Some Properties of Formate Dehydrogenase

THOMAS HÖPNER and ANNEMARIE TRAUTWEIN

Fachgruppe Biochemie, Universität Heidelberg, Germany

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The enzyme was anaerobically isolated and was characterized as a flavoprotein containing at least 1 FMN, 5—8 Fe and 7—8 moles labile sulfide per mole (M.W. appr. 300,000). It catalyzes the oxidation of formate by NAD, O₂ (forming H₂O₂) and dyes and the oxidation of NADH by O₂ (forming H₂O) and dyes. It is irreversibly inhibited by formate.

Formate dehydrogenase (FDH, Formate: NAD oxidoreductase, E.C. 1.2.1.2.) from Pseudomonas ovaltaticus has been introduced by JOHNSON et al. 1, QUAYLE 2 and HÖPNER and KNAPP 3 as a tool for the enzymatic assay of formate. The enzyme catalyzes the following two types of reactions:

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\begin{align*}
\text{HCOO}^- + \text{NAD}^+ + \text{H}_2\text{O} & \rightleftharpoons \text{HCO}_3^- + \text{NADH} + \text{H}^+ \\
\text{HCOO}^- + \text{O}_2 + \text{H}_2\text{O} & \rightleftharpoons \text{HCO}_3^- + \text{H}_2\text{O}_2
\end{align*}
\]

(1) (2)

In addition the enzyme catalyzes the oxidation of NADH by O₂. The stoichiometry of the first reaction is proved by the formation of the amount of NADH which under anaerobic conditions exactly corresponds to the amount of formate used. In this reaction NAD is replaceable by a large number of redox dyes. For the second reaction the stoichiometry is proved a) kinetically by the relation of the velocities of oxygen consumption with and without catalase (ratio 1:2) and b) by the amount of oxygen used for the oxidation of a given amount of formate with and without catalase (0.5 and 1 mole O₂ per mole formate, respectively).

The enzyme was isolated from formate/pyruvate grown bacteria 4 by means of conventional steps (ammonium sulfate fractionations, chromatography on DEAE-cellulose and hydroxylapatite, and finally sucrose density gradient centrifugation on Sephadex G-150) at pH 5.6 under anaerobic conditions. In the last step two enzymatically active species were separated from each other from which the main species exhibits a molecular weight of about 300,000 ("FDH I"), the minor of about 200,000 daltons ("FDH II") as found by disc electrophoresis which delivered the same pattern for both species consisting of two major and three minor bands which were visible at exactly the same positions a) on account of the enzyme's self colour, b) after staining for activity by formate/NAD/phenazin methosulfate/nitrotetrazolium blue and c) after staining for protein by amido black. Besides these bands no protein bands could be detected.

During aerobic aging of FDH I an apparently irreversible conversion to a second species, presumably FDH II, takes place. Compared to FDH I in this species the relation between the velocities of the formate: NAD oxidoreductase reaction and the reactions involving nonphysiological electron acceptors is altered favouring the latter. After aerobic isolation the amount of FDH II is markedly enhanced (Fig. 1).

Requests for reprints should be sent to THOMAS HÖPNER, Fachgruppe Biochemie der Universität, D-6900 Heidelberg, Im Neuenheimer Feld 7.
iron and sulfide as indicated by a coincidence of loss of colour and activity. It is assumed, therefore, that the isolated enzyme is not fully saturated with its low molecular components. Besides iron no metals exceeding 0.1 mole/mole could be detected by X-ray fluorescence analysis.

FDH is inactivated by light irradiation. It is inactivated by its substrate formate in a slow reaction of which the velocity is dependent on the concentration of formate (Fig. 2). In both cases the inactivation concerns only the reactions which involve formate and not the NADH diaphorase activity. A reactivation could not be achieved hitherto.

![Fig. 2. Inactivation of FDH by formate. The numbers at the curves indicate the formate concentrations (mM) in the samples. Half live times may be estimated from the (broken) 50% inactivation line. 25 °C. 0.05 M histidine hydrochloride buffer pH 5.6. Aerobic.](image)

The spectrum of FDH I (Fig. 3) shows a broad decrease of extinction between 320 and 600 nm with shoulders at 360 and 420 nm. After addition of formate two phases of bleaching are observed. A quick reaction which leads to curve 4 of Fig. 3 occurs within some minutes and is easily reversed by oxygen. Further bleaching coincides with the inactivation as shown in Fig. 2 and is only partially reversed by oxygen. Reduction by dithionite clearly leads to a different product (Fig. 3 curve 2) which is rapidly reoxidized by oxygen (curve 3).

![Fig. 3. Spectra of FDH. 1. ——— 4.85 mg/ml FDH in 0.05 M histidine hydrochloride buffer pH 5.6. 2. ——— same as 1, reduced by 0.16 mM dithionite. 3. ——— same as 2, reoxidized by O₂. 4. ——— same as 1, 10 min after addition of formate (8 mM). 5. ——— supernatant of 1 after acidification by perchloric acid. 5 x enlarged. Conditions: anaerobic, 0—3 °C.](image)

Besides the soluble FDH *Pseudomonas oxalaticus* contain an insoluble formate oxidase activity which easily can be isolated by differential centrifugation. The particles are extremely oxygen sensitive and are protected best by addition of formate. There is no formate inactivation. Various treatments did not cause any release of soluble FDH.

**Discussion**

FDH is a new member of the intensely studied family of metallo sulfur flavoproteins covering as far as characterized 10 enzymes from various sources, among them familiar ones as xanthine oxidase from milk and succinate dehydrogenase from heart mitochondria. The physiological role of FDH presumably is to provide NAD(P)H for syntheses while the particles are considered to contain the respiratory chain. Studies to confirm this are in progress.

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