THE STRUCTURES OF THE NITROGENASE PROTEINS - AN OVERVIEW

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At this Congress there is a half-day formal session on the function of nitrogenases together with a workshop on the same subject. Therefore the object of this overview is to provide an analysis of the structural data upon which much of the interpretation of mechanistic experiments is now based.

Protein crystallographers admit that the resolution of the data obtained for most protein structures is insufficient for them to define the exact positions of atoms. Accordingly they fit 'models' to the electron densities obtained: if the intensity is high then perhaps metal or sulfur atoms are indicated; the shape of electron densities associated with specific amino acid side chains is usually characteristic and the bond lengths are known and can be fitted with some accuracy. However, when unknown structures, particularly of metal clusters, are met then there must be an element of guesswork. It is possible by using different X-ray wavelengths to highlight specific atoms and thus, by difference Fourier analysis, to identify their positions with some certainty. Another important source of information is EXAFS spectroscopy which provides accurate atom-to-atom distances, although if there is more than one similar interaction in a cluster only averages can be indicated. A variety of other spectroscopic methods can also provide useful data.

Currently only two groups, Rees from Caltech and Bolin from Purdue, have produced crystallographic data on the nitrogenases; the former has worked largely with Azotobacter vinelandii nitrogenase, and the latter largely with Clostridium pasteurianum nitrogenase, although each group has worked on the other species. Below we attempt to synthesise the data from both groups to indicate those aspects where there is agreement and those where there still remain some differences.

All molybdenum nitrogenases consist of two metallo-proteins: the iron protein, a $\gamma_2$ dimer of equivalent subunits containing a single Fe₄S₄ cluster; and the molybdenum-iron protein, which is an $\alpha_2\beta_2$ tetramer containing two molybdenum and about 30 iron and acid labile sulfur atoms distributed into two types of cluster, the iron-molybdenum cofactor and the P
cofactor and the P cluster. It is well established that the Fe protein passes electrons to the MoFe protein in a reaction which requires hydrolysis of MgATP to MgADP. Within the MoFe protein the substrates N₂, H⁺, C₂H₂, etc. are almost certainly reduced at the FeMoco site. Alternative nitrogenases are also known where molybdenum is replaced by vanadium or possibly just by iron (Eady, 1995). These alternative nitrogenases contain an extra subunit in the larger protein which is apparently essential for N₂ reduction but not other substrate reductions (Waugh et al., 1995).

**Fig. 1** The structure of the Fe protein from *A. vinelandii*, drawn using MOLSCRIPT and the coordinates of Georgiadis et al., 1992.

The structure of Av2 (the Fe protein from *A. vinelandii*) (Georgiadis et al., 1992) is shown in Fig. 1. The two subunits are related by two-fold symmetry axis which passes through the Fe₄S₄ cluster which is bonded to four cysteine residues, two provided by each subunit. Each subunit consists of a single α/β domain with an eight-stranded β sheet flanked by nine α-helices. In the structure (Fig. 1) an ADP molecule is shown. This was observed as an approximately half occupied site in the Av2 structure but apparently not in the Cp2 structure. Its phosphate end is associated with a previously identified (Robson, 1984) Walker motif A, GXXXXGKS/T, phosphate binding site (Walker et al., 1982) which binds molybdate in the Av2 structure. The ADP spans the two subunits and there is room for two such molecules, with complementary binding sites in each subunit. These are almost certainly the two MgATP or MgADP sites identified in binding experiments with the Fe proteins from a number of species (Howard, Rees, 1994). Recent site-directed mutagenesis experiments which probe these sites are fully consistent with this view (Seefeldt, Mortenson, 1993).