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Crystallization and preliminary X-ray analysis of a bifunctional catalase-phenol oxidase from *Scytalidium thermophilum*

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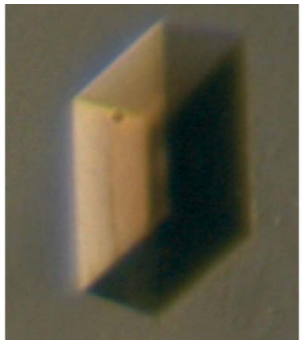


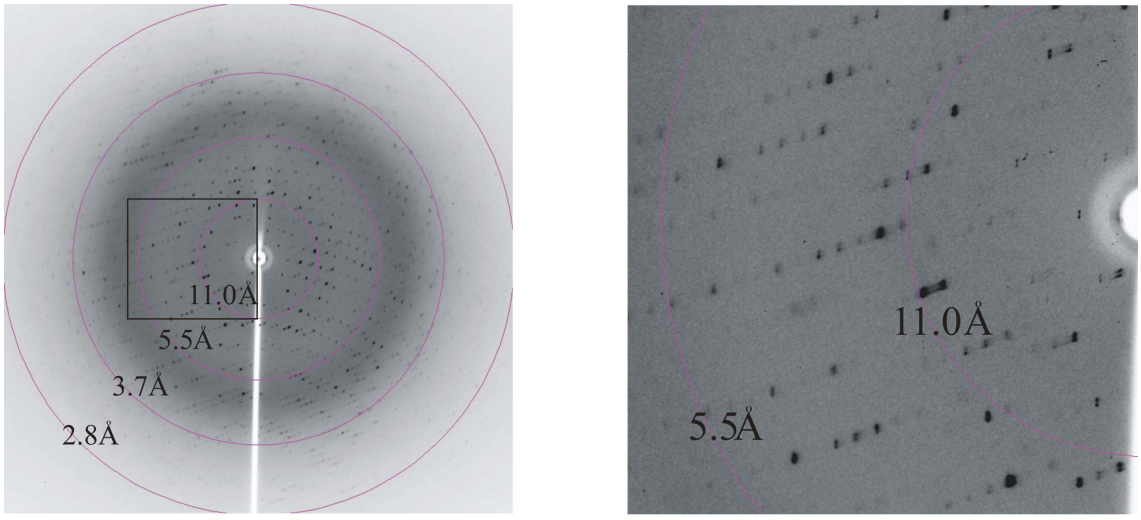
Catalase-phenol oxidase from *Scytalidium thermophilum* is a bifunctional enzyme: its major activity is the catalase-mediated decomposition of hydrogen peroxide, but it also catalyzes phenol oxidation. To understand the structural basis of this dual functionality, the enzyme, which has been shown to be a tetramer in solution, has been purified by anion-exchange and gel-filtration chromatography and has been crystallized using the hanging-drop vapour-diffusion technique. Streak-seeding was used to obtain larger crystals suitable for X-ray analysis. Diffraction data were collected to 2.8 Å resolution at the Daresbury Synchrotron Radiation Source. The crystals belonged to space group $P2_1$ and contained one tetramer per asymmetric unit.

1. Introduction

Catalases (EC 1.11.1.6) belong to a group of haem-containing metalloenzymes which are usually homotetramers; their major function is the decomposition of hydrogen peroxide (H_2O_2) to dioxygen and water (Goldberg & Hochman, 1989). Catalases have been classified into three groups: monofunctional haem (typical) catalases, catalase-peroxidases and manganese catalases (Zamocky & Koller, 1999). A secondary function of catalases has been reported to be the oxidation of hydrogen donors such as methanol, ethanol, formic acid and phenols, with the concomitant consumption of peroxide, called peroxidic activity (Aebi, 1984). A further secondary function of catalases, namely an oxidase activity in the absence of hydrogen peroxide, has been reported by Vetrano *et al.* (2005) for mammalian catalases, although the enzyme was not further characterized. The first study describing the characterization of a bifunctional catalase–oxidase enzyme and demonstrating that it possessed peroxide-independent phenol oxidase activity was reported by Sutay Kocabas *et al.* (2008) for *Scytalidium thermophilum* catalase. Since *S. thermophilum* catalase showed both catalase and phenol oxidase activities, it was named catalase-phenol oxidase (CATPO).

Nonhaem phenol oxidases have, however, been reported to show catalase activity. Jolley *et al.* (1972) observed a reaction between mushroom tyrosinase and hydrogen peroxide that yielded the spectroscopically observable product oxytyrosinase. Recently, Yamazaki *et al.* (2004) and Garcia-Molina *et al.* (2005) have also reported that mushroom tyrosinase exhibits catalase activity. Catalase-like activity of isoenzymes from sweet potato (*Ipomea batatas*) has also been observed (Gerdemann *et al.*, 2001). In contrast, there are only two reports of catalases displaying oxidative activity (Vetrano *et al.*, 2005; Sutay Kocabas *et al.*, 2008).

Catalases have been the subject of many structural studies. The first catalase structures solved were those of a monofunctional catalase from *Penicillium vitale* at 2.0 Å resolution (Vainshtein *et al.*, 1986) and of bovine liver catalase at 2.5 Å resolution (Fita *et al.*, 1986). The haem in the bovine liver enzyme is a haem b, while subsequent studies on the *P. vitale* catalase revealed the presence of a haem d, which is also present in *Escherichia coli* catalase hydroperoxidase II (Murshudov *et al.*, 1996). Currently, over 75 structures of catalases have been deposited in the PDB, compared with only ten structures of catechol and phenol oxidases.



(*a*) (*b*)

Figure 1
X-ray diffraction pattern of CATPO (*a*) and a close-up view of the boxed low-resolution region from the same image (*b*).

The purpose of the current study was to purify and crystallize the dual-function catalase-phenol oxidase from *S. thermophilum* in order to provide a structural basis from which to understand the mechanistic nature of its two catalytic activities. CATPO is a homotetramer with a molecular weight of 320.0 kDa and 717 residues per subunit.

2. Materials and methods

2.1. Protein cultivation and purification

S. thermophilum (type culture *Humicola insolens*; ATCC No. 16454) was cultivated and CATPO was purified tenfold from the culture supernatant with 46% yield by anion-exchange and gel-filtration chromatography techniques as previously described (Sutay Kocabas *et al.*, 2008).

2.2. Crystallization

Purified CATPO at a concentration of 3 mg ml^{-1} in 50 m*M* Tris–HCl pH 8.0 was used to set up crystallization screening trials using Crystal Screens 1 and 2, Index, MembFac, SaltRx, Natrix (Hampton Research, USA) and Wizard 1 and 2 (Emerald Biosystems, USA). 60 µl crystallization solution was pipetted into each well of a 96-well plate. 0.5 µl purified enzyme solution was mixed with 0.5 µl well solution using an Oryx4 liquid-handling robot (Douglas Instruments, USA) and crystallization trials were carried out using the sitting-drop vapour-diffusion technique. Plates were covered with adhesive sealing film (Hampton Research, USA) and incubated at 277, 291 and 303 K.

2.3. Data collection and processing

Prior to data collection, a CATPO crystal (200 × 120 × 70 µm) was coated in Paratone-N oil (Hampton Research, USA) and flash-cooled in liquid nitrogen. Native X-ray diffraction data were recorded on a MAR 165 CCD C-TRAIN fluorescence detector at 100 K on the macromolecular crystallography beamline 10.1 at the Daresbury Synchrotron Radiation Source (SRS; Daresbury, England). Diffraction data were recorded at a wavelength of 1.196 Å to a resolution of 2.8 Å. A total of 191 images were recorded with an oscillation angle of 0.5° and an exposure time of 1 s per image. Data were processed with *DENZO* and *SCALEPACK* from the *HKL*-2000 suite (Otwinowski & Minor, 1997). A diffraction image of CATPO is shown in Fig. 1(*a*), with a close-up view of the low-resolution region shown in Fig. 1(*b*).

3. Results and discussion

The first crystal was observed in 28%(*w*/*v*) polyethylene glycol (PEG) 1500, 100 m*M* bis-tris buffer pH 5.0 at 291 K. Using this initial crystallization condition, the addition of different salts and additives and the use of different types of PEG and crystallization techniques (hanging drop, sitting drop, with and without paraffin oil) at various pH values and temperatures were tested. The streak-seeding technique was also used to prevent cluster formation and improve crystal size. A diffraction-quality CATPO crystal was obtained using a crystallization solution containing 24%(*w*/*v*) PEG 2000, 10 m*M* NaCl, 10 m*M* $CaCl_2$ and 3% 6-aminocaproic acid in 100 m*M* bis-tris buffer pH 6.5 at 291 K with the hanging-drop vapour-diffusion technique after applying streak-seeding. Crystallization droplets were prepared on siliconized cover slips by mixing 1 µl protein solution at 3 mg ml^{-1}

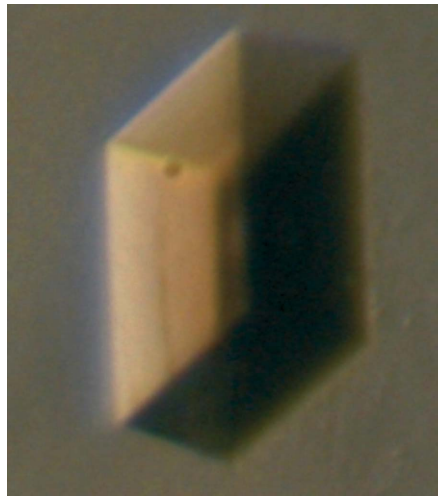


Figure 2
Crystal of CATPO. The approximate dimensions of the crystal are 200 × 120 × 70 µm.

Table 1
Crystallographic summary for catalase-phenol oxidase from *S. thermophilum*.

Values in parentheses are for the outermost shell of the resolution range.

Synchrotron beamline	Daresbury station 10.1
Wavelength (Å)	1.196
Space group	$P2_1$
Resolution range (Å)	50.0–2.8 (2.9–2.8)
Unit-cell parameters (Å, °)	a = 95.7, b = 122.6, c = 117.9, β = 116.0
No. of observed reflections	74955
No. of unique reflections	33958 (860)
Completeness (%)	56.1 (14.3)
Redundancy	2.2 (1.2)
$\langle I/\sigma(I)\rangle$	11.9 (3.2)
$R_{\rm merge}$† (%)	9.6 (18.3)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

and 1 µl reservoir solution and were equilibrated against 1 ml of the same reservoir solution. CATPO crystals appeared after 3 d at 291 K and were harvested for data collection after two weeks. Crystals were monoclinic with a brownish-green colour (Fig. 2).

The recorded data were highly anisotropic, with multiple lattices. In addition, the crystal was highly mosaic. The resultant spot profiles were poor and Fig. 1(*b*) shows one of the best images from the recorded data. As a consequence of these factors, the final data set could only be processed to a completeness of 56% while retaining good statistics (Table 1).

Cell-content analysis using the program *MATTHEWS_COEF* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994) suggested that four subunits of CATPO were present in the asymmetric unit, with a Matthews coefficient of 1.94 Å^3 Da^{-1} and a corresponding solvent content of 37%. In order to obtain better and more complete data, work is in progress to grow better quality crystals and to improve the cryoprotectant conditions.

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