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Crystallization and preliminary X-ray analysis of catalase–peroxidase from the halophilic archaeon *Haloarcula marismortui*

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Catalase–peroxidases are bifunctional enzymes found in many microorganisms. Crystals of catalase–peroxidase from the halophilic archaeon *Haloarcula marismortui* were obtained using the hanging-drop vapour-diffusion method. The rhombic plate-shaped crystals were grown from purified protein solution using $(NH_4)_2SO_4$ as precipitant at 293 K. The crystal belongs to the monoclinic system, space group $C2$, and diffracted beyond 2.0 Å resolution.

1. Introduction

Catalase functions as a member of the oxidative defence system in microorganisms by removing H_2O_2 before cellular components are damaged. It contains a haem as the catalase centre and is thus unique from a number of non-haem catalases. Based on structural and functional differences, haem-containing catalases can be classified into two groups: monofunctional catalases and catalase–peroxidases (Loewen *et al.*, 2000). Bovine liver catalase is one of the monofunctional enzymes and is a typical homotetramer composed of ~60 kDa subunits; its three-dimensional structure has been discussed (Murthy *et al.*, 1981).

Catalase–peroxidases are distributed in many microorganisms and exhibit bifunctional activity. In *Mycobacterium tuberculosis* the activation of the antitubercular drug isoniazid is believed to result from the peroxidase activity of the catalase–peroxidase (Ramaswamy & Musser, 1998; Chouchane *et al.*, 2000). Most catalase–peroxidases are oligomers composed of ~80 kDa identical subunits. Their primary structures have no similarity to those of the monofunctional catalases, but show significant homologies with those of plant peroxidases and yeast cytochrome *c* peroxidase (Zamocky *et al.*, 2000). Two similar domains recognized in the sequence of the catalase–peroxidase suggest that the enzyme evolved through duplication of the gene encoding the peroxidase (Zamocky *et al.*, 2000). It shows a narrow pH dependence and reacts with 3-amino-1,2,4-triazole, a specific inhibitor of catalase. However, the three-dimensional structures of catalase–peroxidases are not yet available, although a couple of structures of monofunctional catalases have been solved.

Halobacterial catalase–peroxidase (*Hm*CP) purified from *H. marismortui* is a bifunctional enzyme that is a homodimer of 81 kDa identical subunits and contains one haem *b* per subunit. As generally observed for halobacterial enzymes, it is a highly acidic protein and requires a high concentration of salts for stability and enzymatic activity (Mevarech *et al.*, 2000). The structure of this enzyme will aid in understanding the relationship between structure and bifunctional activity and will provide a structural basis for understanding the 'haloadaptation' phenomenon generally observed in the halobacterial enzymes. The present paper describes the crystallization and preliminary X-ray studies of halobacterial catalase–peroxidase from *H. marismortui*.

2. Purification and crystallization

Catalase–peroxidase was purified from *H. marismortui* ATCC43049 as described previously (Fukumori *et al.*, 1985; Cendrin *et al.*, 1994) with some modifications. As the starting material for the purification of the enzyme, a soluble fraction was prepared from cultivated archaeal cells by freeze–thaw disruption, DNase treatment and hypercentrifugation. Successive chromatographic fractionations were then performed on columns of butyl-Toyopearl 650M (Tosoh Co., Tokyo, Japan), Sephacryl S-300 (Pharmacia, Uppsala, Sweden) and octyl-Sepharose (BioRad, Richmond, CA, USA) in the presence of at least 2 *M* KCl. *Hm*CP eluted from the gel-filtration column as a dimer as estimated from size markers. Finally, carefully replicated $(NH_4)_2SO_4$ fractionation yielded *Hm*CP of sufficient purity for crystallization. The purity was checked by SDS–PAGE and the A_{406nm}/A_{280nm} ratio (0.73 or greater). The enzyme was dialyzed against 10 m*M* Tris–HCl buffer pH 8.0 containing 2.0 *M* $(NH_4)_2SO_4$ and 0.5 *M* KCl and was concentrated using a

Table 1
Data-collection statistics.

Values in parentheses refer to the outer shell.

	Native	K_2PtCl_4
X-ray source	PF BL6A	PF BL18B
Space group	*C*2	*C*2
Unit-cell parameters (Å,°)	*a* = 317.7, *b* = 82.1, *c* = 75.1, β = 100.2	*a* = 318.9, *b* = 82.6, *c* = 75.9, β = 100.8
No. measured reflections	478920 (67434)	334880 (47739)
No. unique reflections	128103 (18576)	92942 (13447)
Resolution (Å)	2.0 (2.11–2.00)	2.2 (2.32–2.20)
R_{merge}† (%)	5.3 (14.0)	5.3 (29.1)
Completeness (%)	99.9 (99.7)	99.2 (98.6)
$I/\sigma(I)$	9.1 (5.0)	11.9 (2.4)
Mosaicity (°)	0.4	0.4

† $R_{merge} = \sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the measured intensity of an individual refection and $\langle I\rangle$ is the average intensity of the symmetry-related measurements of this reflection.

Centricon 25 (Amicon Inc., Beverly, MA, USA) to 40–50 mg ml^{-1}. The concentration of *Hm*CP was determined from the optical density at 406 nm using 1.64 mg^{-1} ml as the extinction coefficient. Crystallization was performed by the hanging-drop vapour-diffusion method. The concentrated sample (2 µl) was not mixed with reservoir solution and was equilibrated against 1.0 ml of 3.0–3.2 *M* $(NH_4)_2SO_4$ at 293 K. After incubation of one to two weeks, rhombic and plate-shaped crystals, reddish brown in colour, appeared (Fig. 1). The approximate dimensions of the crystals were 0.5 × 0.2 × 0.1 mm.

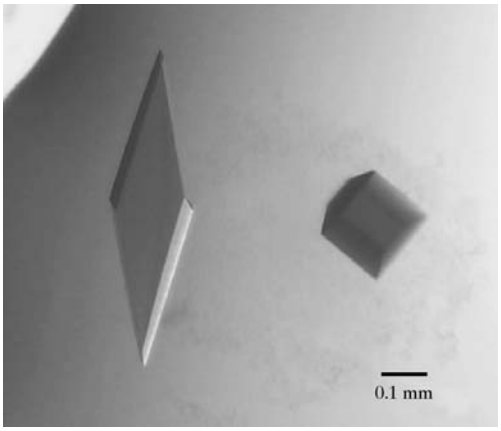

Figure 1
A picture of the *Hm*CP crystals. Crystals are reddish brown in colour and their typical shape is a rhombic plate with approximate dimensions 0.5 × 0.2 × 0.1 mm. The longer diagonal axis of the rhombus corresponds to the crystallographic *a* axis. The crystal on the right was viewed from the *a*-axial direction.

3. Data collection and X-ray analysis

The X-ray diffraction data were collected using synchrotron radiation and the ADSC Quantum 4R CCD detector at the Photon Factory in Tsukuba. Data collection at 293 K resulted in an incomplete data set owing to extensive crystal decay. Intensity images collected at 100 K using cryoprotectants such as glycerol and sucrose resulted in mosaic spreads of greater than 1.5° and were unsuitable for further data collection. Utilization of Li_2SO_4 as a cryoprotectant was expected to suppress the increase of mosaicity on flash-freezing, as several molar ionic salts have been used to form aqueous glasses in spectroscopy (Hays & Fennema, 1982) and in crystallography (Rubinson *et al.*, 2000), and Li_2SO_4 has anions in common with the precipitant $(NH_4)_2SO_4$. A crystal was soaked in 0.1 *M* Tris–HCl pH 8.0, 2.5 *M* $(NH_4)_2SO_4$, 0.5 *M* KCl and 1.0 *M* Li_2SO_4 as cryoprotectant for 30 min and was then flash-frozen directly in the cold nitrogen-gas stream from the cryostat (Oxford Cryosystems, England) operated at 100 K.

The measurements of intensity data were undertaken using an oscillation angle of 1.0°, a wavelength of 1.0 Å and a camera distance of 170 mm. Freezing the *Hm*CP crystal reduces the radiation damage so that nearly complete data can be obtained from a single crystal. Such stability is very important if multiple isomorphous data is to be collected. Flash-frozen crystals diffracted beyond 2.0 Å resolution. This implies that salts of lithium may be effective cryoprotectants for X-ray data collection from crystals grown at high salt concentrations. Data were processed using the interactive program *DPS*/*MOSFLM*/*CCP*4 (Rossmann & van Beek, 1999; Leslie, 1999; Collaborative Computational Project, Number 4, 1994) and data-collection statistics are shown in Table 1. Assuming a molecular weight of 81 kDa for each subunit, the *Hm*CP crystal contains one dimmer per asymmetric unit with a V_M of 2.96 Å^3 Da^{-1}. These values are within the normal range for protein crystals (Matthews, 1968).

To determine the crystal structure, molecular replacement was employed using the coordinates of yeast cytochrome *c* peroxidase (PDB code 1cca) which exhibits 41% similarity to the N-terminal half of *Hm*CP. However, no reasonable solution was obtained.

A heavy-atom derivative was prepared by soaking crystals in mother liquor containing 2 m*M* K_2PtCl_4 for 1 d. The intensity data of the derivative was collected at the Photon Factory synchrotron-radiation facility with a CCD detector. The isomorphous difference Patterson map was calculated using the program *FFT* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994) and gives prominent Pt—Pt self vectors on the Harker section.

Determination of the crystal structure will be carried out by the isomorphous heavy-atom replacement method. A search for further heavy-atom derivatives is now in progress.

References

Cendrin, F., Jouve, H. M., Gaillard, J., Thibault, P. & Zaccai, G. (1994). *Biochim. Biophys. Acta*, **1209**, 1–9.
Chouchane, S., Lippai, I. & Magliozzo, R. S. (2000). *Biochemistry*, **39**, 9975–9983.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Fukumori, Y., Fujiwara, T., Okada-Takahashi, Y., Mukohata, Y. & Yamanaka, T. (1985). *J. Biochem. (Tokyo)*, **98**, 1055–1061.
Hays, D. L. & Fennema, O. (1982). *Arch. Biochem. Biophys.* **213**, 1–6.
Leslie, A. G. W. (1999). In *Crystallographic Computing*. Oxford University Press.
Loewen, P. C., Klotz, M. G. & Hassett, D. J. (2000). *ASM News*, **66**, 76–82.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mevarech, M., Frolow, F. & Gloss, L. M. (2000). *Biophys. Chem.* **86**, 155–164.
Murthy, M. R. N., Reid, T. J. III, Sicignano, A., Tanaka, N. & Rossmann, M. G. (1981). *J. Mol. Biol.* **152**, 465–499.
Ramaswamy, S. & Musser, J. M. (1998). *Tuber. Lung Dis.* **79**, 23–29.
Rossmann, M. G. & van Beek, C. G. (1999). *Acta Cryst.* D**55**, 1631–1640.
Rubinson, K. A., Ladner, J. E., Tordova, M. & Gilliland, G. L. (2000). *Acta Cryst.* D**56**, 996–1001.
Zamocky, M., Janecek, S. & Koller, F. (2000). *Gene*, **256**, 169–182.