HETEROGENEITY OF CATALASE IN MORRIS HEPATOMAS
AS REVEALED BYIsoELECTRIC FOCUSING AND
DEAE-CELLULOSE COLUMN CHROMATOGRAPHY*1

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Synopsis

The heterogeneity pattern of catalase in Morris hepatomas 7316A, 7800, 5123C,
7794A, 9618A, 16, and 9618B was examined by isoelectric focusing using Amphol-
line-carrier ampholyte and DEAE-cellulose column chromatography. Catalase
in the liver of normal adult rats was fractionated by isoelectric focusing into 5
major components each having its own isoelectric point at pH 6.95, 6.75, 6.50,
6.10, and 5.90. In all the hepatomas examined, the acidic components of catalase
having isoelectric points at pH 6.10 and 5.90 were found to disappear, while basic
components of the enzyme showed a definite appearance. Chromatographic anal-
ysis of the enzyme using DEAE-cellulose column showed that a considerable
portion of the enzyme activity failed to be adsorbed on the ion exchanger, which
may well support the above-mentioned appearance of the basic components of
the enzyme. The isoelectric focusing pattern as well as the chromatographic elu-
tion characteristics of catalase in the hepatomas closely resembled those in the liver of
fetal and newborn rats. It is suggested that the alteration in the heterogeneity of
hepatomas may be ascribed to the dedifferentiating nature of cancer.

INTRODUCTION

Alterations in the isozyme or heterogeneity pattern of various enzymes in hepatomas
have been studied extensively; the reports largely suggested that the pattern in poorly
differentiated tumors resembled that in fetal liver. On the other hand, investigations on
a series of Morris hepatomas of various degrees of differentiation revealed that highly
differentiated hepatomas exhibited the isozyme pattern of certain enzymes closely re-
sembling those in adult liver, suggesting that, in highly differentiated tumors, an altera-
tion in the isozyme pattern may not be a necessary postulate of cancer.3–5,31,34)

On the other hand, catalase in the liver was reported to exist in multiple forms and/or
isozymes,7–20,25–27,30) and changes in the heterogeneity pattern of catalase in the liver of
rats and mice were observed to occur during growth of the animals.12,18,30) In spite of the
fact that the precise function of catalase in the liver has not been fully clarified as yet,
the enzyme is considered as one of the phenotypic markers of hepatocytes related to

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the cell differentiation, and thus might be expected to reflect differences between normal and neoplastic cells.

In these respects, the present experiments were undertaken with several lines of Morris hepatomas of various growth rates, in order to clarify whether alterations in the heterogeneity pattern of catalase correlated with the rate of growth or represented a dedifferentiating nature as neoplasms.

**Materials and Methods**

Morris hepatomas used in the present experiments were selected to have relatively high catalase activity and also to cover a range of intermediate and slow growth rates. The fast growing hepatomas were not used because of their almost no or very low catalase activity.\(^2\) The tumors were transplanted intramuscularly into both thighs of male rats of Buffalo strain weighing about 200 g. They were fed on a commercial diet (Oriental, NMF) and received drinking water freely. After the tumor sizes reached about 1.5~2.0 cm in diameter, the animals were killed by decapitation between 10:00 and 11:00 a.m. Immediately after the tumor was excised, the necrotic and hemorrhagic areas together with fibrous tissues were removed.

For isoelectric focusing of catalase, the tumor tissues were homogenized in 4 volumes of ice-cold distilled water in a glass homogenizer with a Teflon pestle. The homogenate was subjected to sonication for 7 min at 200 W using a Kubota KMS-250 ultrasonic generator, and then centrifuged at 105,000g for 60 min at 0°. The supernatant solution therefrom was concentrated by the use of a collodion bag (Sartorius Membrane Filter) for 2 hr at 0°. A portion of this enzyme solution containing 15 mg of protein was subjected to isoelectric focusing principally according to the method of Vesterberg and Svensson, using an electrofocusing column (LKB Produkter, 8100-10) and Ampholine-carrier ampholyte (LKB Produkter, pH range: 5~8). The details of the procedure were described in our previous reports. Electrolysis was done for 40 hr at 0~1°. One-ml fractions were obtained at a flow rate of 1 ml/min. The pH of each fraction was determined at 0~1° with a Beckman Digital pH-meter. Catalase activity of the fractions was then measured according to the method of Adams.

For chromatographic analysis on DEAE-cellulose column, 10% tissue homogenate was made with 0.01M phosphate buffer (pH 7.4) containing 0.3% Triton X-100. The homogenate was centrifuged at 105,000g for 60 min, and (NH\(_4\))\(_2\)SO\(_4\) was added with stirring to the supernatant to a concentration of 30%. The precipitate was separated by centrifugation and then resuspended in an original volume of 0.01M phosphate buffer (pH 7.4). After being centrifuged at 30,000g for 30 min, the supernatant solution was dialyzed against 3 changes of ice-cold 0.01M phosphate buffer (pH 7.4) for 20 hr at 0°. Appropriate amounts of this enzyme solution containing 50~150 mg of protein were applied to a column (2.4 × 30 cm) of DEAE-cellulose equilibrated with 0.01M phosphate buffer (pH 7.4). Five-ml fractions were obtained at a flow rate of 60 ml/hr, after successive elution with the phosphate buffer, and the same buffer containing 0.05M, 0.1M, and 0.2M NaCl.

Protein content of the specimens for isoelectric focusing and chromatography was determined by the method of Lowry et al. with crystalline bovine serum albumin as the standard. Measurement of catalase activity of the tissue homogenate was made on the
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specimens appropriately diluted with 1% acacia solution containing 0.25% Triton X-100.

RESULTS

Catalase Activity

Catalase activity of the hepatomas used is shown in Table I. The activity in hepatoma 7794A and 16 was found to be higher than that in normal liver, while the activities in other tumors were more or less lower than that in the liver. The individual variety of the level of the activity was not found to be correlated with the number of the transplant generation.

<table>
<thead>
<tr>
<th>Hepatoma</th>
<th>Catalase activity(a)</th>
<th>Transplant generations</th>
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<tbody>
<tr>
<td>7316A</td>
<td>20.7±1.40 (7)</td>
<td>54~59</td>
</tr>
<tr>
<td>7800</td>
<td>71.2±9.02 (9)</td>
<td>60~63</td>
</tr>
<tr>
<td>5123C</td>
<td>44.5±2.51 (8)</td>
<td>89~95</td>
</tr>
<tr>
<td>7794A</td>
<td>159.0±9.51 (7)</td>
<td>35~37</td>
</tr>
<tr>
<td>9618A</td>
<td>84.3±3.08 (7)</td>
<td>8, 9</td>
</tr>
<tr>
<td>16</td>
<td>184.0±19.20 (5)</td>
<td>6, 8</td>
</tr>
<tr>
<td>9618B</td>
<td>97.5, 127.0 (2)</td>
<td>6, 7</td>
</tr>
<tr>
<td>Normal adult liver(b)</td>
<td>136.4±6.90 (8)</td>
<td></td>
</tr>
</tbody>
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Figures in parentheses are the number of determinations.
\(a\) Catalase activity is expressed in terms of catalase coefficient (k)/min/100 mg dry tissues. Values of mean ± SE (standard error of the mean).
\(b\) Livers obtained from male Buffalo rats weighing between 200 and 300 g.

Isoelectric Focusing Pattern of Catalase Activity

The typical patterns of pI-heterogeneity of catalase in normal adult liver and the hepatomas are shown in Figs. 1, 2, and 3. As shown in Fig. 1, it was found that catalase in the liver of adult rats was separated into 5 major components, each having an isoelec-
Fig. 2. Isoelectric focusing pattern of catalase in Morris hepatomas of intermediate growth rate.
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electric point at about pH 6.95, 6.75, 6.50, 6.10, and 5.90, as was described in our previous reports. Repeated examinations showed that these pH values were accurate within an error of about pH $\pm 0.05$.

Fig. 2 shows the heterogeneity pattern in the hepatomas with intermediate growth rate. Hepatoma 7316A exhibited a single peak activity at pH 6.95, with a trailing skirt covering a pH range from about 7.5 to 6.5. Hepatoma 7800, on the other hand, showed

**Fig. 3.** Isoelectric focusing pattern of catalase in Morris hepatomas of slow growth rate
5 major peaks at pH about 7.21, 7.07, 6.90, 6.70, and 6.52, with about the same level of the activity for each fraction. Hepatoma 5123C showed 3 peaks of activity at about pH 7.38, 7.23, and 7.05, while 7794A tumor showed 2 peaks at pH 7.32 and 7.18. In hepatoma 7794A, the level of the activity in both fractions was higher than that seen in any peak fractions in normal liver.

Fig. 4. DEAE-cellulose column chromatography of catalase in the liver of normal adult and 5-day-old rats (applied amount: adult, 84 mg; 5-day-old, 80 mg)
Fig. 5. DEAE-cellulose column chromatography of catalase in Morris hepatomas (applied amount: 7916A, 50 mg; 7918A, 60 mg; 9618A, 100 mg)
Fig. 3 shows the typical patterns of the heterogeneity in the slow growing hepatomas. Hepatoma 9618A showed 5 peaks of activity at about pH 7.23, 7.03, 6.95, 6.73, and 6.53. Hepatoma 16 showed a single peak of activity exhibiting a gentle slope with a shoulder at pH around 6.95. The skirts of this peak were seen spreading over a pH range from about 7.5 to 6.5. Hepatoma 9618B showed 2 peaks at pH 7.14 and 6.95, with a broad range of activity from pH 7.5 to 6.5.

Chromatographic Pattern of Catalase Activity on DEAE-cellulose Column

Fig. 4 illustrates a typical chromatographic elution pattern of catalase in the normal adult and 5-day-old rat liver. In the adult liver, it was found that all the activity of the enzyme was adsorbed on the DEAE-cellulose and the predominant portion thereof was eluted from the column with 0.05M NaCl. Furthermore, two smaller peaks of activity were obtained by stepwise elution with 0.1M and 0.2M NaCl. In the newborn liver, on the other hand, a portion of the activity was not adsorbed on DEAE-cellulose and passed through the column with the starting buffer solution. The main peak was obtained likewise with 0.05M NaCl, but very low and almost no activity was recovered from fractions eluted with 0.1M and 0.2M NaCl, respectively.

Fig. 5 shows the chromatographic pattern of the enzyme from hepatomas of both intermediate and slow growth rates. In all of these tumors, a considerable portion of the enzyme activity was not adsorbed on DEAE-cellulose and passed through the column when the column was washed with the starting buffer solution. The main peak was obtained likewise with 0.05M NaCl, but almost no or significantly low activity was found in the fractions eluted from the column with 0.1M and 0.2M NaCl, as in the case of newborn liver.

Discussion

It has been suggested that hepatic catalase exists in multiple forms with respect to characteristics revealed by immunolectrophoresis, polyacrylamide or starch gel electrophoresis, DEAE-cellulose column chromatography, or isoelectric fractionation using Ampholine-carrier ampholyte. Furthermore, as reported by Holmes, Koyama, and by Patton and Nishimura, certain changes in the heterogeneity pattern of catalase were observed to occur in the liver of mice and rats during fetal and postnatal growth, suggesting that changes may have a certain relationship to the cell differentiation and growth.

In the present experiments, it is clearly noted that acidic components of catalase having isoelectric points at pH about 6.10 and 5.90 are missing in the hepatomas, resembling the enzyme in fetal and newborn livers, but obviously different from that in adult liver. In addition to the lack of acidic components, an appearance of basic components is also emphasized as a characteristic of the hepatomas which seems to be shared in common with fetal and newborn livers but not with adult liver. The presence of the basic components of the enzyme in the hepatomas is assumed to be supported by the present experiments on DEAE-cellulose column chromatography which revealed that a considerable portion of the enzyme was not adsorbed on DEAE-cellulose.

As to the nature of chromatographic and electrophoretic heterogeneity of catalase, several reports have suggested that the heterogeneity may be the results of an oxidoreductive interconversion or a conformational change of the enzyme molecule that occurs
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during the purification of the enzyme or simply by coming in contact with an ion-exchanger such as DEAE-Sephadex. However, our unpublished data on DEAE-cellulose column chromatography of liver catalase which was carried out in the presence of Cleland's reagent suggested that the lack of catalase which was not adsorbed on DEAE-cellulose did not result from oxidation of the enzyme.

Heterogeneity or isozyme pattern of several enzymes in a variety of hepatomas has been reported to be altered depending on the growth rate and/or the degree of differentiation of the tumors. In other words, the pattern in poorly differentiated hepatomas shows salient deviations from normal adult liver, whereas that in highly differentiated hepatomas presents striking similarities to the pattern in the tissue of origin. However, the appearance of a fetal and newborn pattern of the heterogeneity of catalase in Morris hepatomas seems to be ascribable to the general characteristic of hepatomas, irrespective of variations in their growth rate or the degree of differentiation. In fact, hepatoma 7316A and 16 showed a similar pattern of the pI-heterogeneity, in spite of the fact that the former tumor was less differentiated and grew much faster than the latter. Hepatoma 9618A has been regarded as a minimal deviation hepatoma with the same morphological features of chromosomes as in normal hepatocytes, and as having isozymes of pyruvate kinase, glucose-ATP phosphotransferases, or adenylate kinase closely resembling those in normal adult liver. The fact that pI-heterogeneity of catalase in hepatoma 9618A showed a fetal pattern might therefore be assessed as one of the most outstanding biochemical traits to characterize this tissue as a hepatoma.

As to the heterogeneity of catalase in neoplastic cells, Nishimura et al. reported an immunoelectrophoretic study on leucocytes in leukemic patients and found disappearance of a normal antigen of catalase and an appearance of an abnormal antigen of the enzyme. The abnormal antigen was furthermore identified as the fetal liver antigen of catalase. These observations on the heterogeneity of catalase in leukemic cells are considered to be coincident with the present findings on Morris hepatomas.

It was reported that particulate (peroxisomal) and soluble catalase of the liver showed different characteristics with respect to an isoelectric focusing pattern, to an elution pattern on DEAE-cellulose column chromatography, and also to an electrophoretic pattern on starch gel. Except for hepatoma 7794A, the hepatomas examined were observed to contain peroxisomes, though varying in number, and the catalase activity that was bound to peroxisomes comprised about 50~90% of the total activity. Thus, it is suggested that pI-heterogeneity of catalase in the hepatomas might not correlate with intracellular distribution of the enzyme, in contrast to the enzyme in normal adult liver.

As to the nature of the multiplicity of liver catalase, it has been suggested that an epigenetic modification of the enzyme might be responsible for the ontogenic alterations of the heterogeneity, and also for the differences in the heterogeneity pattern between peroxisomal and soluble enzyme. However, the exact intracellular mechanisms by which catalase in the hepatomas is modified and undergoes changes to exhibit more basic isoelectric points remain to be investigated.
REFERENCES