Isozyme Patterns of Branched-Chain Amino Acid Transaminase in Various Rat Hepatomas

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SUMMARY

Branched-chain amino acid transaminase (EC 2.6.1.6) from various rat tissues was separated by DEAE-cellulose column chromatography into three types of isozymes (Enzymes I to III). Enzyme I was found in all tissues examined, whereas Enzyme II was present only in the liver and Enzyme III was present only in the brain.

Yoshida ascites hepatomas are rapidly growing and poorly differentiated, and in all those examined we found Enzymes I and III instead of Enzymes I and II, as in normal liver. Similarly, in primary malignant tumors of the liver induced by p.o. administration of 0.06% 3'-methyl-4-dimethylaminoazobenzene, Enzymes I and III were present and Enzyme II was absent. Conversely, benign tumors showed isozyme patterns similar to that of normal liver. Morris hepatomas showed diverse isozyme patterns. Hepatomas 5123tc and 7793 contained all three enzymes, hepatoma 7777 contained only Enzyme I (fetal pattern), and hepatomas 7794A and 7316A contained Enzymes I and II (normal adult pattern). No relation was found between the presence of Enzyme III and the growth rates of these tumors.

These observations indicate that hepatomas show characteristic patterns of isozymes of the transaminase. This may be caused by changes of gene expression during oncogenesis.

INTRODUCTION

Recent progress in the study of isozymes has made it possible not only to distinguish similar enzymes in different tissues but also to detect qualitative abnormalities in the isozyme patterns of tumor cells. It has been found that various enzymes of carbohydrate metabolism divert from the liver type to the muscle type in hepatomas (6—9, 27, 28, 32, 34, 38). The isozyme patterns in hepatomas were also found to resemble those in fetal liver and were considered to represent disturbance of differentiation due to neoplastic transformation of the cells (25, 31, 32).

However, there have been very few reports on the isozyme patterns of enzymes involved in amino acid metabolism in tumors (14, 16, 18, 22, 29). We reported recently (23) that there is a specific transaminase for branched-chain amino acids, and we found 3 types of isozyme (Enzymes I to III) of this transaminase in various rat tissues. Enzyme I was found in all tissues examined, while Enzymes II and III were found only in liver and brain, respectively. Enzymes I and III transaminated all 3 amino acids equally well, while Enzyme II showed activity only with leucine (2). Adult liver contained Enzymes I and II, while fetal liver contained only Enzyme I (12). The activity of the latter was very high, but during development it decreased, and at birth Enzyme II activity appeared and increased rapidly. The activity of Enzyme II was also found to increase in liver during regeneration. However, no Enzyme III appeared in liver during either development or regeneration.

Recently, we observed that Yoshida ascites hepatoma AH 130 contained Enzymes I and III instead of Enzymes I and II, and the Enzyme III from this tumor was indistinguishable either chromatographically or immunologically from that found in normal brain (23).

These findings prompted us to see whether the presence of Enzyme III was a common character in hepatomas. Various hepatomas were examined, including Yoshida ascites hepatomas, primary hepatomas induced by feeding 3'-Me-DAB,2 and Morris hepatomas. The present work shows that poorly differentiated tumors contain Enzymes I and III instead of the normal liver pattern of Enzymes I and II, while noncancerous tumors have the isozyme pattern of normal liver. Morris hepatomas have various isozyme patterns.

MATERIALS AND METHODS

Hepatomas. Male Wistar strain rats weighing 100 to 150 g were used in experiments on transplantation of Yoshida ascites hepatomas and Yoshida sarcoma. They were also used for induction of primary tumors by feeding 3'-Me-DAB. Adult, male Buffalo strain rats weighing 200 g were used in experiments on transplantation of Morris hepatomas. Various strains of transplantable hepatoma were generously supplied by Dr. Takashi Sugimura, of the Cancer Research Center, Tokyo, Japan, Dr. Tetsuo Ono, of the Cancer Institute, Tokyo, Japan, and Dr. Nobuhiko Katunuma, of the Institute for Enzyme Research.

Yoshida ascites hepatomas and sarcoma were suspended in 0.9% NaCl solution and transplanted by i.p. injection, and animals were sacrificed about 1 week later. Morris hepatomas were transplanted into thigh muscle or s.c. tissue of the back.

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2 The abbreviation used is: 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene.

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For induction of hepatomas by 3'-Me-DAB, male Wistar strain rats weighing 100 to 130 g were fed ad libitum for 1 to 5 months on a diet of the following composition: 0.06 g 3'-Me-DAB, 1.0 g vitamin mixture (containing vitamin B_2, 0.25 mg), 4.0 g salt mixture, 8.0 g corn oil, 0.2 g choline-HCl, 15 g casein, 36 g sucrose, and 36 g starch. Then animals were given laboratory chow for the rest of the total feeding period of 7 months. Standard laboratory chow was obtained from the Kansai Institute for Experimental Animals, Osaka, Japan, and other nutritional materials were obtained from Tanabe Amino Acid Research Foundation, Osaka, Japan.

Preparation of Enzyme Extracts. Ascites fluid containing tumor cells was collected, diluted about 5 times with 0.25 M sucrose solution, and centrifuged at 1000 x g for 5 min. The packed cells were washed twice with sucrose solution. Morris hepatomas and tumors induced by 3'-Me-DAB were separated from normal tissue, and necrotic parts were carefully trimmed off.

All samples were homogenized in a Teflon-glass homogenizer in 10 volumes of cold 0.25 M sucrose solution. The homogenates were centrifuged at 10,000 x g for 15 min, and the resulting supernatant fluids were used as enzyme preparations.

DEAE-cellulose Chromatography. The crude supernatant fluids described above were dialyzed against 5 x 10^{-3} M potassium phosphate buffer (pH 7.8) containing 1 x 10^{-4} M EDTA and 1 x 10^{-3} M 2-mercaptoethanol. The dialyzed preparations were applied to DEAE-cellulose columns equilibrated with the same buffer. Two sizes of column were used, depending on the amount of the sample available. With Yoshida ascites hepatomas, such as those shown in Chart 1 and Table 1, extract containing about 0.8 g protein was applied on a column (2.5 x 40 cm), and the column was washed with 50 ml of the same buffer. Then, enzymes were eluted with a linear concentration gradient of buffer obtained by putting 500 ml of 5 x 10^{-3} M buffer in the mixing chamber and 500 ml of 3 x 10^{-3} M buffer in the reservoir. Ten-nl fractions of eluate were collected. For extracts of Morris hepatomas and tumors induced by 3'-Me-DAB, such as those shown in Tables 2 and 3, about 0.4 g protein was applied on a column (1.5 x 25 cm). The column was eluted in the same way, with 250-ml volumes of the 2 concentrations of buffer, and 5-ml fractions of eluate were collected.

Enzyme Assay. The activity of branched-chain amino acid transaminase was assayed spectrophotometrically by a slight modification of the method described previously (11). For assay of Enzyme II in the crude extract, 160 µmoles of L-leucine were added to the reaction mixture. Leucine is rather insoluble, so it was dissolved in 0.5 ml of 0.1 M pyrophosphate buffer before addition to the reaction mixture. Enzyme II activity was calculated by subtracting the activity with 20 µmoles of either valine or isoleucine (activity of Enzyme I) from that with 160 µmoles of leucine (total activity). One unit of enzyme activity is defined as the amount of enzyme forming 1 nmole of ketoacid/min, and specific activity is expressed as units/mg protein. Protein was measured by the method of Lowry et al. (19).

Activity in fractions of eluate from columns is expressed as the absorbance of the phenylhydrazone formed from the keto acid at 440 nm (its absorption maximum). The proportions of

Isozyme Patterns of Yoshida Ascites Hepatomas and Sarcoma. Normal adult liver contained Enzymes I and II, but Yoshida ascites hepatoma AH 130 contained Enzymes I and III (2, 23). The isozyme patterns of several other types of Yoshida ascites hepatomas were examined by DEAE-cellulose column chromatography, and the active fractions obtained were also identified by use of antisera against Enzymes I and III, respectively. A typical example is shown in Chart 1. As shown in Table 1, all strains examined contained Enzymes I and III instead of Enzymes I and II. It was also found that these hepatomas contained much more Enzyme III than Enzyme I and that the specific activities of crude extracts, which contained Enzymes I and III, were very much higher than that of normal liver.

Yoshida sarcoma, which is thought to be derived from hepatocytes, contained Enzymes I and III. Mouse Ehrlich ascites tumor, which is derived from mouse mammary tumor, contained only Enzyme I (result not shown in this paper).

Morris Hepatomas. There are many reports that the isozyme patterns of Morris hepatomas deviate less from normal than those of rapidly growing hepatomas, such as Yoshida hepatomas (7, 38). Table 2 shows that the isozyme patterns of branched-chain amino acid transaminase varied considerably in different strains of Morris hepatoma. Only Enzyme I was present in hepatomas 7777 and in 1 of the 2 samples of hepatoma 7795 examined. Both Enzyme I and II were found in normal adult liver and ascites hepatoma AH 130 on DEAE-cellulose chromatography. The enzyme activities in the fractions of eluate were identified by incubating samples with 20 µmoles of isoleucine (Ileu) or leucine (Leu).
in hepatomas 7794A and 7316A, and all 3 enzymes were found in hepatomas 5123tc and 7793 and in the other sample of hepatoma 7795 examined. The presence of Enzyme I only and a combination of Enzymes I and II are similar to the patterns found in normal fetal and adult liver, respectively. However, all 3 enzymes together have not been found in any normal tissue yet examined. Also, unlike poorly differentiated Yoshida hepatomas, none of the Morris hepatomas examined contained a combination of Enzymes I and III.

No definite relation was found between the presence of Enzyme III and the growth rates of the tumors. Moreover, the specific activity varied considerably in extracts of different tumors but showed no relation with the growth rates of the tumors.

The 2 samples of hepatoma 7795 examined had different isozyme patterns, 1 containing all 3 enzymes and the other containing only Enzyme I. This difference may be due to a difference in the propagation times of the samples: the sample with all 3 enzymes had been passaged for 15 months less than that with only Enzyme I. This suggests that the phenotype of the isozyme pattern in a single tumor strain may not be stable and that it can be altered by aging, probably toward a less differentiated type. However, this phenomenon cannot be common, because 3 samples of hepatoma 7316A with different propagation times had similar isozyme patterns. The 2nd and 3rd samples shown in Table 2 had been passaged for 4 and 5 months, respectively, longer than the 1st sample.

The transplantation site, i.e., i.m. or s.c., did not affect the isozyme pattern. These findings agree well with results on hexokinase isozymes (EC 2.7.1.1) reported by Weinhouse (38).

Enzyme III did not appear in the liver of rats bearing either Yoshida or Morris hepatomas, and the specific activities of Enzymes I and II in the host liver were not significantly different from normal. Thus, 3 samples of host liver bearing Yoshida hepatoma AH 130 showed a mean specific activity for leucine of 0.98 ± 0.15, while the mean value for normal liver was 1.0 ± 0.2, as shown in Table 1.

**Primary Tumors Induced by Feeding 3'-Me-DAB.** The established tumors examined had been transplanted for many generations, so their isozyme patterns might have changed.

### Table 1

<table>
<thead>
<tr>
<th>Tissue or tumor</th>
<th>Activity for leucine (units/mg protein)</th>
<th>Distribution of enzymes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver (10)</td>
<td>1.0 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>I (25–30)&lt;sup&gt;c&lt;/sup&gt; II (70–75) III 0</td>
</tr>
<tr>
<td>Yoshida ascites hepatoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH 130 (9)</td>
<td>8.9 ± 2.1</td>
<td>0 (60–80)</td>
</tr>
<tr>
<td>60 C</td>
<td>15.4</td>
<td>50 0 50</td>
</tr>
<tr>
<td>66 F</td>
<td>10.8</td>
<td>30 0 70</td>
</tr>
<tr>
<td>143 A</td>
<td>10.7</td>
<td>35 0 65</td>
</tr>
<tr>
<td>7974</td>
<td>43.6</td>
<td>25 0 75</td>
</tr>
<tr>
<td>Yoshida sarcoma</td>
<td>7.6</td>
<td>35 0 65</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number of specimens examined is shown in parentheses. No number indicates the values for a single specimen.

<sup>b</sup> Mean value ± S.D.

<sup>c</sup> Range of values.

### Table 2

<table>
<thead>
<tr>
<th>Morris hepatoma</th>
<th>Activity for leucine (units/mg protein)</th>
<th>Distribution of enzymes (%)</th>
<th>Growth rate (1/cm/mo.)</th>
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</thead>
<tbody>
<tr>
<td>7777</td>
<td>3.2</td>
<td>100 0 0</td>
<td>0.2</td>
</tr>
<tr>
<td>5123tc</td>
<td>0.6</td>
<td>3 78 19</td>
<td>0.3</td>
</tr>
<tr>
<td>7316A</td>
<td>2.3</td>
<td>50 50 0</td>
<td>0.35</td>
</tr>
<tr>
<td>7316A</td>
<td>7.1</td>
<td>68 32 0</td>
<td>0</td>
</tr>
<tr>
<td>7316Ab</td>
<td>0.4</td>
<td>10 90 0</td>
<td>0</td>
</tr>
<tr>
<td>7795</td>
<td>1.3</td>
<td>17 41 42</td>
<td>0.4</td>
</tr>
<tr>
<td>7795</td>
<td>5.9</td>
<td>100 0 0</td>
<td>0</td>
</tr>
<tr>
<td>7793</td>
<td>0.5</td>
<td>28 16 56</td>
<td>0.45</td>
</tr>
<tr>
<td>7794A</td>
<td>1.3</td>
<td>46 54 0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Reported by Dr. H. P. Morris (21).

<sup>b</sup> Transplanted s.c. Other tumors were transplanted i.m.

### Table 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mo. of 3'-Me-DAB administration</th>
<th>No. of rats</th>
<th>No. of tumors induced</th>
<th>Activity for leucine (units/mg protein)</th>
<th>Distribution of enzymes (%)</th>
<th>Histological type&lt;sup&gt;d&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>20</td>
<td>4</td>
<td>2.0</td>
<td>66 0 34</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>3.6</td>
<td>0 20</td>
<td>A</td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>0.5</td>
<td>80 0 20</td>
<td>A</td>
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</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>0.9</td>
<td>10 90 0</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>55 45 0</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>1.2</td>
<td>28 72 0</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>5 95 0</td>
<td>B</td>
<td></td>
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<tr>
<td>9</td>
<td>0.4</td>
<td>11 89 0</td>
<td>B</td>
<td></td>
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<tr>
<td>10</td>
<td>0.4</td>
<td>8 92 0</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>11</td>
<td>2.2</td>
<td>65 0 35</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.3</td>
<td>14 14 72</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1.7</td>
<td>50 50</td>
<td>E (C &lt; D)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>3.3</td>
<td>14 0 86</td>
<td>E (C &gt; D)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> A, cholangiocellular fibrosis; B, hepatocellular adenoma; C, hepatocellular carcinoma; D, cholangiocellular carcinoma; E, mixed type.
during transplantation and may not have been due to carcinogenesis per se. For a test of this possibility, we induced primary tumors in the livers of rats by p.o. administration of 3'-Me-DAB. For avoidance of the complication of toxic effects of the carcinogen on the isozyme pattern, the rats were given only laboratory chow for a considerable time after administration of the carcinogen. As shown in Table 3, the frequency of tumor induction increased with the period of carcinogen administration. Histological examination showed that administration of the carcinogen for various periods induced cholangiocellular fibrosis (Experiments 1 to 4), malignant hepatomas and mixed type tumors (Experiments 11 to 14), and benign hepatocellular adenomas (Experiments 5 to 7, 9, and 10). The histological appearances of these tumors are shown in Figs. 1 to 3. The isozyme patterns of these tumors showed that benign hepatocellular adenomas (Experiments 5 to 7, 9, and 10) had the isozyme pattern of normal liver, while the others contained Enzymes I and III. In nontumorous parts of the livers of these rats, the specific activity of Enzyme II, but not that of Enzyme I, tended to decrease gradually during carcinogen administration; however, no Enzyme III was detected in these parts (Chart 2). The appearance of a combination of Enzymes I and III in tumors with cholangiocellular fibrosis will be discussed in a later section, but it should be noted that many results presented in this paper indicate that the presence of a combination of Enzymes I and III may be a fairly specific characteristic in hepatic carcinogenesis.

**DISCUSSION**

In hepatomas the isozyme patterns involved in carbohydrate metabolism resemble those in fetal liver or adult muscle. However, the patterns of our transaminase did not; i.e., most of the tumors studied contained Enzymes I and III, although some Morris hepatomas contained only Enzyme I. The appearance of the brain type isozyme of aldolase (EC 4.1.2.13) (C type) has also been reported in some strains of hepatomas (33). The kidney-type isozymes of glutaminase (EC 3.5.1.2), phosphofructokinase (EC 2.7.1.11), and the isoprotein of ferritin have been found in hepatomas (16–18, 35). Moreover, the isozymes of various enzymes in hepatomas change to patterns that have not been identified with those of any known tissue. These include the patterns of DNA polymerase (EC 2.7.7.7) (15, 24), aspartate transaminase (EC 2.6.1.1) (22, 29), thymidine kinase (EC 2.7.1.21) (10), carbamylphosphate synthetase (EC 2.7.2.5) (14), and adenylate kinase (EC 2.7.4.3) (5). The isoproteins of α-fetoglobulin and ferritin can also be included in this category (1, 26). Furthermore, recent reports have shown that the isozyme of pyruvate kinase (EC 2.7.1.40) found in hepatomas was not identical with the muscle type (4, 35, 36). These results indicate that biochemical dedifferentiation in hepatomas does not necessarily represent a change to the type found in muscle, nor is it a reflection of the type in fetal liver at a certain period of development.

Enzyme II is found only in adult liver and is inducible under various physiological conditions (13, 30), so it seems to be a functional enzyme in leucine metabolism. Many functional enzymes, such as serine dehydrase (EC 4.2.1.13), tryptophan pyrrolase (EC 1.13.1.12), and tyrosine transaminase (EC 2.6.1.5) are not found in fetal liver or in poorly differentiated hepatomas (25). Enzyme II disappears from malignant hepatomas, but some Morris hepatomas still retain this enzyme.

The appearance of Enzyme III in tumors with cholangiocellular fibrosis induced by 3'-Me-DAB suggests that this enzyme may be present in normal bile duct cells and that the proportion of this enzyme may increase as these cells proliferate. However, this is unlikely because the appearance of Enzyme III was accompanied by loss of Enzyme II, although many normal hepatocytes were still seen. Therefore, it seems likely that dedifferentiation occurred in these tumors and that the malignant pattern of the isozymes was fixed at an early stage during administration of the carcinogen. Similar observations were made on pyruvate kinase, aldolase, and α-fetoglobulin during carcinogenesis (37, 39), although Endo *et al.* (6) reported that acquisition of a new type of isozyme at an early stage of carcinogenesis is irreversibly fixed. Therefore, it is uncertain at present whether the mechanisms of appearance of Enzyme III at early and later stages of carcinogen administration are the same.

These changes in the isozyme patterns in tumor cells may be due to aberration of gene expression, but another possible explanation is that they are due to selection of a cell population. This possibility would be disproved by finding hybrid isozymes of transaminase like those of aldolase (20), but such hybrids have not yet been found. We do not yet know whether single cells can contain all 3 enzymes, although the present work has shown that some hepatomas contained all 3. Recently, we found that tissue culture cells isolated and cloned from rat liver contained only Enzyme I; however, when transformed in medium containing 4-nitroquinoline oxide or p-dimethylaminoazobenzene, they produced Enzyme III (unpublished data).

Many transaminases have different isozymes in the supernatant and mitochondrial fractions, and the isozymes in these 2 fractions differed quantitatively in tumors and in normal liver (22, 29). Enzymes I and II were also present in
both fractions, but in hepatomas Enzyme II disappeared from both fractions, and Enzyme III appeared only in the supernatant fraction (23). The Enzyme I's in the 2 fractions had different properties (3), and so it is possible that the isozymes of subcellular fractions may be controlled by different genes, and their aberrations in the respective fractions of hepatomas may occur independently.

It has been found that livers of tumor-bearing animals have isozyme patterns which tend to deviate from normal to a dedifferentiated pattern (31, 39). However, we could find no significant change in the isozyme pattern of this transaminase in livers of rats bearing tumors, although Enzyme II tended to decrease in nontumorous parts of the liver in animals receiving 3'-Me-DAB (Chart 2).

ACKNOWLEDGMENTS

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REFERENCES


34. Tanaka, T., Harano, Y., Sue, F., and Morimura, H. Crystallization,
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Figs. 1 to 3. Sections of tumors induced by feeding of 3'-Me-DAB.

Fig. 1. Tumor of Experiment 1. Marked cholangiocellular fibrosis is observed. H & E, x 40 (A) and x 100 (B).

Fig. 2. Tumor of Experiment 6. Cellular pleomorphism and benign type hepatoma cells (hepatocellular adenoma) are seen. H & E, x 40 (A) and x 100 (B).

Fig. 3. Tumor of Experiment 13. This appears to be a mixed type of cholangiocellular and hepatocellular carcinomas. H & E, x 100 (A) and x 200 (B).
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