying in length from three to six sugar units) and a fatty acyl chain on the C-2 position of the nonreducing sugar (Figure 12.10).

Three of the *nod* genes (*nodA*, *nodB*, and *nodC*) encode enzymes (NodA, NodB, and NodC, respectively) that are required for synthesizing this basic structure (Stokkermans et al. 1995):

1. NodA is an *N*-acyltransferase that catalyzes the addition of a fatty acyl chain.
2. NodB is a chitin-oligosaccharide deacetylase that removes the acetyl group from the terminal nonreducing sugar.

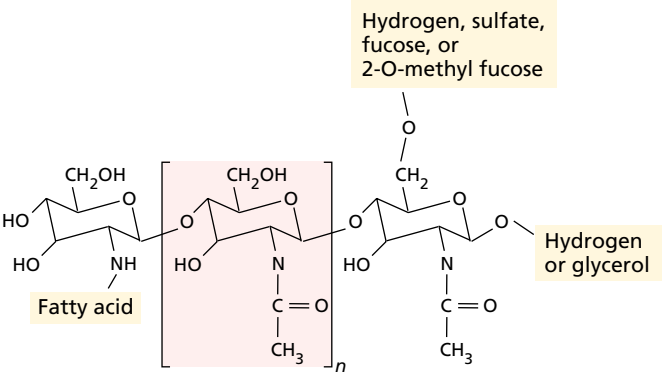


FIGURE 12.10 Nod factors are lipochitin oligosaccharides. The fatty acid chain typically has 16 to 18 carbons. The number of repeated middle sections (*n*) is usually 2 to 3. (After Stokkermans et al. 1995.)

3. NodC is a chitin-oligosaccharide synthase that links *N*-acetyl-D-glucosamine monomers.

Host-specific *nod* genes that vary among rhizobial species are involved in the modification of the fatty acyl chain or the addition of groups important in determining host specificity (Carlson et al. 1995):

- NodE and NodF determine the length and degree of saturation of the fatty acyl chain; those of *Rhizobium leguminosarum* bv. *viciae* and *R. meliloti* result in the synthesis of an 18:4 and a 16:2 fatty acyl group, respectively. (Recall from Chapter 11 that the number before the colon gives the total number of carbons in the fatty acyl chain, and the number after the colon gives the number of double bonds.)
- Other enzymes, such as NodL, influence the host specificity of Nod factors through the addition of specific substitutions at the reducing or nonreducing sugar moieties of the chitin backbone.

A particular legume host responds to a specific Nod factor. The legume receptors for Nod factors appear to be special lectins (sugar-binding proteins) produced in the root hairs (van Rhijn et al. 1998; Etzler et al. 1999). Nod factors activate these lectins, increasing their hydrolysis of phosphoanhydride bonds of nucleoside di- and triphosphates. This lectin activation directs particular rhizobia to appropriate hosts and facilitates attachment of the rhizobia to the cell walls of a root hair.

Nodule Formation Involves Several Phytohormones

Two processes—infection and nodule organogenesis—occur simultaneously during root nodule formation. During the infection process, rhizobia that are attached to the root hairs release Nod factors that induce a pronounced curling of the root hair cells (Figure 12.11A and B). The rhizobia become enclosed in the small compartment formed by the curling. The cell wall of the root hair degrades in these regions, also in response to Nod factors, allowing the bacterial cells direct access to the outer surface of the plant plasma membrane (Lazarowitz and Bisseling 1997).

The next step is formation of the **infection thread** (Figure 12.11C), an internal tubular extension of the plasma membrane that is produced by the fusion of Golgi-derived membrane vesicles at the site of infection. The thread grows at its tip by the fusion of secretory vesicles to the end of the tube. Deeper into the root cortex, near the xylem, cortical cells dedifferentiate and start dividing, forming a distinct area within the cortex, called a *nodule primordium*, from which the nodule will develop. The nodule primordia form opposite the protoxylem poles of the root vascular bundle (Timmers et al. 1999) (See **Web Topic 12.1**).

Different signaling compounds, acting either positively or negatively, control the position of nodule primordia. The nucleoside uridine diffuses from the stele into the cortex in the protoxylem zones of the root and stimulates cell division (Lazarowitz and Bisseling 1997). Ethylene is synthesized in the region of the pericycle, diffuses into the cortex, and blocks cell division opposite the phloem poles of the root.

FIGURE 12.11 The infection process during nodule organogenesis. (A) Rhizobia bind to an emerging root hair in response to chemical attractants sent by the plant. (B) In response to factors produced by the bacteria, the root hair exhibits abnormal curling growth, and rhizobia cells proliferate within the coils. (C) Localized degradation of the root hair wall leads to infection and formation of the infection thread from Golgi secretory vesicles of root cells. (D) The infection thread reaches the end of the cell, and its membrane fuses with the plasma membrane of the root hair cell. (E) Rhizobia are released into the apoplast and penetrate the compound middle lamella to the subepidermal cell plasma membrane, leading to the initiation of a new infection thread, which forms an open channel with the first. (F) The infection thread extends and branches until it reaches target cells, where vesicles composed of plant membrane that enclose bacterial cells are released into the cytosol.

The infection thread filled with proliferating rhizobia elongates through the root hair and cortical cell layers, in the direction of the nodule primordium. When the infection thread reaches specialized cells within the nodule, its tip fuses with the plasma membrane of the host cell, releasing bacterial cells that are packaged in a membrane derived from the host cell plasma membrane (see Figure 12.11D). Branching of the infection thread inside the nodule enables the bacteria to infect many cells (see Figure 12.11E and F) (Mylona et al. 1995).

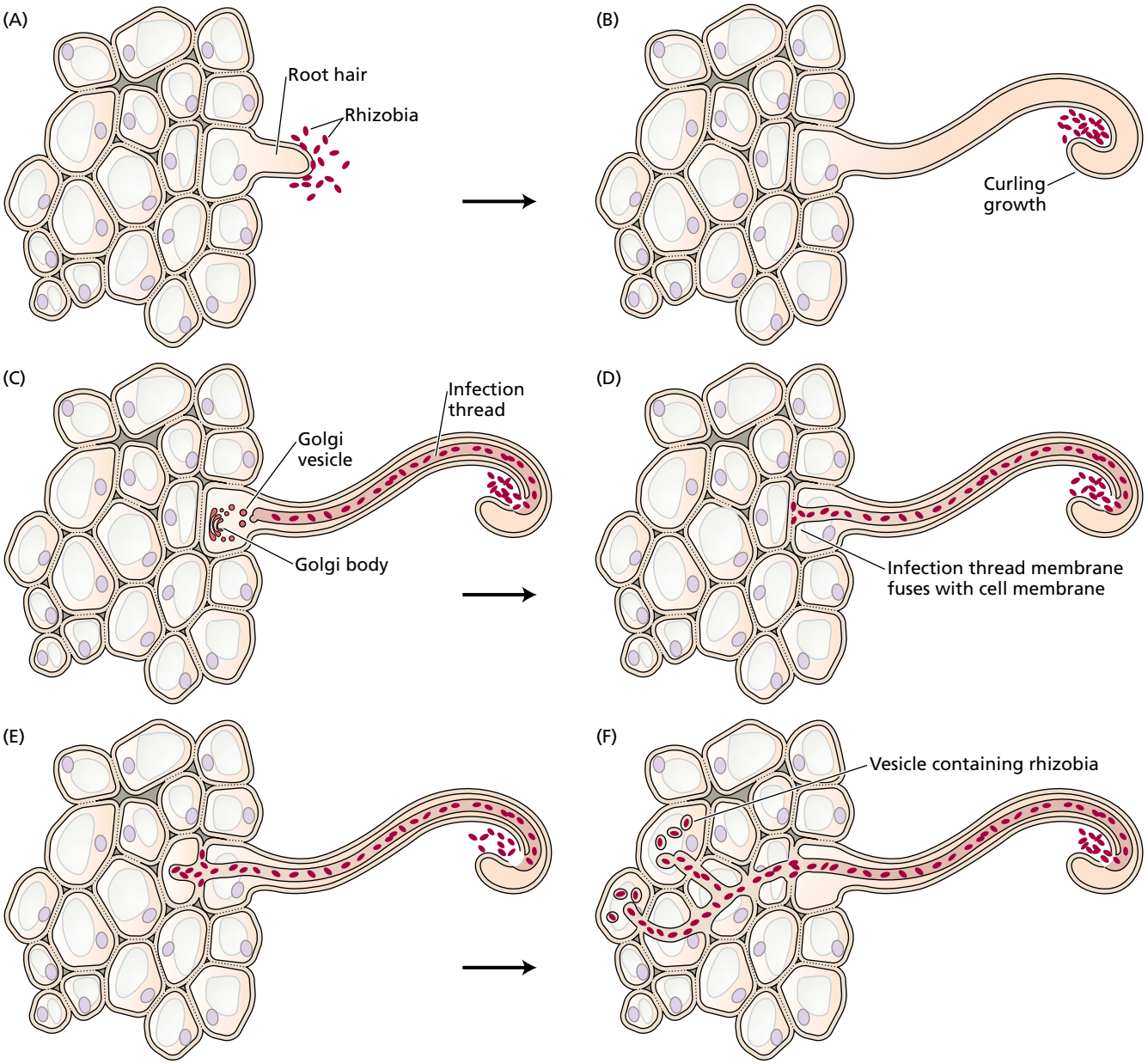
At first the bacteria continue to divide, and the surrounding membrane increases in surface area to accommodate this growth by fusing with smaller vesicles. Soon thereafter, upon an undetermined signal from the plant, the bacteria stop dividing and begin to enlarge and to differentiate into nitrogen-fixing endosymbiotic organelles called **bacteroids**. The membrane surrounding the bacteroids is called the *peribacteroid membrane*.

The nodule as a whole develops such features as a vascular system (which facilitates the exchange of fixed nitrogen produced by the bacteroids for nutrients contributed by the plant) and a layer of cells to exclude O_2 from the root nodule interior. In some temperate legumes (e.g., peas), the nodules are elongated and cylindrical because of the presence of a *nodule meristem*. The nodules of tropical legumes, such as soybeans and peanuts, lack a persistent meristem and are spherical (Rolfe and Gresshoff 1988).

The Nitrogenase Enzyme Complex Fixes N_2

Biological nitrogen fixation, like industrial nitrogen fixation, produces ammonia from molecular nitrogen. The overall reaction is

$$N_2 + 8\ e^- + 8\ H^+ + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i \qquad (12.9)$$



Note that the reduction of N_2 to 2 NH_3, a six-electron transfer, is coupled to the reduction of two protons to evolve H_2. The **nitrogenase enzyme complex** catalyzes this reaction.

The nitrogenase enzyme complex can be separated into two components—the Fe protein and the MoFe protein—neither of which has catalytic activity by itself (Figure 12.12):

- The Fe protein is the smaller of the two components and has two identical subunits of 30 to 72 kDa each, depending on the organism. Each subunit contains an iron–sulfur cluster (4 Fe and 4 S^{2-}) that participates in the redox reactions involved in the conversion of N_2 to NH_3. The Fe protein is irreversibly inactivated by O_2 with typical half-decay times of 30 to 45 seconds (Dixon and Wheeler 1986).
- The MoFe protein has four subunits, with a total molecular mass of 180 to 235 kDa, depending on the species. Each subunit has two Mo–Fe–S clusters. The MoFe protein is also inactivated by oxygen, with a half-decay time in air of 10 minutes.

In the overall nitrogen reduction reaction (see Figure 12.12), ferredoxin serves as an electron donor to the Fe protein, which in turn hydrolyzes ATP and reduces the MoFe protein. The MoFe protein then can reduce numerous substrates (Table 12.4), although under natural conditions it reacts only with N_2 and H^+. One of the reactions catalyzed by nitrogenase, the reduction of acetylene to ethylene, is used in estimating nitrogenase activity (see **Web Topic 12.2**).

The energetics of nitrogen fixation is complex. The production of NH_3 from N_2 and H_2 is an exergonic reaction

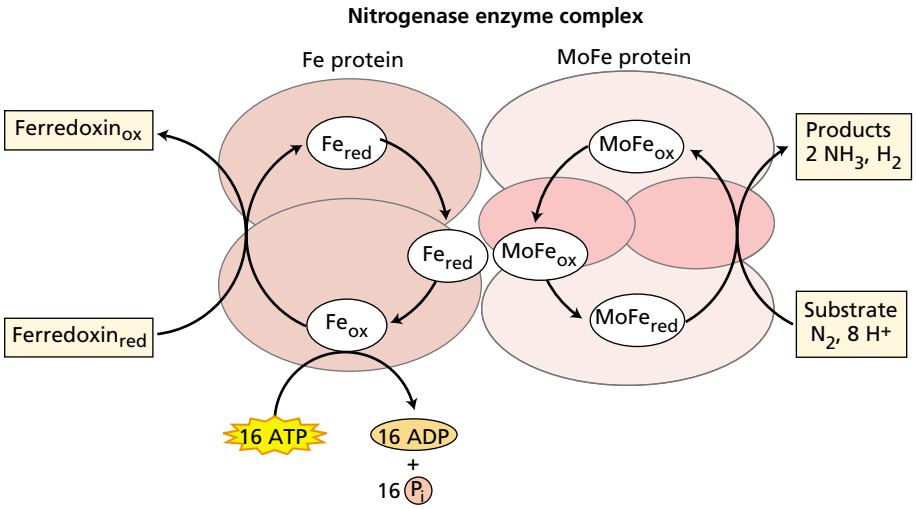


FIGURE 12.12 The reaction catalyzed by nitrogenase. Ferredoxin reduces the Fe protein. Binding and hydrolysis of ATP to the Fe protein is thought to cause a conformational change of the Fe protein that facilitates the redox reactions. The Fe protein reduces the MoFe protein, and the MoFe protein reduces the N_2. (After Dixon and Wheeler 1986, and Buchanan et al. 2000.)

(see Chapter 2 on the website for a discussion of exergonic reactions), with a $\Delta G^{0\prime}$ (change in free energy) of –27 kJ mol^{-1}. However, industrial production of NH_3 from N_2 and H_2 is *endergonic*, requiring a very large energy input because of the activation energy needed to break the triple bond in N_2. For the same reason, the enzymatic reduction of N_2 by nitrogenase also requires a large investment of energy (see Equation 12.9), although the exact changes in free energy are not yet known.

Calculations based on the carbohydrate metabolism of legumes show that a plant consumes 12 g of organic carbon per gram of N_2 fixed (Heytler et al. 1984). On the basis of Equation 12.9, the $\Delta G^{0\prime}$ for the overall reaction of biological nitrogen fixation is about –200 kJ mol^{-1}. Because the overall reaction is highly exergonic, ammonium production is limited by the slow operation (number of N_2 molecules reduced per unit time) of the nitrogenase complex (Ludwig and de Vries 1986).

Under natural conditions, substantial amounts of H^+ are reduced to H_2 gas, and this process can compete with N_2 reduction for electrons from nitrogenase. In rhizobia, 30 to 60% of the energy supplied to nitrogenase may be lost as H_2, diminishing the efficiency of nitrogen fixation. Some rhizobia, however, contain hydrogenase, an enzyme that can split the H_2 formed and generate electrons for N_2 reduction, thus improving the efficiency of nitrogen fixation (Marschner 1995).

TABLE 12.4
Reactions catalyzed by nitrogenase

$N_2 \rightarrow NH_3$	Molecular nitrogen fixation
$N_2O \rightarrow N_2 + H_2O$	Nitrous oxide reduction
$N_3^- \rightarrow N_2 + NH_3$	Azide reduction
$C_2H_2 \rightarrow C_2H_4$	Acetylene reduction
$2\ H^+ \rightarrow H_2$	H_2 production
$ATP \rightarrow ADP + P_i$	ATP hydrolytic activity

Source: After Burris 1976.

Amides and Ureides Are the Transported Forms of Nitrogen

The symbiotic nitrogen-fixing prokaryotes release ammonia that, to avoid toxicity, must be rapidly converted into organic forms in the root nodules before being transported to the shoot via the xylem. Nitrogen-fixing legumes can be divided into amide exporters or ureide exporters on the basis of the composition of the xylem sap. Amides (principally the amino acids asparagine or glutamine) are exported by temperate-region legumes, such as pea (*Pisum*), clover (*Trifolium*), broad bean (*Vicia*), and lentil (*Lens*).

Ureides are exported by legumes of tropical origin, such as soybean (*Glycine*), kidney bean (*Phaseolus*), peanut (*Arachis*), and southern pea (*Vigna*). The three major ureides are allantoin, allantoic acid, and citrulline (Figure 12.13). Allantoin is synthesized in peroxisomes from uric acid, and allantoic acid is synthesized from allantoin in the endoplasmic reticulum. The site of citrulline synthesis from the amino acid ornithine has not yet been determined. All three compounds are ultimately released into the xylem and transported to the shoot, where they are rapidly catabolized to ammonium. This ammonium enters the assimilation pathway described earlier.

SULFUR ASSIMILATION

Sulfur is among the most versatile elements in living organisms (Hell 1997). Disulfide bridges in proteins play structural and regulatory roles (see Chapter 8). Sulfur participates in electron transport through iron–sulfur clusters (see Chapters 7 and 11). The catalytic sites for several enzymes and coenzymes, such as urease and coenzyme A, contain sulfur. Secondary metabolites (compounds that are not involved in primary pathways of growth and develop-

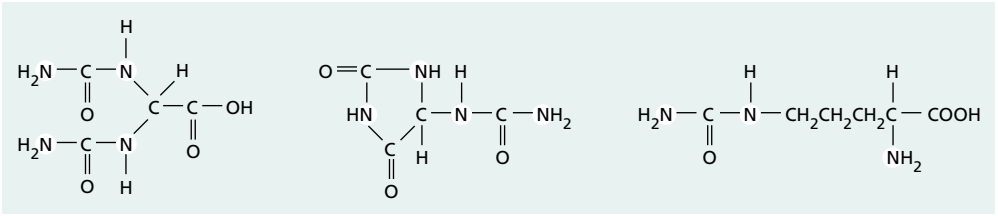


FIGURE 12.13 The major ureide compounds used to transport nitrogen from sites of fixation to sites where their deamination will provide nitrogen for amino acid and nucleoside synthesis.

ment) that contain sulfur range from the rhizobial Nod factors discussed in the previous section to antiseptic alliin in garlic and anticarcinogen sulforaphane in broccoli.

The versatility of sulfur derives in part from the property that it shares with nitrogen: *multiple stable oxidation states*. In this section we discuss the enzymatic steps that mediate sulfur assimilation, and the biochemical reactions that catalyze the reduction of sulfate into the two sulfur-containing amino acids, cysteine and methionine.

Sulfate Is the Absorbed Form of Sulfur in Plants

Most of the sulfur in higher-plant cells derives from sulfate (SO_4^{2-}) absorbed via an H^+–SO_4^{2-} symporter (see Chapter 6) from the soil solution. Sulfate in the soil comes predominantly from the weathering of parent rock material. Industrialization, however, adds an additional source of sulfate: atmospheric pollution. The burning of fossil fuels releases several gaseous forms of sulfur, including sulfur dioxide (SO_2) and hydrogen sulfide (H_2S), which find their way to the soil in rain.

When dissolved in water, SO_2 is hydrolyzed to become sulfuric acid (H_2SO_4), a strong acid, which is the major source of acid rain. Plants can also metabolize sulfur dioxide taken up in the gaseous form through their stomata. Nonetheless, prolonged exposure (more than 8 hours) to high atmospheric concentrations (greater than 0.3 ppm) of SO_2 causes extensive tissue damage because of the formation of sulfuric acid.

Sulfate Assimilation Requires the Reduction of Sulfate to Cysteine

The first step in the synthesis of sulfur-containing organic compounds is the reduction of sulfate to the amino acid cysteine (Figure 12.14). Sulfate is very stable and thus needs to be activated before any subsequent reactions may proceed. Activation begins with the reaction between sulfate and ATP to form 5′-adenylylsulfate (which is sometimes referred to as adenosine-5′-phosphosulfate and thus is abbreviated APS) and pyrophosphate (PP_i) (see Figure 12.14):

$$SO_4^{2-} + \text{Mg-ATP} \rightarrow \text{APS} + PP_i \qquad (12.10)$$

The enzyme that catalyzes this reaction, ATP sulfurylase, has two forms: The major one is found in plastids, and a minor one is found in the cytoplasm (Leustek et al. 2000). The activation reaction is energetically unfavorable. To drive this reaction forward, the products APS and PP_i must be converted immediately to other compounds. PP_i is hydrolyzed to inorganic phosphate (P_i) by inorganic pyrophosphatase according to the following reaction:

$$PP_i + H_2O \rightarrow 2\ P_i \qquad (12.11)$$

The other product, APS, is rapidly reduced or sulfated. Reduction is the dominant pathway (Leustek et al. 2000).

The reduction of APS is a multistep process that occurs exclusively in the plastids. First, APS reductase transfers two electrons apparently from reduced glutathione (GSH) to produce sulfite (SO_3^{2-}):

$$\text{APS} + 2\ \text{GSH} \rightarrow SO_3^{2-} + 2\ H^+ + \text{GSSG} + \text{AMP} \qquad (12.12)$$

where GSSG stands for oxidized glutathione. (The *SH* in GSH and the *SS* in GSSG stand for S—H and S—S bonds, respectively.)

Second, sulfite reductase transfers six electrons from ferredoxin (Fd_{red}) to produce sulfide (S^{2-}):

$$SO_3^{2-} + 6\ Fd_{red} \rightarrow S^{2-} + 6\ Fd_{ox} \qquad (12.13)$$

The resultant sulfide then reacts with *O*-acetylserine (OAS) to form cysteine and acetate. The *O*-acetylserine that reacts with S^{2-} is formed in a reaction catalyzed by serine acetyltransferase:

$$\text{Serine} + \text{acetyl-CoA} \rightarrow \text{OAS} + \text{CoA} \qquad (12.14)$$

The reaction that produces cysteine and acetate is catalyzed by OAS thiol-lyase:

$$\text{OAS} + S^{2-} \rightarrow \text{cysteine} + \text{acetate} \qquad (12.15)$$

The sulfation of APS, localized in the cytosol, is the alternative pathway. First, APS kinase catalyzes a reaction of APS with ATP to form 3′-phosphoadenosine-5′-phosphosulfate (PAPS).

$$\text{APS} + \text{ATP} \rightarrow \text{PAPS} + \text{ADP} \qquad (12.16)$$

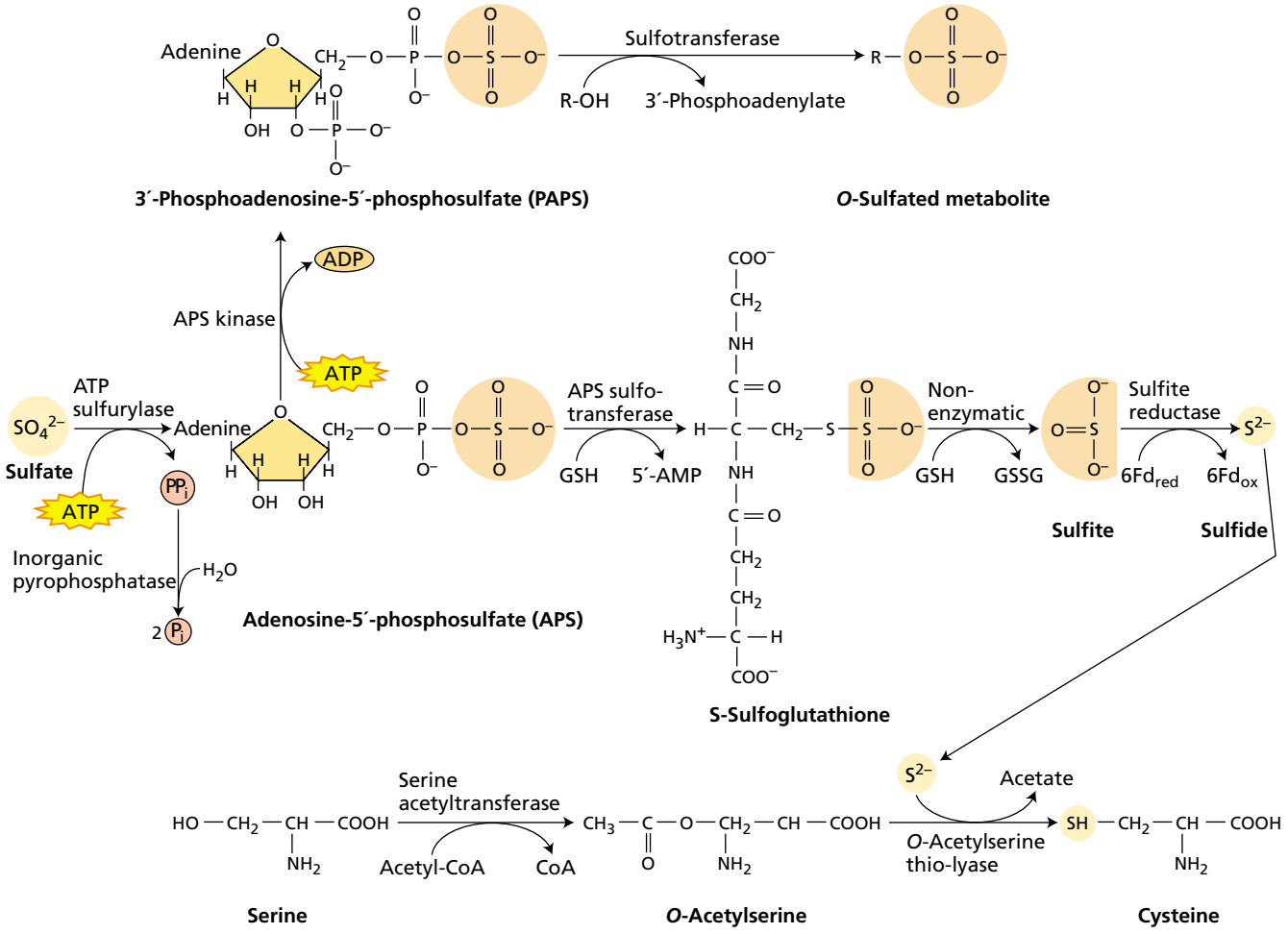


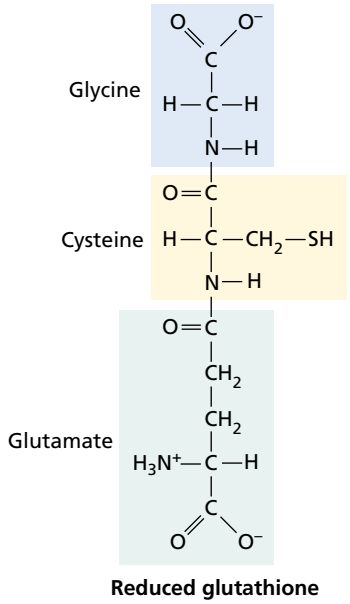
FIGURE 12.14 Structure and pathways of compounds involved in sulfur assimilation. The enzyme ATP sulfurylase cleaves pyrophosphate from ATP and replaces it with sulfate. Sulfide is produced from APS through reactions involving reduction by glutathione and ferredoxin. The sulfide or thiosulfide reacts with *O*-acetylserine to form cysteine. Fd, ferredoxin; GSH, glutathione, reduced; GSSG, glutathione, oxidized.

Sulfotransferases then may transfer the sulfate group from PAPS to various compounds, including choline, brassinosteroids, flavonol, gallic acid glucoside, glucosinolates, peptides, and polysaccharides (Leustek and Saito 1999).

Sulfate Assimilation Occurs Mostly in Leaves

The reduction of sulfate to cysteine changes the oxidation number of sulfur from +6 to –4, thus entailing the transfer of 10 electrons. Glutathione, ferredoxin, NAD(P)H, or *O*-acetylserine may serve as electron donors at various steps of the pathway (see Figure 12.14).

Leaves are generally much more active than roots in sulfur assimilation, presumably because photosynthesis provides reduced ferredoxin and photorespiration generates serine that may stimulate the production of *O*-acetylserine (see Chapter 8). Sulfur assimilated in leaves is exported via the phloem to sites of protein synthesis (shoot and root apices, and fruits) mainly as glutathione (Bergmann and Rennenberg 1993):



Glutathione also acts as a signal that coordinates the absorption of sulfate by the roots and the assimilation of sulfate by the shoot.

Methionine Is Synthesized from Cysteine

Methionine, the other sulfur-containing amino acid found in proteins, is synthesized in plastids from cysteine (see **Web Topic 12.3** for further detail). After cysteine and methionine are synthesized, sulfur can be incorporated into proteins and a variety of other compounds, such as acetyl-CoA and *S*-adenosylmethionine. The latter compound is important in the synthesis of ethylene (see Chapter 22) and in reactions involving the transfer of methyl groups, as in lignin synthesis (see Chapter 13).

PHOSPHATE ASSIMILATION

Phosphate (HPO_4^{2-}) in the soil solution is readily absorbed by plant roots via an H^+–HPO_4^{2-} symporter (see Chapter 6) and incorporated into a variety of organic compounds, including sugar phosphates, phospholipids, and nucleotides. The main entry point of phosphate into assimilatory pathways occurs during the formation of ATP, the energy "currency" of the cell. In the overall reaction for this process, inorganic phosphate is added to the second phosphate group in adenosine diphosphate to form a phosphate ester bond.

In mitochondria, the energy for ATP synthesis derives from the oxidation of NADH by oxidative phosphorylation (see Chapter 11). ATP synthesis is also driven by light-dependent photophosphorylation in the chloroplasts (see Chapter 7). In addition to these reactions in mitochondria and chloroplasts, reactions in the cytosol also assimilate phosphate.

Glycolysis incorporates inorganic phosphate into 1,3-bisphosphoglyceric acid, forming a high-energy acyl phosphate group. This phosphate can be donated to ADP to form ATP in a substrate-level phosphorylation reaction (see Chapter 11). Once incorporated into ATP, the phosphate group may be transferred via many different reactions to form the various phosphorylated compounds found in higher-plant cells.

CATION ASSIMILATION

Cations taken up by plant cells form complexes with organic compounds in which the cation becomes bound to the complex by noncovalent bonds (for a discussion of noncovalent bonds, see Chapter 2 on the web site). Plants assimilate macronutrient cations such as potassium, magnesium, and calcium, as well as micronutrient cations such as copper, iron, manganese, cobalt, sodium, and zinc, in this manner. In this section we will describe coordination bonds and electrostatic bonds, which mediate the assimilation of several cations that plants require as nutrients, and the special requirements for the absorption of iron by roots and subsequent assimilation of iron within plants.

Cations Form Noncovalent Bonds with Carbon Compounds

The noncovalent bonds formed between cations and carbon compounds are of two types: coordination bonds and electrostatic bonds. In the formation of a coordination complex, several oxygen or nitrogen atoms of a carbon compound donate unshared electrons to form a bond with the cation nutrient. As a result, the positive charge on the cation is neutralized.

Coordination bonds typically form between polyvalent cations and carbon molecules—for example, complexes between copper and tartaric acid (Figure 12.15A) or magnesium and chlorophyll *a* (Figure 12.15B). The nutrients that are assimilated as coordination complexes include copper, zinc, iron, and magnesium. Calcium can also form coordination complexes with the polygalacturonic acid of cell walls (Figure 12.15C).

Electrostatic bonds form because of the attraction of a positively charged cation for a negatively charged group such as carboxylate (—COO^-) on a carbon compound. Unlike the situation in coordination bonds, the cation in an electrostatic bond retains its positive charge. Monovalent cations such as potassium (K^+) can form electrostatic bonds with the carboxylic groups of many organic acids (Figure 12.16A). Nonetheless, much of the potassium that is accumulated by plant cells and functions in osmotic regulation and enzyme activation remains in the cytosol and the vacuole as the free ion. Divalent ions such as calcium form electrostatic bonds with pectates (Figure 12.16B) and the carboxylic groups of polygalacturonic acid (see Chapter 15).

In general, cations such as magnesium (Mg^{2+}) and calcium (Ca^{2+}) are assimilated by the formation of both coordination complexes and electrostatic bonds with amino acids, phospholipids, and other negatively charged molecules.

Roots Modify the Rhizosphere to Acquire Iron

Iron is important in iron–sulfur proteins (see Chapter 7) and as a catalyst in enzyme-mediated redox reactions (see Chapter 5), such as those of nitrogen metabolism discussed earlier. Plants obtain iron from the soil, where it is present primarily as ferric iron (Fe^{3+}) in oxides such as $Fe(OH)^{2+}$, $Fe(OH)_3$, and $Fe(OH)_4^-$. At neutral pH, ferric iron is highly insoluble. To absorb sufficient amounts of iron from the soil solution, roots have developed several mechanisms that increase iron solubility and thus its availability. These mechanisms include:

- Soil acidification that increases the solubility of ferric iron.
- Reduction of ferric iron to the more soluble ferrous form (Fe^{2+}).
- Release of compounds that form stable, soluble complexes with iron (Marschner 1995). Recall from Chapter 5 that such compounds are called iron chelators (see Figure 5.2).

Roots generally acidify the soil around them. They extrude protons during the absorption and assimilation of

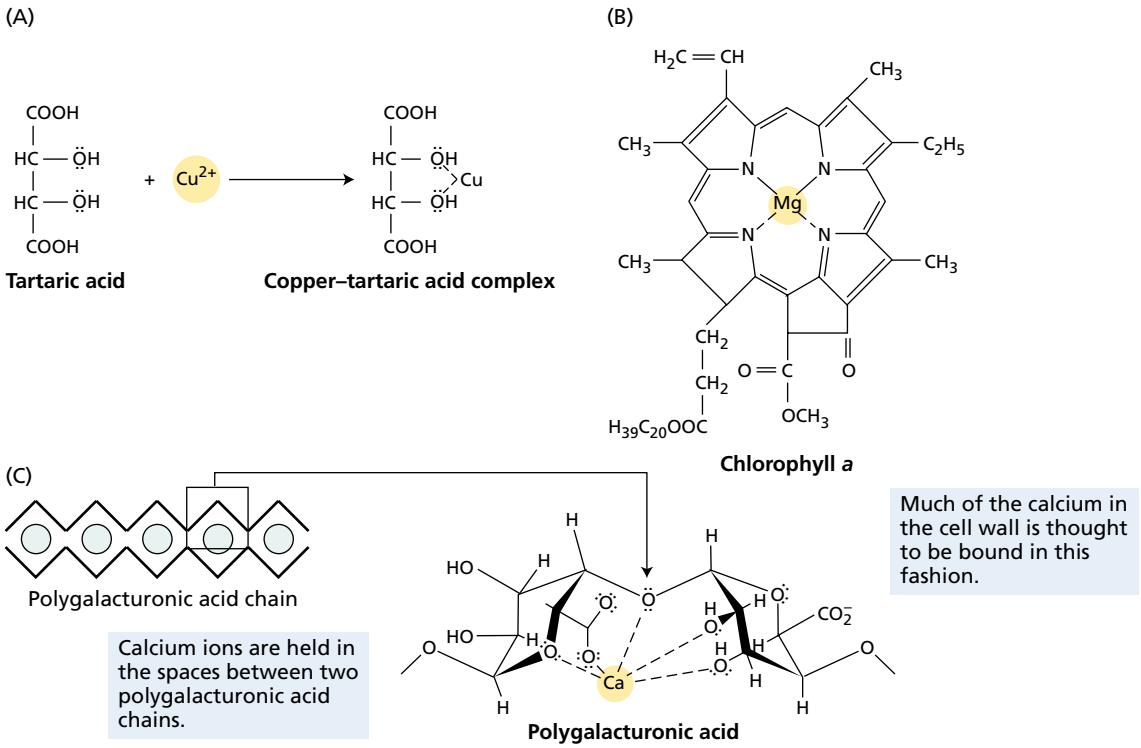


FIGURE 12.15 Examples of coordination complexes. Coordination complexes form when oxygen or nitrogen atoms of a carbon compound donate unshared electron pairs (represented by dots) to form a bond with a cation. (A) Copper ions share electrons with the hydroxyl oxygens of tartaric acid. (B) Magnesium ions share electrons with nitrogen atoms in chlorophyll *a*. Dashed lines represent a coordination bond between unshared electrons from the nitrogen atoms and the magnesium cation. (C) The "egg box" model of the interaction of polygalacturonic acid, a major constituent of pectins in cell walls, and calcium ions. At right is an enlargement of a single calcium ion forming a coordination complex with the hydroxyl oxygens of the galacturonic acid residues. (After Rees 1977.)

cations, particularly ammonium, and release organic acids such as malic acid and citric acid that enhance iron and phosphate availability (see Figure 5.4). Iron deficiencies stimulate the extrusion of protons by roots. In addition, plasma membranes in roots contain an enzyme, called *iron-chelating reductase*, that reduces ferric iron to the ferrous form, with NADH or NADPH serving as the electron donor. The activity of this enzyme increases under iron deprivation.

Several compounds secreted by roots form stable chelates with iron. Examples include malic acid, citric acid, phenolics, and piscidic acid. Grasses produce a special class

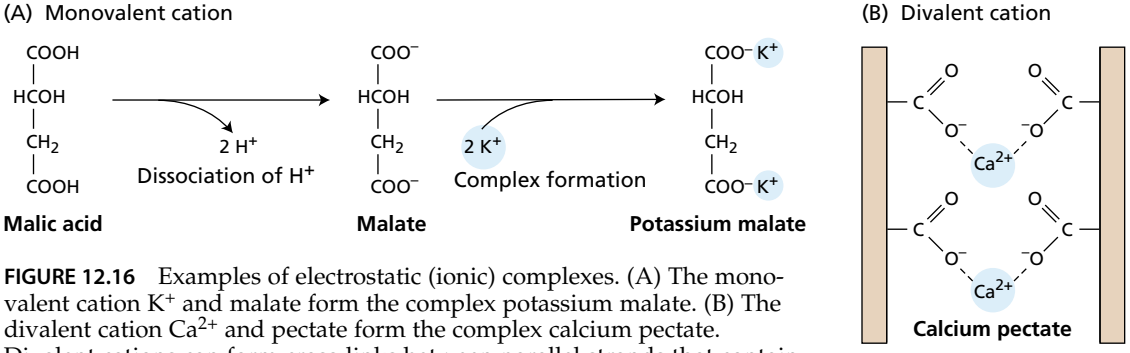


FIGURE 12.16 Examples of electrostatic (ionic) complexes. (A) The monovalent cation K^+ and malate form the complex potassium malate. (B) The divalent cation Ca^{2+} and pectate form the complex calcium pectate. Divalent cations can form cross-links between parallel strands that contain negatively charged carboxyl groups. Calcium cross-links play a structural role in the cell walls.

of iron chelators called *phytosiderophores*. Phytosiderophores are made of amino acids that are not found in proteins, such as mugineic acid, and form highly stable complexes with Fe^{3+}. Root cells of grasses have Fe^{3+}–phytosiderophore transport systems in their plasma membrane that bring the chelate into the cytoplasm. Under iron deficiency, grass roots release more phytosiderophores into the soil and increase the capacity of their Fe^{3+}–phytosiderophore transport system.

Iron Forms Complexes with Carbon and Phosphate

Once the roots absorb iron or an iron chelate, they oxidize it to a ferric form and translocate much of it to the leaves as an electrostatic complex with citrate.

Most of the iron in the plant is found in the heme molecule of cytochromes within the chloroplasts and mitochondria (see Chapter 7). An important assimilatory reaction for iron is its insertion into the porphyrin precursor of heme. This reaction is catalyzed by the enzyme ferrochelatase (Figure 12.17) (Jones 1983). In addition, iron–sulfur proteins of the electron transport chain (see Chapter 7) contain nonheme Fe covalently bound to the sulfur atoms of cysteine residues in the apoprotein. Iron is also found in Fe_2S_2 centers, which contain two irons (each complexed with the sulfur atoms of cysteine residues) and two inorganic sulfides.

Free iron (iron that is not complexed with carbon compounds) may interact with oxygen to form superoxide anions (O_2^-), which can damage membranes by degrading unsaturated lipid components. Plant cells may limit such damage by storing surplus iron in an iron–protein complex called **phytoferritin** (Bienfait and Van der Mark 1983). Phytoferritin consists of a protein shell with 24 identical subunits forming a hollow sphere that has a molecular mass of about 480 kDa. Within this sphere is a core of 5400 to 6200 iron atoms present as a ferric oxide–phosphate complex.

How iron is released from phytoferritin is uncertain, but breakdown of the protein shell appears to be involved. The level of free iron in plant cells regulates the de novo biosynthesis of phytoferritin (Lobreaux et al. 1992).

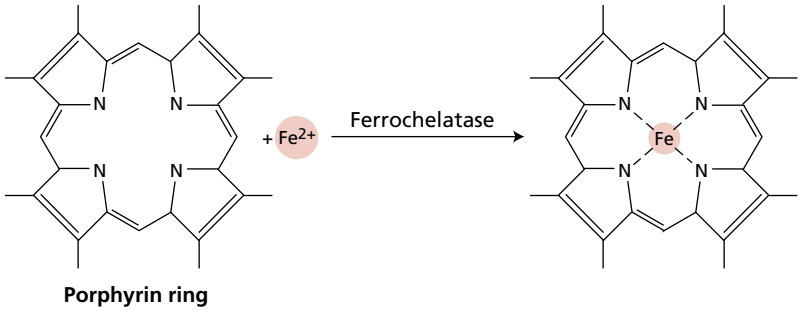


FIGURE 12.17 The ferrochelatase reaction. The enzyme ferrochelatase catalyzes the insertion of iron into the porphyrin ring to form a coordination complex. See Figure 7.37 for illustration of the biosynthesis of the porphyrin ring.

OXYGEN ASSIMILATION

Respiration accounts for the bulk (about 90%) of the oxygen (O_2) assimilated by plant cells (see Chapter 11). Another major pathway for the assimilation of O_2 into organic compounds involves the incorporation of O_2 from water (see reaction 1 in Table 8.1). A small proportion of oxygen can be directly assimilated into organic compounds in the process of *oxygen fixation.*

In oxygen fixation, molecular oxygen is added directly to an organic compound in reactions carried out by enzymes known as *oxygenases*. Recall from Chapter 8 that oxygen is directly incorporated into an organic compound during photorespiration in a reaction that involves the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the enzyme of CO_2 fixation (Ogren 1984). The first stable product that contains oxygen originating from molecular oxygen is 2-phosphoglycolate.

In general, oxygenases are classified as dioxygenases or monooxygenases, according to the number of atoms of oxygen that are transferred to a carbon compound in the catalyzed reaction. In **dioxygenase** reactions, both oxygen atoms are incorporated into one or two carbon compounds (Figure 12.18A and B). Examples of dioxygenases in plant cells are lipoxygenase, which catalyzes the addition of two atoms of oxygen to unsaturated fatty acids (see Figure 12.18A), and prolyl hydroxylase, the enzyme that converts proline to the less common amino acid hydroxyproline (see Figure 12.18B).

Hydroxyproline is an important component of the cell wall protein extensin (see Chapter 15). The synthesis of hydroxyproline from proline differs from the synthesis of all other amino acids in that the reaction occurs after the proline has been incorporated into protein and is therefore a posttranslational modification reaction. Prolyl hydroxylase is localized in the endoplasmic reticulum, suggesting that most proteins containing hydroxyproline are found in the secretory pathway.

Monooxygenases add one of the atoms in molecular oxygen to a carbon compound; the other oxygen atom is converted into water. Monooxygenases are sometimes referred to as *mixed-function oxidases* because of their ability to catalyze simultaneously both the oxygenation reaction and the oxidase reaction (reduction of oxygen to water). The monooxygenase reaction also requires a reduced substrate (NADH or NADPH) as an electron donor, according to the following equation:

$$A + O_2 + BH_2 \rightarrow AO + H_2O + B$$

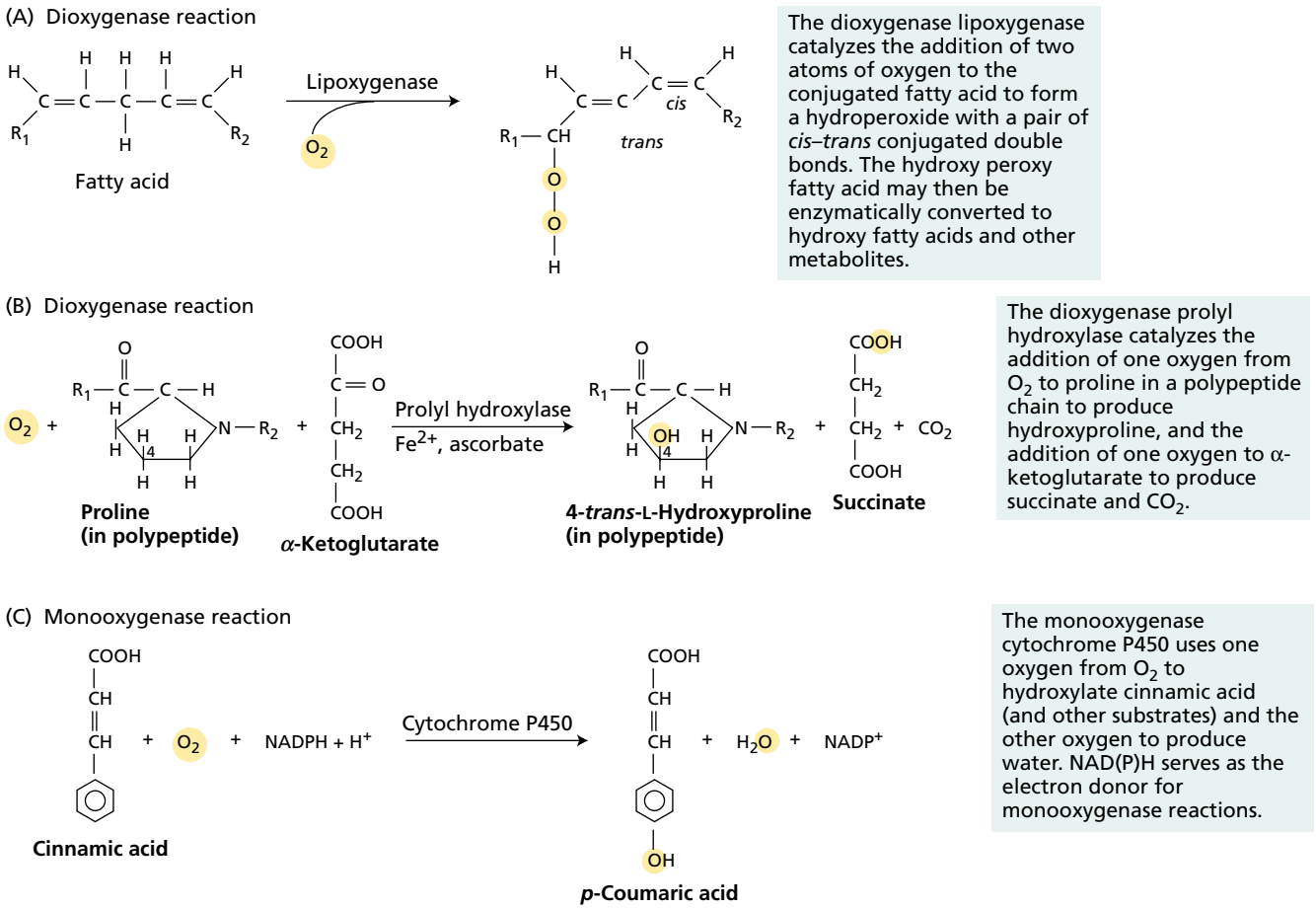


FIGURE 12.18 Examples of the two types of oxygenase reactions in cells of higher plants.

where A represents an organic compound and B represents the electron donor.

An important monooxygenase in plants is the family of heme proteins collectively called cytochrome P450, which catalyzes the hydroxylation of cinnamic acid to *p*-coumaric acid (Figure 12.18C). In monooxygenases, the oxygen is first activated by being combined with the iron atom of the heme group; NADPH serves as the electron donor. The mixed-function oxidase system is localized on the endoplasmic reticulum and is capable of oxidizing a variety of substrates, including mono- and diterpenes and fatty acids.

THE ENERGETICS OF NUTRIENT ASSIMILATION

Nutrient assimilation generally requires large amounts of energy to convert stable, low-energy inorganic compounds into high-energy organic compounds. For example, the reduction of nitrate to nitrite and then to ammonium requires the transfer of about ten electrons and accounts for about 25% of the total energy expenditures in both roots and shoots (Bloom 1997). Consequently, a plant may use one-fourth of its energy to assimilate nitrogen, a constituent that accounts for less than 2% of the total dry weight of the plant.

Many of these assimilatory reactions occur in the stroma of the chloroplast, where they have ready access to powerful reducing agents such as NADPH, thioredoxin, and ferredoxin generated during photosynthetic electron transport. This process—coupling nutrient assimilation to photosynthetic electron transport—is called **photoassimilation** (Figure 12.19).

Photoassimilation and the Calvin cycle occur in the same compartment but only when photosynthetic electron transport generates reductant in excess of the needs of the Calvin cycle (e.g., under conditions of high light and low CO_2), does photoassimilation proceed (Robinson 1988). High levels of CO_2 inhibit photoassimilation (Figure 12.20 see **Web Essay 12.1**). As a result, C_4 plants (see Chapter 8) conduct the majority of their photoassimilation in mesophyll cells, where the CO_2 concentrations are lower (Becker et al. 1993).

The mechanisms that regulate the partitioning of reductant between the Calvin cycle and photoassimilation warrant investigation because atmospheric levels of CO_2 are

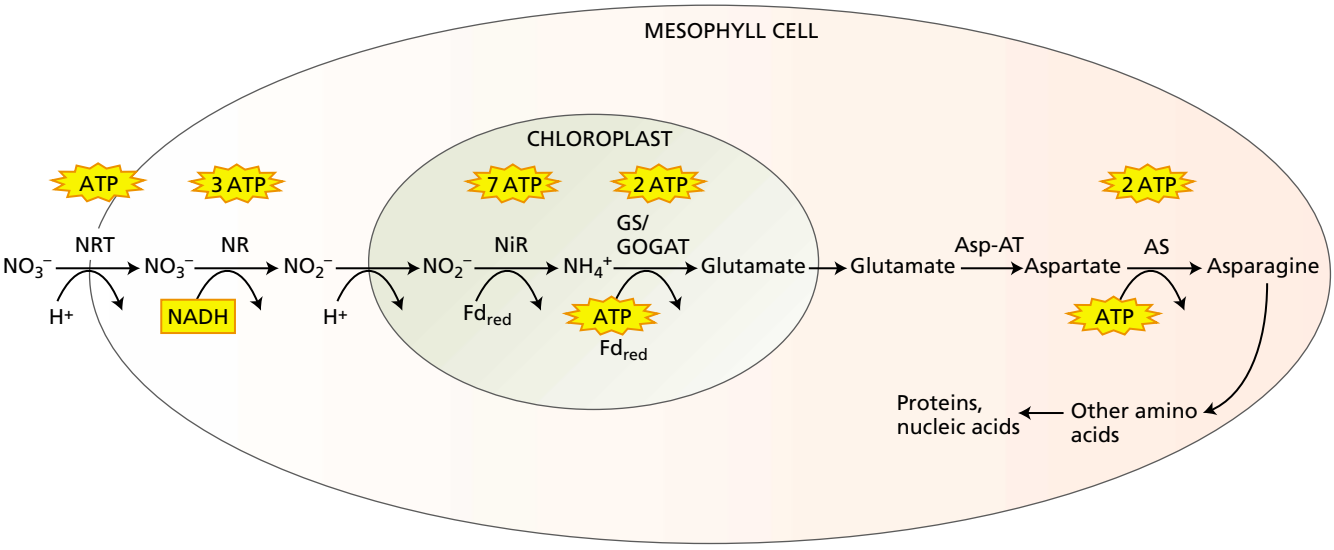


FIGURE 12.19 Summary of the processes involved in the assimilation of mineral nitrogen in the leaf. Nitrate translocated from the roots through the xylem is absorbed by a mesophyll cell via one of the nitrate–proton symporters (NRT) into the cytoplasm. There it is reduced to nitrite via nitrate reductase (NR). Nitrite is translocated into the stroma of the chloroplast along with a proton. In the stroma, nitrite is reduced to ammonium via nitrite reductase (NiR) and this ammonium is converted into glutamate via the sequential action of glutamine synthetase (GS) and glutamate synthase (GOGAT). Once again in the cytoplasm, the glutamate is transaminated to aspartate via aspartate aminotransferase (Asp-AT). Finally, asparagine synthetase (AS) converts aspartate into asparagine. The approximate amounts of ATP equivalents are given above each reaction.

expected to double during the next century (see Chapter 9), so this phenomenon may affect plant–nutrient relations.

SUMMARY

Nutrient assimilation is the process by which nutrients acquired by plants are incorporated into the carbon constituents necessary for growth and development. These processes often involve chemical reactions that are highly energy intensive and thus may depend directly on reductant generated through photosynthesis.

For nitrogen, assimilation is but one in a series of steps that constitute the nitrogen cycle. The nitrogen cycle encompasses the various states of nitrogen in the biosphere and their interconversions. The principal sources of nitrogen available to plants are nitrate (NO_3^-) and ammonium (NH_4^+).

The nitrate absorbed by roots is assimilated in either roots or shoots, depending on nitrate availability and plant

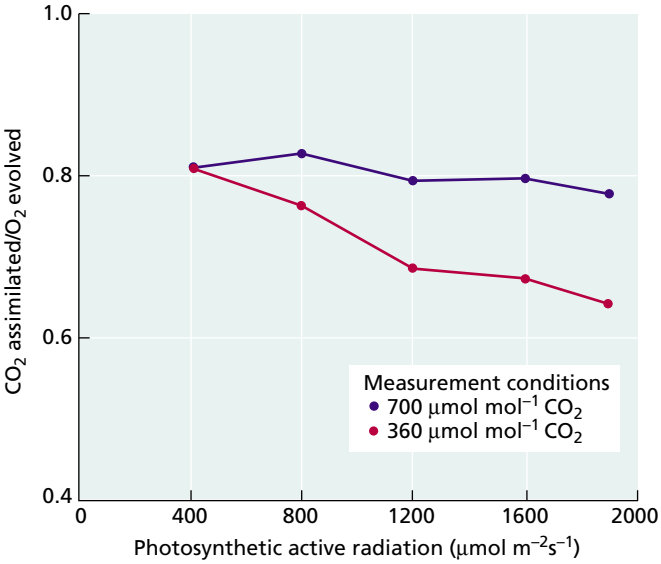


FIGURE 12.20 The assimilatory quotient (AQ = CO_2 assimilated/O_2 evolved) of wheat seedlings as a function of light level (photosynthetic active radiation). Nitrate photoassimilation is directly related to assimilatory quotient because transfer of electrons to nitrate and nitrite during photoassimilation increases O_2 evolution from the light-dependent reactions of photosynthesis, while CO_2 assimilation by the light-independent reactions continues at similar rates. Therefore, plants that are photoassimilating nitrate exhibit a lower AQ. In measurements carried out at ambient, 360 μmol mol^{-1} CO_2 concentrations (red trace), the AQ decreased as a function of incident radiation, indicating that photoassimilation rates increased. At elevated (700 μmol mol^{-1} CO_2, blue trace) the AQ remains constant at all light levels used, indicating that the CO_2-fixing reactions are competing for reductant, and inhibit photoassimilation. (After Bloom et al. 2002.)

species. In nitrate assimilation, nitrate is reduced to nitrite (NO_2^-) in the cytosol via the enzyme nitrate reductase; then nitrite is reduced to ammonium in root plastids or chloroplasts via the enzyme nitrite reductase.

Ammonium, derived either from root absorption or generated through nitrate assimilation or photorespiration, is converted to glutamine and glutamate through the sequential actions of glutamine synthetase and glutamate synthase, which are located in the cytosol and root plastids or chloroplasts.

Once assimilated into glutamine or glutamate, nitrogen may be transferred to many other organic compounds through various reactions, including the transamination reactions. Interconversion between glutamine and asparagine by asparagine synthetase balances carbon metabolism and nitrogen metabolism within a plant.

Many plants form a symbiotic relationship with nitrogen-fixing bacteria that contain an enzyme complex, nitrogenase, that can reduce atmospheric nitrogen to ammonia. Legumes and actinorhizal plants form associations with rhizobia and *Frankia*, respectively. These associations result from a finely tuned interaction between the symbiont and host plant that involves the recognition of specific signals, the induction of a specialized developmental program within the plant, the uptake of the bacteria by the plant, and the development of nodules, unique organs that house the bacteria within plant cells. Some nitrogen-fixing prokaryotic microorganisms do not form symbiotic relationships with higher plants but benefit plants by enriching the nitrogen content of the soil.

Like nitrate, sulfate (SO_4^{2-}) must be reduced by assimilation. In sulfate reduction, an activated form of sulfate called 5′-adenylylsulfate (APS) forms. Sulfide (S^{2-}), the end product of sulfate reduction, does not accumulate in plant cells, but is instead rapidly incorporated into the amino acids cysteine and methionine.

Phosphate (HPO_4^{2-}) is present in a variety of compounds found in plant cells, including sugar phosphates, lipids, nucleic acids, and free nucleotides. The initial product of its assimilation is ATP, which is produced by substrate-level phosphorylations in the cytosol, oxidative phosphorylation in the mitochondria, and photophosphorylation in the chloroplasts.

Whereas the assimilation of nitrogen, sulfur, and phosphorus requires the formation of covalent bonds with carbon compounds, many macro- and micronutrient cations (e.g., K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Co^{2+}, Na^+, Zn^{2+}) simply form complexes. These complexes may be held together by electrostatic bonds or coordination bonds.

Iron assimilation may involve chelation, oxidation–reduction reactions, and the formation of complexes. In order to store large amounts of iron, plant cells synthesize phytoferritin, an iron storage protein. An important function of iron in plant cells is to act as a redox component in the active site f enzymes, often as an iron–porphyrin complex. Iron is inserted into a porphyrin group in the ferrochelatase reaction.

In addition to being utilized in respiration, molecular oxygen can be assimilated in the process of oxygen fixation, the direct addition of oxygen to organic compounds. This process is catalyzed by enzymes known as oxygenases, which are classified as monooxygenases or dioxygenases.

Nutrient assimilation requires large amounts of energy to convert stable, low-energy, inorganic compounds into high-energy organic compounds. A plant may use one-fourth of its energy to assimilate nitrogen. Plants use energy from photosynthesis to assimilate inorganic compounds in a process called photoassimilation.

Web Material

Web Topics

12.1 Development of a root module

Nodule primordia form opposite to the protoxylem poles of the root vascular bundles.

12.2 Measurement of Nitrogen Fixation

Acetylene reduction is used as an indirect measurement of nitrogen reduction.

12.3 The Synthesis of Methionine

Methionine is synthesized in plastids from cysteine.

Web Essay

12.1 Elevated CO_2 and Nitrogen Photoassimilation

In leaves grown under high CO_2 concentrations, CO_2 inhibits nitrogen photoassimilation because it competes for reductant.

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