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Crystallization and preliminary X-ray studies of nitrogenase component 1 (the MoFe protein) from *Klebsiella pneumoniae*

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Abstract

Two crystal forms of component 1 (the MoFe protein) of nitrogenase from *Klebsiella pneumoniae* have been isolated and characterized. The triclinic form has cell dimensions $a = 76.0$, $b = 109.6$, $c = 144.6$ Å, $\alpha = 80.3$, $\beta = 74.9$ and $\gamma = 69.6°$, diffracts to around 3.0 Å and has two molecules in the asymmetric unit. The monoclinic form belongs to space group $P2_1$ with $a = 76.6$, $b = 127.8$, $c = 109.1$ Å and $\beta = 104.6°$ (frozen at 100 K), diffracts to 1.5 Å and has one molecule in the asymmetric unit. At this resolution the outstanding questions concerning the structure and the operation of the enzyme, in particular the linkage between the Fe_4S_4 units in the P clusters, the true geometry of the apparently trigonal Fe atoms in the FeMoco and the reduction site itself, should be answerable.

1. Introduction

The nitrogenase enzyme system is used by bacteria to reduce gaseous dinitrogen to ammonia for growth. The overall stoichiometry of the reduction is,

$$N_2 + 8H^+ + 8e^- + 16\text{MgATP} \rightarrow 2NH_3 + H_2 + 16\text{MgADP} + 16P_i.$$

The conventional system consists of two metalloproteins, the molybdenum–iron (MoFe) protein and the iron (Fe) protein. The MoFe protein (component 1) is an $\alpha_2\beta_2$ tetramer with a molecular mass of ~220 kDa, containing two pairs of multimetal clusters, named FeMoco and the P cluster. FeMoco has the stoichiometry $MoFe_7S_9$:homocitrate and is thought to be the cluster at which dinitrogen is reduced (Hawkes, McLean & Smith, 1984). The P cluster has a stoichiometry of Fe_8S_8 or Fe_8S_7 and consists of two Fe_4S_4 cubes linked either by a single corner S or by an S—S bond. The function of the P clusters is obscure but they may be on the electron-transfer path from the Fe protein to the FeMoco. The Fe protein (component 2) is smaller (molecular mass ~60 kDa), contains a single Fe_4S_4 cluster and is the obligate electron donor to component 1 in a reaction in which ATP is hydrolyzed. The structures of both proteins have been elucidated to medium resolution, in the case of the MoFe protein from *Azotobacter vinelandii* (Av1) (Kim & Rees, 1992; Chan, Kim & Rees, 1993) to 2.2 Å and *Clostridium pasteurianum* (Cp1) to 2.2 Å (Bolin, Campobasso, Muchmore, Morgan & Mortenson, 1993) and 3.0 Å (Kim, Woo & Rees, 1993).

2. Crystallization

Component 1 of nitrogenase from *Klebsiella pneumoniae* (Kp1) was extracted and purified as described earlier (Eady, Smith, Cook & Postgate, 1972) followed by fast protein liquid chromatography (Yousafzai, Buck & Smith, 1996). All solutions used for crystallization were purged with N_2 prior to use and all manipulations were carried out in a temperature-controlled (290±2 K) glove box, where O_2 levels were kept below 1 part per million. Sodium dithionite, used in all manipulations outside the glove box to preserve the protein, was not required inside and thus not used. The protein solution, Kp1 in 50 m*M* Tris pH 8.0, was filtered successively through two low protein-binding filters (0.45 and 0.2 µm, Gelman Sciences) to remove aggregates. The crystals were grown and stored in a water-cooled box inside the glove box at 288±0.5 K.

The crystallization conditions published for Av1 (Kim & Rees, 1992) and Cp1 (Weininger & Mortenson, 1982) were used as a guide for initial screening tests. Crystals of the triclinic form were found over a wide range of conditions and were optimized at 14% PEG 6000, 0.14 *M* $MgCl_2$ in 50 m*M* Tris pH 8.0 with 10 mg ml^{-1} Kp1 (final concentrations), using the liquid–liquid diffusion technique, in 1 mm siliconized glass tubes. These crystals grew as badly formed needles, up to 2 mm in length, and always appeared after a quantity of amorphous precipitate had collected, suggesting that the protein solutions used were not homogeneous. Very small crystals with a different morphology were also seen, but these could only be optimized after the protein purification had improved. These were finally optimized at 14% PEG 6000, 0.4 *M* $MgCl_2$ in 50 m*M* Tris pH 8.0 and ~3 mg ml^{-1} Kp1 (final concentrations). These crystals were single, often only one or two per tube, and very well formed, with no or very little amorphous precipitate evident. Analysis of this protein indicated a higher metal content than previously obtained (1.8–1.9 *versus* 1.5–1.6, compared with a theoretical content of 2.0 Mo per molecule) indicating a higher level of homogeneity.

3. Results

Data were collected to 3.0 Å (effective limit 4.0 Å, four crystals) on a San Diego Multiwire System mounted on a Rigaku RU-200B generator on the first needle-like crystal form of Kp1. The size of the crystal was approximately 1.5 × 0.2 × 0.2 mm. Temperature control was achieved by blowing a constant stream of room temperature air over the capillary. Extensive searching of reciprocal space failed to show any axes with symmetry, indicating a triclinic space group. Volume analysis indicated two molecules in the asymmetric unit. *TRACER* (Collaborative Computational Project, Number 4, 1994) a cell reduction and lattice Bravais lattice check program, failed to find any higher symmetry space groups. Further data were collected on Station 9.6 at Daresbury to 2.8 Å (effective 3.0 Å), processed using *MOSFLM* (Leslie, Brick & Wonacott, 1986) and scaled with *ROTAVATA/AGROVATA* (Collaborative Computational Project,

Number 4, 1994) giving 116 364 reflections, 79 322 unique, $R_{merge} = 7.2\%$. The positions of the two molecules were located using the molecular replacement technique, *AMoRe* (Collaborative Computational Project, Number 4, 1994), with the $\alpha\beta$ subunits from Av1 as the search model, these having 73 and 68% identity with Kp1. The four correct solutions were in the top five rotation-function solutions, the top peak only being 30% above the noise level though. *TNT* (Tronrud, Ten Eyck & Matthews, 1987) gave an R factor of 46.8% after rigid-body fitting of the four halves of the molecules.

The second crystal form was initially examined at the cryocrystallography course in Heidelberg (EMBL, 1995). The data obtained indicated a monoclinic cell and diffraction to at least 1.8 Å at 100 K, after a ~2 min soak in mother liquor containing 25% ethylene glycol as the cryo-protectant. Data collection took place on Station 9.6, with the crystal frozen to 100 K. Diffraction was observed to 1.5 Å with the data taken to a maximum of 1.6 Å. The data were processed in the same manner as above, resulting in a 75% complete data set, with $\langle I \rangle / \sigma \langle I \rangle$ of 3.4 at the edge and $R_{merge} = 4.7\%$ on 402 207 reflections, 198 442 unique. Owing to lack of $0k0$ data it was not possible to distinguish between $P2$ and $P2_1$, however molecular replacement was only successful in the latter, using the same search model. The two correct solutions were the only peaks in the rotation-function list higher than 50% of the highest peak. Rigid-body refinement with *X-PLOR* (Brünger, 1992) using all reflections gave an R factor of 45%. Refinement is in progress. Early indications show that this structure (Lawson, 1996) has the same P cluster structure as that published for Cp1 by Bolin *et al.* (1993).

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