

## Crystallization and Preliminary X-ray Diffraction Analysis of Catalase HPII from *Escherichia coli*

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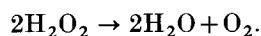
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Green crystals of the hexameric catalase HPII from *Escherichia coli* have been obtained by the hanging-drop method. The crystals belong to the monoclinic space group *P*2 with  $a=123 \text{ \AA}$ ,  $b=132 \text{ \AA}$ ,  $c=93 \text{ \AA}$ ,  $\beta=112.5^\circ$ . There are three subunits in the asymmetric unit. The crystals diffract at least to  $3.2 \text{ \AA}$  resolution and are suitable for further X-ray diffraction studies.

Catalase (EC 1.11.1.6;  $\text{H}_2\text{O}_2:\text{H}_2\text{O}_2$ -oxidoreductase) is an enzyme that has been found in most aerobic organisms. It decomposes hydrogen peroxide to molecular oxygen and water:



Crystals of catalase from a variety of sources have been described and some of them were used for structural investigations by X-ray diffraction and electron microscopy. The spatial organization of a non-heme hexameric catalase from *Thermus thermophilus* (Vainshtein *et al.*, 1985), and three tetrameric catalases, from *Penicillium vitale* (Vainshtein *et al.*, 1986), from beef liver (Murthy *et al.*, 1981), and recently from *Micrococcus lysodeikticus* (Yusifov *et al.*, 1989), are known at high resolution.

Two different catalases from *Escherichia coli* have been purified and characterized (Claiborne & Fridovich, 1979; Loewen & Switala, 1986). HPI (hydroperoxidase I) is a tetrameric enzyme containing the normal protoheme IX prosthetic group, but with larger than normal 84 kDa subunits. It is also unique in having an associated peroxidase activity (Claiborne & Fridovich, 1979). HPII is a monofunctional catalase but is unusual in most other respects including its hexameric structure, the heme-d-like prosthetic group that gives the enzyme its characteristic green colour and the 93 kDa size of the subunit (Loewen & Switala,

1986). More recently, the gene encoding the subunit of HPII, *katE*, has been cloned (Mulvey *et al.*, 1988) and a structure has been proposed for the HPII heme (Chiu *et al.*, 1989).

We now report the crystallization of catalase HPII. The enzyme was purified as described by Loewen & Switala (1986) from strain UM255 (*pro leu rpsL hsdR endI lacY katG2 katE12::Tn10*) transformed with pAMkatE22 (Mulvey *et al.*, 1988), a plasmid containing *katE*. In one representative preparation, 320 mg of enzyme with a specific activity of 9950 units per milligram of protein was isolated from 82 g of cell paste. For crystallization, solutions of the lyophilized protein were prepared at a concentration of 10 to 15 mg/ml in 50 mM-Tris·HCl buffer (pH 7.0). Crystals were obtained using the hanging-drop vapor diffusion method at 4°C using polyethylene glycol as precipitant. Best crystals were obtained from 10 to 20  $\mu\text{l}$  droplets under the following initial conditions: 7 mg of protein per ml, 7% (w/v) polyethylene glycol 3350, 0.5 M-LiCl, 3.5 mM- $\text{NaN}_3$  in 50 mM-Tris·HCl (pH 7.0). The equilibrating reservoir consisted of 1 ml of 15% polyethylene glycol 3350, 1 M-LiCl similarly buffered. Crystals were obtained as prisms reaching a size of 0.6 mm  $\times$  0.3 mm  $\times$  0.05 mm over one to two months. Typical crystals diffracted to about 3.2  $\text{\AA}$  resolution and were stable in the X-ray beam for about 12 hours. The most likely space

group was determined to be *P2* from inspection of the symmetry in precession photographs with unit cell constants  $a=123 \text{ \AA}$  ( $1 \text{ \AA}=0.1 \text{ nm}$ ),  $b=132 \text{ \AA}$ ,  $c=93 \text{ \AA}$ ,  $\beta=112.5^\circ$ . With one molecule of the native protein ( $M_r=532,000$ ) in the unit cell a value of  $2.6 \text{ \AA}^3/\text{Da}$  is obtained for  $V_m$  that falls within the range of volume-to-mass ratios commonly observed (Matthews, 1986). Hence, there is probably one oligomeric protein per unit cell and three subunits per asymmetric unit, the crystal 2-fold axis being coincident with a molecular dyad axis. This would correspond to a solvent content of about 53%. This crystal form appears to be suitable for further crystallographic investigation.

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