Structure and Function in Nitrogenase Proteins

Scott D. Rein Literature Seminar April 13, 1995

The reduction of N_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_2 to ammonia, when catalyzed by a molybdenum-nitrogenase, has the following stoichiometry [3]:

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

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 *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_4S_3 subunit bridged by three μ_2-S atoms to an $MoFe_3S_3$ subunit . The terminal Fe of the Fe_4S_3 subunit is bound to a sulfur of a cysteine in the α subunit and the molybdenum is bound to a nitrogen of a histidine in the α 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_4S_4 cubanes connected by two μ_2-S atoms between iron centers and a disulfide bond between two of the cluster sulfurs. The P-clusters connect the α and β domains [3-6], with one cubane bound to an α subunit and the other bound to a β 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_4S_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).

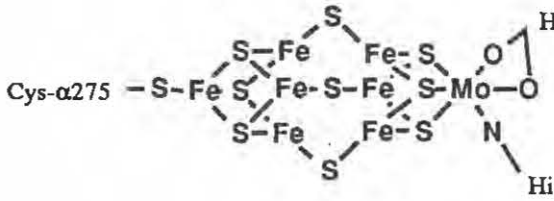

Figure 1
FeMo Cofactor

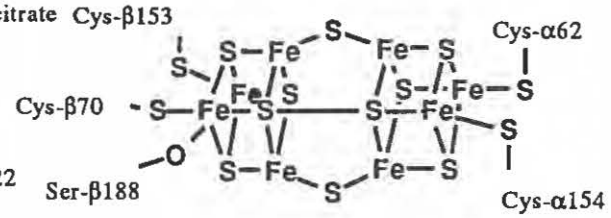

Figure 2
P cluster

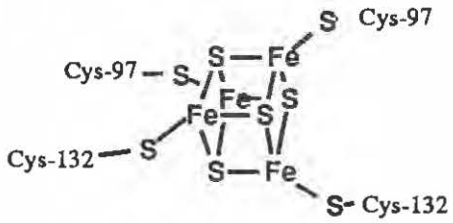

Figure 3
Fe-cofactor

Developing an understanding of the way N_2 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-N_2 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 N_2 to be coordinated to more than two metal centers [4c,10]. The specific coordination site of N_2 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 N_2 are known that produce ammonium when reduced [17,25,26]. For example, $[Vdmpe_2(N_2)_2]$ {dmpe= bis(dimethylphosphino)ethane$^+$}, forms NH_4Cl when treated with dry HCl in THF (tetrahydrofuran) in 30% yield [25]. The cubane $(Me_4N)[(DMF)_3VFe_3S_4Cl_3]\cdot 2DMF$ is a catalyst for the reduction of hydrazine, believed to be an intermediate in nitrogen reduction to NH_3, using cobaltocene as a reducing agent and 2,6-lutidine hydrochloride as the proton source. An essentially quantitative production of NH_3 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 occuring cofactors. This remains a goal for the synthestic inorganic chemist.

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