Nitrogenases in Bacteria

Nitrogenases are enzymes found in certain bacteria and archaea; they fix atmospheric nitrogen (N2) typically by catalysing the reaction that reduces dinitrogen to form ammonia. Bacteria that contain nitrogenases are known as diazotrophs.

The best known family of diazotrophs are the rhizobia, a symbiotic bacteria found in the root nodules of leguminous plants (peas, beans and clover) and which provide nitrogen for the plant. Symbiotic bacteria such as this usually obtain sugars from the plant and in return produce ammonia which is used by the bacteria itself. Any excess ammonia produced is released in to the plant.

Nitrogenases are utilised for the most part by sowing leguminous plants such as peas or clover as part of a crop rotation to fix nitrogen in the soil that can later be used by follow-on crops.[1]

The basic reaction to produce ammonia involves the reduction of dinitrogen to an amine. This however, is difficult because of the high activation energy (944KJmol^-1)[2] required to break the NΞN triple bond that binds the two nitrogen molecules. Dinitrogen is the most inert of diatomic molecules, so even with a negative reaction enthalpy the reaction still needs to be catalysed. A basic equation for this reduction reaction is given below.

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$$

When catalysed by the enzyme, nitrogenase, the reaction requires 2 molecules of ATP (a chemical energy source) being hydrolysed to ADP per electron.[3]

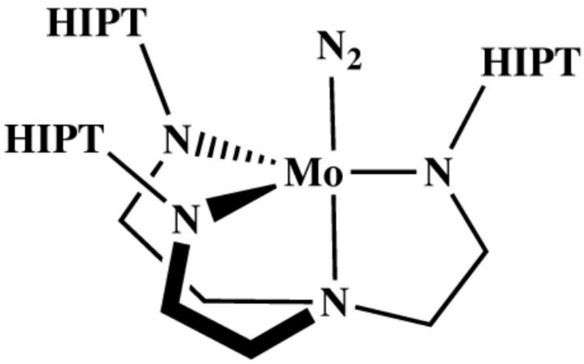
Nitrogen constitutes nearly 80% of our atmosphere and is the fourth most abundant element in the biosphere, hence it represents an inexhaustible supply if it can be used by the plant. As mentioned above it is also inactive in diatomic form - ‘Fixed’ nitrogen is needed for the synthesis of amino acids and other essential compounds in biological organisms.

For a plant to grow, fixed nitrogen must either be taken up from the soil or the plant must have symbiotic bacteria that contain the necessary nitrogenases. Most of the nitrogen found in arable crops today is from ammonia produced by the Haber-Bosch process on an industrial scale with a relatively inexpensive iron catalyst. This method of synthesis requires extremely high temperatures and pressures to produce the ammonia, and needs hydrogen which is usually obtained from natural gas. The ammonia made from the process is then used as fertiliser after removing water or conversion into compounds such as urea or ammonium nitrate.

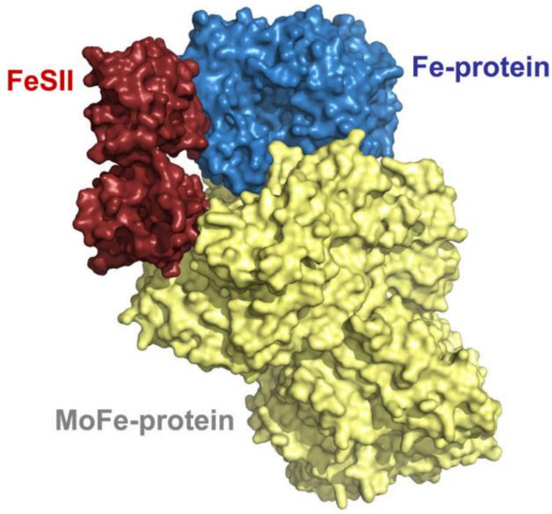
Reduction of atmospheric dinitrogen with nitrogenase requires a highly controlled and specific biological environment, with a range of other co-factors and enzymes such as ferredoxin involved. Metalloproteins like nitrogenase are very delicate when removed from this specific environment, which has meant that study of the enzyme is particularly difficult.[4]

The nitrogenase acts as a biological catalyst, binding to the dinitrogen, weakening the N≡N triple bond and allowing the reduction to ammonia to occur under ambient conditions.

[Picture of model dinitrogen complex MoN2]



Drawing of MoN2[8]



Model structure of A.vinelandii nitrogenase[9]

The exact mechanism of catalysis by the enzyme is unknown, not least because it is hard to obtain the nitrogen crystals that attach to nitrogenase[5]. All the mechanisms that are currently being studied are only hypothetical. It is known however that electrons are transferred from the iron protein to the molybdenum-iron protein for the process[4]. It is thought that nitrogen attaches to the iron atoms in the molybdenum-iron protein, as carbon monoxide can inhibit the nitrogenase by binding to these atoms[6]. In nitrogenase, the reaction occurs rapidly and so the evidence for this process is difficult to follow. As more model dinitrogen complexes are generated, possible geometry and n2 binding will be better understood.

As can be seen, nitrogen fixation is an important process to provide organisms with all the nitrogen they need for proteins and synthesis of various other biologically important molecules. With a growing population, the demand for this resource from agriculture also increases[7]. For over a century, this demand has been met for the most part by use of the industrial Haber-Bosch process, and a limited use of biological nitrogen fixation in legumes by way of crop rotation.

With the global concerns regarding the environment and energy use and efficiency, biological nitrogen fixation using symbiotic bacteria could meet our nitrogen needs more economically and in a greener way. Azotic's technologies provide one solution to improving the production of fixed nitrogen. Let’s hope production of fixed nitrogen improves further still in future!

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