This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Catalase

by

Iman Ahmad

B-180 Medical Laboratories
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2001

8. March 2001

Abbreviations

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

Table of content *Page number*

Abstract

Catalase is an enzyme, which can function either in the catabolism of H_2O_2 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_2O_2 by the following two reactions:

$$2\ H_2O_2 \xrightarrow[\text{catalytic reaction}]{\text{CAT}} 2\ H_2O + O_2$$

$$H_2O_2 + SH_2 \xrightarrow[\text{peroxidatic reaction}]{\text{CAT}} 2\ H_2O + S$$

Where (S) is any one of a number of hydrogen-donating substrates including ethanol, methanol, formate, nitrite, and quinones [2]. H_2O_2 is produced in cells by a number of enzymatic reactions including those catalyzed by SOD, which converts $O_2^{\bullet -}$ to H_2O and O_2 and non-enzymatically by the autooxidation of compounds such as thiol and ascorbate. Under normal physiological conditions, catalase controls the H_2O_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_2O_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_2O_2 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^{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^{73} 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].

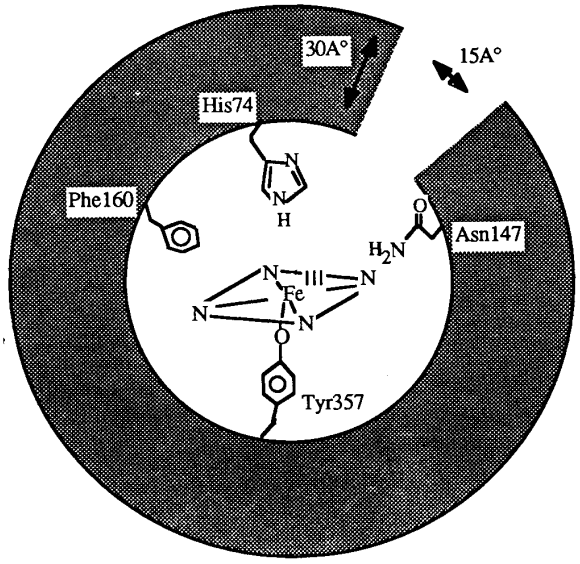

Figure 1. Schematic drawing of the active site of catalase [4].

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_2O_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_2O_2 that they generate *in vivo* can not be disposed of by catalase, unless H_2O_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 1. Catalase activity in human tissues. Results are expressed as enzyme activity/mg protein. Two individuals denoted A and B, were used as sources of tissue sample [2].

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

Catalase reaction mechanism

Catalase is an enzyme that can function in two distinct modes. The catalytic mode is responsible for H_2O_2 breakdown, which is thought to occur in two stages [2,8]:

$$H_2O_2 + \text{Fe(III)-CAT} \longrightarrow H_2O + \text{O=Fe(V)-CAT} \quad (k_1 = 1.7 \times 10^7\ M^{-1}s^{-1})$$

$$H_2O_2 + \text{O=Fe(V)-CAT} \longrightarrow \text{Fe(III)-CAT} + H_2O + O_2 \quad (k_2 = 2.6 \times 10^7\ M^{-1}s^{-1})$$

(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 hetrolytically 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 H_2O_2 is present. Formation of compound I leads to characteristic changes in the absorbance spectrum of catalase (Figure 2) [7].

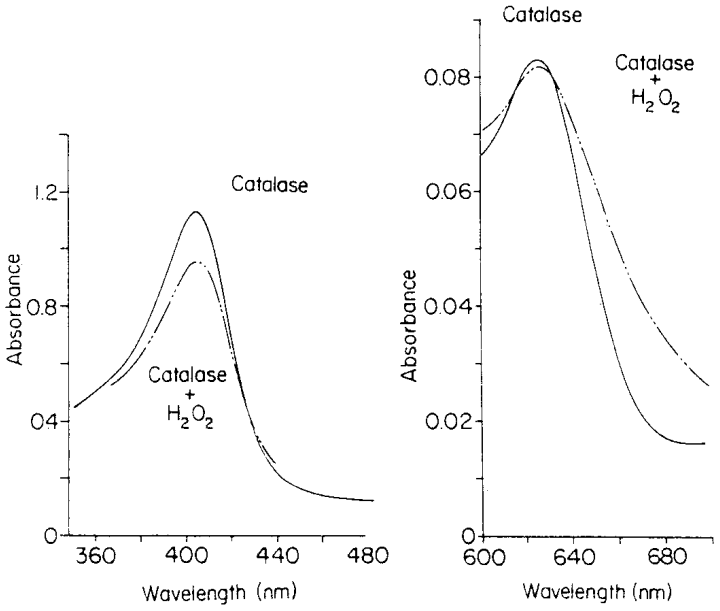

Figure 2. The optical spectra of the free enzyme and its enzyme-substrate compound. The decrease in absorbance at 405 nm in the region of the γ-band or Soret band is characteristic of the "primary" green enzyme-substrate compound [7].

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 O_2 from a single H_2O_2 molecule in a two-electron oxidation step. This is the peroxidatic mode of catalase action [8].

$$O{=}Fe(V){-}CAT + RCH_2OH \longrightarrow Fe(III){-}CAT + RC(=O){-}H + H_2O$$

$$(k_3 = 0.2 - 1 \times 10^3)$$

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 (NO_2^-) into nitrate (NO_3^-) *in vitro* [7].

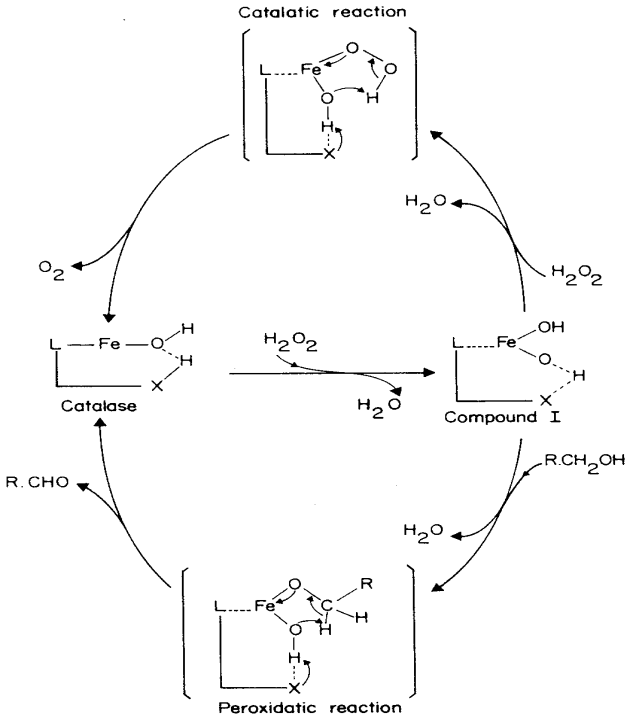

Figure 3. Proposed mechanism of the reaction of catalase compound I. Two cycles of catalase: the upper portion indicates the catalytic cycle and the lower portion indicates the peroxidatic cycle. The formation of compound I by reaction with peroxide is indicated across the middle of the diagram [7].

At any moment only a fraction of catalse heme is bound to H_2O_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_2O_2 generation. For example, one expects a low H_2O_2 steady-state concentration in those organs having a high catalase content, such as liver and kidney and a high H_2O_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_2O_2 in the reaction system. For a given concentration of H_2O_2, the initial rate of its removal is proportional

to the concentration of catalase. Decomposition of H_2O_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_2O_2 and PBN.

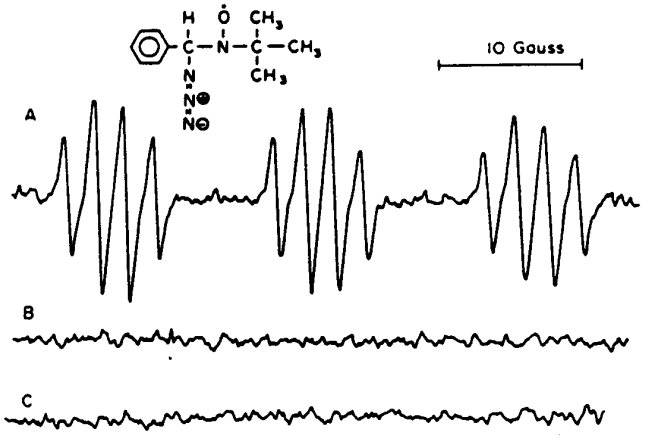

Figure 4. The EPR spectrum of the PBN-azide radical adduct from catalase and H_2O_2. A, the incubation contained sodium azide (60 mM), PBN (70 mM), H_2O_2 (350 μM), DETAPAC (10 μM), and catalase (5000 units/ml) in phosphate buffer (pH 7.6). The spectrometer settings were; scan range 50 G; modulation amplitude, 0.33 G; time constant, 0.5 s; gain, 2.5×10^4; microwave power, 20 mW; and scan time, 4 min. B, identical with A, but catalase omitted. C, identical with A, but using heat denatured catalase [11].

Summary

Catalase is an example of a particularly efficient enzyme. It has two-enzymatic function depending on the concentration of H_2O_2 available. If the steady-state concentration of H_2O_2 in the system is high, the enzyme acts catalytically *e.g.* remove H_2O_2 to form H_2O and O_2. However, at low concentration of H_2O_2 and in the presence of a suitable hydrogen donor (*eg.* methanol, ethanol) catalase acts peroxidically, it removes H_2O_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_2O_2. Although it is clear that all catalases share a general structure, some differ in the number and identity of domains.

References

1) Sumner JB, Dounce AL. (1937) Crystalline catalase. *J Biol Chem*. **121**:417-424.

2) Halliwell B, Gutteridge JMC. (1999) Free *Radicals in Biology and Medicine*. 3rd ed. New York: Oxford University Press. pp:134-140.

3) Murthy MR, Reid TJ 3d, Sicignano A, Tanaka N, Rossmann MG. (1981) Structure of beef liver catalase. *J Mol Biol.* **152** (2):465-499.

4) Belal R, Momenteau M, Meunier B. (1989) Why an oxygen and not a nitrogen atom as proximal ligand in catalase ? Hydrogen peroxide dismutationcatalayzed by synthetic iron and manganese porphyrins. *New J Chem*. **13**:853-862.

5) Fita I, Rossmann MG. (1985) The active center of catalase. *J Mol Biol*. **185** (1):21-37.

6) Reid TJ 3d, Murthy MR, Sicignano A, Tanaka N, Musick WD, Rossmann MG. (1981) Structure and heme environment of beef liver catalase at 2.5 A resolution. *Proc Natl Acad Sci USA*. **78** (8):4767-4771.

7) Chance B, Sies H, Boveris A. (1979) Hydroperoxide metabolism in mammalian organs. *Physiol Rev*. **59** (3):527-625.

8) Sichak SP, Dounce AL. (1986) Analysis of the peroxidatic mode of action of catalase. *Archives of Biochem & Biophys*. **249** (2):286-295.

9) Darr D, Fridovich I. (1986) Irreversible inactivation of catalase by 3-amino-1,2,4-triazole. *Biocheml Pharmacol.* **35** (20):3642.

10) Percy ME. (1984) Catalase: an old enzyme with a new role? *Can J Biochem Cell Biol.* **62** (10): 1006-1014.

11) Kalyanaraman B, Janzen EG, Mason RP. (1985) Spin trapping of the azidyl radical in azide/catalase/H_2O_2 and various azide/peroxidase/ H_2O_2 peroxidizing systems. *J Biol Chem.* **260** (7):4003-4006.