Nitrogen Fixation

It is a process where N_2(gas) converted into NH_3 or other ions which can be taken by the Biosystem.

To meet the consumption of Nitrogen fertilizers demand the World is producing about 10^4 tons of NH_3 fertilizers per day through Habor - Bosch process which requires drastic condition :—

$$N_{2(g)} + 3H_{2(g)} \longrightarrow 2NH_{3(g)}$$

Catalyst = Fe
Temp. = 450 - 600°C
Pressure = 200 - 600 atm

But N_2 - fixation by Nitrogenase i.e Fe and Mo metal centre containing enzyme goes on at ambient temperature and pressure.

Kinetic and Thermodynamic Aspects :-

Molecular nitrogen is very inert towards ordinary chemical reaction due to

i) Bond energy $N \equiv N$ ⟶ 945 KJ mol^{-1}

ii) " distance " ⟶ 1.1 A^0

iii) Stretching frequency ⟶ 2331 cm^{-1} / 2143 cm^{-1} (CO)

iv) Ionisation Energy ⟶ 1503 KJ/mol (for atom)

v) Electron affinity $N \rightarrow N^-$ — very less

vi) Low polarizability

(a) The highest occupied molecular orbital (HOMO) is purely σ-character

(b) LUMO is π antibonding in character (Hence reluctant to receive electron)

only strong RA. like Li_3N and Mg_3N_2 etc.

↳ due to

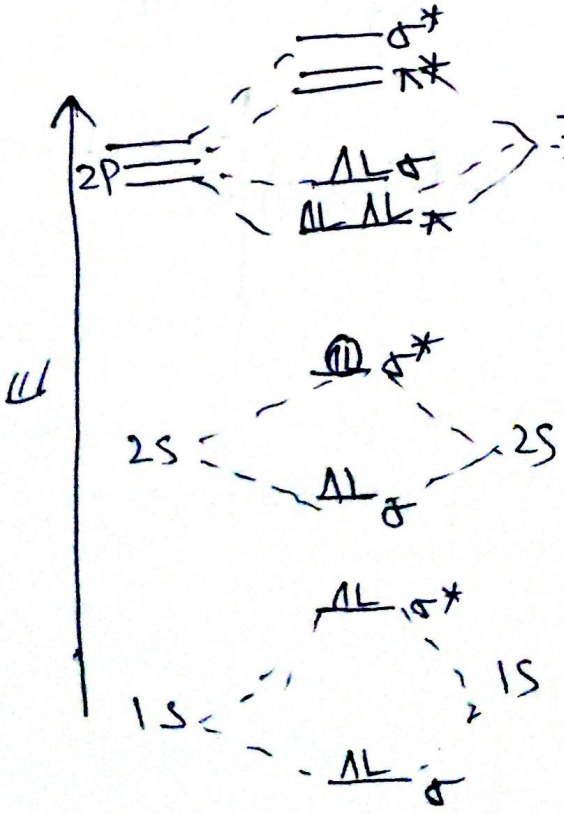
i) very high bond energy

ii) High energy gap between HOMO & LUMO

iii) Antibonding character of LUMO

iv) σ bonding character of HOMO

v) Low polarizability of N_2



MO diagram of N_2

Low chemical reactivity of N_2 is due to the following reasons! —

$N \equiv N$ → bond distance = 1.1 Å

1. very high bond energy. = 945 KJ/mol
2. High energy gap between HOMO & LUMO
3. Antibonding character of LUMO — highly reluctant to receive electron
4. σ-bonding character of HOMO
5. Low polarizability of N_2
6. stretching frequency = 2331 cm^{-1}
7. Ionisation energy = 1503 KJ/mol (for atom)

Conversion of N_2 to NH_3 passes through several intermediates like N_2H_2 (diimine), N_2H_4.

3. The standard reduction Potential of the involved process are given below! —

$$N_2 + 2H^+ + 2e^- \rightleftharpoons N_2H_2 \quad (E_0' = -1.0 - -1.5V)$$

$$N_2 + 5H^+ + 4e^- \rightleftharpoons N_2H_5^+ \quad (E_0' = -0.7V)$$

$$N_2 + 6H^+ + 6e^- \rightleftharpoons 2NH_3 \quad (E_0' = -0.34\ V \text{ at } pH = 7.4)$$

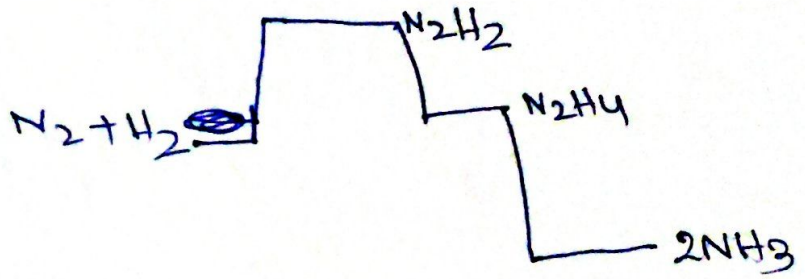
Such low E^0 — values indicates that only strong R.A. can transfer electron to N_2. Ferridoxin (Fe_4S system) having low E^0 value can transfer e^- to N_2.

Li_3N, Mg_3N_2

∴ N_2 to NH_3 conversion must have kinetic barrier

∴ unstable intermediate forms stabilized by complexation process

∴ So complexation can help conversion.



Possible reaction pathways for red^n of N_2 to NH_3

Conversion of N_2 to NH_3 passes through several intermediates like

$$N_2 + 2H^+ + 2e \rightleftharpoons N_2H_2$$ (diimine)

Standard R.P ($E_0' = -1.0$ to -1.5 V)

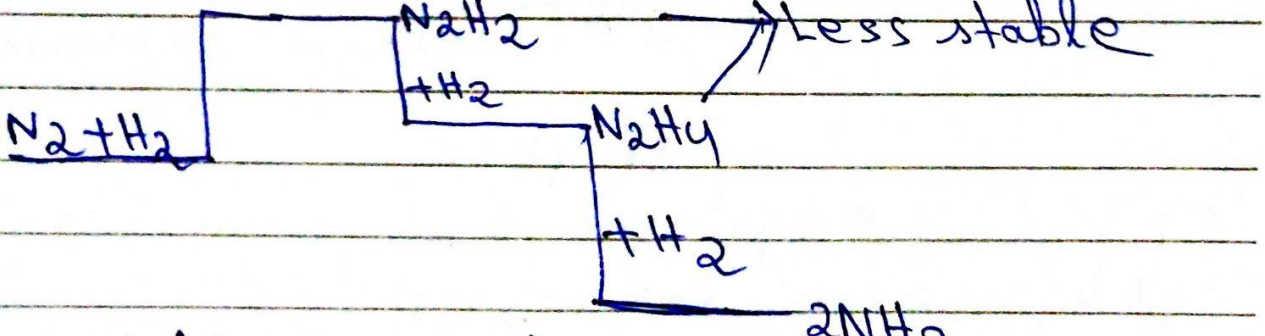
$$N_2 + \underbrace{4H^+}_{5H^+} + 4e \rightleftharpoons N_2H_4 / N_2H_5^+ \quad (E_0' = -0.7\text{ V})$$

$$N_2 + 6H^+ + 6e \rightleftharpoons 2NH_3 \quad (E_0' = -0.34\text{ V at pH 7.4})$$

⇒ small E_0 values

⇒ Only strong R.A can transfer electron to N_2

⇒ Conversion of N_2 to NH_3 must have kinetic barrier



⇒ Unstable intermediates forms stabilized by complexation process.

⇒ Complexation can help conversion.

Possible Reaction Pathways for red^n of N_2 to NH_3

Conversion of N_2 to NH_3 passes through several intermediates like

Standard Red^n Potential

$N_2 + 2H^+ + 2e^- \rightleftharpoons N_2H_2$ (diimine) $\rightarrow$ ($E_0' = -1.0$ to -1.5 V)

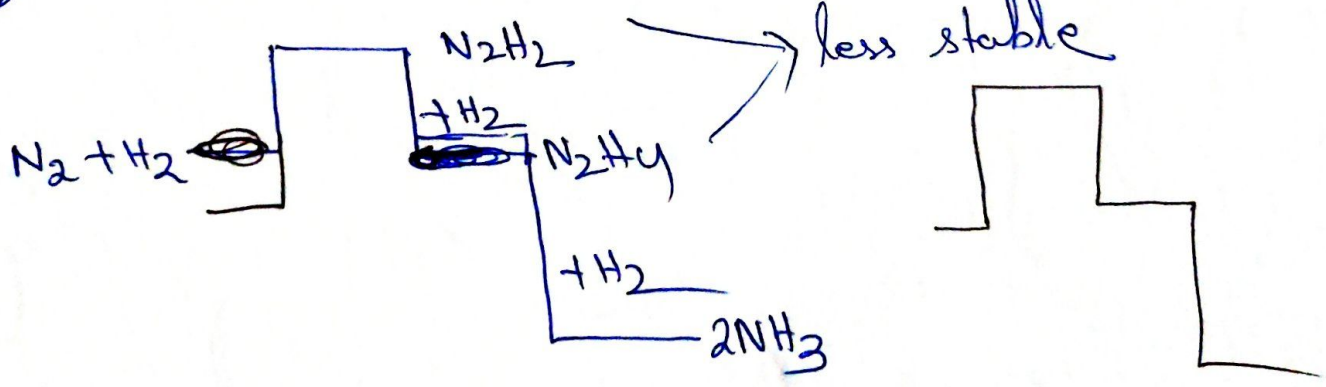
$N_2 + 4H^+ + 4e^- \rightleftharpoons N_2H_4/N_2H_5^+$ (hydrazine) $\rightarrow$ ($E_0' = -0.7$ V)

$N_2 + 6H^+ + 6e^- \rightleftharpoons 2NH_3 \rightarrow (E_0' = -0.34$ V at pH 7.4)

$\Rightarrow$ Small E^0 values.

$\Rightarrow$ Indicates that only strong R.A can transfer electron to N_2

$\Rightarrow$ Conversion of N_2 to NH_3 must have kinetic barrier



$\Rightarrow$ Unstable intermediates becomes stabilized by complexation process.

$\Rightarrow$ Complexation can help conversion.

Possible reaction pathways for red^n of N_2 to NH_3

Conversion of N_2 to NH_3 passes through several intermediates like

$$N_2 + 2H^+ + 2e \rightleftharpoons N_2H_2$$ (diimine) — Standard R.P ($E_0' = -1.0$ to -1.5 V)

$$N_2 + 4H^+ + 4e \rightleftharpoons N_2H_4 \,|\, N_2H_5^+ \quad (E_0' = -0.7\text{ V})$$

$$N_2 + 6H^+ + 6e \rightleftharpoons 2NH_3 \quad (E_0' = -0.34\text{ V at pH 7.4})$$

⇒ small E_0 values

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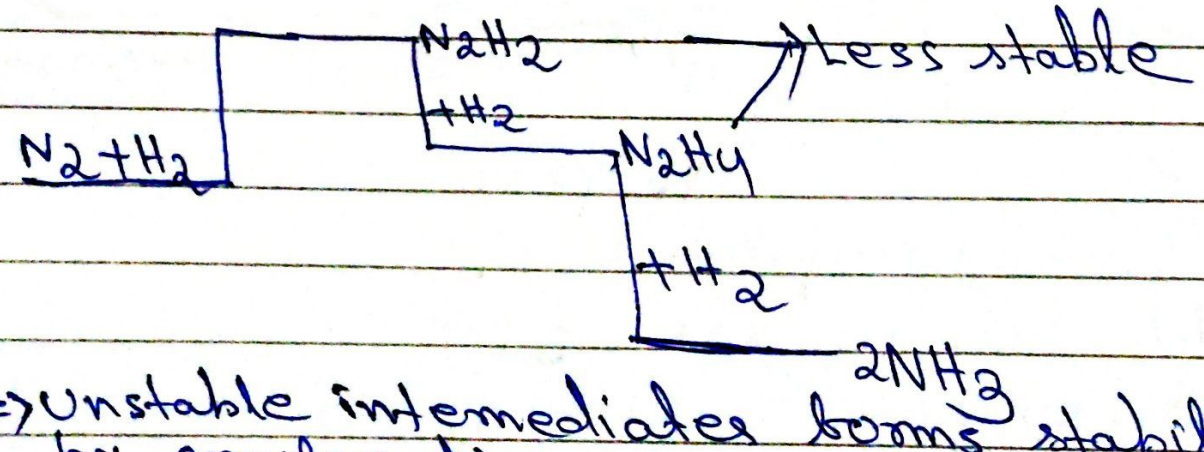
⇒ Conversion of N_2 to NH_3 must have kinetic barrier



⇒ Unstable intermediates forms stabilized by complexation process.

⇒ Complexation can help conversion.

Biological Nitrogen Fixation

It requires ① Source of electrons
② Source of energy for the activation of electrons and N_2.

Source of [redacted] Electrons

Due to oxidation of pyruvates

Pyruvates are generated from carbohydrates in respiration process or other sources. These electrons are carried to the system receiving site of nitrogenase by the Fe-S system.

Source of Energy

The energy required for the activation of electrons and N_2 is provided by the hydrolysis of ATP to ADP

$$ATP + H_2O \longrightarrow ADP + PO_4^{3-} + Energy$$

12 to 14 molecules of ATP are required to reduce one molecule of N_2.

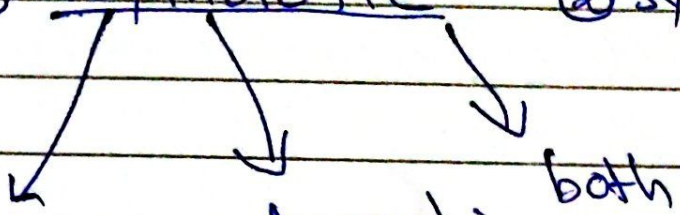
$$N_2 + 6H^+ + 6e + 12ATP + 12H_2O \longrightarrow 2NH_3 + 12ADP + 12PO_4^{3-} + Energy$$

$$N_2 + 8H^+ + 8e + 16ATP + 16H_2O \longrightarrow 2NH_3 + 16ADP + 16PO_4^{3-} + H_2 + Energy$$

12ATP | 14ATP | 16ATP

Biological N_2-fixation is done by some certain prokaryotic organisms called diazotrophs. These are three types:-

① Asymbiotic ② Symbiotic ③ Others



Aerobic (Azotobacter)

Anaerobic (clostridium pasturianum)

② Symbiotic

Rhizobium, Bradyrhizobium

Above these are present in the red root nodules of the leguminous plant such as soyabeans, peas, peanuts etc. The red colour inside the nodules due to leghemoglobin i.e a plant O_2-binding protein analogous to animal hemoglobin.

③ Others

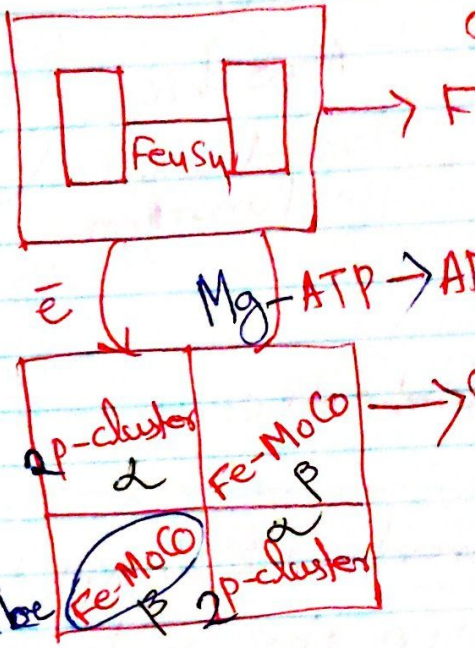
Blue green algae, Anabaena azollae

Nitrogenase

Nitrogenase fix N_2 and it is isolated from Rhizobium Lupini & clostridium pasterianum. It contains two proteins

(a) Iron protein (b) Mo-Fe protein

Component I Component II



Component I — oxidised form is diamagnetic
reduced form paramagnetic

→ Fe-protein

→ Component II

FeMoCo — 2 paramagnetic unit.

p-cluster - 2 diamagnetic unit.

FeMoCo is cofactors small in size can be detached from the protein/enzyme.

→ both proteins contain equal no. of Fe and S^{2-} & Fe as Fe_4S_4 provide a system for electron storage.

→ $2\alpha\beta$ subunits.

→ Electron transfer from Fe-protein to Mo-Fe protein occurs in the presence of Mg-ATP.

→ Suitable R-A is dithionite.

Properties of Nitrogenase Proteins

(42)

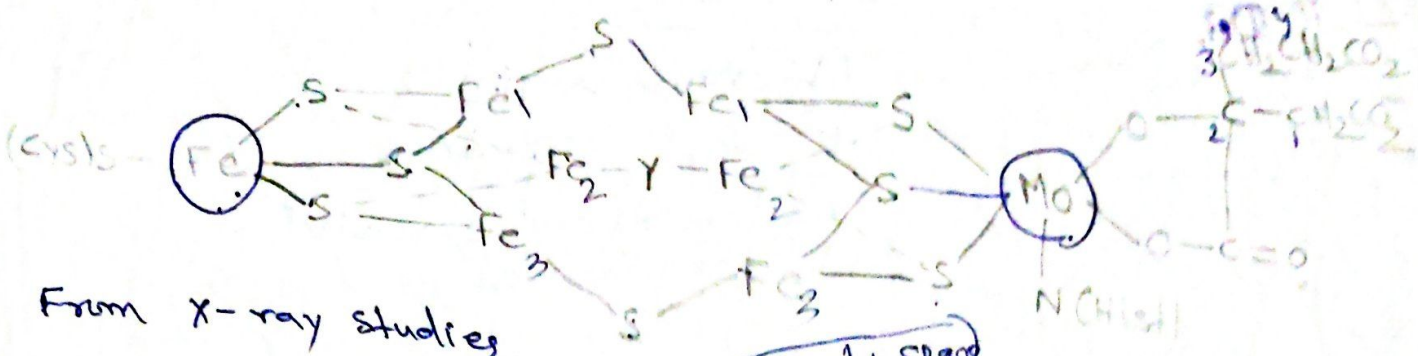
Property	Fe-Protein	Mo-Fe Protein
colour	yellow	brown
M.M.	≃ 65,000	≃ 2,00,000 to 2,20,000
No. of metal atoms	4 Fe atoms	2 Mo + 24 Fe (atoms)
No. of 'S' atoms	4	24
Structure	monomer	tetramer
Specific activity	460-530 nm	250 nm
sensitivity to O_2	irreversible	reversible

~~2) Some evidences 2MoFe; 6S; 2MoCo~~

~~Star~~ Structural Aspects

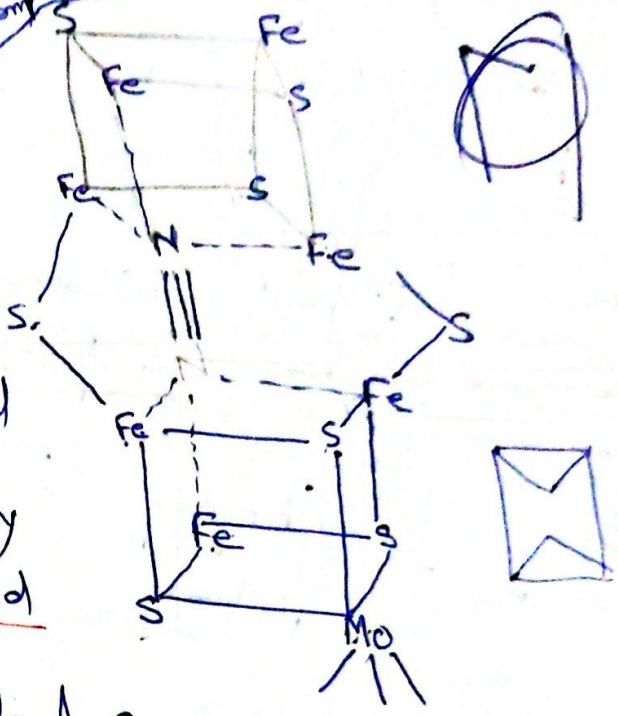
43 42

① [FeMoCo]



From X-ray studies,

1) Fe_7MoS_8 consists of 2 voided, ~~large~~ empty cubane MuS_4 units each missing one S^{2-} ion



3) Fe_3 face, facing each other and eclipsed

2) Six central Fe centres are probably arranged as a trigonal prism.

4) Fe_4S_3 and Fe_3MoS_3 bridged by 2 S^{2-} ions and unknown ligand Y

5) Mo centre is six Coordinated (distorted Oh)
=> 2O donors from a homocitric acid is 2-hydroxyl-1,2,4-butanetricarboxylic acid

6) Oxidised form of Mo probably +4

7) αβ Consists of one FeMoCo and two p-clusters

8) Two FeMoCo separated by 60-70 Å

9) but p-clusters are @ 15Å away from FeMoCo

10) The two FeMoCo are independently separated.

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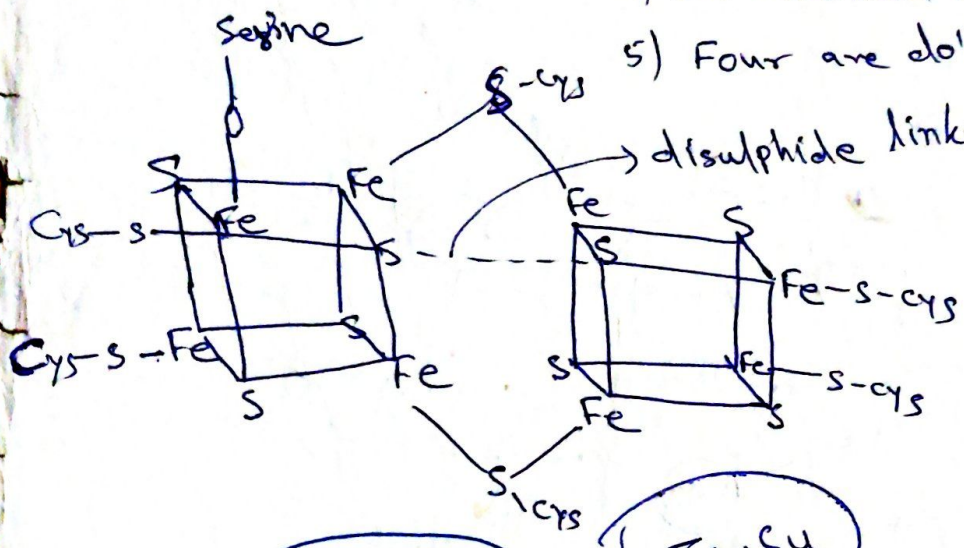
P-cluster with one FeMoCo

P-Clusters on FeMoCo

↓

4 Fe_4S_4 / 1 $2Fe_8S_8$

1) Four Fe_4S_4 cluster called p-clusters not behaved identically
2) Serve as electron pool for red^n of N_2 or called reservoir of low potential electrons which are used by FeMo
3) Each FeMo associated with 2 P-Clusters different from Fd.
4) On oxidised form high spin ($S = 7/2$) present
5) Four are do't behave identically.



$2Fe_4S_4$ + $2Fe_4S_4$

associated with one FeMoS (Fe_8S_8 + Fe_8S_8)

- 1) Fe_8S_8 is 2P-cluster one FeMoS
- is cubane units
- 2) Bridged by 2S of Cyst(2)
- 3) disulphide linkage is believed to be potentially redox active in nitrogenase activity
- 4) One cubane is Fe_4S_4
- 5) other in which Fe is 5-coordinated unique

Fe-Protein

1) Two identical subunits connected by Fe_4S_4 cluster
2) Fe_4S_4 undergoes a single one-electron change
3) Oxidised form – dia, Red – para
4) Fe-protein acts as an ATP binding site, delivers the electrons to FeMo protein, it can bind two ATP units
5) Acts as an electron source
6) FeMo acts as an substrate binding site.

The activity of nitrogerase is mainly due to the combined effect contributed by both proteins. Both proteins appear to contain sulfide ions equal to the number of Fe atoms. The iron is present mainly as Fe_4S_4 cubane clusters, which provide a system for electron storage.

Each ($\alpha\beta$) of this component II contains a paramagnetic FeMO cluster with two diamagnetic p-cluster about 0.15A° apart from the Fe-MO cluster. The two Fe-MO cluster was separated by 60-70A°

The component I (Fe-protein) consists of two identical subunits bridged by Ferredoxin.

In fact to drive the nitrogerase reaction both reducing agent and ATP-binding agent are required. The conformational change in Fe-Protein is due to hydrolysis of Mg-ATP which is responsible for the change in Redox Potential from -0.29V to -0.4V. It thermodynamically, allows the reduction of ($N_2 + 6H^+ + 6\bar{e} \rightleftharpoons 2NH_3$ $E^0 = -0.34V$)

The NH_3 formed in this process can be incorporated into amino-acids such as Glutamates and to nucleic acids.

Inorder to prevent the oxygen bound in the root's environment from reaching the nitrogerase, the plant produces a special form of hemoglobin called leghb. This protein has a affinity to O_2 and it's binding to the O_2 originated around the root prevents it from reaching the Nitrogenase complex.

present mainly as Fe_4S_4 cubane clusters.

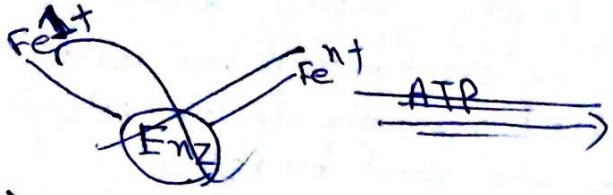
~~* Crystal str. studies~~

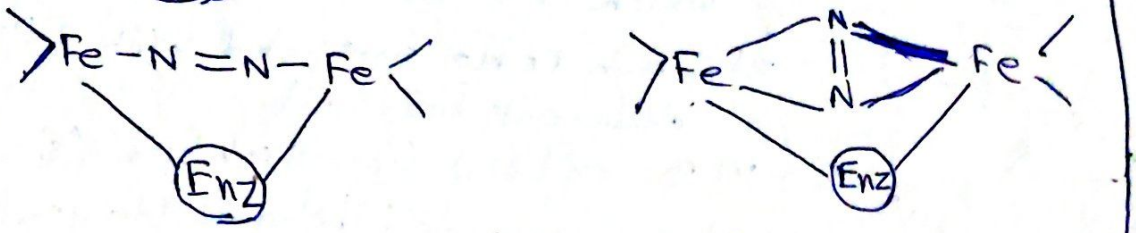
Possible Reaction pathway for Red^n of N_2 to NH_3

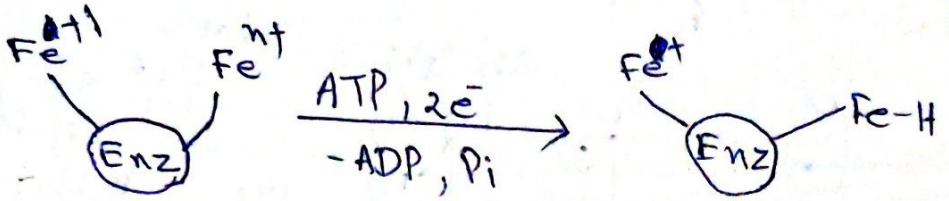
1) Taking $\rangle Fe - Y - Fe\langle$ segment which is the active site by crystal str. studies for N_2 binding

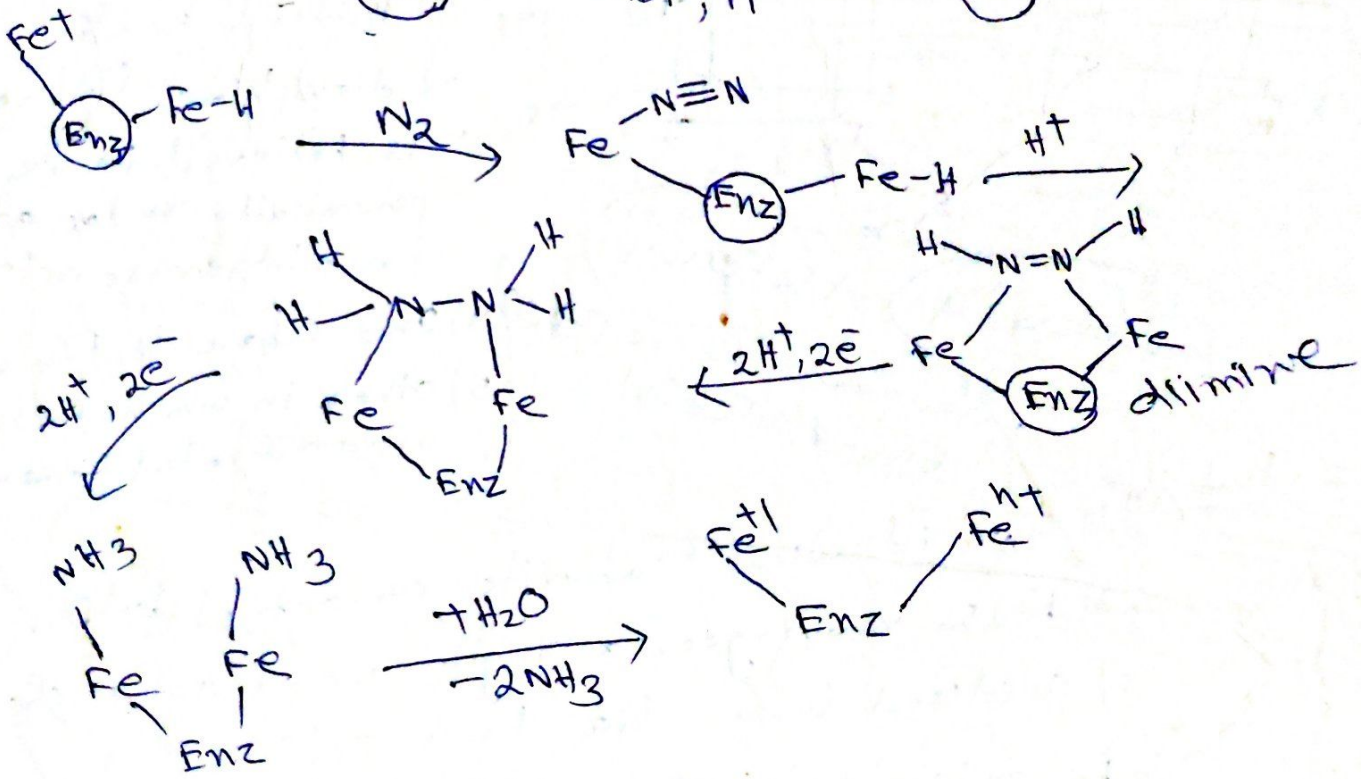
2) * N_2 replaces Y, $Fe - N \equiv N - Fe$, end on binding.

3) Formation of active site through an ATP hydrolysis. This ATP-induced reduction leads to Fe^{n+} to $Fe^{(n-2)+}$









Evidence

1) Isolation of symmetrical red^n products, i.e diimine ($HN=NH$) and Hydrizine H_2N-NH_2, considering the proposed dizene bridged dinuclear complex

$Fe - NH = NH - Fe$

(47) N_2-red^n is energetically costly (48)

*The transfer of electrons during the nitrogenase reaction requires ATP dependent protein conformational changes and the dissociation of the Fe-protein from the MoFe-protein after each electron transfer.

*During the reaction cycle, two molecules of ATP bind to the Fe-Protein and are hydrolysed as an electron passes to the MoFe-Protein.

Mg-ATP

*ATP hydrolysis induces a conformational change in the Fe-protein that alters its redox-potential from −0·29 to −0·40V, making the electron capable of N_2-reduction.

$N_2 + 6H^+ + 6e^- \rightleftharpoons 2NH_3$ is −0·34V

N_2-reduction is in 3-discrete steps

✓Actual red^n occurs on the MoFe-protein

✓Each step involves an electron pair

$$N\equiv N \xrightarrow{2H^+ + 2e^-} H-N=N-H \xrightarrow{2H^+ + 2e^-} \underset{\text{diimine}}{} \xrightarrow{\text{Hydrazine}} \xrightarrow{2H^+ + 2e^-} 2NH_3.$$

$$N_2 + 6H^+ + 6e^- + 12ATP + 12H_2O \rightarrow 2NH_3 + 12ADP + 12P_i$$

$$N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \rightarrow \quad + H_2$$