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Preliminary X-ray crystallographic study of DsrD protein from the sulfate-reducing bacterium *Desulfovibrio vulgaris*

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DsrD (dissimilatory sulfite reductase D) protein encoded by the *dsr* operon of the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough has been crystallized using the vapour-diffusion method with ammonium sulfate as a precipitating agent. The crystals diffract to 1.7 Å resolution and belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 60.54 (6), b = 65.20 (4), c = 46.41 (3) Å. The crystal contains two DsrD molecules per asymmetric unit, giving a Matthews coefficient (V_M) of 2.6 Å^3 Da^{-1}. A gold-derivative ($NaAuCl_4$) crystal has been successfully prepared.

1. Introduction

DsrD protein is encoded by the *dsr* operon of the sulfate-reducing eubacterium *D. vulgaris* Hildenborough and is composed of only 78 amino-acid residues (Karkhoff-Schweizer *et al.*, 1995). The *dsr* operon also contains the *dsrAB* genes for dissimilatory sulfite reductase (DsrAB; desulfoviridin), which is an $\alpha_2\beta_2$ tetramer with a molecular mass of 180 kDa (Aketagawa *et al.*, 1985). DsrD is expressed constitutively in *D. vulgaris* Hildenborough (Hittel & Voordouw, 2000). A function for the DsrD molecule as a sulfite-binding protein for DsrAB was suggested because of its relatively high content of positively charged lysine residues (Karkhoff-Schweizer *et al.*, 1995). However, a recent spectroscopic study of purified DsrD failed to indicate binding of either sulfite or sulfide with high affinity (Hittel & Voordouw, 2000). Since *BLAST* and *FASTA* searches with the amino-acid sequence of DsrD indicated no strong similarity to protein sequences other than those of DsrD homologs in other sulfate-reducing bacteria, the real function of the DsrD protein in dissimilatory sulfite reduction is still unknown. However, it should have an important role, such as a transcription factor for DsrAB. We have started the X-ray structural study of DsrD in order to clarify its function with the high-resolution three-dimensional structure.

2. Methods and results

DsrD was purified by a method reported recently (Hittel & Voordouw, 2000). Purified DsrD in 40 m*M* Tris–HCl buffer pH 7.2 was concentrated in Centriprep-3 and Centricon-3 ultrafiltration tubes (Amicon) and stored at 193 K until use in crystallization. Purity was checked by SDS–PAGE. Crystallization of DsrD was achieved at 293 K by the hanging-drop vapour-diffusion method. The drops were prepared by mixing a protein solution (40–60 mg ml^{-1}) and 100% saturated ammonium sulfate (final drop volume 10 µl) in a 1:2 ratio. The drops were equilibrated against 0.5 ml 73–75% saturated ammonium sulfate. Suitable crystals for X-ray diffraction study were obtained within one week. The dimen-

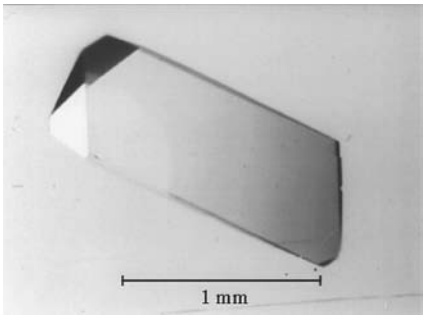

Figure 1
A crystal of the DsrD protein.

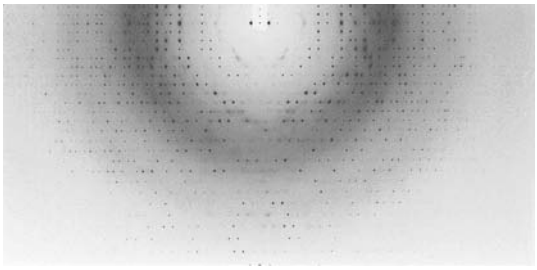

Figure 2
An diffraction pattern of DsrD crystal with a rotation range of 2.0°. The image was obtained using a screenless Weissenberg camera (crystal-to-film distance 286.5 mm) at the BL-6A beamline of the Photon Factory (KEK). The diffraction limit to the edge of the image is 1.6 Å.

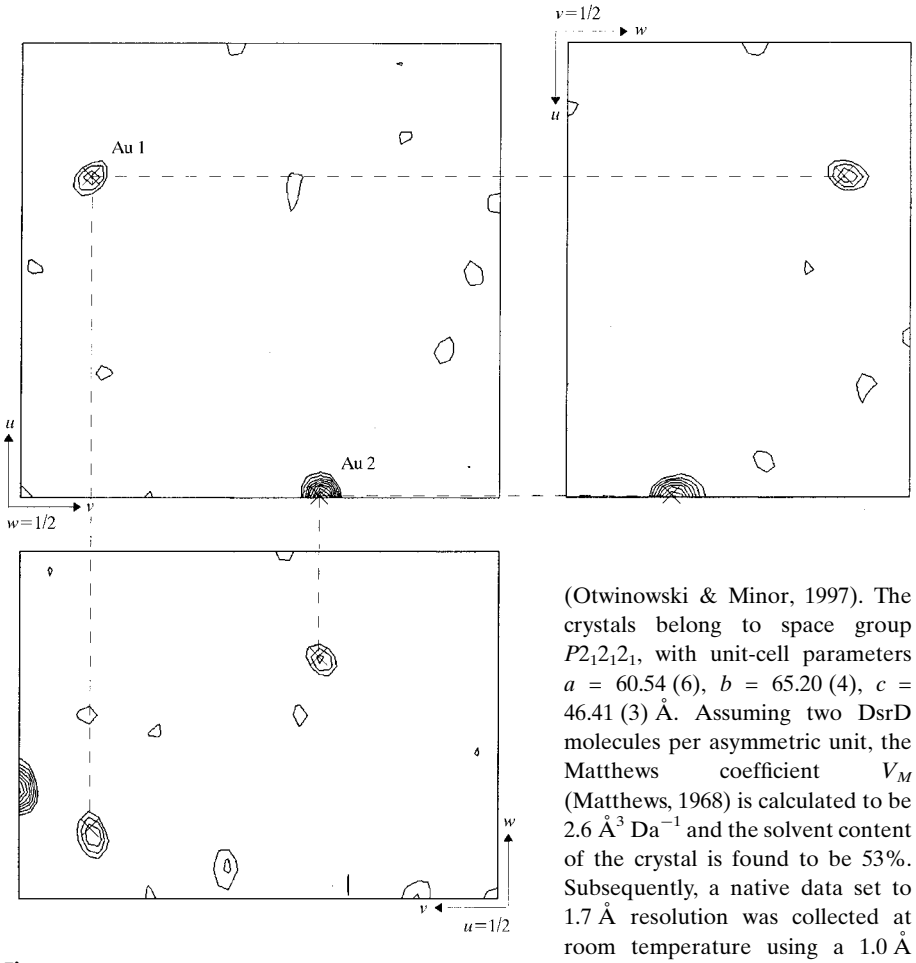

Figure 3
Harker sections of the difference Patterson map calculated from the gold derivative and native crystals of DsrD protein at 2.0 Å (contours 1σ). Two consistent peaks are marked Au1 and Au2.

sions of the crystals obtained were typically 0.3 × 0.3 × 1.0 mm (Fig. 1).

Diffraction data of the native crystals were collected at room temperature to 2.0 Å resolution using a Cu $K\alpha$ rotating-anode source with a Rigaku R-AXIS IV image-plate system. Data processing of 111 445 measured reflections led to 12 938 unique reflections with an overall $R_{\rm merge}$ of 0.049 using the programs *DENZO* and *SCALEPACK* from the *HKL* program package (Otwinowski & Minor, 1997). The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 60.54 (6), b = 65.20 (4), c = 46.41 (3) Å. Assuming two DsrD molecules per asymmetric unit, the Matthews coefficient V_M (Matthews, 1968) is calculated to be 2.6 Å^3 Da^{-1} and the solvent content of the crystal is found to be 53%. Subsequently, a native data set to 1.7 Å resolution was collected at room temperature using a 1.0 Å wavelength X-ray beam on beamline BL-6A of the Photon Factory (KEK). A screenless Weissenberg camera equipped with a 0.1 × 0.1 mm collimator and a cassette of 286.5 mm radius (Sakabe *et al.*, 1996) was used for the data collection. Fig. 2 shows a typical X-ray diffraction pattern. The intensity data, consisting of 68 048 measured reflections and 20 881 unique reflections, were scaled and merged to give an overall $R_{\rm merge}$ of 0.035 using the *HKL* package; the overall completeness (to 1.70 Å) and completeness in the outer shell (1.76–1.70 Å) of the final indexed structure factors were calculated to be 94.3 and 86.6%, respectively.

Searches for heavy-atom derivatives were performed on the native crystals by the conventional soaking method. A diffraction data set of a gold derivative (1 mM $NaAuCl_4$; 72 h soaking) was collected to 2.0 Å resolution at 293 K on beamline BL-6A of the Photon Factory (KEK). The derivative crystal [$P2_12_12_1$, unit-cell parameters a = 60.19 (5), b = 65.25 (9), c = 46.53 (4) Å] was isomorphous to the native crystal. Difference Patterson synthesis at 2.0 Å resolution calculated using the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) indicated two major gold-binding sites in the asymmetric unit (Fig. 3). The positions of two Au atoms, probably binding to two different DsrD molecules, were refined with the program *MLPHARE* (Collaborative Computational Project, Number 4, 1994), considering the anomalous dispersion effect of the Au atom. Interpretation of the electron-density map treated by an electron-density modification method such as the solvent-flattening procedure is now in progress.

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