# Nitrogen Metabolism

The biosynthetic pathways leading to amino acids and nucleotides share a requirement for nitrogen. Biologically useful nitrogen compounds are generally scarce in nature, most organisms maintain economy in their use of ammonia, amino acids and nucleotides. Free amino acids, purines and pyrimidines formed during metabolic turnover of proteins and nucleic acids are often reused.

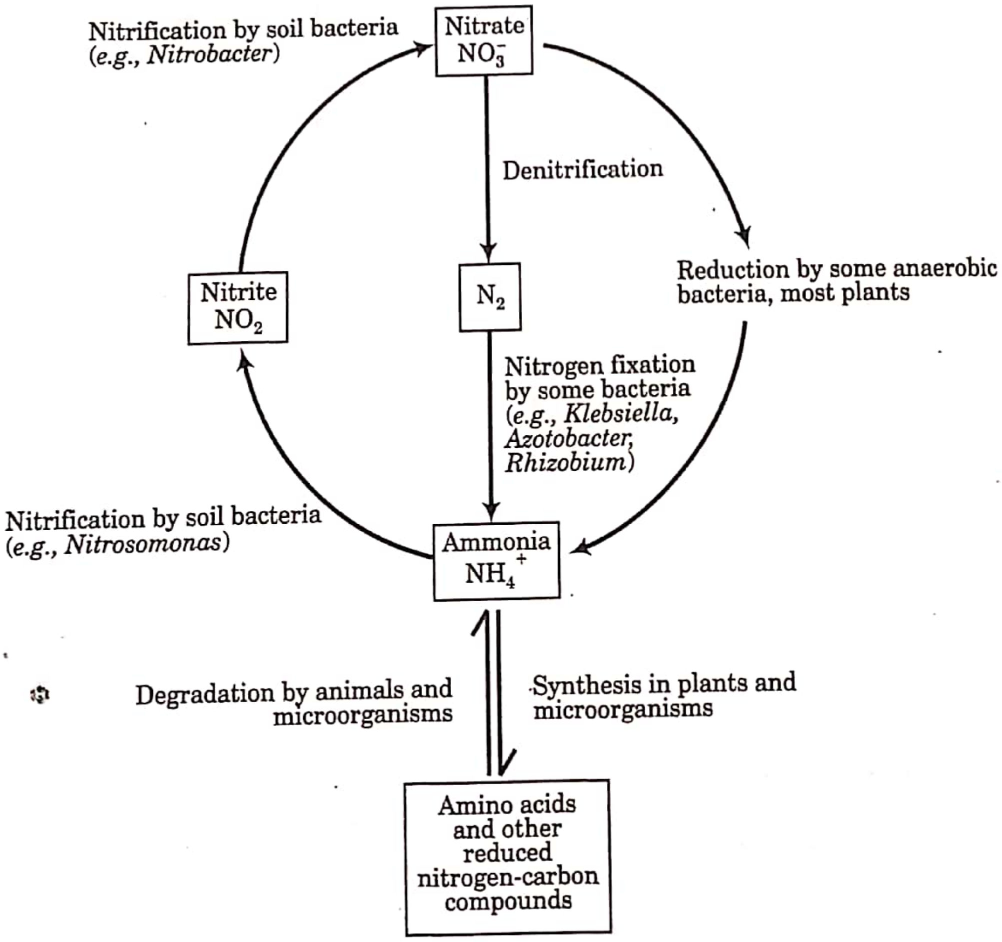


**Fig. 16.53** The nitrogen cycle

## Nitrogenase Enzyme Complex Fixes $N_2$

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 $N_2$.

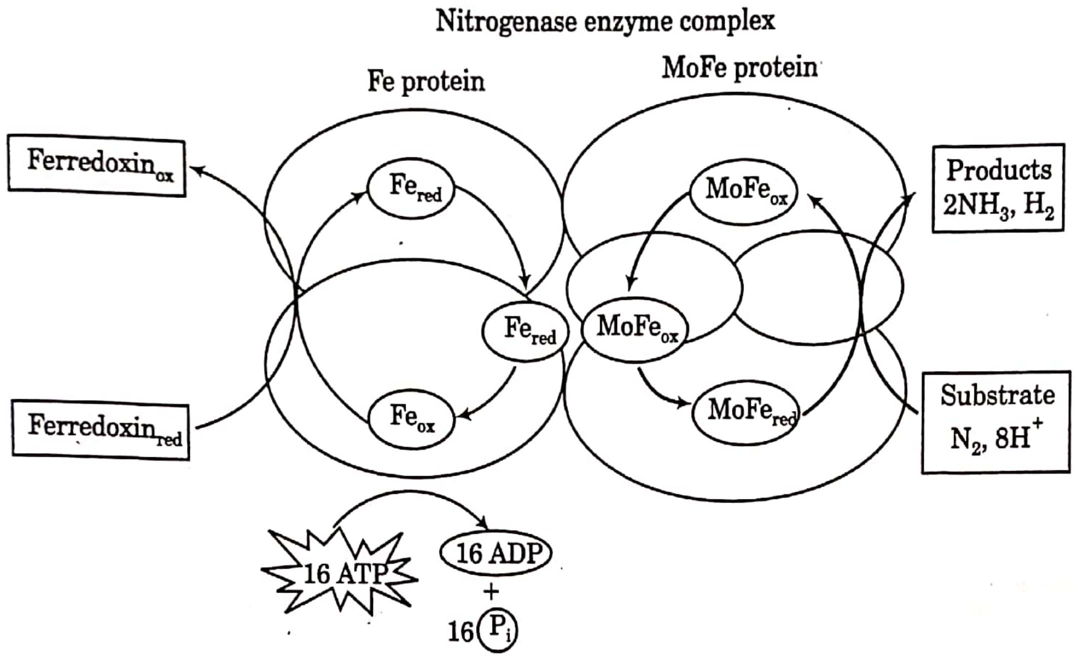


**Fig. 16.54** Nitrogenase enzyme complex

## Nitrate and Ammonium Assimilation

Many mineral nutrients absorbed from the soil by the roots 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 nitrogen and sulphur requires a complex series of biochemical reactions that are among the most energy-requiring reactions in living organisms:

1. 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.
2. 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.

| Free Living Nitrogen Fixing Organisms | |
|---|---|
| Archaebacteria | |
| Methanogens | *Methanococcus volate* |
| Eubacteria | |
| Heterotrophs | |
| Anaerobes | *Clostridium pasteurianum* |
| Facultative anaerobes/ | *Klebsiella pneumoniae* |
| Microaerobes | *Azotobacter vinelandii* |
| Aerobes | *Azospirillum lipoferum* |
| Autotrophs | |
| Chemotrophic bacteria | *Thiobacillus ferriooxidans* |
| Photosynthetic bacteria | *Rhodospirillum rubrum* |
| Cyanobacteria | |
| Unicellular | *Gloeothece* sp. |
| Filamentous | *Oscillatoria* sp. |
| Heterocystous | *Anabaena*, *Nostoc* sp. |

| Symbiotic Nitrogen Fixing Organisms | |
|---|---|
| **Name** | **Host** |
| Rhizobiaceae | Legumes and Parasponia |
| *Azorhizobium* | |
| *Bradyrhizobium* | |
| *Photorhizobium* | |
| *Rhizobium* | |
| *Sinorhizobium* | |
| Actinomycetales | |
| *Frankia* | |
| Cyanobacteria | *Gunnera* (angiosperm) |
| | *Macrozamia* (gymnosperm) |
| | *Azolla* (pteridophyte) |
| | *Blasia* (bryophyte) |
| | *Rhizalenia* (diatom) |
| | *Lichens* |
| | *Siphonochaliana* (sponge) |

| Associations Between Host Plants and Rhizobia | |
|---|---|
| **Plant host** | **Rhizobial symbiont** |
| *Parasponia* (a non-legume, formerly called Trema) | *Bradyrhizobium* sp. |
| Soyabean (*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* var. *phaseoli*; *Rhizobium tropicii*; *Rhizobium etli* |
| Clover (*Trifolium*) | *Rhizobium leguminosarum* var. *trifolii* |
| Pea (*Pisum sativum*) | *Rhizobium leguminosarum* var. *viciae* |
| *Aeschenomene* (aquatic) | *Photorhizobium* (photosynthetically active rhizobia that form stem nodules, probably associated with adventitious roots) |

In contrast to nitrate, high levels of ammonium are toxic to both plants and animals. Ammonium dissipates transmembrane proton gradients that are required for both photosynthetic and respiratory electron transport.

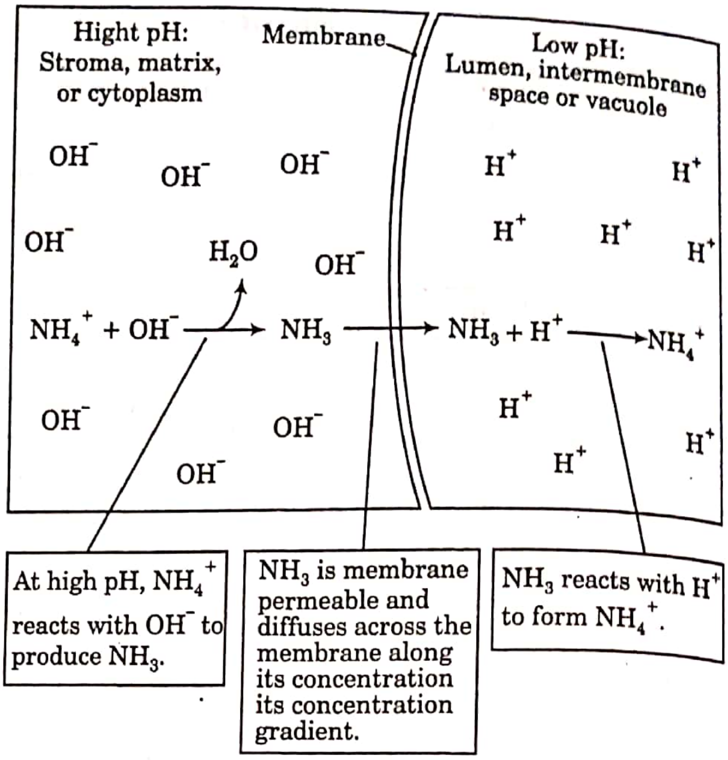


**Fig. 16.55** $NH_4^+$ toxicity can dissipate pH gradients

## Nitrate Reductase

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.

$$NO_3^- + NAD(P)H + H^+ + 2e^- \longrightarrow NO_2^- + NAD(P)^+ + H_2O$$

### Nitrate Reductases

The nitrate reductases of higher plants are composed of two identical subunits, each containing three prosthetic groups: **FAD (Flavin Adenine Dinucleotide)**, **haeme**, and **a molybdenum complexed** to an organic molecule called a **pterin.**

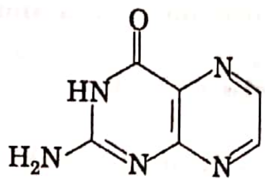


**Fig. 16.56** A pterin (fully oxidized)

Nitrate reductase is the main molybdenum-containing protein in vegetative tissues and one symptom of molybdenum deficiency is the accumulation of nitrate. The three-domain model for nitrate reductase function coordinately. The FAD-binding domain accepts two electrons from NADH or NADPH. The electrons then pass through the haeme domain to the molybdenum complex, where they are transferred to nitrate.

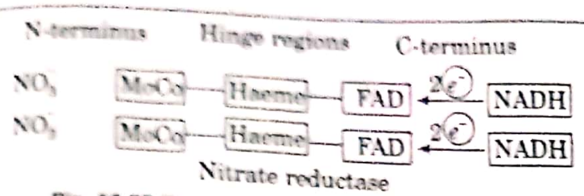


Fig. 16.57 Flow of electrons in nitrate reductase

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. The reverse direction, darkness and $Mg^{2+}$ stimulate a protein kinase that **phosphorylates** the same serine residues, which then interact with a inhibitor protein, and thereby inactivate nitrate reductase. **Regulation of nitrate reductase activity** through phosphorylation and dephosphorylation provides more rapid control than can be achieved through synthesis or degradation.

## Nitrite Reductase Converts Nitrite to Ammonium

Plant cells immediately transport the nitrite generated by nitrate reduction from the cytosol into chloroplasts in leaves and plastids in roots. In these organelles, the enzyme nitrite reductase reduces nitrite to ammonium.

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

Chloroplasts and root plastids contain different forms of the enzyme, but both forms consist of a single polypeptide containing two prosthetic groups: an iron–sulphur cluster ($Fe_4S_4$) and a specialized heme. These groups acting together bind nitrite and reduce it directly to ammonium.

Nitrite reductase is encoded in the nucleus and synthesized in the cytoplasm with an N-terminal transit peptide that targets it to the plastids.

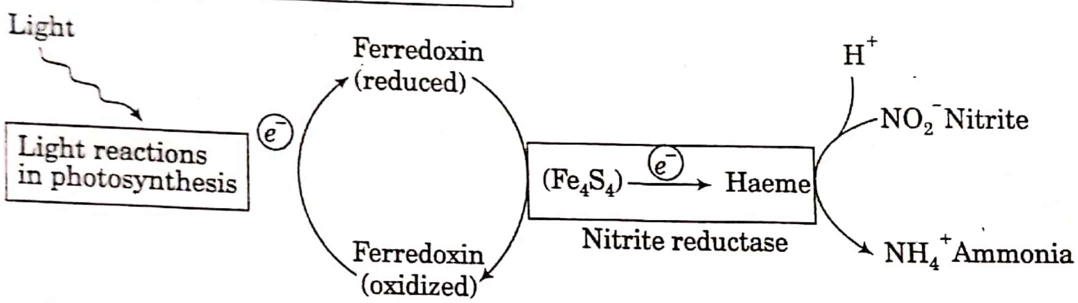


Fig. 16.58 Model for coupling of photosynthetic electron flow, *via* ferredoxin, to the reduction of nitrite by nitrite reductase

## Plants 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. 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. 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 into amino acids.

The primary pathway for conversion of ammonium to amino acids involves the sequential actions of **glutamine synthetase** and **glutamate synthase.**

- **Glutamine synthetase** (GS) combines ammonium with glutamate to form glutamine.

$$\text{Glutamate} + NH_4^+ + \text{ATP} \rightarrow \text{Glutamine} + \text{ADP} + P_i$$

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. Plants contain two types of GOGAT: One accepts electrons from **NADH**; the other accepts electrons from **ferredoxin (Fd)**.

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

$$\text{Glutamine} + \text{2-oxoglutarate} + Fd_{red} \rightarrow 2\ \text{glutamate} + Fd_{ox}$$

The **NADH type of the enzyme** (NADH-GOGAT) is located in plastids of non-photosynthetic tissues such as roots or vascular bundles of developing leaves.

The **ferredoxin-dependent type** of glutamate synthase (Fd- GOGAT) is found in chloroplasts and serves in photorespiratory nitrogen metabolism.

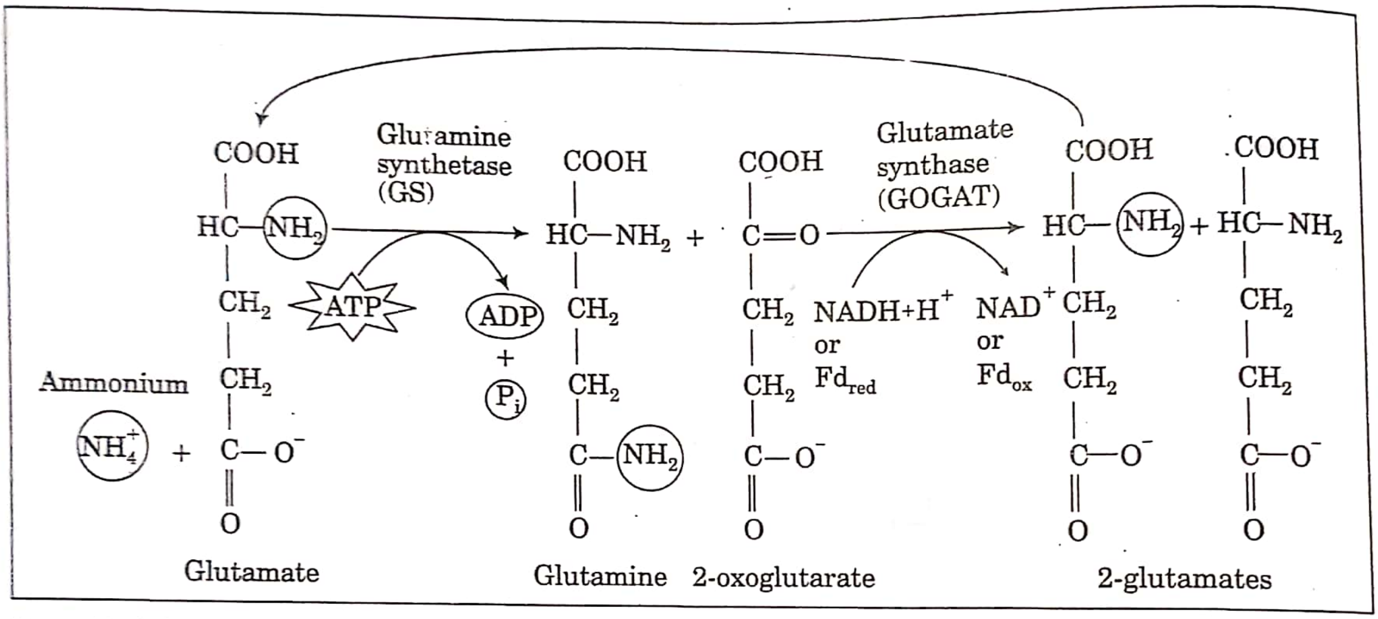


**Fig. 16.59** The GS-GOGAT pathway that forms glutamine and glutamate. A reduced cofactor is required for the reaction: ferredoxin in green leaves and NADH in non-photosynthetic tissue.