Acta Cryst. (1996). D**52**, 194–196

Preliminary crystallographic studies of dimethylsulfoxide reductase from *Rhodobacter capsulatus*

S. Bailey,[a] A. S. McAlpine[a], E. M. H. Duke,[a] N. Benson[b] and A. G McEwan[b]† *at* [a]*CLRC Daresbury Laboratory, Daresbury, Warrington, Cheshire WA4 4AD, England, and* [b]*Centre for Metalloprotein Spectroscopy and Biology, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England*

(Received 7 June 1995; accepted 12 June 1995)

Abstract

Dimethylsulfoxide reductase from the photosynthetic bacterium *Rhodobacter capsulatus* has been crystallized in two similar forms which are suitable for X-ray structure determination. Both crystals forms belong to space group $P4_122$ or $P4_322$, with cell dimensions $a = b = 80.81$, $c = 229.75$ Å (type I crystals) or $a = b = 89.30$, $c = 230.05$ Å (type II crystals) and one molecule in the asymmetric unit. Diffraction has been observed to at least 2.0 Å in type I crystals and to 2.6 Å in type II crystals. Dimethylsulfoxide reductase from *Rhodobacter* is the simplest molybdenum oxotransferase known and this makes it an ideal model to study the structure and function of this class of enzymes.

1. Introduction

Enzymes containing a pterin molybdenum cofactor have a variety of important roles and are found throughout the biological world (Rajagopalan, 1991). These enzymes are molybdenum oxotransferases which catalyse the transfer of an O atom to or from a substrate in a reaction which is linked to the transfer of two electrons. Detailed structural information from X-ray crystallography has not been published for any of these molybdenum enzymes, although the structure of a hyperthermophilic tungstopterin enzyme, aldehyde ferrodoxin oxidoreductase, has recently been published (Chan, Mukund, Kletzin, Adams & Rees, 1995). As a consequence, attempts to describe the ligands which bind the Mo atom have had to rely on EPR spectroscopy and a variety of other spectroscopic approaches (Bray, 1988). Most molybdenum oxotransferases are large proteins which contain, in addition to the molybdenum cofactor, a number of redox centres. The simplest molybdenum oxotransferase which has been described is the dimethylsulfoxide (DMSO) reductase from photosynthetic bacteria of the genus *Rhodobacter*. This enzyme is located in the periplasm where it catalyses the final step in an anaerobic respiratory chain (McEwan, Wetzstein, Ferguson & Jackson, 1985). *Rhodobacter* DMSO reductase is water soluble and can be purified as a monomer (M_r = 82 000) which contains a pterin molybdenum cofactor as its only prosthetic group (Satoh & Kurihara, 1987; McEwan, Ferguson & Jackson, 1991).

New insights into the molecular properties of molybdenum oxotransferases have been made using DMSO reductase. EPR spectroscopic studies have provided new information about structures of signal giving species (Bennett, Benson, McEwan & Bray, 1994) and DMSO reductase was the first enzyme in which the molybdenum cofactor was shown to be in a dinucleotide form, linked to guanosine monophosphate (Johnson, Bastian & Rajagopalan, 1991). Recently, direct support for O-atom transfer catalysed by DMSO reductase has been obtained (Schultz, Hills & Holm, 1995). The simplicity of DMSO reductase makes it an ideal model to study the structure and function of molybdenum oxotransferases. Here we describe preliminary crystallographic studies of the DMSO reductase of *R. capsulatus*.

2. Crystallization and data collection

R. capsulatus, strain H123, was grown phototrophically under conditions described by McEwan *et al.* (1991). Purification was a modification of the method of McEwan *et al.* (1991) which has recently been described. (Bennett *et al.*, 1994). The enzyme is pure as determined by sodium dodecyl sulfate polyacrylamide-gel electrophoresis (Schagger & von Jagow, 1987). Enzyme activity was confirmed as described previously (McEwan *et al.*, 1985) using dithionite reduced methyl viologen as an electron donor.

Initial crystallization conditions were screened using the sparse-matrix method (Jancarik & Kim, 1991). DMSO reductase yielded small crystals under a number of different conditions. Further investigation of these conditions indicated that the most promising conditions were vapour diffusion against 24–28% PEG 4000 in 0.1 *M* sodium citrate buffer pH 5.0–5.6 plus 20% ethanol. Typically, 8 µl of protein at 7 mg ml^{-1} in 5 m*M* sodium phosphate buffer, are mixed with 5 µl of the equilibrating solution. Crystals appear after a few days and continued to grow as tetragonal needles up to a maximum size of 0.15 × 0.15 × 1.0 mm.

Data have been collected from both crystal forms at the synchrotron radiation source at the CLRC's Daresbury Laboratory. X-ray analysis indicated cell dimensions of 80.81 (0.01) × 80.81 (0.01) × 229.63 (0.01) Å for crystals grown between pH 5.0 and 5.3 (type I) and 89.32 (0.02) × 89.32 (0.02) × 230.05 (0.03) Å for crystals grown at pH 5.3–5.6 (type II). The crystal forms appear very similar when examined under a microscope although type I crystals tend to be hollow at the ends of the crystals (Fig. 1). Rotation photographs from the crystals are shown in Fig. 2. Type I crystals diffract strongly to beyond 2.0 Å resolution and are stable in the X-ray beam. Type II crystals diffract to 2.6 Å resolution and these crystals are more radiation sensitive than type I crystals. The data merge in point group 4/*mmm* and examination of the *h*0*l* and 00*l* reflections indicates that the space group for both crystal types is $P4_122$ or its enantiomorph $P4_322$. Data-collection statistics for native data are given in Table 1.

Assuming one molecule per asymmetric unit, the crystal volume per unit molecular weight is 2.28 Å^3 Da^{-1} for type I crystals and 2.8 Å^3 Da^{-1} for type II crystals which is near the average value found for protein crystals (Matthews, 1968) and indicates solvent contents of 46 and 56% for types I and II,

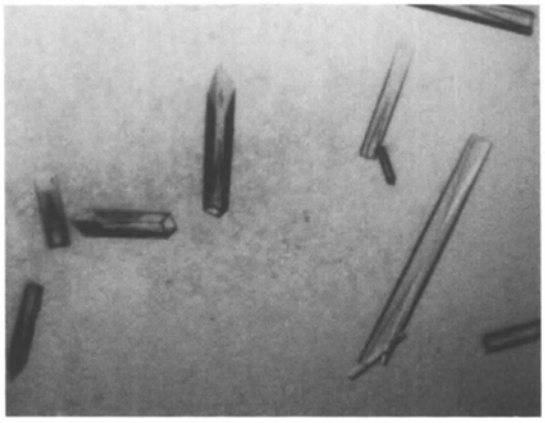
† Present address: Department of Microbiology, University of Queensland, Brisbane, Queensland 4072, Australia.

Table 1. *Data-collection statistics*

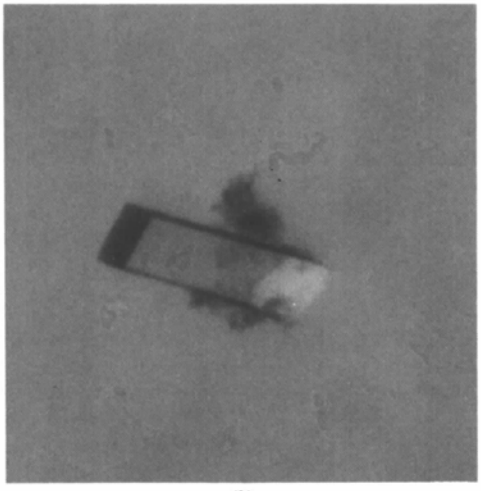
	Type I crystals	Type II crystals
No. of measured reflections	106688	50815
No. of independent reflections	23438	18109
No. of crystals	1	3
Resolution (Å)	2.6	3.0
R_{merge} (%)*	6.4 (12.9)	10.6 (19.7)
Completeness (%)	97.3 (97.8)	94.0 (87.3)
$I/\sigma(I)$	9.6 (5.5)	4.9 (2.5)

* Defined as $R = \sum |I(k) - \langle I \rangle| / \sum I(k)$, where $I(k)$ and $\langle I \rangle$ represent the diffraction intensity values of individual measurements and the corresponding mean values. The summation is over all measurements. Values given in parentheses refer to reflections in the outer resolution shell, 2.74–2.6 Å for type I crystals and 3.21–3.0 Å for type II crystals.

respectively. Both crystal types have been soaked in a variety of heavy-atom solutions and investigation of possible heavy-atom derivatives is continuing. Although type II crystals do not diffract as well, the potential for averaging over the two crystal forms encourages us to continue to work with both types of crystal.



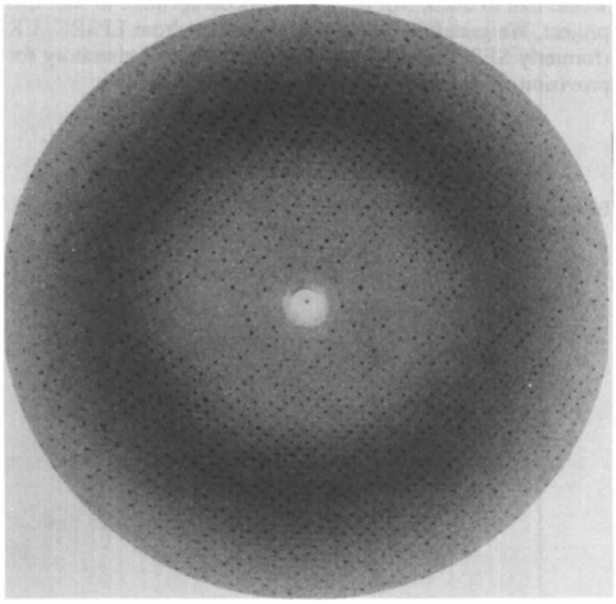
(*a*)



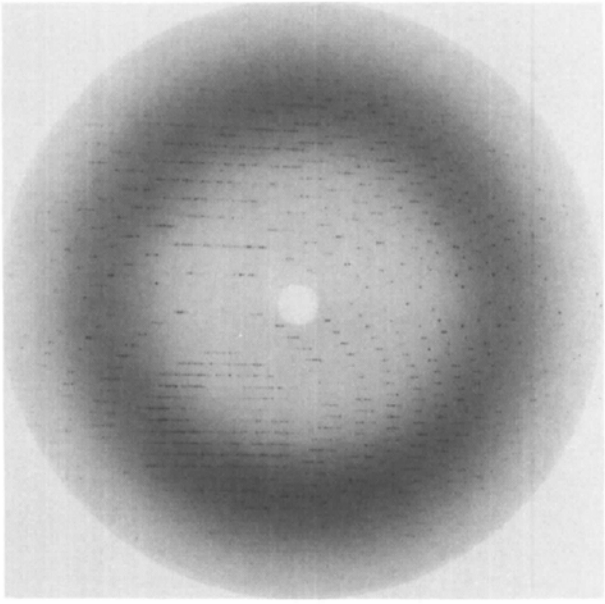
(*b*)

Fig. 1. (*a*) Type I and (*b*) type II crystals of DMSO reductase from *R. capsulatus*. The crystals are approximately 0.15 × 1.0 mm (type I) and 0.1 × 0.5 mm (type II).

Spectroscopic and biochemical methods have so far failed to give definitive details of the nature of the pterin molybdenum cofactor and its interactions with the protein. This information is likely to be essential in order to understand the mechanism of catalysis. The reported crystals give the opportunity for



(*a*)



(*b*)

Fig. 2. 1.5° oscillation photographs from crystals of DMSO reductase from *R. capsulatus*. (*a*) Type I crystals: data were collected on station 9.5 of the SRS at the CLRC's Daresbury Laboratory using the 90 mm MAR imaging plate at a distance of 220 mm from the crystal at $\lambda = 0.92$ Å allowing diffraction to 2.3 Å at the edge of the plate. (*b*) Type II crystals: data were collected on station 9.6 of the SRS at the CLRC's Daresbury Laboratory using the 150 mm MAR imaging plate at a distance of 400 mm from the crystal at $\lambda = 0.87$ Å allowing diffraction to 2.44 Å at the edge of the plate.

a detailed structural analysis to underpin the spectroscopic studies being carried out on this protein.

We thank Miroslav Papiz and Pierre Rizkallah for their help during data collection, and Peter Lindley, David Garner and Miroslav Papiz for encouragement and useful discussions. SB would like to thank Bill Hunter for encouragement to start this project. We gratefully acknowledge support from EPSRC, UK (formerly SERC) for AM and from Daresbury Laboratory for provision of beamtime and other facilities.

References

Bennett, B., Benson, N., McEwan, A. G. & Bray, R. C. (1994). *Eur. J. Biochem.* **225**, 321–331.

Bray, R. C. (1988). *Quart. Rev. Biophys.* **21**, 299–329.

Chan, M. K., Mukund, S., Kletzin, A., Adams, W. W. W. & Rees, D. (1995). *Science*, **267**, 1463–1469.

Jancarik, J. & Kim, S. H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Johnson, J. L., Bastian, N. R. & Rajagopalan, K. V. (1991). *Proc. Natl Acad. Sci. USA*, **87**, 3190–3194.

McEwan, A. G., Ferguson, S. J. & Jackson, J. B. (1991). *Biochem. J.* **207**, 305–307.

McEwan, A. G., Wetzstein, H. G., Ferguson, S. J. & Jackson, J. B. (1985). *Biochim. Biophys. Acta*, **806**, 410–417.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Rajagopalan, K. V. (1991). *Adv. Enzymol. Relat. Areas Mol. Biol.* **64**, 215–290.

Satoh, T. & Kurihara, F. N. (1987). *J. Biochem.* **102**, 191–197.

Schagger, H. & von Jagow, G. (1987). *Anal. Biochem.* **166**, 368–379.

Schultz, B. E., Hills, R. & Holm, R. H. (1995). *J. Am. Chem. Soc.* **117**, 827–828.