

# Crystallization and preliminary X-ray analysis of the catalase–peroxidase KatG from *Burkholderia pseudomallei*

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The bifunctional catalase–peroxidase KatG encoded by the *katG* gene of *Burkholderia pseudomallei* has a predicted subunit size of 81.6 kDa. It shows high sequence similarity to other catalase–peroxidases of bacterial, archaeobacterial and fungal origin, including 64% identity to KatG from *Mycobacterium tuberculosis* and lesser sequence similarity to members of the plant peroxidase family. Crystals from this protein were grown in 16–20% PEG 4000, 20% 2-methyl-2,4-pentanediol and 0.1 M sodium citrate pH 5.6 by the hanging-drop vapour-diffusion method at 293 K. These crystals diffracted beyond 1.8 Å resolution and belong to space group  $P2_12_12_1$ , with unit-cell parameters  $a = 100.9$ ,  $b = 115.6$ ,  $c = 175.2$  Å. The data are consistent with either a monomer or a dimer in the crystal asymmetric unit.

Received 26 June 2002  
Accepted 30 September 2002

## 1. Introduction

The bifunctional catalase–peroxidases (CPx) are found in a wide variety of organisms including bacteria, archaeobacteria and fungi (Nicholls *et al.*, 2001). They are protective enzymes that degrade hydrogen peroxide either catalytically ( $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$ ) or peroxidatically ( $\text{H}_2\text{O}_2 + 2\text{AH} \rightarrow 2\text{H}_2\text{O} + 2\text{A}^+$ ), thereby preventing hydroxyl-radical-induced cellular damage. The rapidity of the catalytic reaction results in the catalase–peroxidases appearing to be predominantly catalases, despite their sequences suggesting a close relation to the plant peroxidases. Indeed, the close relation to the plant peroxidases was confirmed when the Trp105Phe variant of *Escherichia coli* HPI (hydroperoxidase I) proved to be a predominant peroxidase, with a 1000-fold lower catalytic activity and a three-fold higher peroxidatic activity (Hillar *et al.*, 2000).

The catalase–peroxidases gained significant notoriety in 1992 when it was confirmed that mutation of the *katG* gene encoding the *Mycobacterium tuberculosis* catalase peroxidase imparted isoniazid resistance (Zhang *et al.*, 1992). Given the importance of isoniazid as an anti-tuberculosis drug and the prevalence of *katG*-induced resistance, interest in determining the structure of KatG grew, with several groups worldwide attempting to solve its structure. Unfortunately, attempts to crystallize a number of different catalase–peroxidases were without success until very recently, when the crystallization of the

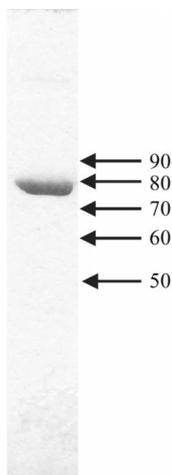
enzymes from *Haloarcula marismortui* (Yamada *et al.*, 2001) and *Synechococcus* (Wada *et al.*, 2002) and of the C-terminal domain of *E. coli* HPI were reported (Carpena *et al.*, 2002).

The large size of the CPx subunits, which contains two distinct sequence-related domains, relative to the plant peroxidases may have been the result of a gene duplication and fusion event (Welinder, 1991). The N-terminal domain is the active domain, containing haem and active-site residues that when modified affect enzyme activity. The C-terminal domain has less sequence similarity, does not seem to bind haem and does not have the well conserved active-site motif characteristic of peroxidases. The haem occupancy for many catalase–peroxidases is partial, with an average of 0.5 haem per subunit in a heterogeneous mixture of dimers and tetramers with 0, 1 and 2 or 1, 2 and 3 haems, respectively (Hillar *et al.*, 2000). In this paper, we report that the CPx from *Burkholderia pseudomallei* (BpCPx) purifies with an apparently higher haem-to-subunit ratio. This more homogeneous protein proved amenable to rapid crystallization and the crystals diffracted to 1.8 Å.

## 2. Experimental results

### 2.1. Protein purification and characterization

The plasmid pBG306 was constructed by inserting a 3.0 kbp *SmaI*–*SfiI* fragment containing the *B. pseudomallei katG* open reading frame (GenBank Accession No.

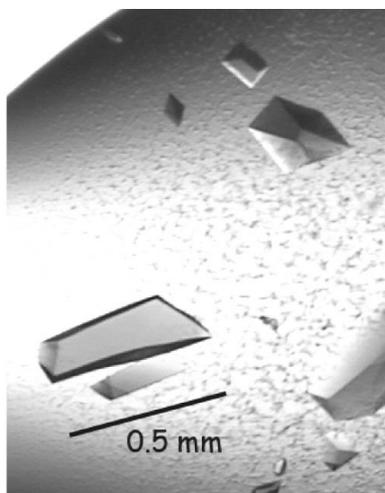


**Figure 1**  
Purified *BpCPx* separated on a 10% SDS polyacrylamide gel. The arrows indicate the location of size markers (values are in kDa).

AY040244) into the plasmid pBBR-*Cm* (Kovach & Elzer, 1995) and transforming into UM262 (Loewen *et al.*, 1990). Cultures were grown with shaking in LB medium supplemented with  $4 \mu\text{g ml}^{-1}$  chloramphenicol and  $40 \mu\text{g ml}^{-1}$  haem (Sigma) for 16 h at 301 K. Following harvesting, the cells were lysed using a French press. *BpCPx* was purified by fractionation with streptomycin sulfate, ammonium sulfate, elution from DEAE cellulose with a linear gradient of 0–0.5 M NaCl and elution from hydroxylapatite with a linear gradient of 5–200 mM potassium phosphate pH 7. The purified protein presented a single band on a denaturing SDS polyacrylamide gel (Fig. 1) and stained for both catalytic and peroxidatic activities on a non-denaturing gel (not shown). The catalase specific activity was 2200 units per milligram of protein (one unit of catalase decomposes  $1 \mu\text{M}$  of  $\text{H}_2\text{O}_2$  in 1 min at 310 K) and the final  $A_{407}/A_{280}$  ratio was 0.60.

## 2.2. Crystallization and data collection

Initially, Hampton Crystal Screen 1 and 2 kits were used for screening; after optimization, thin tetragonal crystal plates were obtained from purified *BpCPx* within 3–5 d at 293 K using the hanging-drop diffusion method with  $1 \mu\text{l}$  of a  $22 \text{ mg ml}^{-1}$  protein solution and  $1 \mu\text{l}$  of reservoir solution containing 16–20% PEG 4K, 20% MPD and 0.1 M sodium citrate pH 5.6. These crystals yielded low-resolution data when collected using a home radiation source, which allowed their characterization as tetragonal with unit-cell parameters  $a = 177$ ,  $b = 177$ ,  $c = 99 \text{ \AA}$ . A second crystal form, consisting of brownish parallelepiped-shaped crystals



**Figure 2**  
Orthorhombic crystals of *B. pseudomallei* KatG (*BpCPx*).

reaching  $0.5 \times 0.2 \times 0.2 \text{ mm}$  in size (Fig. 2), was also obtained in a few days under identical crystallization conditions but with a higher protein-to-reservoir solution ratio, being prepared with  $1 \mu\text{l}$  reservoir solution and  $2 \mu\text{l}$   $22 \text{ mg ml}^{-1}$  protein solution. Crystals from this second form diffracted beyond  $1.9 \text{ \AA}$  resolution when using a synchrotron source. The unit cell, characterized using  $1^\circ$  rotation diffraction images, was consistent with the primitive orthorhombic space group  $P2_12_12_1$ , with unit-cell parameters  $a = 100.9$ ,  $b = 115.6$ ,  $c = 175.2 \text{ \AA}$ . A data set was collected from a single flash-cooled crystal at the BM7B beamline of the DESY Hamburg Outstation on a MAR Research imaging-plate detector using radiation of  $0.85 \text{ \AA}$  wavelength (Fig. 3). Diffraction data were indexed and integrated using the program *DENZO* and merged using the program *SCALEPACK* (Otwinowski & Minor, 1996) (Table 1).

The self-rotation function calculated using the program *MOLREP* (Vagin & Teplyakov, 1997) did not show significant peaks besides those corresponding to the crystallographic twofold axis. In turn, native Patterson maps calculated using the *CCP4* package (Collaborative Computational Project, Number 4, 1994) proved the absence of translational symmetries. Therefore, the orthorhombic *BpCPx* crystals appear to contain only one protein subunit in the crystal asymmetric

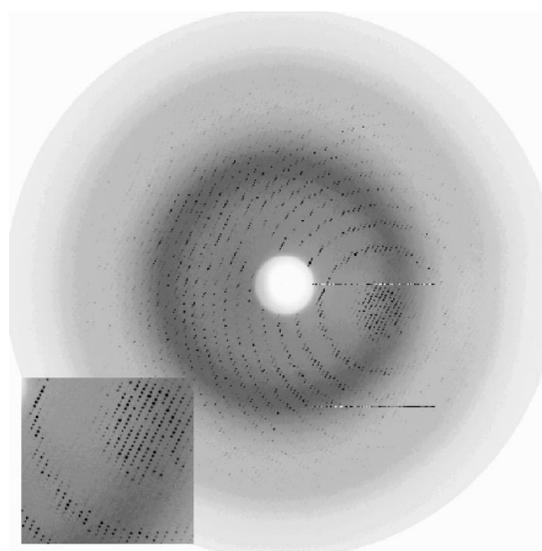
**Table 1**  
Data-collection statistics.

Values in parentheses are for the highest resolution shell.	
Space group	$P2_12_12_1$
Resolution ( $\text{\AA}$ )	1.8
Unit-cell parameters ( $\text{\AA}$ )	$a = 100.9$ , $b = 115.6$ , $c = 175.2$
No. of measured reflections	663193
No. of unique reflections	186738 (9316)
Completeness (%)	99.0 (99.9)
$R_{\text{merge}}^\dagger$ (%)	6.4 (46.4)
Average $I/\sigma(I)$	20.2 (2.9)
Mosaicity	0.3

$$^\dagger R_{\text{merge}} = \sum |I_i - \langle I \rangle| / \sum I_i.$$

unit, which would correspond to a volume solvent content percentage of 80% (Matthews coefficient  $6.26 \text{ \AA}^3 \text{ Da}^{-1}$ ). However, a non-crystallographic twofold axis in a direction almost parallel (within a few degrees) to one of the crystallographic twofold axis could still exist and be undetected by both the self-rotation and native Patterson. Two subunits in the crystal asymmetric unit would correspond to a 60% solvent content (Matthews coefficient  $3.13 \text{ \AA}^3 \text{ Da}^{-1}$ ) and would be compatible with the existence of a molecular dimer. The possibility of molecular tetramers seems incompatible with the present crystal packing analysis.

Crystal structure determination is now in progress by molecular replacement using the programs *AMoRe* (Navaza, 1994) and *BEAST* (Read, 2001). Search models for the N-terminal region of *BpCPx* have been derived from yeast cytochrome *c* peroxidase (Finzel *et al.*, 1984). Search models for the C-terminal region of *BpCPx* have been



**Figure 3**  
Diffraction image, collected at BM7B in DESY (Hamburg), from an orthorhombic crystal of *BpCPx*. The image edge, where faint spots are still visible, corresponds to a resolution of  $1.58 \text{ \AA}$ .

derived from the results from crystals of KatG\_2D from *E. coli* HPI (Carpena *et al.*, 2002; Carpena *et al.*, unpublished results).

### 3. Conclusions

The catalase–peroxidase from *B. pseudo-mallei* has been crystallized from sodium citrate pH 5.6 and the crystals diffract beyond 1.8 Å. The diffraction data is well suited to high-resolution studies, which are in progress. The data is compatible with either one subunit in the crystal asymmetric unit, corresponding to solvent content of 80%, or to a dimer of subunits, corresponding to a solvent content of 60%.

This work was supported by grants BIO099-0865 from DGICYT to IF and OGP9600 from the Natural Sciences and Engineering Research Council of Canada (NSERC) to PCL.

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