Kinetic Properties of Alcohol Dehydrogenase in Hepatocellular Carcinoma and Normal Tissues of Rat

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SUMMARY

It was previously reported that the properties of alcohol dehydrogenase of a rat hepatocellular carcinoma (Becker H-252), a tumor of intermediate growth rate, were different from those of the liver enzyme, suggesting different isozymes. To determine whether the degree of differentiation affected the isozyme of alcohol dehydrogenase, a fast-growing, poorly differentiated tumor and one that is well differentiated and of intermediate growth rate were studied. Alcohol dehydrogenase from Morris hepatoma 7288ctc, a fast-growing, poorly differentiated tumor, had properties similar to those found with the Becker H-252 tumor, including a high $K_m$ for ethanol and acetaldehyde and the absence of substrate inhibition. By contrast, alcohol dehydrogenase from the well-differentiated Morris hepatoma 5123C had properties similar to those of the liver enzyme. Thus, alcohol dehydrogenase is another example of an enzyme the isozyme composition of which changes with neoplastic de-differentiation. Further studies, including gel electrophoresis, substrate specificity patterns, and interaction with antibodies to alcohol dehydrogenase, are required to determine the factors responsible for the biochemical defect that occurs at the molecular level during carcinogenesis and whether the alcohol dehydrogenase isozymes in the Becker H-252 and Morris 7288ctc hepatomas are identical. A survey of several normal rat tissues revealed that only the stomach contains this unique isozyme of alcohol dehydrogenase.

INTRODUCTION

The properties of alcohol dehydrogenase in the 100,000 × g supernatant fraction of Becker H-252 rat hepatocellular carcinoma, a moderately well-differentiated tumor of intermediate growth rate induced in ACI rats (1, 14) are different from those of the liver (5). Whereas liver alcohol dehydrogenase has a $K_m$ for ethanol of about 0.3 mM and is subject to substrate inhibition, the $K_m$ of the tumor enzyme is about 220 mM, and no substrate inhibition is observed (5). Alcohol dehydrogenase activity in the Becker H-252 tumor is NAD$^+$ dependent, is inhibited by pyrazole, and is also inhibited by an antibody to pure horse liver alcohol dehydrogenase (5). The liver and tumor enzymes differ in electrophoretic mobility, susceptibility to heat treatment, pH optimum, some catalytic properties, and substrate specificity (5). It appears that the liver and tumor enzymes are different isozymes (5).

Neoplastic transformation is often associated with the appearance of isozymes that are different from those of the parent tissues (for reviews, see Refs. 6, 15, and 16). In slow-growing, poorly differentiated hepatocellular carcinomas, the isozyme pattern is nearly identical with that of the adult liver, whereas with decreased differentiation and increased growth rate, there is a loss of the liver isozymes, which are replaced by isozymes that are of low activity or absent in the normal adult liver (15, 16). It was therefore of interest to determine whether the degree of differentiation of hepatocellular carcinoma correlates with the presence of this isozyme of alcohol dehydrogenase.

The properties of alcohol dehydrogenase from rat stomach of male ACI rats are identical with those of the enzyme in Becker H-252 hepatocellular carcinoma (5). Therefore, we surveyed kinetic properties of alcohol dehydrogenase in several different tissues of ACI rats to determine whether the liver or tumor isozyme was present.

MATERIALS AND METHODS

Tumors. Morris hepatomas 7288ctc, a poorly differentiated rapid-growing transplanted hepatocellular carcinoma (3) and 5123C, a well-differentiated intermediate growing tumor (7, 12), were kindly provided by Dr. E. Farber. The tumors were transplanted into male Buffalo rats by s.c. or i.p. injection and were allowed to grow at these sites for 2 to 3 weeks (7288ctc) or 8 weeks (5123C). Since these tumors do not involve the normal liver, tumor and liver supernatant fractions may be prepared from the same rat, the latter serving as a control for the former.

Preparations. Rats were decapitated and the livers were removed, care being taken to avoid any contamination with the i.p. tumor. Homogenates of liver and tumor (1:4) were prepared in 0.25 M sucrose-0.01 M Tris-HCl (pH 7.4)-0.001 M EDTA. The homogenates were centrifuged at 10,000 × g for 10 min. The resulting supernatant fluid was then centrifuged at 100,000 × g for 60 min. This supernatant fraction was used as the source of liver or tumor alcohol dehydrogenase. Similar procedures were used to prepare supernatant fractions of stomach, kidney, testes, intestine, spleen, and pancreas from male ACI rats. In the case of stomach, only the glandular portion of the stomach was removed. The mucosa was not separated from submucosa and muscle for these experiments, hence the activity of alcohol dehydrogenase in the mucosa of the stomach is obviously much...
higher than the activity reported here for the whole stomach. Tissues were rinsed with 0.9% NaCl solution, sliced, and gently blotted before homogenization.

**Assays.** All enzyme assays were performed in a cuvet with a final volume of 3.0 ml at 25°. Changes in absorbance were monitored at 340 nm with a Gilford recording spectrophotometer. The reaction was initiated by addition of enzyme (supernatant). In the oxidative direction, the assay system contained 75 mM sodium pyrophosphate buffer (pH 9.6), 75 mM semicarbazide, 25 mM glycine, 1.5 mM NAD^+", and varying concentrations of ethanol. In the reductive direction, the assay system contained 100 mM sodium phosphate buffer (pH 7.0), 0.3 mM NADH, and varying concentrations of acetaldehyde or m-nitrobenzaldehyde. All rates were corrected for blanks, which contained all components except the substrate. Protein was determined by the method of Lowry et al. (10) using bovine serum albumin as the standard. The variability between experiments was less than 20%.

**RESULTS**

**Alcohol Dehydrogenase Activity in Morris Hepatomas and Liver.** Supernatant fractions of livers from rats implanted with the 2 Morris hepatomas showed no increase in alcohol dehydrogenase activity when the concentration of ethanol was raised from 5 to 500 mM (Table 1). Some decrease in activity was observed at higher ethanol concentrations, indicating substrate inhibition of the enzyme (5). The \( K_m \) for ethanol for the liver enzyme was about 0.3 to 0.5 mM. In a similar manner, alcohol dehydrogenase activity of the well-differentiated Morris hepatoma 5123C also did not increase with an increasing concentration of ethanol (Table 1). The \( K_m \) for ethanol for the tumor enzyme was about 0.6 mM. However, in the less differentiated Morris hepatoma 7288ctc, alcohol dehydrogenase activity was dependent on the concentration of ethanol, with striking increases in the rates of ethanol oxidation at higher concentrations of ethanol (Table 1). No evidence for substrate inhibition of this tumor enzyme was apparent. The \( K_m \) value for ethanol for tumor 7288ctc was about 285 mM, a value comparable to that previously found for the Becker H-252 tumor enzyme [220 mM (5)]. Ethanol oxidation by the supernatant fraction of hepatoma 7288ctc was NAD^+ dependent and sensitive to pyrazole, an inhibitor of alcohol dehydrogenase.

To support these results, alcohol dehydrogenase activity was assayed in the reverse direction, using acetaldehyde plus NADH as substrates. It was previously observed with supernatant fractions of liver that the rate of reduction of acetaldehyde did not vary as the concentration of acetaldehyde increased from 0.6 to 12 mM (5). By contrast, with the Becker H-252 hepatoma under these conditions, the rate of reduction of acetaldehyde increased from 75 to 300% (5). Using livers of rats implanted with Morris hepatoma 7288ctc, the rate of reduction of acetaldehyde also did not vary as the concentration of acetaldehyde increased 20-fold (Table 2). However, with Morris hepatoma 7288ctc, the rate of reduction of acetaldehyde depended on the acetaldehyde concentration, the activity increasing at elevated levels of acetaldehyde (Table 2). No such increase in activity was observed with Morris hepatoma 5123C.

**Alcohol Dehydrogenase Activity in Supernatant Fractions of Rat Tissues.** In previous experiments with ACI rats, the kinetic and electrophoretic data suggested that the alcohol dehydrogenases of stomach and Becker hepatoma H-252 were identical (5). Previous experiments showed that striking differences in activity between liver and tumor or stomach supernatants were obtained with m-nitrobenzaldehyde as the substrate, the rate with the tumor was 32 times greater than that with the liver (5). Therefore, both acetaldehyde and m-nitrobenzaldehyde were used as substrates in a survey of rat tissues. As shown in Table 3, unlike the stomach enzyme, the rate of acetaldehyde reduction by liver, kidney, intestine, and testes was not changed as the concentration of acetaldehyde was raised. Similarly, whereas m-nitrobenzaldehyde reductase activity in the stomach was virtually abolished by pyrazole, indicating the involvement of alcohol dehydrogenase. Similar results were obtained when ethanol and NAD^+ were used as substrates, although activity in some tissues was low and difficult to assay. Under these conditions, alcohol dehydrogenase activity was not detected in the spleen or pancreas.

**Table 1**

<table>
<thead>
<tr>
<th>Ethanol concentration (mM)</th>
<th>Activity (nmoles NADH formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
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<tr>
<td>0.5</td>
<td></td>
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<tr>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>38.4</td>
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<tr>
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<td>25.5</td>
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<td>27.4</td>
</tr>
<tr>
<td>357.0</td>
<td>31.2</td>
</tr>
<tr>
<td>510.0</td>
<td>25.4</td>
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</table>

**Table 2**

<table>
<thead>
<tr>
<th>Acetaldehyde concentration (mM)</th>
<th>Activity (nmoles NADH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>0.6</td>
<td>125.4</td>
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<tr>
<td>1.2</td>
<td>130.2</td>
</tr>
<tr>
<td>1.8</td>
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Similar results were also obtained with male Sprague-Dawley rats; only the stomach appeared to contain this kinetically unique isozyme of alcohol dehydrogenase.

### DISCUSSION

In dedifferentiation of the liver, normal liver isozymes are often replaced by other isozymes not found to any great extent in the normal adult liver (15, 16). For example, the normal liver isozymes of hexokinase, glucokinase, aldolase, pyruvate kinase, and glycogen phosphorylase are replaced by isozymes normally not present to any significant extent in rapidly growing, poorly differentiated tumors, whereas in slow-growing, highly differentiated hepatocellular carcinomas, the isozyme pattern resembles that of adult liver (for review, see Refs. 15 and 16).

We previously observed that the properties of alcohol dehydrogenase in Becker H-252 transplantable hepatocellular carcinoma, a tumor of intermediate differentiation, are conspicuously different from those of the liver enzyme (5). Similar results were obtained with Becker H-242, a tumor similar to Becker H-252. Morris hepatomas 5123C and 7288ctc are well characterized, the former being a well-differentiated tumor (3, 7, 12) whereas the latter is less differentiated (3). The data presented here demonstrate that the isozyme pattern of alcohol dehydrogenase varies with the differentiation of the tumor, and this enzyme can now be included in the group undergoing isozyme alterations during neoplasia. The activity of alcohol dehydrogenase in less differentiated tumors (Morris 7288ctc, Becker H-252) is associated with an isozyme that has different properties from the normal adult liver enzyme, whereas the properties of alcohol dehydrogenase in the more highly differentiated tumor (5123C) resemble those of adult liver. A more detailed study of the properties of alcohol dehydrogenase in Morris hepatoma 7288ctc, including gel electrophoresis, pH optima, substrate specificity patterns, kinetic analysis of pyrazole inhibition, stability to heat treatment, and possible cross-reactivity with antibodies to alcohol dehydrogenase is required to understand the factors responsible for the biochemical defect occurring at the molecular level in carcinogenesis. Such studies may also indicate whether the alcohol dehydrogenase isozymes in Becker H-252 and Morris hepatoma 7288ctc are identical. A similar study with Becker H-252 transplantable hepatocellular carcinoma revealed that the tumor enzyme differed from the liver enzyme in its migration pattern on 2 different gels, pH optima, substrate specificity pattern, stability to heat treatment, and $K_i$ for inhibition by pyrazole (5).

Alcohol dehydrogenase from the stomach has kinetic and electrophoretic properties similar to those in less differentiated tumors (2, 5). Other rat tissues (e.g., kidney, testes, and intestine) possess alcohol dehydrogenase activity with kinetic properties similar to those of the liver enzyme, rather than that of the stomach. In adults, the isozyme pattern in lung or kidney is electrophoretically similar to that of liver (13), whereas the enzyme from the retina is reported to be electrophoretically distinct (8). Since the major function of alcohol dehydrogenase in the retina may be the oxidation of...
vitamin A alcohol to the aldehyde, an isozyme different from that of the liver enzyme may have evolved.

The physiological function of stomach alcohol dehydrogenase is not known. Although endogenous ethanol was found in all sections of the alimentary tract of the rat, particularly in the stomach (9), the high \( K_m \) of stomach alcohol dehydrogenase for ethanol probably precludes a significant function of the stomach enzyme in endogenous ethanol metabolism. The stomach enzyme also shows good activity with long-chain, unsaturated, and aromatic alcohols; these may represent substrates for the enzyme. It is possible that the stomach enzyme may play a role in metabolizing exogenously administered alcohol. In rats given 10% ethanol in their drinking water, the concentration of ethanol in the stomach after 1 hr is about 0.24 M (9). At this concentration, ethanol is a good substrate for stomach alcohol dehydrogenase. Indeed, it has been reported that rat stomach slices can oxidize \([^{14}C] ethanol to \(^{14}CO_2\) (4). The availability of NAD\(^+\), mitochondrial activity, and the capacity of substrate shuttles may all represent rate-limiting factors for the oxidation of ethanol in the stomach.

The different \( K_m \) values for ethanol for isozymes present in stomach and liver explain why the reaction velocity of the stomach enzyme was reported to be twice that of the liver enzyme (11), since the assays were performed at an ethanol concentration of 533 mM, a level that leads to substrate inhibition of the liver enzyme, but not of the stomach enzyme (5.) Ethanol oxidation by stomach slices was reported to be only 20% that of liver slices, whereas alcohol dehydrogenase activity of the stomach was 3 times greater than that of the liver (4). In that study, the concentration of ethanol was 533 mM. Thus the stomach enzyme showed high activity, whereas the liver enzyme displayed substrate inhibition. However, in experiments with tissue slices, the final concentration of ethanol was 2.6 mM, a level well below the \( K_m \) of the stomach enzyme for ethanol but greater than the \( K_m \) of the liver enzyme. Hence, rates of \( CO_2 \) production from ethanol would be much lower for stomach than those for liver. The finding that different isozymes of alcohol dehydrogenase exist in normal rat tissues suggests that assaying alcohol dehydrogenase activity or alcohol metabolism at only 1 substrate concentration may be misleading.

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REFERENCES

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