Molecular Symmetry of the MoFe Protein of Nitrogenase

STRUCTURAL HOMOLOGY/NITROGEN FIXATION/X-RAY CRYSTALLOGRAPHY

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X-ray diffraction data to 2.4-Å resolution have been collected for native monoclinic crystals of the MoFe protein of nitrogenase from Clostridium pasteurianum. The MoFe protein is an αβ4 tetramer of 220,000 molecular weight with 1 molecule in the crystallographic asymmetric unit. A 6-Å resolution rotation function shows the orientation of the crystallographic diad and pseudo mutually perpendicular diads representing 3-fold relationships between α and β chains. Hence, at least at low resolution, there exists structural homology between these two polypeptide chains.

A class of bacteria exists which is capable of reducing atmospheric dinitrogen (N2) to ammonia. The enzyme which is common to all nitrogen-fixing bacteria and plays the central role in the reduction of dinitrogen is nitrogenase. The reaction catalyzed by nitrogenase is

\[ N_2 + 8H^+ + 8e^- + nATP \rightarrow 2NH_3 + H_2 + nADP + nP_i \]

where \( n \) is between 12 and 24 (1).

During catalysis, nitrogenase is composed of two proteins, the Fe protein and the MoFe protein. The molecular weight of the Fe protein is 59,700 for Clostridium pasteurianum. It is composed of two identical subunits and contains one FeS4 center. The MoFe protein has a molecular weight of 220,000, contains two α and two β subunits, two Mo atoms, 28-32 Fe atoms, and 24-32 acid-labile sulfurs. The molecular weight of the α and β subunits is 60,000 and 50,000, respectively (2). The MoFe protein contains an extrudable MoFe cofactor, which has a metal to sulfur ratio of 1:Mo:8Fe:6S (3).

In order to understand the mechanism of catalysis and its relation to structure, the three-dimensional crystal structure determination of the MoFe protein of nitrogenase from C. pasteurianum has been initiated. The crystals have the symmetry of a P21 space group with cell dimensions \( a = 69.99 \text{ Å}, \)

\( b = 151.05 \text{ Å}, \)

\( c = 122.04 \text{ Å} \) and \( β = 110^\circ 20' \) and with 1 molecule in the asymmetric unit (4). Native data were used to determine the orientation of the molecule within the cell and to search for a possible relationship between the α and β subunits.

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EXPERIMENTAL PROCEDURES

Native data have been collected by oscillation photography using focused (5) CuKα radiation from an Elliot rotating anode X-ray generator. In order to assure that the O2-sensitive MoFe protein crystals would remain in an anaerobic environment, mounting procedures were developed which excluded atmospheric oxygen from the mounted crystals (4, 6). The crystals were kept between 14 and 18 °C during data collection.

The crystal to film distance was 75 mm, permitting the recording of data to 2.4-Å resolution. A complete set of data was collected rotating the crystals through a total angle of 90° about the a*-axis. The crystals were oscillated through 1.25° for each film exposure and with an overlap of 0.25° between films. A total of 90 film packs were collected from 22 different crystals.

The films were processed on an Optronics P1000 film scanner using a 100-µm raster step size and analyzed by techniques reported elsewhere (7, 8). The final scaling included all reflections with intensities greater than 1 standard deviation. The overall R-factor, defined as

\[ R = \frac{\sum \sum |I_i - k_iI_o|}{\sum \sum I_o} \times 100, \]

where \( I_o \) is the intensity of reflection \( h \) on film \( i \), \( I_i \) is the mean of measurements for reflection \( h \), and \( k_i \) is the scale factor for reflections

**TABLE I**

Error in intensity measurements

Internal agreement refers to error computed from differences of specific intensity measurement with respect to their mean. Counting statistics refer to the error estimate based on noise level on the films with respect to a mean background and the best fit of a profile. Precise definition for internal agreement and counting statistics were given in Ref. 8. \( I = 162 \) on an arbitrary scale.

| Size | 0 | 1 | 2 | 5 | 10 |
|------|---|---|---|---|----|
| Internal agreement | 7 | 11 | 14 | 18 | 27 |
| Counting statistics | 20 | 24 | 26 | 31 | 42 |
| Number of reflections | 5464 | 8609 | 7702 | 5213 | 7205 |

**TABLE II**

Percentage of observed data

Resolution ranges

| Resolution ranges | 1/oranges | Overloads | Total |
|-------------------|------------|-----------|-------|
| 1.0-1.50 | 0 | 0 | 0 | 0 | 0 | 18 | 100 |
| 1.50-2.00 | 0 | 0 | 1 | 0 | 3 | 8 | 12 | 100 |
| 2.00-2.50 | 0 | 0 | 1 | 1 | 1 | 3 | 87 | 5 | 100 |
| 2.50-3.00 | 0 | 0 | 2 | 2 | 2 | 7 | 85 | 2 | 100 |
| 3.00-3.50 | 0 | 0 | 3 | 3 | 9 | 8 | 1 | 100 |
| 3.50-4.00 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 4.00-4.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4.50-5.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5.00-5.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5.50-6.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.00-6.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.50-7.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7.00-7.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7.50-8.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8.00-8.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8.50-9.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9.00-9.50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9.50-10.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Overloads refer to reflections which contained optical densities greater than an arbitrarily selected limit of around 2.
RESULTS AND DISCUSSION

The rotation function has shown the orientation of the molecular dimer axis between αβ subunit pairs to be inclined at an angle of 20° to the b-axis in an orientation which projects onto the c-axis in the ac plane. The secondary peaks reveal a low resolution homology between the α and β subunits.

The two different kinds of subunits in the MoFe protein of nitrogenase were not previously known to have any homology. The amino acid sequence of the α chain, as derived from the corresponding nucleotide sequence (11, 12) and the partial protein sequence, representing 20% of the total chains of the α and β subunits (13), had previously been determined. However, no obvious sequence homology between the α and β subunits was observed among the various peptides at this stage. Homology of structure as shown by the current results does not necessarily imply observable homology of amino acid sequence, since the conformation of polypeptide folds are almost invariably more conserved than sequence (14).

The data collected here, and the information about the noncrystallographic symmetry, is essential for further progress in the structure determination of this enzyme. The molecular diad will be an aid in locating heavy atom sites and extremely useful for improving phases by use of molecular replacement averaging (15).

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