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Investigating the active centre of the *Scytalidium thermophilum* catalase

Almost all monofunctional haem catalases contain a highly conserved core containing the active site, which is connected to the exterior of the enzyme by three channels. These channels have been identified as potential routes for substrate flow and product release. To further investigate the role of these molecular channels, a series of mutants of *Scytalidium thermophilum* catalase were generated. The three-dimensional structures of four catalase variants, N155A, V123A, V123C and V123T, have been determined at resolutions of 2.25, 1.93, 1.9 and 1.7 Å, respectively. The V123C variant contains a new covalent bond between the S atom of Cys123 and the imidazole ring of the essential His82. This variant enzyme has only residual catalase activity and contains haem *b* instead of the normal haem *d*. The H82A variant demonstrates low catalase and phenol oxidase activities (0.2 and 20% of those of recombinant wild-type catalase–phenol oxidase, respectively). The N155A and N155H variants exhibit 4.5 and 3% of the wild-type catalase activity and contain haem *d*, showing that Asn155 is essential for catalysis but is not required for the conversion of haem *b* to haem *d*. Structural analysis suggests that the cause of the effect of these mutations on catalysis is the disruption of the ability of dioxygen substrates to efficiently access the active site. Additional mutants have been characterized biochemically to further probe the roles of the different channels. Introducing smaller or polar side chains in place of Val123 reduces the catalase activity. The F160V, F161V and F168V mutants show a marked decrease in catalase activity but have a much lower effect on the phenol oxidase activity, despite containing substoichiometric amounts of haem.

1. Introduction

Haem catalases (EC 1.11.1.6) are one of the most studied groups of enzymes. They are found in almost all aerobic organisms, including bacteria, fungi, plants and animal cells (Mueller *et al.*, 1997; Loewen *et al.*, 1993). Although the broad range of biological functions of catalases still remains unclear, their main function is the decomposition of hydrogen peroxide into water and oxygen (Bhaskar *et al.*, 2005).

Catalases have been studied for over 100 years (Nicholls *et al.*, 2001), with the result that examples of the enzyme have been isolated, purified and characterized from many different organisms. The crystal structures of 14 haem-containing catalases have now been solved (Díaz *et al.*, 2012), revealing a common highly conserved core in all enzymes. The active centre consists of a haem with a tyrosine ligand on the proximal side and a conserved histidine and an aspartate flanking the putative peroxide-binding site on the distal side (Chelikani *et al.*, 2003). The haem prosthetic group of monofunctional catalases is either a noncovalently bound iron protoporphyrin IX (haem *b*) or an oxidized form of protoporphyrin IX (haem *d*) (Loewen *et al.*, 1993; Bravo *et al.*, 1995; Murshudov *et al.*, 1996; Maté *et al.*, 2001). It is assumed that the oxidized form of haem *b* is generated self-catalytically by the catalase and requires some degree of catalase activity (Maté *et al.*, 1999). Three channels, the main channel oriented perpendicular to the plane of the haem, the lateral channel approaching the plane of the haem and the central channel leading from the distal side of the haem to the central cavity, connect the

deeply buried active site to the exterior of the enzyme (Díaz *et al.*, 2004; Chelikani *et al.*, 2003).

The main channel is believed to be a primary route for the access of peroxide to the active site (Chelikani *et al.*, 2003; Switala & Loewen, 2002), and studies of various catalase variants from different sources with mutations in the residues lining the channel (Zamocky *et al.*, 1995) as well as molecular-dynamics simulations (Kalko *et al.*, 2001; Amara *et al.*, 2001) support this idea. The lateral or minor channel approaches the haem from above, adjacent to the essential asparagine, and emerges on the molecular surface at a location corresponding to the NADP(H)-binding pocket in catalases that bind a nicotinamide cofactor (Maté *et al.*, 2001). The function of this channel remains unknown (Díaz *et al.*, 2004), although molecular-dynamics simulations suggest that water can exit the protein through this channel (Sevinc *et al.*, 1999).

A number of highly conserved residues are situated in the main channel. These include the essential histidine and a conserved valine and aspartate (His128, Val169 and Asp181 in hydroperoxidase II), which are situated 4, 8 and 12 Å from the haem, respectively (Chelikani *et al.*, 2003). The histidine residue is essential for catalysis (Melik-Adamyany *et al.*, 2001) and the side chain of the valine residue narrows the channel to a diameter of about 3 Å, preventing any molecule larger than H₂O and H₂O₂ from gaining access to the active site. The presence of a negatively charged side chain at this position has been found to be critical for catalysis (Chelikani *et al.*, 2003).

Although catalases have been studied for many years, a peroxide-independent oxidative activity of catalases has recently been recognized (Vetrano *et al.*, 2005; Sutay Kocabas *et al.*, 2008; Yuzugullu *et al.*, 2013). For example, the *Scytalidium thermophilum* catalase (CATPO) has been shown to oxidize *o*-diphenolic and some *p*-diphenolic compounds in the absence of hydrogen peroxide (Ögel *et al.*, 2006; Sutay Kocabas *et al.*, 2008; Yuzugullu *et al.*, 2013). This and other studies have led to the proposal that this secondary oxidative activity may be a general characteristic of catalases.

As part of an ongoing study of haem catalases, CATPO variants with mutations of His82, Asn155, Val123, Phe160, Phe161 and Phe168 were produced. Variants at other positions were also created in order to further investigate the role of the protein structure and molecular channels in catalysis. In total, 11 mutant variants of CATPO have been biochemically characterized and the structures of four of them, N155A, V123A, V123C and V123T, determined at 2.25, 1.93, 1.9 and 1.7 Å resolution, respectively, are reported here.

2. Materials and methods

2.1. Materials

Standard chemicals and biochemicals were obtained from Sigma. Molecular-size markers and DNA ladder were obtained from BioLabs. Site-directed mutagenesis was performed using the QuikChange approach.

2.2. Strains and plasmids

The plasmid pET28a-CATPO (Yuzugullu *et al.*, 2013) including a hexa-His tag and a TEV cleavage site was used as the source of the *catpo* gene. *Escherichia coli* XL-1 Blue was used as a general cloning strain and the BL21 Star (DE3) strain was used for expression of the mutant *catpo* constructs and isolation of the mutant CATPO proteins.

Table 1
Oligonucleotides used in site-directed mutagenesis of catpo.

Table with 3 columns: Mutant, Sequence change, Oligonucleotide†. Rows include H82A, N155A, N155H, V123A, V123C, V123G, V123S, V123T, F160V, F161V, F168V with their respective sequence changes and oligonucleotide sequences.

† The sequence in bold is the codon that has been modified.

2.3. Site-directed mutagenesis

Single-point mutations were introduced into the catpo coding region by a QuikChange approach with mutagenic primer pairs using the approach described by Stratagene but with KOD DNA polymerase (Novagen) for 25 cycles before DpnI digestion. The PCR primers containing the desired mutations were purchased from Sigma and are listed in Table 1.

2.4. Protein overexpression and purification

pET28a-CATPO was freshly transformed into E. coli BL21 Star (DE3) cells and a single colony was inoculated into 10 ml LB medium supplemented with 50 µg ml⁻¹ kanamycin and incubated overnight at 310 K with shaking (200 rev min⁻¹) (Yuzugullu et al., 2013). This overnight culture was used to inoculate 1 l LB with 50 µg ml⁻¹ kanamycin in a 2.4 l conical flask and was grown at 310 K (200 rev min⁻¹) to an OD600 of 0.6–0.8. IPTG was added (to a final concentration of 0.1 mM) and incubation was continued at 303 K (120 rev min⁻¹) for 24 h in order to achieve semi-anaerobic conditions. The cells were then harvested by centrifugation for 10 min at 6000 rev min⁻¹ and the pellets were frozen at 193 K until use. Following thawing of the pellet, the cells were lysed using 100 ml lysis buffer [50 mM Na HEPES, 25% (w/v) sucrose, 1% (v/v) Triton-X 100, 5 mM MgCl₂] per litre of original culture. The resulting suspension was centrifuged at 10 000 rev min⁻¹ for 30 min and the supernatant was either used as a crude enzyme solution for activity assays or was dialyzed overnight against 20 mM sodium phosphate buffer pH 7.4, 0.5 M NaCl. The dialyzed fraction was filtered (0.2 µm) and loaded onto a HiTrap Chelating HP column (1 ml; GE Healthcare) pre-charged with Ni²⁺ and pre-equilibrated with 20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole pH 7.4 using a Gradifrac Purifier (GE Healthcare, USA). Recombinant hexa-His-CATPO was purified by affinity chromatography by applying a wash step (20 mM sodium phosphate, 0.5 M NaCl, 100 mM imidazole pH 7.4) followed by elution as 1 ml fractions over a 50 ml linear gradient from 0.1 to 0.5 M imidazole. Each fraction was tested for protein purity by SDS-PAGE and the concentration of the pooled fractions was determined by the Bradford assay using bovine serum albumin as a standard.

2.5. Enzyme-activity assay

Catalase and phenol oxidase activity assays were performed using a temperature-controlled spectrophotometer (Shimadzu UV-2401). All assays were performed in triplicate in 100 mM sodium phosphate buffer pH 7.0 at 333 K. Specific activity assays for catalase activity were carried out using 10 mM H₂O₂ as the substrate and monitoring the decrease in absorbance at 240 nm. Enzyme activity

Table 2

Crystallographic data-collection and refinement statistics.

Values in parentheses are for the outermost shell.

Variant	N155A	V123A	V123C	V123T
PDB code	4b31	4b40	4b2y	4b5k
Diamond beamline	I24	I24	I04-1	I24
Wavelength (Å)	0.97	0.97	0.92	0.97
Space group	C2	C2	C2	C2
Unit-cell parameters				
<i>a</i> (Å)	201.0	201.7	201.3	201.4
<i>b</i> (Å)	121.3	121.8	121.2	122.0
<i>c</i> (Å)	125.6	125.4	125.0	125.7
Resolution (Å)	113.3–2.25 (2.31–2.25)	29.4–1.93 (1.98–1.93)	29.01–1.90 (1.95–1.90)	28.92–1.70 (1.74–1.70)
<i>R</i> _{merge} [†] (%)	11.1 (47.0)	8.0 (41.6)	8.9 (43.7)	9.6 (40.9)
<i>R</i> _{p.i.m.} [‡] (%)	7.1 (30.8)	5.2 (26.8)	5.6 (27.1)	6.2 (29.1)
Observed reflections	431129	672140	728570	967195
Unique reflections	127403	202855	209169	297340
Completeness (%)	99 (99.1)	98.7 (99.8)	98.5 (99.6)	99.1 (97.1)
Multiplicity	3.4 (3.3)	3.3 (3.3)	3.5 (3.6)	3.3 (2.7)
<i>I</i> / <i>σ</i> (<i>I</i>)	10.4 (3.2)	10 (3.0)	11.9 (3.1)	9.8 (2.4)
Refinement				
<i>R</i> factor (%)	15.4 (27.7)	16.7 (25.3)	15.2 (20.2)	15.4 (25.6)
<i>R</i> _{free} [§] (%)	20.8 (35.5)	19.9 (31.4)	18.1 (24.4)	18.0 (29.5)
No. of protein atoms	22213	21979	21683	22181
No. of solvent molecules	1357	1682	1804	2019
No. of ligand atoms	176	176	172	176
Average overall <i>B</i> factor (Å ²)	15.3	22.4	17.8	19.8
R.m.s.d., bond lengths [¶] (Å)	0.0173	0.0103	0.0116	0.0132
R.m.s.d., bond angles [¶] (°)	1.96	1.53	1.81	1.65
Ramachandran analysis ^{††} (%)				
Residues in most favoured regions	96.03	96.09	96.5	96.4
Outliers	0.47	0.50	0.31	0.34

[†] $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$. [‡] *R*_{p.i.m.} is the precision-indicating (multiplicity-weighted) *R*_{merge} relative to *I*⁺ or *I*[−]. [§] *R*_{free} was calculated with a randomly set aside 5% of the reflections. [¶] Based on the ideal geometry values of Engh & Huber (1991). ^{††} Ramachandran analysis was performed using the program *MolProbity* (Chen *et al.*, 2010).

was determined using the initial rate of the reaction and an extinction coefficient at 240 nm for H₂O₂ of 39.4 M^{−1} cm^{−1} (Merle *et al.*, 2007). One unit of activity was defined as the amount of enzyme that catalyzed the decomposition of 1 μmol of H₂O₂ per minute. Phenol oxidase activity was determined by monitoring the increase in absorbance at 420 nm using 100 mM catechol as the substrate. Enzyme activity was determined using the initial rate of the reaction and an extinction coefficient at 420 nm of 3450 M^{−1} cm^{−1} for oxidized catechol (Ögel *et al.*, 2006), where one unit of activity corresponded to the formation of 1 nmol of product per minute.

2.6. UV–Vis spectra

All spectroscopic measurements were performed on a Shimadzu UV-2401 UV–Vis spectrophotometer at room temperature in a 1 cm quartz cuvette between 400 and 750 nm.

2.7. Crystallization, data collection and refinement

Crystals of the CATPO variants were obtained by hanging-drop vapour diffusion using mother liquor consisting of 6–16% (w/v) PEG 400, 0.2 M potassium chloride, 0.01 M calcium chloride, 0.05 M sodium cacodylate pH 5.0–5.6. The crystals were monoclinic, belonging to space group C2 with one CATPO tetramer molecule in the asymmetric unit, and had a solvent content of 44%. Diffraction data were collected at 100 K from crystals that had been transferred into a solution containing 20% (v/v) PEG 400 and flash-cooled in a nitrogen cryostream, giving the unit-cell parameters listed in Table 2. The diffraction data were autoindexed and integrated using *XDS* (Kabsch, 2010) and merged using *SCALA* (Evans, 1997). Structure determination was carried out by difference Fourier methods using the structure of recombinant wild-type CATPO with ligands and solvent removed as a model (Yuzugullu *et al.*, 2013). Structural

models were built and refined using *Coot* and *REFMAC5* (Emsley *et al.*, 2010; Murshudov *et al.*, 2011). The final structures of the N155A, V123A, V123C and V123T variants of CATPO were determined at 2.25, 1.93, 1.9 and 1.7 Å resolution, respectively. The asymmetric units of the four CATPO variants analysed in this study each contained a CATPO homotetramer. The N-terminal 20 residues of all subunits in each of the four variants were disordered, as in the wild-type enzyme (Yuzugullu *et al.*, 2013), and were not included in the refined structures. The structure factors and coordinates for each structure have been deposited in the Protein Data Bank under accession codes 4b31 for the N155A mutant, 4b40 for the V123A mutant, 4b2y for the V123C mutant and 4b5k for the V123T mutant. All figures were prepared using *PyMOL* (<http://www.pymol.org/>).

3. Results

We have previously shown that changing the conserved histidine 82 to an asparagine resulted in extremely low catalase and reduced phenol oxidase activities in CATPO (Yuzugullu *et al.*, 2013). Here, the replacement of histidine by the smaller residue alanine caused a similar reduction in activity (Table 3) and also resulted in the presence of a *b*-type protohaem in the active site in place of haem *d* (Table 4).

Asn155, a conserved asparagine, is situated at the base of the main channel of all catalases and is approximately 9 Å from the haem. The replacement of asparagine by alanine in HP11 catalase (Melik-Adamyany *et al.*, 2001) results in a decrease in catalase activity, suggesting that this asparagine plays a role in catalysis or substrate binding. In this study, the N155A mutant was deficient in both catalase and phenol oxidase activities (Table 3). Spectroscopic measurements and the structure show that the haem is still oxidized to haem *d* (Figs. 1 and 2; Table 4), consistent with previously reported

results for this variant of HP11 catalase (Melik-Adamyan *et al.*, 2001). However, the structure showed that the chain of ordered water

Table 3
Specific activities of purified CATPO variants and type of haem present.
Nd, not detectable.

Variant	Specific activity		% of WT activity†		Haem
	Catalase ($\mu\text{mol mg}^{-1} \text{min}^{-1}$)	Phenol oxidase ($\text{nmol mg}^{-1} \text{min}^{-1}$)	Catalase	Phenol oxidase	
Wild type	18713 $\pm$ 935	213 $\pm$ 5	100	100	<i>d</i>
H82A	33	42 $\pm$ 7	0.4	46	<i>b</i>
N155A	537 $\pm$ 3	78 $\pm$ 3	4.5	56	<i>d</i>
N155H	241 $\pm$ 15	66 $\pm$ 3	3	71	<i>d</i>
V123A	3827 $\pm$ 132	61 $\pm$ 5	60	87	<i>d</i>
V123C	40 $\pm$ 2	26 $\pm$ 2	0.2	14	<i>b</i>
V123G	18084 $\pm$ 183	222 $\pm$ 3	70	81	<i>d</i>
V123S	4127 $\pm$ 494	128 $\pm$ 4	31	84	<i>d</i>
V123T	9254 $\pm$ 607	113 $\pm$ 8	89	92	<i>d</i>
F160V	65 $\pm$ 6	19 $\pm$ 1	1.2	103	Nd
F161V	153 $\pm$ 14	155 $\pm$ 2	2	175	Nd
F168V	18 $\pm$ 1	15 $\pm$ 3	1.7	140	Nd

† Percentage of the wild-type activity, corrected for haem content.

molecules that fill the main channel is disrupted in the N155A mutant, suggesting that the loss of activity arises from a reduced ability of peroxide to access the active site. To further probe the role of Asn155, the effect of a bulkier side chain at this location was investigated by the construction of an N155H variant. Purification and biochemical characterization of this variant revealed the

Table 4
Spectroscopic characterization of CATPO-variant haem content.

Variant	Rz (A_{406}/A_{280})	A_{590}/A_{406}	A_{715}/A_{406}	A_{535}/A_{406}	A_{630}/A_{406}
WT	0.7	0.15	0.04	—	—
H82A	0.2	—	—	0.15	0.1
N155A	0.5	0.20	0.06	—	—
N155H	0.4	0.18	0.05	—	—
V123A	0.6	0.15	0.03	—	—
V123C	1	—	—	0.1	0.07
V123G	0.7	0.17	0.05	—	—
V123S	0.6	0.18	0.06	—	—
V123T	0.8	0.18	0.06	—	—
F160V	0.09	—	—	—	—
F161V	0.09	—	—	—	—
F168V	0.07	—	—	—	0.2

Figure 1
UV-Vis spectra of CATPO variants. (a) Spectra of the H82A and N155A variants. (b) Spectra of the F160V, F161V and F168V variants. (c) Spectra of the V123A, V123C, V123G, V123S and V123T variants. The spectra were obtained with 1 mg ml⁻¹ catalase in 20 mM sodium phosphate pH 7.4 at room temperature. The wild-type CATPO (WT_CATPO) spectrum is shown in each panel, adjusted to have an equivalent value at the Soret peak for each set of mutants. (d) SDS-PAGE gels of the CATPO variants stained with Coomassie Blue, showing that each mutant was purified to homogeneity.

presence of haem *d* and of 3 and 71% of the wild-type catalase and oxidase activities, respectively (Tables 3 and 4). These data suggest that Asn155 facilitates catalysis, but is not essential (Loewen *et al.*, 1993).

Val123 is a conserved valine that is present in the main channel of all catalases and is sited approximately 9 Å from the haem. Its side chain causes a constriction or narrowing of the channel to a diameter of approximately 3 Å that prevents any molecules much larger than H₂O and H₂O₂ from gaining access to the active site. To determine whether Val123 plays a similar role in CATPO, a series of mutations were created at this position: V123A, V123G, V123S, V123T and V123C. Characterization of mutants containing smaller uncharged residues showed that the V123A variant has 60% of the wild-type catalase activity and 87% of its oxidase activity and that the V123G variant has a similar activity profile (Table 3). Introducing the possibility of hydrogen bonding in the V123S and V123T variants also resulted in a slightly decreased efficiency of the catalase reaction (Table 3). All variant enzymes apart from H82A and V123C

contained haem *d* (Fig. 1; Tables 3 and 4). The crystal structures of the V123A and V123T variants showed a broadly similar chain of ordered waters in the main channel to that observed for wild-type CATPO (Fig. 2). In the structure of the V123A variant, W6 and W8, water molecules that sit to one side of the channel at the constriction point created by Val123, are missing and a new water molecule is observed to bind in the main channel in the wider pocket created by the V123A mutation. In the V123T variant W7 and W8 are missing entirely and no additional water is bound.

As previously reported for the same mutation in HP11 catalase (Maté *et al.*, 1999), mutation of Val123 to cysteine resulted in more marked changes in both the structure and activity of CATPO. V123C had only 0.2% of the wild-type catalase activity but retained 14% of its phenol oxidase activity (Table 3) and contained haem *b* (Table 4). In addition, as previously observed for HP11 catalase (Maté *et al.*, 1999), this variant contains an unusual covalent bond between the essential histidine (His82) and the substituted residue (Cys123; Fig. 3). The His82 imidazole ring is rotated ~30° relative to its position in

Figure 2

Comparison of the haem distal side of wild-type CATPO (a) and the N155A (b), V123A (c), V123C (d) and V123T (e) variants. The corresponding electron densities are shown for the five cases. For clarity, only the catalytically important residues His82, Asn155 and Phe168 on the haem distal side are explicitly shown. Also displayed are the conserved residues lining the channel: Val123, Asp135, Phe160 and Phe161. The ring of hydrophobic residues that includes Val123 defines the narrowest point in the major channel. Haem *d* is evident in wild-type CATPO (a) and the N155A (b), V123A (c) and V123T (e) variants, while haem *b* is only evident in the V123C variant (d). Changes in solvent organization are evident among the structures. Water molecules in the recombinant wild-type enzyme and their equivalents in the variants are labelled numerically: W1–W11. Water molecules in the variant structures that do not correspond to waters in recombinant wild-type CATPO are labelled alphabetically: WA–WD.

Figure 3

The environment of the haem centre of wild-type CATPO (a) and the V123C mutant (b). The $2F_o - 2F_c$ electron-density map is shown in blue. The covalent bond formed between the S atom of the mutated Cys123 and the C atom of the imidazole ring of His82 is shown. The structure of wild-type CATPO contains haem *d*, while the V123C variant contains unmodified protohaem (haem *b*). The proximal ligand of the haem iron, Tyr369, is also illustrated.

wild-type CATPO and this places the C^ε of the imidazole ring just 2 Å from the cysteine S atom, a distance that is consistent with a covalent bond, and the electron density clearly supports this. The formation of the His–Cys covalent bond is likely to result in an increase in the p*K*_a of His82 as well as altered imidazole–haem packing, explaining the reduced catalytic activity observed in this variant and the lack of conversion of haem *b* to haem *d*. As in the other Val123 variants the water structure in the main channel is disrupted, and a similar solvent arrangement to V123A is observed (Fig. 2).

A cluster of conserved hydrophobic residues, Phe160, Phe161 and Phe168, are also located in the main channel of all catalases and are approximately 10, 12 and 9 Å from the haem, respectively. It has been proposed that like Val123 these bulky side chains are responsible for the exclusion of larger organic substrates from the active site (Zamocky *et al.*, 1995). In this study, although these proteins express well and can be purified, spectroscopic characterization indicates that very little haem is bound (Fig. 1; Table 4), which is reflected in the very low catalase activity that is observed (Table 3). Even when corrected for haem content, these variants still show <2% of the wild-type catalase activity. Consistent with this observation, Phe168 sits directly above the haem, with its side chain oriented parallel to the tetrapyrrole system, and has also been suggested to be involved in haem incorporation (Zamocky *et al.*, 1995). Interestingly, although it has a similar haem content and catalase activity to the F160V and F168V mutants, the F161V mutant showed nearly twice the wild-type phenol oxidase activity (Table 3). Unfortunately, no crystals of these mutants have been obtained and the reason for this unexpected level of phenol oxidase activity despite the low haem content remains unclear, although one possibility might be poor containment of peroxide, reducing its effective concentration at the active site below that required for the reduction of compound I (Dominguez *et al.*, 2010; Jha *et al.*, 2011).

4. Conclusions

We report here the biochemical characterization of a series of main-channel mutants of the *S. thermophilum* catalase as well as the structural characterization of four of these mutants. Differences

in the pattern of ordered solvent molecules observed in the main channel in the different mutants suggests that the altered catalase and phenol oxidase activities of the mutants may be caused by a differential disruption of the access of dioxygen species to the active site, as has previously been proposed for equivalent mutations in monofunctional catalases derived from other sources (Carpena *et al.*, 2003; Chelikani *et al.*, 2003; Maté *et al.*, 1999; Melik-Adamyany *et al.*, 2001). The data reported here suggest that the mode of dioxygen-species binding and access to the active site of CATPO is similar to that in other monofunctional catalases.

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