Structure and Function in Nitrogenase Proteins

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The reduction of N\textsubscript{2} to ammonia by nitrogen-fixing bacteria is an essential biological process [1]. Nitrogen-fixing bacteria generate approximately 60\% of the world's fixed nitrogen, an essential ingredient in agriculture [2]. The mechanism of nitrogen fixation is, however, poorly understood. In order to better understand this mechanism, studies of nitrogenase and nitrogenase model compounds have been undertaken.

Nitrogen-fixing bacteria use two-protein enzymes called nitrogenases as catalysts for the reduction of nitrogen to ammonia [3]. The reduction of N\textsubscript{2} to ammonia, when catalyzed by a molybdenum-nitrogenase, has the following stoichiometry [3]:

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\text{N}_2 + 8e^- + 8H^+ \rightarrow 2 \text{NH}_3 + \text{H}_2
\]

There are three classes of nitrogenases, each coded by different genes. The first class is the molybdenum-nitrogenases. Genes for nitrogenases of this class are present, but not always expressed, in all nitrogen-fixing bacteria. The molybdenum-nitrogenases are the most thoroughly characterized class of nitrogenases. The second class of nitrogenases are called the ‘alternate’ nitrogenases or the vanadium-nitrogenases. These are very similar to molybdenum-nitrogenases. The vanadium-nitrogenases are, however, coded by separate genes. The gene is expressed when molybdenum is absent [3]. These alternate nitrogenases show less activity than the molybdenum-nitrogenases. They are sufficiently active, however to sustain culture growth. There is evidence for the existence of a third nitrogenase that contains only Fe and neither molybdenum nor vanadium. This enzyme has not yet been isolated.

The crystal structures of the two proteins comprising the molybdenum-nitrogenase in \textit{Azotobacter vinelandii} were solved to 2.7 Å resolution by Kim and Rees in 1992 [4a,b] and subsequently refined to 2.2 Å resolution [4c]. The larger protein, referred to as the MoFe protein or component one, is an \(\alpha_2\beta_2\) tetramer. There are two types of metal complexes, the FeMo-cofactor and the P-cluster (Fig 1 and 2), in the MoFe protein. Each MoFe protein contains two of each of these complexes. The FeMo-cofactor is believed to be the active site of the enzyme [3-7]. It contains a Fe\textsubscript{4}S\textsubscript{3} subunit bridged by three \(\mu_2\)-S atoms to an \(\text{MoFe}_3\text{S}_3\) subunit. The terminal Fe of the Fe\textsubscript{4}S\textsubscript{3} subunit is bound to a sulfur of a cysteine in the \(\alpha\) subunit and the molybdenum is bound to a nitrogen of a histidine in the \(\alpha\) subunit. The coordination sphere of molybdenum is completed by a bidentate ligand, a homocitrate molecule. The terminal iron center is four-coordinate. The remaining iron centers are three-coordinate and in a trigonal planar configuration. The distances between the three-coordinate iron centers in different subunits are unusually short at 2.5-2.6 Å. The second type of metal cluster in the MoFe protein is the P-cluster (Fig 2). The P-cluster is involved in the transfer of electrons from the Fe protein to the FeMo cofactor. The P-cluster consists of two Fe\textsubscript{4}S\textsubscript{4} cubanes connected by two \(\mu_2\)-S atoms between iron centers and a disulfide bond between two of the cluster sulfurs. The P-clusters connect the \(\alpha\) and \(\beta\) domains [3-6], with one cubane bound to an \(\alpha\) subunit and the other bound to a \(\beta\) subunit. All irons in the P-cluster are four-coordinate. The Fe protein is a dimer consisting of two identical subunits bridged through a Fe\textsubscript{4}S\textsubscript{4} metal cubane (Fig 3) called the Fe-cofactor [3,8]. The second protein in nitrogenases are called the Fe protein or component two. It acts as a source of electrons for the MoFe protein, each Fe protein being capable of providing one electron. Each iron center is four-coordinate (bound to three sulfurs in the cube and to the sulfur of one cysteine).
Developing an understanding of the way N₂ binds to the FeMo-cofactor is central to understanding the mechanism of nitrogen fixation, and the study of model compounds has demonstrate possible binding configurations. A number of metal-N₂ binding modes are known. The nitrogen may bind end-on or side-on either to one metal center or bridging between two different metal centers [9]. An additional possibility is for the N₂ to be coordinated to more than two metal centers [4c, 10]. The specific coordination site of N₂ in the FeMo-cofactor is as yet unknown. The three-coordinate iron center and the molybdenum/vanadium centers are both suspected binding sites. Model systems have been developed that have structural or chemical environments similar to the FeMo or FeV cofactors [6, 9-27]. Models containing molybdenum, vanadium, and iron bound to N₂ are known that produce ammonium when reduced [17, 25, 26]. For example, [Vdmpe₂(N₂)₂] (dmpe = bis(dimethylphosphino)ethane⁺), forms NH₄Cl when treated with dry HCl in THF (tetrahydrofuran) in 30% yield [25]. The cubane (Me₄N)[(DMF)₃VF₄[S₄Cl₃]-2DMF is a catalyst for the reduction of hydrazine, believed to be an intermediate in nitrogen reduction to NH₃, using cobaltocene as a reducing agent and 2,6-lutidine hydrochloride as the proton source. An essentially quantitative production of NH₃ was achieved [27].

Steps have been taken in understanding the mechanism of nitrogen fixation by nitrogenases. Complete understanding, however, awaits the synthesis of a functional, model compound that has the same structure and chemical environment as the naturally occurring cofactors. This remains a goal for the synthestic inorganic chemist.

References


