Structure and heme environment of beef liver catalase at 2.5 Å resolution

(polyptide fold/proximal tyrosine/amino triazole complex/crystallography)

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ABSTRACT Most of the amino acid side chains of beef liver catalase were clearly identifiable in the 2.5 Å resolution electron-density map, and the results are in good agreement with the sequence [Schroeder, W. A., Shelton, J. R., Shelton, J. B., Robberson, B. & Apell, G. (1989) Arch. Biochem. Biophys. 131, 653-655]. The tertiary structure of one subunit consists of a large antiparallel β-pleated sheet domain with helical insertions, followed by a smaller domain containing four α-helices. The heme group is buried at least 20 Å below the molecular surface and is accessible by a channel lined with hydrophobic residues. The proximal ligand is tyrosine-357, while histidine-74 and asparagine-147 are the important residues on the distal side of the heme. The inhibitor 3-amino-1,2,4-triazole, which has been shown to covalently bond to histidine-74, can be built into the heme cavity with its N(2) atom coordinated to the heme iron.

Catalase (hydrogen peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) occurs in almost all aerobically respiring organisms and in part serves to protect cells from the toxic effects of hydrogen peroxide. The subcellular location of liver catalase is restricted to the peroxisomes, and the enzyme is probably incorporated into these organelles during their biogenesis (1). The properties of catalase have been reviewed by numerous authors including Deisseroth and Dounce (2) and Schonbaum and Chance (3). The overall reaction catalyzed by the enzyme can be written as

\[ \text{ROOH + HQOH = QO + ROH + H}_2\text{O}, \]  

where \( R \) is H or an alkyl or aryl group and \( \text{HQOH} \) is a two-electron donor in which \( Q \) is O, C==O, or H(CH\( n \))\( \text{CH}_2 \)\( \text{CH}_2 \)\( \text{CH}_2 \)\( \text{n} = 1, 2, \text{or} 3 \). This reaction proceeds by two steps: (i) oxidation of the enzyme (E) by a peroxide

\[ \text{E-H}_2\text{O} + \text{ROOH} = \text{E-O} + \text{ROH} + \text{H}_2\text{O}, \]  

apocatalase

and (ii) oxidation of the substrate

\[ \text{E-O + HQOH = E-H}_2\text{O + QO}. \]  

Compound I possesses two oxidation equivalents above the native enzyme and contains a highly reactive oxygen bound to the iron (4). Oxidation of the enzyme is probably achieved by raising the heme iron from an Fe(III) to an Fe(IV) state and by forming a radical on the heme (5). Substrate oxidation is operationally divided into "catalytic" activity when \( Q \) is an oxygen atom and "peroxidatic" activity when \( Q \) is one of the other groups listed above. Reaction 3 can also proceed, albeit more slowly, in the presence of one-electron donors via steps involving the one-oxidation equivalent, compound II and, in the presence of \( \text{H}_2\text{O}_2 \) via the three-oxidation equivalent, compound III (6). Compounds II and III are enzyme-peroxide derivatives with formal oxidation states Fe(IV) and Fe(VI), respectively (3).

The proximal fifth ligand of the heme has been postulated as a carboxyl group (7, 8), tyrosine (9), histidine (10), or as being nonnitrogenous (11). The identity of the sixth ligand has been suggested to be a hydroxide ion (12, 13), a water molecule (9, 12, 15), or a protein group (8, 15).

Beef liver catalase contains four identical subunits (16), each of \( M = 57000 \) and equipped with a high-spin Fe(III)-protoporphyrin-IX (17-19). However, approximately two of the four heme groups are degraded to a high-spin Fe(III)-bilaevdin complex (20, 21). The monomer consists of 506 amino acid residues with an additional 10-15 residues occurring at the COOH terminus of a small fraction of the subunits. The complete amino acid sequence has been determined, although the amino terminus is blocked by an as yet unidentified group (ref. 22; W. A. Schroeder, personal communication).

Catalase was one of the first enzymes to be crystallized (20). Electron microscopy (23, 24), electron diffraction (25-27), and low angle x-ray scattering (28) all show a slightly ellipsoidal molecule with an average radius of 40 Å. Single-crystal forms of catalase reported to date (29-37) contain one or more molecules per asymmetric unit except for bacterial catalase (35), the catalase of Penicilium citrate (36), and a trinodal form of beef liver catalase (37), all of which incorporate one crystallographic 2-fold axis in the molecule. We describe here the preliminary results of a high-resolution structure determination of the beef liver catalase crystals reported by Eventoff et al. (37). Comparison with the high-resolution results for P. citrate catalase (36) is not yet possible but will undoubtedly be of considerable interest.

MATERIALS AND METHODS

Beef liver catalase was purified by a method similar to that of Sumner and Dounce (20). Purity of the final catalase solution was determined by NaDODSO₄/polyacrylamide gel electrophoresis and by measuring A at 279 and 405 nm. Catalase activity was calculated from the rate of \( \text{H}_2\text{O}_2 \) decomposition and expressed by the Kat. I. value (39), which was 20,000-35,000 g\(^{-1}\) min\(^{-1}\) (40). Catalase concentration was determined from the extinction coefficient at 405 nm, which was 420 OD units cm\(^{-1}\) mM\(^{-1}\) (of the tetramer). Crystals were grown by dissolving catalase in 1.71 M NaCl/10 mM Tris, pH 8.8, to 35 mg/ml. Ali-

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The multiple isomorphous replacement map was averaged (46, 47) between the two noncrystallographically related subunits. The molecular envelope was then defined and used for a further 10 cycles of molecular replacement (48). The final electron density map was displayed in a Richards optical comparator (49) with sections cut perpendicular to the molecular P axis at intervals of 0.75 Å. α-Helix and β-sheet regions were well defined and side chains were easily recognizable, permitting a polypeptide chain tracing consistent with the amino acid sequence (22). A detailed description of the structure will be published elsewhere (50).

RESULTS

The Polypeptide Fold. The molecule is dumbbell shaped (Fig. 1) with a length of 90 Å and a waist of 50 Å diameter. This is in good agreement with unpublished results of Rossmann (1961), who analyzed the structure amplitudes of disordered horse erythrocyte catalase (29).

The heme group could be recognized as a large flattened density having a maximum 38% higher than the next highest protein peak. The shape of this density (Fig. 2) determines both the position of the pyrrole rings and the propionic side chains, which are directed toward the molecular interior. Each propionic acid is neutralized: one interacts with arginine-71 and arginine-111 and the other interacts with histidine-361 and arginine-364. The heme Fe coordinates in the reference subunit are 17, 5, and 14 Å, and the heme orientation is given by...
the direction cosines of $-0.321$, $-0.718$, and $0.617$ with respect to the molecular $P$, $Q$, and $R$ axes. The heme is well buried, 20 Å below the molecular surface at a distance of only 23 Å from the molecular center. This is particularly fascinating in light of the large turnover number of 100,000 per sec per active center, making catalase one of the few enzymes whose reaction rate approaches the limit set by substrate diffusion (51). The buried nature of the heme is, however, consistent with the unsuccessful attempts to replace the heme in catalase (19) and the pH of 2.4 required to completely extract the heme (52). This contrasts to most other known heme proteins.

The NH$_2$-terminal 70 residues form an arm that extends from the globular region of one subunit. A helical part of this arm is situated parallel to the helix containing the essential tyrosine-357 of the $R$-axis-related subunit. The hydrophobic residues methionine-60 and phenylalanine-63 on this helical extension line the heme pocket of the neighboring subunit. This gives structural understanding to the biosynthesis of catalase, which requires the association of heme groups with apo-monomers before assembly into tetramers (53). The intricate weave of the arm with other subunits and the activity of the crystals show that the active molecule must be a tetramer (54). An NH$_2$-terminal arm also occurs in lactate dehydrogenase (55) and in some spherical viruses (56, 57), where it is similarly involved in subunit interactions.

Amino acid residues 70-440 (22) form a large antiparallel $\beta$-pleated sheet of eight strands with a curled-over edge and helical insertions between the strands. The heme group abuts onto one side of this $\beta$-barrel.

The remaining part of the structure consists of a four-helical region. Unlike the structure for $P$. vitale catalase (38), there is no additional "flavodoxin"-like domain at the COOH end of the chain. However, beef liver catalase has $\approx$108 fewer residues than the fungal catalase. The electron density within the helical region is weaker and more diffuse than that in other parts of the molecule. The tracing of the polypeptide chain (Fig. 3) is, as far as can be seen from the available diagrams, consistent with the larger domain of $P$. vitale catalase (38).

The Heme Environment. There is one major channel through which the substrate must diffuse to the active center. The channel is 30 Å long, with a maximum width of 15 Å at its mouth, and opens toward the molecular $R$ axis in the restricted waist of the molecule. The hydrophilic residues aspartate-127, glutamine-167, and lysine-172 are located at the channel entrance. Hydrophobic residues (valines-73 and -115, alanine-116, glycine-117, proline-128; phenylalanines-152, -153, -160, and -163, isoleucine-164, and leucine-198) define the channel. These strict constraints are undoubtedly reflected in the reduction of the rate of formation of compound I by about an order of magnitude for every additional carbon in the R group (Eq. 2) (3).

FIG. 3. Stereo diagram of chain tracing and heme group of one catalase subunit relative to the molecular $P$, $Q$, and $R$ axes. This diagram was prepared by a rough measurement of points (not corresponding to C atoms) along the polypeptide chain on a small electron-density map. (See Table 1.)
which led Chance (58) to conclude that the heme was buried.

Histidine-74 can be modified by 3-amino-1,2,4-triazole, which irreversibly inhibits the enzyme (59). The imidazole ring of histidine-74 is well defined, almost parallel to the heme plane, at a distance of 3.0 Å. The N2 of histidine-74 is 4.5 Å from the Fe atom. The properties of the aminotriazole-inhibited enzyme indicate that the site of modification is associated with the distal side of the prosthetic group (59, 60). Both asparagine-147 and phenylalanine-160 are close to the heme on the same side as histidine-74. The carboxamide of asparagine-147 is 7.0 Å from the Fe atom. Hence, residues histidine-74, asparagine-147, and phenylalanine-160 are associated with the distal side of the heme. The proximal side of the heme is far more confined, being closely surrounded by valine-145, histidine-211, proline-239, arginine-353, and alanine-356. The electron density and heme orientation and position clearly show that tyrosine-357 occupies the fifth coordination site, with the phenolate oxygen 1.5 Å from the heme iron. The three-dimensional heme environment is shown in Fig. 4.

The occurrence of a tyrosine group as proximal heme ligand has not been found in other heme proteins with known tertiary structure except for some mutant hemoglobins (61, 62). Nevertheless, Yonetani and Yamamoto (11) have suggested a nonnitrogenous ligand while Nicholls (9) has indeed suggested a tyrosine as the fifth ligand on the basis of spectroscopic comparison with other heme proteins. The proximity of arginine-353 (~3 Å) to the phenolic oxygen of tyrosine-357 suggests that this oxygen may well be deprotonated. This phenolate group will tend to stabilize the heme iron in the ferric state, making the formation of an O2 complex with Fe(II) catalase less likely (61, 62). In addition, anionic ligands, such as the phenolate group (63), increase the rate of heme autoxidation. The special environment of the heme Fe provided by the L2 tyrosine is consistent with visible and EPR spectra (18).

Model building (Fig. 5) shows that the aminotriazole adduct of histidine-74 can be built into the active center pocket after a slight rotation of the imidazole ring about the C8—C9 bond. Irrespective of whether the N4 (60) or the N3 atom is the point of covalent attachment to the C(5) position of the aminotriazole, the N(2) atom can occupy the sixth coordination position of the heme iron. Thus, when the aminotriazole is bound, no further substitution is possible at the heme or the histidine, resulting in enzyme inhibition. This model of catalase inhibition by aminotriazole-related compounds is consistent with the observations of Margolish et al. (59) that (i) the ring system of the aminotriazole and its analogues is not essential, (ii) the primary amino group attached to C(3) of the aminotriazole must be unsubstituted (because of steric hindrance with the heme), and (iii) an N atom at position 2 of the aminotriazole is essential. A hydroperoxide coordinated to the iron would be in a suitable orientation, consistent with peroxide oxygen geometry (64), to associate with histidine-74. Model building shows that such a complex might be formed without changing the position of the imidazole group, as found in the apoenzyme. This reactive species will then oxidize a substrate as indicated in reaction 3.

CONCLUSION

It is not our purpose here to propose a complete description of the mechanism of catalase action. However, it is possible to provide a structural framework. The preference for small substrates is the result of steric hindrance within the channels leading to the buried heme groups. The heme reactivity is enhanced by the phenolate ligand in the fifth position, which may help the oxidation of Fe(III) to Fe(IV) and the removal of an electron from the heme during the formation of compound I. Both the addition of the oxidant to create compound I and the addition of the subsequent reductant probably involve heme iron adducts containing two atoms. The constraints on peroxidatic activity differ remarkably from those observed in cytochrome c peroxidase (65), in which the proximal ligand is a histidine and the aquo ligand can hydrogen bond to another histidine. Although metmyoglobin and methemoglobin have some ability to catalyze the conversion of H2O2 to H2O and O2 (66), the heme environment in these proteins also differs from that of catalase. The far greater efficiency of catalase may in part be due to the interaction of histidine-74 and asparagine-147 with intermediates.

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