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

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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 $P2$ with $a=123$ Å, $b=132$ Å, $c=93$ Å, $\beta=112{\cdot}5°$. There are three subunits in the asymmetric unit. The crystals diffract at least to 3·2 Å resolution and are suitable for further X-ray diffraction studies.

Catalase (EC 1.11.1.6; $H_2O_2:H_2O_2$-oxidoreductase) is an enzyme that has been found in most aerobic organisms. It decomposes hydrogen peroxide to molecular oxygen and water:

$$2H_2O_2 \rightarrow 2H_2O+O_2.$$

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*::Tn*10*) 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 µl 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-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 × 0·3 mm × 0·05 mm over one to two months. Typical crystals diffracted to about 3·2 Å resolution and were stable in the X-ray beam for about 12 hours. The most likely space

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group was determined to be $P2$ from inspection of the symmetry in precession photographs with unit cell constants $a=123$ Å (1 Å = 0·1 nm), $b=132$ Å, $c=93$ Å, $\beta=112{\cdot}5°$. With one molecule of the native protein ($M_r=532{,}000$) in the unit cell a value of 2·6 Å^3/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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