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STUDIES ON THE HETEROGENEITY OF CATALASE IN RAT LIVER

II. Catalase pI-Isozyme Pattern and Its Relation to Intracellular Distribution of the Enzyme

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INTRODUCTION

Although a number of investigations have been reported on the multiple forms of catalase (E. C. 1.11.1.6) in various species and tissues, the nature of the multiplicity has not been precisely defined. Holmes and Masters (11) stated that hepatic catalase was divisible into five components of activity by elution electrohoresis on polyacrylamide gel. Nishimura, et al. (22) and Patton and Nishimura (23) showed that liver catalase of rats consisted of six distinct immunoelectric sub-components. Recent studies have shown that liver catalase of adult rats was resolved into several forms with different isoelectric points by electrofocusing (6, 14, 21). On the other hand, heterogeneous patterns of catalase of human and horse erythrocytes and beef liver tissues were shown by Cantz, et al. (2) and Mörikofer-Zwez, et al. (19, 20) and Heidrich (7) and Heidrich and Hannig (8), to be correlated with an oxidoreductive state.

The heterogeneous pattern of liver catalase has been demonstrated to correlate with the intracellular distribution of the enzyme. Higashi and Shibata (9), Higashi, et al. (10) isolated catalase fractions from rat liver tissues by DEAE-cellulose column chromatography and suggested that catalase in the peroxisomal fraction corresponded to one of the four fractions (catalase III), while the other two (catalase I and II) were found in the soluble fraction of the cells. Holmes and Masters (12) described multiple forms of catalase which were various depending on the tissue and subcellular distribution, by polyacrylamide gel electrophoresis. Feinstein and Peraino (4) recently showed soluble and particulate fractions of catalase activity in mouse liver were different isoelectric points on isoelectric focusing, and suggested that the soluble and particulate catalase might be a different molecular species.

In the present study, examinations were undertaken to fractionate the soluble and particulate forms of catalase in rat liver on isoelectric focusing. Soluble and particulate forms of the enzyme was separated in 0.25 M sucrose solution for examining correlation between catalase pI-isozyme pattern and intracellular distribution of the enzyme. Furthermore, distilled water was used as a homogenizing medium to determine changes of the pI-isozyme pattern after disrupting in part the peroxisomal membranes.

MATERIALS AND METHODS

Animals

Male adult rats were used as described in a previous study (14).

Preparation of 0.25 M Sucrose Solution of Various pHs

0.25 M sucrose solution was adjusted to appropriate pH value with NaOH.

Preparation of the Enzyme Solution for Isoelectric Fractionation

For the measurement of soluble catalase, the liver tissues were rinsed with PBS, and homogenized in a Potter-Elvehjem homogenizer with 4 vol. of ice-cold 0.25 M sucrose (pH 6.0) or ice-cold distilled water, and then centrifuged at 48,000 x g for 30 minutes at 0°C, using a Beckman Model L2-65B ultracentrifuge. For the measurement of particulate catalase, livers were rinsed, homogenized with 4 vol. of ice-cold 0.25 M sucrose (pH 6.0) or ice-cold distilled water, and centrifuged at 48,000 x g for 30 minutes at 0°C. The supernatant was discarded, and the resulting pellet was washed once in the centrifuge with cold 0.25 M sucrose (pH 6.0), when water-particulate fraction was prepared. After the washing, the pellet was resuspended in ice-cold distilled water.

The suspention was treated with sonication for 7 minutes at 200 W with a Kubota KMS-250 ultrasonic generator. Finally, the sonicated suspention was centrifuged at 105,000 x g for 30 minutes at 0°C. The clear supernatant was concentrated and dialyzed against cold distilled water using collodion bags. A portion of the enzyme solution containing 15 mg of protein was submitted to isoelectric focusing. Protein was measured according to the method of Lowry, et al. (16).

Isoelectric Fractionation by Ampholine-Carrier Ampholites

The same as in the previous reports (13, 14).

RESULTS

Influences of Various pHs of 0.25 M Sucrose Solution on Release of Catalase from Cytoplasmic Particles in Rat Liver

As shown in Fig. 1, the degree of release of catalase from cytoplasmic particles in the liver was influenced by pHs of 0.25 M sucrose solution as the homogenizing medium. In pH 6.0 sucrose solution, 25% to 30% of the total catalase activity in the whole homogenate was found in the supernatant fraction, in pH 7.4 sucrose solution, about 40% of the total catalase activity was found in the cell sap, and furthermore, in pH 10.5 sucrose solution, 50%

to 70% of the activity was present in the cell sap.

Fractionation of Soluble and Particulate Liver Catalase on Isoelectric Focusing

It was reported previously (14) that catalase in adult rat liver subjected to isoelectric focusing in a pH range between pH 5 and pH 8, exhibited five catalatically active major components, pIs of which were at pH 6.95, 6.75, 6.50, 6.10 and 5.90, respectively. Fig. 2a showed that the sucrose-soluble fraction consisted mainly of three components, which were at pH 6.95, 6.77 and 6.53. Sucrose-particulate fraction showed two different isoelectric points which were at pH 6.05 and 5.90, as shown in Fig. 2b. It appears likely from these data that adult rat liver catalase was divided into two groups, neutral and acidic, as pointed out by Nakamura, et al. (21).

When fractionation of soluble and particulate of catalase was made in distilled water, approximately 50% of total catalase activity was found in the soluble fraction. It was shown in Fig. 3a that the water-soluble fraction was separated into five different catalase fractions. The catalase pI-isozyme pattern appearing in the supernatant fraction showed that it contained both neutral and acidic catalase. On the other hand, it was observed that water-particulate fraction consisted of acidic catalase alone, as in the

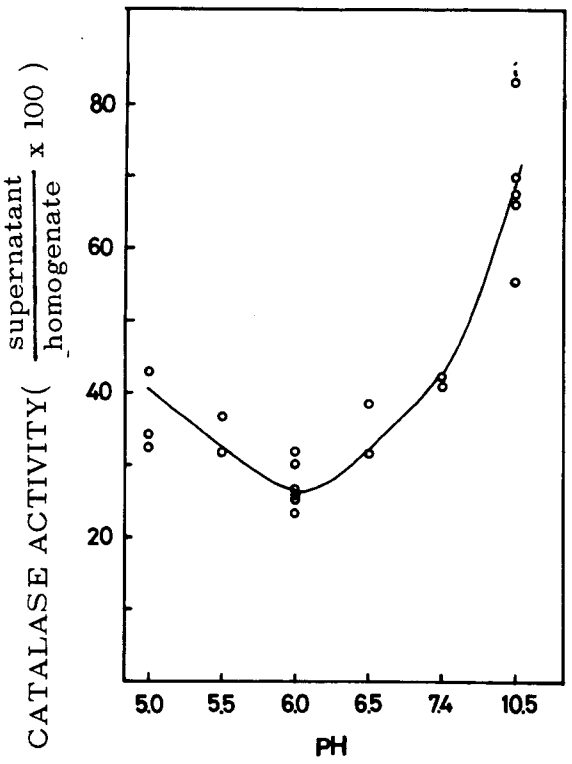

Fig. 1. Influences of various pHs of 0.25 M sucrose solution on release of catalase from cytoplasmic particles in rat liver.

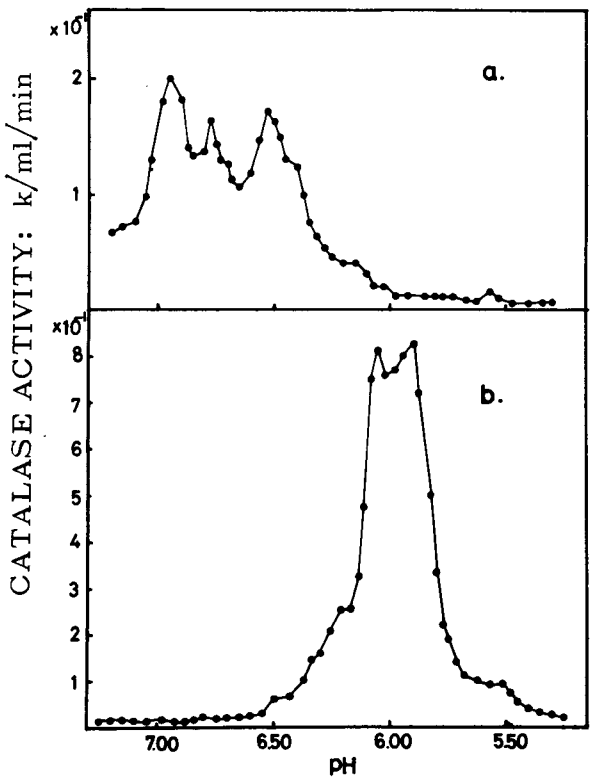

Fig. 2. Isoelectric focusing pattern of sucrose-soluble and particulate fractions of catalase in rat liver.

a: sucrose-soluble fraction
b: sucrose-particulate fraction

Enzyme activity is expressed in terms of catalase coefficient (k)/ml/min.

ase of the sucrose-particulate fraction (Fig. 3b).

DISCUSSION

Influences of fractionation media on the release of catalase bound to cytoplasmic particles of hepatic cells were reported by some investigators (1, 5, 17, 18). Fujiwara (5) showed that more than two-thirds of the total catalase activity in rat and mouse liver were found in the supernatant fraction when 0.25 M sucrose solution was used as the medium. On the other hand, Miller (18) reported that catalase activity of cell sap in mouse liver prepared in 0.25 M sucrose showed a mere one-fifth value of the total enzyme activity after the treatment with 0.5% deoxycholate. Furthermore, Mannering, et al. (17) discussed the role of the distribution of catalase between soluble and particulate fractions of the hepatic cell on the in vivo oxidation of C^{14}-methanol in mice, rats, guinea pigs and monkeys. In the present experiment, it was clearly shown that catalase activity in cell sap prepared in 0.25 M sucrose was influenced by various pHs of homogenizing media.

It is known that liver catalase exists in two different forms, namely free and particulate bound. Concerning this, Feinstein and Peraino (4) reported that catalase of mouse liver had at least two independent fractions, and that the soluble form showed an isoelectric point at pH 8.0 - 8.5, while the particulate form showed

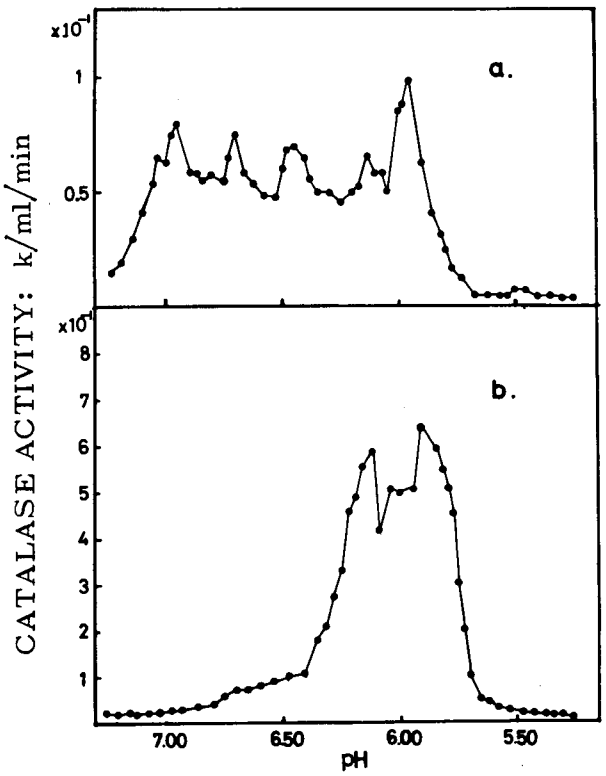

Fig. 3. Isoelectric focusing pattern of water-soluble and particulate fractions of catalase in rat liver.

a: water-soluble fraction
b: water-particulate fraction

Enzyme activity is expressed in terms of catalase coefficient (k)/ml/min.

an isoelectric point at pH 6.7. Insofar as the fractionation of the soluble and particulate forms of catalase with the aid of isoelectric focusing is concerned, our results are considered to be essentially in agreement with those described by Feinstein and Peraino (4), except for values of pHs of isoelectric points of both soluble and particulate forms of the enzyme. This incongruity is probably due to the fact that pIs were measured on a 3 - 10 pH gradient which was considered not to be sufficiently sensitive for the fractionation. On the other hand, Higashi and Shibata (9), and Higashi, et al. (10) reported the existence of four forms of catalase in rat liver by DEAE-cellulose column chromatography, and demonstrated that catalase I and II were found in sucrose-supernatant fraction, whereas, catalase III was involved in sucrose-peroxisomal fraction. According to the present data, sucrose-soluble fraction is neutral catalase, while the sucrose-particulate fraction is anodic one's. When the tissues were homogenized in cold distilled water, a part of acidic catalase found in cell sap may be great enough to account for the leakage from the peroxisomes by osmotic shock. It is suggested that our data coincide with those obtained by the DEAE-cellulose chromatograms. (9, 10).

Since the mammalian liver catalase has been postulated to consist of four subunits by some investigators (24 - 26), the presence of two different homozygotes should produce three additional hetero-

zygotes. These heterozytoes were observed in gel electrophoresis by Holmes and Masters (11). Our experiments reported here also showed that rat liver catalase consisted of five different isoelectric points, as well as in the case of catalase pI-isozyme patterns in Tetrahymena pyriformis (14). In fetal and early newborn rat liver, acidic components of catalase was shown to be absent (15), in spite of the fact that peroxisomes in that stage were observed to exist with a regularity (27), and also to contain a catalase as examined by the method of cytochemical electronmicroscopy (3). Similar findings were observed on Morris hepatomas in our laboratory (15). In the hepatomas, 10 - 20% (7793, 7316A and 9618A tumors) to 20 - 30% of catalase activity (9633 and 8994 tumors) were recovered as in the soluble fraction, when the tumors were homogenized in 0.25 M sucrose solution at pH 6.0 - 7.4. The presence of neutral catalase and absence of acidic catalase activities might be characteristic of these tumors. These findings suggest that catalase pI-isozyme patterns do not necessarily correlate with the intracellular distribution of the enzyme.

On the other hand, Heidrich (7), and Heidrich and Hannig (8) reported the existence of five enzymatically active fractions in commercial preparations of beef liver catalase on acrylamide slab electrophoresis, and stated the possibility of interconversion between these fractions as artifacts by either oxidation or reduction.

In addition, they obtained catalase from the light mitochondrial fraction of beef liver and showed that this native form of catalase consisted of only a single band form on disc electrophoresis, showing the same electrophoretic mobility as that of the reduced form of the enzyme. After this particle fraction was ultrasonified, catalase was shown to be resolved into five active bands. Similar findings were observed on erythrocyte catalase by Cantz, et al. (2) and Mörikofer-Zwez, et al. (19, 20), and they postulated that this conversion was caused by the formation of disulphide bridges or conformers. On these points, Holmes and Masters (12) studied the tissues and subcellular distribution of rat liver and kidney catalase, and stated that the existence of multiple forms of catalase was not always sufficiently explained by the oxidoreductive interconversions.

We clearly showed the differences in heterogeneous nature between the soluble and particulate fraction of liver catalase in adult rats. As to whether the heterogeneity of catalase may be defined by differences in intracellular distribution is another problem.

SUMMARY

The degree of release of catalase from cytoplasmic particles in the liver of rats was influenced by pHs of 0.25 M sucrose as the homogenizing medium. About 25 - 30%, 40%, and

50 - 70% of total catalase activity was recovered from the soluble fraction at pH 6.0, 7.4 and 10.5, respectively. Using the 0.25 M sucrose of pH 6.0, it was found that catalase in the soluble fraction consisted of three components (pI-6.95, 6.77 and 6.53), i.e. neutral catalase; while that in the particulate fraction contained two components (pI-6.05 and 5.90), i.e. acidic catalase. Using homogenates prepared in distilled water, the soluble fraction was separated into both neutral and acidic catalase, while the particulate fraction consisted of only acidic catalase.

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