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Nitrogenase Enzyme: A Review

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ABSTRACT

Biological nitrogen fixation is accomplished through the catalytic action of complex enzyme system known as nitrogenase and it is the only known family of enzymes which accomplishes this process. The strength of Nitrogen molecule is because of its N-N triple bond. To break nitrogen atoms requires breaking of all three chemical bonds. So Nitrogen fixation using nitrogenase requires rather large inputs of energy to drive the process. Nitrogenase consists of two major protein components, namely, dinitrogenase (MoFe-protein) and dinitrogenase reductase (Fe-protein). 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 (O_2), they can only operate in an anaerobic environment. Only certain microorganisms can synthesize the nitrogenase enzyme. Some of these microbes are free-living in soils and water, while others occur in mutualisms with fungi or plants.

Keywords: Diazotrops, Nitrogen fixation, Nitrogenase protein.

INTRODUCTION

The nitrogenase enzyme catalyses the reduction of N_2 to NH_3 accompanied by reduction of protons to H_2. The enzyme consists of Fe and MoFe component proteins which can be purified separately[1]. The MoFe protein contains two types of metal-sulfur clusters, the **P-clusters**, and the Fe, Mo cofactor known as **FeMoco**, believed to be the site of substrate binding. Although the structure of FeMoco within crystalline resting-state nitrogenase was determined in 1992, the sites of substrate and inhibitor coordination are still poorly understood[2].

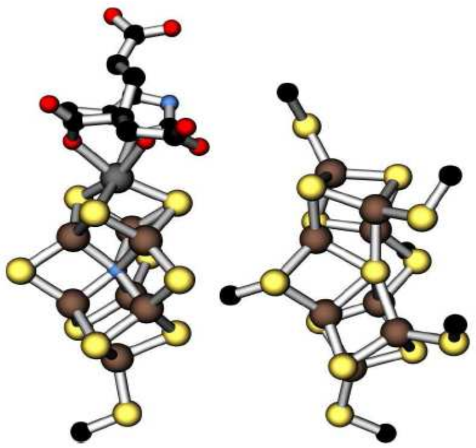


Figure 1: The FeMo-cofactor and its ligands (left) and the P-cluster with the sulfur bonds of cysteine residues (right). Brown: Fe, Yellow: S, Gray: Mo, Black: C, Blue: N,Red: O.

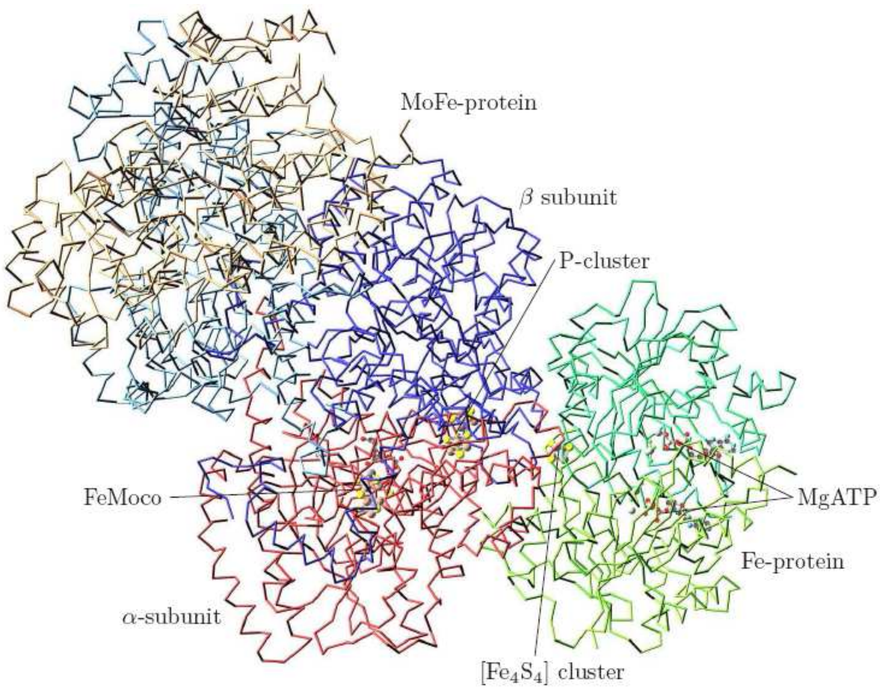


Figure 2: Complex between the MoFe-protein (left) and the Fe-protein (right). Only the backbones of the protein chains are shown. The cofactors are only shown in one half of the

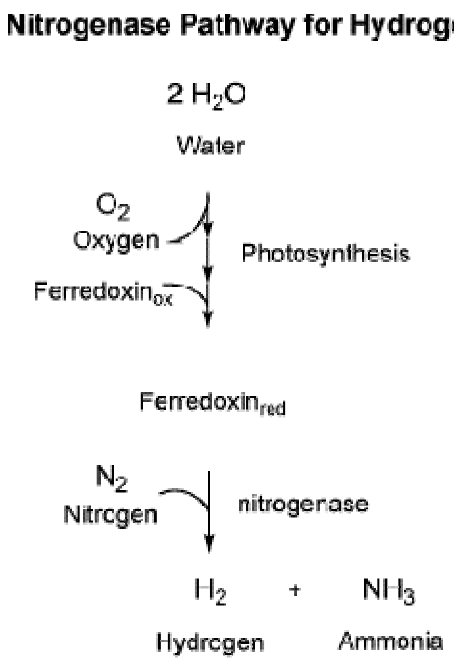
Complex Formation

The electron transfer from the Fe-protein to the MoFe-protein occurs via formation of a complex between them[3]. The two proteins are arranged such that they minimize the distance between the [Fe4S4] cluster of the Fe-protein and the P-cluster in the MoFe-protein. The distance is ¼15°A. A short distance is required to facilitate electron transport. The Fe-protein is only able to hydrolyze MgATP when bound in this complex. It has been possible to stabilize this complex, crystallize it, and determine its structure by replacing MgATP by MgADP·AlF4 and thus suppress hydrolyzation[4,5].

MoFe-protein.

Nitrogenase Pathway

Found in nitrogen-fixing bacteria and archaea, nitrogenase's primary biological function generates ammonia from dinitrogen. This activity is essential for all organisms because it sequesters nitrogen gas in a biologically accessible form. Throughout the process of ammonia generation by nitrogenase, hydrogen is formed. The enzyme has several forms, which utilize different metals in the catalytic site. These metals evolve various amounts of hydrogen. The three metals characterized are molybdenum, vanadium, and iron. They generate 1, 3, 7.5 moles of hydrogen per mole of nitrogen, respectively. This enzyme is powered by ATP; it requires 2 ATP per hydrogen atom produced. Both the electrons and the ATP in this system are provided through photosynthesis. Inherently, photosynthesis generates oxygen gas, while concurrently generating protons and electrons. The electrons pass through a series of electron carriers to finally reduce ferredoxin or flavodoxin. (These require approximately 4 electrons for reduction). In addition to the large energy requirements, a further obstacle for industrial use of nitrogenase is its oxygen sensitivity[6].



Nitrogenase Structure and Turnover Cycle [7]

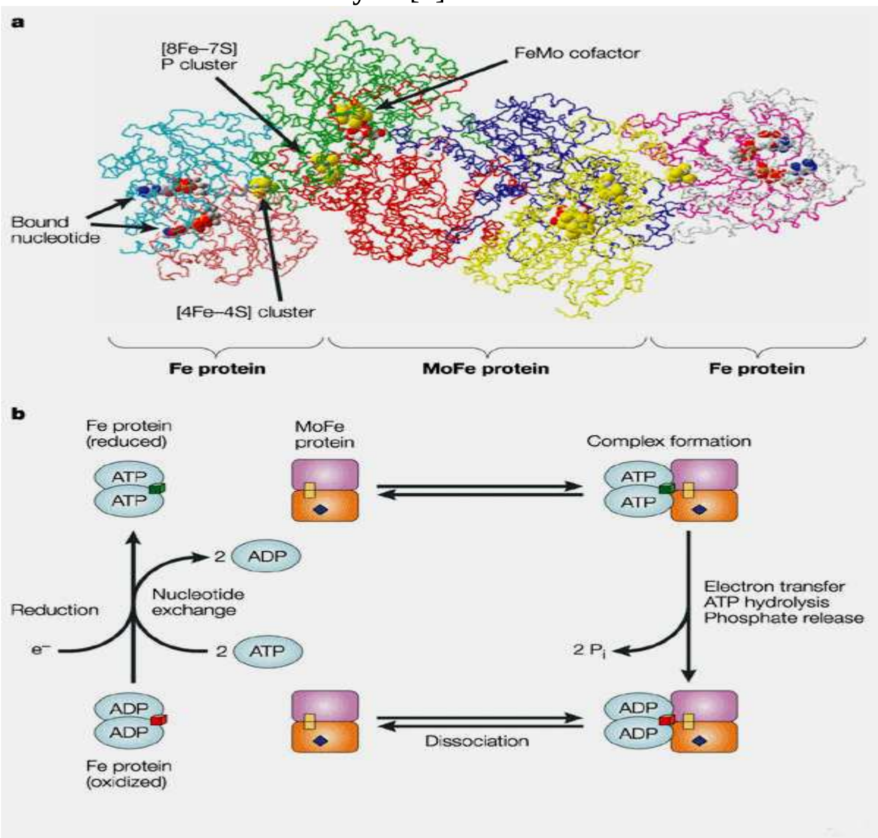


Figure 3: Nitrogenase Structure and Turnover Cycle

Structure of the complex that is formed between the component Fe and MoFe proteins of Azotobacter vinelandii nitrogenase, stabilized by the transition-state analogue for ATP hydrolysis, ADP–AlF_4^-. The subunits of the two Fe protein dimers are coloured in cyan, brown, magenta and grey. The α-subunits of the MoFe protein are coloured in green and yellow, with subunits in red and blue. The oxygen-sensitive metalloclusters and bound nucleotides are shown in space-fill. For simplicity, arrows indicating these features are only shown on one half of the symmetrical complex. b| Schematic representation of the nitrogenase Fe protein cycle. The Fe protein dimer is shown in light blue with the cube representing the [4Fe–4S] cluster coloured green to indicate the reduced form and red to represent the oxidized form. The α and subunits of the MoFe protein are depicted as orange and pink, respectively, the yellow squares represent the P cluster and the blue diamond represents the FeMo cofactor. Changes in the oxidation state of the MoFe protein are not shown.

Factors affecting nitrogenase activity

i) Oxygen Sensitivity

One of the most striking properties of nitrogenase is its sensitivity to oxygen. The Fe protein is irreversibly damaged by oxygen, while the MoFe protein is relatively insensitive. There are two protective mechanisms operating, conformational protection and respiratory protection. In conformational protection, the enzyme undergoes a conformational change by which it becomes insensitive to oxygen. In this condition it is unable to catalyze dinitrogen fixation. In respiratory protection there is an oxygen scavenging process operating as a result of high respiratory activity[8]. This permits the enzyme to maintain a catalytically active form. Azospirillum spp are aerobic bacteria which require low partial pressure of oxygen (pO2) for expression of hydrogenase activity. Under fully aerobic conditions, the fixation of nitrogen is not possible unless nitrogenase is protected. Azospirillum chroococcum and Azotobacter vinelandii have been used for nitrogen fixation studies under conditions of high pO2. Azotobacter can carry out nitrogen fixation in air because of respiratory and conformational protection of its nitrogenase, and because of the location of the enzyme within the cell. Another mechanism by which certain bacteria can express nitrogenase activity is microaerophily. Azospirillum requires microaerophilic conditions for nitrogen fixation. A. brasilense does not grow with N2 as the sole source of nitrogen under aerobic conditions. In Azospirillum, nitrogen fixation takes place at optimal rate between 0.005-0.007 atmospheres (0.507 and 0.709 k Pa) [3,9].

ii) Temperature and pH

In Azospirillum, the optimum temperature for H2-depentent growth is 32-40°C. This is similar to the optima of tropical nitrogen fixing bacteria. Nitrogenase activity of Azospirillum is sensitive to temperatures below 15°C. This accounts for the higher incidence of these bacteria in tropical countries. For Azospirillum, the optimal pH for N2 dependent growth is 6.8-7.8. These requirements are met with at the surface or within the cells of roots. Roots of grasses provide optimal pH conditions to bacteria even when the soil pH is low[5,10].

iii) Soil Moisture

In the Broad balk experiments it was observed that soil cores from wet areas showed high nitrogenase activity. It was thus inferred that nitrogenase activity is correlated with soil moisture. The rate of acetylene reduction was found to increase exponentially with linear increase in soil moisture. A similar correlation has been found between nitrogen fixation and soil core moisture of grasslands[11]. The changed pO_2 in turn affects nitrogenase activity this view is supported by the fact that higher rates of acetylene reduction are observed in soil cores incubated under N_2 compared with incubation under air[12]. These observations support the hypothesis that nitrogen fixation in non nodular plants may be increased in wet soils. Nitrogenase activity in wetland grasses has been reported to be much higher than that of plants growing in mesic or dry soils. The much higher rates of acetylene reduction in soil cores of marsh plants and paddy rice, as compared with those of agriculturally important cereal grasses, may be explained by the leaching hypothesis. High levels of combined nitrogen are inhibitory to nitrogen fixation. Because of denitrification and leaching of nitrates, the available combined nitrogen content of wet soils is low. The lowered combined nitrogen content of the soil increases nitrogen fixation[13].

Organisms that synthesize nitrogenase [14,15]

- Free-living diazotrophs, e.g.
  - Cyanobacteria (by means of differentiated heterocysts)
  - Azotobacteraceae
- Symbiotic diazotrophs, e.g.
  - Rhizobia
  - Frankia

Fixation of atmospheric N_2

Nitrogen fixation generally refers to the natural process, either biological or abiotic, by which nitrogen (N_2) in the atmosphere is converted into ammonia. This process is essential for life because fixed nitrogen is required to biosynthesize the basic building blocks of life, e.g. nucleosides for DNA and RNA and amino acids for proteins. Formally, nitrogen fixation also refers to other abiological conversions of nitrogen, such as its conversion to nitrogen dioxide. Nitrogen fixation is utilized by numerous prokaryotes, including bacteria, actinobacteria, and certain types of anaerobic bacteria. Microorganisms that fix nitrogen are called diazotrophs. Some higher plants, and some animals (termites), have formed associations (symbioses) with diazotrophs. Nitrogen fixation also occurs as a result of non-biological processes. These include lightning, industrially through the Haber - Bosch process, and combustion. Biological nitrogen fixation was discovered by the Dutch microbiologist Martinus Beijerinck[14].

Biological nitrogen fixation

Biological nitrogen fixation (**BNF**) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase[15]. The formula for BNF is:

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

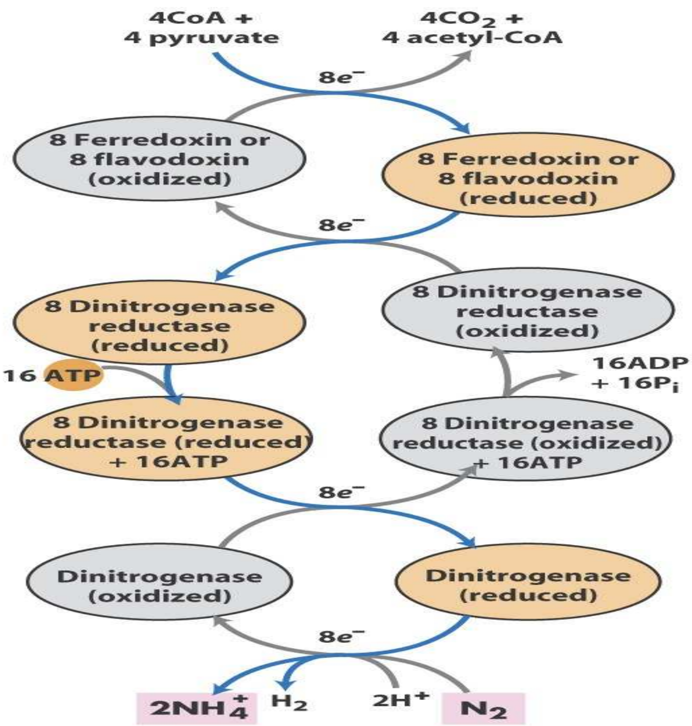


Figure 4: fixation of atmospheric N_2

The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2. In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway. Enzymes responsible for nitrogenase actions are very susceptible to destruction by oxygen. (In fact, many bacteria cease production of the enzyme in the presence of oxygen) [15]. Many nitrogen-fixing organisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with a protein such as Leghemoglobin[16]

Microorganisms that fix nitrogen [17]

- Diazotrophs
- Cyanobacteria
- Azotobacteraceae
- Rhizobia
- Frankia

Chemical nitrogen fixation

Nitrogen can also be artificially fixed for use in fertilizers, explosives, or in other products. The most common method is the Haber process. Artificial fertilizer production is now the largest source of human-produced fixed nitrogen in the Earth's ecosystem.[17] The Haber process requires high pressures (around 200 atm) and high temperatures (at least 400 °C), routine conditions for industrial catalysis. This highly efficient process uses natural gas as a hydrogen source and air as a nitrogen source[18].

CONCLUSION

Microorganisms like Cyanobacteria, Azotobacters etc. are the natural nitrogen fixers. Natural process of Nitrogen fixation is accomplished by complex but important enzyme system known as nitrogenase. This important biological fixation of nitrogen into ammonia makes it accessible for synthesis of nucleotide, DNA, RNA, amino acid and protein. Nitrogenase enzyme's primary biological function generates ammonia from dinitrogen. This activity is essential for all organisms because it sequesters atmospheric nitrogen gas in a biologically accessible form. Artificial agricultural fertilizer production is now the largest source of fixed nitrogen in the Earth's ecosystem.

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