The Nitrogenase Enzyme Complex

Biological N_2 fixation is mediated by the enzyme complex nitrogenase. It is most appropriately called the "nitrogenase complex" because it consists of two protein components each composed of multiple subunits. The nomenclature of the nitrogenase complex is as follows (Evans and Burris, 1992):

…s reserve of orga… could be circula… trogen. These org… …ei Winogradsk… atmospheric… biolog-

- The overall complex is known as **nitrogenase.**
- The molybdenum-iron (MoFe) protein is *dinitrogenase* (the "type species" substrate is dinitrogen; enzymes conventionally are named relative to their substrate).
- The iron (Fe) protein is designated *dinitrogenase reductase;* the general consensus is that its function is the reduction of dinitrogenase.

Characteristics of the two proteins are summarized in Table 13–1. A diagrammatic representation of the nitrogenase complex is shown in Figure 13–2. What sets the nitrogenase complex apart is that it:

- consists of two proteins, the MoFe protein (dinitrogenase) and the iron protein (dinitrogenase reductase),
- is destroyed by oxygen,
- contains iron and molybdenum or vanadium,
- needs Mg^{2+} ions to be active,
- converts ATP to ADP when functioning,
- is inhibited by ADP,
- reduces dinitrogen and several other small triply bonded molecules, and
- reduces H^+ to H_2 even when dinitrogen is present.

The overall reaction for biological N_2 fixation using nitrogenase is shown in Figure 13–2. Two MgATP are required for each electron transferred from dinitrogenase reductase to dinitrogenase; thus the reaction shows a requirement of 16 molecules of ATP (112 kcal). Under natural conditions, however, probably 20 to 30

Table 13–1 **Characteristics of dinitrogenase (the MoFe protein) and dinitrogenase reductase (the Fe protein).**

Dinitrogenase
  Mol. wt.: 220,000–270,000
  Has 4 subunits: 2 approximately 50,000 mol. wt.
    2 approximately 59,000 mol. wt.
  2 molybdenum atoms per molecule
  22–24 iron atoms per molecule
  Half-life in air: up to 10 min.

Dinitrogenase reductase
  Mol. wt.: 55,000–66,000
  Has 2 subunits: 27,500–34,000
  No molybdenum
  4 iron atoms per molecule
  4 labile sulfur atoms per molecule
  Half-life in air: 0.5–0.75 sec.

Adapted from Postgate (1987). Used with permission.

- The overall complex is known as **nitrogenase.**
- The molybdenum-iron (MoFe) protein is *dinitrogenase* (the "type species" substrate is dinitrogen; enzymes conventionally are named relative to their substrate).
- The iron (Fe) protein is designated *dinitrogenase reductase;* the general consensus is that its function is the reduction of dinitrogenase.

Characteristics of the two proteins are summarized in Table 13–1. A diagrammatic representation of the nitrogenase complex is shown in Figure 13–2. What sets the nitrogenase complex apart is that it:

- consists of two proteins, the MoFe protein (dinitrogenase) and the iron protein (dinitrogenase reductase),
- is destroyed by oxygen,
- contains iron and molybdenum or vanadium,
- needs Mg^{2+} ions to be active,
- converts ATP to ADP when functioning,
- is inhibited by ADP,
- reduces dinitrogen and several other small triply bonded molecules, and
- reduces H^+ to H_2 even when dinitrogen is present.

The overall reaction for biological N_2 fixation using nitrogenase is shown in Figure 13–2. Two MgATP are required for each electron transferred from dinitrogenase reductase to dinitrogenase; thus the reaction shows a requirement of 16 molecules of ATP (112 kcal). Under natural conditions, however, probably 20 to 30

***Table 13–1* Characteristics of dinitrogenase (the MoFe protein) and dinitrogenase reductase (the Fe protein).**

Dinitrogenase

Mol. wt.: 220,000–270,000
Has 4 subunits: 2 approximately 50,000 mol. wt.
2 approximately 59,000 mol. wt.
2 molybdenum atoms per molecule
22–24 iron atoms per molecule
Half-life in air: up to 10 min.

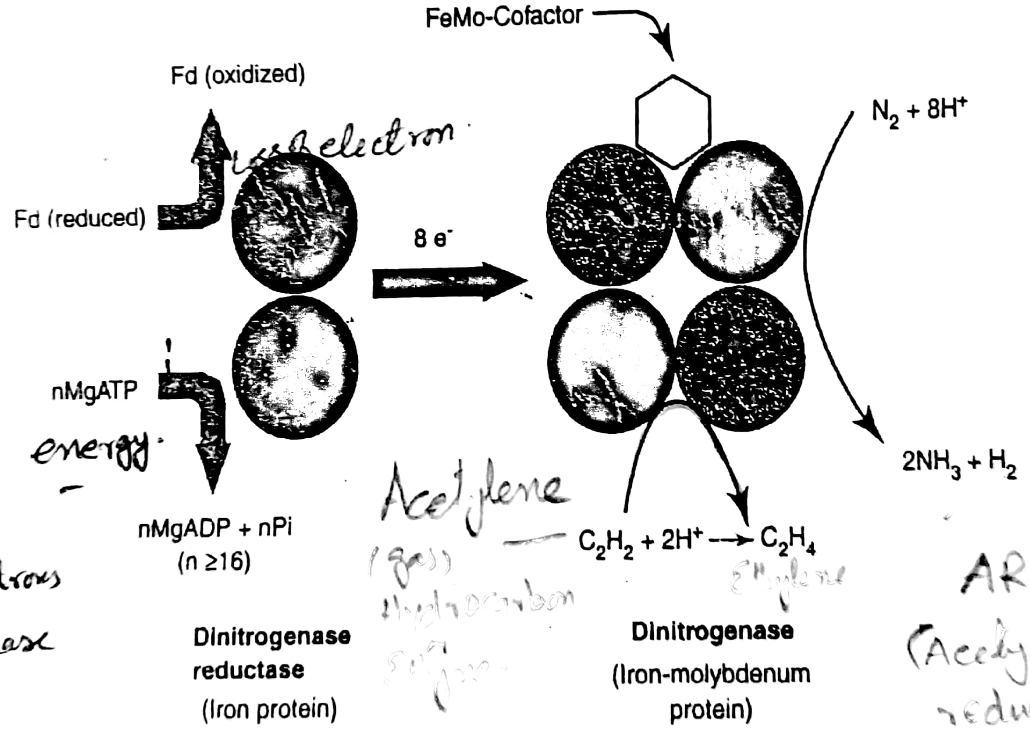
Dinitrogenase reductase

Mol. wt.: 55,000–66,000
Has 2 subunits: 27,500–34,000
No molybdenum
4 iron atoms per molecule
4 labile sulfur atoms per molecule
Half-life in air: 0.5–0.75 sec.

Adapted from Postgate (1987). Used with permission.

MgATP are needed as the process is less efficient than when observed under optimum laboratory conditions (Burris and Roberts, 1993). A consensus on the general model for the mechanism of nitrogenase has evolved over quite a few years. The mechanism can be summarized as follows (Evans and Burris, 1992):

- Dinitrogenase reductase (the Fe protein) accepts electrons from a low-redox donor, such as reduced ferredoxin (Fd_{red}) or flavodoxin, and binds two MgATP.
- It transfers electrons, one at a time, to dinitrogenase (the MoFe protein).
- Dinitrogenase reductase and dinitrogenase form a complex, the electron is transferred, and two MgATP are hydrolyzed to two MgADP + Pi (phosphate). inorganic
- Dinitrogenase reductase and dinitrogenase dissociate, and the process is then repeated.
- ★ When dinitrogenase has collected enough electrons, it binds a molecule of dinitrogen, reduces it, and releases ammonium.
- Dinitrogenase then accepts additional electrons from dinitrogenase reductase to repeat the cycle.



This energy is used to transfer electrons from dinitrogenase reductase to dinitrogenase

Overall reaction:

$$N_2 + 8H^+ + 8e^- + 16MgATP \longrightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

Figure 13–2 The nitrogenase enzyme complex consists of two protein components, dinitrogenase reductase (the Fe protein) and dinitrogenase (the MoFe protein). The dinitrogenase reductase gathers electrons from low-redox carriers such as ferredoxin and flavodoxin and transfers them to dinitrogenase. Nitrogen is bound to the dinitrogenase protein where it is reduced.

3

In each cycle of N_2 fixation, dinitrogenase and dinitrogenase reductase bind together, MgATP is hydrolyzed, and an electron is transferred. The dissociation of the MoFe protein–Fe protein complex is the rate limiting step of the process. In fact, the nitrogenase complex is "remarkably slow—it takes 1.25 sec for a molecule of enzyme to form two of NH_3. The two proteins have to come together and separate 8 times to reduce one N_2 molecule" (Postgate, 1987). A consequence of the slowness of nitrogenase is that N_2-fixing bacteria must synthesize a lot of the protein. Nitrogenase can commonly account for 10% of the cell's proteins, and levels up to 40% have been recorded (Postgate, 1994).

Returning to the reaction in Figure 13–2 of biological N_2 fixation, we observe that for every $8e^-$ transferred via the nitrogenase complex, $2e^-$ are consumed in the formation of H_2. The production of H_2 that accompanies the fixation of N_2 is obligatory. One H_2 (requiring 4 MgATP) is released for each N_2 reduced to $2NH_3$ (requiring 12 MgATP). Thus, 25% of the energy from MgATP is "lost" in the production of H_2. Interestingly, some diazotrophs contain an uptake hydrogenase that allows them to oxidize some of the H_2 and to generate a reduced electron carrier or MgATP. This can then be used in the N_2 fixation reaction, thereby recapturing some of the energy lost.

The physiological requirements for a free-living diazotroph to fix dinitrogen are summarized in Table 13–2. These requirements stem in large part from the unique properties and requirements of the nitrogenase complex, including its exceptional sensitivity to molecular oxygen, the metal content of the complex (iron and molybdenum or vanadium), and the need for adequate supplies of reducing power and MgATP.

Substrates for Nitrogenase

The principal substrate for nitrogenase is dinitrogen (N≡N). Note that the two atoms of nitrogen are joined by a triple bond. In addition to reducing protons to H_2

H^+

Table 13–2 **Physiological needs for N_2 fixation by free-living diazotrophic microbes.**

Trace elements
  Molybdenum or vanadium, iron—for nitrogenase
  Magnesium—for production of MgATP
ATP—a minimum of 16 ATP per N_2 fixed. Probably 20–30 under natural conditions.
  Needed for nitrogenase activity and for nitrogenase synthesis. The high ATP requirement means an abundant supply of energy-yielding substrates must be readily available for vigorous N_2 fixation.
Acceptable temperature
  Most diazotrophs are mesophiles.
  Many will not grow on media at 37°C.
  Nitrogenase activity falls off rapidly at about 40°C.
Oxygen excluded from the enzyme complex
  Nitrogenase is destroyed by O_2.
Source of low-redox reductant
  Restricted to the naturally occurring ferredoxins and flavodoxins.
Reduce hydrogen evolution—up to 35% of the ATP diverted to nitrogenase may be consumed in H_2 evolution.

Obligate anaerobe:- An organism that die in the presence of oxygen.

Heterotroph:- An organism that absorbs organic carbon in order to be able to produce energy to maintain his life.

Chemoautotrophic bacteria:- The bacteria that use inorganic energy sources such as hydrogen sulphide, elemental sulphur, ferrous iron, molecular hydrogen and ammonia. They break the chemical bonds of inorganic substances that does not contain carbon in order to get their energy.

Photoautotroph:- These organisms derive their energy for food synthesis from light and are capable of using CO_2 as their principal source of carbon. e.g photosynthetic bacteria and green plants.

Photoheterotrophs:- photo = light hetero = another troph:- nutrition :- These are the organisms that use light for energy but can not use CO_2 as their sole carbon source. They use compounds from the environment to satisfy their carbon requirements. These compounds include carbohydrates, fatty acids and alcohols. e.g purple non-sulphur bacteria, green non-sulphur bacteria

Anaerobic organism:- Any organism that does not require oxygen for growth.

Microaerophile:- A microorganism that requires lower levels of oxygen than are present in the atmosphere to survive.

~~Factd~~ **Facultative anaerobe**:- An organism that makes ATP by aerobic respiration if oxygen is present but it is capable of switching to fermentation (anaerobic respiration) if oxygen is absent.

Obligate aerobe:- An organism which can not make ATP in the absence of oxygen.