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# THE SEPARATION OF PYRUVATE-FERREDOXIN OXIDOREDUCTASE FROM *CLOSTRIDIUM PASTEURIANUM* INTO TWO ENZYMES CATALYZING DIFFERENT REACTIONS

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## Summary

The ferredoxin requiring cleavage of pyruvate to acetyl-CoA and $CO_2$ is catalyzed by pyruvate ferredoxin oxidoreductase (pyruvate:ferredoxin oxidoreductase (CoA-acetylating), EC 1.2.7.1). The same enzyme is thought to catalyze the reversal of this reaction, i.e. the synthesis of pyruvate from acetyl-CoA and $CO_2$ in the presence of reduced ferredoxin. Evidence is presented that the forward and reverse reactions are catalyzed not by one, but by two proteins that are clearly separable by Sephadex G-200 gel filtration.

In a series of publications, Raeburn and Rabinowitz [1,2] and Uyeda and Rabinowitz [3,4] have proposed that pyruvate ferredoxin oxidoreductase (pyruvate:ferredoxin oxidoreductase (CoA-acetylating), EC 1.2.7.1) catalyzes the following reversible reaction:

$$\text{Pyruvate} + \text{CoA} + \text{Ferredoxin}_{ox} \rightleftharpoons \text{Acetyl-CoA} + CO_2 + \text{Ferredoxin}_{red}$$

Uyeda and Rabinowitz [3] have presented evidence that the oxidoreductase contains, in addition to thiamine pyrophosphate, a chromophore that can be either reduced or oxidized. By their mechanism, the same enzyme, when reduced, synthesizes pyruvate and when oxidized, decarboxylates pyruvate to acetyl-CoA and $CO_2$. Both reactions are thought to involve, as common intermediate, enzyme bound hydroxyethyl thiamine pyrophosphate. Although some purification of this enzyme has been achieved [3] the enzyme is unstable and rapidly inactivated by manipulation. In this report we show that

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pyruvate ferredoxin oxidoreductase isolated from *Clostridium pasteurianum* consists of two enzymes, one of which catalyzes the oxidative decarboxylation of pyruvate and the exchange reaction, the other catalyzes reductive carboxylation of acetate and $CO_2$ to pyruvate.

*C. pasteurianum* strain W-5 (A.T.C.C. 6013) was grown under nitrogen-fixing conditions in 20-l fermenter vessels in the medium of Carnahan et al. [5] containing 5.0 g of $CaCO_3$ /l. The cells were harvested during log phase and stored frozen. Packed cells (5 g) were suspended in 10 ml of 50 mM potassium phosphate (pH 7.5) containing 50 mM 2-mercaptoethanol and sonicated five times for 15 s at 1-min intervals (to prevent heat buildup) in a Bronson Sonifier Cell Disruptor (Heat Systems Co., Melville, L.I., New York) at 90—100 W. The enzyme-containing protein was isolated from high-speed, cell-free supernatant as described previously [6]. Active protein (42 mg), was put in Sephadex G-200 equilibrated with 50 mM potassium phosphate, pH 7.5 containing 10 mM dithiothreitol. Each fraction (2.5 ml) collected from the Sephadex column was assayed separately for these reactions:

$$\text{CoASH} + \text{Pyruvate} + \text{FAD} \rightarrow \text{Acetyl CoA} + CO_2 + FADH_2 \quad (1)$$

$$\text{Acetyl-CoA} + CO_2 + \text{Ferredoxin}_{red} \rightarrow \text{Pyruvate} + \text{Ferredoxin}_{ox} + \text{CoASH} \quad (2)$$

$$\text{Pyruvate} + {}^{14}CO_2 \rightarrow [1\text{-}{}^{14}C]\text{Pyruvate} + CO_2 . \quad (3)$$

Reaction 1 was measured by following the reduction of FAD spectrophotometrically at 450 nm in the assay system described previously [6]. Reaction 2, which measures the reductive carboxylation of acetate in the presence of enzyme and ferredoxin (photoreduced with illuminated spinach chloroplasts) was a modification [6] of the assay system of Buchanan [7]. Reaction 3, the pyruvate-$CO_2$ exchange reaction was carried out exactly as described by Raeburn and Rabinowitz [1]. As seen from the results in Fig. 1, the enzyme proteins that catalyze reactions 1, 2 and 3 are readily assayed after elution from Sephadex G-200. The enzyme protein that catalyzes the pyruvate-$CO_2$ exchange (Reaction 3) co-elutes from Sephadex G-200 with the pyruvate decarboxylating enzyme (Reaction 1).

It therefore seems reasonable to assume that the enzyme that catalyzes Reaction 1 is the same enzyme that catalyzes the exchange reaction. The enzyme that catalyzes the reductive carboxylation of acetate, Reaction 2, is clearly separable from the other two activities. The separation of the two enzymes is considerably improved by increasing the length of the Sephadex column (Fig. 1, inset), however, the enzymes are increasingly unstable with longer column lengths and considerable activity is lost.

Gehring and Arnon [8] have successfully separated 2-oxoglutarate synthase and pyruvate synthase from the cell free extract of *Chlorobium thiosulfatophilum*. This report shows that in *C. pasteurianum*, as in rumen microorganisms [6] the reductive synthesis and oxidative decarboxylation of pyruvate are not the result of a single reversible reaction but are catalyzed by two clearly separable proteins and that the assigned nomenclature for the previously described enzymes [9] is probably incorrect.

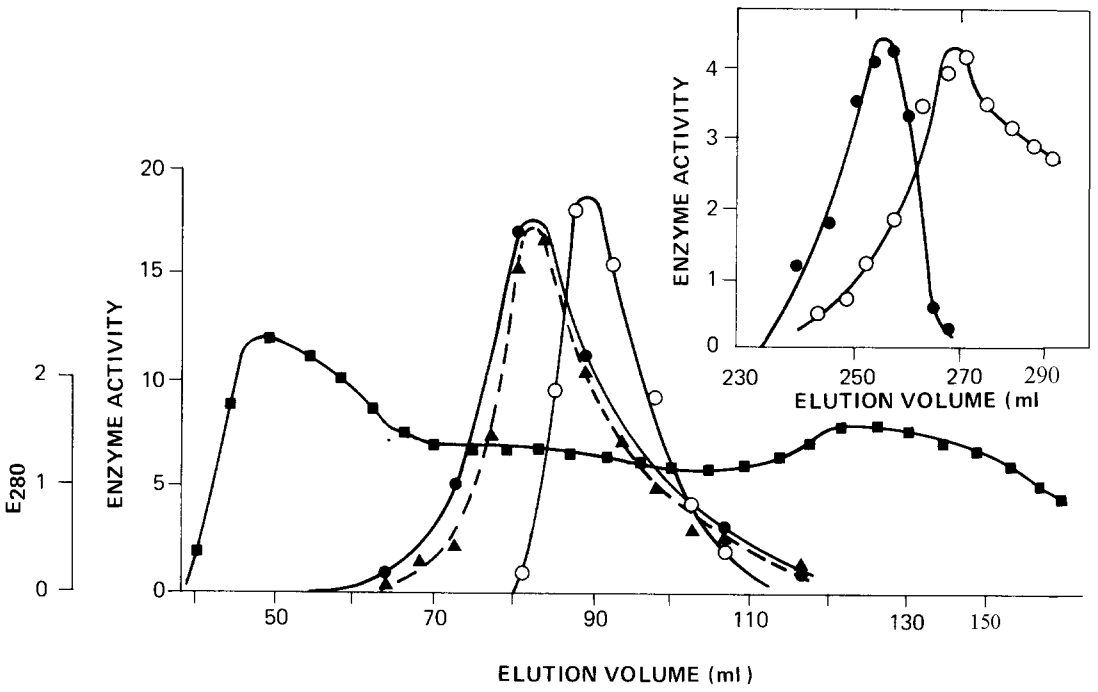


Fig. 1. Separation of the carboxylation and decarboxylation activities of pyruvate ferredoxin oxidoreductase on Sephadex G-200 (2.5 X 38.0 cm). Acetate carboxylation (○—○) (nmol pyruvate synthesized/ml per 30 min incubation); pyruvate decarboxylation (●—●) (μmol pyruvate decarboxylated/ml per 10 min); pyruvate-$CO_2$ exchange reaction (▲—▲) (dpm X $10^{-4}$ in pyruvate/ml per 30 min). Assays were done as described in text. Protein concentration (■—■) was monitored at 280 nm. Inset: repeat separation on Sephadex G-200 (2.5 X 80.0 cm).

## References


1 Raeburn, S. and Rabinowitz, J.C. (1971) Arch. Biochem. Biophys. 146, 9—20
2 Raeburn, S. and Rabinowitz, J.C. (1971) Arch. Biochem. Biophys. 146, 21—33
3 Uyeda, K. and Rabinowitz, J.C. (1971) J. Biol. Chem. 246, 3111—3119
4 Uyeda, K. and Rabinowitz, J.C. (1971) J. Biol. Chem. 246, 3120—3125
5 Carnahan, J.E., Mortensen, J.E., Mower, H.F. and Castle, J.E. (1960) Biochim. Biophys. Acta 44, 520—525
6 Bush, R.S. and Sauer, F.D. (1976) Biochem. J., in the press
7 Buchanan, B.B. (1969) J. Biol. Chem. 241, 4218—4223
8 Gehring, U. and Arnon, D.I. (1972) Biol. Chem. 247, 6963—6969
9 Enzyme Nomenclature (1972) p. 74. Elsevier Scientific Publishing Co., Amsterdam