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# S-FORMYL GLUTATHIONE AS A SUBSTRATE OF BACTERIAL FORMATE DEHYDROGENASE

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NAD-dependent formate dehydrogenase from the methylotrophic bacterium Achromobacter parvulus is active with S-formyl glutathione as a substrate. The hydrolytic activity of highly purified enzyme preparations in the absence of the cofactor cannot be detected. From several independent lines of evidence it is concluded that the dehydrogenase activity with S-formyl glutathione and NAD should be ascribed to formate dehyrogenase.

## INTRODUCTION

NAD-dependent formate dehydrogenase is a terminal enzyme in the dissimilative pathway of methanol oxidation in methylotrophs. The homogeneous preparations of formate dehydrogenases from the methylotrophic bacterium Achromobacter parvulus (I,2) and the methylotrophic yeast Candida methylica (3) were obtained and characterized in this laboratory.

Recently controversy has arisen concerning the nature of the true substrate of formate dehydrogenase. Studying several strains of methylotrophic yeasts Van Dijken et al (4) found that partially purified preparations of formate dehydrogenases oxidized S-formyl glutathione (GSF). They suggested that the true physiological substrate of formate dehydrogenase was not the formate but GSF, the product of the preceding enzymatic reaction, catalyzed by glutathione-dependent formaldehyde dehydrogenase.

Abbreviations used: GSF - S-formyl glutathione, GSH - reduced glutathione

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In contrast, highly purified preparations of formate dehydrogenases from methylotrophic yeasts Candida boidinii (5) and Kloeckera sp. No. 2201 (6) did not oxidize GSF. The presence of the special enzyme S-formyl glutathione hydrolase which was isolated and characterized was essential for the dehydrogenase activity (5,6). The related results were obtained with the enzymes isolated from pea Pisum sativum (7). It was shown that the presence of the specific hydrolase was necessary for the effective utilization of the GSF in the formate dehydrogenase--catalyzed reaction.

This work presents an evidence that GSF is oxidized in the presence of formate dehydrogenase from the methylotrophic bacterium.A.parvulus with relatively high rates compared to formate. The GSF is not hydrolyzed by the purified enzyme preparations in the absence of the cofactor.

## MATERIALS AND METHODS

Formate dehydrogenase was isolated from the methylotrophic bacterium A.parvulus as described earlier (I,2). The enzyme was homogeneous as judged by disc-electrophoresis in polyacrylamide gel. Formate dehydrogenase from the methylotrophic yeast C. methylica was isolated by a simplified procedure, which included ion-exchange chromatography on DE-52 cellulose (3). According to electrophoretic data the enzyme preparations beside formate dehydrogenase, contained two minor impurities, not active with formate.

GSF was synthesized by the method of Votila (8). The mixed anhydride of formic and acetic acids was obtained from acetyl chloride and formate (9). The content of GSF in final preparations ranged from 20 to 50% of the total amount of the glutathione present in solution.

The GSF concentration was determined spectrophotometrically with 5,5'-dithiobis(2-nitrobenzoate) (8). At the beginning of the experiment the solution of GSF was passed through a column (0,9 x 60 cm) of Sephadex G-10, equilibrated with water. The purity of GSF was verified by isotachoelectrophoretic technique (the leading buffer was 5 mM β-alanin-HCI, pH 3.8; the terminating buffer - 5 mM caproic acid, pH 5.5, containing 0.3% hydroxypropyl methyl cellulose) on the Tachophor 2127 (LKB, Sweden). According to isotachoelectrophoretic data the GSF preparations did not contain formate. The sensitivity of the method is sufficient to detect 0.I nmole of formate in a 10 µl sample.

The activity of formate dehydrogenase was determined spectrophotometrically with saturating substrate concentrations at 340 nm in Reaction Rate Analyzer model 2086 (LKB, Sweden) in

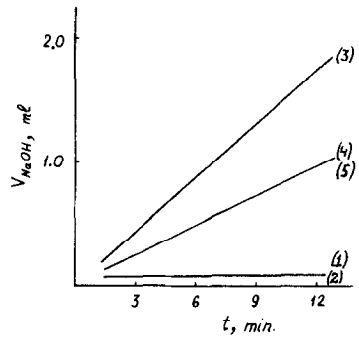


**Fig.1. Reaction catalyzed by formate dehydrogenase from A.parvulus (14 μmol/min) and formate dehydrogenase from C.methylica (3 μmol/min) followed on pH-stat, at 22°, pH 7.0. Concentrations of the substrates used were as follows: GSF:0.7 mM; NAD:1.5 mM. (1) GSF, control; (2) GSF + bacterial enzyme; (3) same as (2) + NAD; (4) GSF + yeast enzyme; (5) same as (4) + NAD.**

0.05 M phosphate buffer, pH 7.0, 37° or titrimetrically on pH-stat TT2 equipped with autoburette ABI 12 (Radiometer, Denmark).

## RESULTS AND DISCUSSION

The GSF is stable for at least 40 min in the presence of purified formate dehydrogenase from A.parvulus. That follows from the titrimetric measurements and from the constant value of the absorbance at 235 nm (the absorption maximum for GSF, $\Delta\varepsilon = 3300\ M^{-1}cm^{-1}$ (8)). Addition of 1.3 mM NAD to the reaction mixture initiates the enzymatic reaction, which is manifested by the absorbance changes at 235 and 340 nm and is readily detected on pH-stat (Fig.I). In the case of the enzyme preparation from C.methylica GSF is hydrolyzed even in the absence of the cofactor (Fig.I). The addition of NAD in saturating amounts has no effect on the reaction rate.

The ability of enzyme preparations from the yeast C.methylica to hydrolyse GSF is probably determined by the presence of the specific S-formyl glutathione hydrolase as in the case of other methylotrophic yeasts (5,6). On the other hand the formate dehydrogenase from A.parvulus does not hydrolyze GSF in the absence of the cofactor. In the presence of NAD the rates of

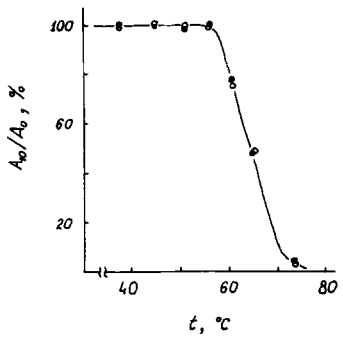


Fig.2. Temperature inactivation of formate dehydrogenase from A.parvulus. Residual enzyme activity was measured as described in Materials and Methods with GSF (o) and formate (•) as substrates after 10 min. incubation at desired temperature.

GSF hydrolysis and NADH accumulation calculated on the basis of absorbance changes at 235 and 340 nm respectively are equal, that points to the simultaneous character of those processes. The homogeneity of the enzyme used and the absence of hydrolytic activity in the bacterial formate dehydrogenase allow us to suggest the absence of a second hydrolytic enzyme in preprations of bacterial formate dehydrogenase. The activity with GSF and NAD should thus be ascribed totally to the formate dehydrogenase.

The conclusion that GSF is a substrate of formate dehydrogenase is substantiated by some indirect evidences. The kinetic parameters of formate dehydrogenase from A.parvulus with GSF as substrate were determined. The $K_m$ value was 0.9±0.1 mM and the specific activity was 60±3% of the activity with formate for several enzyme preparations and several lots of GSF with different GSH content. The constant ratio of $V_{GSF}/V_{formate}$ suggests that both types of activities are catalyzed by the same protein.

The fact that the oxidation of GSF and formate is catalyzed by the same protein is validated by the heat inactivation experiments with formate dehydrogenase (Fig.2). According to Neben et al.(5) S-formyl glutathione hydrolase is rather labile and is inactivated at temperatures above 40°. As it follows

from Fig.2, the formate dehydrogenase is active with GSF well above this value and both activities with formate and GSF are lost simultaneously.

All the data available show that contrary to the yeast enzyme, which activity with the GSF is determined by the presence of the specific S-formyl glutathione hydrolase, the formate dehydrogenase from methylotrophic bacterium A.parvulus is capable of oxidizing this substrate.

The results of the investigation into the kinetic mechanism of formate dehydrogenase with GSF and substrate specificity of the enzyme will be published.

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