SOLUBILIZATION AND SOME PROPERTIES OF FORMIC DEHYDROGENASE FROM ESCHERICHIA COLI

Eiji Itagaki, Takeshi Fujita and Ryo Sato

Institute for Protein Research, Osaka University, Kita-ku, Osaka, Japan

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Wrigley and Linnane (1961) have recently reported the solubilization and partial purification of a formic dehydrogenase (FDH)-cytochrome b_1 complex from the particulate fraction of aerobically grown Escherichia coli. We have also independently succeeded in solubilizing FDH from anaerobically grown E. coli (Itagaki, Fujita and Sato, 1960) and found that the solubilized dehydrogenase was inseparably associated with cytochrome b_1. Unlike the Wrigley-Linnane preparation, however, the solubilized preparation obtained in our laboratory seems to have lost a factor required for the electron transfer from FDH to cytochrome b_1. The nature of the lost factor has not yet been clarified, but it has been found that the link between FDH and the cytochrome could be restored by the addition of either vitamin K_3 or a lipid fraction extracted from E. coli cells. This paper describes the solubilization procedure employed in our laboratory and reports some properties of the solubilized preparation.

E. coli cells grown anaerobically at 33° for 5 hours in a complex medium supplemented with 0.35 % each of KNO_3 and sodium formate were washed, ground at 0° with alumina and extracted with 0.05 M phosphate, pH 7.4. The extract was centrifuged at 2,000 × g for 20 minutes to remove alumina and cell debris. The particulate fraction was then collected from the supernatant by

centrifugation at 78,000 x g for 30 minutes, washed once and resuspended in distilled water.

FDH was solubilized from the washed particulated fraction by treating with both deoxycholate and snake venom (Trimeresurus flavoviridis). The solubilization mixture contained in final concentrations the followings: E. coli particles, 1~1.5 mg N per ml; sodium deoxycholate, 0.1 %; snake venom, 0.05 %; ethylenediamine tetracetate, 10^{-5} M; and tris buffer of pH 8.0, 0.05 M. This mixture was incubated at 0° for about 16 hours in a nitrogen atmosphere. Approximately 60 % of the total FDH activity was converted by this treatment into a form which could not be sedimented by centrifugation at 105,000 x g for 60 minutes. The enzyme thus solubilized could be precipitated by neutralized ammonium sulfate between 45 and 60 % saturation. The precipitated enzyme was further purified by adsorption onto fresh calcium phosphate gel in 0.05 M tris buffer, pH 7.0, and by subsequent elution with 0.2 M phosphate-0.2 M citrate, pH 7.1.

The eluate thus obtained was a reddish amber solution and represented a 9-fold purification over the particulate preparation. It catalyzed the oxidation of formate with methylene blue as hydrogen acceptor; the activity being 15~20 μmoles CO_2 liberated/minute/mg N when measured as described by Peck and Gest (1957). Janus green, toluidine blue, thionine and ferricyanide could also serve as acceptors, though less effective than methylene blue. Methyl viologen and phenazine methosulfate, on the other hand, were quite inactive. Although 2,6-dichlorophenol indophenol acted as an effective acceptor for the particulate dehydrogenase, it was completely inactive toward the solubilized enzyme. Apparently, a factor involved in the indophenol reduction had been damaged by the solubilization procedure. The soluble

preparation could oxidize neither DPNH nor succinate in contrast to the intact particles.

Fig. 1 shows the absorption spectra of the purified FDH preparation. It is evident from the characteristic peaks at 559 (α), 530 (β) and 427 mμ (Soret) in the dithionite-reduced spectrum that the preparation contains cytochrome $\underline{b}_1$ as does the Wrigley-Linnane preparation. The reduced pyridine hemochromogen prepared from the purified enzyme according to the method described by Appleby and Morton (1959) showed three absorption maxima at 557, 525 and 418 mμ indicating that protoheme is the prosthetic group of the cytochrome. From the height of α-band in the pyridine hemochromogen spectrum, it was estimated that the protoheme content was about 1~3 mμmoles per mg protein. This value is about the same order to that reported for the cytochrome $\underline{b}_1$ content of the Wrigley-Linnane preparation. Although Wrigley and Linnane have detected as much as 30 % of polyribonucleotide in their preparation, it does not seem likely that our preparation contains so much polynucleotide in view of the fact that only a symmetrical peak at 280 mμ due to protein was observed in the ultraviolet region of the spectrum (Fig. 1).

The cytochrome in the purified preparation could be reduced by formate only at negligibly slow rates; sometimes no reduction could be observed at all. This is another important difference that distinguishes our preparation from the Wrigley-Linnane preparation. Since the cytochrome in the particulate fraction could be rapidly and fully reduced by formate, it appears likely that a factor functionally mediating between FDH and cytochrome $\underline{b}_1$ had been destroyed or removed during the solubilization and/or purification. It was in fact found, as can be seen from Fig. 2, that the reduction of cytochrome $\underline{b}_1$ by formate could be greatly stimu-

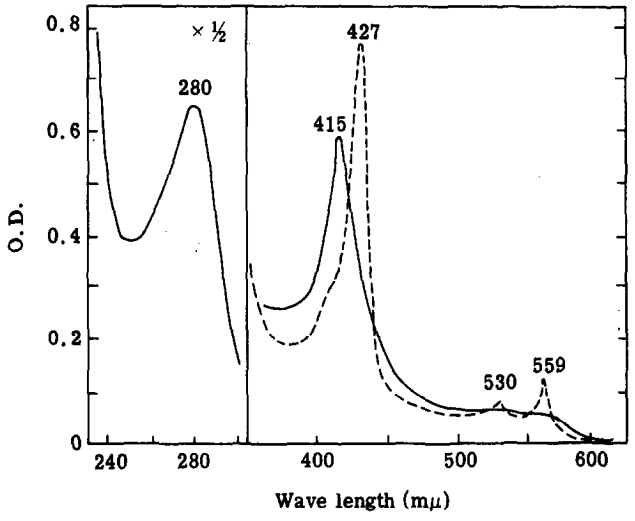

Fig. 1. Absorption spectra of purified preparation. pH 7.1. 5.8 mg protein per ml.
——— Oxidized form; ----- Reduced with dithionite.

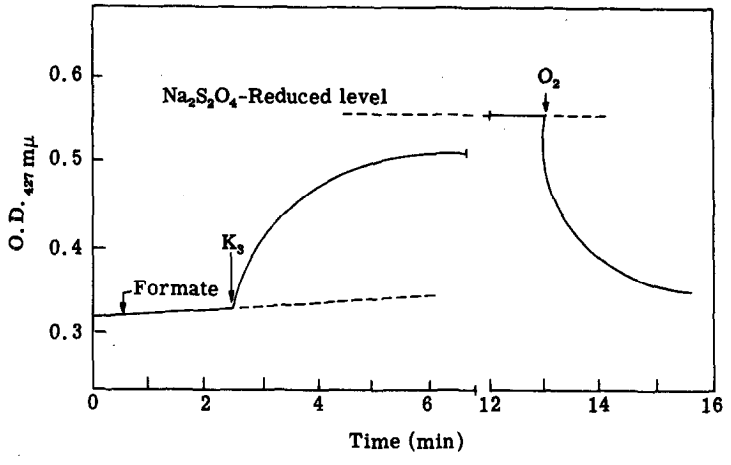

Fig. 2. Reduction and reoxidation of cytochrome $\underline{b}_1$ in purified FDH. Room temperature, pH 7.1. Reduction phase was carried out anaerobically in a Thunberg type cuvette. To 1.9 ml mixture containing 3.8 mg protein were added 5 μmoles sodium formate (0.05 ml) and 75 mμmoles vitamin K_3 (0.05 ml in acetone) as indicated.

lated by the addition of small amounts of vitamin K_3; the cytochrome being fully reduced under anaerobic conditions. Neither DPNH nor succinate could reduce the cytochrome even in the presence of vitamin K_3. A preliminary attempt was then made to extract the natural mediating factor from E. coli cells, and a lipid fraction obtained by extraction with ethanol-ether (1:1 by volume) was capable of replacing vitamin K_3 in stimulating the reduction of cytochrome b_1 in the solubilized system if the lipid was finely dispersed with the aid of 0.5 % deoxycholate. Work is going on in our laboratory to purify the active factor in the lipid fraction.

Fig. 2 also shows that cytochrome b_1 in the purified preparation could be oxidized by oxygen. This oxidation was insensitive to 10^{-3} M KCN indicating the autoxidizability of the cytochrome.

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