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Crystallization and preliminary X-ray diffraction studies of catalase–peroxidase from *Synechococcus* PCC 7942

Kei Wada,[a]* Toshiji Tada,[a] Yoshihiro Nakamura,[a] Takayoshi Kinoshita,[b] Masahiro Tamoi,[c] Shigeru Shigeoka[c] and Keiichiro Nishimura[a]

[a]Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka 599-8570, Japan, [b]Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd, Tsukuba, Ibaraki 300-2698, Japan, and [c]Department of Food and Nutrition, Faculty of Agriculture, Kinki University, Nakamachi, Nara 631-8505, Japan

Correspondence e-mail: keiwada@biochem.osakafu-u.ac.jp

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The recombinant catalase–peroxidase of *Synechococcus* PCC 7942 overexpressed in *Escherichia coli* was purified and crystallized by the hanging-drop vapour-diffusion method using sodium formate as a precipitant. The crystals belonged to the tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 109.3$, $c = 202.0$ Å. The calculated V_M value based on a dimer in the asymmetric unit was 1.9 Å^3 Da^{-1}. A native data set was collected to 2.3 Å resolution from a frozen crystal using synchrotron radiation at SPring-8.

1. Introduction

Active oxygen species including superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radicals are produced as byproducts in both aerobic respiration and oxygenic photosynthesis in organisms. Owing to the high reactivity of active oxygen species with biomaterials, most organisms have evolved sophisticated and efficient enzyme systems to avoid oxidative damage (Asada, 1994). Animals, plants and most microorganisms contain H_2O_2-scavenging enzymes as independent catalases and peroxidases, whereas some bacteria and fungi contain an enzyme exhibiting both catalase and peroxidase activities, which is called catalase–peroxidase (Hochman, 1993).

Recently, a catalase–peroxidase was isolated from the cyanobacterium *Synechococcus* PCC 7942 (*S.* 7942) and its nucleotide sequence was analyzed (Mutsuda *et al.*, 1996). The gene encodes a 720-residue haem enzyme with a molecular mass of 80 kDa. The enzyme exists in a dimeric form consisting of two identical subunits. The deduced amino-acid sequence shows a high homology with catalase–peroxidases from *E. coli* HP I (55.8%; Triggs-Raine *et al.*, 1988), *Salmonella typhimurium* (55.5%; Loewen & Stauffer, 1990) and *Mycobacterium tuberculosis* (52.9%; Zhang *et al.*, 1992).

The catalase–peroxidase of *S.* 7942 contains two His residues, one distal (position 94) and the other proximal (position 262), both of which are considered to be essential for its activity (Mutsuda *et al.*, 1996). The amino-acid sequences around the distal and proximal His regions are also observed in the regions of catalase–peroxidases belonging to class I peroxidases of other microorganisms (Kaput *et al.*, 1982) and plants (Mittler & Zilinskas, 1991; Ishikawa *et al.*, 1996). The *S.* 7942 enzyme has a dominant catalase activity equal to or greater than that of typical monofunctional catalases. Interestingly, the enzyme is not inhibited by 3-amino-1,2,4-triazole (Mutsuda *et al.*, 1996), which is an irreversible inhibitor of typical catalases. Furthermore, the turnover number (k_{cat}) for H_2O_2 in the catalatic activity of the enzyme is markedly higher than that of other catalase–peroxidases (Mutsuda *et al.*, 1996; Johnsson *et al.*, 1997; Obinger *et al.*, 1997; Regelsberger *et al.*, 1999).

Clarification of the three-dimensional structure would be one of the most effective ways to understand these unique characteristics of the catalase–peroxidase from *S.* 7942. A comparison of its structure with those of peroxidases and catalases should provide some important information about the mechanism of the bifunctional reaction. However, there is no structural report of a catalase–peroxidase and no enzymes are known that have high sequence homology with the catalase–peroxidases. We undertook an analysis of the structure by means of X-ray crystallography. Here, we report the crystallization and preliminary X-ray analysis of the catalase–peroxidase of *S.* 7942.

2. Experimental

2.1. Purification

All purification steps were carried out using an FPLC system from Amersham-Pharmacia at 277 K. The recombinant catalase–peroxidase was overexpressed in *E. coli* BL21(DE3)plysS (Mutsuda *et al.*, 1996). *E. coli* cells were harvested, resuspended in 30 ml of 50 m*M* potassium phosphate buffer pH 7.0 and sonicated. The lysate was centrifuged to obtain a crude enzyme. The supernatant was loaded onto a HiLoad 26/10 Q Sepharose ion-

Table 1
Summary of crystal parameters and data-collection statistics.

Values in parentheses are for the last resolution shell (2.42–2.30 Å).

Resolution (Å)	2.3
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 109.3$, $c = 202.0$
R_{merge} (%)	7.4
$I/\sigma(I)$	6.5 (1.6)
No. of reflections	335521
Unique reflections	92049
Completeness (%)	89.6 (78.2)
Mosaicity (°)	0.3

exchange column equilibrated with phosphate buffer and eluted with a linear gradient of KCl (0–0.6 *M*). To the active fractions, ammonium sulfate was added at 30% saturation and precipitate was removed by centrifugation. The resulting supernatant was chromatographed on a HiLoad 16/10 Phenyl Sepharose column equilibrated with phosphate buffer containing 30% saturated ammonium sulfate and eluted with a descending linear gradient of 30–0% saturated ammonium sulfate. The ammonium sulfate in the active fractions was removed by excess dialysis against phosphate buffer. The resulting enzyme solution was applied to a Mono-Q HR 5/5 column equilibrated with phosphate buffer and eluted with a linear gradient of 0–0.6 *M* KCl. The purified enzyme was collected and the purity was confirmed by SDS–PAGE.

2.2. Crystallization

The purified protein was dialyzed against 50 m*M* HEPES buffer pH 7.0 and concentrated for crystallization with an Ultrafree (Millipore) filter. All crystallization trials were carried out using the hanging-drop vapour-diffusion method at 277 K. The initial trials were performed using the commercially available sparse-matrix screening kits Crystal Screen I and II from Hampton Research (Jancarik & Kim, 1991) by mixing 1 µl of protein solution with 1 µl of various reservoir solutions and equilibrating the drops over their respective reservoirs. The initial conditions found to be the best were optimized by varying the concentrations of protein, precipitants and buffer systems, including the concentration and pH.

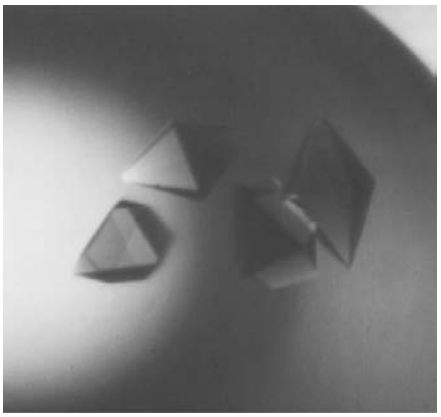

Figure 1
A typical crystal of the catalase–peroxidase from *S.* 7942. The dimensions of the crystals are approximately 0.5 × 0.25 × 0.25 mm.

2.3. Data collection and analysis

Preliminary X-ray diffraction studies were carried out using an R-AXIS IIc image-plate area detector mounted on a Rigaku RU-300 rotating-anode source operating at 40 kV, 100 mA with Cu $K\alpha$ radiation. A complete data set was collected at 100 K on a MAR CCD detector using synchrotron radiation of wavelength 1.0 Å at the BL-41XU station of SPring-8, Japan. The crystal-to-detector distance was 230 mm and 45 images were recorded at 1° intervals with an exposure time of 1 s per image. The intensity data were processed with the program *MOSFLM* (Steller *et al.*, 1997) and scaled using the program *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The purification of the enzyme yielded approximately 3 mg of protein, the purity of which was evaluated to be over 95% by Coomassie brilliant-blue stained gels. In the initial crystallization trials, small crystals were obtained from a drop containing sodium formate as a precipitant. The crystallization conditions finally established involved a protein solution of 12 mg ml^{-1} in 50 m*M* HEPES pH 7.0 and a reservoir solution consisting of 4.3 *M* sodium formate and 100 m*M* sodium citrate at pH 6.3. A 3 µl drop consisting of 2 µl of protein solution and 1 µl of reservoir solution was equilibrated against 0.5 ml of the above-mentioned reservoir solution. Crystals formed within one week and grew to maximum dimensions of 0.5 × 0.25 × 0.25 mm in one month (Fig. 1).

The crystals mounted in thin-walled glass capillaries were easily damaged and only diffracted to about 3.4 Å resolution when exposed to Cu $K\alpha$ radiation at room temperature. A complete data set was collected to a resolution of 2.3 Å using synchrotron radiation at 100 K. A typical X-ray diffraction pattern of the catalase–peroxidase is shown in Fig. 2. The crystal class was determined to be tetragonal space group $P4_12_12$ (or its enantiomorph $P4_32_12$) based on the symmetry and the systematic absences of the reflections, with unit-cell parameters $a = b = 109.3$, $c = 202.0$ Å. Detailed crystal parameters and data-collection statistics are shown in Table 1. Assuming a dimer in the asymmetric unit, the V_M value as defined by Matthews (1968) was 1.9 Å^3 Da^{-1}, corresponding to a solvent content of 35%.

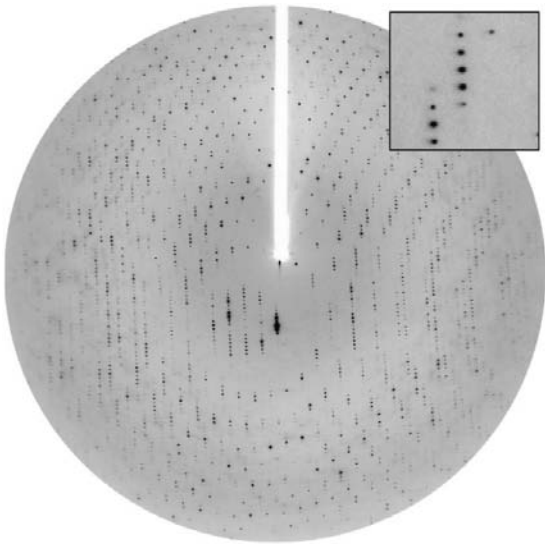

Figure 2
A diffraction image of the catalase–peroxidase from *S.* 7942. Magnified spots are shown in the upper right corner.

We have now started overexpression and crystallization of the selenomethionine derivative of the catalase–peroxidase. The phase problem will be solved by multi-wavelength anomalous dispersion (MAD) experiments.

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