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Crystallization and preliminary X-ray analysis of clade I catalases from *Pseudomonas syringae* and *Listeria seeligeri*

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Haem-containing catalases are homotetrameric molecules that degrade hydrogen peroxide. Phylogenetically, the haem-containing catalases can be grouped into three main lines or clades. The crystal structures of seven catalases have been determined, all from clades II and III. In order to obtain a structure of an enzyme from clade I, which includes all plant, algae and some bacterial enzymes, two bacterial catalases, CatF from *Pseudomonas syringae* and Kat from *Listeria seeligeri*, have been crystallized by the hanging-drop vapour-diffusion technique, using PEG and ammonium sulfate as precipitants, respectively. Crystals of *P. syringae* CatF, with a plate-like morphology, belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 60.6, b = 153.9, c = 109.2 Å, β = 102.8°. From these crystals a diffraction data set to 1.8 Å resolution with 98% completeness was collected using synchrotron radiation. Crystals of *L. seeligeri* Kat, with a well developed bipyramidal morphology, belong to space group $I222$ (or $I2_12_12_1$), with unit-cell parameters a = 74.4, b = 121.3, c = 368.5 Å. These crystals diffracted beyond 2.2 Å resolution when using synchrotron radiation, but presented anisotropic diffraction, with the weakest direction perpendicular to the long c axis.

1. Introduction

Catalase is an ubiquitous enzyme present in nearly all aerobic organisms. It has a protective role in removing hydrogen peroxide before it or its more reactive hydroxide radical breakdown products can react with and damage other cellular components. The catalatic reaction occurs in two stages, both of which employ hydrogen peroxide either as an oxidizing or as a reducing substrate. Hydrogen peroxide reacts during the first reaction stage (reaction 1) with the haem iron to form a water molecule and an oxyferryl species called compound I [$Por^{+\cdot}(Fe^{IV}{=}O)$]. It carries two oxidizing equivalents, one on the iron and one as a delocalized porphyrin cation radical. This reaction intermediate is subsequently reduced in the second stage (reaction 2) by a second molecule of hydrogen peroxide, giving rise to molecular oxygen, a second water molecule and the native enzyme.

$$Por(Fe^{III}) + H_2O_2 \rightarrow Por^{+\cdot}(Fe^{IV}{=}O) + H_2O, \quad (1)$$

$$Por^{+\cdot}(Fe^{IV}{=}O) + H_2O_2 \rightarrow Por(Fe^{III}) + O_2 + H_2O. \quad (2)$$

The ubiquity of catalases and their utility as a diagnostic tool for organism identification have resulted in the enzyme being the focus of extensive biochemical, physiological and structural studies for over 100 y (Deisseroth & Dounce, 1970; Nicholls *et al.*, 2001). One of the most remarkable aspects of haem-containing catalases is the extremely rapid reaction rate of up to 10^6–10^7 s^{-1} at high (>1 *M*) substrate hydrogen peroxide concentrations (Ogura, 1955). In fact, such rapid rates of catalysis are maintained for only short periods of time because peroxide concentrations over 300 m*M* cause a rapid inactivation of small-subunit (∼500 residues per subunit) catalases (Ogura, 1955; Sevinc *et al.*, 1997).

To date, the sequences of more than 190 haem-containing catalases have been determined, making phylogenetic comparisons possible and reliable. Such comparisons have resulted in the identification of a number of highly conserved residues, a highly conserved core sequence and the grouping of catalases into three main lines or clades consistent with biological origins (Klotz *et al.*, 1997). Clade I catalases are all small-subunit enzymes identified in plants, algae and bacteria; clade II catalases are all large-subunit enzymes, 700–800 residues per subunit, identified in either fungi or bacteria; clade III catalases are all small-subunit enzymes identified in bacteria, archaea, protozoa, fungi and animals. The abundance of catalase in some tissue types and the availability of various clones from others have facilitated the isolation of large quantities

of pure enzyme useful for a variety of physical studies including crystal structure determination.

The structures of seven haem-containing catalases have been solved to date including enzymes from bovine liver (BLC; Murthy *et al.*, 1981; Fita & Rossmann, 1985; Fita *et al.*, 1986), *Penicillium vitale* (PVC; Vainshtein *et al.*, 1981, 1986), *Micrococcus lysodeikticus* (MLC; Murshudov *et al.*, 1992), *Proteus mirabilis* (PMC; Gouet *et al.*, 1995), *Escherichia coli* (HPII; Bravo *et al.*, 1995, 1999), *Saccharomyces cerevisiae* (CATA; Berthet *et al.*, 1997; Maté, Zamocky *et al.*, 1999) and, most recently, human erythrocytes (HEC; Maté, Ortiz-Lombardia *et al.*, 1999; Ko *et al.*, 2000; Putnam *et al.*, 2000). Despite the widely varied sources of the enzymes, all seven structures present a highly conserved core structure surrounding a deeply buried haem in the active site.

All of the catalases for which the structures have been solved are from either clade II, including the large-subunit PVC and HPII, or clade III, including the small-subunit PMC, MLC, BLC, CATA and HEC. There is a notable absence of solved structures from Clade I enzymes which, from sequence similarities, are expected to retain the catalase fold, though with variations related to their phylogenetic divergence. There are currently sequences available for over 70 clade I enzymes, but most reside in plants, making their purification problematic. There are, however, 14 bacterial clade I enzymes; in this paper we report the crystallization and unit-cell organization of two of them, CatF from *Pseudomonas syringae* (Klotz *et al.*, 1995) and Kat from *Listeria seeligeri* (Haas *et al.*, 1991). The catalase from *L. seeligeri* is a cytoplasmic catalase (Haas *et al.*, 1991), whereas CatF, which is found in all strains of *P. syringae* pv. syringae and some other pathovars, resides in the periplasm as a secreted catalase as well as in the cytoplasm of its host (Klotz & Hutcheson, 1992; Klotz *et al.*, 1995).

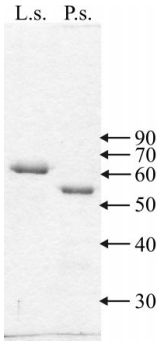

Figure 1
Molecular-weight determination of Kat from *L. seeligeri* (L.s.) and CatF from *P. syringae* (P.s.). The arrows on the right, with sizes in kDa, reflect the location of size markers.

2. Experimental results

CatF encoded on the plasmid pEC3E56 cloned from *P. syringae* pv. syringae strain 61 (Klotz *et al.*, 1995) and Kat encoded on the plasmid pAHA1 cloned form *L. seeligeri* (Haas *et al.*, 1991) were expressed in the catalase-deficient strain UM255 (Mulvey *et al.*, 1988). The catalases were purified essentially as described in Loewen & Switala (1986), except that DEAE cellulose replaced DEAE Sephadex. The proteins were dialysed into 50 m*M* potassium phosphate pH 7 and concentrated by filtration for further characterization. Purified CatF with a specific activity of 78 800 units per milligram of protein was confirmed by SDS–polyacrylamide gel electrophoresis (Fig. 1) and electrospray mass spectrometry to be a homotetramer of 57 000 Da subunits. *L. seeligeri* Kat, with a specific activity of 62 900 units per milligram of protein, migrated on SDS–polyacrylamide gels as a 62 000 Da protein (Fig. 1) consistent with earlier observations (Haas *et al.*, 1991), despite its predicted size of 55 289 Da. This discrepancy may be a result of its acidic pI affecting its rate of migration. One unit of catalase is defined as the amount that decomposes 1 µmol of H_2O_2 in 1 min at 310 K.

Samples of CatF protein at initial concentrations of 6 mg ml^{-1} were used in extensive crystallization screenings with vapour-diffusion methods. Small crystals with a plate-like morphology were obtained using 12% PEG 4000 as precipitant in 0.1 *M* sodium cacodylate buffer at pH 6. Crystals, always smaller than 0.1 × 0.1 × 0.03 mm in size, belong to the monoclinic space group $P2_1$, with unit-cell parameters $a = 60.6$, $b = 153.9$, $c = 109.2$ Å, $\beta = 102.8^\circ$. Diffraction images to 1.8 Å resolution were collected using synchrotron radiation at the ESRF from crystals cooled to liquid-nitrogen temperature. The cryobuffer was the same as the crystallization buffer but containing 30% 2-methyl-2,4-pentanediol. Images were processed and scaled with *DENZO* and *SCALEPACK* (Otwinowski, 1993), giving a diffraction data set with an overall completeness and $R_{\rm merge}$ of 98 and 8.4%, respectively. Considerations of the packing density and the results from the self-rotation function (Fig. 2) support the presence of four catalase subunits (one molecule with 222 symmetry) in the asymmetric unit, which would correspond to a crystal solvent content volume of 40% (Matthews, 1968). Structure determination by molecular replacement with *AMoRe* (Navaza & Saludjian, 1994) and non-crystallographic symmetry averaging is in progress using as an initial model a modified HPII subunit in which 90 residues from the amino-terminal region and 160 residues from the carboxy-terminal domain have been omitted and sequence changes incorporated to bring HPII in line with the CatF sequence.

Extensive crystallization screenings of the *L. seeligeri* KatA with vapour-diffusion methods were undertaken with samples of protein at concentrations of ∼10 mg ml^{-1}. Crystals of 0.2 × 0.2 × 0.2 mm in size with a well developed bipyramidal morphology were obtained using as precipitant 2.1–2.4 *M* ammonium sulfate with HEPES buffer at pH 7.5. The cryobuffer was identical but was supplemented with 30% 2-methyl-2,4-pentanediol. The crystals, belonging to space group $I222$ (or $I2_12_12_1$) with unit-cell parameters $a = 74.4$, $b = 121.3$, $c = 368.5$ Å, probably contain four protein subunits in the asymmetric unit with a volume solvent content of 45%. Kat crystals presented conspicuous anisotropic diffraction, with resolution beyond 2.2 Å along c^* but of only

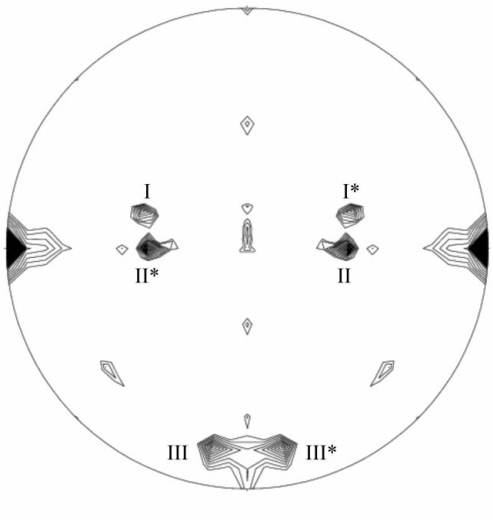

Figure 2
Representation of the $\kappa = 180^\circ$ section of the self-rotation function from the CatF crystals. The largest peak corresponds to the twofold crystallographic symmetry along the *b* axis. Three perpendicular non-crystallographic dyad axis (indicated as I, II and III in the figure) are also clearly defined.

4 Å in the perpendicular directions. Anisotropic diffraction is probably a consequence of the dominant character of packing forces along the long *c* axis (Maté, Ortiz-Lombardia *et al.*, 1999; Hendrickson & Sheriff, 1987).

3. Conclusions

The crystals reported in this paper will provide structural information at almost atomic resolution for CatF, a representative of the clade I catalases, which are the only remaining family of haem-containing catalases for which no structural information is yet available. This will make possible a structural comparison with clade II and III catalases to determine if any structural differences are associated with the sequence differences that have given rise to the phylogenetic divergence. In turn, this will provide insights into the evolution of the haem-containing catalases which will supplement the purely sequence-based conclusions. Because of the anistropic distortion in the crystals of KatA, a complete structure may not be possible but information about specific parts of the molecules may be obtained. Catalases have been found to contain a variety of residues with unique covalent modifications (Nicholls *et al.*, 2001) and the structure of CatF reported here may reveal yet more such modifications, providing further information about the catalytic processes occurring within catalases.

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References

Berthet, S., Nykyri, L. M., Bravo, J., Berthet-Colominas, C., Alzari, P., Koller, F. & Fita, I. (1997). *Protein Sci.* **6**, 481–483.
Bravo, J., Maté, M. J., Schneider, T., Switala, J., Wilson, K., Loewen, P. C. & Fita, I. (1999). *Proteins*, **34**, 155–166.
Bravo, J., Verdaguer, N., Tormo, J., Betzel, C., Switala, J., Loewen, P. C. & Fita, I. (1995). *Structure*, **3**, 491–502.
Deisseroth, A. & Dounce, A. L. (1970). *Physiol. Rev.* **50**, 319–374.
Fita, I. & Rossmann, M. G. (1985). *Proc. Natl Acad. Sci. USA*, **82**, 1604–1608.
Fita, I., Silva, A. M., Murthy, M. R. N. & Rossmann, M. G. (1986). *Acta Cryst.* B**42**, 497–515.
Gouet, P., Jouve, H. M. & Dideberg, O. (1995). *J. Mol. Biol.* **249**, 933–954.
Haas, A., Brehm, K., Kreft, J. & Goebel, W. (1991). *J. Bacteriol.* **173**, 5159–5167.
Hendrickson, W. A. & Sheriff, S. (1987). *Acta Cryst.* A**43**, 121–125.
Klotz, M. G. & Hutcheson, S. W. (1992). *Appl. Environ. Microbiol.* **58**, 2468–2473.
Klotz, M. G., Kim, Y. C., Katsuwon, J. & Anderson, A. J. (1995). *Appl. Microbiol. Biotechnol.* **43**, 656–666.
Klotz, M. G., Klassen, G. R. & Loewen, P. C. (1997). *Mol. Biol. Evol.* **14**, 951–958.
Ko, T. P., Safo, M. K., Musayev, F. N., Di Salvo, M. L., Wang, C., Wu, S. H. & Abraham, D. J. (2000). *Acta Cryst.* D**56**, 241–245.
Loewen, P. C. & Switala, J. (1986). *Biochem. Cell Biol.* **64**, 638–646.
Maté, M. J., Ortiz-Lombardia, M., Marina, A. & Fita, I. (1999). *Acta Cryst.* D**55**, 1066–1068.
Maté, M. J., Zamocky, M., Nykyri, L. M., Herzog, C., Alzari, P. M., Betzel, C., Koller, F. & Fita, I. (1999). *J. Mol. Biol.* **286**, 135–149.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mulvey, M. R., Sorby, P. A., Triggs-Raine, B. L. & Loewen, P. C. (1988). *Gene*, **73**, 337–345.
Murshudov, G. N., Melik-Adamyan, W. R., Grebenko, A. I., Barynin, V. V., Vagin, A. A., Vainshtein, B. K., Dauter, Z. & Wilson, K. (1992). *FEBS Lett.* **312**, 127–131.
Murthy, M. R. N., Reid, T. J., Sicignano, A., Tanaka, N. & Rossmann, M. G. (1981). *J. Mol. Biol.* **152**, 465–499.
Navaza, J. & Saludjian, P. (1994). *Acta Cryst.* A**50**, 157–163.
Nicholls, P., Fita, I. & Loewen, P. C. (2001). *Adv. Inorg. Chem.* **51**, 51–106.
Ogura, Y. (1955). *Biochem. Biophys.* **57**, 288–300.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Putnam, C. D., Arval, A. S., Bourne, Y. & Tainer, J. A. (2000). *J. Mol. Biol.* **296**, 295–309.
Sevinc, M. S., Maté, M. J., Switala, J., Fita, I. & Loewen, P. C. (1999). *Protein Sci.* **8**, 490–498.
Vainshtein, B. K., Melik-Adamyan, W. R., Barynin, V. V., Vagin, A. A. & Grebenko, A. I. (1981). *Nature (London)*, **293**, 411–412.
Vainshtein, B. K., Melik-Adamyan, W. R., Barynin, V. V., Vagin, A. A., Grebenko, A. I., Borisov, V. V., Bartels, K. S., Fita, I. & Rossmann, M. G. (1986). *J. Mol. Biol.* **188**, 49–61.