FORMIC ACID DEHYDROGENASE-CYTOCHROME b_1 COMPLEX FROM ESCHERICHIA COLI.*

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Formic acid dehydrogenase of Escherichia coli has long been recognised as being firmly bound to the particulate fraction of the cell and closely associated with the cytochrome system (Gale 1939; Iida & Taniguchi 1959). The present communication describes the isolation and some properties of a solubilized formic dehydrogenase-cytochrome b_1 complex from E. coli.

E. coli cells were grown aerobically at 30° on a medium containing beef extract, peptone, glucose and phosphate. The harvested, washed cells were disintegrated at 0° in 0·02M potassium phosphate buffer pH 6·8 by high speed homogenization for 10 minutes with ballotini glass beads in a specially designed blendor (Linnane & Vitols, in preparation). All subsequent manipulative operations were also at 0°. Glass beads and large cell fragments were removed by centrifugation at 5000 x g for 20 minutes, the particulate material remaining in suspension was then collected by centrifugation at 55,000 x g for 30 minutes, washed and resuspended in 0·01M KCl. This fraction, denoted as the " cell residue", contained the cytochrome system of the cell together with formic, succinic and DPNH

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oxidases and the nitrate reductase system.

Formic dehydrogenase together with some of the cytochrome b_1 was released from the cell residue by treatment with deoxycholate and ammonium sulphate. Deoxycholate solution and 0·4M tris(hydroxymethyl)aminomethane buffer, pH 8·0, were added to a suspension of the cell residue to give a final concentration of 15 mg protein/ml, 1 mg deoxycholate/mg protein and 0·1M buffer. Saturated ammonium sulphate solution was then added to 40% saturation and after stirring for 45 minutes the mixture was centrifuged at 55,000 x g for 30 minutes and the precipitate discarded. To the supernate, solid ammonium sulphate was added to 75% saturation and the precipitate sedimented by centrifugation. This precipitate was finally suspended in 0·01M KCl and dialyzed for 18 hours against several changes of 0·01M KCl. The material which precipitates during dialysis was removed by centrifugation at 105,000 x g for 30 minutes.

The resulting supernate was a highly coloured amber solution which at this stage behaved as a soluble protein or macromolecular complex. Dialysis for 5 days caused no active protein to precipitate and no significant loss of enzyme activity. After such dialysis, the level of deoxycholate as determined by the method of Mosbach *et al* (1954) was only 0·003 mg/mg protein which would contribute little to solubilization of the preparation. Centrifugation at 127,000 x g (average) for 60 minutes in a swinging bucket head did not lead to sedimentation of the enzyme nor was any stratification of colour observed in the tube.

The activities of the classically insoluble enzymes present in the cell residue and in the soluble preparation are shown in Table I. The oxidase activities of the enzyme systems are not tabulated since the soluble preparation was essentially without oxidase activity. The procedure resulted in the

quantitative extraction of the formic dehydrogenase and a twenty fold purification of the enzyme. Only small amounts of the other insoluble enzymes were released by this procedure.

TABLE 1.

Enzyme Activities of Cell Residue and Soluble Preparation.

	Cell Residue		Soluble Preparation	
Substrate	Specific Activity	Total Units	Specific Activity	Total Units
Formate	1·0	1230	22	1120
Succinate	0·11	135	0	-
DPNH	0·21	258	0·54	28
Formate-nitrate	0·12	147	0	-
Reduced benzyl viologen-nitrate	0·25	307	0·08	4

Cell residue, total protein 1230 mg, from which was prepared the soluble preparation, total protein 51 mg. Specific activity = μM substrate decomposed/min./mg protein (biuret determination). Total units = specific activity x total protein.

All assays at 30°. Formic and succinic dehydrogenases were assayed manometrically by measurement of acid production in bicarbonate buffer with ferricyanide as acceptor. DPNH dehydrogenase was assayed spectrophotometrically with potassium ferricyanide as acceptor. The nitrate reductase activity was estimated as described by Iida *et al* (1959), with formate or reduced benzyl viologen acting as hydrogen donor.

The amber colour of the preparation was accounted for by the presence of a cytochrome, reducible by formate but not by succinate or DPNH. The difference spectrum of the preparation showed α, β and γ peaks at 560, 532 and 429 mμ which have been reported to be characteristic of cytochrome b_1 (Smith 1954). Figure 1 illustrates a typical experiment, in which cytochrome b_1 reduction by formate was about 70% complete compared with that obtained with dithionite. The percentage

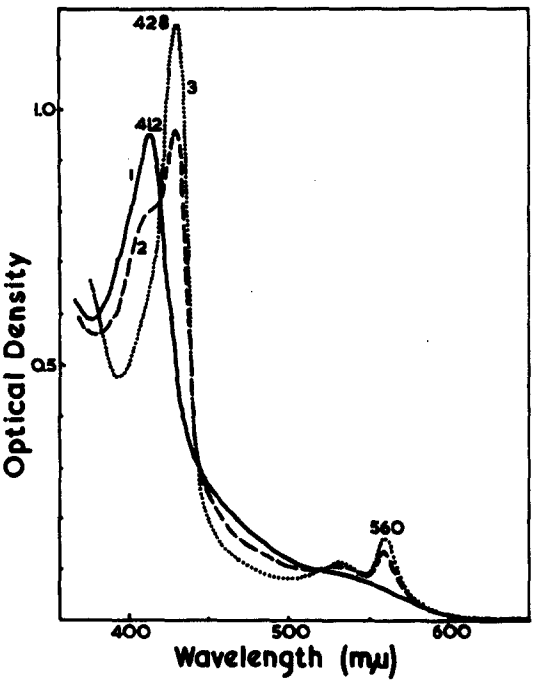

Figure 1. Direct absorption spectra of the soluble formic dehydrogenase-cytochrome b_1 complex. The 4 cm light path cuvette contained 1·6 mg protein/ml of 0·01M KCl. 1. oxidized spectrum; 2. preparation reduced by formate; 3. dithionite reduced preparation.

reduction of the cytochrome by formate usually varied from 50-90% depending upon the particular preparation and its age. The cytochrome b_1 content of most preparations was about 0·7 mμ moles/mg protein, using the extinction coefficient for mammalian cytochrome b reported by Chance (1952). The presence of flavin reducible by formate is evidenced by the trough at about 450 mμ in Figure 1. Flavin levels determined as described by Green et al (1955) were of the order of 0·4 and 0·6 mμ moles of flavin/mg protein for acid extractable and total flavin respectively.

The chemical composition of an exhaustively dialyzed salt free preparation was about 66% protein, 3-5% lipide and 30% polyribonucleotide or ribonucleic acid. Acid hydrolysis of the extracted polynucleotide followed by paper chromatography showed the presence of guanine, cytosine, uracil and adenine,

together with a still unidentified fifth compound presumably another base.

Work is proceeding on the further purification of formic dehydrogenase and on the significance of the RNA content of the formic dehydrogenase-cytochrome b_1 complex.

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