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Crystallization and preliminary X-ray analysis of *Escherichia coli* p20, a novel thiol peroxidase

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Escherichia coli p20 is a thioredoxin-dependent thiol peroxidase. This protein represents a novel group of antioxidant enzymes that are widely expressed in various pathogenic bacteria and show distant yet significant sequence homology with peroxiredoxins. *E. coli* p20, overexpressed in *E. coli*, was crystallized with PEG 4000 and 2-propanol as precipitants using the hanging-drop vapour-diffusion method. Diffraction data were collected to 2.2 Å resolution using synchrotron radiation. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 38.97, b = 58.97, c = 127.59 Å. The asymmetric unit contains two p20 molecules, with a corresponding V_M of 2.06 $Å^3\ Da^{-1}$ and a solvent content of 40.4%.

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1. Introduction

Reactive oxygen species (ROS), such as the superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\cdot}$), are formed as intermediates during the univalent reduction of oxygen to water and are also produced during β-oxidation of fatty acids and upon exposure to radiation, metals and redox drugs (Nordberg & Arnér, 2001). ROS can cause molecular damage to various cellular components such as lipids, proteins and nucleic acids, leading to cell death, and are thus considered to be highly toxic byproducts of oxygen metabolism and of no biological significance. However, recent findings show that ROS also act as signal transducing molecules, with significant roles in the activation of transcription factors leading to gene expression. To minimize the damaging effects of ROS, aerobic organisms have evolved both non-enzymatic and enzymatic antioxidant defences (Ahmad, 1995). Enzymatic defenses include superoxide dismutases, which convert $O_2^{\cdot-}$ to H_2O_2, and catalases, gluthathione peroxidases and peroxiredoxins, which convert H_2O_2 to H_2O. H_2O_2 itself is not very reactive, but can be further reduced to the extremely reactive $HO^{\cdot}$. Therefore, all aerobic cells are equipped with H_2O_2-removing enzymes.

The p20 protein, isolated from the periplasmic space of *Escherichia coli*, is a thioredoxin-dependent thiol peroxidase that catalyzes the reduction of H_2O_2 or alkyl hydroperoxides (Cha *et al.*, 1995). It displays an antioxidant activity that protects glutamine synthetase from inactivation by a thiol/Fe^{III}/O_2 mixed-function oxidation system. The p20 protein is alternatively called scavengase (Wan *et al.*, 1997). Sequence analysis indicates that *E. coli* p20, with 168 amino-acid residues, represents a group of antioxidant enzymes that are widely expressed in various pathogenic bacteria including *Haemophilus influenzae*, *Streptococcus* spp., *Vibrio cholerae*, *Mycobacterium tuberculosis* and *Helicobacter pylori*. These bacterial p20 proteins are highly homologous (38–64% identity and 58–73% similarity), with similar molecular sizes. It has been found that *E. coli* p20 contains two functionally essential cysteine residues (Cys61 and Cys95) that are conserved in other p20 proteins and does not self-associate in a non-reducing gel (Cha *et al.*, 1996; Zhou *et al.*, 1997). Otherwise, detailed knowledge of the catalytic mechanism of these proteins is limited at present.

It has been suggested that the bacterial p20 proteins may be a novel subfamily of peroxiredoxins (Prxs) as they show distant yet significant sequence identity to them (12–22%; Zhou *et al.*, 1997). Prxs are a large and diverse family of peroxidases that are present in both prokaryotes and eukaryotes. They catalyze the reduction of H_2O_2 or alkyl hydroperoxides and are also involved in cell differentiation, proliferation, immune response and apoptosis (Rhee *et al.*, 2001). Recent X-ray structures have shown that Prxs are novel members of the thioredoxin-fold superfamily (Hirotsu *et al.*, 1999; Schröder *et al.*, 2000; Alphey *et al.*, 2000; Declercq *et al.*, 2001; Wood *et al.*, 2002). Prxs are distinct from other peroxidases in that they have no cofactors, such as metals or prosthetic groups, but utilize the thiol group of the cysteine residue for catalysis. Prxs are divided into two categories, the 1-Cys and 2-Cys Prxs, based on the number of cysteinyl residues directly involved in catalysis. All Prxs, whether of the 1-Cys or the 2-Cys type, contain a conserved Cys in the N-terminal portion, which is oxidized by peroxides to a cysteine sulfenic acid (Cys-SOH). In the 2-Cys Prxs, the Cys-

SOH intermediate from one subunit is attacked by a Cys residue located in the C-terminus of the other subunit. The structural characteristics of 2-Cys Prxs are illustrated by the X-ray structure of *Salmonella typhimurium* AhpC (Wood *et al.*, 2002). AhpC, with 187 residues, is an obligate homodimer in the oxidized form. The active site is composed of the peroxidatic Cys46 and the resolving Cys165 from the other subunit of the antiparallel dimer, resulting in two symmetrical active sites per dimer. In general, the disulfide formed in the oxidized Prxs is reduced by a thiol-containing donor molecule, such as thioredoxin, glutathione, trypanothione or AhpF, completing the catalytic cycle. However, unlike 2-Cys Prxs, the catalytic mechanism of 1-Cys Prxs still remains to be elucidated (Declercq *et al.*, 2001).

Like the 2-Cys Prxs, *E. coli* p20 also contains two functional Cys residues, but their relative positions are quite different. In addition, p20 is not dimerized in a non-reducing gel, while the 2-Cys Prxs are dimerized. These differences suggest that the structural homology may be low between the two classes of peroxidases even if the bacterial p20 proteins can be classified as members of the Prx subfamily. Structural information on *E. coli* p20 will certainly help to identify the characteristics of these novel bacterial peroxidases. In an effort to elucidate its three-dimensional structure, we have crystallized *E. coli* p20 and report here the crystallization conditions and preliminary X-ray crystallographic data.

2. Materials and methods

2.1. Overexpression and purification

The p20 gene was amplified from *E. coli* K-12 genomic DNA by the polymerase chain reaction (PCR) using the oligonucleotides 5′-CGATC**CATATG**TCACAAACCGTTCATTTCC-3′ and 5′-CGC**GGATTC**TTATGCTTTCAGTACAGCCAGAGC-3′ as the forward and reverse primers, respectively. The *Nde*I and *Bam*HI restriction-enzyme sites that were incorporated into the two primers are shown in bold. The amplification was carried out using standard protocols. Purified PCR products were digested with *Nde*I and *Bam*HI and cloned into the *Nde*I/*Bam*HI-digested expression vector pT7-7. The plasmid encoding p20 was transformed into *E. coli* strain BL21 (DE3). The cells were grown in Luria–Bertani (LB) medium containing 100 μg ml^{-1} ampicillin and induced with 1 m*M* isopropyl-β-thiogalactopyranoside (IPTG) at 303 K. After 4 h induction, the cells were harvested by centrifugation and stored frozen at 203 K. Frozen cells were suspended in 50 m*M* Tris–HCl pH 8.0 containing 2 m*M* phenylmethylsulfonyl fluoride and 1 m*M* EDTA and disrupted by sonication. The p20 protein was purified to homogeneity through DEAE-cellulose, Sephadex G75, phenyl-Sepharose CL-4B, Sephadex G50 and Q-Sepharose column chromatography, following the published protocol with a minor modification (Cha *et al.*, 1995). Determination of the antioxidant activity of p20 was performed using the glutamine synthetase protection assay technique (Kim *et al.*, 1988). The purified protein was concentrated for crystallization using an Amicon YM-10 membrane.

2.2. Crystallization and data collection

Crystallization of p20 was carried out using the hanging-drop vapour-diffusion method. Initial searches for the crystallization conditions were performed using 24-well Linbro tissue-culture plates (ICN) and JBScreen Crystal Screening Kits (Jena Bioscience) at 293 K. Hanging drops were prepared by mixing equal volumes (2 μl) of protein solution (10 mg ml^{-1} in 50 m*M* Na HEPES pH 7) and reservoir solution and were allowed to equilibrate against 0.5 ml of reservoir solution.

For cryogenic freezing of the crystals, 3 μl of 50% PEG 4000 was added to the hanging drops. In about 1 min, crystals were mounted in nylon loops and flash-frozen at 100 K in a nitrogen-gas stream. X-ray diffraction data were collected at 100 K with a MacScience 2030 image-plate detector at the BL-6B experimental station of Pohang Light Source (PLS), South Korea. The synchrotron X-ray wavelength was 1.12714 Å. The crystal-to-detector distance was set to 250 mm, the exposure time per frame to 3 min and the oscillation range to 1.5° for data collection. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

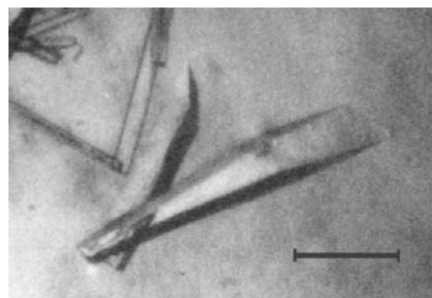

Figure 1
Crystals of *E. coli* p20. The bar represents 0.3 mm.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.28–2.20 Å).

X-ray wavelength (Å)	1.12714 (Pohang Light Source BL-6B)
Temperature (K)	100
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 38.97, b = 58.97, c = 127.59
Resolution limit (Å)	2.2
No. of measured reflections	108838 (4855)
No. of unique reflections	15079 (1479)
Completeness (%)	98.7 (98.9)
$I/\sigma(I)$	19.3 (8.9)
R_{merge}† (%)	6.1 (19.2)
Redundancy	7.2 (3.3)

† $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of the reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over the i measurements of the reflection h.

3. Results and discussion

Recombinant *E. coli* p20 was overexpressed in *E. coli* in a soluble form with a yield of ~10 mg of homogeneous protein per litre of culture. Rod-shaped crystals were obtained using a reservoir solution containing a mixture of 10% PEG 4000 and 20% 2-propanol as precipitant at 293 K in 3 d. Initial crystals were smaller than 0.2 mm in length. Seeding techniques with macroseeds were essential to obtain large single crystals. With this procedure, single crystals grew routinely to dimensions of 0.10 × 0.15 × 0.85 mm (Fig. 1). Diffraction data were collected to 2.2 Å resolution with synchrotron X-rays. A total of 108 838 measured reflections were merged into 15 079 unique reflections with an $R_{\rm merge}$ (on intensity) of 6.1%. The merged data set is 98.7% complete to 2.2 Å. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 38.97, b = 58.97, c = 127.59 Å. The presence of two molecules of p20 in the asymmetric unit gives a crystal volume per protein mass $V_{\rm M}$ of 2.06 Å^3 Da^{-1} and a corresponding solvent content of 40.4% (Matthews, 1968). The data-collection statistics are summarized in Table 1. There is no prominent peak in the self-rotation map, indicating that the two independent molecules in the asymmetric unit may not be related by non-crystallographic symmetry. Searches for heavy-atom derivatives are in progress.

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