Formaldehyde Dehydrogenase from Bakers’ Yeast*

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In the course of studies of formaldehyde metabolism in yeast (1), an enzyme was found that catalyzed the oxidation of formaldehyde in the presence of glutathione and diphosphopyridine nucleotide. A similar enzyme was discovered independently by Strittmatter and Ball in liver (2). This paper describes the partial purification and some of the properties of yeast formaldehyde dehydrogenase. Some evidence bearing on the role of glutathione in the reaction is given.

EXPERIMENTAL PROCEDURE

Analytical Procedures—Protein was determined by the biuret test (3) or the method of Warburg and Christian (4). Formaldehyde and formate were estimated according to the procedures of MacFadyen (5) and Grant (6), respectively. Free sulfhydryl groups were measured by the method of Grunert and Phillips (7). Determinations of α-amino groups were done according to the method of Moore and Stein (8). The Lipmann and Tuttle (9) procedure was used for thiol ester determinations. Formyloxamic acid was prepared by the method of Oddo and Deleo (10). The millimolar absorbancy of its ferric salt in the Beckman spectrophotometer at 540 μm was 0.75.

Enzyme Assays—A unit of enzyme is the amount necessary to oxidize 1 μmole of aldehyde per minute under the conditions of the assay. Specific activity is expressed as units per mg of protein.

The activity of the enzyme in crude extracts was measured manometrically. Warburg flasks contained, in a 3-ml volume, GSH, 6.5 μmoles; DPN, 1.2 μmoles; KHC03, 200 μmoles; and enzyme. The flasks were placed in a 30° bath and flushed with carbon dioxide gas to start the reaction. After a 2-minute period, HCHO (1 μmole) was added to start the reaction. The presence of hydroxylamine in the assay system eliminated interference from alcohol dehydrogenase (11).

The system used to study DPNH oxidation consisted of 100 μmoles of triethanolamine·Cl buffer, 0.1 μmole of DPNH, and enzyme and substrate in a 1-ml volume.

Preparations of Compounds S-Formylglutathione was synthesized by the anhydride method used by Wieland and Köppe (12) for the synthesis of S-lactylglutathione. The acylating agent was formic acid (98 to 100%). Analytical data of one preparation indicated that from 7.5 μmoles of GSH, 6.5 μmoles of thiol ester were obtained. The product contained 6.5 μmoles of free α-amino groups.

S-Acetylglutathione was obtained commercially or synthesized by the method of Wieland and Köppe (12) with glacial acetic acid as the acylating agent. S-Lactylglutathione was prepared enzymically with glyoxalase I (13). It was purified by chromatography on Dowex 1-Cl (14).

Purification of Enzyme

Step 1. Extraction—Fleischmann’s bakers’ yeast was air-dried and ground in a ball mill. The yeast powder, 300 g, was extracted with 0.066 x disodium phosphate, 900 ml, for 2 hours at 37° and then for 3 hours at room temperature. The mixture was centrifuged at 4° for 80 minutes at 3000 × g. The extract was heated for 15 minutes at 55°, cooled rapidly, and centrifuged at 4° for 10 minutes.

Step 2. Precipitation with Acetone—The enzyme solution, 410 ml, was maintained at 0° to −2° while cold acetone, 205 ml, was added. The precipitate that formed was removed by centrifugation at 4000 × g for 15 minutes at 0°. Cold acetone, 205 ml, was added to the supernatant solution, and the precipitate was removed by centrifugation, dissolved in a minimal amount of cold water, and dialyzed overnight against distilled water at 4°.

Step 3. Precipitation with Ammonium Sulfate—To the dialyzed solution from Step 2, 30 g of solid ammonium sulfate were added per 100 ml of solution. The preparation was centrifuged at 0° for 15 minutes at 12,000 × g, and the precipitate was discarded. To the supernatant fluid were added an additional 18 g of ammonium sulfate per 100 ml of solution. After centrifugation, the precipitate was dissolved in a minimal amount of cold water. This fraction could be stored for at least several months at −20° without loss of activity. At this stage, most of the alcohol dehydrogenase had been removed, but residual DPNH oxidase, thiolasease, and glyoxalase I and II activities interfered with further investigations of certain properties of the dehydrogenase.
### TABLE I

Purification of formaldehyde dehydrogenase from bakers' yeast

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Units</th>
<th>Protein</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>450</td>
<td>0.38</td>
<td>44</td>
<td>0.0086</td>
</tr>
<tr>
<td>Heated extract</td>
<td>410</td>
<td>0.34</td>
<td>26</td>
<td>0.013</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>128</td>
<td>1.36</td>
<td>24</td>
<td>0.087</td>
</tr>
<tr>
<td>Ammonium sulfate precipitate</td>
<td>14</td>
<td>0.60</td>
<td>75</td>
<td>0.088</td>
</tr>
<tr>
<td>Combined eluates from calcium</td>
<td>54</td>
<td>0.64</td>
<td>1</td>
<td>0.64</td>
</tr>
</tbody>
</table>

### TABLE II

HCHO disappearance and DPNH formation

<table>
<thead>
<tr>
<th></th>
<th>0.07</th>
<th>0.04</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔHCHO</td>
<td>-0.20</td>
<td>-0.23</td>
<td>-0.26</td>
</tr>
<tr>
<td>ΔDPNH</td>
<td>0.21</td>
<td>0.24</td>
<td>0.27</td>
</tr>
</tbody>
</table>

* Control experiments with bovine serum albumin indicated that, when the protein concentration exceeded 0.1 mg per ml, a considerable portion of HCHO was bound nonspecifically. This protein bound HCHO did not react either in the chromotropic acid test (see zero time control) or in the enzymic reaction.

### Results and Discussion

Formaldehyde was oxidized completely to formic acid by the enzyme. Glutathione was required for activity; no reaction was observed upon substitution of cysteine, 2,3-dimercaptopropanol, or thioglycolate. DPN was necessary and could not be replaced by TPN. DPNH was formed to the same extent that formaldehyde disappeared (Table II). During the course of the reaction, no decrease of sulphydryl groups or α amino groups was observed. Methyglyoxal and glyoxal, as well as formaldehyde, caused DPN to be reduced in the presence of the enzyme. Aldehydes found inactive as substrates were acetaldelyde, glycolaldehyde, glyoxalic acid, benzaldehyde, and glucosone.

The enzyme was active between pH 6 and 8.5, with an optimum at pH 8.0. The $K_m$ for DPN was $6.8 \times 10^{-4}$ M. As shown in Table III, HCHO inhibited the enzyme under some conditions. This inhibition could be overcome by the addition of adequate amounts of glutathione. These findings made it difficult to obtain meaningful $K_m$ values for the substrates and favor the idea that the actual substrate of the reaction is the thiohemiacetal of glutathione and formaldehyde. The spontaneous formation of such addition compounds is well known (16), and the thiohemiacetal of glutathione and formaldehyde was suggested as substrate for liver formaldehyde dehydrogenase (2).

If the substrate for the reaction is indeed the postulated thiohemiacetal, the oxidation product would be a thiol ester. Hydroxamate determinations did not reveal accumulation of a thiol ester intermediate. S-Formylglutathione, the proposed product of the reaction, was synthesized. In the presence of the enzyme, it caused a rapid oxidation of DPNH, but not of TPNH. At levels of 0.034 to 0.15 mM S-formylglutathione, the initial rates of DPNH oxidation were proportional to the thiol ester and enzyme concentrations. With the highest concentration of thiol ester used, the initial rate of DPNH oxidation was 2.5 times the rate of formaldehyde oxidation measured under the standard assay conditions. S-Acetylglutathione, synthetic or commercial,
and \(S\) lactylglutathione, enzymically prepared, did not result in DPNH oxidation, nor was either inhibitory.

Formaldehyde was produced during the enzymic reaction with \(S\)-formylglutathione. For example, upon oxidation of 0.062 \(\mu\) mole of DPNH, 0.052 \(\mu\) mole of formaldehyde accumulated. When a solution of the synthetic thiol ester was incubated without enzyme at pH 8.5 for 15 minutes at 37\(^\circ\), conditions that resulted in complete loss of hydroxamate color, its activity in the enzymic reaction with DPNH was also lost.

Synthetic \(S\)-formylglutathione was hydrolyzed rapidly by even the most highly purified enzyme preparations obtained; \(S\)-acetylglutathione was hydrolyzed very slowly, and \(S\)-lactylglutathione not at all. The presence of a specific glutathione thiolesterase distinct from glyoxalase II is thus indicated (17). The nonaccumulation of \(S\)-formylglutathione during the oxidation of formaldehyde may be explained by the presence of the thiolesterase. Attempts to separate the thiolesterase activity from the formaldehyde-glutathione dehydrogenase activity have not been successful to date.

**SUMMARY**

This paper concerns the purification and properties of an enzyme from bakers' yeast that catalyzes the reduction of diphosphopyridine nucleotide (DPN) in the presence of formaldehyde and glutathione. Formate was produced in equal amount with DPNH. Methylglyoxal and glyoxal, but not acetaldehyde or other aldehydes tested, were able to replace formaldehyde. Addition of \(S\)-formylglutathione, prepared chemically, resulted in the rapid oxidation of DPNH with the formation of an equivalent amount of formaldehyde. This reaction was dependent on the integrity of the thiol ester linkage. It is suggested that the substrate of the dehydrogenase reaction is formaldehyde-glutathione thiohemiacetal and that \(S\)-formylglutathione is an intermediate in the reaction.

**REFERENCES**

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