Nitrogen Fixation

PG Third Semester

Bioinorganic Chemistry-V

Lecture 11 & 12

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Contents

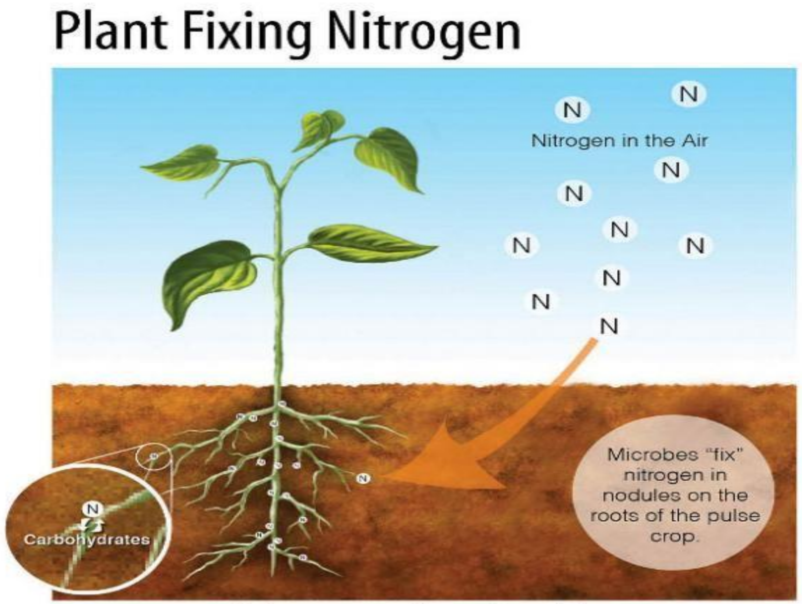
- Nitrogen Fixation
- Structure of nitrogenase
- Its role in di-nitrogen fixation

Books/References used and suggested

- Bioinorganic Chemistry by Bertini, Gray, Lippard and Valentine
- Inorganic Biochemistry by Cowan
- Bioinorganic Chemistry by A. K. Das
- Oxford Chemistry Primer by Fenton

Nitrogen Fixation

- A key reaction of biological system, converts atmospheric dinitrogen (N_2) to other N-containing species (nitrate/nitrite/other essential compounds)
- Fixed nitrogen is essential for the synthesis of amino acids and nucleic acid.
- Natural systems cannot provide adequate amount of fixed nitrogen for agriculture and animal husbandry.
- Industrial processes have been developed to fix nitrogen chemically.



Pulse crop with root nodules

Haber-Bosch Process

- Dinitrogen is almost chemically inert (bond energy 945 $kjmol^{-1}$) and activation energy is prohibitively high (kinetically unfavourable).
- Breaking and reducing N≡N triple bond is a challenging task.
- Requires about 300-500 $^{\circ}$C and 300 atmospheric pressure with Haber's catalyst, usually metallic Fe or its oxide (thermodynamically favourable).

$$N_2 + 3H_2 \rightarrow 2NH_3 \quad \Delta H = -50\ kjmol^{-1}, \quad \Delta G = -16.6\ kjmol^{-1}$$

- At pH=7, E° value is easily accessible to biological reductant such as low potential ferredoxins

$$N_2 + 8e^- + 6H^+ \rightarrow 2NH_4^+ \quad E^{\circ} = -280\ mV$$

Biological nitrogen fixation

- Nitrogen fixation with the help of microorganisms (nitrogen fixing bacteria).
- Two types - symbiotic and non symbiotic
- **Non symbiotic -** Fixation by free living organisms (aerobic, anaerobic and blue green algae)

  Free living aerobic : *Azotobacter vinelandii*

  Free living anaerobic : *Clostridium pasteurianum*

  Facultative aerobes: *Klebsiella pneumoniae*

  Free living photosynthetic: *Rhodobacter capsulatus* - purple,

  Blue green algae: *Anabaena cylindrica* (cyanobacterium)

- **Symbiotic -** Fixation by microorganisms in soil living symbiotically inside the plants.

  Nodule formation in leguminous plants: *Rhizobium* and *Bradyrhizobium*

  Nodule formation in non-leguminous plants: *Frankia*

  Non nodulation : *Anabaena azollae*

Symbiotic Nitrogen Fixation

- Rhizobium (gram negative, aerobic) is present in nodules on the roots of legumes (peas, beans, clover, alfalfa and soya)
- Red colour inside the nodules is due to leghaemoglobin (a plant O_2-binding protein)
- The reaction takes place at 0.8 atmospheric pressure and ambient temperature.
- Intensive efforts have been made to determine the bacterial mechanism.
- The nitrogen fixing bacteria contains an enzyme called nitrogenase.
- Nitrogenase catalyses the reduction of N_2 to NH_3 in a reaction coupled to the hydrolysis of 16 ATP molecules and production of H_2.

$$N_2 + 16MgATP^{2-} + 8e^- + 8H^+ \rightarrow 2NH_3 + 16MgADP^- + 16H_2PO_4^- + H_2$$

- Fe-Mo nitrogenase is mostly studied - reduction of N_2 probably occurs at Mo-site
- Fe-V nitrogenase - V plays (other metal can also) the role of Mo
- Fe-Fe nitrogenase - similar metals can also effect fixation

Biochemistry of nitrogen fixation

Basic requirements

- Nitrogenase and hydrogenase enzyme
- Protective mechanism against Oxygen (leghaemoglobin binds oxygen tightly and protects nitrogenase that cannot operate in presence of oxygen.)
- Ferredoxin
- Hydrogen releasing systems or electron donor (pyruvic acid or glucose/sucrose
- Constant supply of ATP
- Coenzymes and cofactors TPP, CoA, inorganic phosphate and Mg^{2+}

Nitrogenase enzyme

- Active in anaerobic condition
- Consists of two protein subunits

  Non heme Fe-protein : P clusters (dinitrogen reductase, smaller)

  Fe-Mo protein : Di-nitrogenase (larger)
- Fe-protein reacts with ATP and reduces second subunit which ultimately reduces N_2 to NH_3,

$$N_2 + 6e^- + 6H^+ \rightarrow 2NH_3$$

- Larger protein is an $\alpha_2\beta_2$ tetramer with molecular weight 220,000-240,000 D. Contains 2-Mo atoms, almost 30-Fe atoms and about 30-labile (inorganic) sulphur atoms.
- P-cluster has the molecular weight of 57,000-73,000 D with an Fe_4S_4 cluster (dimer of two identical subunits bridged by an Fe_4S_4 cluster). It acts as a redox centre.

Fe-Mo nitrogenase

- The protein free cofactor is soluble and contains Mo and Fe (1 Mo, 7-8 Fe and 4-6 S^{2-} ions)
- Recombination of the cofactor with inactive nitrogenase restores the activity.
- Reaction: $N_2 + 16MgATP^{2-} + 8e^- + 8H^+ \rightarrow 2NH_3 + 16MgADP^- + 16\ PO_4^{3-} + H_2$
- Electrons required for the reaction are transferred to nitrogenase by reduced form of ferredoxins and flavodoxins.
- The source of these electrons is the oxidation of pyruvate (2-oxo-propionate).
- The e^-s first transferred to the Fe-protein, the reduced form of which forms a complex with Mg-ATP and Fe-Mo-protein.
- The reducing e^- is transferred to Fe-Mo-protein and then to N_2 in a series of steps accompanied by H^+ transfer from H_2O to N_2 produces NH_3, Mg-ADP and *i*-PO_4^{3-}.
- Regeneration of proteins.

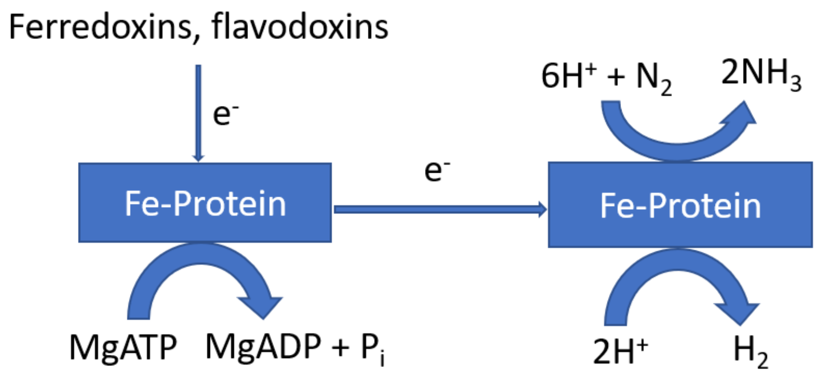
- The energy released from hydrolysis of ATP drives the reaction.

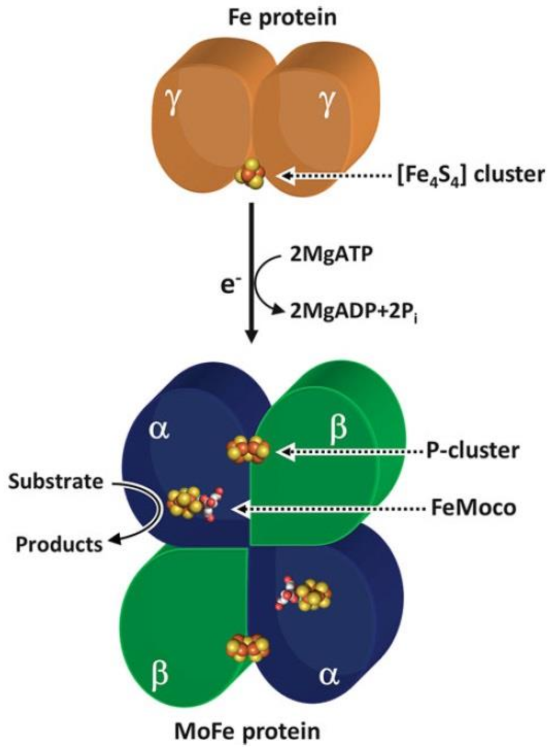
$$ATP \rightarrow ADP + PO_4^{3-} \qquad \Delta G = -30.5\ kjmol^{-1}$$

- Fe-Mo protein contains the site of N_2 binding (Fe-Mo cofactor) where the N_2 is reduced.

- P cluster is believed to assist the reduction of N_2 by transfer of electrons.

$$N_2 + 16MgATP^{2-} + 8e^- + 8H^+ \rightarrow 2NH_3 + 16MgADP^- + 16\ PO_4^{3-} + H_2$$

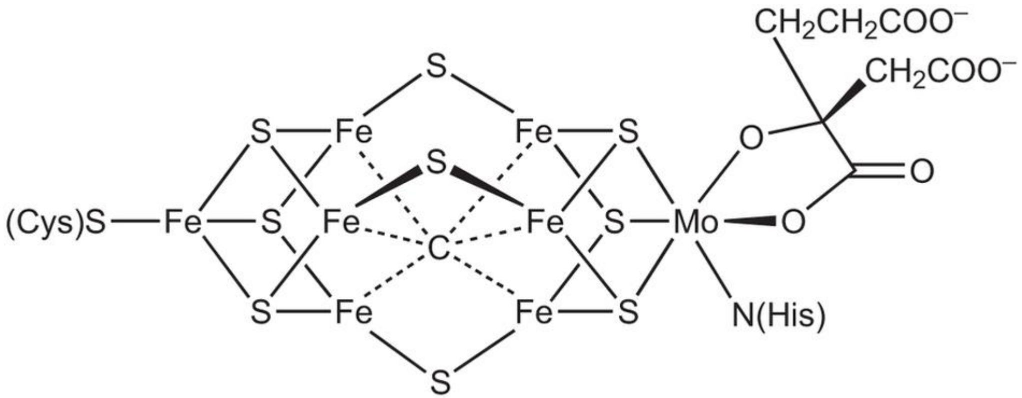




Pathways proposed for nitrogen fixation

Fe-Mo-protein

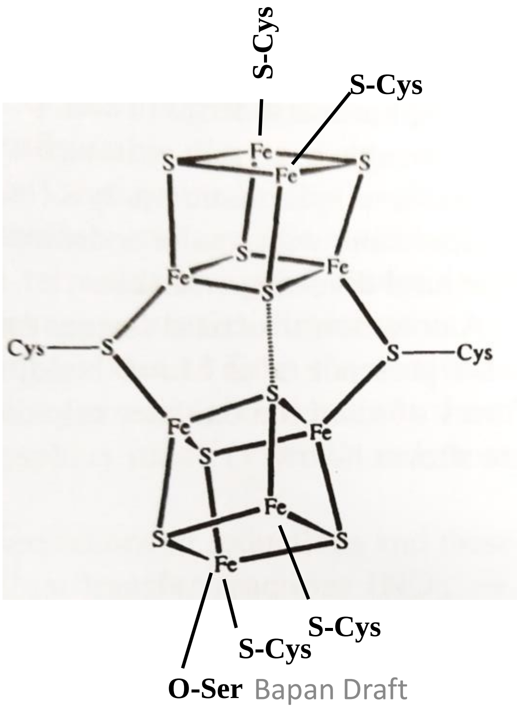
- Two Fe-Mo cofactors are located at about 70 Å apart.
- It is an $\alpha_2\beta_2$ tetramer with molecular weight of 220000-240000 D (2Mo, 30Fe & 30 labile S^{2-})
- Consists of cuboidal [Fe_4S_4] and [Fe_3MoS_3] fragments linked by two sulphide bridges
- A third bridge might be derived from O or N donor.
- The cluster is bound to the protein via a cysteine at Fe and a histidine at the Mo.
- The coordination sphere of the six coordinated Mo (probably in IV OS) is completed by the homocitrate anion.



Fe-Mo cofactor

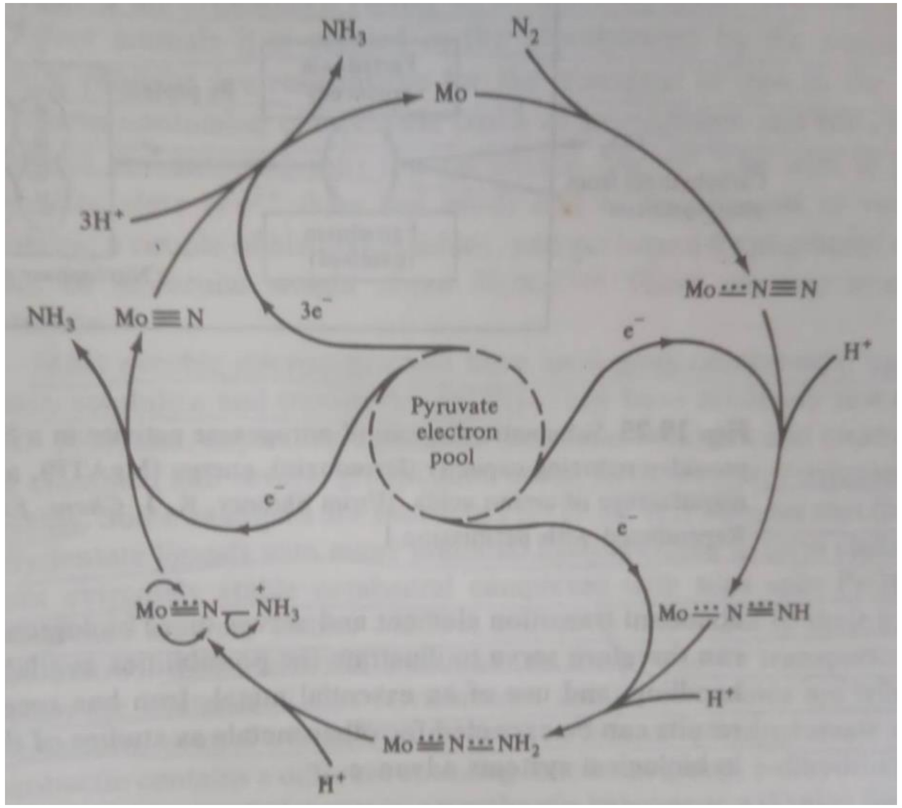
P-cluster

- It is smaller protein with molecular weight 57000-73000 D
- Contains a pair of $[Fe_4S_4]$ units linked by two bridging cysteine thiolate group
- A disulphide bridge formed between S atoms of each $[Fe_4S_4]$ cubane cluster.
- Unusual, EPR studies suggests its oxidized form to be high spin with S=7/2 and Mossbauer spectra reveals inequivalent Fe-population indicating $[Fe_4S_4]$ cluster to be distorted or asymmetric.

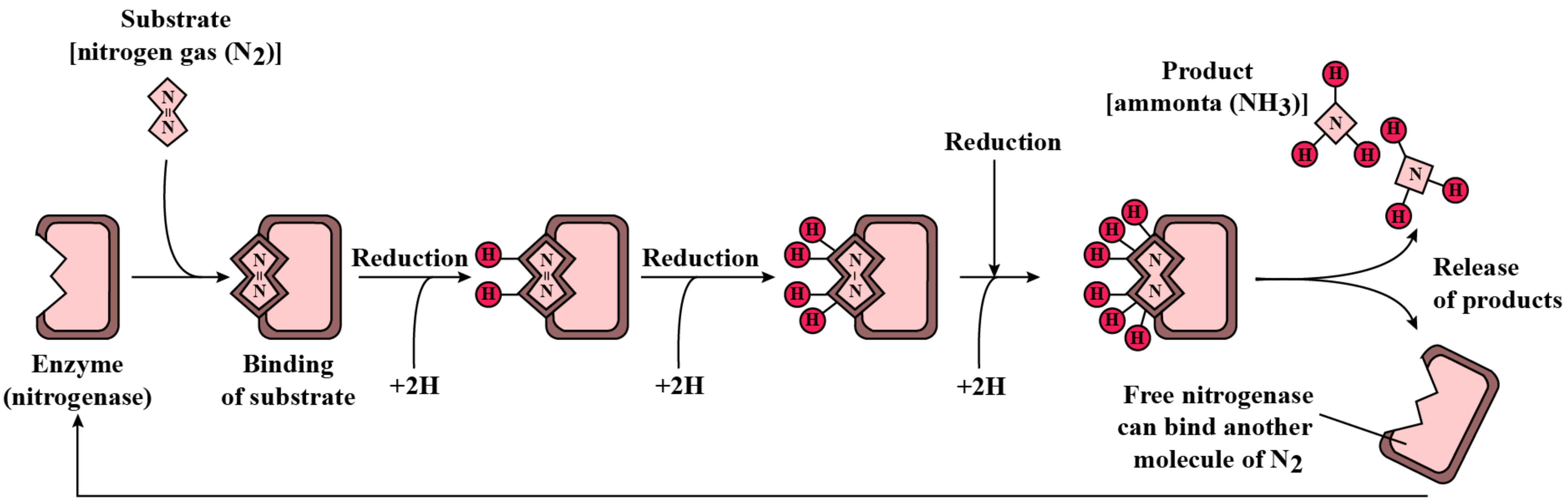


Mechanism of nitrogen fixation

- The active site for dinitrogen binding involves Mo atom.
- The coordination sphere consists of several S-atoms at distances of about 2.35 Å
- The source of reductive capacity is pyruvate, and e^-s are transferred via ferredoxin to nitrogenase
- Two Mo(III) atoms cycling through Mo(VI) would provide six electrons required for the reduction.
- Alternatively, there should be ready flow of electrons, and the Mo may stay in the one or two oxidation states that most readily bind dinitrogen and its intermediate reductants.



Schematic diagram of nitrogenase activity in bacterial cell



Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found found in nitrogen - fixing bacteria