Formaldehyde Dehydrogenase from Human Liver

PURIFICATION, PROPERTIES, AND EVIDENCE FOR THE FORMATION OF GLUTATHIONE THIOL ESTERS BY THE ENZYME*

LASSE UOTILA$ AND MARTTI KOIVUSALO

From the Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10 A, SF-00170 Helsinki 17, Finland

SUMMARY

We have purified formaldehyde dehydrogenase (EC 1.2.1.1) 1390-fold from human liver. The final preparation, which has a specific activity of 3.20 i.u. per mg of protein (25°) is homogeneous according to electrophoretic criteria. S-Formylglutathione rather than formate is formed from formaldehyde and reduced glutathione in the reaction catalyzed by purified formaldehyde dehydrogenase. The enzyme is not strictly NAD-specific; NADP can also be used although NADP has a much higher $K_v$ value than NAD, especially at high pH values. S-Formylglutathione is reduced by the enzyme to formaldehyde with either NADH or NADPH as cofactors. Methylglyoxal and some other ketoaldehydes also can be used as the substrates of formaldehyde dehydrogenase. The product obtained from methylglyoxal is probably S-pyruvylglutathione. S-Formylglutathione is hydrolyzed very actively in crude human liver preparations. All of this activity is removed during the purification of formaldehyde dehydrogenase.

Formaldehyde dehydrogenase has an apparent molecular weight of 81,400 according to gel filtration and a subunit molecular weight of 39,500 according to dodecyl sulfate gel electrophoresis. Isoelectric focusing experiments gave an isoelectric point, pI of 6.35.

The enzyme is very sensitive to mercaptide-forming reagents. NAD and NADH protect the enzyme. NAD and especially NADH stabilize the enzyme during storage and against denaturation at high temperatures.

Formaldehyde is known to be oxidized by a specific NAD-linked formaldehyde dehydrogenase (EC 1.2.1.1) in beef and chicken livers (1), human and animal retinas (2), yeast (3−5), human, monkey, and rat livers (6), and Pseudomonas methanica (7). The enzyme from all of these sources needs GSH as a specific cofactor and is inactive with acetaldehyde and several other aldehydes. Thus far, no highly purified preparation of this enzyme has been available. Also, the role of GSH has remained unclear and the physiological significance of the enzyme is unsettled.

In the present work, formaldehyde dehydrogenase has been obtained in an electrophoretically homogeneous form from human liver. It is shown that in the formaldehyde dehydrogenase reaction, S-formylglutathione is formed from formaldehyde and GSH, instead of formate. This reaction is freely reversible. The hydrolysis of S-formylglutathione is catalyzed very actively in the human liver. This activity, mostly caused by a specific thiol esterase, is totally removed by our purification procedure. The purification and properties of this thiol esterase are described in the following report (8).

EXPERIMENTAL PROCEDURE

Materials

NAD(H) and NADP(H) were purchased from Boehringer, Mannheim; GSH, GSSG, sulphydryl reagents, and S-methylglutathione, from Sigma. 2,4,6-Trinitrobenzene sulfonate, o-phenanthroline, α,α'-dipyridyl, S-hydroxyquinoline, methylglyoxal, glyoxal, and phenylglyoxal were obtained from Fluka, Switzerland. Methylglyoxal was purified by steam distillation. Kethoxal (α-ethoxy-α-ketobutyraldehyde) was obtained from Nutritional Biochemicals. Hydroxypyruvaldehyde was prepared according to Reeves and Ajl (9) and S-hexylglutathione, by the method of Vines and Wadd (10). Formaldehyde was prepared by hydrolyzing hexamethylenetetramine with $H_2SO_4$ and standardized after steam distillation by the chromotropic acid method (11). DE22 and DE32 ion exchange celluloses were obtained from Whatman; Sephadex G-100 and quaternary aminoethyl (QAE)-Sephadex A-50, from Pharmacia, and hydroxylapatite (Hypatite C), from Clarkson. The preparation of S-pyruvylglutathione is described in the accompanying paper (8). The other thiol esters of glutathione were prepared according to the method of Uotila (12).

Methods

Preparation of Subcellular Fractions

A sample of one human liver obtained 4 hours after death was fractionated according to Schneider and Hogeboom (13). Formaldehyde dehydrogenase activity of the fractions was determined, after the removal of GSH by dialysis, as described below for crude preparations. Deoxycholate (0.2%, w/v) was added to the mitochondrial and microsomal fractions for solubilization and was included also in the dialysis buffer. Deoxycholate had no effect on the enzyme activity.
on purified formaldehyde dehydrogenase in the concentration used.

**Protein**

Bioiuet method (14) was used in the initial stage. The solutions were cleared for the measurement by sodium deoxycholate (0.2% (w/v) in the final volume). Later, the method of Lowry et al. (15) or Waddell (16) was used. These methods gave results which agreed well. Bovine serum albumin (Armour) was used as a standard.

**Molecular Weight by Gel Filtration**

A calibrated Sephadex G-100 column (2.5 X 70 cm) was used. The procedures followed those introduced by Andrews (17). The void volume of the column was determined with blue dextran (mol wt = 2 X 10^6). Standard proteins (Boehringer) were: cytochrome c (12,400), chymotrypsinogen (25,000), bovine serum albumin (67,000), aldolase (150,000), and catalase (240,000). The column was equilibrated and eluted with 10 mM Tris-HCl buffer, pH 7.6, containing 150 mM KCl. Catalase was located by measuring the disappearance of H2O2 at 240 nm, caused by the enzyme, cytochrome c from absorbance at 412 nm, and the other standards from absorbance at 220 nm.

**Subunit Molecular Weight**

Dodecyl sulfate gel electrophoresis according to Weber and Osborn (18) was used.

**Dissociation Constant of Adduct**

For the measurement of the rate of the reverse reaction, the assay mixture contained 90 mM sodium pyrophosphate buffer, pH 8.0, 1 mM GSH, 1.2 mM formaldehyde, enzyme, and 0.67 mM NAD. Formation of NADH was followed at 340 nm with a Gilford apparatus (model 220) attached to a Beckman model DU spectrophotometer thermostated at 25°. No blank was needed with the formaldehyde for purified preparations, but when ketoaldehydes were tested, a blank without enzyme was always included. In some experiments, NADP (2 mM) replaced NAD. When preparations from Steps 1 and 2 were studied, a blank without GSH was included and subtracted from the total rate. This blank was interpreted to measure the unspecific aldehyde dehydrogenase activity of the sample with formaldehyde, and the additional activity obtained with GSH was calculated as formaldehyde dehydrogenase. The activity with and without GSH was not changed if 2 mM mercaptoethanol was also included. Therefore, the activation of unspecific aldehyde dehydrogenase(s) by GSH probably did not occur under these conditions. The assay mixtures for the early steps also contained 3 mM pyrazole to block alcohol dehydrogenase activity.

For the measurement of the rate of the reverse reaction, the assay mixture contained 90 mM potassium acetate buffer, pH 5.7, 1.3 mM S-formylglutathione, and 0.1 mM NADH or 0.2 mM NADPH. Blanks without enzyme and without substrate were always used.

The hydrolysis of S-formylglutathione was measured at 240 nm as described in the accompanying paper (8). The activities were calculated as international units (micromoles per min) by using cM = 6220 cm⁻¹ for NADH at 340 nm and aM = 3800 cm⁻¹ for S-formylglutathione at 240 nm (12).

**Disc Electrophoresis**

The methods followed those of Davis (19), except for the omission of the sample gel. Electrophoreses were run at two pH values, 8.9 and 7.5 according to Maurer (20). Part of the gels were stained with 1% Amido black and destained with 7% acetic acid. The rest of the gels were sliced into about 40 pieces. The usual assay mixture for the forward reaction of formaldehyde dehydrogenase (1 ml) was then added to the pieces and the tubes were incubated, after mixing, for a suitable interval (20 to 60 min) at 25°. The activity was located by measuring A₄₅₀ from the supernatants. The assay mixture was then removed and the pieces were stained for protein as above.

**Determination of Formate**

The formaldehyde dehydrogenase synthetase method of Rabinowitz (21) was used with modifications which are described in the following report (8). The enzyme (from Clostridium acidi-urici, 115 i.u. per mg) was a generous gift from Professor Jesse C. Rabinowitz, University of California, Berkeley.

**Determination of Dissociation Constant of the Adduct**

It has been supposed that a hemimercaptal spontaneously formed from formaldehyde and GSH is the true substrate of formaldehyde dehydrogenase (1, 3, 4). The dissociation constant of this compound was therefore determined by the method of Kermack and Matheson (22). Six equimolar concentrations of GSH and formaldehyde were used (between 0.8 and 4 mM), and the resulting absorbance decreases owing to hemimercaptal formation compared in pairs. The dissociation constant thus obtained is 1.5 ± 0.3 mM at pH 8.0. In these conditions, the molar absorbances at 240 nm of GSH, formaldehyde, and the adduct were 578, 0.68, and about 10 cm⁻¹, respectively.

**Purification of Formaldehyde Dehydrogenase from Human Liver**

The livers of persons killed in accidents were obtained 4 to 24 hours after death. The activity in livers obtained during this period was fairly constant. Freshly prepared tissue and tissue stored for several months at -20° gave similar results. All of the steps were performed at 0-4°.

**Step 1. Preparation of Initial Extract**—The liver tissue was homogenized with 3 volumes of 50 mM potassium phosphate buffer, pH 7.4, containing 5 mM mercaptoethanol in a Waring Blender and centrifuged at 25,000 X g in a Sorvall RC-2B refrigerated centrifuge for 60 min. The precipitate was discarded.

**Step 2. Ammonium Sulfate Fractionation**—The protein fraction precipitated between 45 and 67% of saturation with ammonium sulfate (0°) was taken. The pH was maintained at 7.4 with 2 M NH₄. The precipitates were collected by centrifuging as above and dissolved in a minimal amount of 10 mM Tris-HCl buffer, pH 7.4, containing 5 mM mercaptoethanol.

**Step 3. DEAE Cellulose Chromatography**—The enzyme solution was dialyzed overnight against 10 mM Tris-HCl, pH 7.6, containing 5 mM mercaptoethanol with at least two changes of buffer. After centrifugation, the enzyme was applied to a DEAE-cellulose column (DE22, 5.0 X 85 cm) and the column was washed with 1000 ml of equilibration buffer (10 mM Tris-HCl, pH 7.6, + 5 mM mercaptoethanol). Then the column was eluted with a linear gradient (total volume, 4000 ml) from the initial buffer to 40 mM Tris-HCl, pH 7.0, containing 5 mM mercaptoethanol. A second gradient was applied from the latter buffer to the same buffer also containing 20 mM KCl (total volume, 2000 ml). In addition to the formaldehyde dehydrogenase activity, the fractions were analyzed for the hydrolysis of S-formylglutathione and S-laeylglutathione. The enzyme eluted from formaldehyde dehydrogenase most of the activity toward S-formylglutathione, whereas the activity with S-laeylglutathione, which represents glyceraldehyde II in human liver (24), was eluted together with formaldehyde dehydrogenase (Fig. 1).
FIG. 1. Purification of formaldehyde dehydrogenase on a DE22 cellulose column (Step 3). Formaldehyde dehydrogenase activity (●, multiplied by 200) and thiol esterase activities with S-lactylglutathione (△) and S-formylglutathione (○) as substrates are presented. Alcohol dehydrogenase activity was not bound in the column under the conditions used (see text), and was eluted between Fractions 20 and 50 (not illustrated). Unspecific aldehyde dehydrogenase activity was bound more strongly than the proteins shown in the figure. For details see "Experimental Procedure."

Fig. 2. Purification of formaldehyde dehydrogenase on a DE32 cellulose column (Step 4). Glyoxalase II (△, divided by 3, measured by S-lactylglutathione) and formaldehyde dehydrogenase (●) activities are presented. For details see "Experimental Procedure."

toethanol. The enzyme was then applied to a DEAE-cellulose column (DE32, 3.0 x 35 cm). A linear gradient (total volume, 3000 ml) from the equilibration buffer (10 mM Tris-HCl, pH 7.6, + 2 mM mercaptoethanol) to 40 mM Tris-HCl, pH 7.0, + 2 mM mercaptoethanol was used as an eluant. In these conditions, most of the glyoxalase II activity was not bound to the column (Fig. 2). The pooled formaldehyde dehydrogenase preparation was concentrated in an Amicon ultrafiltration cell with a PM-10 membrane, and then dialyzed against 10 mM Tris-HCl, pH 7.6.

Step 6. Preparative Isoelectric Focusing—The formaldehyde dehydrogenase preparation was applied to an isoelectric focusing column (LKB, Stockholm, model 8101, volume, 110 ml) in the light gradient solution where it replaced a comparable amount of water. Both gradient solutions contained 1 mM DTT. The procedures followed those described by Vesterberg (25). Focusing was continued for 50 hours at 4°C. Initial voltage was 400 volts and the final voltage, 700 volts. When the column was emptied, the effluent was divided into fractions of about 2 ml. These were assayed for activity, protein, and pH (Fig. 3). The five most active fractions were pooled.

Step 6. Sephadex G-100 Gel Chromatography—The preparation was concentrated to 4 ml and then passed through a Sephadex G-100 gel column (2.5 X 45 cm), equilibrated, and eluted with 10 mM potassium phosphate buffer, pH 6.8, containing 2 mM mercaptoethanol. The enzyme was eluted in the mid-part of the protein peak. Fractions (2 ml) containing at least one-half of the activity of the maximal fraction were taken.

Step 7. Hydroxyapatite Chromatography—The pooled preparation was applied to a hydroxyapatite column (1.5 X 30 cm) equilibrated with 10 mM potassium phosphate, pH 6.8, containing 2 mM mercaptoethanol. The column was eluted with a linear 700-ml gradient from the initial buffer to 140 mM potassium phosphate, pH 6.8, and then washed with 500 mM potassium phosphate. Formaldehyde dehydrogenase was eluted at about 90 mM phosphate (Fig. 4).

Step 8. QAE-Sephadex Chromatography—The pooled formaldehyde dehydrogenase preparation was dialyzed against 10 mM Tris-HCl buffer, pH 7.6, containing 2 mM mercaptoethanol (enzyme was dialyzed for 9 hours; new dialysis buffer was changed every 3 hours) after a preceding 10-fold concentration. Then, the enzyme was applied to a QAE-Sephadex A-50 column (1.0 X 26 cm) equilibrated with the buffer used for dialysis. After sample application, the column was eluted with a linear 400-ml gradient from the equilibration buffer to the same buffer also containing

*The abbreviations used are: DTT, dithiothreitol; PMB, p-hydroxymercuribenzoate; Nbsn, 5,5'-dithiobis(2-nitrobenzoate); MalNEt, N-ethylmaleimide.

Fig. 3. Preparative isoelectric focusing of formaldehyde dehydrogenase (△) on a hydroxyapatite column (Step 5). (○), pH gradient. For details see "Experimental Procedure."

Fig. 4. Purification of formaldehyde dehydrogenase on a hydroxyapatite column (Step 7). For details see "Experimental Procedure."

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**Table I**

Purification of formaldehyde dehydrogenase from human liver (560 g)

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Volume</th>
<th>Total activity</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Purification factor</th>
<th>Yield</th>
<th>Hydrolysis of formyl-SC/dehydrogenase act.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Supernatant of homogenate</td>
<td>1570</td>
<td>167</td>
<td>74100</td>
<td>0.0023</td>
<td>1.0</td>
<td>100</td>
<td>770</td>
</tr>
<tr>
<td>(2) Ammonium sulfate (45 - 57%)</td>
<td>232</td>
<td>123</td>
<td>26200</td>
<td>0.0047</td>
<td>2.0</td>
<td>73.5</td>
<td>755</td>
</tr>
<tr>
<td>(3) DE 22</td>
<td>360</td>
<td>43.5</td>
<td>1230</td>
<td>0.035</td>
<td>15.2</td>
<td>26.0</td>
<td>13.0</td>
</tr>
<tr>
<td>(4) DE 32</td>
<td>160</td>
<td>27.6</td>
<td>176</td>
<td>0.157</td>
<td>68.2</td>
<td>16.5</td>
<td>0.7</td>
</tr>
<tr>
<td>(5) Isoelectric focusing</td>
<td>10.3</td>
<td>26.6</td>
<td>n.d. b</td>
<td>n.d. b</td>
<td>n.d. b</td>
<td>n.d. b</td>
<td>n.d. b</td>
</tr>
<tr>
<td>(6) Sephadex G-100</td>
<td>21.4</td>
<td>8.82</td>
<td>5.50</td>
<td>1.60</td>
<td>697</td>
<td>5.3</td>
<td>0</td>
</tr>
<tr>
<td>(7) Hydroxylapatite</td>
<td>143</td>
<td>4.95</td>
<td>1.80</td>
<td>2.75</td>
<td>1190</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td>(8) QAE-Sephadex A 50</td>
<td>70</td>
<td>1.90</td>
<td>0.660 (L)c</td>
<td>3.17 (L)c</td>
<td>0.585 (W)c</td>
<td>3.25</td>
<td>0.600 (L)c = 0.585 (W)c</td>
</tr>
</tbody>
</table>

- The ratio between the hydrolytic rate of S-formylglutathione and the formaldehyde dehydrogenase activity (forward rate) of the preparations in conditions described under “Methods.”
- n.d. = not determined.
- *L* = protein measurement according to Lowry et al. (15), *W* = according to Waddell (16).

60 mM KCl. The fractions were analyzed rapidly and those fractions containing formaldehyde dehydrogenase were pooled.

**RESULTS**

Comments on Purification—Table I summarizes the isolation of formaldehyde dehydrogenase from human liver. About 1400-fold purification was obtained. In addition to the formaldehyde dehydrogenase activity, the pooled preparations were assayed for the hydrolysis of S-formylglutathione. It is seen from Table I that in the crude solutions the rate of hydrolysis of S-formylglutathione was 770 times the rate of formaldehyde dehydrogenase. Most of the hydrolysis activity was removed in Step 3. This activity was caused by a specific S-formylglutathione hydrolase (8). Glyoxalase II, however, is also active with S-formylglutathione (24), and its activity is still in excess of formaldehyde dehydrogenase. The next step (DE32) decreased the hydrolytic activity below the dehydrogenase activity (Table I), and the succeeding isoelectric focusing finally removed all of the hydrolysis activity. Thus, formaldehyde dehydrogenase itself does not catalyze the hydrolysis of S-formylglutathione. Alcohol and aldehyde dehydrogenase activities which interfered in the assay of formaldehyde dehydrogenase in crude preparations (see “Methods”) were removed completely in the first column chromatography on DE22 cellulose (Step 3).

**Disc Electrophoresis**—Electrophoreses run at both pH 8.9 and pH 7.5 showed that only one protein band was present in the final preparation from Step 8 (Fig. 5, a and b) and activity determinations from sliced gels showed that this protein band contained all of the formaldehyde dehydrogenase activity.

**Dodecyl Sulfate Gel Electrophoresis**—After treatment with mercaptoethanol and sodium dodecyl sulfate, formaldehyde dehydrogenase moved slightly faster than the alcohol dehydrogenase. The subunit molecular weight calculated from the linear plot of eight standards (Fig. 6) was 39,500 ± 900 (n = 3). The purest formaldehyde dehydrogenase preparation contained no impurities visible in sodium dodecyl sulfate electrophoresis (Fig. 5c).

**Molecular Weight**—The elution volume of formaldehyde dehydrogenase in a calibrated Sephadex G-100 column corresponded to an apparent molecular weight of 81,400 ± 2,800 (n = 5). Preparations from Steps 1 to 5 were tested for molecular weight. No change occurred during purification. Goodman and Tephly (6) have reported that in crude preparations of human liver alcohol, aldehyde and formaldehyde dehydrogenases were eluted together in a Sephadex G-200, all having apparent molecular weights of over 250,000. We could not repeat this result.
which does not contain this enzyme. Somes, and 1.9% in the nuclear fraction. Formaldehyde dehydrogenase is thus a cytosolic enzyme. S-Formylglutathione hydrolase, studied from the same liver, gave a similar distribution.

Distribution of Formaldehyde Dehydrogenase—Besides human liver, we have found and partially purified (through Steps 1 to 4) formaldehyde dehydrogenase from the following sources: human brain; rat brain, kidney, heart muscle, and skeletal muscle; rabbit brain; bovine brain and adrenal gland; sheep liver; and mouse ascites tumor cells. No tissue has been found. Both the Sephadex G-100 and G-200, alcohol and formaldehyde dehydrogenases had practically identical elution volumes corresponding to a molecular weight of about 80,000, and only unspecific aldehyde dehydrogenase had a molecular weight of about 200,000. Lowering the concentration of the elution buffer to 10 mM did not change the result.

Comparison of the results of gel filtration and sodium dodecyl sulfate electrophoresis suggests that formaldehyde dehydrogenase consists of 2 identical subunits with a molecular weight of about 40,000.

Isoelectric Point—In addition to preparative isoelectric focusing experiments several analytical focusings were done for the preparations from Steps 3 and 4. A single peak was always obtained. The enzyme is isoelectric at pH 6.35 ± 0.15 (n = 4).

Intracellular Distribution—Of the formaldehyde dehydrogenase activity of liver homogenate, 92% was found in a 105,000 X g supernatant, 1.5% in the mitochondria, 2.9% in the microsomes, and 1.9% in the nuclear fraction. Formaldehyde dehydrogenase is thus a cytosolic enzyme. S-Formylglutathione hydrolase, studied from the same liver, gave a similar distribution.

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Substrate and Cofactor Specificity—Mammalian formaldehyde dehydrogenase has been considered specific for formaldehyde (1, 6). The enzyme purified by us also used some ketoaldehydes as substrates. The rate obtained with methylglyoxal was not much lower than the rate obtained with formaldehyde (Table II). Hydroxypyruvlate, ketoaldehy, and glyoxal reacted more slowly, whereas phenylglyoxal was not used at all. The reactions with ketoaldehydes were similarly NAD- and GSH-dependent, but was the reaction with formaldehyde. Acetalddehyde, propionaldehyde, glycolaldehyde, n- and t-glyceraldehyde, benzaldehyde, and phenylacetaldehyde were all inactive.

It was not possible to replace GSH by mercaptoethanol, TTYT, coenzyme A, cysteine, or homocysteine.

In contrast to the previous results on formaldehyde dehydrogenase (1-7), the enzyme purified by us is not strictly NAD-specific. Under specified conditions, the enzyme also used NADP. The pH curves obtained with a fixed concentration of NAD and NADP were markedly different (Fig. 7). At pH 8.0, which was the optimum with NAD in accordance with the results of Goodman and Tephly (6), NADP was a poor cofactor. The latter was optimal at pH 6.0. At a low pH, NADP had a higher maximum velocity than did NAD. Thus, the activity with NADP is not explained as the presence of some NAD in the commercial NADP sample used by us, or as the conversion of NADP to NAD by some factor present in the enzyme sample. GSH was needed specifically in the reaction obtained with NADP, as in the case of NAD.

Reversibility of Reaction—Purified formaldehyde dehydrogenase preparations catalyzed (1) the oxidation of NADH and NADPH in the presence of S-formylglutathione. The pH curves for this reverse reaction differed greatly from those of the forward reaction (Fig. 7). For studies of the reverse reaction only preparations from Steps 6 to 8, totally free from NADH oxidase, NADPH oxidase, and glutathione reductase activities, were used. Glutathione reductase easily causes interference because of the presence of some GSSG in the S-formylglutathione preparations. The reaction rate obtained with NADH was about the same at pH 8 as the forward rate with formaldehyde + NAD. The rate of the reverse reaction was increased at the lower pH values and at pH 5.7, optimal for the reverse reaction, the rate was 3.9 times that of the optimal forward rate with formaldehyde (Fig. 7). With NADPH, no reaction at all was seen at pH 8. However, NADPH was used below pH 7 (Fig. 7). The oxidation of NADPH was also optimal around pH 5.7, and then twice as rapid as the forward rate with formaldehyde + NAD at pH 8.0. The form of the pH curves for

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>S0.5</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>0.009</td>
<td>100</td>
</tr>
<tr>
<td>Methylglyoxal</td>
<td>0.080</td>
<td>85</td>
</tr>
<tr>
<td>Glyoxal</td>
<td>0.056</td>
<td>10</td>
</tr>
<tr>
<td>Hydroxypyruvlate</td>
<td>0.03</td>
<td>61</td>
</tr>
<tr>
<td>Kethoxal (β-ethoxy-α-ketobutyraldehyde)</td>
<td>1.43</td>
<td>37</td>
</tr>
</tbody>
</table>

* Values expressed indicate the half-saturation concentrations of total aldehyde. Thus, the real substrate form, either hemimeric with GSH, or the free aldehyde, has not been taken into account.

In contrast to the previous results on formaldehyde dehydrogenase, the enzyme purified by us is not strictly NAD-specific. Under specified conditions, the enzyme also used NADP. The pH curves obtained with a fixed concentration of NAD and NADP were markedly different (Fig. 7). At pH 8.0, which was the optimum with NAD in accordance with the results of Goodman and Tephly (6), NADP was a poor cofactor. The latter was optimal at pH 6.0. At a low pH, NADP had a higher maximum velocity than did NAD. Thus, the activity with NADP is not explained as the presence of some NAD in the commercial NADP sample used by us, or as the conversion of NADP to NAD by some factor present in the enzyme sample. GSH was needed specifically in the reaction obtained with NADP, as in the case of NAD.

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the reverse reactions was influenced by the denaturation of formaldehyde dehydrogenase around pH 5. In the absence of a pyridine nucleotide, the purified enzyme was unstable at pH values as high as 5.7. In contrast, formaldehyde dehydrogenase was well preserved in alkaline buffers up to pH 11.

Fig. 7. Dependence of the activity of formaldehyde dehydrogenase (Step 7) on pH. The following activities are illustrated: forward reaction with formaldehyde, GSH and NAD (Δ), and with formaldehyde, GSH, and NADP (■); reverse reaction with S-formylglutathione and NADH (○), and with S-formylglutathione and NADPH (□). Substrate concentrations were as described under “Experimental Procedure.”

<table>
<thead>
<tr>
<th>Experiment and electron donor</th>
<th>Pyridine nucleotide oxidized (nmoles)</th>
<th>Formaldehyde formed (nmoles)</th>
<th>Recovery of formaldehyde (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. NADH</td>
<td>107</td>
<td>120</td>
<td>112</td>
</tr>
<tr>
<td>II. NADH</td>
<td>98</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td>III. NADPH</td>
<td>69</td>
<td>80</td>
<td>116</td>
</tr>
<tr>
<td>IV. NADPH</td>
<td>78</td>
<td>72</td>
<td>92</td>
</tr>
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</table>

No reverse reaction could be shown by substituting formate for S-formylglutathione. Also, pretreatment of S-formylglutathione with a highly active preparation of S-formylglutathione hydrolyase (8) caused the disappearance of all of the activity. The stoichiometry of the reverse reaction was studied by determining the amount of formaldehyde formed by the chromotropic acid method (11). The amount of formaldehyde was in full agreement with the amount of oxidized pyridine nucleotide with either NADH or NADPH as the electron donor (Table III).

S-Pyruvylglutathione was reduced also by formaldehyde dehydrogenase in the presence of either NADH or NADPH. The rate with 1 mM S-pyruvylglutathione and 0.1 mM NADH in acetate buffer (pH 5.7) was about the same as the rate of the forward reaction with formaldehyde and NAD at optimal pH (8.0). However, \( K_m \) for S-pyruvylglutathione appeared to be high (4.0 mM) and the maximum velocity from a Lineweaver-Burk plot (26) approached the maximum velocity with S-formylglutathione. In contrast, all of the following thiol esters were inactive at 1 mM concentration: S-acetylglutathione, S-propionylglutathione, S-glycolylglutathione, S-glyceroylglutathione, S-mandelylglutathione, S-succinylglutathione, and S-acetoacetylglutathione. None of them at 1 mM concentration inhibited the rate obtained in the standard assay with S-formylglutathione.

Michaelis Constants for Substrates—It is not known whether formaldehyde dehydrogenase is a three-substrate enzyme (GSH, aldehyde, and NAD) or whether there are only two substrates, NAD, and the hemimercaptal which both formaldehyde (27) and ketoaldehydes (28) form nonenzymically with GSH.

Half-saturation concentrations and the maximum velocities of the aldehydes active in the formaldehyde dehydrogenase reaction, obtained by using constant GSH and NAD, are collected in Table II. Linear Lineweaver-Burk plots were obtained only for formaldehyde and methylglyoxal, whereas with the other ketoaldehydes, this plot was curved. However, instead of using total aldehyde concentrations, either the concentration of free aldehyde or the concentration of aldehyde-GSH-hemimercaptal should be used, depending on which one of these is the real substrate of the enzyme. When the true concentrations of free and bound aldehyde were calculated using \( K_{diss} = 1.5 \text{ mM at pH 8} \) for formaldehyde and GSH (see “Experimental Procedure”) and \( K_{diss} = 3.0 \text{ mM for methylglyoxal and GSH (29), the following apparent Michaelis constants were obtained: } K_m = 6.5 \text{ PM for S-hydroxymethylglutathione and } 4.7 \mu M \text{ for free formaldehyde and } K_m = 30 \mu M \text{ for methylglyoxal-GSH-hemimercaptal or 36 } \mu M \text{ for free methylglyoxal. At the used concentrations (GSH = 2 mM, aldehyde = 5 to 120 } \mu M \text{ the ratio of free and bound aldehyde was fairly constant when calculated from the dissociation constants reported. Therefore, the Lineweaver-Burk plots were always linear with these two aldehydes when either hemimercaptal, free aldehyde, or total aldehyde was plotted as the substrate.}

The unlinearity of the Lineweaver-Burk plots with the other ketoaldehydes may, among other possible explanations, be due to the inability to calculate the true substrate concentrations because the dissociation constants of the hemimercaptals of these aldehydes with GSH have not been determined.

NAD had a low apparent \( K_m \) value at pH 8 and the value at pH 5.7 was slightly higher (Table IV). NADP had a 1000 times higher \( K_m \) than did NAD at pH 8, but only 40 times higher at pH 5.7 (Table IV). The maximum velocity with NADP was higher than that with NAD at pH 5.7.

NADH had a very low apparent \( K_m \) in the reverse reaction especially at low pH. NADPH had a 10-fold higher \( K_m \) than NADH at pH 5.7. No reaction was detected with NADPH at pH 8, but the concentrations of NADPH over 0.3 mM were not tested.
The concentration of substrates which were kept constant are given in parentheses. Buffer concentration was 90 mM.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Substrate</th>
<th>$K_m$</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>GSH (2 mM HCHO, 1.33 mM NAD)</td>
<td>8.1</td>
<td>100</td>
</tr>
<tr>
<td>pyro-</td>
<td>NAD (1.2 mM HCHO, 2 mM GSH)</td>
<td>9.0</td>
<td>100</td>
</tr>
<tr>
<td>phos-</td>
<td>NADP (1.2 mM HCHO, 2 mM GSH)</td>
<td>9500</td>
<td>40</td>
</tr>
<tr>
<td>(pH 8.0)</td>
<td>NADH (1.3 mM formyl-SG)</td>
<td>16.0</td>
<td>105</td>
</tr>
<tr>
<td>Potassium</td>
<td>Formyl-SG (0.1 mM NADH)</td>
<td>300</td>
<td>440</td>
</tr>
<tr>
<td>phos-</td>
<td>Formyl-SG (0.02 mM NADPH)</td>
<td>280</td>
<td>230</td>
</tr>
<tr>
<td>(pH 5.7)</td>
<td>Pyruvyl-SG (0.1 mM NADH)</td>
<td>4000</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>NADH (1.3 mM formyl-SG)</td>
<td>4.2</td>
<td>440</td>
</tr>
<tr>
<td></td>
<td>NADPH (1.3 mM formyl-SG)</td>
<td>45.0</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>NAD (1.2 mM HCHO, 2 mM GSH)</td>
<td>13.6</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>NADP (1.2 mM HCHO, 2 mM GSH)</td>
<td>570</td>
<td>33</td>
</tr>
</tbody>
</table>

$^a$ 100% value has been given to the system where the enzyme is saturated with respect to formaldehyde, GSH, and NAD at pH 8.0.

Abbreviations used are: formyl-SG, S-formylglutathione and pyruvyl-SG, S-pyruvylglutathione.

The apparent $K_m$ value for S-formylglutathione was about 0.3 mM with either NADH or NADPH as the second substrate and, for S-pyruvylglutathione with NADH, the $K_m$ was 4.0 mM. Impurities, especially GSH, present in the thiol esters may make these $K_m$ values too high. The pyridine nucleotides and thiol esters all gave linear Lineweaver-Burk kinetics.

Product of Formaldehyde Dehydrogenase Reaction—Formaldehyde dehydrogenase partially purified from other sources (1-7) oxidized formaldehyde to formate. Because our purified preparations catalyzed the formation of formaldehyde from S-formylglutathione but not from formate, and had no hydrolytic activity towards synthetic S-formylglutathione, S-formylglutathione was the probable reaction product. Product identification was attempted with enzyme preparations which were free from all of the hydrolase activity. The enzyme used in these experiments was purified to Step 4 (see above) and was then, after concentration, filtered through a Sephadex G-100 gel. Mercaptoethanol was omitted in gel filtration to ensure the inactivation of traces of S-formylglutathione hydrolase and also because mercaptoethanol increases the rate of nonenzymic decomposition of S-formylglutathione (8).

The possible formation of a thiol ester was tested by measuring the absorbance increase at 240 nm during the formaldehyde dehydrogenase reaction. Measurements at 240 nm are complicated by the high absorbance given by NAD and especially by the difference in the absorbances between NAD and NADH (30), which is able to quench most of the absorption increase expected from the formation of a thiol ester bond. Therefore, our reaction mixture in these experiments contained 0.1 mM buffer (various pH values), formaldehyde or methylglyoxal, GSH, NAD, sodium pyruvate, lactic dehydrogenase, and purified formaldehyde dehydrogenase. Measurements at 240 nm showed that the lactic dehydrogenase added to the system containing the other components was able instantaneously to transform all of the NADH back to NAD. Thus, a cycle was formed in which aldehyde, GSH, and pyruvate were converted to the possible thiol ester and lactate whereas the other components were catalytic.

It is seen from Fig. 8 that in the described conditions, a product absorbing at 240 nm was formed and no absorption increase was obtained in the absence of GSH. Other blank experiments showed that formaldehyde, formaldehyde dehydrogenase, pyruvate, and lactic dehydrogenase were also necessary for any significant absorption increase to occur at 240 nm. Slight absorption increase without NAD was obtained probably due to the presence of some NAD(N) in the lactic dehydrogenase preparation used. Because NAD was catalytic, only a very small amount of it was needed. When the absorption increase at 240 nm was almost stopped, a small amount of the purified S-formylglutathione hydrolase (8) was added to the cuvettes. The product obtained from formaldehyde was rapidly decomposed, as is seen from the almost instantaneous decrease of the absorbance to the original level (Fig. 8). The product obtained from methylglyoxal was also hydrolyzed by S-formylglutathione hydrolase but much more slowly. Glyoxalase II partially purified from human liver (to Step 4 according to Ref. 24) also catalyzed the hydrolysis of both the product obtained from formaldehyde and that obtained from methylglyoxal, and the hydrolysis velocities of these two products were about the same. It is further seen from Fig. 8 that if S-formylglutathione hydrolase was added to the usual assay mixture of these experiments before formaldehyde dehydrogenase, no absorption increase at all was obtained. This corresponds to the result obtained for crude human liver formaldehyde dehydrogenase preparations (containing both hydrolase and formaldehyde dehydrogenase activity).

The results presented in Fig. 9 show that the formation of a thiol ester, as calculated from the initial velocities at three differ-
ent pH values, was compatible with the pH curve obtained for the forward reaction of formaldehyde dehydrogenase by the usual assay method, i.e. by recording the formation of NADH at 340 nm (Fig. 7). However, the reaction was non-linear at high pH values, and at pH 8, the absorbance soon decreased back to the original level, whereas at pH 5.7 the absorbance after 2 hours was still slowly increasing (Fig. 9). Our experience of the stability of a chemically prepared S-formylglutathione (2) is in accord with the curves of Fig. 9.

The results presented in Figs. 8 and 9 do not necessarily show that a thiol ester of glutathione is formed, because a formyl thiol ester of the enzyme might also cause absorbance at 240 nm. This possibility was tested by filtering the low molecular weight components of the mixture through a PM-10 membrane (Amicon ultrafiltration cell, at 4°C). The absorbance of the ultrafiltrate at 240 nm was decreased on treatment with S-formylglutathione hydrolase by exactly the same amount as was the total absorption increase by formaldehyde dehydrogenase and the decrease caused by the hydrolase without ultrafiltration. Thus, no part of the absorbance was caused by the thiol ester of the enzyme.

The extremely rapid hydrolysis of the product of formaldehyde dehydrogenase by the highly specific S-formylglutathione hydrolase (8) suggests that the product is S-formylglutathione. An attempt was made to obtain further evidence on the nature of the product from paper chromatography. With our enzyme preparations, the absorption increase at 340 nm stopped at 0.5 to 0.6 absorbance unit; this was at least partially due to product inhibition. Therefore 200 ml of the reaction mixture were collected. The thiol ester was freed from the enzymes by ultrafiltration. Then 2-N-hydroxylamine neutralized to pH 7 (10 ml) was added. The hydroxamate was, after concentration under reduced pressure, freed from NAD and salt by adding 20 volumes of acetone, centrifuging, and concentrating the supernatant (31). This was repeated several times. After the last concentration, part of the supernatant was spotted on Whatman No. 3MM paper together with a standard sample of formylhydroxamate prepared from ethyl formate by the procedure of Hestrin (32) and purified as above. Both hydroxamates gave a single spot, with \( R_f = 0.42 \) in 1-butanol-methylethylketone-NH₃-water (5:3:1:1) (31). These values are in full agreement with the values for formylhydroxamate reported by Sly and Stadtman (31). No spots were obtained for a blank containing all of the substrates without enzyme, and otherwise similarly treated and purified.

The product of the hydrolase-free formaldehyde dehydrogenase was further studied through formate determinations (21). The absorption increase at 240 nm was followed, as in Fig. 8, from a mixture in acetate buffer at pH 5.7. Samples were taken at suitable intervals from the mixture and from blanks without substrates and without enzymes. When the absorption increase stopped, the mixture was cooled rapidly and separated from enzymes by ultrafiltration. Samples were taken from the ultrafiltrate, which was then treated with S-formylglutathione hydrolase. Thiol ester hydrolysis was followed at 240 nm. Further samples were taken from the hydrolase-treated solution, and the formate content of samples was determined with the modified procedure (8). No formate was present in the samples taken during the first 30 min. During the rest of the time (40 min) some formate appeared, but this was only 10 to 20% of the amount of thiol ester present, calculated from the absorbance at 240 nm. On treatment of the ultrafiltrate with hydrolase, 88.2 nmoles of formate were liberated. The amount of S-formylglutathione hydrolyzed, calculated from the decrease of the absorbance at 240 nm, was 91.2 nmoles. We conclude that purified formaldehyde dehydrogenase catalyzes the formation of S-formylglutathione from formaldehyde and that no formate is enzymically formed in this reaction.

Product and Substrate Inhibition—The forward reaction with formaldehyde, GSH, and either NAD or NADP as substrates was inhibited by NAD-formylglutathione, NADH, and NADPH. The inhibition by S-formylglutathione was stronger at pH 5.7 than at pH 8.0. As expected from \( K_m \) values, NADH easily inhibited the activity with NADP and NADPH inhibited only when in high excess the activity with NAD, the other combinations being between these extremes.

The reverse reaction with either NADH or NADPH was inhibited by NAD and NADP. NAD was a strong inhibitor, especially with NADP as the substrate, and NADP was weak, especially with NADH. GSH was a weak inhibitor of the reverse reaction. Further addition of formaldehyde resulted in a much stronger inhibition especially at high pH (8.0), and formaldehyde alone was also an efficient inhibitor (Table V). However, one of the substrates, S-formylglutathione, was contaminated with free GSH. If MAMNEt was added to the assay mixture in slight excess over GSH before the further addition of formaldehyde and enzyme, formaldehyde had no effect on the reverse reaction at least up to 2 mM (Table V). MAMNEt prevents the nonenzymic formation of S-hydroxymethylglutathione from GSH and formaldehyde due to the alkylation of the —SH group of GSH. This result suggests that the hemimercaptal is bound to the active center of the enzyme rather than free formaldehyde. MAMNEt was at the concentration used in this experiment without any effect on the enzyme itself (see below).

Human liver formaldehyde dehydrogenase was less sensitive to an excess of free formaldehyde than has been reported for the yeast enzyme (3, 4). Formaldehyde up to 3 mM was uninhibitory at all GSH concentrations. When GSH was 1 mM, formaldehyde began to inhibit at about 12 mM. Formaldehyde (20 mM) inhibited 20% and at 40 mM, 60%. Methylglyoxal had a similar effect. As described above (see "Methods"), we have noticed that a high excess of formaldehyde results in further
modification of GSH after the initial formation of S-hydroxymethylglutathione. Thus, some of the inhibition may be caused by the modification of the substrate in addition to the reaction of the enzyme with free formaldehyde.

**Thermosensitivity**—Incubation of formaldehyde dehydrogenase for 15 min at 37° caused a loss of 40% of activity. At 50°, the enzyme was inactivated in a few minutes (Fig. 10a). However, the addition of NAD or NADH resulted in the stabilization of the enzyme. At 60°, where the enzyme alone was instantaneously inactivated (Fig. 10b), NAD was a rather ineffective stabilizer. However, NADH was still able to protect the enzyme completely. In the presence of NADH, 40% of the activity measured with NAD or NADH, but the effects were inferior to those of NADH and NAD.

The protective effect of NADH allowed us in preliminary experiments to separate the dehydrogenase activity from most S-formylglutathione hydrolase activity in crude preparations. Incubation for 10 min at 61° caused the loss of most of both activities. If NADH (0.5 mM) was added, 99% of the dehydrogenase activity was preserved, but only 0.4% of the thiol esterase activity remained.

**Stability**—Formaldehyde dehydrogenase was stable in intact frozen human liver, and preparations from Steps 1 to 3 were kept for 2 years at -20° with only a slight loss of activity. At 0°, preparations from Step 3 lost one-half of their activity in 1 month. More purified preparations were less stable. One freezing at -20° resulted in full inactivation of the preparations from Steps 7 and 8. They were, however, well preserved on rapid freezing at -70°. The pure enzyme in 2 mM mercaptoethanol lost 50% of its activity at -70° during 2 months. The stability of the enzyme from Step 8 was studied more thoroughly at 0° (Fig. 11). Overnight dialysis to remove mercaptoethanol destroyed 60% of the original activity. The thiol-free enzyme was then rapidly totally inactivated. Addition of bovine serum albumin, mercaptoethanol, or DTT did not appreciably increase the stability of the enzyme. Addition of glycerol or NAD increased the half-life of the enzyme from less than 1 day to about 1 month. NADH, however, was even more efficient, and the enzyme saturated with it was fully stable for at least 2½ months at 0°.

**Effect of —SH Reagents on Formaldehyde Dehydrogenase**—Purified preparations were very sensitive to mercurial-forming —SH reagents (Table VI). If the enzyme before the addition of HgCl₂, PMB, or p-chloromercuriphenyl sulfonate was saturated with NAD or NADH, higher inhibitor concentrations were needed (Fig. 12). NADII protected the enzyme more effectively than did NAD. Two oxidizing agents, Nbs and GSSG, were without effect on formaldehyde dehydrogenase (Table VI). Of the alkylation agents, iodosocetate was more effective than the neutral alkylator MalNEt. A long incubation time and a
pyrophosphate. Piperazine-N'-2-ethanesulfonic acid buffer was used instead of start the reaction. In the case of AgNO₃, N-2-hydroxyethyl-piperezine-N'-2-ethanesulfonic acid buffer was used instead of pyrophosphate.

The effect of PMB is shown in Fig. 11. With Nbs₂ and MalNEt longer incubation times were studied, as explained below.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgCl₂</td>
<td>0.00010</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>0.00015</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>0.00020</td>
<td>90%</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>0.0003</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>0.0006</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>0.0010</td>
<td>100%</td>
</tr>
<tr>
<td>Nbs₂</td>
<td>1.0</td>
<td>0%</td>
</tr>
<tr>
<td>GSSG</td>
<td>7.0</td>
<td>19%</td>
</tr>
<tr>
<td>MalNEt</td>
<td>15.0</td>
<td>25%</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>1.0</td>
<td>9%</td>
</tr>
<tr>
<td>(+NAD)</td>
<td>3.0</td>
<td>46%</td>
</tr>
<tr>
<td>(+NAD)</td>
<td>8.0</td>
<td>82%</td>
</tr>
<tr>
<td>Arsenite</td>
<td>16.0</td>
<td>100%</td>
</tr>
</tbody>
</table>

* With Nbs₂ and MalNEt, activity was followed during 90 min at 25° by taking samples to the assay mixture from incubation mixtures with and without inhibitor. With Nbs₂, no inhibition developed. With MalNEt, slight inhibition was seen after a lag of 20 min. The inhibition given was obtained after 90 min both at pH 8.0 and pH 6.6.

Fig. 12. Effect of p-hydroxymercuribenzoate (PMB) on the forward (□), formaldehyde, GSH, and NAD) and reverse (○, S-formylglutathiones and NADH) reaction of formaldehyde dehydrogenase at pH 8. These curves were obtained by adding PMB to the enzyme before the pyridine nucleotide. ■, NADH (0.20 mM) was added to the enzyme before PMB; reverse reaction was measured. ●, NADH (0.20 mM) was added to the enzyme before PMB; reverse reaction was measured.

The present results show that the previously described formaldehyde dehydrogenase activity which oxidized formaldehyde to formate is caused by two enzymes, a dehydrogenase which catalyzes the formation of S-formylglutathione from formaldehyde and GSH and a thiol esterase which hydrolyzes S-formylglutathione. Thus, formaldehyde dehydrogenase resembles the glyoxalase system, in which the first enzyme also transforms an aldehyde and GSH to a thiol ester of GSH and the second enzyme hydrolyzes this thiol ester. The principal difference is that in the glyoxalase I reaction, the substrate is modified through an intramolecular hydride shift (34, 35), whereas in the present system, an NAD-linked oxidation occurs. Evidence has been reported that in the glyoxalase I reaction, a hemimercaptal of glyoxalase II and the instability of S-formylglutathione at neutral to slightly alkaline pH values where formaldehyde dehydrogenase has its pH optimum.

No other dehydrogenase has been described previously which can catalyze the formation of glutathione thiol esters. However, two bacterial dehydrogenases are involved in the formation of thiol esters of CoA (30, 39) from aldehydes. One of them, catalyzing the formation of acetyl-CoA from acetaldehyde (30), catalyzes a reversible reaction like formaldehyde dehydrogenase.
In contrast, those aldehyde dehydrogenase reactions in which direct oxidation of aldehyde to acid occurs are irreversible (40).

In the present work, formaldehyde dehydrogenase has been purified to homogeneity and the obtained specific activity is about 15 times higher than that previously obtained from human liver (6). Formaldehyde dehydrogenase appears to consist of 2 identical subunits of molecular weight 40,000 like several other dehydrogenases. The isoelectric point is 6.35.

The inhibition results found with formaldehyde dehydrogenase are not typical for an aldehyde dehydrogenase because arsenite and dithiobenzoic acid are not effective as inhibitors. HgCl₂ and PMR were, however, inhibitory at low concentrations. NAD and NADH protected the enzyme from -SH reagents.

The physiological significance of formaldehyde dehydrogenase is not clear at the present time. A role in the detoxication of compounds, which can be converted to formaldehyde in the metabolism (41). Formaldehyde can, however, be actively oxidized also by the unspecific NAD-linked aldehyde dehydrogenase (42). Formaldehyde dehydrogenase reaction is easily reversible and below pH 8, the reverse reaction has the higher velocity.

Formaldehyde dehydrogenase can also metabolize methylglyoxal and other α-ketoaldehydes, which Szent-Györgyi et al. (43) have proposed to have a role in the regulation of cell division and growth together with glyoxalase. Besides formaldehyde dehydrogenase and glyoxalase, two additional enzymes, α-ketoaldehyde dehydrogenase (44) and the unspecific aldehyde dehydrogenase, can also metabolize methylglyoxal. The existence of so many enzymes capable of destroying methylglyoxal apparently does not give support to the theory mentioned above. In the reverse reaction, formaldehyde dehydrogenase can produce methylglyoxal from S-pyruvylglutathione. The physiological occurrence of S-formylglutathione or S-pyruvylglutathione remains, however, to be demonstrated.

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REFERENCES

5. SAHL, H., AND WAGNER, F. (1973) Arch. Mikrobiol. 90, 263-268
12. UOTILA, L. (1973) Biochemistry 12, 3938-3943
17. ANDREWS, P. (1965) Biochem. J. 95, 595-600
24. UOTILA, L. (1973) Biochemistry 12, 3944-3951
42. KENNENKEN, F. M., AND OSBORN, M. J. (1959) Advan. Enzymol. 21, 309-446
44. MONDER, C. (1967) J. Biol. Chem. 242, 4603-4609
Formaldehyde Dehydrogenase from Human Liver: PURIFICATION, PROPERTIES, AND EVIDENCE FOR THE FORMATION OF GLUTATHIONE THIOL ESTERS BY THE ENZYME
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