Stereospecificity of the Oxidation of Ethanol by Catalase*

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SUMMARY

(R)-[1-3H]ethanol and (S)-[1-3H]ethanol were oxidized to acetaldehyde using bovine hepatic catalase. The acetaldehyde evolved from the S but not the (R)-[1-3H]ethanol contained tritium. Thus the stereospecificity of catalase for alcohol is the same as that of alcohol dehydrogenase.

The primary action of catalase, the ability to decompose hydrogen peroxide with the evolution of oxygen, was originally described by Thénoud in 1911 (1). Keilin and Hartree (2) later showed that catalase was also capable of oxidizing a wide variety of compounds including ethanol in the presence of a hydrogen peroxide-generating system. The present study was undertaken to determine the stereospecificity of this catalytic oxidation of ethanol to acetaldehyde.

EXPERIMENTAL PROCEDURE

Materials

Acetaldehyde from Eastman Kodak was stored frozen in aqueous solution at -20°. Sodium (R,S)-[2-3H]lactate (Ne/T-025) was purchased from Sigma: lactic dehydrogenase-L1625 from beef heart type III, yeast alcohol dehydrogenase-10711, glucose oxidase-N10125 type II and catalase-C100 from beef liver twice recrystallized. Nicotinamide adenine dinucleotide-15300 was obtained from Boehringer, and the 3-acetyl pyridine analog of nicotinamide adenine dinucleotide-A5251 (AP-NAD) from Sigma. Dimetol reagent was made by dissolving dimethylglyoxime in 20 ml of water in a Buchner funnel and dried at 90° for 1 hour. All counts were converted to disintegrations per min by internal standardization.

Methods

Preparation of (R)-[1-3H]Ethanol and (S)-[1-3H]Ethanol—The methods described herein are similar to those reported by Leewus et al. (3) for preparation of the deuterated derivatives. (R)-[1-3H]Ethanol was prepared by the enzymatic reduction of acetaldehyde using sodium (R,S)-[2-3H]lactate with lactate dehydrogenase and alcohol dehydrogenase. In a stopped flask 1 mg of lactic dehydrogenase, 0.65 mg of alcohol dehydrogenase, 1.56 mg of NAD, 9.65 mg of lithium (R,S)-lactate, 7 mCi of sodium (R,S)-[2-3H]lactate, and 82 mg of acetaldehyde were incubated at 37° in 9.65 ml of phosphate buffer, pH 7.4, 0.1 M. After 3 hours the reaction was terminated by the method of Somogyi (4).

1 AP-NAD was preferred to NAD because of its more favorable equilibrium.

Results of the oxidation of the tritiated ethanols are expressed as the specific activity dpm X 10^-6 per mmole of the alcohol incubated with alcohol dehydrogenase and the specific activity of the acetaldehyde produced.

<table>
<thead>
<tr>
<th>Ethanol specific activity</th>
<th>Acetaldehyde specific activity</th>
<th>Ratio of specific activity acetaldehyde to specific activity ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-[1-3H]ethanol</td>
<td>1.4</td>
<td>0.26</td>
</tr>
<tr>
<td>(S)-[1-3H]ethanol</td>
<td>1.79</td>
<td>1.81</td>
</tr>
</tbody>
</table>

(S)-[1-3H]Ethanol was prepared in two steps. In the first step, 9.6 mg of lithium (R,S)-lactate with 8 mCi of sodium (R,S)-[2-3H]lactate was oxidatively decarboxylated to [1-3H]acetaldelyde (5). This was collected in 9 ml of ice-cold water. In the second step, the aqueous [1-3H]acetate was added to 10.7 ml of phosphate buffer, pH 7.4, 0.2 M, containing 160 mg of lithium (R,S)-lactate, 0.6125 mg of NAD, 0.145 mg of alcohol dehydrogenase, and 1 mg of lactic dehydrogenase. This was incubated in a stoppered Erlenmeyer flask at 37° for 3 hours, after which the reaction was terminated (4).

Both ethanols were collected in aqueous solution by distillation. Contaminating acetaldehyde was removed by combination with semicarbazone and the ethanols were redistilled under reduced pressure at 20°. The enantiomers were contaminated by less than 0.6% of tritiated acetaldehyde. When necessary, this contamination can be reduced to much less by repeated purification with 0-carboxy-2,4-dinitrophenylhydrazine.

Proof of Structure—The putative structure was tested by oxidation to acetaldehyde using alcohol dehydrogenase with AP-NAD as coenzyme. The acetate was treated with pyrophosphate buffer, pH 9.0, 0.06 M. The acetaldehyde was distilled into dimethylamine and precipitated overnight as dimetol and acetaldehyde. (5). This was washed with 500 ml of water in a Buchner funnel and dried at 60° under reduced pressure. The precipitate was dissolved in Aquasol and counted. The specific activity of the acetaldehyde (Table I) approximated closely to that of the parent ethanol in the case of (S)-[1-3H]ethanol. In contrast, when (R)-[1-3H]ethanol was the substrate, only 1.8% of the tritium was retained in the acetaldehyde. This small fraction of the total tritium content may represent tritium in the S position and suggests the possibility that alcohol dehydrogenase does not show absolute stereospecificity. Each of the enantiomers was oxidized to acetic acid by chromic acid. The acetic acid from each contained less than 0.6% of tritiated acetaldehyde. When necessary, this contamination can be reduced to much less by repeated purification with 0-carboxy-2,4-dinitrophenylhydrazine.
TABLE II
Catalytic oxidation of ethanol

Specific activities of the ethanol and the acetaldehydes formed from them are expressed as dpm X 10⁻⁴ per mmole. Corrections have been made for the contributions of contaminating tritiated acetaldehyde (not more than 0.6% of the added dpm) in the [1-³H]ethanol preparations.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Position</th>
<th>Per cent ethanol oxidized</th>
<th>Ethanol specific activity</th>
<th>Acetaldehyde specific activity</th>
<th>Ratio of specific activity acetaldehyde to specific activity ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>95</td>
<td>1.37</td>
<td>0.028</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>90</td>
<td>1.81</td>
<td>1.09</td>
<td>0.93</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>75</td>
<td>1.20</td>
<td>0.032</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>84</td>
<td>1.31</td>
<td>1.32</td>
<td>1.01</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>77</td>
<td>1.20</td>
<td>0.030</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>75</td>
<td>1.31</td>
<td>1.28</td>
<td>0.97</td>
</tr>
</tbody>
</table>

8 mg of ethanol with 0.1 μCi of tritium, 1 mg of catalase, 0.2 mg of glucose oxidase, and 20 mg of glucose were incubated at 25°C in 12 ml of potassium phosphate buffer, pH 5.9, 0.2 M, for 15 hours. The flask was connected to a distillation apparatus and 5 ml were distilled into 8 ml of ice-cold dimetol reagent and the weighed precipitate was counted as described above. Identity of the dimetoacetaldehyde was confirmed by melting point determination (139°C). Appropriate control experiments without catalase and with added carrier acetaldehyde were performed to allow correction for contaminating [1-³H]acetaldehyde. A 0.1 ml aliquot was removed from the flask at termination, deproteinated, and assayed for ethanol (6) to determine the percentage of oxidation. In a separate experiment it was shown that the ethanol disappearance could be entirely accounted for by the accumulation of acetaldehyde assayed by the method of Gupta and Robinson (7).

RESULTS

The results of catalytic oxidation are shown in Table II. The specific activity of the resultant acetaldehyde on oxidation of the (S)-[1-³H]ethanol approximated closely to that of the ethanol. With the (R)-[1-³H]ethanol it was less than 3% of that of the ethanol. These results parallel those obtained with oxidation by alcohol dehydrogenase (Table I). To eliminate the possibility of contaminating alcohol dehydrogenase, the following experiment was performed. Catalase was added to a pyrophosphate buffer, pH 9.4, 0.075 M, containing ethanol, NAD, and semicarbazone and was incubated for 30 min at 37°C. No change in optical density at 340 nm was observed after the addition of catalase. In a control flask to which alcohol dehydrogenase was added, a change in optical density of 0.67 was observed.

DISCUSSION

The catalytic oxidation experiments provide the first information on the stereospecificity of catalase. Table II indicated that the pro-R-hydrogen atom is selectively removed during catalatic oxidation. Fisher et al. (8) showed that alcohol dehydrogenase has an identical stereospecificity. This strict stereospecificity of catalase is remarkable in view of its broad spectrum for substrates including formaldehyde and nitrite (2). The results are clearly not an artifact of alcohol dehydrogenase contamination as shown by the experiment cited with NAD at pH 9.4. Furthermore, it is inconceivable that alcohol dehydrogenase could achieve almost complete oxidation of ethanol at pH 5.9 in the absence of a trapping agent for acetaldehyde. This stereospecificity of catalase may explain at least in part the high degree of orientation of the ethanol molecule with catalase that Sizer (9) predicted was necessary on thermodynamic grounds.

Note Added in Proof—After we had submitted this paper a communication (10) came to our attention that also reports that the stereospecificity of catalase for ethanol is the same as that of alcohol dehydrogenase.

REFERENCES

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