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Crystallographic properties of the MoFe proteins of nitrogenase from *Clostridium pasteurianum* and *Azotobacter vinelandii*

(x-ray diffraction/nitrogen fixation/iron–sulfur proteins)

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ABSTRACT **Preliminary x-ray diffraction data from single crystals of the MoFe proteins of nitrogenase from *Clostridium pasteurianum* and *Azotobacter vinelandii* have been obtained. Both protein crystals belong to the $P2_1$ space group. The MoFe protein crystals from *C. pasteurianum* and *A. vinelandii* diffract to angles corresponding to resolutions as great as 2.4 Å and 3.0 Å, respectively. The cell dimensions of the MoFe protein crystals from the two species have been determined from precession photographs.**

A variety of bacteria exist that are capable of reducing molecular nitrogen (N_2) to ammonia (NH_3). This overall process, known as biological nitrogen fixation, is catalyzed by the enzyme system nitrogenase according to the general reaction

$$n\text{ATP} + N_2 + 6H^+ + 6e^- \rightarrow 2NH_3 + n\text{ADP} + nP_i.$$

In all cases examined nitrogenase consists of two separate and distinct proteins; the MoFe protein and the Fe protein. The Fe proteins (1–3), composed of two subunits with one [4Fe–4S] cluster, have molecular weights of 59,674 and 64,000 for *Clostridium pasteurianum* (*Cp*) and *Azotobacter vinelandii* (*Av*), respectively. The MoFe protein is isolated as an $\alpha_2\beta_2$ dimer. Molecular weights for the MoFe proteins of *Cp* and *Av* are approximately 220,000 and 250,000, respectively. Under physiological conditions ferredoxin (or flavodoxin) donates an electron to the Fe protein, which, when complexed with MgATP, transfers its electron to the MoFe protein. In the latter electron transfer, ATP is hydrolyzed to ADP. Presently it is thought that the reduction of N_2 occurs at a site on the MoFe protein that in the appropriate conformation combines N_2, protons, and electrons to produce ammonia.

The exact details of the catalysis of N_2 reduction by nitrogenase are still unknown, although a considerable amount of structural, kinetic, and biochemical data on the system are available. EPR (4–7) and Mössbauer spectroscopy (8, 9) have elucidated not only the nature of the oxidation states during various stages of catalysis but also, to some extent, the number and kind of metal clusters in nitrogenase. Recent studies also have demonstrated that the MoFe protein has four [4Fe–4S] clusters (10). In addition, extended x-ray absorption fine structure (EXAFS) has given considerable information about the kinds of atoms in the environment of Mo and their distances from the Mo (11, 12). Information from the EXAFS analysis is limited, however, because any atoms greater than 3.5 Å from the Mo are not identifiable, no information about stereochemistry of the atoms in the MoFe cofactor is revealed, and the relative positioning of the [4Fe–4S] clusters and MoFe cofactor in the MoFe protein cannot be determined. Ideally, to understand how nitrogenase functions during catalysis, a three-dimensional model scaled to atomic resolution must be available. For this reason we have grown crystals of the MoFe protein of *Cp* and *Av* and embarked on an x-ray analysis of their diffraction patterns. The three-dimensional structure determination will reveal not only the secondary and tertiary structure of the proteins but also the kind, position, and orientation of their associated metal clusters.

The MoFe protein from *Cp* was purified by a modified version of the original procedure (13). All steps were performed under N_2. The MoFe protein from *Av* was purified with the same procedure used for the MoFe protein of *Cp*, but the cells were broken with a Gaulin homogenizer (14). The proteins were crystallized at pH 7.5 in the presence of approximately 5% (wt/vol) polyethylene glycol 6000 and 0.2–0.4 M $MgCl_2$. During the entire purification processes and subsequent crystallization steps, anaerobic conditions were strictly maintained.

X-ray data reported here were obtained with a precession camera using Cu K_α radiation obtained with nickel filters. Manipulation of the crystals was performed in an anaerobic glove box purchased from Vacuum Atmospheres (Hawthorne, CA). Also, the crystals were kept at approximately 18°C during data collection.

Crystals of the MoFe protein from *Cp* and *Av* are shown in the photomicrographs of Fig. 1. An oscillation photograph of a crystal (Fig. 2) of the MoFe protein from *Cp* indicates that the diffraction extends to angles further than those corresponding to 2.4-Å resolution. However, the crystals of the MoFe protein from *Av* extend only to angles corresponding to 3.0-Å resolution. From precession photographs of the MoFe protein crystals from *Cp* the Laue symmetry is *mm* and the b^* axis contains only those reflections at which $k = 2n$. On the basis of this information the space group is $P2_1$. Similarly, precession photographs from the crystals of the MoFe protein from *Av* show *mm* Laue symmetry. At low resolution (less than 6 Å) for the 0*kl* zone only reflections at which $k = 2n$ are present. At higher resolution (greater than 5.0 Å) the extinctions indicate the $P2_1$ space group but at lower resolution the extinctions indicate a pseudo-*C* centering or a *C*2 space group (Fig. 3). All cell dimensions are listed in Table 1.

The most plausible numbers of molecules per unit cell for the crystals from both *Cp* and *Av* were determined by calculating values for V_m, the ratio of unit cell volume to total protein mass of the unit cell (15). When values of V_m are calculated for the crystals of the MoFe protein from *Cp*, V_m equals 2.7 when the number of molecules in the unit cell is 2, and, for the crystals of the MoFe protein from *Av*, V_m equals 2.3 when the number of molecules in the unit cell is 4.

To confirm the calculated number of molecules per unit cell, the densities of the crystals and their mother liquors were mea-

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Abbreviations: *Cp*, *Clostridium pasteurianum*; *Av*, *Azotobacter vinelandii*.

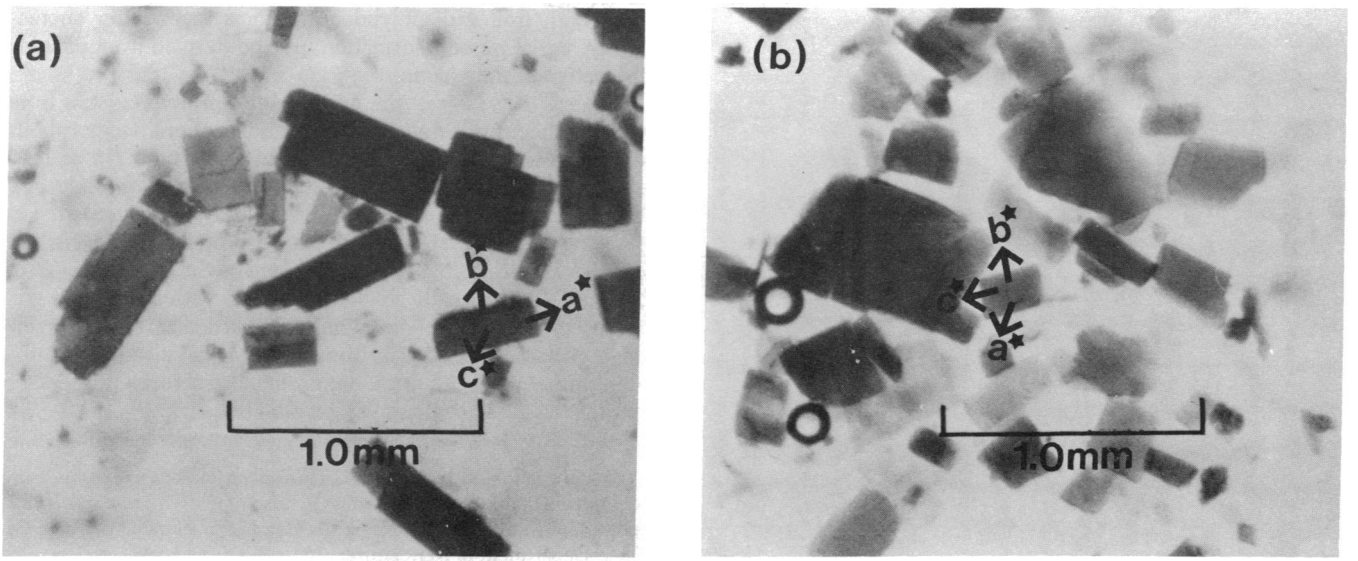

FIG. 1. (*a*) Photomicrograph of the crystals of the MoFe protein from *Cp*. The crystals are brown, as is the protein in solution. The arrow indicating the a^* direction makes an angle of 70° with the c^* direction, which makes an angle of 90° with the flat face of the crystal. (*b*) Photomicrograph of the crystals of the MoFe protein *Av*. The crystals are brown, as is the protein in solution. The arrow indicating the a^* direction makes an angle of 75° with the c^* direction, which lies in the plane of the flat face of the crystal.

sured in a gradient density column of bromobenzene and xylene. From these density measurements the numbers of molecules per unit cell were calculated to be 1.3 and 3.3 for the MoFe protein crystals from *Cp* and *Av*, respectively. If one assumes that the number of molecules per unit cell is normally measured on the low side due to adherence of mother liquor to the crystals, two and four molecules per unit cell for the crystals from *Cp* and *Av*, respectively, seem to be reasonable values. In addition, because the MoFe protein crystals from *Av* lie in a pseudo-*C*2 space group, the pseudo-asymmetric unit of the

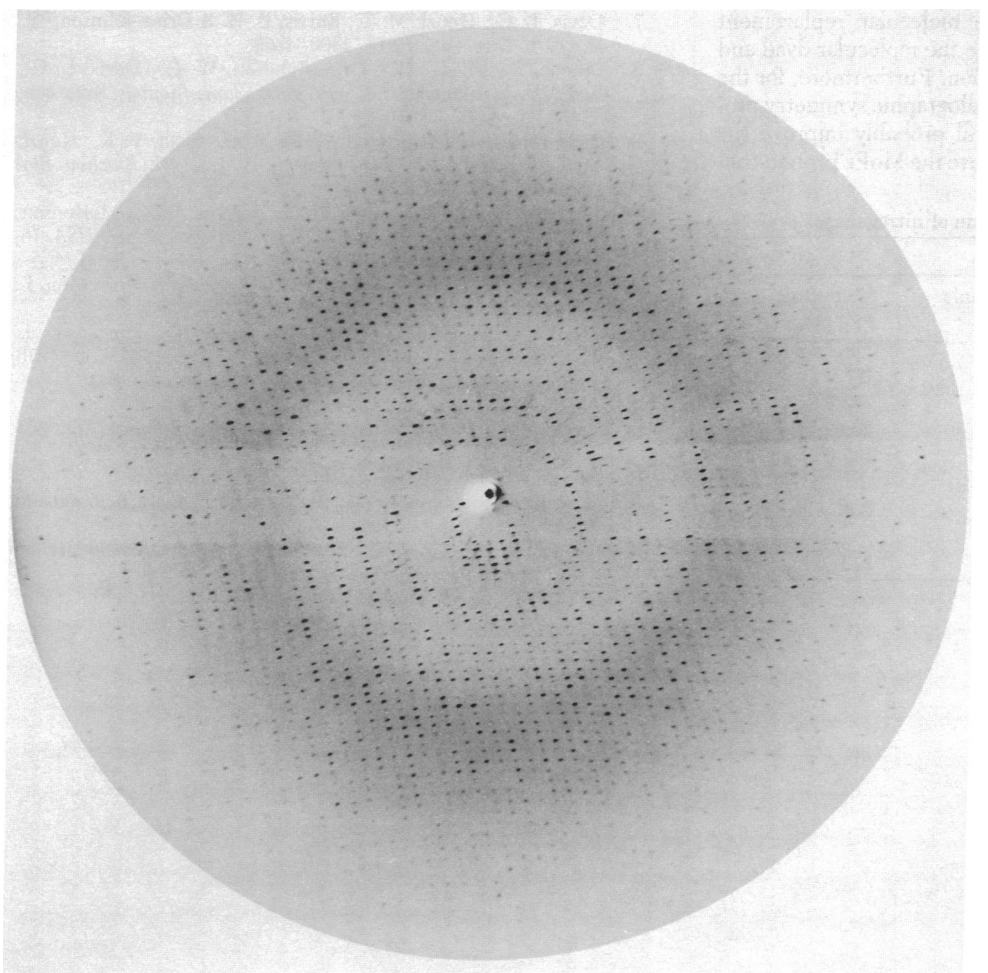

FIG. 2. Oscillation photograph of a MoFe protein crystal from *Cp*. The oscillation range is 1.25° starting at 4° from the *h*0*l* zone. The spindle axis is the a^* axis. The crystal-to-film distance is 75 mm and Cu K_α radiation from a rotating anode was used. The diffraction extends to the edge of the film, which is an angle corresponding to 2.4-Å resolution.

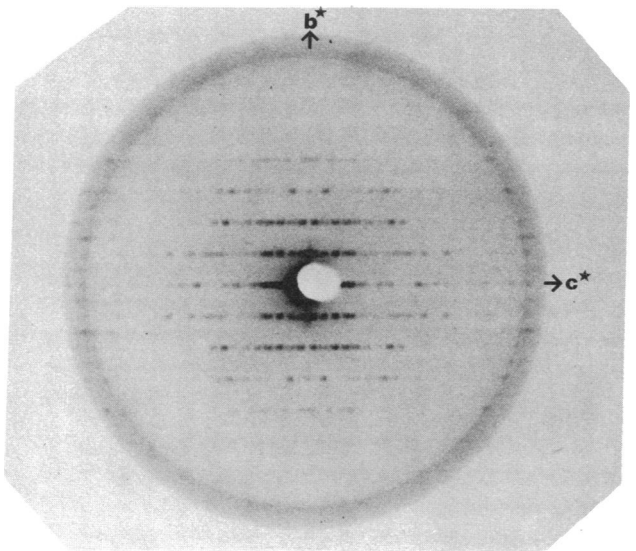

FIG. 3. A 9° precession photograph of the 0*kl* zone of a crystal of the MoFe protein from *Av*. The crystal-to-film distance is 75 mm and Cu k_α radiation from a stationary anode was used. The reciprocal cell axes b^* and c^* are designated in the conventional manner.

Av crystals contains one molecule. This result is important because it simplifies structure determination.

To aid in the determination and refinement of phase angles, molecular averaging and anomalous scattering data will be used. The MoFe protein has a molecular subunit configuration of $\alpha_2\beta_2$ and preliminary rotation function calculations indicate a molecular twofold axis (16). Thus the molecular replacement method (17) can be used to determine the molecular dyad and to aid and improve phase determination. Furthermore, for the crystals from *Av*, additional noncrystallographic symmetry provided by the pseudo-*C* centering will probably improve the molecular averaging technique. Because the MoFe protein contains both iron and molybdenum atoms that scatter anomalously, initial phase information may be derived from data for the native protein alone.

Table 1. Crystal forms of MoFe protein of nitrogenase

Property	*Cp*	*Av*
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1$	$P2_1$, pseudo-*C*2
Unit cell dimensions, Å		
a	69.9	153.9
b	151.0	72.9
c	122.0	208.2
β, degrees	110.3	105.0
Volume of unit cell, $Å^3 \times 10^{-6}$	1.2	2.3
No. of molecules in asymmetric unit	1	2, 1

Recently the data of the native MoFe protein crystals from *Cp* have been collected on an oscillation camera. Isomorphous derivatives of the MoFe protein crystals of both *Cp* and *Av* should be sought. Also, as the result of our search for other crystal forms of the MoFe proteins, we have recently succeeded in growing single crystals of the MoFe protein from *Klebsiella pneumoniae* (*Kp*). Information gathered on the crystals from *Cp*, *Av*, and *Kp* eventually should provide us with a complete picture of the structure of nitrogenase. In particular, the extremely high quality of the crystals from *Cp* have convinced us that the determination of the three-dimensional structure of the MoFe protein from nitrogenase will be attained.

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1. Mortenson, L. E. & Thorneley, R. N. F. (1979) *Annu. Rev. Biochem.* **48**, 387–418.
2. Dalton, H. & Mortenson, L. E. (1972) *Bacteriol. Rev.* **36**, 231–260.
3. Zumft, W. G. (1976) *Struct. Bond.* **29**, 1–65.
4. Zumft, W. G., Palmer, G. & Mortenson, L. E. (1973) *Biochim. Biophys. Acta* **292**, 413–421.
5. Mortenson, L. E., Zumft, W. G. & Palmer, G. (1973) *Biochim. Biophys. Acta* **292**, 422–435.
6. Smith, B. E., Lowe, D. J. & Bray, R. C. (1973) *Biochem. J.* **135**, 331–341.
7. Davis, L. C., Henzl, M. T., Burris, R. H. & Orme-Johnson, W. H. (1979) *Biochemistry* **18**, 4860–4869.
8. Münck, E., Rhodes, H., Orme-Johnson, W. H., Davis, L. C., Brill, W. J. & Shah, V. K. (1975) *Biochim. Biophys. Acta* **400**, 32–53.
9. Zimmerman, R., Münck, E., Brill, W. J., Shah, V. K., Henzl, M. T., Rawlings, J. & Orme-Johnson, W. H. (1978) *Biochim. Biophys. Acta* **537**, 185–207.
10. Kurtz, D. M., Jr., McMillan, R. S., Burgess, B. K., Mortenson, L. E. & Holm, R. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4986–4989.
11. Cramer, S. P., Hodgson, K. O., Gillum, W. O. & Mortenson, L. E. (1978) *J. Am. Chem. Soc.* **100**, 3398–3407.
12. Cramer, S. P., Gillum, W. O., Hodgson, K. O., Mortenson, L. E., Stiefel, E. I., Chisnell, J. R., Brill, W. J. & Shah, V. K. (1978) *J. Am. Chem. Soc.* **100**, 3814–3819.
13. Mortenson, L. E. (1972) *Methods Enzymol.* **29**, 446–456.
14. Shah, V. K. & Brill, W. J. (1973) *Biochim. Biophys. Acta* **305**, 445–454.
15. Matthews, B. W. (1968) *J. Mol. Biol.* **33**, 491–497.
16. Yamane, T., Weininger, M. S., Mortenson, L. E. & Rossmann, M. G. (1982) *J. Biol. Chem.* **257**, in press.
17. Rossmann, M. G. (1972) *The Molecular Replacement Method* (Gordon and Breach, New York).