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Electron-Transferring Flavoprotein of *Peptostreptococcus elsdenii* That Functions in the Reduction of Acrylyl-Coenzyme A[1]

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In *Peptostreptococcus elsdenii*, a three-component flavoprotein electron transfer system catalyzes the oxidation of lactate and the reduction of crotonyl-coenzyme A (CoA). Spectral evidence showed that D-lactate dehydrogenase, when reduced by D-lactate, was able to reduce butyryl-CoA dehydrogenase but only in the presence of the electron-transferring flavoprotein. Reduced nicotinamide adenine dinucleotide could replace reduced D-lactate dehydrogenase. A reconstituted system, containing the three partially purified enzymes, excess D-lactate, and a limiting amount of crotonyl-CoA, reduced the crotonyl-CoA to butyryl-CoA, but only if all components were present. The electron-transferring flavoprotein activity, purified 22-fold, was separated into two major flavoprotein components, A and B, after polyacrylamide gel electrophoresis. Elution of the proteins and subsequent kinetic assays of the eluates showed that component B catalyzes the reduction of butyryl-CoA dehydrogenase by reduced D-lactate dehydrogenase, whereas component A does not. Both A and B catalyzed the reduction of butyryl-CoA dehydrogenase by reduced nicotinamide adenine dinucleotide. The results suggest that the D-lactate dehydrogenase-dependent reduction involves a heretofore unrecognized component of the electron-transferring protein group which may utilize an unusual flavin, 6-hydroxy-7,8-dimethyl-10-(ribityl-5'-adenosine diphosphate)-isoalloxazine.

Peptostreptococcus elsdenii is an obligate anaerobic bacterium which ferments D,L-lactate principally to acetate, propionate, butyrate, and valerate (6). The principal electron acceptors formed in the fermentation are acrylyl-coenzyme A (CoA), crotonyl-CoA, and small amounts of longer chain length, α,β-unsaturated acyl-CoA's. The principal electron-donating reactions are the oxidation of D-lactate to pyruvate and the oxidation of pyruvate to acetyl-CoA and CO_2 (R. L. Baldwin, Ph.D. thesis, Michigan State University, East Lansing, 1962) (1, 10).

The path of electrons generated in the oxidation of pyruvate to α,β-unsaturated acyl-CoA has been established by Baldwin and Milligan (1). Electrons are transferred to ferredoxin and then to nicotinamide adenine dinucleotide (NAD). Reduced NAD (NADH) then reduces α,β-unsaturated acyl-CoA in a reaction requiring butyryl-CoA dehydrogenase and another protein fraction, "Fraction II." Recently, an electron-transferring flavoprotein which catalyzes the reduction of butyryl-CoA dehydrogenase or dyes by NADH was isolated from *P. elsdenii* (15). A second protein which exhibits only the NADH dehydrogenase activity was also purified. More detailed studies showed that these proteins differ only in the structure of the flavin cofactor. The enzyme catalyzing the former activity (ETF-NADH) contains predominantly flavin adenine dinucleotide (FAD), whereas the latter contains about 50% FAD and 50% modified flavins, largely 7-methyl-8-hydroxy-10-(ribityl-5'-adenosine diphosphate [ADP])-isoalloxazine (8-OH-FAD) (16).

In contrast, the path of electrons from lactate to α,β-unsaturated acyl-CoA has not been established. Baldwin and Milligan (1) partially purified a D-specific lactate dehydrogenase from extracts of *P. elsdenii*, but its role in electron transport was not determined. This paper describes the path of electrons derived from the oxidation of D-lactate to the reduction of α,β-unsaturated acyl-CoA in the lactate fermentation of *P. elsdenii*, and reports the existence of

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an electron-transferring flavoprotein different from one previously known for NADH. The relationship of NADH oxidation to this pathway was also established.

MATERIALS AND METHODS

Butyryl-CoA dehydrogenase. *P. elsdenii* (B159 strain, ATCC 17752) was grown anaerobically at 37 C for 12 h in deep culture on corn steep liquor and lactic acid as described by Ladd and Walker (9). Metals were also added as described for a defined medium (4). Log-phase cells were collected by continuous centrifugation, resuspended in an equal weight of water, and disrupted by two passages through a laboratory submicron dispersor (Manton-Gaulin Co., Everett, Mass.) at 1600 lb/in^2. Deoxyribonuclease (2 to 5 μg) was added to the preparation to lower the viscosity. A crude extract was obtained by centrifugation at 18,000 $\times$ *g* for 20 min to remove cell debris.

The butyryl-CoA dehydrogenase prepared from this extract was approximately 30% pure and contained only a single flavoprotein (H. Brockman, Ph.D. thesis, Michigan State University, East Lansing, 1971). This enzyme, which catalyzes the reduction of acrylyl- and crotonyl-CoA to propionate and butyrate (2), has recently been purified to homogeneity (7) and characterized (8) in another laboratory. The yellow form of the enzyme was generated from the green form by reduction with dithionite, followed by reoxidation with air.

D-Lactate dehydrogenase. The details of the purification and properties of D-lactate dehydrogenase from *P. elsdenii* are presented in an accompanying paper (3). The preparations employed in these studies were at least 50% pure based on polyacrylamide gel electrophoresis and optical scanning of the gels.

A unit of D-lactate dehydrogenase activity is that amount of enzyme which will reduce 1 μmol of ferricyanide per min in an assay containing 160 μmol of D-lactate, 0.5 μmol of potassium ferricyanide, and 10 μmol of potassium phosphate (pH 7.0), at 24 C in a total volume of 0.2 ml.

Assay of electron-transferring flavoprotein. To facilitate purification of electron-transferring flavoprotein, a spectrophotometric assay was developed which measured the rate of bleaching of the yellow form of butyryl-CoA dehydrogenase at 450 nm. Reduced D-lactate dehydrogenase was the electron donor and bleaching was catalyzed by a limiting amount of electron-transferring flavoprotein. Figure 1 shows the linear dependence of the rate of loss of absorbance at 450 nm on the amount of electron-transferring flavoprotein added to the assay. A unit of activity is defined as that amount of electron-transferring flavoprotein which gives a rate of bleaching of butyryl-CoA dehydrogenase of 1.0 absorbance unit per min at 450 nm at 37 C and pH 7.0.

Chemical preparation. Crotonyl-CoA and butyryl-CoA were prepared by the anhydride method of Stadtman (14).

Fatty acid determination. Butyrate formation was measured on acidified reaction mixtures on a dual-column gas chromatograph (Packard Instrument Co.) using 10% free fatty acid, aqueous phase, on Chromosorb W, 80–100 mesh (Wilkins Instrument Co.). Isobutyric acid was added as an internal standard.

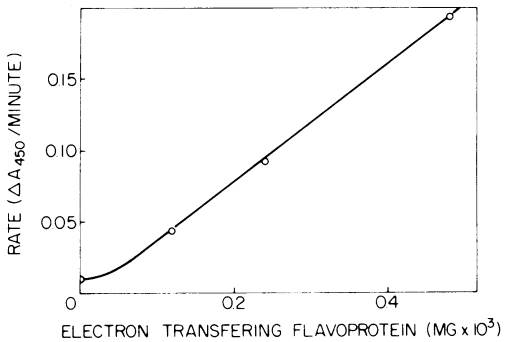

FIG. 1. *Dependence of the rate of electron transport on the amount of electron-transferring flavoprotein added. Each cuvette contained 8 nmol of yellow bytyryl-CoA dehydrogenase (molecular weight, 150,000), 80 μmol of* D*-lactate, 13.8 μmol of potassium phosphate (pH 7.0), and 2.4 units of* D*-lactate dehydrogenase in a total volume of 0.2 ml. Electron-transferring flavoprotein (22-fold purified) was added as indicated. Each assay was deaerated and maintained at 37 C. The initial velocity was plotted against the amount of electron-transferring flavoprotein added.*

Spectrophotometric measurements. All absorption spectra were obtained on a Cary 15 spectrophotometer using 2-ml cells with a light path of 1 cm. To obtain anaerobic conditions, the top of each cell was sleeved with 0.75-inch (about 1.9-cm ID) gum rubber tubing and, after addition of all but the last component, the top half of a septum from a Vacutainer (Becton-Dickinson & Co.) was inserted into the tubing. A syringe needle from a manifold system was inserted through the septum into the airspace over the liquid. Using this arrangement, the cuvette was partially evacuated and refilled with argon. This procedure was repeated several times to insure complete deaeration of the sample. The reaction was initiated by the addition of the last component through the septum from a Hamilton microsyringe.

Spectrophotometric rate measurements were obtained with a Gilford 2000 spectrophotometer. The cells were 0.5-ml microcuvettes of 1-cm light path equipped with rubber caps and deaerated as described above.

Electrophoresis. Polyacrylamide gels (7.5% cross-linking) were run at pH 8.3 as described by Davis (5). Stacking and spacer gels were not used. After electrophoresis in quartz tubes, the gels were scanned directly at 280 and 450 nm with a Gilford recording spectrophotometer equipped with a linear transport attachment.

RESULTS

Components of the system. The reduction of D-lactate by D-lactate dehydrogenase constitutes the first reaction of the pathway. As shown

in an accompanying paper (3), the flavin moiety of this enzyme is readily reduced by D-lactate, suggesting that the enzyme is an intermediate carrier of reducing equivalents. The ultimate acceptor is α,β-unsaturated acyl-CoA, which is reduced by the reduced form of butyryl-CoA dehydrogenase (7). Preliminary attempts to directly couple these two reactions using purified dehydrogenases were unsuccessful, suggesting that additional component(s) were required. Accordingly, we chromatographed a sample of extract from *P. elsdenii* and tested the resulting fractions for the ability to catalyze this transfer. One protein peak did contain this activity, which was designated ETF-LAC to distinguish it from other electron-transferring flavoprotein (ETF) activities associated with the butyryl-CoA dehydrogenase of *P. elsdenii* (15).

Figure 2 shows that a reconstituted system containing the three protein components plus D-lactate and crotonyl-CoA was able to reduce all the crotonyl-CoA. In the absence of any one component, the reaction did not proceed at a significant rate.

Purification of ETF-LAC. The purification of the enzyme(s) catalyzing this transfer of reducing equivalents was undertaken using a spectral assay based on the bleaching of butyryl-CoA dehydrogenase by reduced D-lactate dehydrogenase (see Materials and Methods).

Crude extract was applied to a column (2.5 by 25 cm) of diethylaminoethyl-cellulose, equilibrated with 0.1 M potassium phosphate buffer (pH 6.0), and the enzyme was eluted with a potassium phosphate buffer gradient from 0.1 to 0.3 M (pH 6.0). The peak activity fractions were pooled and brought to 70% saturation with ammonium sulfate. The resulting precipitate was dissolved in a minimum volume of 0.1 M phosphate buffer (pH 6.0) and chromatographed on Sephadex G-100 (2.5 by 90 cm) equilibrated with the same buffer. The ETF-LAC activity eluted as a small peak immediately after a much larger protein peak. The larger peak did not contain or stimulate the ETF-LAC activity. A summary of the purification of ETF-LAC from 30 g of *P. elsdenii* cells is presented in Table 1. Since the enzyme was highly labile, further purification steps were not routinely employed. The lability of the purified material was not decreased and was, in fact, enhanced by the addition of FAD or riboflavin 5'-phosphate (FMN) (1 μM), dithiothreitol (1 mM), ethylenediaminetetraacetic acid (10 mM), or bovine serum albumin (10 mg/ml).

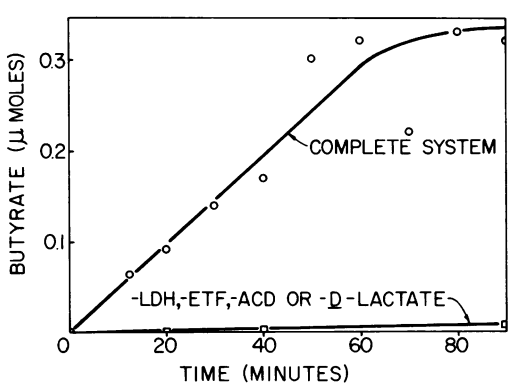

FIG. 2. *Butyrate production by the reconstituted electron transport system. Each sample in the complete system contained 0.49 mg of yellow butyryl-CoA dehydrogenase (ACD), 6 μg of electron-transferring flavoprotein (ETF), 80 μmol of D-lactate, 0.3 μmol of crotonyl-CoA, 2.75 μmol of potassium phosphate (pH 7.0), and 0.29 unit of D-lactate dehydrogenase (LDH), in a total volume of 0.176 ml. Each reaction was deaerated and maintained at 37 C. Butyrate was determined as described in Materials and Methods.*

Flavin analysis of the preparation by thin-layer chromatography (3) showed that the predominant flavin co-chromatographed with FAD. A small amount of another yellow, fluorescing material, neither FAD nor FMN, was also present.

Figure 3 demonstrates spectrally the ETF-LAC activity of our purified preparation. Curve 1 shows the spectrum of the oxidized, yellow form of butyryl-CoA dehydrogenase with D-lactate present. This spectrum was essentially unaltered by addition of D-lactate dehydrogenase. If ETF-LAC was also present, the spectrum was rapidly bleached (curve 3). Similar results were obtained when D-lactate and D-lactate dehydrogenase were replaced by NADH. Curve 2 shows the bleaching observed when the ETF-LAC preparation was present. These results indicate that our preparation contains at least two activities, ETF-LAC and ETF-NADH.

TABLE 1. *Purification of electron-transferring flavoprotein from P. elsdenii*

Step	Total units	Sp act (U/mg)	Recovery (%)	Purification (fold)
Crude extract	9,600	4	100	1
Diethylaminoethyl-cellulose, 0.1–0.3 M KPO_4^-, pH 6.0		36		9
G-100 Sephadex, 0.1 M KPO_4^-, pH 6.0	1,936	88	20	22

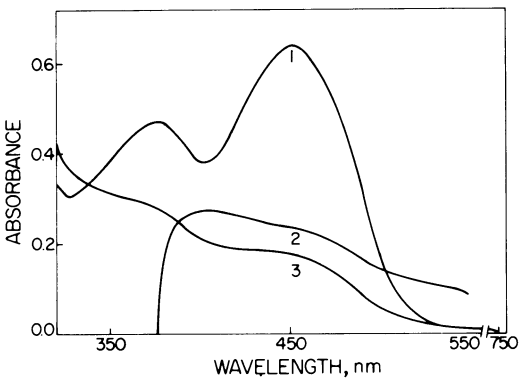

FIG. 3. *Bleaching of the yellow form of butyryl-CoA dehydrogenase by reduced D-lactate dehydrogenase or NADH. Each sample contained 3.5 mg of yellow butyryl-CoA dehydrogenase and 0.023 mmol of potassium phosphate (pH 7.0), in a volume of 0.5 ml, plus the following additions. Curve 1, ± 0.2 mmol of D-lactate; curve 2, + 55 μmol of NADH and 0.9 μg of electron-transferring flavoprotein; curve 3, + 0.078 unit of D-lactate dehydrogenase, 0.2 mmol of D-lactate, and 0.9 μg of electron-transferring flavoprotein. The reference cell for each sample contained 0.5 ml of 0.05 M potassium phosphate buffer (pH 7.0) plus the additions indicated above. Each sample was deaerated and maintained at 24 C. The spectra were recorded 30 min after the addition of the last component.*

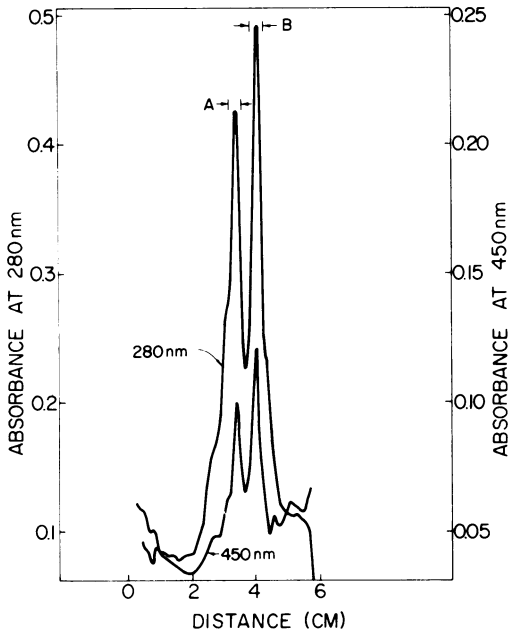

FIG. 4. *Absorbance tracings of electron-transferring flavoprotein after polyacrylamide disc gel electrophoresis. The 90-μg sample of enzyme was incubated for 1 h at 4 C in 0.01 M dithiothreitol prior to electrophoresis at 4 C.*

Substitution of the green butyryl-CoA dehydrogenase for the yellow form gave essentially the same results.

To determine the relationship between these activities and the proteins present in our ETF-LAC preparation, we combined polyacrylamide gel electrophoresis with ETF-LAC and ETF-NADH assays of the resulting bands. Figure 4 shows absorbance tracings of a polyacrylamide gel of 90 μg of electron-transferring flavoprotein on Sephadex G-100. The 280-nm trace (protein) indicated that this preparation contains roughly equal amounts of two proteins and the 450-nm trace suggested that both of these proteins are flavoproteins.

Gel slices containing each protein band, designated A and B in Fig. 4, were excised, cut into small pieces, and incubated in 0.05 ml of potassium phosphate buffer (pH 6.0) for 2 h at 4 C. A 0.02-ml volume of each eluate was then assayed for ETF-LAC and ETF-NADH activities (Table 2). The ETF-NADH assay was based on the spectral data shown in Fig. 3 and was similar to the ETF-LAC assay. However, it was not determined if the observed rates were proportional to the amount of enzyme added. Although the resolution of these proteins was incomplete, the results indicated that the material from band B is both necessary and sufficient to catalyze electron transfer from reduced D-lactate dehydrogenase to oxidized butyryl-CoA dehydrogenase, whereas that from band A is not. In contrast, material from both bands A and B contained the ETF-NADH

TABLE 2. *Electron-transferring flavoprotein activities of protein bands eluted from a polyacrylamide disc gel*[a]

Protein band	ΔA_{460}/min[b]	
	D-Lactate[c]	NADH[c]
A	0.0 (0.005)	0.026 (0.014)
B	0.015 (0.005)	0.038 (0.014)

[a] The assay mixture with D-lactate as the source of electrons contained the following: 0.35 mg of yellow butyryl-CoA dehydrogenase, 6.75 μmol of potassium phosphate buffer (pH 7.0), 80 μmol of D-lactate, 0.26 unit of D-lactate dehydrogenase, and 0.02 ml of gel eluate in a total volume of 0.2 ml. The mixture was deaerated and the assay was run at 37 C. With NADH as the electron donor, D-lactate and D-lactate dehydrogenase were replaced by 11 μmol of NADH and 1 μmol of potassium phosphate buffer (pH 7.0).

[b] The rates shown are corrected for the blank rates without electron-transferring flavoprotein. The blank rates are given in parentheses.

[c] Source of electrons.

activity. This was confirmed by immersing the gels in a solution of NADH and 2-*para*-indophenyl-3-*para*-nitrophenyl-5-phenyl tetrazolium chloride and showing that the region of bands A and B became stained.

The relation between the two proteins present in the purified ETF-LAC preparation was further deduced from spectral evidence. Curve 1 of Fig. 5 shows the spectrum 20-fold purified ETF-LAC from Sephadex G-100. It exhibits the normal maxima at 375 and 450 nm with atypical shoulders at 360 and 425 nm. In the presence of D-lactate, the spectrum is not altered, but if D-lactate dehydrogenase is also present in both the sample and reference cells, a partial bleaching of the spectrum occurs (curve 2). The 16% absorbance decrease in the 450-nm region shows that some bleaching of flavoprotein(s) in the preparation occurs but that the process does not proceed to completion. The bleaching does suggest, however, that one of the proteins acts as carrier of reducing equivalents from D-lactate dehydrogenase. Alternatively, the ability of NADH to serve as a source of reducing equivalents results in a much greater bleaching (curve 3), suggesting that both proteins are reduced, directly or indirectly, by NADH. The large negative absorbance at lower wavelengths presumably reflects an excess of NADH in the reference cell after the reduction of the flavins in the sample.

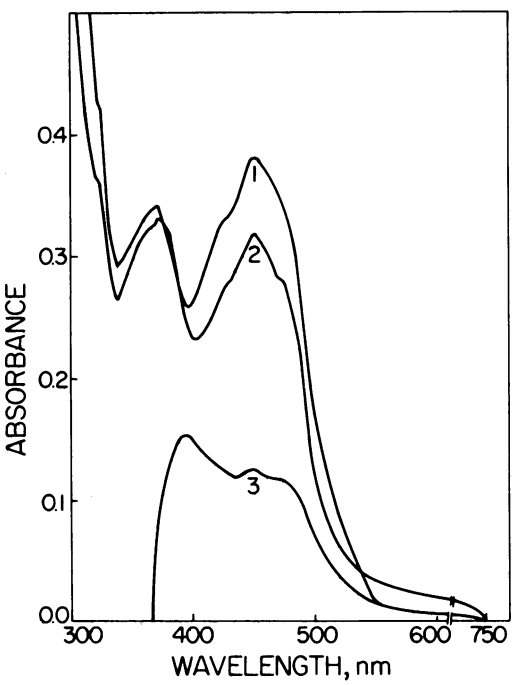

FIG. 5. *Bleaching of electron-transferring flavoprotein by reduced* D-*lactate dehydrogenase or NADH. Each sample contained 0.145 mg of electron-transferring flavoprotein and 25 μmol of potassium phosphate (pH 6.0), in a total volume of 0.5 ml plus the following additions. Curve 2, 20 μmol of* D-*lactate and 6.5 units of* D-*lactate dehydrogenase; curve 3, 55 μmol of NADH. The reference cell for each sample contained 0.5 ml of 0.05 M potassium phosphate buffer (pH 6.0), plus the additions described above. Each sample was deaerated and maintained at 24 C. The spectra were recorded 5 min after the addition of the last component.*

DISCUSSION

The foregoing experiments indicate the existence in *P. elsdenii* of a soluble, electron-transferring system between D-lactate and α,β-unsaturated acyl-CoA. The ability of D-lactate to reduce D-lactate dehydrogenase established the first reaction of the sequence and provided a means of producing reduced D-lactate dehydrogenase in situ (3). D-Lactate dehydrogenase reduced in this manner was incapable of reducing either the green or yellow form of butyryl-CoA dehydrogenase unless a heretofore unrecognized activity, designated ETF-LAC, was also present.

ETF-LAC activity was defined and assayed as the ability to catalyze transfer of reducing equivalents from reduced D-lactate dehydrogenase to oxidized butyryl-CoA dehydrogenase. The purified enzyme also contained ETF-NADH and NADH dehydrogenase activities. Polyacrylamide gel electrophoresis of this material revealed two major protein bands, each of which contained ETF-NADH and NADH dehydrogenase activities.

This separation of two flavoprotein bands containing these activities is similar to the results obtained by Whitfield and Mayhew (15) who purified ETF-NADH from *P. elsdenii* by a procedure similar to ours. Both our preparation and theirs exhibit spectral maxima at 450 and 375 nm with atypical shoulders at 360 and 425 nm. Whitfield and Mayhew showed that the two bands, separated by electrophoresis, as well as an NADH dehydrogenase isolated from *P. elsdenii*, differ only in the relative proportion of various flavins present. In addition to FAD, variable amounts of 6-hydroxy-7,8-dimethyl-10-(ribityl-5'-ADP)-isoalloxazine (6-OH-FAD) and 7 - methyl - 8 - hydroxy - 10 - (ribityl - 5' - ADP)-isoalloxazine (8-OH-FAD) were found. These flavins produce the atypical spectra which characterize the ETF-NADH and NADH dehydrogenase preparations (16). In particular, the shoulders at 360 and 425 nm are attributed to 6-OH-FAD. As reported here, our preparation also contained a chromophore which was

neither FAD nor FMN. These properties, summarized in Table 3, lead us to believe that the preparations are quite similar, it not identical.

An involvement of 6-OH-FAD in ETF-LAC activity is suggested by a comparison of the catalytic properties of ETF-LAC and ETF-NADH activities. Addition of FAD to an ETF-NADH preparation stimulates its activity 1.7- to 2.1-fold as measured after chromatography on Sephadex G-25 (15). Enzyme treated in this manner is 80 to 100% stable for months. In contrast, our ETF-LAC activity is not stimulated by FAD and incubation with this cofactor actually enhances activity loss during storage. Also, chromatography on Sephadex G-25 routinely results in a 20 to 40% loss of activity. These differences in activity and stability, coupled with the small and variable amounts of 6-OH-FAD present in ETF-NADH preparations, suggest (i) that the flavin, presumably 6-OH-FAD, required for ETF-LAC activity is not as tightly bound to the apoprotein as is FAD, and (ii) that replacement of this flavin by FAD results in a loss of ETF-LAC activity. These differences and similarities between the catalytic properties of ETF-LAC and ETF-NADH preparations are summarized in Table 3.

The atypical spectral characteristics, the presence of FAD and novel flavin, and behavior on polyacrylamide gels suggest that the preparations are nearly identical. Conversely, those properties based on catalytic activity show that the two electron-transferring activities are distinct and that the presence of ETF-LAC activity is correlated with the presence of 6-OH-FAD in the polyacrylamide gels. Thus, the data support the hypothesis (16) that the various electron-transferring flavoprotein activities in *P. elsdenii* share a common or very similar apoprotein, but differ in the flavin required for activity. This difference in flavin composition results in species with different electrophoretic mobilities.

The proposed relationship between the electron transfer enzymes and the other enzymes of lactate metabolism in *P. elsdenii* is shown in Fig. 6. Initially, D- and L-lactate are interconverted by a lactate racemase which has been partially purified and characterized (D. L. Schnieder, Ph.D. thesis, Michigan State University, East Lansing, 1969). D-Lactate is oxidized by D-lactate dehydrogenase, generating two reducing equivalents which are transferred to butyryl-CoA dehydrogenase, presumably via the 6-OH-FAD-containing electron-transferring flavoprotein. Pyruvate dehydrogenase complex oxidizes pyruvate to acetyl-CoA and CO_2 (S. G. Mayhew, and J. L. Peel, Biochem. J. **100**:80p, 1966; and reference 13), and donates reducing equivalents to a ferredoxin-type carrier. Rubredoxin (S. G. Mayhew and J. L. Peel, Biochem. J. **100**:80p, 1966) and flavodoxin (11) have been isolated from *P. elsdenii* and presumably can function in this reaction. The reducing equivalents from reduced ferredoxin may produce hydrogen gas or they may reduce NAD (1). NADH can then donate to butyryl-CoA dehydrogenase via the FAD-containing electron-

TABLE 3. *Comparison of electron-transferring factors for lactic dehydrogenase (ETF-LAC) and NADH (ETF-NADH)*

Property	ETF-LAC[a]		ETF-NADH[b]	
Absorbance maxima (nm)	372, 453		375, 400, 600	
Atypical absorbance shoulders (nm)	358, 426		360, 425	
Bleaching at 450 nm (% of A_{460})				
By NADH	68		83	
By reduced D-LDH	16		ND[c]	
Stability	Poor		Excellent	
Stimulation by FAD(%)	0		70–100	
Cofactor	FAD + unidentified		FAD + 6-OH-FAD, 8-OH-FAD	
Catalytic properties of bands on disc gels				
NADH dehydrogenase	+[d]	+[e]	+[d]	+[e]
ETF-NADH	+[d]	+[e]	+[d]	+[e]
ETF-LAC	−[d]	+[e]	ND	ND
High 6-OH-FAD	ND	ND	−[d]	+[e]

[a] Data from this paper.
[b] Data from references 14, 22, and 23.
[c] ND, Not determined.
[d] Slow.
[e] Fast

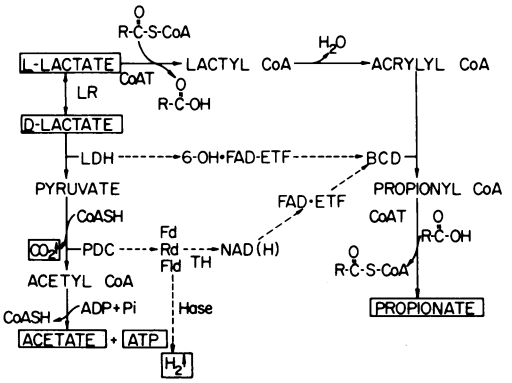

FIG. 6. *Lactate metabolism and electron transport in P. elsdenii. Abbreviations for enzyme names: LR, lactate racemase; LDH, D-lactate dehydrogenase; PDC, pyruvate dehydrogenase complex; PCS, the phosphorclastic system; CoAT, coenzyme A transferase; Fd, ferredoxin; Rd, rubredoxin; Fld, flavodoxin; Hase, hydrogenase; TH, ferredoxin:NAD oxidoreductase; ETF, electron-transferring flavoprotein; 6-OH-FAD, 6-hydroxy-7,8-dimethyl-10-(ribityl-5'-ADP)-isoalloxazine; BCD, butyryl-CoA dehydrogenase. Starting materials and final products are indicated by boxes. Solid arrows indicate reaction pathways, while dashed lines indicate the transfer of reducing equivalents.*

transferring flavoprotein (15). The transfer of electrons between the various species of electron-transferring flavoproteins is, as yet, unclear because completely pure species have not been used. However, our data would suggest that FAD-ETF is not reduced by reduced 6-OH-FAD·ETF.

CoA transferase, which activates lactate for the synthesis of acrylyl-CoA, has been purified to homogeneity (K. K. Tung and W. A. Wood, Fed. Proc. **30**:1266, 1971). The transferase also utilizes propionate and butyrate. The remaining reaction, the elimination of water from lactyl-CoA, remains to be established.

ACKNOWLEDGMENTS

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