Biochemistry of Nitrogen fixation –Nitrogenase complex

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Types of Nitrogenase complex

- **The biological reduction of di-nitrogen to ammonia is catalysed by nitrogenase enzyme complex are of four types.**
- **The most common form of nitrogenase, referred to as Mo-nitrogenase or conventional nitrogenase, contains a prosthetic group with molybdenum, FeMoCo.**
- **Some bacteria, such as *Azotobacter* and several photosynthetic nitrogen fixers (including some cyanobacteria), carry additional forms of nitrogenase whose cofactor contains vanadium (V-nitrogenase) or only iron (Fe-nitrogenase).**
- **The nitrogenases from all studied systems have very similar properties. These three have nearly similar characteristic features which differs only by the heterometal atom present in the active site metal cluster (Mo, V or Fe).**
- **The fourth class isolated from *Streptomyces thermoautotrophicus* is a superoxide dependent nitrogenase.**
- **The Mo-nitrogenases are the most important and best studied enzyme.**

Structure of Molybdenum containing dinitrogenase complex

- **Consisting of contains two metallocomponents:**
  - **dinitrogenase [molybdenum–iron (MoFe) protein]**
  - **Dinitrogenase reductase (Fe protein)**
- **Dinitrogenase (MoFe protein): is an α2β2 tetramer of the *nifD* and *nifK* gene products and has a molecular weight of approximately 240 kDa (Figure 1).**
  - **The MoFe protein also contains two iron-sulfur clusters, known as P-clusters, located at the interface between the α and β subunits and two FeMo cofactors, within the α subunits.**
  - **The core (Fe_8S_7) of the P-cluster takes the form of two [Fe_4S_3] cubes linked by a central sulfur atom.**
  - **Each FeMo cofactor (Fe_7MoS_9C) consists of two non-identical clusters: [Fe_4S_3] and [$MoFe_3S_3$], which are linked by three sulfide ions. Another constituent of the cofactor is homocitrate, which is linked via oxygen atoms of the hydroxyl group to molybdenum.**
- **Dinitrogenage reductase (Fe-protein) or NifH protein (60-64 kDa): is a γ2 dimer of the *nifH* gene product, and it contains a single 4Fe4S cluster that bridges the two proteinsubunits (Figure 2).**

Structure of Dinitrogenase complex

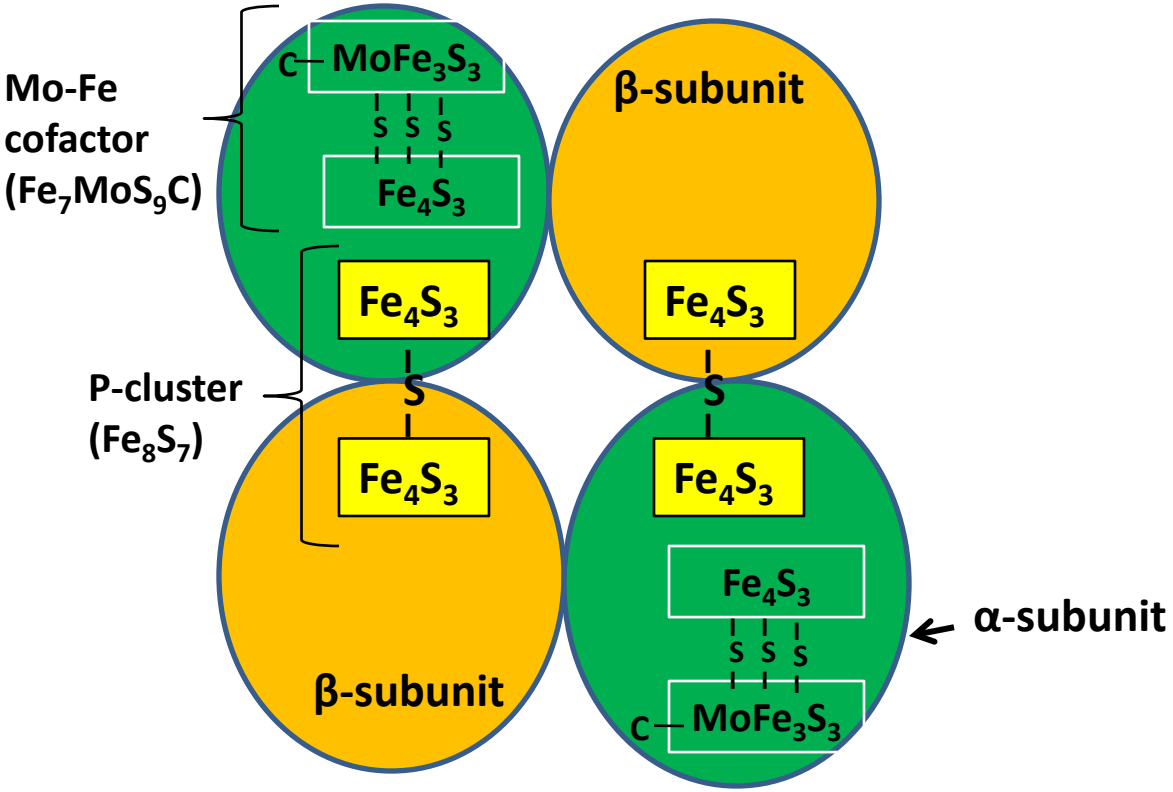


Figure 1. Dinitrogenase
C = homocitrate

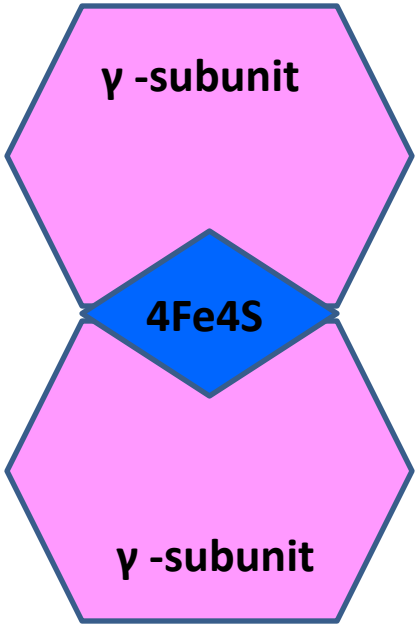


Figure 2. Dinitrogenase reductase

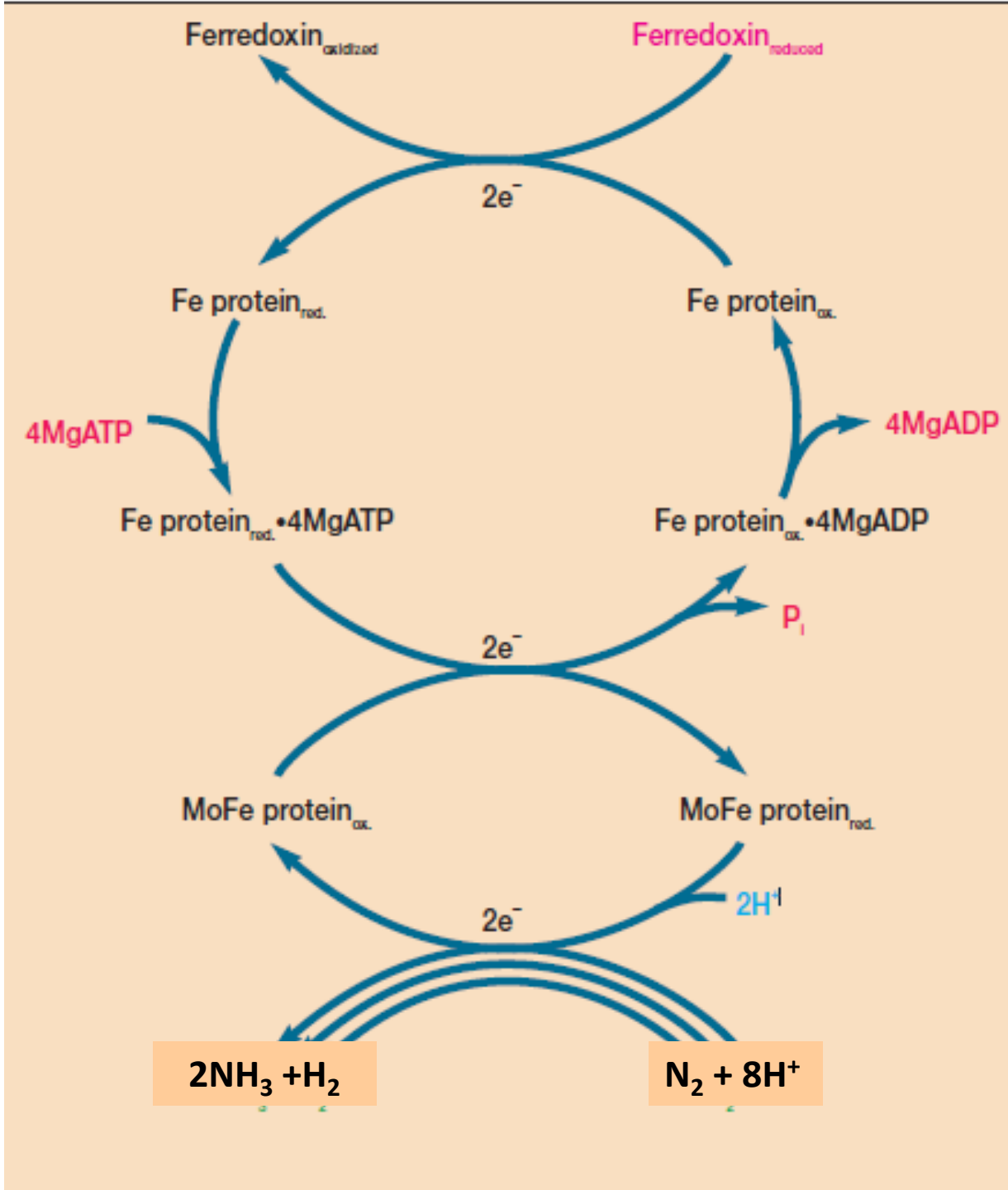
Biochemistry of N_2 Fixation (Figure 3)

- The reduction of molecular nitrogen to ammonia is quite exergonic, but the reaction has a high activation energy because molecular nitrogen is an unreactive gas with a triple bond between the two nitrogen atoms. Therefore nitrogen reduction is expensive and requires a large ATP expenditure. At least 8 electrons and 16 ATP molecules, 4 ATPs per pair of electrons, are required.

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

- The electrons come from ferredoxin that has been reduced in a variety of ways: by photosynthesis in cyanobacteria, respiratory processes in aerobic nitrogen fixers, or fermentations in anaerobic bacteria. For example, *Clostridium pasteurianum* (an anaerobic bacterium) reduces ferredoxin during pyruvate oxidation, whereas the aerobic *Azotobacter* uses electrons from NADPH to reduce ferredoxin.

Figure 3. Mechamism/ Biochemistry of N_2 fixation



- **Fe protein is first reduced by ferredoxin, then it binds ATP.**
- **ATP binding changes the conformation of the Fe protein and lowers its reduction potential, enabling it to reduce the MoFe protein.**
- **ATP is hydrolyzed when this electron transfer occurs.**
- **Electrons from the Fe protein enter the MoFe protein at the P-clusters, which then transfer the electrons to the FeMo cofactors.**
- **Each FeMo cofactor then acts as a site for nitrogen fixation, with N_2 binding in the central cavity of the cofactor.**
- **The reduction of N_2 to NH_3 occurs in three steps, each of which requires an electron pair (Figure4). Six electron transfers take place, and this requires a total 12 ATPs per N_2 reduced. However, nitrogenase also reduces protons to H_2, a reaction which consumes two electrons. Therefore, the total cost of N_2 reduction is 8 electrons transferred and 16 MgATP hydrolysed.**

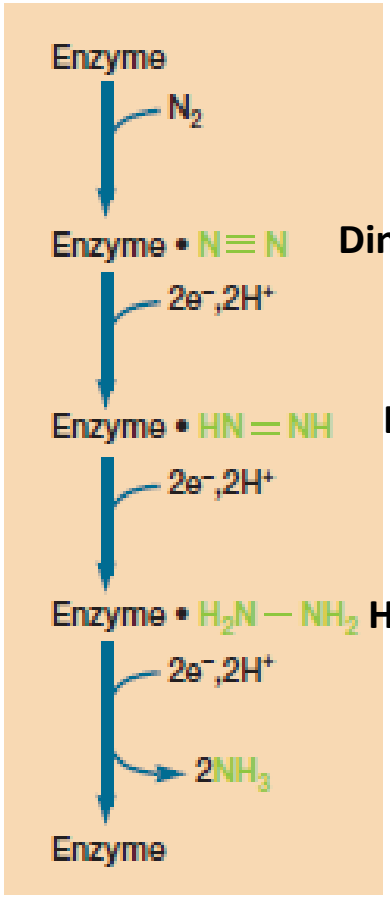
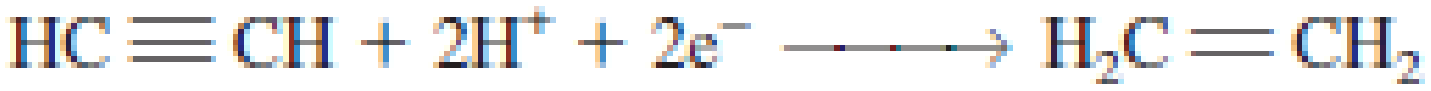


Figure 4.

- Symbiotic nitrogen fixing bacteria can consume almost 20% of the ATP produced by the host plant.
- Nitrogenase can reduce a variety of molecules containing triple bonds (e.g., acetylene, cyanide, and azide).



- The rate of reduction of acetylene to ethylene is even used to estimate nitrogenase activity.

Questions

- Explain in detail the biochemistry of nitrogen fixation.
- Explain the structure of nitrogenase enzyme complex.
- What are the types of nitrogenase enzyme complex. Explain the structure of most common type of nitrogenase enzyme.