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Crystallization and preliminary X-ray analysis of human thioredoxin peroxidase-B from red blood cells

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Two different crystal forms of human thioredoxin peroxidase-B have been grown by vapour diffusion using polyethylene glycol 400 as a precipitant. Monoclinic $P2_1$ crystals were grown from freshly purified protein, whilst orthorhombic $P2_12_12_1$ crystals were grown from purified protein that had been stored in ammonium sulfate, but otherwise under the same conditions. The diffraction from both crystal forms was observed to extend to beyond 2.0 Å resolution using synchrotron radiation. Complete native data sets to 1.8 and 3.7 Å have been collected from the monoclinic and orthorhombic crystals, respectively.

1. Introduction

Thioredoxin peroxidase-B (TxP-B), previously known as natural-killer enhancing factor-B (NKEF-B), is an intracellular member of the peroxiredoxins, an emerging family of antioxidant enzymes with monomeric masses of 20–29 kDa (Chae, Robison *et al.*, 1994). Their biological interest lies in their ability to combat oxidative stress by degrading hydrogen peroxide and organic hydroperoxides to water or to the corresponding alcohol (Jacobson *et al.*, 1989; Chae, Chung *et al.*, 1994; Bruchhaus *et al.*, 1997; Shau *et al.*, 1997). More recently, peroxiredoxins have been shown to play roles in tyrosine kinase signalling (Wen & Van Etten, 1997) and in apoptosis (Ichimiya *et al.*, 1997; Zhang *et al.*, 1997).

The red blood cell (RBC) contains at least three distinct peroxiredoxins within its life cycle (Nemoto *et al.*, 1990; Shau *et al.*, 1994), although TxP-B appears to be the most predominant within fully differentiated RBCs (Schröder *et al.*, 1998). TxP-B exhibits peroxidase activity and prevents monocyte attachment to endothelial cells in response to treatment with oxidized lipoproteins and lipopolysaccharides, with implications in atherosclerosis (Shau *et al.*, 1997). TxP-B appears to be identical to calpromotin (Moore & Shriver, 1993; Moore, 1997), an enzyme which is essential for the calcium-dependent efflux of potassium ions from RBCs (Plishker *et al.*, 1992). In common with other peroxiredoxins, TxP-B readily forms disulfide-linked dimers under oxidizing conditions and oligomers under reducing conditions with an approximate molecular mass of 350 kDa (Shau *et al.*, 1993; Schröder *et al.*, 1998).

2. Experimental

Mature RBCs were sourced from screened out-of-date blood packs. Native human TxP-B was purified from RBCs using a procedure

Table 1
Processing statistics for both crystal forms.

Parameter	Data set	
	In house, orthorhombic	EMBL (Hamburg), monoclinic
Wavelength (Å)	1.542	0.9076
Oscillation range (°)	0.1	0.7
Exposure time (s)	40	240
Resolution range (Å)	34–3.66	20–1.76
Highest resolution shell (Å)	3.8–3.66	1.79–1.76
Mosaicity (°)	0.1	0.3
Number of measured reflections	116395	956097
Number of unique reflections	25981	206236
Completeness (all data) (%)	93.1	99.9
Completeness (highest resolution shell) (%)	90.8	99.9
$I > 2\sigma(I)$ (%)	80.0	81.2
$\langle I\rangle/\sigma\langle I\rangle$	38.5	19.18
R_{sym}(%) (all data)†	7.4	5.7
R_{sym}(%) (highest resolution shell)†	16.2	39.8

† $R_{sym} = \sum|I - \langle I\rangle|/\sum I$, where I is the intensity of reflection.

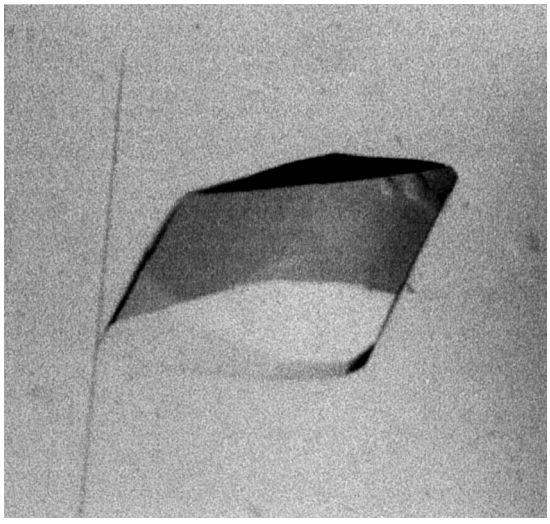

Figure 1
Monoclinic crystals of human TxP-B.

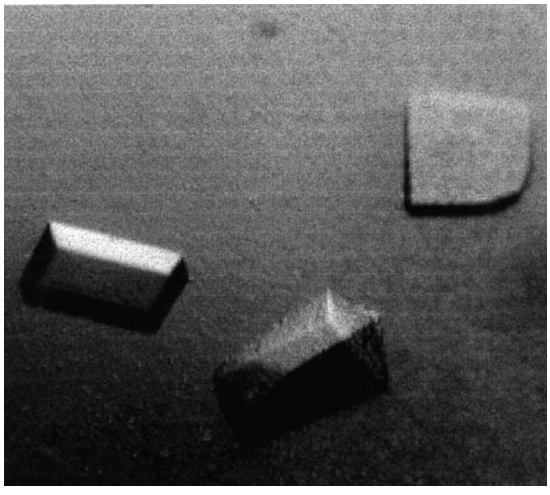

Figure 2
Orthorhombic crystals of human TxP-B.

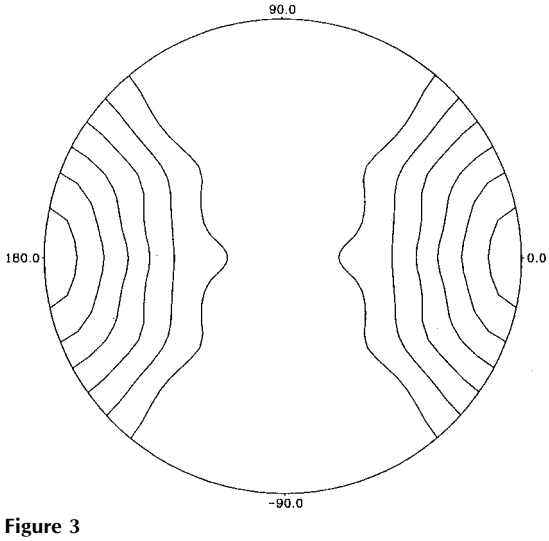

Figure 3
Self-rotation function calculated from orthorhombic data, calculated for $\kappa = 180^\circ$.

derived for porcine TxP-B (Schröder, 1994; Schröder *et al.*, 1998). For crystallization, the purified TxP-B was concentrated to 15 mg ml^{-1} in 25 m*M* Tris–HCl pH 7.5, 5 m*M* EDTA, 10 m*M* dithioerythreitol by ultrafiltration using Centricon 10 concentrators (Millipore, Watford, UK). Crystallization trials were set up using the hanging-drop vapour-diffusion method. Concentrations were estimated using the extinction coefficient derived for pig TxP-B, $E_{280}^{1\%} = 10$ (Schröder, 1994). The hanging drops [2 µl protein and 2 µl mother liquor containing 16%(*v*/*v*) PEG 400, 100 m*M* Tris–HCl pH 7.5, 10%(*v*/*v*) 2-propanol, 10 m*M* dithioerythreitol] were suspended in sealed compartments above 1 ml of the same mother liquor and allowed to equilibrate at 290 K. Data were collected using both in-house and synchrotron radiation. In-house data were collected from a single capillary-mounted orthorhombic crystal at 290 K, using a Siemens Hi-Star area detector and rotating-anode generator operated at 50 kV and 100 mA and producing Cu $K\alpha$ radiation. Synchrotron data were collected from a single cryocooled monoclinic crystal at 100 K on station X11 at EMBL (Hamburg), using an MAR Research 345 imaging-plate scanner (300 mm).

3. Results and discussion

The purified protein migrated as a single band on SDS polyacrylamide gel electrophoresis, with an apparent mobility equivalent to a molecular mass of 22 kDa. The isoelectric point was determined as 5.8 using the PHAST system (Pharmacia LKB, Milton Keynes, UK). Since the protein was found to be N-terminally blocked, fragments were generated with trypsin using standard procedures at the Aberdeen protein-sequencing facility. Two tryptic fragments of the purified protein were micro-sequenced (43 residues, representing 22% of the total polypeptide) and shared complete identity with residues 94–135 of the deposited sequence for TxPB_HUMAN (SwissProt accession number P31945). The purified protein was therefore positively identified as TxP-B and distinct from the two closely related RBC peroxiredoxins, TxP-A and MER5.

Typically one to five crystals per hanging drop began to appear within 2 d. Crystals grown from freshly prepared protein measured up to 0.7 mm in the largest dimension (Fig. 1) and their space group was determined as monoclinic $P2_1$ with unit-cell parameters $a = 88.9$, $b = 107.0$, $c = 119.5$ Å and $\beta = 110.9^\circ$. Crystals grown from protein which had been stored for a number of months as an ammonium sulfate precipitate measured up to 0.5 mm in the largest dimension (Fig. 2) and their space group was determined as orthorhombic $P2_12_12_1$ with $a = 57.2$, $b = 185.3$ and $c = 226.5$ Å. In-house diffraction data were collected to 3.7 Å from a single orthorhombic crystal and processed using *SAINT* (*SAINT*, 1993). The requirements of the *SAINT* software limited the oscillation range to 0.1° because the mosaicity of the crystal was low (0.1°). With synchrotron radiation, frozen orthorhombic crystals were observed to diffract to at least 2.0 Å. Synchrotron data to 1.8 Å were collected from a single monoclinic crystal that had been cryocooled in 30%(*v*/*v*) PEG 400, 100 m*M* Tris–HCl pH 7.5, 10%(*v*/*v*) 2-propanol, 10 m*M* dithioerythreitol, and processed using *DENZO* (Otwinowski & Minor, 1997). The asymmetric unit of either crystal form is likely to contain 6–14 molecules, assuming that the solvent content falls within the range 30–75%.

Self-rotation functions (Collaborative Computational Project, Number 4, 1994) calculated from the monoclinic data over a range of integration radii and resolution limits were difficult to interpret (data not shown). The self-rotation function may have been obscured by the relatively strong anisotropy of the data, since the ratio of eigenvalues of the anisotropic distribution of the structure factors was 0.51:1.0:0.38, as estimated by the program *SFCHECK* (A. Vagin, personal communication). Self-rotation functions calculated from the orthorhombic data gave strong features at κ values of 60, 120 and 180°, consistent with the presence of a sixfold molecular axis almost parallel to the *a* axis (Fig. 3) and six twofold molecular axes normal to the sixfold (Fig. 4). There were no strong features which could indicate pseudotranslation within the native Patterson map. The ratio of eigenvalues of the anisotropic distribution of the

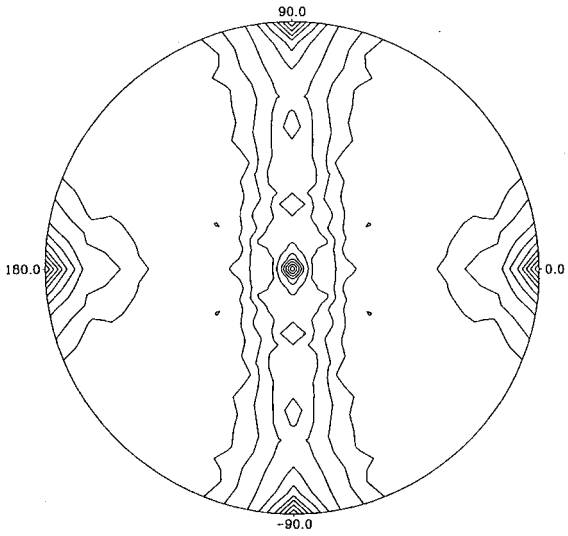

Figure 4
Self-rotation function calculated from orthorhombic data, calculated for $\kappa = 60^\circ$.

structure factors for the orthorhombic data is estimated as 1.00:0.92:0.91, which suggested that the orthorhombic data is significantly less anisotropic than the monoclinic data. It is therefore plausible from the self-rotation functions for the orthorhombic cell that the oligomeric state of the protein is a dodecamer with 622 point symmetry. A search for suitable heavy-atom derivatives for phasing purposes is currently in progress.

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