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The Fine Print:

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Catalase

by

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Abbreviations

Å, angstrom
Arg, arginine
Asn, asparagine
CAT, catalase
His, histidine
NADPH, nicotinamide adenine dinucleotide phosphate
Phe, phenylalanine
Pro, proline
Tyr, tyrosine
Val, valine
Abstract

Catalase is an enzyme, which can function either in the catabolism of H₂O₂ or in the peroxidatic oxidation of small substrates such as ethanol, methanol or elemental mercury. Catalase has four subunits. Each subunit contains a heme group. This heme group is responsible for carrying out catalase’s activity. This paper provides a brief review of catalase structure (beef liver catalase), catalytic reaction, localization and distribution of the enzyme, and regulation of catalase.

Introduction

Mammalian catalase is probably one of the best-studied enzymes in existence. It was first crystallized from beef liver by Sumner and Dounce in 1937 [1]. Catalase has a molecular weight of about 240,000 Da, and is composed of four identical subunits, each containing a
protoporphyrin ring and a central iron (Fe) atom. Catalase catalyses the destruction of H\textsubscript{2}O\textsubscript{2} by the following two reactions:

\[
\begin{align*}
2 \text{H}_2\text{O}_2 & \xrightarrow{\text{CAT}} 2 \text{H}_2\text{O} + \text{O}_2 \\
\text{H}_2\text{O}_2 + \text{SH}_2 & \xrightarrow{\text{CAT}} 2 \text{H}_2\text{O} + \text{S}
\end{align*}
\]

Where (S) is any one of a number of hydrogen-donating substrates including ethanol, methanol, formate, nitrite, and quinones [2]. H\textsubscript{2}O\textsubscript{2} is produced in cells by a number of enzymatic reactions including those catalyzed by SOD, which converts O\textsubscript{2}\textsuperscript{-} to H\textsubscript{2}O and O\textsubscript{2} and non-enzymatically by the autooxidation of compounds such as thiol and ascorbate. Under normal physiological conditions, catalase controls the H\textsubscript{2}O\textsubscript{2} concentration so that this does not reach toxic levels that could bring about oxidative damage in cells.

**Catalase structure**

The structure of catalase from many different species has been studied by X-ray diffraction. Although it is clear that all catalases share a general structure, some differ in the number and identity of domains. In this paper, beef liver catalase will be used as a model for catalase structure. Functional catalase is a tetramer of four identical subunits. Each monomer harbors a single heme and NADPH. The NADPH’s lie on the surface, whereas the heme are embedded in the middle of each monomer, about 20 Å below the molecular surface and 23 Å from the center of the tetramer [3]. This NADPH may serve to protect the enzyme from oxidation by its H\textsubscript{2}O\textsubscript{2} substrate. Heme consists of a protoporphyrin ring and a central iron (Fe) atom. A protoporphyrin ring is made up of four pyrole rings linked by methene bridges. The
iron can either be in the ferrous (Fe$^{2+}$) or the ferric (Fe$^{3+}$) oxidation state. Each heme is exposed through a funnel-shaped channel 30 Å long and 15 Å wide [3,4]. The channel is linked with hydrophilic residues at the entrance and with hydrophobic residues as the channel descends, constricting toward the heme (Figure 1) [4]. This narrow channels, thus preventing most molecules larger than H$_2$O from gaining access [4]. The proximal and distal sides of the heme are quite different environment. The proximal side is crowded with residues Val$^{145}$, His$^{217}$, Pro$^{335}$, Arg$^{353}$, Ala$^{356}$ and Tyr$^{357}$ [5,6]. The phenolic side chain of Tyr$^{357}$ acts as the 5$^\text{th}$ heme iron ligand as seen in Figure 1, the other four being nitrogens of the heme protoporphyrin ring. Tyr$^{357}$ is exposed tightly to the Fe; the Fe-phenolic oxygen distance is 1.9 Å. As a probable consequence, the phenolic oxygen is deprotonated due to the electron withdrawing power of Fe. Arg$^{353}$ may also promote ionization of Tyr$^{357}$ by lowering the effective pKa of the tyrosine phenol (the two side chains are only 3.5 Å apart) [5,6]. In contrast to the heme’s proximal side, its distal side (facing the channel) is much less confined. It consists of many residues, some of which are contributed by the beta-barrel. Phe$^{160}$ is stacked parallel to one of the heme pyrole rings and Val$^{173}$ makes hydrophobic contact with a different pyrole ring [6]. His$^{74}$ is also parallel to the heme, with bond angles normally allowed for only glycine residues. This confirmation is stabilized by interaction with Arg$^{111}$ and Thr$^{114}$ and probably relates directly to enzymatic activity [5,6].

![Figure 1](image-url). Schematic drawing of the active site of catalase [4].
Localization and distribution of catalase

Catalase is present in most aerobic cells. However, few do not have catalase such as the bacterium Bacillus popilliae, Mycoplasma pneumoniae and the blue-green alga Gloeocapsa [2]. A few anaerobic bacteria, such as Propionibacterium shermanii, contain catalase but most do not. In many cases, the enzyme is localized in subcellular organelle such as the peroxisomes (microbodies) of liver and kidney or in much smaller aggregates such as the microperoxisomes found in a variety of other cells [7]. Peroxisomes contain many of the cellular enzymes that generate H$_2$O$_2$, such as glycolate oxidase and flavoprotein dehydrogenases [2]. Mitochondria (in liver), chloroplasts and the endoplasmic reticulum contain little, if any catalase. Hence, any H$_2$O$_2$ that they generate in vivo can not be disposed of by catalase, unless H$_2$O$_2$ diffuses to the peroxisomes [2]. The brain, heart and skeletal muscle contain only low amounts; however, the activity does vary between muscles and even in different regions of the same muscle Table 1 [2].

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Individual</th>
<th>Catalase activity (mg$^{-1}$ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td>A</td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1500</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>A</td>
<td>990</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1300</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>A</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>110</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>B</td>
<td>300</td>
</tr>
<tr>
<td>Kidney medulla</td>
<td>A</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>220</td>
</tr>
<tr>
<td>Spleen</td>
<td>A</td>
<td>56</td>
</tr>
<tr>
<td>Lymph node</td>
<td>A</td>
<td>120</td>
</tr>
<tr>
<td>Pancreas</td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>120</td>
</tr>
<tr>
<td>Lung</td>
<td>A</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>180</td>
</tr>
<tr>
<td>Heart</td>
<td>A</td>
<td>54</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>A</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>25</td>
</tr>
<tr>
<td>Brain (gray matter)</td>
<td>A</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3</td>
</tr>
<tr>
<td>Brain (white matter)</td>
<td>A</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>20</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>A</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>560</td>
</tr>
</tbody>
</table>
Catalase reaction mechanism

Catalase is an enzyme that can function in two distinct modes. The catalytic mode is responsible for $\text{H}_2\text{O}_2$ breakdown, which is thought to occur in two stages [2,8]:

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{Fe(III)-CAT} & \rightarrow \text{H}_2\text{O} + \text{O} = \text{Fe(V)-CAT} \quad (k_1 = 1.7 \times 10^7 \text{ M}^{-1}\text{s}^{-1}) \\
\text{H}_2\text{O}_2 + \text{O} = \text{Fe(V)-CAT} & \rightarrow \text{Fe(III)-CAT} + \text{H}_2\text{O} + \text{O}_2 \quad (k_2 = 2.6 \times 10^7 \text{ M}^{-1}\text{s}^{-1})
\end{align*}
\]

(III and V indicates formal oxidation states of Fe)

In this reaction Fe(III)-CAT represents the native catalase molecules and O=Fe(V)-CAT represents catalase compound I, which was first described by Chance et al. [7]. Peroxide upon entering the heme cavity, is severely sterically hindered and must interact with His$^{74}$ and Asn$^{147}$ [5]. It is in this position that the first stage of catalysis takes place. Transfer of a proton from one oxygen of the peroxide to the other, via His$^{74}$, elongates and polarizes the O-O bond, which eventually breaks heterolytically as a peroxide oxygen is coordinated to the iron center. This coordination displaces water and forms Fe(V)=O plus a heme radical. The radical quickly degrades in another one electron transfer to rid of the radical electron, leaving the heme ring unaltered. During the second stage, in a similar two electron transfer reaction, Fe(V)=O reacts with a second hydrogen peroxide to produce the original Fe(III)-CAT, another water and a mole of molecular oxygen. The heme reactivity is enhanced by the phenolate ligand of Tyr$^{357}$ in the 5$^{th}$ iron ligand position [5], which may aid in the oxidation of Fe(III) to Fe(V) and the removal of an electron from the heme ring. The efficiency of catalase may be due to the interaction of His$^{74}$ and Asn$^{147}$ with reaction intermediates. This mechanism is supported by experimental evidence indicating modification of His$^{74}$ with 3-amino-1,2,4-triazole, which inhibits the enzyme by hindering substrate-binding [9]. Its inhibitory action is exerted on compound I, so aminotriazole
will only inhibit catalase if H₂O₂ is present. Formation of compound I leads to characteristic changes in the absorbance spectrum of catalase (Figure 2) [7].

![Figure 2](image.png)

Figure 2. The optical spectra of the free enzyme and its enzyme-substrate compound. The decrease in absorbance at 405 nm in the region of the γ-band or Soret band is characteristic of the “primary” green enzyme-substrate compound [7].

Alternatively, catalase compound I can react with a limited number of hydrogen donors, such as ethanol or methanol, or with elemental mercury and oxidize these substrates by utilizing the O₂ from a single H₂O₂ molecule in a two-electron oxidation step. This is the peroxidatic mode of catalase action [8].

\[
O = \text{Fe(V)}- \text{CAT} + \text{RCH}_2\text{OH} \rightarrow \text{Fe(III)}- \text{CAT} + \text{R} \text{C} - \text{H} + \text{H}_2\text{O}
\]

\[(k_3 = 0.2 \times 10^3)\]

Generalized scheme for the chemistry of the catalase reaction is shown in Figure 3, which indicates two cycles of catalase. Catalase can also oxidize nitrite (NO₂⁻) into nitrate (NO₃⁻) *in vitro* [7].
Figure 3. Proposed mechanism of the reaction of catalase compound I. Two cycles of catalase: the upper portion indicates the catalytic cycle and the lower portion indicates the peroxidatic cycle. The formation of compound I by reaction with peroxide is indicated across the middle of the diagram [7].

At any moment only a fraction of catalase heme is bound to H$_2$O$_2$ in the form of compound I, and that fraction reaches a maximal level determined by the ratio of the rate constants $k_1$ and $k_2$ [7]. Thus, the physiological or pathological variation of catalase concentration in different organs and tissues will lead to different steady–state level of H$_2$O$_2$ generation. For example, one expects a low H$_2$O$_2$ steady-state concentration in those organs having a high catalase content, such as liver and kidney and a high H$_2$O$_2$ concentration in other organs such as heart and brain, possessing a low catalase content [7,10].

**Catalase detection**

Catalase activity can be measured by monitoring the disappearance of H$_2$O$_2$ in the reaction system. For a given concentration of H$_2$O$_2$, the initial rate of its removal is proportional
to the concentration of catalase. Decomposition of H$_2$O$_2$ can be followed by the loss of its light absorbance at 240 nm, or by measuring the release of O$_2$ by using an oxygen electrode [2].

Furthermore, catalase can oxidize other small species, e.g. azide to Azidyl radical, which has been observed by EPR [11]. Figure 4 shows an EPR spectrum consisting of triplet of quartets by adding catalase to a solution containing sodium azide, H$_2$O$_2$ and PBN.

![Figure 4](image.png)

**Summary**

Catalase is an example of a particularly efficient enzyme. It has two-enzymatic function depending on the concentration of H$_2$O$_2$ available. If the steady-state concentration of H$_2$O$_2$ in the system is high, the enzyme acts catalytically e.g. remove H$_2$O$_2$ to form H$_2$O and O$_2$.

However, at low concentration of H$_2$O$_2$ and in the presence of a suitable hydrogen donor (eg. methanol, ethanol) catalase acts peroxidically, it removes H$_2$O$_2$ but oxidizes its substrate.

Catalase present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of H$_2$O$_2$. Although it is clear that all catalases share a general structure, some differ in the number and identity of domains.


References


