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CATALASE: A REPERTOIRE OF UNUSUAL FEATURES

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ABSTRACT

Catalases are antioxidant enzymes which catalyze the breakdown of hydrogen peroxide to water and oxygen, and are one of the oldest enzymes to be studied biochemically. The first crystal structure of a catalase appeared in the year 1980 and it revealed the tetrameric nature of the enzyme and presence of channels accessing the deeply buried active site heme. An interesting feature of the tetrameric structure is the characteristic interweaving or arm exchange of the subunits. The recent elucidation of the crystal structure of transport proteins (porins, aquaporins) showed that these proteins are also tetrameric in nature and posses channels. However, recent specific investigations focusing on the roles for these channels, in the mechanism of enzyme action of catalases, revealed significant similarities with that observed for the transport of water and/or glycerol, in aquaporins.

KEYWORDS

Aquaporins, Catalases, Channels.

INTRODUCTION

Catalases are ubiquitous antioxidant enzymes and irrespective of their origin catalyze the same basic reaction, the breakdown of hydrogen peroxide into water and oxygen. Hydrogen peroxide is a reactive oxygen species produced as a by-product of aerobic respiration. The importance of catalases is clearly established by the presence of a series of pathologies related to their malfunction which comprise among others, increased susceptibility to apoptosis (1), inflammation (2), accelerated aging and mutagenesis (3), stimulation of wide spectrum of tumors (4). Catalase from human erythrocytes is found to protect hemoglobin by removing over half of the hydrogen peroxide generated in normal erythrocytes, which are exposed to substantial oxygen concentrations (5).

Classification of catalases

The diversity among catalases enabled them to be organized into four main groups: monofunctional catalases (typical catalases), bi-functional catalase-peroxidases, non-heme catalases and minor catalases (6). The most widespread type of catalase is the monofunctional class, examples of which are found in most aerobic organisms. They are typically homotetrameric with, either small subunits (55 to 65 kDa) associated with heme b or large subunits (80 to 84 kDa) associated with heme d. The active center of catalases consists of a heme group deeply buried inside the molecular tetramer. Access to this heme is through long channels, which connect the molecular surface to the active site. Hydroperoxidase II (HPII) is the main catalase produced in the enterobacterium *Escherichia coli* and is the largest catalase crystallized to date, it is used as a common reference for all the catalase structures (7).

Heme in catalases

The prosthetic group of catalases is a noncovalently bound iron - protoporphyrin IX or hemin, except for a mutant of HPII were the heme is presumed to be covalently attached to the protein (8). While hemin is ubiquitous in a wide variety of electron transfer proteins and enzymes, catalases are unique in having two types of metalloporphyrins, heme b and heme d, the latter represents a metallochlorin in which a pyrrole ring is reduced (9). Infact, in large subunit enzymes such as HPII, heme b is initially bound during assembly and it is subsequently oxidized by the catalase itself during the early rounds of catalysis (10). One of the most intriguing features of heme in catalases is that it can exist in two orientations relative to the active site residues. The stereospecificity of heme binding is assumed to be a function of the residues that line the heme pocket. This might explain

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why attempts to force a change in heme orientation in HPII by mutating residues that interact with the heme were unsuccessful (11).

Resistance of Catalases to Proteolysis

The resistance of beef liver catalase (BLC) to chymotrypsin and pepsin was reported early in the 1960's (35). Recently the importance of extended domains and the characteristic "interweaving" exhibited by heme catalases has been the subject of intense investigation(s) (11, 12, 13, 14). However, due to lack of sufficient information on the tetramerization process, no concrete conclusions have been drawn concerning the role of these extended N and C-terminal domains. Attempts to address this question by molecular genetic approaches have also been unsuccessful (11, 13). Recent proteolytic studies on catalases, focusing on catalase HPII did yield significant insight(s) into the roles of these additional domains (15, 16). HPII exhibits unusual resistance not only to proteases like trypsin and chymotrypsin with greater substrate specificity, but it is resistant even to the broad substrate range protease, proteinase K. Globular proteins generally exhibit some level of resistance to proteolysis, at least initially, because the tertiary structure of the protein chain imparts sufficient inflexibility that it cannot fit into the protease active site. What makes HPII unusual is its resistance to cleavage even at a very high (1 to 1) ratio of protein to protease at 37^{0}C (15). This property complements another unusual property of HPII, its enhanced thermal stability (17). Are these two unusual properties of HPII, enhanced thermal stability and protease resistance, related in some way? Although there is no direct answer for this. However, resistance to thermal denaturation is unusual in an enzyme from a bacterium that does not survive exposure to temperatures anywhere near the 83^{0}C required to inactivate the enzyme. What is strange is the retention of thermal stability, when there seems to be no benefit to the host organism. On the other hand, the reason for enhanced resistance to proteolysis in HPII can be easily found in simple bacterial physiology. The expression of *katE* (encodes HPII) is induced in early stationary phase, and HPII accumulates in stationary phase cells. This is a period of rapid protein turnover as cells adapt to a period of slowed metabolism, and it is here that resistance to proteolysis is important to HPII because it allows the enzyme to survive and function in its role as a protective enzyme.

Diversity in Structures of Catalases

Eight heme-containing catalase structures have been reported. They include, the small subunit clade 3 enzymes from, BLC (18), *Micrococcus luteus* (MLC) (19), *Proteus mirabilis* (PMC) (20), *Sacchoromyces cerevisiae* (CATA) (21), and human erythrocytes (HEC) (22), and the large subunit clade 2 enzymes from *Penecillium vitale* (PVC) (23), *Escherichia coli* (HPII) (7, 24) and CatF the catalase from *Pseudomonas syringae* (25). The crystal structures of the two non-heme catalases one from *Thermus thermophilus* (TTC) (26) and the second from *Lactobacillus plantarum* (LPC) (27) reveal that the catalytic center is a dimanganese group. The enzyme is a homo-hexameric structure of approximately 30 kDa monomers. Catalases irrespective of whether they contain heme or not, share a unique structural feature; the presence of long channels that access the active sites of these enzymes.

Role of Channels in Enzyme Catalysis

The 2003 Nobel Prize in Chemistry awarded to Prof's Peter Agre and Roderick Mackinnon, for their structural work on water and ion channels, illustrates two important facts. First, it shows how contemporary biochemistry reaches down to the atomic level and secondly, it emphasis the role of channels in the fundamental processes of life (28). Long before, any structural studies have been started on other types of protein channels (for example, aquaporins, porins, ion channels), structural studies carried out on catalases in the labs of Prof's Michael Rossmann and Boris Vanshtein (in the early 80's), showed that access to the deeply buried active site heme in catalases is through two or more channels that extend to the protein exterior. However, specific studies focusing on the role of these channels in enzyme catalysis are lacking. This can be explained in part, owing to the rigid conceptual framework of enzyme catalysis centering around an active site. The concept of a buried active site accessible by long channels and the physiological significance associated with it are not realized, until recently (6, 29).

Role of Channels in Catalases

The crystal structure of HPII (at 1.9Å) revealed the presence of two main channels accessing the deeply buried active site (24). One channel reaches the active site perpendicular to the plane of the heme (~50Å long) and is commonly referred as the "main channel". A second channel, the "lateral channel", approaches laterally to the plane of the heme and is shorter than the main channel (~30Å long). However, little experimental evidence is currently available to assign any specific roles to these channels, or to describe the pathway of substrate access to the active site. It is tempting to speculate that these channels may be acting as separate inlet and outlet channels to allow the rapidly evolving oxygen to be efficiently removed without interfering with the incoming H_2O_2. Indeed the high turnover rates of catalases (10^6/second) are indicative of separate channels, rather than of an enzyme which requires a conformational change to

become active. The above model presupposes a "flow through mechanism" whereby the enzyme acts as a channel allowing the substrate to pass through it. It represents a novel concept normally associated only with transport proteins more specifically to aquaporins (AQPs).

Aquaporins are a large family of integral membrane proteins selective for the transport of water (aquaporins) or water plus glycerol (aqua-glyceroporins). When one looks at the architecture of the channels and turnover rates, very interesting similarities can be noticed between AQPs and catalases in general. Both catalases and AQPs are tetrameric in nature. Water or glycerol is shown to pass through aquaporins in a single file accounting for their high turnover rates of 10^9/second (30). The recent crystal structures of HPII mutants indicated the presence of a chain of water molecules in the main channel extending from the molecular surface to the heme distal pocket (31). The author's interpret that it might reflect the organization of hydrogen peroxide substrate when entering the native enzyme, giving credibility to the theory of a single file transport of substrate that can account for the turnover rates seen in catalases. The main channel in catalases show a constriction in the lower part (represented by V169 in HPII) just before the channel opens into the distal side of heme pocket. This region of the channel is surrounded by hydrophobic residues, which appear to have a major role in controlling substrate access, similar to the selectivity filter noticed in the aquaporins GlpF, which allows "only" glycerol molecules to pass through in a single file (32).

A similar mechanism is noticed in human red cell AQP1 were water selectivity is due to a constriction, which spans about 3Å in diameter in the channel. At this juncture the role of lateral channel in catalases assumes significance. It has long been considered in catalases that the lateral channel is the principal route for exhaust of products but no structural evidence supporting the above assumption is available. On the contrary, enlarging the opening of the lateral channel either by mutagenesis or proteolysis seems to actually increase the catalytic activity of the enzyme three-fold (16). However, whether the increase in activity noticed, is due to increased channel volume or because of the efficient removal of the products could not be ascertained. To further complicate matters, molecular dynamic simulations support the concept of hydrogen peroxide entering the enzyme through the main channel, oriented perpendicular to the plane of the heme, but do not agree to the route of product exhaust (33, 34).

CONCLUSION

With the growing importance of the crucial roles of the access (and egress) channels in the mechanism of enzyme action, contemporary biochemistry is now becoming increasingly dependent on 3-dimensional structural visualizations to unlock the fundamental process of life. Although preliminary experiments show some specific roles for these channels in catalases, more detailed studies are warranted. Elucidating the role of these channels will not only decipher the enigma behind the functionally important high turnover rates, but will verify the concept of "flow through

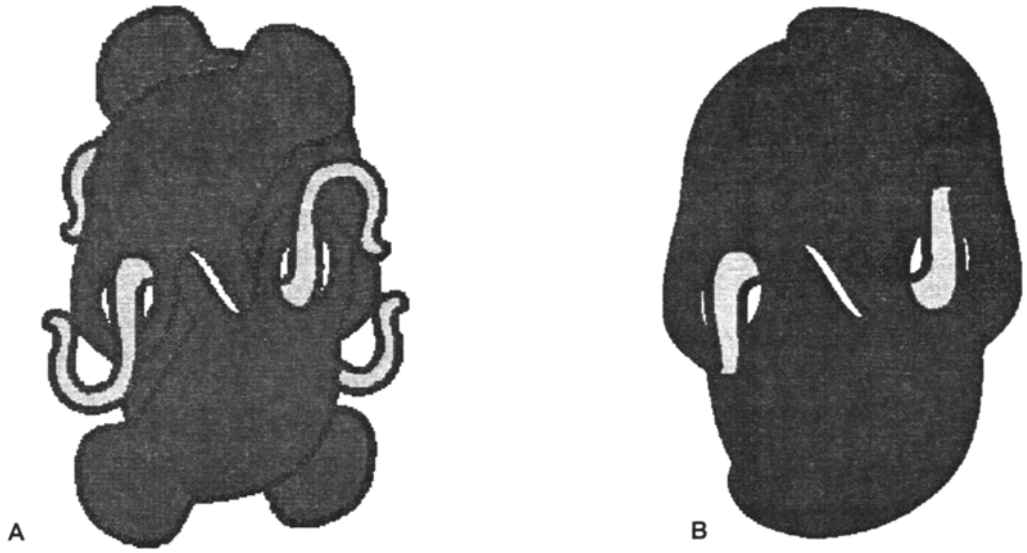

Fig. 1. Cartoon representation of catalases, a large subunit enzyme is shown in Panel A and a small subunit enzyme is shown in Panel B. The extensive interweaving of the N-termini and the extended C-terminal domain can be clearly noticed in the large subunit enzymes

mechanism". An important implication of the enzyme acting as a channel is that it limits the exposure time of catalases to the reactive hydrogen peroxide, thereby reducing the chance of unwanted or damaging affects to the protein. In this short communication, taking the example of catalases, an attempt has been made to potray the diverse and ubiquitous nature of these channels, from membrane bound proteins (i.e., AQPs) to soluble proteins (such as catalases).

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