

REVIEW

Human catalase: looking for complete identity

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ABSTRACT

Catalases are well studied enzymes that play critical roles in protecting cells against the toxic effects of hydrogen peroxide. The ubiquity of the enzyme and the availability of substrates made heme catalases the focus of many biochemical and molecular biology studies over 100 years. In human, this has been implicated in various physiological and pathological conditions. Advancement in proteomics revealed many of novel and previously unknown features of this mysterious enzyme, but some functional aspects are yet to be explained. Along with discussion on future research area, this mini-review compile the information available on the structure, function and mechanism of action of human catalase.

KEYWORDS human catalase, structure and function, mechanism of action, futuristic research area

INTRODUCTION

The ability of aerobic respiration using electron transport chain and other haem-containing proteins increased the efficiency of energy production in eukaryotic system. Among many other advantages of this evolutionary breakthrough, generation of reactive oxygen species (ROS) is also very useful phenomenon (Goth et al., 2004; Oktyabrsky and Smirnova, 2007), which if left unchecked would seriously affect an organism's viability. These ROS include hydrogen peroxide, superoxide anion radicals, singlet oxygen, hydroxyl radicals and nitric oxide. More specifically, the role of hydrogen peroxide (H_2O_2) has been implicated in death induction (Sancho et al., 2003).

To combat the destructive effects of ROS and make oxidative cellular metabolism possible, aerobic organisms developed protective antioxidant enzymes such as catalase, superoxide dismutase, peroxiredoxin and glutathione

peroxidase, among which, catalase is a well-known crucial enzyme to scavenge H_2O_2 . This haem-containing protein is the most efficient enzyme and can decompose millions of hydrogen peroxide ($\sim 10^7$ M/Sec) molecules every second (Young and Woodside, 2001) into molecular oxygen and water without the production of free radicals (Fig. 1). Even under anaerobic conditions, catalase is considered necessary to certain parasitic microorganisms for protection against H_2O_2 produced by host organisms (Rocha et al., 1996).

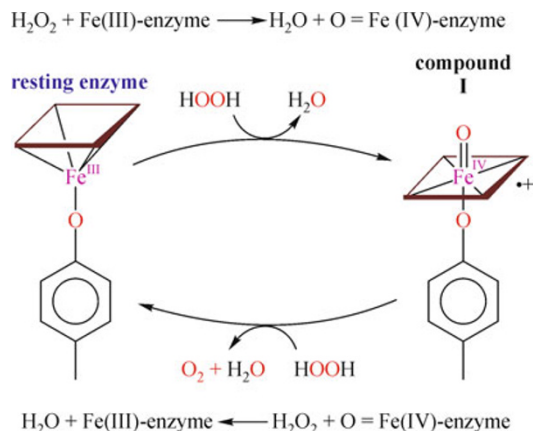


Figure 1. Two-stage mechanism of catalase action. The reaction cycle of the catalase begins with the high spin ferric (Fe^{III}) state, which reacts with peroxide molecule to form compound I intermediate, a porphyrin π -cation radical containing Fe^{IV} . One of the protons of the hydrogen peroxide molecule is being removed from one end of the molecule and placed at the other end. The proton is transferred via a histidine residue in the active site. This action polarizes and breaks the O-O bond in hydrogen peroxide. In the next step, a second hydrogen peroxide molecule is used as a reductant to regenerate the enzyme, producing water and oxygen. Oxidation of an electron donor (here second H_2O_2) returns compound I, a highly-oxidising $Fe(IV)$ species, to the native resting state $Fe(III)$ (Andersson et al., 1991).

Catalases include three types: typical catalases or monofunctional such as mammal type catalases, bifunctional catalase-peroxidases, and pseudo catalase. The mammal type catalases are commonly isolated from animals, plants, fungi and bacteria, and their molecular features are similar: they are composed of four equal-size subunits containing 2.5–4 protohemes-IX per tetramer, with a molecular mass of 225–270 kDa. Typically, the monofunctional catalases display minor peroxidase activity, and the target molecules are limited to small organic substrates. Based on the subunit size, this group can be further divided into catalases with small (55–69 kDa) and large (75–84 kDa) subunits. In addition to the size, difference also exists in heme prosthetic group, with heme b present in small-subunit enzymes (e.g., bovine liver catalase (BLC)) and heme d present in large-subunit enzymes (e.g., *E. coli* HP11). Generally, the monofunctional catalases are active as tetramers, but dimers, hexamers and even an unusual heterotrimer structure (from *Pseudomonas aeruginosa*) were also found (Peter et al., 2000). Catalases exhibit a broad optimum pH range of 5–10; these are glycoproteins that are resistant to treatment with organic solvents and are inhibited by 3-amino-1,2,4-triazole (Clairborne et al., 1979; Nadler et al., 1986; Kim et al., 1994; Brown-Peterson et al., 1995; Terzenbach et al., 1998).

The second group—catalase-peroxidases, have been isolated from bacteria and fungi, resemble plant and fungal peroxidases in sequence but have larger subunits (~80 kDa). They display several distinguished properties in comparison to typical catalases: they are reduced by dithionite, they are not glycoproteins, their activity is pH-dependent, and they are more sensitive to heat, organic solvents and H₂O₂, but they are insensitive to 3-amino-1,2,4-triazole (Nadler et al., 1986; Yumoto et al., 1990; Hochman and Goldberg, 1991; Brown-Peterson and Salin, 1993; Maricinkeviciene et al., 1995; Fraaije et al., 1996). These haem-containing catalases are bifunctional, acting as both catalase and peroxidase, and can use a variety of organic substances as hydrogen donor. Because the catalase-peroxidases contain two similar, fused domains—one active and the other inactive, the genes encoding these enzymes appear to evolve through a duplication event. They bear no resemblance to the monofunctional catalases in sequence, but the catalase-peroxidases do contain heme b and are active as dimers or tetramers. The third group, non-heme catalases, contains only three characterized and sequenced enzymes from different bacterial species. Activity is derived from a manganese-rich reaction center rather than a heme group, and thus, they are called “pseudo-catalases” or non-haem manganese-containing catalases (Peter et al., 2000).

In mammalian tissues, catalase activity is highest in liver and erythrocytes, relatively high in kidney and adipose tissue, intermediate in lung and pancreas, and very low in heart and brain (Deisseroth and Dounce, 1970; Schonbaum and Chance, 1976; Aebi and Wyss, 1978; Kang et al., 1996). In

human, catalase is absent in vascular smooth muscle cells and endothelial cells (Shingu et al., 1985); however, it is found free in the cytosol of mature erythrocytes. Its activity has also been shown in human milk that is about 10 times higher than the level determined in cow's milk (Friel et al., 2002). In recent reviews, the biochemical history of catalase has been tabulated (Kirkman and Gaetani, 2006; Zamocky et al., 2008).

PHYSIOLOGICAL AND PATHOLOGICAL SIGNIFICANCE

The enzyme catalase has a predominant role in controlling the concentration of H₂O₂ (Gaetani et al., 1996; Mueller et al., 1997) and other cytotoxic oxygen derivatives (Renato et al., 1982). The very rigid and stable structure of tetrameric catalases makes them more resistant to pH, thermal denaturation and proteolysis than most other enzymes. Their stability and resistance to proteolysis is an evolutionary advantage, especially since they are produced during the stationary phase of cell growth when levels of proteases remain high and rate of protein turnover is rapid.

Catalase protects hemoglobin by removing over half of the hydrogen peroxide generated in normal human erythrocytes, which are exposed to substantial oxygen concentrations (Gaetani et al., 1989). It has been implicated as an important factor in inflammation (Halliwell and Gutteridge, 1984), mutagenesis (Vuillame, 1987), prevention of apoptosis (Sandstrom et al., 1993; Islam et al., 1997; Yabuki et al., 1999), and stimulation of a wide spectrum of tumors (Miyamoto et al., 1996). Shih Ho et al. (2004) suggested that the role of catalase in antioxidant defense is dependent on the type of tissue and the model of oxidant-mediated tissue injury.

Addition of exogenous catalase usually attenuated apoptosis induction as well as the toxicity of antitumor drugs. It shows both, direct antiapoptotic action of catalase as an antioxidant and indirect proapoptotic action as a suppressor of protective proteins (HSP70 and HSP27) in specialized cells (Sancho et al., 2003). Catalase protects pancreatic β -cells from damage by H₂O₂ (Tiedge et al., 1997; Tiedge et al., 1998). It was suggested that deficiency of catalase and oxidant damage contribute to the development of diabetes (Góth, 2000; Góth and Eaton, 2000). Low catalase activities have been reported in patients with schizophrenia and atherosclerosis (Góth and Vitai, 1996). It also plays an important role in sperm survival within female tract (Lapointe et al., 1998). Loss of catalase leads to the human genetic disease known as acatalasemia, or Takahara's disease (Ogata, 1991). The structure of catalase gene and diseases associated with mutation and polymorphism has also been described (Goth et al., 2004). Catalase was shown to be effective in inhibiting the degeneration of neurons (Busciglio and Yankner, 1995; Mann et al., 1997). A growth promoting factor derived from human erythrocytes with a wide target-cell

spectrum was also identified as catalase (Takeuchi et al., 1995). In brain, the reaction of ethanol with catalase is an important source of acetaldehyde (Mason et al., 1997; Zimatkin et al., 1998), which is implicated in the neurological effects of alcohol in humans (Hunt, 1996). Treatment of rats with the catalase inhibitor, 3-amino-1,2,4-triazole (3AT), decreases voluntary ethanol consumption (Aragon and Amit, 1992). There are also several studies on catalases from agents that cause human disease in relation to protection against the oxidative bursts of macrophages (Archibald et al., 1986; Bishai et al., 1994).

Reduced catalase activity in Xeroderma pigmentosum cells could be directly related to impaired DNA repair (Quilliet et al., 1997) and elevation of catalase activity to an optimum level provides protection against doxorubicin-induced cardiac injury (Kang et al., 1996). It has been proposed that catalase, at acid pH and in the presence of iodide or low concentrations of hydrogen peroxide (H_2O_2), can exert a bactericidal effect similar to that of neutrophil myeloperoxidase. Erythrocyte catalase protects heterologous somatic cells against challenge by high levels of exogenous H_2O_2 , e.g., in areas of inflammation (Agar et al., 1986).

STRUCTURE

The ubiquity of the enzyme and the availability of the substrates (H_2O_2 and alkyl peroxides) have made heme catalases the focus of many biochemical and molecular biology studies (Kani et al., 2004). The crystal structures of eight heme-containing monofunctional catalases have been solved, including those from animal (BLC) and human erythrocytes catalase (HEC) (Fita et al., 1986; Vainshtein et al., 1986; Murshudov et al., 1992; Bravo et al., 1995; Gouet et al., 1995; Mate et al., 1999; Putnam et al., 2000; Ko et al., 2000; Carpena et al., 2003), revealing a highly conserved β -barrel core structure in all enzymes. Because of their large molecular size, catalases have been exploited in a number of crystal-growth experiments (Sato et al., 1993; Malkin et al., 1995). The crystal structure of human erythrocyte catalase (HEC) has been determined (Putnam et al., 2000). It was purified and crystallized in three different forms: orthorhombic, hexagonal and tetragonal (Ko et al., 2000).

HEC is a tetrameric protein of 244 kDa containing 1997 amino acid residues in four identical subunits (named A, B, C, D) of 59.7 kDa, four heme groups, 393 water molecules and four NADPH molecules (Bonaventura et al., 1972; Kirkman and Gaetani, 1984; Fita and Rossmann, 1985). The polypeptide chain of each subunit has residues 4–502. An additional residue, Glu503, was included as an alanine in subunit B. Each subunit can be conceptually divided into four domains: β -barrel, N-terminal threading arm, wrapping loop and C-terminal helices (Fig. 2). The extensive hydrophobic core of each subunit is generated by an eight-stranded anti parallel β -barrel ($\beta 1$ –8), surrounded by a number of α -helices

(Fita and Rossmann, 1985). The N-terminal threading arm (residues 5–70) intricately connects two subunits by hooking through a long wrapping loop (residues 380–438) around another subunit. Finally, a helical domain at one face of the β barrel is composed of four C-terminal helices ($\alpha 16$, $\alpha 17$, $\alpha 18$, and $\alpha 19$) and four helices derived from residues between $\beta 4$ and $\beta 5$ ($\alpha 4$, $\alpha 5$, $\alpha 6$, and $\alpha 7$). Like BLC, the HEC binds NADPH.

Two arm-exchanged dimers, which are related by the Q-axis of the P, Q, R molecular axes as defined for BLC (Fita et al., 1986), assemble to form the 222-symmetric tetramer (Ko et al., 2000) that is roughly square with overall dimensions $100 \text{ \AA} \times 100 \text{ \AA} \times 70 \text{ \AA}$. Tetramerization forces the N-terminal threading arms from the arm-exchanged dimer to cover the heme active site for the other pair of dimers (related by the R-axis). This human and other catalase structures both suggest that tetramerization is essential for function and that tetramer assembly may proceed through arm-exchanged dimers as heme can still be loaded. The heme packs perpendicularly against the $\beta 2$, $\beta 3$ and $\beta 4$ and is held between a loop preceding the first β strand, an extended loop (residues 332–336), two α -helices from the subunit ($\alpha 4$ and $\alpha 12$), and $\alpha 2$ from a subunit in the other arm-exchanged dimer pair. Tetramerization is important to ensure that the active site is sequestered and that the enzyme is competent to complete the reaction cycle rather than allow generation of hydroxyl radicals from exposed heme.

The 1.5 \AA structure of the peroxiacetic acid (PAA) treated catalase reveals that human catalase is extensively hydrated. Throughout the protein, water fills in packing defects between the four domains of the subunit, and between subunits within the tetramer. There were 393 bound waters in the current HEC model (Matthews, 1968). Only the hydrophobic β -barrel and the immediate vicinity of the active site are substantially devoid of these structural water molecules.

The tetrameric enzyme contains a central cavity and several channels that reach the active-site heme groups (Fita and Rossmann, 1985; Gouet et al., 1996; Sevinc et al., 1999; Diaz et al., 2004); access to these deeply buried heme is via narrow channels restrict access to small molecules, explaining, at least in part, the weak peroxidatic activity among catalases (Peter et al., 2000). Spectral analysis of mammalian catalases confirmed the presence of a high spin pentacoordinate heme as active site. A water molecule on the distal side and one tyrosine residue at proximal side present as ligand with the heme (Sharma et al., 1989).

Human catalase binds NADPH at a cleft between the helical domain and the β -barrel on the surface of the molecule. The 19 amino-acid residues of BLC involved in contact with NADPH are also conserved in HEC. The acidic side chain of Asp213 forms hydrogen bonds with the phosphate of the NADPH molecule. Ko et al. showed that the HEC can be fully active with NADPH removed (Ko et al., 2000). Phlorizine, a bacterial product, have tendency to bind with NADPH

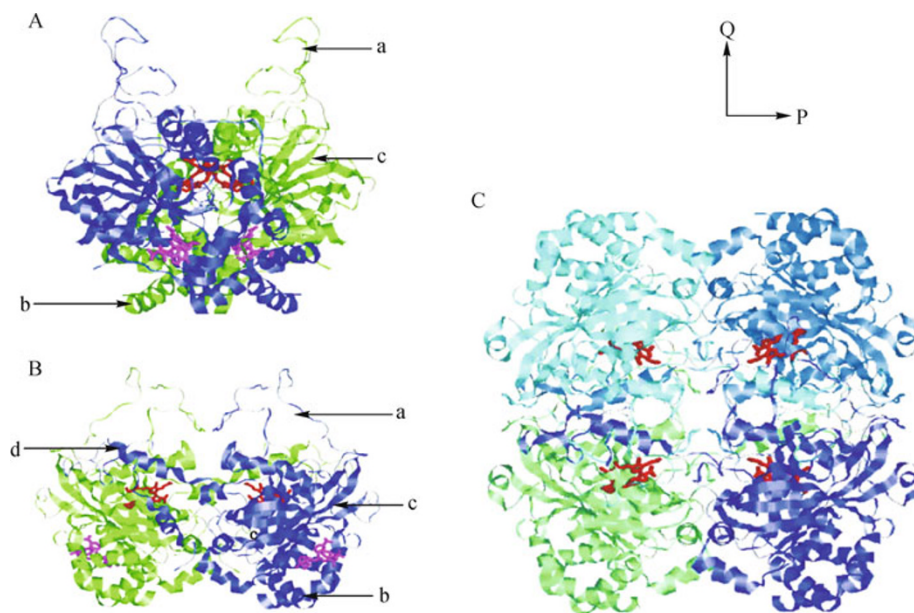


Figure 2. Structure of human erythrocyte catalase. (A and B) Wrapping loop (a), C-terminal helices (b), β -barrel (c) and N-terminal threading arm (d) in arm exchanged dimers. Heme molecules are shown in red color and NADPH molecules are in pink (not shown in tetramer). Dimer 'A' after 90° rotation around axis-Q appears as 'B'. Two dimers exchange their wrapping loops to form active tetramer 'C'. Structures were obtained with the assistance of RasMOL software based on published data (Putnam et al., 2000). Axis R is perpendicular to axis P and Q towards readers' side.

binding site. This property is used to separate catalase by affinity column chromatography (Kitlar et al., 1994).

MECHANISM OF ACTION

Catalase is intensively studied because it is easy to isolate from tissues like liver and blood, which facilitates its purification to provide sufficient protein for detailed biochemical studies. The heme chromophore provided a convenient tool for the workers who intend to study the reaction mechanism, eventually lead to the characterization of two distinct stages in the reaction pathway. Even with the technology advancement in proteomics, a clear understanding of how catalase maintains high selectivity for small-uncharged polar substrate hydrogen peroxide while at the same time exhibiting turnover rates in excess of $10^6/s$ remained elusive (Nicholls et al., 2001; Chelikani et al., 2003). Its turnover number is close to the theoretical rate at which the reactants can diffuse together.

Putnam et al. (2000) explained the selectivity and proposed mechanism of action for human catalase, which includes substrate selection, reactions of compound-I leading to the resting state enzyme or the tyrosine radical, the roles of the 3AT and cyanide inhibitors; it was termed as molecular ruler mechanism for peroxide selection. Initial concentration of hydrogen peroxide at the active site takes advantage of the molecular ruler generated by the narrow, hydrophobic tunnel that promotes occupancy of one peroxide molecule and

blocks the passage of large molecules. According to this mechanism amino acids like Val, Phe, Tyr create hydrophobic interaction a $\sim 25 \text{ \AA}$ deep channel from the surface to the buried active site. $2\text{--}3 \text{ \AA}$ width allows only water, H_2O_2 and few other small molecules to reach the haem molecule. At the time only four molecules of water can be present in this channel. Among them one molecule gets attached at the beginning and another one at the end of the hydrophobic channel by H-bonds. The space between these water molecules in hydrophobic channel is too long to be bridged by the rest of two water molecules and too short to accommodate an additional fifth water molecule. This gap can be filled with one H_2O_2 replacing one H_2O among four present in hydrophobic channel. The low dipole moment of H_2O_2 is more suitable than water to enter in hydrophobic environment of channel. In this way H_2O_2 is selected and concentrated at the active site through use of a narrow hydrophobic channel with two fixed water sites next to the active site (His75 and Asn148) and at the other end of the hydrophobic channel (Asp128 and Gln168).

Haem-containing catalases break down hydrogen peroxide by a two-stage mechanism in which hydrogen peroxide alternately oxidises and reduces the haem iron at the active site (Fig. 1). Both the resting state and compound I of catalase are neutral. At low H_2O_2 concentrations and in the presence of one-electron donors, compound I may undergo a one-electron reduction toward the so-called compound II intermediate, which transforms back to the resting state by

another one-electron reduction step. Formation of compound II and III has been described elsewhere (Kirkman et al., 1999; Rovira, 2005). The haem group in catalase is vital to the reaction, because Fe(III) bound at the center of porphyrin ring can be oxidised to the very oxidised and less common Fe(IV), or ferryl species. This is enhanced by the presence of a nearby tyrosine residue, which is a ligand to the iron in the haem group. The tyrosine is in the ionized phenolate form (O^-) and it has lost its proton due to the electron-withdrawing power of the haem ring and of a nearby arginine residue.

Collectively, presence of amino acid residues at one plane of haem group where H_2O_2 molecules reached from hydrophobic channel (His75 and Asn148), presence at opposite plane (Tyr370), and the volume and shape of the hydrophobic region play very important role in the heterolytic cleavage of H_2O_2 in water and O_2 . They ensure optimum substrate access and electrostatic effects during catalysis (Chelikani et al., 2003). Good geometry for both iron coordination and hydrogen bond formation would require stretching of the peroxide bond, furthering the complex toward the cleavage transition state. The roles of His75 and Asn148 in the formation and polarization of this peroxide complex is the step disrupted by 3-amino-1,2,4-triazole (3AT), and prevention of iron ligation by peroxide is most likely the mechanism of action of cyanide. Less synthesis of heme brings a decrease in catalytic activity (Muppala et al., 2000).

High turnover number of catalase suggests the presence of both inlet and outlet routes to prevent interference between incoming H_2O_2 and exhausting O_2 . Amara et al. (2001) proposed that the major channel is used for both substrate entry and product exit. The exit of product molecules (H_2O or O_2) while substrate (H_2O_2) is entering, is facilitated by presence of cavities located in the near vicinity of the active site. In these cavities, product molecules can stay and wait for substrate molecule to be passed.

ROLE OF NADPH

Catalase is a major NADPH binding protein within human erythrocytes (Kirkman et al., 1984, 1986). X-ray crystallographic studies revealed that each HEC subunit contains (NADPH)b (bound NADPH on HEC surface) in an unusual configuration (Fita and Rossmann, 1985). The binding sites have the relative affinities $NADPH > NADH > NADP^+ > NAD^+$. This reduced dinucleotide is not essential for activity of catalase. The function of the bound NADPH is not fully understood (Kirkman et al., 1984), but three hypothesis were proposed.

The first possibility is that the NADPH decreases the susceptibility of catalase that is inactivated by low concentrations of its toxic substrate, H_2O_2 . When reaction of compound I with suitable reductants, is frustrated, catalase appears to oxidize bound NADPH as the preferred reductant (Kirkman et al., 1999). When NADPH is unavailable, the one-electron

reduction of the porphyrin π -cation by an electron from Tyr370 appears to be the next best alternative. Thus, Tyr and NADPH serve as alternative reduction pathways for catalase trapped in compound I at low peroxide concentrations or with NADPH oxidation being more rapid (Olson and Bruce 1995; Ivancich et al., 1996; Hoffschir et al., 1998; Putnam et al., 2000).

According to next possibility, the (NADPH)b of bovine liver catalase is a remnant of a system in which (NADPH)b was once a necessary intermediate in the prevention of compound II formation by NADPH (present in solution at near vicinity), but the system has evolved so that NADPH bypasses (NADPH)b and provides its reducing equivalents directly for compound II prevention (Gaetani et al., 2005).

Lastly, the purified samples of human and bovine catalase were found to bind and release NADPH, suggesting that catalase may also function as a regulatory protein, releasing $NADP^+$ when the cell is under peroxidative stress. This release would augment the removal of H_2O_2 by the glutathione reductase-glutathione peroxidase mechanisms (Kirkman et al., 1984) to operate more efficiently when the cell is under peroxidative stress. The initial step of that mechanism is catalyzed by glucose-6-phosphate dehydrogenase and is rate limiting in human erythrocytes. Catalase represents a reservoir of 11–12 μM NADPH. Although this represents only one-third of the total NADPH of the erythrocyte, these concentrations exceed the 0–5 μM concentration of unbound $NADP^+$ in normal erythrocytes (Kirkman et al., 1980, 1982; Gaetani et al., 1983).

The finding of (NADPH)b was followed by the finding that added NADPH largely prevents or reverses the formation of compound II by bovine liver or human catalase in the presence of H_2O_2 (Jouve et al., 1986; Kirkman et al., 1987). The protective action of NADPH occurs at concentrations as low as 2 μM (Kirkman et al., 1987). A subsequent study revealed that the action of NADPH is more one of prevention than of reversal (Kirkman et al., 1999). Several groups of authors have proposed that the action of (NADPH)b or NADPH is via electron tunneling (Almarsson et al., 1993; Bicout et al., 1995; Gouet et al., 1995; Olson et al., 1995). The tunnel to the haem group is large enough to accommodate H_2O_2 , but much too small for NADPH. The human erythrocyte, however, has most of its NADP in the form of NADPH, whereas its NAD is nearly all in the form of NAD^+ (Canepa et al., 1991). Therefore, NADPH is known to be effective, and to be oxidized, in preventing the inactivation of catalase exposed to H_2O_2 , but the function of (NADPH)b remains unclear.

INHIBITION

Purdue and Paul (1996) identified a novel peroxisomal targeting sequence (PTS) at the extreme COOH terminal of human catalase that is necessary to targeting to peroxisomes

in human fibroblasts. Deletion of these residues or alteration of the penultimate asparagine to aspartate abolished localization of human catalase to yeast peroxisomes. Catalase assembly can also occur outside of the peroxisome.

Loss of catalase activity by a variety of chemicals including H_2O_2 has been reported (Margoliash et al., 1960; Vetrano et al., 2005; Gibbons et al., 2006). Oxidation by H_2O_2 does not directly affect the active site domain of catalase but brings about conformational changes (necessary for catalysis) by oxidation of amino acid residues. Binding of cyanide clearly blocks heme access to other potential iron ligands (Putnam et al., 2000). 3-amino-1,2,4-triazole (3AT) does not react directly (Jackson et al., 1985) but the near vicinity of active site.

Pyocyanin decreases cellular catalase activity via both transcriptional regulation and direct inactivation of the enzyme (O'Malley et al., 2003). Exogenous nitric oxide also inhibits catalase activity but the effects are reversible in the absence of these agents (Sigfrid et al., 2003).

FUTURE RESEARCH AREAS

In this mini-review, we summarized the information available on human catalase. When started working on human catalase, we realized that there is a need of compiled information to understand its structure, function and mechanism of action so that further experiment can be designed. The

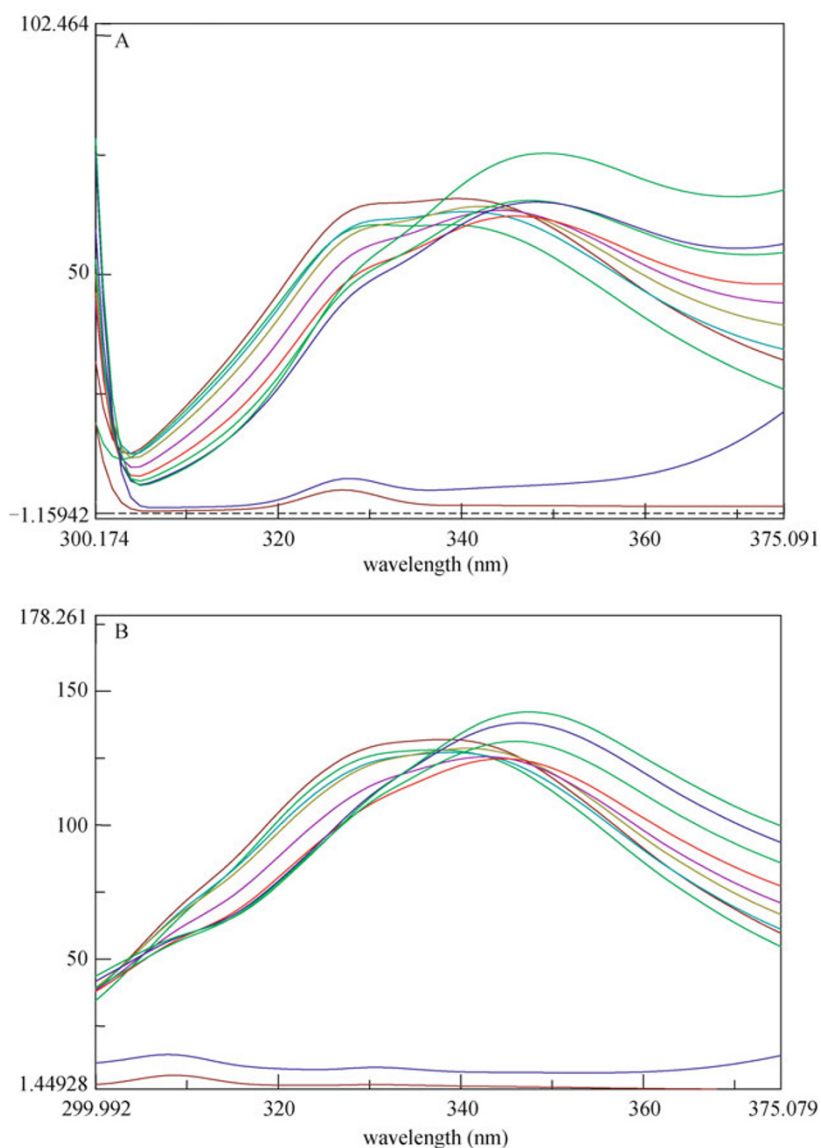


Figure 3. Fluorescent spectra of catalase (BLC) in Na-phosphate buffer (50 mM, pH 7.4) with various concentration of urea (1–8 M). (A) Tryptophan spectra, λ_{ex} 295. (B) Tyrosine and tryptophan combine spectra, λ_{ex} 280. Y-axis denotes fluorescent intensity units. In both spectra, fluorescence peak shifted towards 350 nm with increasing molarity of urea.

information on gray areas is also important. This enzyme plays a significant role in normal function of body ambience, and thus, better understanding can contribute a lot in field of medicine.

Few studies emphasized that the quantity of this enzyme in animal cells is a balance between the rate of synthesis and the rate of degradation (Ganschow and Schimke, 1969). It is thought to be synthesized from single gene and built up from only one type of subunit; exist in heterogenous form with respect to their conformations and association status in biologic system. Prakesh et al. (2002) observed an enzymatically active, folded dimer of native BLC, which is not reported previously. Safo et al. (2001) reported tetragonal crystal of HEC in which 20 residue N-terminal segments corresponding to the first exon of the human catalase gene was omitted. N-terminal segment is essential for dimerization hence for catalytic activity. A new oxidase activity has recently been characterized in catalase (Vetrano et al., 2005). From protein unfolding study in our laboratory, we recently found that bovine liver catalase (of Sigma) produce hydroxyl radicals in presence of 1–8 M urea (unpublished data). This hydroxyl radical generation by catalase has also been shown in physiologic conditions. The existences of active dimers or monomers of catalase tetramer thought to be responsible for this activity.

There are very few articles available on the synthesis of catalase in mammalian systems. The events occur during the synthesis of catalase by which heme is loaded in tetramer and the entry into peroxisomes is not clearly elucidated. The computer simulation proved that SNP (single nucleotide polymorphism) in catalase gene may translate in the form of presence of different amino acid in wild type polypeptide chain. This can cause decrease or complete loss of enzyme activity (Wood et al., 2008). In the face of few available reports, detail screening is required to identify these variations. In a recent review some gray areas related to catalase structure, function and mechanism of action have been discussed (Kirkman and Gaetan, 2007). It shows that the presence of NADPH with enzyme is not completely understood. The conversion of DNA damaging solar radiation into less energetic oxidant species ROS by catalase is novel and previously unrecognized activity (Heck et al., 2003). In erythrocytes, GPx is capable to remove endogenously produced H_2O_2 thus the role of catalase is thought to be to remove exogenous H_2O_2 (Johnson et al., 2010). As erythrocytes circulate in the entire body system, the role of catalase in regulation of systemic redox status (H_2O_2 flux) and comparative load bearing capacity against stress among other antioxidant enzymes like glutathion peroxidase and thioredoxin is yet to be explored.

As Kirkman and Gaetani (2007) titled the mammalian catalase 'a venerable enzyme with new mysteries', the enzyme coming out with new unexplained properties corroborates with the title and presents a scope for further research.

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