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Crystallization and preliminary X-ray diffraction studies of human catalase

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The enzyme catalase (H_2O_2–H_2O_2 oxidoreductase; E.C. 11.1.6) was purified from haemolysate of human placenta and crystallized using the vapour-diffusion technique. Synchrotron-radiation diffraction data have been collected to 1.76 Å resolution. The enzyme crystallized in the space group $P2_12_12_1$, with unit-cell dimensions a = 83.6, b = 139.4, c = 227.5 Å. A molecular-replacement solution of the structure has been obtained using beef liver catalase (PDB code 4blc) as a search model.

1. Introduction

The enzyme catalase (H_2O_2–H_2O_2 oxidoreductase; E.C. 11.1.6) plays an important role in cellular defence against active oxygen species (Aebi, 1984; Halliwell & Gutteridge, 1986; Michiels *et al.*, 1994). Its mechanism of decreasing the hydrogen peroxide concentration has been well described (Jones, 1982; Feinsteins *et al.*, 1971; Almarsson, 1993).

Catalase is found in almost all aerobic organisms (Murthy *et al.*, 1982), and some microbial catalases are used in various industrial processes in which H_2O_2 is utilized for bleaching or disinfecting (Godfrey & West, 1996). Studies in mammalian cells have shown that a lack of or decreased amount of catalase activity is related to many diseases: respiratory distress syndrome (Metnitz *et al.*, 1999), peptic ulcer (Majani & Das, 1998), DNA damage and carcinogenesis (Ohkuma & Kawanishi, 1999), as well as the ageing process (Shah *et al.*, 1999; Casado *et al.*, 1998). Cases of acatalasaemia and hypocatalasaemia have been described (Kishimoto *et al.*, 1992). These studies have shown that gene mutations inhibiting catalase expression or affecting molecules in such a way as to cause instability of tetramer formation decrease the enzyme activity.

Mammalian catalases have been proposed for clinical application in the treatment of many diseases in which oxidative injury has some importance (Greenwald, 1990), such as myocardial ischaemia reperfusion oxidative injury (Simpson *et al.*, 1987; Zughaib *et al.*, 1994), arthritis and inflammatory diseases (Greenwald, 1990), and ageing (Halliwell & Gutteridge, 1986). The human catalase cDNA from kidney has been cloned and its nucleotide sequence has been determined (Quan *et al.*, 1986).

The active mammalian catalase is a homotetramer of 4 × 60 kDa, with one site for haem (Schonbaun & Chance, 1976) and one site for NADP (Kirkiman & Gaetani, 1984; Gouet *et al.*, 1995) per monomer. Crystal structures of several catalases have been described: beef liver (Reid *et al.*, 1981; Fita *et al.*, 1986), *Penicillium vitale* (Melik-Adamyan *et al.*, 1986), *Proteus mirablis* (Gouet *et al.*, 1995) and *Escherichia coli* (Bravo *et al.*, 1995).

In the present work, we describe crystallization and preliminary X-ray diffraction studies of human catalase at 1.76 Å resolution.

Table 1
Crystal data and data-collection statistics.

Statistical values for highest resolution shell (1.79–1.76 Å) are shown in parentheses.

Space group	$P2_12_12_1$
Unit-cell dimensions (Å)	a = 83.6, b = 139.4, c = 227.5
Resolution (Å)	15.0–1.76
No. of observations	604975
No. of unique reflections	232698
$\langle I/\sigma(I)\rangle$	9.9 (1.4)
Multiplicity	2.6 (1.5)
Completeness (%)	88.7 (59.1)
$R_{\rm merge}$† (%)	10.2 (33.4)

† $R_{\rm merge} = \sum_{hkl} |I - \langle I\rangle| / \sum_{hkl} I$.

2. Protein purification

Various procedures for catalase purification from different organisms, different tissues and blood erythrocytes have been described. For this work, catalase was purified from haemolysate of human placenta by combination of the Cohn precipitation method and chromatography (Gonçalves *et al.*, 1999). Essentially, human placentas frozen immediately after childbirth were defrosted, ground and haemolysate was extracted with saline and ethanol. The cellular mass was separated by centrifugation. Haemoglobin was precipitated by ethanol/chloroform and separated by filtration. The clarified solution was submitted to two steps of chromatography: anion-exchange chromatography on Q-Sepharose and affinity chromatography on blue-Sepharose. The purified catalase was desalted, concentrated to 30 mg ml^{-1} in an Amicon system and used for crystallization trials.

3. Crystallization and data collection

Preliminary screening of the crystallization conditions was performed using a sparse-matrix screen at 291 K (Crystal Screen I and II, Hampton Research Corp.). Small crystals were found in the condition number 37 of the Crystal Screen II kit (10% PEG 8000, 8% ethylene glycol, 0.1 *M* HEPES pH 7.5). A search for refined crystallization conditions has been carried out. New crystals were grown at

room temperature using the hanging-drop vapour-diffusion technique, by mixing equal volumes (2 µl + 2 µl) of a protein solution concentrated to 30 mg ml^{-1} and a reservoir solution containing 6–9% PEG 8000, 0.1 *M* MES in the pH range 5.5–8.0. Crystals of two different morphologies appeared after 2 d under similar crystallization conditions and frequently in the same drop: well shaped bipyramidal crystals of dimensions 0.5 × 0.4 × 0.4 mm and rectangular-shaped crystals measuring 0.4 × 0.2 × 0.2 mm. It was subsequently found that the bipyramidal crystals did not diffract X-rays; all the data were therefore collected from the rectangular-shaped crystals.

X-ray diffraction data were collected from crystals immersed for 1 min in a cryo-cooling solution (22% ethylene glycol, 10% PEG 8000, 0.1 *M* HEPES pH 7.5), mounted in a rayon loop and flash-cooled to 80 K in a cold nitrogen stream. Data collection was performed at the Protein Crystallography beamline (Polikarpov, Oliva *et al.*, 1997; Polikarpov, Perles *et al.*, 1997) at the Laboratório Nacional de Luz Síncrotron (Campinas, SP, Brazil), using a MAR345 image plate. The synchrotron-radiation wavelength was set to 1.38 Å to optimize X-ray flux and minimize absorption errors. Several crystals were tested until a good set of diffraction data was obtained. The first image was subjected to the autoindexing routine of *DENZO* (Otwinowski, 1993), from which the best refined solution was a primitive orthorhombic cell. Following an optimum strategy of data collection suggested by the program *marHKL*, a total of 81° of data was collected in steps of 0.5°. The collected images were processed and scaled with the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993). Data sets were collected and processed for two crystals. Details of the significantly better one, which had a mosaic spread of 0.5°, are given in Table 1; the other had a high mosaicity (1.5°) and gave data to a resolution of 2.05 Å. Both showed considerable radiation decay.

Calculations using the Matthews coefficient (Matthews, 1968) suggested the presence of one tetramer per asymmetric unit (V_m = 2.76 Å^3 Da^{-1}), which was subsequently verified by molecular replacement. A *BLAST* search with the human catalase primary sequence (total length of 527 amino-acid residues) against the PDB database showed that beef liver catalase (506 amino-acid residues) represents 91% identity and 95% similarity in primary sequence, followed by *Proteus mirablis* catalase (484 amino-acid residues), which displays 52% primary sequence identity and 65% similarity. The crystal structure of human catalase was solved by molecular-replacement method with the program *AMoRe* (Navaza, 1994), using a tetramer of beef liver catalase (PDB code 4blc) as a search model.

The rotation function was calculated using diffraction data in the resolution range 8.0–3.5 Å using a Patterson radius of 40 Å. The rotation function gave a clear solution [correlation coefficient (CC) of 0.357], where the next highest peak had CC = 0.158. The translation search was performed in the same resolution range using the Crowther & Blow (1967) translation function. The translation search gave a strong peak with CC = 0.614 and an *R* factor of 36.7%, and this solution was then subjected to ten cycles of rigid-body refinement against all data between 8.0 and 3.5 Å resolution (fitting function of *AMoRe*). The resulting *R* factor was 33.4%, with a correlation coefficient of 0.689. Refinement was undertaken using the program *REFMAC* (Murshudov *et al.*, 1997). Three cycles of rigid-body refinement against data in the resolution range 15–1.76 Å were initially performed, treating each of the monomers with its corresponding haem group as a separate rigid body, followed by 40 steps of positional and *B*-factor refinement. Non-crystallographic symmetry restraints have been applied to the monomers, requiring them to be related by local 222 symmetry throughout the refinement. At present, $R_{\rm free}$ and the *R* factor of the model are 29.4 and 25.8%, respectively. No water molecules have so far been introduced. The current model has essentially the same fold as beef liver catalase, but represents significant conformational differences in the C-terminal and N-terminal regions. Further steps of model building and refinement are under way.

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