MSC BOTANY

IIISEMESTER

PHYSIOLOGY:NITROGEN METABOLISM

BIOCHEMISTRY OF NITROGEN FIXATION

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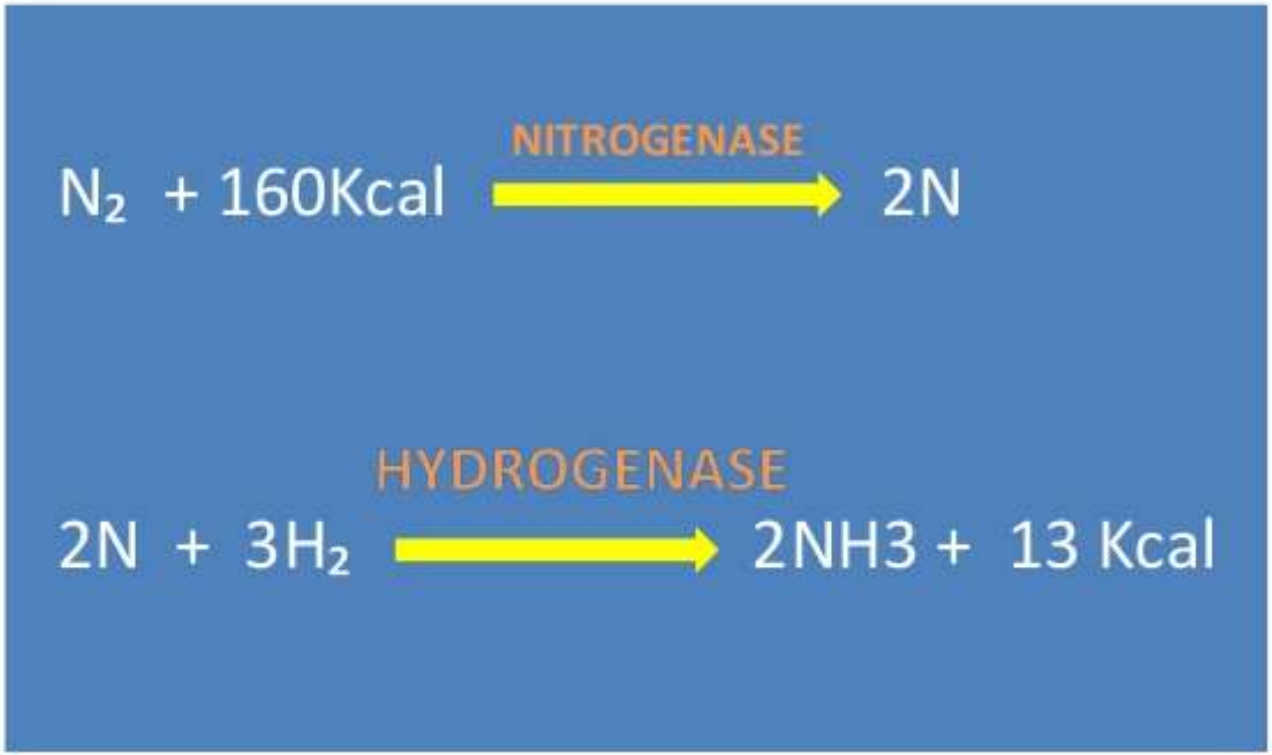
- Dinitrogen is not easily reduced because of the triple bond between nitrogen .
- it is very stable
- Industrial fixation is achieved by high temperature
- Biological fixation is also costly and carried out by ATP

Biochemistry of nitrogen fixation

- N_2 fixers utilize atm. N_2 to synthesize NH3
- In this process , N_2 is first split up into free N_2 atoms by breaking the triple bond , with help of enzyme nitrogenase.
- This reaction is endergonic (energy consuming), it requires an input of nearly 160kcal energy.

- Free nitrogen combines with hydrogen forming NH_3
- This reaction is exergonic (energy releasing)
- Mediated by enzyme hydrogenase & it releases nearly 13 kcal energy.
- BNF requires a net input of 147 kcal energy & an expenditure of nearly 16 mols of ATP per each molecule of nitrogen.


NITROGENASE
N_2 + 160Kcal
2N
HYDROGENASE
2N + $3H_2$
2NH3 + 13 Kcal

The overall reaction of BNF may be represented as follows:

$$N_2 + 8e + 8H^+ + 16\ ATP \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$$

The reduction of N_2 to $2NH_3$ is coupled to the reduction of two protons to evolve H_2. This reaction is catalysed by the nitrogenase enzyme complex.

- Nitrogen is a highly un reactive molecule, which generally requires red-hot Mg for its reduction.
- But under physiological temperature, N2 is made into its reactive form by an enzyme catalyst, nitrogenase.

- The research workers of Central Research Laboratory first isolated the enzyme from the bacteria *C. pasieurianum*.

- They are the bacteria inhabiting the soil; they prefer anerobic environment for their proper growth and development.

- The researchers prepared the extract of these bacteria and searched for the N2 reducing property of the extract.
- The extract converts N2 into NH3.
- The researchers also used radio active labelled N15 in its molecule.

- Since then, Dilworth & Schollhorn et al (1966) have discovered that the enzyme nitrogenase reduces not only the N2 into NH3 but also acetylene into ethylene.

- The ethylene is measured by using gas chromatographic methods.

- Only prokaryote cells are able to fix dinitrogen because only they have the gene coding for the enzyme nitrogenase or dinitrogenase

- This enzyme nitrogenase has been found only in prokaryotes

- **Nitrogenase enzyme structure**
- Nitrogenase enzyme is a multimeric protein complex made up of two proteins of different size
- The larger protein, MoFe protein (component I)
- The smaller protein ,Fe protein (component II) .

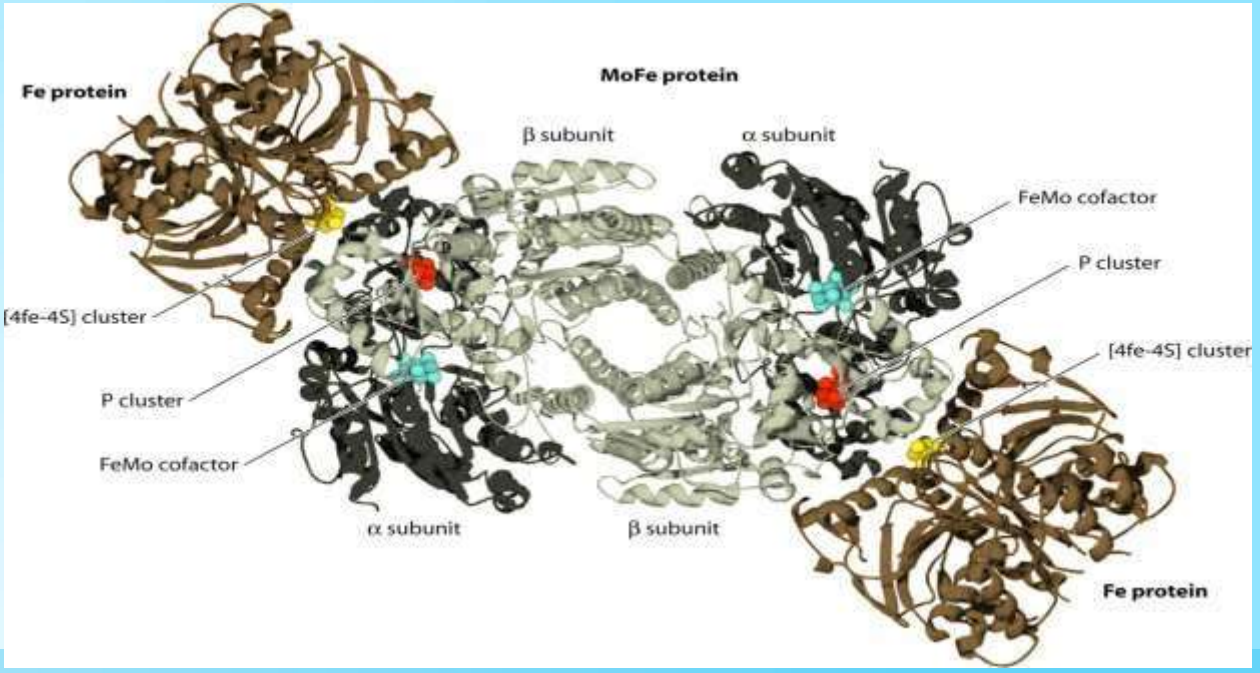
Structure of Nitrogenase Complex

- The absence of any one of these protein units from the nitrogenase causes the failure of N2 reduction.
- Of the two sub-units one is larger and the other is smaller.
- The larger sub-unit is called Mo-Fe protein and the smaller sub-unit is called ferrus protein.

- The MoFe protein has four subunits, with a total molecular mass of 180 to 235 kDa, depending on the species.
- This subunit has the dinitrogenase activity. , so also called dinitrogenase
- It consists of iron, sulphur and additional 2 molybdenum, so called MoFe complex
- It is a tetramer

- Each MoFe protein consist of 2 molybdenum atoms in form of an iron-molybdenum – sulphur cofactor

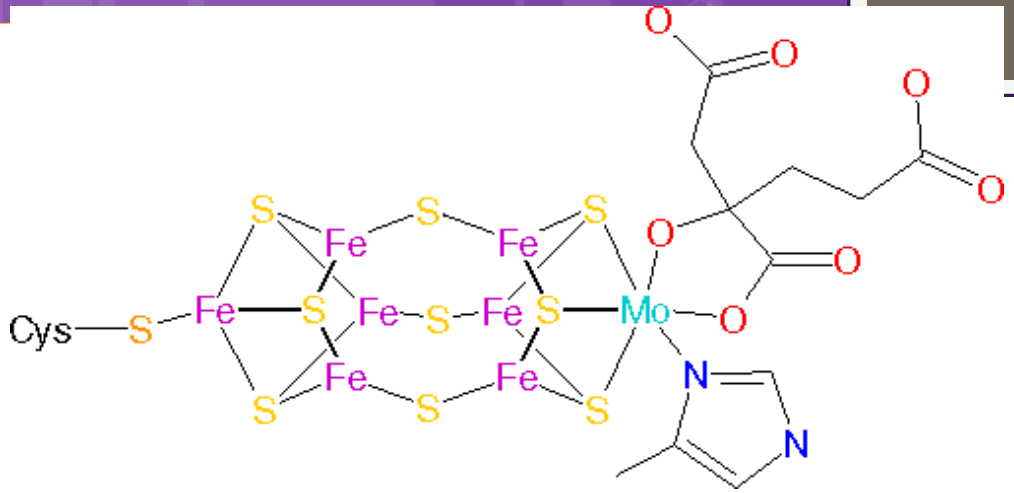
- **Its redox centres has 2 MO, 32 Fe and 30 S per tetramer**



- The smaller protein is a dimer consisting of two identical subunits
- It is called Fe protein because the dimer contains a single cluster of four iron atoms bound to four sulphur groups
- It has a binding site for ATP

Smaller subunit (Coponent II / Nitrogenase reductase / Fe protein)

- Transfers e- from Ferridoxin / Flavodoxin to nitrogenase
- Consists of 2 smaller chains.
- Mol.wt → 60,000 to 60,700 dts
- 2 chains are more or less identical
- Each contains 4 iron & 4 Sulfurs.
- It catalyses the binding of Mg-ATP with the protein.
- The nitrogenase is a binary enzyme.
- The nitrogenase differs from one source to the other in size, structure and activities.


O
O
O
O
O
O
S
Fe
S
Fe
S
Cys
S
Fe
S
Fe
S
Fe
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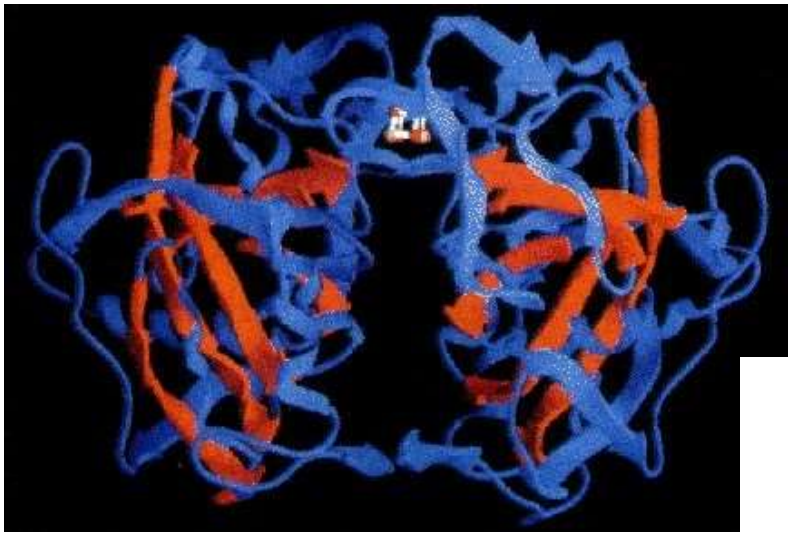
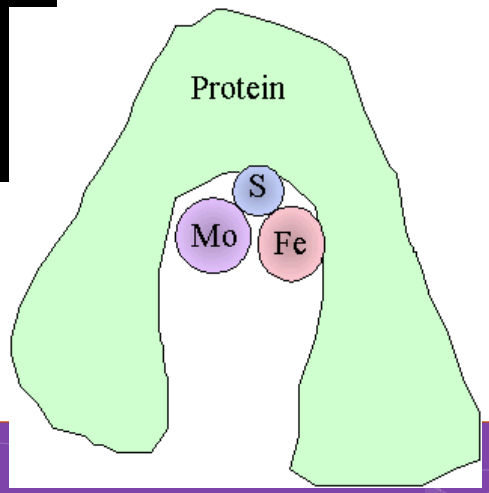
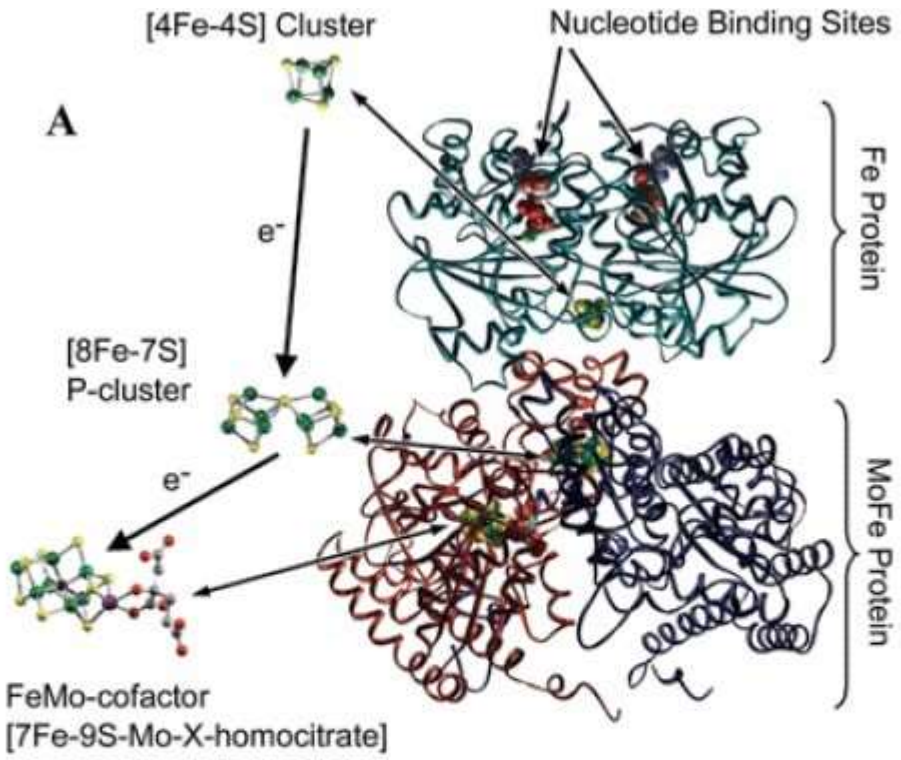
Mo
O
S
Fe
S
Fe
S
N
N




Protein
S
Mo
Fe



Nitrogenase proteins and cofactors. (A) Shown is one half of the nitrogenase complex, with one αβ pair of the MoFe protein $\alpha_2\beta_2$ tetramer and one bound Fe protein dimer. The location of bound nucleotides and the three metal clusters are also shown.

Barney BM, et al., Dalton Trans. 2006, 2277-84.

- The Fe protein has the dinitrogen reductase activity and 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.
- Both the subunits are required for the reduction of N_2 to NH_3 and are susceptible to irreversible inactivation by O_2.

Both components contain molybdenum (Mo) as well.

The iron (Fe) and molybdenum (Mo) in dinitrogenase (MoFe-protein) are contained in a cofactor called MoFe-cofactor or MoFe-co.

The nitrogenase proteins are denatured by exposure to oxygen (O2), they can only operate in an anaerobic environment.


THANK YOU