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Crystallization and preliminary X-ray studies of cold-active protein-tyrosine phosphatase of *Shewanella* sp.

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Cold-active protein-tyrosine phosphatase (PTPase) of *Shewanella* sp. was expressed, purified and crystallized using the hanging-drop vapour-diffusion method at two different pH values (4.6 and 8.5). Both crystals are orthorhombic and belong to space group $P2_12_12_1$, with unit-cell parameters $a = 56.4$, $b = 76.8$, $c = 81.0$ Å (pH 8.5) and $a = 57.1$, $b = 77.0$ and $c = 81.5$ Å (pH 4.6). Diffraction data to 1.82 Å for the pH 8.5 crystal and 2.33 Å for the pH 4.6 crystal were collected on a multiwire area detector using a rotating-anode X-ray generator.

1. Introduction

So-called 'cold-active enzymes' have recently been purified from psychrophilic microorganisms and ectothermic organisms living in low-temperature environments and characterized (Sheridan *et al.*, 2000). They exhibit high catalytic activities at low temperature, with a lower activation enthalpy than those of mesophilic and thermophilic organisms (Lonhienne *et al.*, 2000). It has been suggested that the high catalytic efficiency of these enzymes at low temperature results from the flexibility of their structures (Jaenicke, 1991). Several studies, including the crystal structure of *Alteromonas haloplanctis* cold-active α-amylase (Aghajari *et al.*, 1998), provided evidence that some structural factors might reduce the rigidity of the cold-active enzymes, such as the low content of proline residues in loops and turns, the decrease in the number of interior electrostatic interactions mediated by arginine residues and the exposure of non-polar groups to solvent. However, further evidence of the structural properties responsible for the high catalytic activity at low temperature is needed for a better understanding of cold-active enzymes.

To investigate the relationship between the structure and function of cold-active enzymes, we have previously isolated a cold-active protein-tyrosine phosphatase (PTPase) from *Shewanella* sp. (Tsuruta *et al.*, 1998). This enzyme showed a high catalytic activity at low temperature and its activation enthalpy in the temperature range below 293 K was remarkably lower than at intermediate temperatures above 293 K (Tsuruta *et al.*, unpublished data). It was suggested that this reduction in the enthalpy resulted from a conformational change in the catalytic centre of the enzyme molecule at around 293 K.

Additionally, the catalytic residue of this enzyme is histidine unlike other PTPases (Tsuruta & Aizono, 1999) and this enzyme protein has the conserved amino-acid sequence observed in many protein-Ser/Thr phosphatases, in spite of its PTPase activity (Tsuruta & Aizono, 2000; Zhou *et al.*, 1994). The conserved amino-acid residues are situated in the catalytic centres of protein-Ser/Thr phosphatases and are involved in the cleavage of phosphoester bonds in substrates with the aid of the catalytic residue (histidine) (Zhou *et al.*, 1994). From recent studies on the crystal structure of human protein-Ser/Thr phosphatase, it is understood that the dephosphorylation is a single step involving a metal-ion-activated water molecule (Egloff *et al.*, 1995). On the other hand, many PTPases dephosphorylate phosphotyrosine residues in proteins through a phosphoryl-enzyme intermediate (Guan & Dixon, 1991). Accordingly, the cold-active PTPase may have a different mechanism of catalytic reaction from other PTPases that explains its high catalytic activity at low temperature. A reaction mechanism using a water molecule activated by Mg^{2+} ions similar to that of protein-Ser/Thr phosphatases might be able to produce efficient catalysis at low temperature.

Further investigation of the crystal structure of cold-active PTPase is very important for understanding the structural properties leading the efficient function of this enzyme at low temperature. In this paper, we report the crystallization of cold-active PTPase of *Shewanella* sp. and some results of the preliminary crystallographic studies.

2. Expression and crystallization

The expression vector (pTRCPTP) was constructed with insertion of the gene encoding cold-active PTPase into an *Nco*I site and a *Xho*I site at the each end of the vector pET22b (Novagen). Recombinant cold-active PTPase of *Shewanella* sp. was purified to

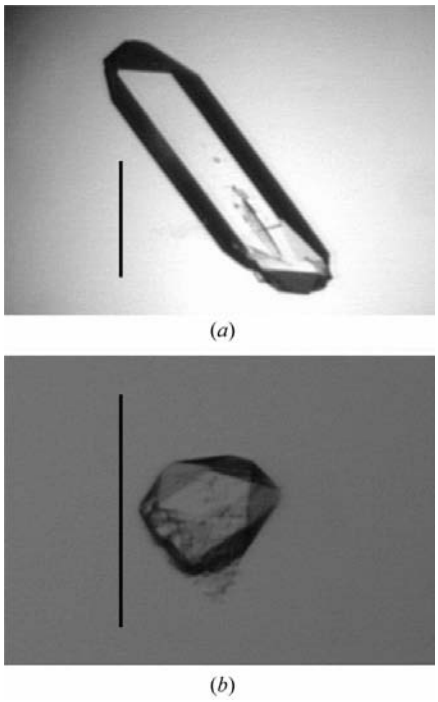

Figure 1
Crystals of cold-active protein-tyrosine phosphatase of *Shewanella* sp. grown from (*a*) 30% polyethylene glycol 4000 and 0.2 *M* sodium acetate in 0.1 *M* Tris–HCl pH 8.5 and (*b*) 30% polyethylene glycol 4000 and 0.2 *M* ammonium acetate in 0.1 *M* sodium acetate buffer pH 4.6. Approximate dimensions are 0.4 × 0.2 × 0.2 mm for the pH 8.5 crystal and 0.1 × 0.1 × 0.1 mm for the pH 4.6 crystal. The scale bars are 0.2 mm long.

homogeneity from *Escherichia coli* AD494 (DE3) with the expression vector pTRCPTP. The enzyme concentration used for crystallization was 4.5 mg ml^{-1}, which was estimated spectrophotometrically on the basis of the molecular extinction coefficient at 280 nm of the native cold-active PTPase, 7.75 × 10^4 cm mol^{-1} l^{-1} (Tsuruta *et al.*, 1998). Crystals were grown using the hanging-drop vapour-diffusion method (McPherson, 1985). For initial crystallization trials, Crystal Screen I kit (Hampton Research) was used as the reservoir solution. 2 µl of the protein solution was mixed with an equal volume of the reservoir solution. Each hanging drop was then vapour-equilibrated against 0.5 ml of the reservoir solution at 277 K.

Crystals (pH 8.5 and pH 4.6) appeared in two weeks under two different conditions (Fig. 1): 30%(*w*/*v*) polyethylene glycol 4000 and 0.2 *M* sodium acetate trihydrate in 0.1 *M* Tris–HCl buffer at pH 8.5 (Crystal Screen I kit No. 22) and 30%(*w*/*v*) polyethylene glycol 4000 and 0.2 *M* ammonium acetate in 0.1 *M* sodium acetate buffer at pH 4.6 (Crystal Screen I kit No. 10). The pH 8.5 and pH 4.6 crystals were octahedral and dodecahedral in shape, with dimensions of 0.4 × 0.2 × 0.2 and 0.1 × 0.1 × 0.1 mm, respectively.

Table 1
Crystal data and X-ray diffraction data-collection statistics for pH 8.5 and pH 4.6 crystals.

Values in parentheses are for the highest resolution shell.

	pH 8.5 crystal	pH 4.6 crystal
X-ray source	Cu *Kα*	Cu *Kα*
Wavelength (Å)	1.54	1.54
Temperature (K)	103	103
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å, °)	*a* = 56.4, *b* = 76.8, *c* = 81.0, *α* = *β* = *γ* = 90	*a* = 57.1, *b* = 77.0, *c* = 81.5, *α* = *β* = *γ* = 90
Resolution (Å)	1.82	2.33
Completeness (%)	91.4 (75.3)	94.2 (72.3)
R_{merge} (%)	6.3 (23.6)	6.8 (21.1)
Total observations	129108	69875
Unique reflections	29747	15009

3. X-ray data collection

The diffraction data from the pH 8.5 and pH 4.6 crystals were collected to 1.82 and 2.33 Å, respectively, with a scan width of 0.25° per frame and an exposure time of 40 s per frame (total of 1120 frames) using Cu *Kα* X-ray radiation using a MacScience M18XHF rotating-anode generator and a Bruker HI-STAR multiwire area detector under a cold nitrogen-gas stream. The crystals were frozen beforehand in liquid nitrogen. The X-ray data were processed with the *SAINT* software package (Siemens). The data set for the pH 8.5 crystal consisted of 129 108 observed reflections, which reduced to 29 747 unique reflections with an R_{merge} of 6.3% on intensities. The completeness of the data is 91.4%. Almost the same data was obtained for the pH 8.5 and pH 4.6 crystals, as shown in Table 1.

Symmetry and systematic absences of the *h*00, 0*k*0 and 00*l* reflections are consistent with the orthorhombic space group $P2_12_12_1$. The unit-cell parameters are *a* = 56.4, *b* = 76.8, *c* = 81.0 Å, *α* = *β* = *γ* = 90° for the pH 8.5 crystal and *a* = 57.1, *b* = 77.0, *c* = 81.5 Å, *α* = *β* = *γ* = 90° for the pH 4.6 crystal. For the pH 8.5 crystal, this results in a unit-cell volume of 0.351 × 10^6 Å^3. Assuming one molecule of 38.5 kDa in the asymmetric unit, the volume per unit mass, V_M, is 2.28 Å^3 Da^{-1}. The value is within the range (1.7–3.5 Å^3 Da^{-1}) usually found for protein crystals (Matthews, 1968). The calculated solvent content is 46.2% and a specific volume of 0.738 cm^3 g^{-1} is estimated from the amino-acid composition (Cohn & Edsall, 1943).

A search for isomorphous heavy-atom derivatives is in progress.

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References

Aghajari, N., Feller, G., Gerday, C. & Haser, R. (1998). *Structure*, **6**, 1503–1516.
Cohn, E. J. & Edsall, J. T. (1943). *Proteins, Amino Acids and Peptides*, pp. 370–381. New York: Reinfold.
Egloff, M.-P., Cohen, P. T. W., Reinemer, P. & Barford, D. (1995). *J. Mol. Biol.* **254**, 942–959.
Guan, K. & Dixon, J. E. (1991). *J. Biol. Chem.* **266**, 17026–17030.
Jaenicke, R. (1991). *Eur. J. Biochem.* **202**, 715–728.
Lonhienne, T., Gerday, C. & Feller, G. (2000). *Biochim. Biophys. Acta*, **1543**, 1–10.
McPherson, A. (1985). *Methods Enzymol.* **114**, 112–125.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Sheridan, P. P., Panasik, N., Coombs, J. M. & Brenchley, J. E. (2000). *Biochim. Biophys. Acta*, **1543**, 417–433.
Tsuruta, H. & Aizono, Y. (1999). *J. Biochem.* **125**, 690–695.
Tsuruta, H. & Aizono, Y. (2000). *J. Biochem.* **127**, 143–149.
Tsuruta, H., Tsuneta, S. T., Ishida, Y., Watanabe, K., Uno, T. & Aizono, Y. (1998). *J. Biochem.* **123**, 219–225.
Zhou, S., Clemens, J. C., Stone, R. L. & Dixon, J. E. (1994). *J. Biol. Chem.* **269**, 26234–26238.