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Abstract

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KEYWORDS: catalse, muscle, bovine heart

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PARTIAL PURIFICATION AND PROPERTIES OF BOVINE HEART CATALASE

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Abstract. Catalase was partially purified (about 380-fold purification) from the post-mitochondrial supernatant of bovine heart and compared with catalases from bovine erythrocytes and bovine liver. The electrophoretic mobility in polyacrylamide gel (pH 8.0) of heart catalase was the same as that of erythrocyte catalase and was smaller than that of the liver enzyme. The heart catalase was indistinguishable from erythrocyte catalase in regard to the molecular weights of subunit polypeptides, the inhibition patterns produced by several catalase inhibitors, and specific activity. The pH-activity curve of heart catalase consisted of a characteristic biphasic pattern with a peak at pH 7.5 and a shoulder at pH 10.

Key words : catalase, muscle, bovine heart

Hydrogen peroxide-destroying enzymes have been demonstrated in various organisms and some of them have been purified to homogeneity (1-6). Catalases from eukaryotes including yeast (6) and mammals (5, 7) consist of four identical subunits, each containing 1 atom of heme iron per molecular weight of about 60,000. The similar structure and properties of catalases indicate the evolutionally homologous origin of this enzyme. In mammals, catalase activity occurs in various tissues. It is not clear whether each tissue has a specific catalase isozyme, although the electrophoretic mobility and antigenicity of liver and erythrocyte enzymes are different (5). The activity level of heart muscle catalase is extremely low compared to that of liver, and its purification has not been reported. This paper reports partial purification of catalase from the supernatant fraction of bovine heart muscle.

MATERIALS AND METHODS

Assay of catalase activity. Catalase activity was assayed by a spectrophotometric method. The sample cell contained 1 ml of 9 mM H_2O_2 in 10 mM phosphate buffer (pH 7.0). The reaction was started by addition of enzyme to the sample cell and to a reference cell containing 10 mM phosphate buffer only.

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The initial decrease in absorbance at 230 nm was recorded at a constant temperature (25°C). Catalytic activity was expressed in enzyme units. One unit caused one A_{230} unit decrease in 200 seconds under these conditions.

Isolation of bovine heart catalase. All procedures were performed at 0-4°C unless stated otherwise. Bovine heart muscle was minced and washed several times with 0.9% NaCl. The washed muscle was homogenized in 0.25 M sucrose and 50 mM Tris-HCl (pH 7. 5) after brief treatment with pronase at 4°C. Nuclear and mitochondrial fractions were sedimented as described (8) and post-mitochondrial supernatant was used for the enzyme source. The supernatant was shaken with 0.35 volume of ethanol/chloroform (1 : 1 mixture) at room temperature and precipitate formed was removed by centrifugation. The supernatant (containing almost all the catalase activity) was precipitated by ammonium sulfate at 47. 5% saturation. This precipitate was dissolved in 10 mM phosphate buffer (pH 7.0), dialysed against the same buffer, and applied to a column of DEAE-Sephadex A 25 conditioned with the same buffer. The column was eluted by a linear gradient of NaCl (0-0.5 M) in the phosphate buffer. Fractions containing catalase were pooled, concentrated by ultrafiltration (Amicon, XM 50 membrane) and subjected to electrophoresis in 7.5% polyacrylamide gels. The gels were sectioned and extracted with phosphate buffer at 4°C.

Preparation of bovine erythrocyte catalase. Washed bovine erythrocytes were lysed in 2 volumes of distilled water and centrifuged. The hemolysate was directly applied to 7.5% polyacrylamide gels. After electrophoresis, catalase was extracted from the gels as described above.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis under non-denaturating conditions was performed by the method of Davis (9). For subunit analysis and molecular weight determination, gels containing 0.1% sodium dodecylsulfate was used according to Weber and Osborn (10).

Other methods. Protein concentration was determined by the method of Lowry *et al.* (11).

Materials. Crystallized catalase from bovine liver, RNase A, and cytochrome C were purchased from Sigma Chemical Co.

RESULTS

Purification of heart catalase. To see the intracellular distribution of bovine heart catalase, homogenate was fractionated into nuclear, mitochondrial, microsomal, and supernatant fractions. The supernatant fraction showed the highest specific activity (2.5 times the homogenate). Intermediate activities were found in mitochondrial and microsomal fractions (results not shown). This result is consistant with the subcellular distribution pattern of catalase of other species and organs (12).

We used the post-mitochondrial supernatant (See "Materials and Methods") containing peroxisomes and microsomes as well as soluble proteins for purifying heart catalase. The supernatant was first treated with a mixture of ethanol and

chloroform. The optimal volume of the mixture to be added was determined in a small scale experiment. Release of catalase from granular organelles occurs with this treatment. After fractionation with ammonium sulfate the sample was subjected to chromatography on DEAE-Sephadex. The catalase was eluted as a single peak at NaCl concentration of 0.02 M (Fig. 1). About a ten-fold increase

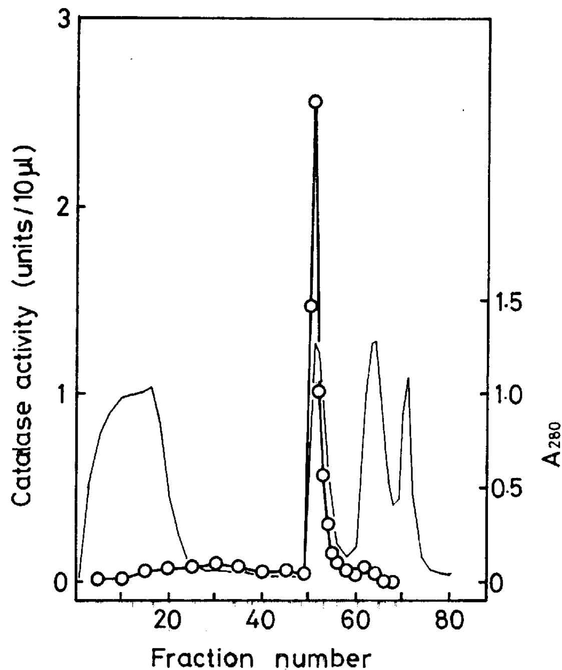

Fig. 1. DEAE-Sephadex chromatography of bovine heart catalase. pH of the dialysed sample was adjusted to 7.5 with NaOH and the sample applied to the column (2.2×30 cm) of DEAE-Sephadex previously conditioned with 10 mM phosphate buffer (pH 7.5). After the column had been washed with the same buffer, adsorbed protein was eluted by applying a linear gradient of NaCl (0–0.5 M) in the phosphate buffer. Fractions of 5 ml were collected and catalase activity was determined immediately. Fractions, No. 50–53 were pooled. Absorbance at 280 nm (—), catalase activity (○).

in specific activity was obtained by this step (Table 1). Pooled fractions containing catalase were further purified by polyacrylamide gel electrophoresis in 20 glass columns (6 mm in diameter). After electrophoresis, gels were frozen at −80°C, cut to 2 mm slices, and extracted with phosphate buffer. Extracts were checked for catalase activity, pooled and concentrated by ultrafiltration. In most of the experiments this enzyme preparation was used. Entire purification steps are summarized in Table 1.

TABLE 1. PURIFICATION OF BOVINE HEART CATALASE

Fraction	Total protein (mg)	Total activity (unit × 10^{-2})	Specific activity (units/mg)	Purification (−fold)	Yield (%)
Post-mitochondrial supernatant	16540	13100	0.79	1.0	100
Ethanol-chloroform treatment	9260	16300	1.76	2.2	124
Ammonium sulfate fractionation	620	5480	8.83	11.1	41.7
DEAE-Sephadex chromatography	17.5	1500	85.6	108	11.4
Polyacryl amide gel electrophoresis	1.1	330	299	377	2.5

Properties of bovine heart catalase. The relative electrophoretic mobility of heart, erythrocyte and liver catalases was compared by electrophoresis in 7.5% polyacrylamide gel at pH 8.6 (Fig. 2). Heart and erythrocyte catalases had the same mobility. Liver catalase moved faster to the anode than the other two at

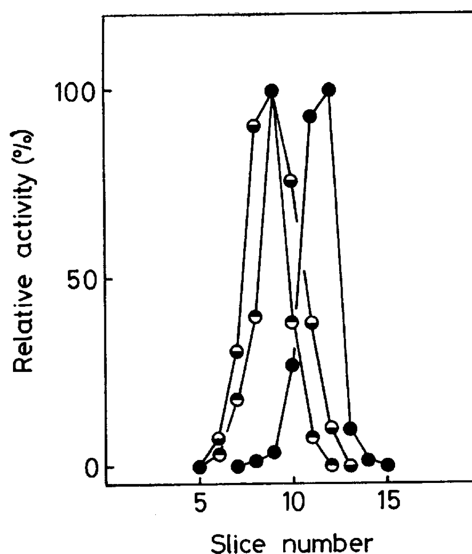

Fig. 2. Comparison of the electrophoretic mobility of catalases. Heart catalase (1.9 units), erythrocyte catalase (7.2 units) and liver catalase (100 units) were applied to 7.5% polyacrylamide gel. Electrophoresis was performed at 2.5 mA/gel for 3 h at room temperature. The gels were frozen at −80°C for 40 min and cut with a set of razor blades to 2 mm-slices. The catalase was extracted into 1 ml of 10 mM phosphate buffer (pH 7.0) overnight at 4°C and assayed. Catalase activity relative to that of peak fractions is shown. ◒, heart catalase; ◓, erythrocyte catalase; ●, liver catalase.

this pH. The same result was obtained when 5% or 10% gel was used.

Heart and erythrocyte catalases were compared using several catalase inhibitors (Table 2). The extent of inhibition by each of these was the same for both enzymes, suggesting that these two enzymes have similar catalytic properties.

TABLE 2. EFFECT OF INHIBITORS ON ERYTHROCYTE AND BOVINE HEART CATALASES

Inhibitors	Concentration (mM)	% activity	
		Heart catalase	Erythrocyte catalase
None	—	100	100
KCN	0.1	80.5	92.8
	0.5	62.5	67.8
NaN_3	0.004	8.5	3.1
	0.02	4.0	0
	0.1	0	0
NaF	0.1	85.4	89.5
	0.5	90.0	87.0
NH_2OH	0.004	26.9	27.4
	0.02	5.8	6.8
	0.1	0	0
NH_2NH_2	0.1	75.3	87.0
	0.5	97.0	78.9
N-Ethylmaleimide	0.1	83.0	93.3
	0.5	12.3	25.3

All inhibitors were dissolved in 10 mM phosphate buffer (pH 7.0). To 1 ml of the inhibitor solutions, heart catalase (0.45 unit) and erythrocyte catalase (0.38 unit) were added. The mixtures were incubated at 25°C for 30 min and the remaining activity was assayed.

Fig. 3 shows variations in the activity of heart catalase with pH. One broad peak around pH 7–8 and one shoulder at pH 10 occurred.

The subunit polypeptides of heart, erythrocyte and liver catalases were compared by polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecylsulfate (Fig. 4). The molecular weight of protomer polypeptide (indicated by arrows) in each of the three enzymes was not significantly different. It was determined to be 61,000±2,000 using molecular weight standards; bovine serum albumin (68,000), RNase A (13,700) and cytochrome c (11,700). Three common polypeptides other than the 61,000 subunit polypeptide were present in heart and erythrocyte enzyme preparations (Fig. 4). These polypeptides were probably derived from contaminating protein with no catalase activity. A comparison of the heme content of these enzyme preparations supported this idea (See "Discussion").

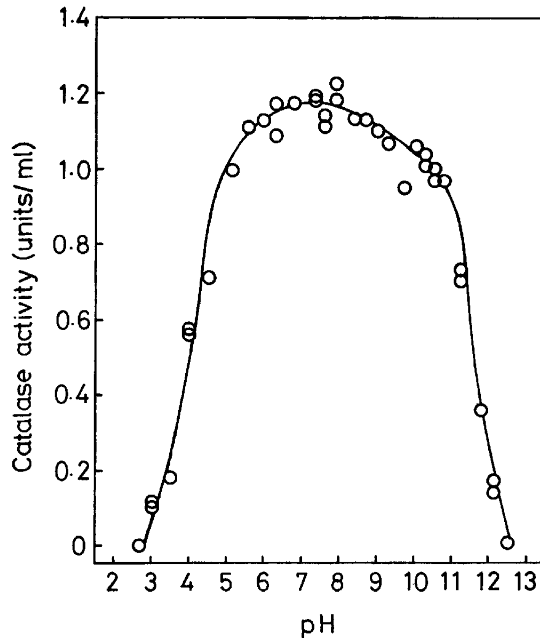

Fig. 3. Effect of pH on the activity of bovine heart catalase. Heart catalase (1.2 units) was mixed with 1 ml of 10 mM phosphate buffer at various pH values (pH 2–13). pH values below 4 and above 9 were adjusted with 1 N HCl or NaOH prior to the addition of the enzyme.

DISCUSSION

Catalase was partially purified from bovine heart muscle and its properties were compared to those of other catalases. This may be the first report dealing with the isolation of muscle catalase, though the purity of the final preparation obtained in this study is insufficient. A comparison of the purity of the catalase preparations is shown in Table 3. Absorption spectra of catalases showed 2 main peaks, one at 278 nm (due to protein) and one at 405 nm (Soret band, due to heme group). Therefore, the absorption ratio (A_{405}/A_{278}) is a measure of the enzyme purity and this value for purified catalase is about 1.10 (5). The purity ("heme purity") of heart catalase and erythrocyte catalase calculated from the absorption ratio was 17% and 41%, respectively. Specific activity corrected for the heme purity was about the same for both enzymes. The "subunit purity" shown in Table 3 is consistent with the heme purity. Since the expression of specific activity used in this paper (units/mg protein) corresponds to about 10 times the value of Kat. f. which is often used as the unit of catalase specific activity, the specific activity of the commercial liver catalase was comparable to that obtained by others (2). The corrected specific activity of heart and erythrocyte

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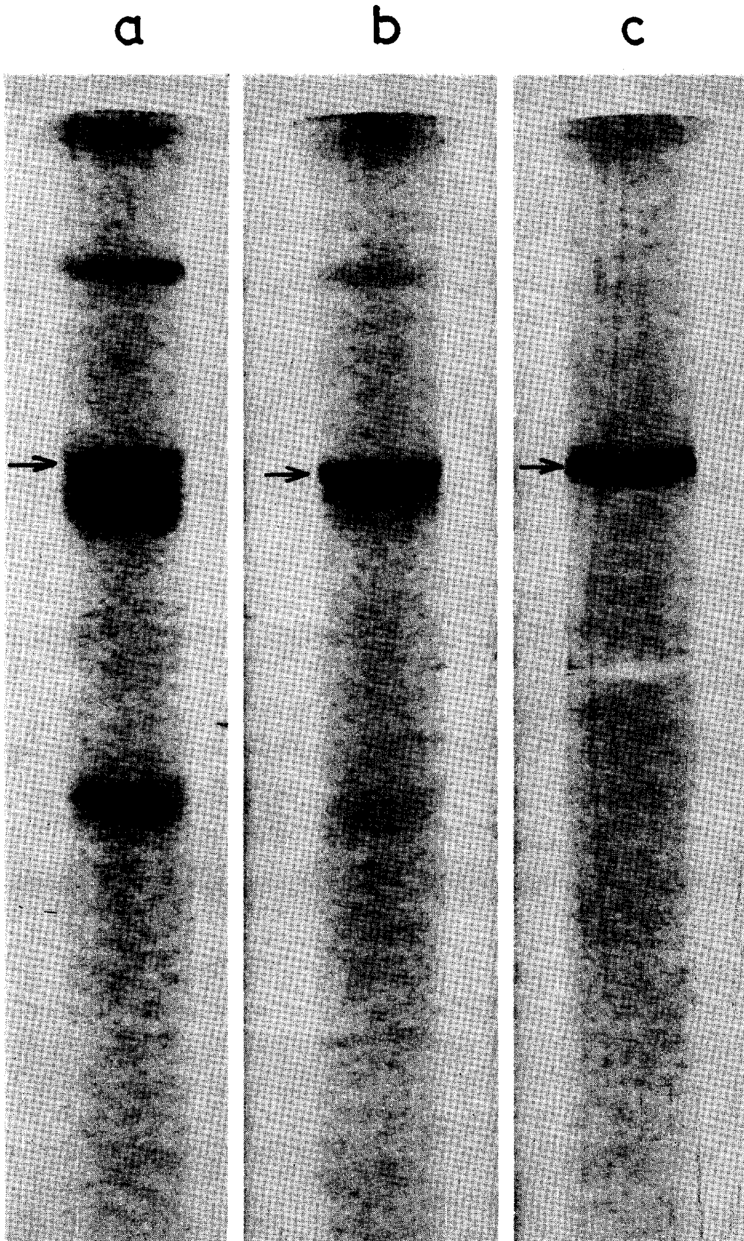

Fig. 4. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of bovine catalases. Samples were dialysed overnight against 10 mM phosphate buffer (pH 7.2), 0.1% sodium dodecylsulfate, 1% 2-mercaptoethanol, 0.002% Bromphenol blue, and 10% glycerine. Aliquots containing 10 μg of protein were applied to 7.5% polyacrylamide gels. Electrophoresis was run at 7 mA/gel for 4.25 h. a, heart catalase; b, erythrocyte catalase; c, liver catalase. The arrows indicate the protomer of catalase.

TABLE 3. PURITY OF CATALASE PREPARATIONS

Catalase	Specific activity (units/mg)	A_{405}/A_{278}	Heme purity[a] (%)	Corrected specific activity[b] (units/mg)	Subunit purity[c] (%)
Heart catalase	299	0.186	16.9	1770	20.4
Erythrocyte catalase	686	0.455	41.4	1660	48.5
Liver catalase	3130	0.733	66.6	4700	100

a Calculated assuming A_{405}/A_{278} of pure catalase is 1.10 (5).
b Specific activity when heme purity is 100%.
c Content of the subunit polypeptide of catalase, calculated from the densitometric tracing of the gels shown in Fig. 4.

enzymes was only about 1/3 that of the liver enzyme. There are some reports claiming that erythrocyte catalase has higher specific activity than liver catalase (5, 13). This discrepancy is probably due to loss of activity in the final purification step of polyacrylamide gel electrophoresis (Table 1). A brown band was always observed at the gel front indicating partial dissociation of the heme groups from the enzyme.

The pH dependency curve of heart catalase strikingly resembles that of yeast catalase (6), both having a shoulder in the alkaline region. Catalases from other tissues have a single pH optimum and a more narrow active pH range (5).

Contamination of erythrocyte catalase in heart catalase was thought to be eliminated by the extensive washing of the minced muscle ("Materials and Methods"). This was supported by the observation that an extremely low concentration of hemoglobin was detected in the post-mitochondrial supernatant.

No differences in the properties of heart and erythrocyte catalases were found in this study. This is not, of course, definitive evidence that these enzymes are the same molecule. More detailed experiments such as determination of amino acid composition and antigenic determinants using more purified preparations are necessary to clarify this problem.

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