Acta Cryst. (1996). D52, 1211–1213

Crystallization and preliminary X-ray crystallographic analysis of the putative [6Fe–6S] prismane protein from *Desulfovibrio vulgaris* (Hildenborough)

ALEXANDER ARENDSEN,[a] YVONNE BULSINK,[a] WILFRED HAGEN,[a] JONATHAN HADDEN,[b] GRAEME CARD,[b] ALAN S. MCALPINE,[b] SUSAN BAILEY, VJACHESLAV ZAITSEV[b] AND PETER LINDLEY[b]* at [a]*Department of Biochemistry, Agricultural University, Wageningen, The Netherlands, and* [b] *CCLRC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, England. E-mail: p.f.lindley@dl.ac.uk*

(*Received 2 May 1996; accepted 11 June 1996*)

Abstract

Crystals of the prismane protein from *Desulfovibrio vulgaris* (Hildenborough) containing a putative [6Fe–6S] cluster have been obtained and X-ray data collected to a resolution of 1.7 Å using synchrotron radiation. The unit cell is orthorhombic with $a = 64.1$, $b = 65.1$ and $c = 154.1$ Å, space group $P2_12_12_1$ (No. 19). The unit cell will readily accommodate four molecules of molecular weight 60 kDa with a corresponding solvent content of approximately 48%.

1. Introduction

Iron–sulfur proteins are found in a wide variety of organisms and are usually involved in electron-transfer processes. These proteins contain one or more iron–sulfur centres, consisting of iron, inorganic sulfur and/or S atoms from cysteine residues organized in a cluster. In size they can vary from around 50 residues, rubredoxin (Watenpaugh, Sieker & Jensen, 1980) to several hundred in for example nitrogenase, (Kim & Rees, 1992*a*,*b*, 1994, Chan, Kim & Rees, 1993), and the redox potentials also show a wide variation (Cammack, 1992; Heering, Bulsink, Hagen & Meyer, 1995). Rubredoxin represents the simplest type of cluster with an Fe atom surrounded by four cysteine S atoms, the plant ferredoxins have two-iron clusters [2Fe–2S] with an additional two cysteine ligands, whereas the larger clusters are comprised of three or four Fe atoms and four S atoms arranged in the shape of a cube and there is often more than one cluster per molecule. In the nitrogenase P cluster, two such [4Fe–4S] are bridged by two cysteine thiol ligands and a disulfide bond. A novel cluster even larger than [4Fe–4S] is supposed to be present at the active site of Fe-only hydrogenases, possibly as a [6Fe–6S] cluster, (Hagen, van Berkel-Arts, Krüse-Wolters, Voordouw & Veeger, 1985). These larger clusters are also proposed in dissimilatory sulfite reductase (Pierik & Hagen, 1991; Arendsen *et al.*, 1993) and carbon monoxide dehydrogenase (Jetten, Pierik & Hagen, 1991). A useful summary of the field of iron–sulfur proteins is that by Johnson (1994).

Hagen, Pierik & Veeger (1989) reported an unusual iron–sulfur protein isolated from *Desulfovibrio vulgaris* (Hildenborough) and suggested a putative [6Fe–6S] cluster on the basis of EPR (electron paramagnetic resonance) measurements and comparison with synthetic clusters of type $[Fe_6S_6(L)_6]^{3-}$ where $L = Cl^-$, Br^-, I^-, RS^- and RO^-. It was noted that the spin concentration for the protein as isolated was substoichiometric and sample dependent and this, together with a later study (Pierik, Hagen, Dunham & Sands, 1992), suggested that the cluster can exist in four different redox states, namely: $[6Fe–6S]^{6+}$ with all the Fe atoms in the fully oxidized Fe^{III} state and spin $S = 0$, the one-electron reduced state $[6Fe–6S]^{5+}$ with a mixture of $S = 1/2$ (10%) and $S = 9/2$ (90%), the two-electron reduced state $[6Fe–6S]^{4+}$ with $S = 0$ or integer, and the three-electron reduced state $[6Fe–6S]^{3+}$ formally containing three Fe^{III} and three Fe^{II} ions and with spin $S = 1/2$. This work was followed by a comprehensive biochemical and biophysical characterization (Pierik, Wolbert, Mutsaers, Hagen & Veeger, 1992), and a primary structure sequence determination (Stokkermans, Pierik *et al.*, 1992). The latter showed that the protein contained nine cysteine residues and that four of these, located towards the N terminus, had a sequence C-2*X*-C-7*X*-C-5*X*-C which could be at least part of a cluster-binding domain. Additional studies using EPR (Stokkermans, Houba *et al.*, 1992), multi-frequency EPR and Mössbauer spectroscopy (Pierik, Hagen, Dunham & Sands, 1992) and low-temperature magnetic circular dichroic spectroscopy (Marritt, Farrar, Breton, Hagen & Thomson, 1995) have shown further evidence for a [6Fe–6S] cluster. Recent resonance Raman studies suggest the presence of an Fe—O—Fe structure, indicating novel functionality for this special cluster (De Vocht, Kooter, Bulsink, Hagen & Johnson, 1996). Crystallographic studies are now in progress at Daresbury Laboratory and the preliminary work is described herein.

2. Experimental

2.1. *Bacterial growth and protein purification*

Desulfovibrio vulgaris (Hildenborough) NCIB 8303 holding an over-producing plasmid (pJSP104, Stokkermans, Pierik *et al.*, 1992) for the putative prismane protein was grown anaerobically as described by van den Berg, Stevens, Verhagen, van Dongen & Hagen (1994). The prismane protein as purified according to the procedure of Pierick, Wolbert *et al.* (1992). The protein was further purified by fast protein liquid chromatography (Pharmacia) on a Q-Sepharose HiLoad column. The protein was finally dialysed against 5 m*M* Tris (pH 8.0), 50 m*M* NaCl and concentrated to 24 mg ml^{-1}. Protein samples were then stored at 203 K prior to use.

2.2. *Crystallization*

The prismane protein crystallized over a range of pH, 5.9–8.0, and PEG 8000 concentrations of 18–24%, using both the sitting and hanging-drop methods. However, the best quality crystals were obtained using the following procedure; (i) 1.3–1.5 µl of protein solution (24 mg ml^{-1} in 5 m*M* Tris PH 8.0, 50 m*M* NaCl) was diluted to 4 µl with 0.1 *M* MES (pH 5.9), 66 m*M* magnesium acetate, (ii) 4 µl samples were set up in crischem plates and equilibrated against 600 µl of well solution

Table 1. *X-ray data for prismane at 1.7Å resolution*

Resolution (Å)	R_{sym}	$I/\sigma(I)$	$N_{symmetry}$	N_{unique}	%Possible collected	N_{total}	% With $I > 3\sigma(I)$
6.61	0.031	11.8	2373	838	79.1	1064	96.1
4.68	0.023	24.9	5706	1867	94.8	2194	99.1
3.82	0.023	18.2	7386	2402	96.5	2841	99.0
3.31	0.025	18.7	9096	2967	98.8	3410	99.0
2.96	0.028	21.1	10240	3364	98.4	3826	98.1
2.70	0.032	15.8	11361	3779	98.9	4232	97.3
2.50	0.039	7.5	12529	4200	100.0	4645	96.8
2.34	0.042	8.1	13353	4516	99.4	4937	96.1
2.21	0.046	9.1	14273	4871	99.9	5264	95.2
2.09	0.052	11.2	15088	5171	100.0	5566	94.5
2.00	0.060	10.8	15773	5399	99.7	5822	91.8
1.91	0.071	9.8	16518	5635	99.8	6087	89.3
1.84	0.090	8.1	17130	5866	100.0	6347	86.2
1.77	0.109	6.4	17758	6053	99.6	6571	82.2
1.71	0.162	3.7	12789	4503	83.3	5532	70.0
	0.036		181423	61431	97.2	68338	91.2

(Average multiplicity = 2.8)

(0.1 *M* MES at pH 5.9, 66 m*M* magnesium acetate), and (iii) plates were incubated at 277 K. Crystals usually appeared within 4 d and grew to a maximum length of 0.7 mm within 10 d as shown in Fig. 1.

2.3. *X-ray data collection*

X-ray data were collected from one crystal mounted in thin-walled glass capillary containing a small volume of mother liquor on station 9.6 of the Synchrotron Radiation Source at CCLRC Daresbury Laboratory, (2 Gev energy with average circulating current of 200 mA). This station derives its synchrotron radiation from a three-pole super-cooled wiggler magnet operating at 5 T and has a platinum-coated cylindrically curved fused quartz mirror and a bent triangular Si(111) monochromator as optical elements. The wavelength selected was 0.87 Å and a distance of 265 mm was set between the sample and a 30 cm diameter MAR-Research image-plate detector system. The sample was cooled to 277 K and a total of 87 1° oscillation images were recorded with exposure times of 60 s per image. All the images were processed using the *MOSFLM* suite of programs (Leslie, 1992) and final scaling and data reduction was achieved using *ROTAVATA* and *AGROVATA* from the CCP4 suite of programs (Collaborative Computational Project, Number 4, 1994).

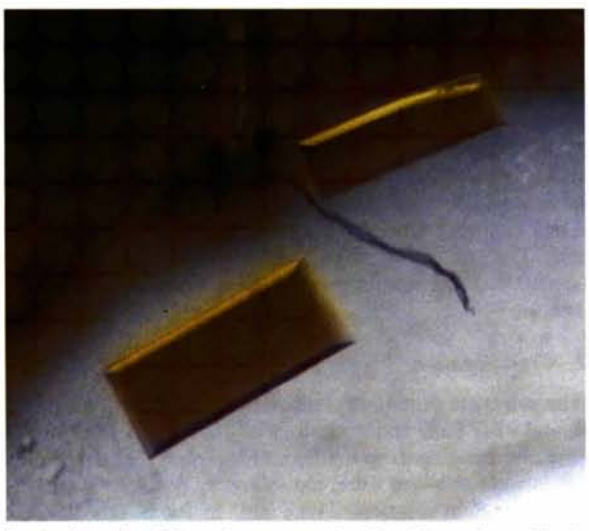

Fig. 1. Crystals of the prismane protein. For X-ray data-collection purposes the prismatic crystals typically have dimensions of 0.1 × 0.3 × 0.7 mm.

3. Results and discussion

Crystals of the prismane protein were shown to diffract at 1.5 Å or even higher, but in order to collect a complete data set at this higher resolution, oscillation ranges considerably smaller than 1° would have had to be used and this was not

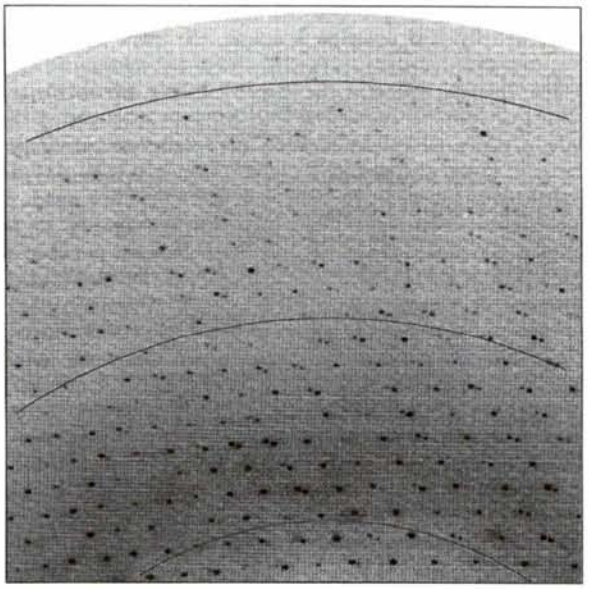

Fig. 2. An outer portion of a typical diffraction pattern of prismane recorded on station 9.6 at the SRS, Daresbury Laboratory; $\lambda = 0.87$ Å, using a MAR-Research image-plate detector system and crystal-to-detector distance = 220 mm. X-ray data clearly extend to 1.5 Å or even higher, but the current working resolution is 1.7 Å.

possible in the synchrotron time available; it may well be the subject of further studies. The statistics of the data set collected to 1.7 Å are given in Table 1 and Fig. 2 shows a typical diffraction image. The native data set is clearly of high quality and a search for putative heavy-atom derivatives indicates that *p*-hydroxymercurybenzoic acid is one likely candidate. Further X-ray analsyis is in progress.

The authors would like to acknowledge the UK inter-research council Biology Program Joint Steering Committee (MRC, BBSRC and EPSRC) which supports the structural biology program at the SRS, Daresbury Laboratory.

References

Arendsen, A. F., Verhagen, M. F. J. M., Wolbert, R. B. G. Pierik, A. J., Stams, A. J. M., Jetten, M. S. M. & Hagen, W. R. (1993). *Biochemistry*, **32**, 10323–10330.

van den Berg, W. A. M., Stevens, A. A. M., Verhagen, M. F. J. M., van Dongen, W. M. A. M. & Hagen, W. R. (1994). *Biochim. Biophys. Acta*, **1206**, 240–246.

Cammack, R. (1992). *Advances in Inorganic Chemistry*, edited by R. Cammack & A. G. Sykes, pp. 281–322. San Diego: Academic Press.

Chan, M. K., Kim, J. & Rees, D. C. (1993). *Science*, **260**, 792–794.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

De Vocht, M L., Kooter, I. M., Bulsink, Y. B. M., Hagen, W. R. & Johnson, M. K. (1996). *J. Am. Chem. Soc.* **118**, 2766–2767.

Hagen, W. R., van Berkel-Arts, A., Krüse-Wolters, K. M., Voordouw, G. & Veeger, C. (1985). *FEBS Lett.* **203**, 59–63.

Hagen, W. R., Pierik, A. J. & Veeger, C. (1989). *J. Chem. Soc. Faraday Trans. J.* **85**, 4083–4090.

Heering, H. A., Bulsink, Y. B. M., Hagen, W. R. & Meyer, T. E. (1995). *Biochemistry*, **34**, 14675–14686.

Jetten, M. S. M., Pierik, A. J. & Hagen, W. R. (1991). *Eur. J. Biochem.* **202**, 1291–1297.

Johnson, M. K. (1994). *Encyclopedia of Inorganic Chemistry*, edited by R. B. King, pp. 1896–1915. Chichester: John Wiley.

Kim, J. & Rees, D. C. (1992*a*). *Science*, **257**, 1677-1682.

Kim, J. & Rees, D. C. (1992*b*). *Nature (London)*, **360**, 553–560.

Kim, J. & Rees, D. C. (1994). *Biochem. Perspect. Biochem.* **33**, 389–397.

Leslie, A. G. W. (1992). In *Jnt CCP4 ESF-EACMB Newslett. Protein Crystallogr.* Vol. 26. Warrington: Daresbury Laboratory.

Marrit, S. J., Farrar, J. A., Breton, J. L. J., Hagen, W. R. & Thomson, A. J. (1995). *Eur. J. Biochem.* **232**, 501–505.

Pierik, A. J. & Hagen, W. R. (1991). *Eur J. Biochem.* **195**, 505–516.

Pierik, A. J., Hagen, W. R., Dunham, W. R. & Sands, R. H. (1992). *Eur. J. Biochem.* **206**, 705–719.

Pierik, A. J., Wolbert, R. B. G., Mutsaers, P. H. A., Hagen, W. R. & Veeger, C. (1992). *Eur. J. Biochem.* **206**, 697–704.

Stokkermans, J. P. W. G., Houba, P. H. J., Pierik, A. J., Hagen, W. R., Van Dongen, W. M. A. M. & Veeger, C. (1992) *Eur. J. Biochem.* **210**, 983–988.

Stokkermans, J. P. W. G., Pierik, A. J., Wolbert, R. B. G., Hagen, W. R., Van Dongen, W. M. A. M. & Veeger, C. (1992). *Eur. J. Biochem.* **208**, 435–442.

Watenpaugh, K. D., Sieker, L. C. & Jensen, L. H. (1979). *J. Mol. Biol.* **131**, 509–522.