ENZYMATIC FORMATION OF LACTATE AND ACETATE FROM α-HYDROXYGLUTARATE[1]

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Received May 16, 1963

It has recently been reported (Reeves and Ajl, 1962 a,b,c) that the enzyme, α-hydroxyglutarate synthetase, which catalyzes the stoichiometric condensation of propionyl-CoA and glyoxylate to form α-hydroxyglutaric acid, occurs in cell-free extracts obtained from Escherichia coli which have been grown aerobically in a simple mineral-salts medium with propionate as the sole carbon source. Further studies in our laboratory have shown that these same extracts also contain an enzyme(s), previously not described, which catalyzes the cleavage of this compound to lactate and acetate.

METHODS

E. coli was grown and cell-free extracts prepared as previously described (Reeves and Ajl, 1962 a). Enzyme activity was demonstrated in a reaction mixture which contained: 400 μM Tris.HCl buffer, pH 7.5;260 μM disodium α-hydroxyglutarate; 2.0 ml of extract (34 mg protein) and water to a total volume of 6.0 ml. Tubes in which either the α-hydroxyglutarate was omitted or which contained boiled extract were used as controls. Incubation was for 2 hours at 30 C under an atmosphere of nitrogen. The reaction

1 Supported by grants from the National Institute of Health (AI 03866) and the National Science Foundation (G 23434)

2 N.I.H. Research Career Development Award Investigator (AI-K3-6928)

was terminated by the addition of 1.0 ml each of 15% sodium tungstate and 50% H_2SO_4, the protein removed by centrifugation and the supernatant adjusted to pH 2-3 with 10 M KOH. This was then continuously extracted with ether for 24 hours. The ether was evaporated, the residue dissolved in 0.5 ml of water and aliquots spotted on Whatman No. 1 filter paper.

RESULTS

The papers were chromotographed, using the ascending technique, at 26 C in one of several different solvent systems. The results are shown in the following table:

TABLE 1

CHROMATOGRAPHIC CHARACTERIZATION OF ACETATE AND LACTATE

Solvent *	R_f Experimental	R_f Authentic Lactate	R_f Authentic Acetate
I	0.60	0.60	-
II	0.86	0.85	-
III	0.46	-	0.47
IV	0.27	-	0.27

* I = Ethanol:Buffer (Aqueous solution of ammonia and ammonium carbonate, 1.5 N with respect to each) - (7:3 v/v).

II = Ethyl acetate: Formic acid: Water (10:2:3)

III = Ethanol: Water: 0.88 Ammonia (35:13:2 v/v) containing thymol blue (0.03% w/v).

IV = n-Propanol: Conc. NH_4OH (7:3)

Spots with R_f's corresponding to authentic lactate and acetate were found in aliquots of the ether extract from the tube containing the com-

plete reaction mixture. No spots corresponding to either lactate or acetate were found in aliquots from either of the two control tubes. The spot at R_f 0.60 in solvent system No. 1 was further identified as lactate by the o-hydroxydiphenyl and hydrogen sulfide tests (Feigl, 1960). This spot also gave no reaction with keto acid reagents such as semicarbazide or 2,4-dinitrophenylhydrazine.

In order to further identify acetate and lactate as the products of the enzymatic reaction, silicic acid columns were employed. The silicic acid columns were prepared and used as follows: 3.0 gms of Mallinckrodt Chromatographic Grade Silicic Acid (100 mesh) was mixed with 2.0 ml of 0.05 M H_2SO_4 in a mortar and slurried with 20.0 ml of 10% n-butanol (v/v) in water saturated chloroform (CB-10). The mixture was transferred to a glass column (10 mm x 320 mm) containing a small cotton plug in the bottom and allowed to pack by gravity. Aliquots (0.2 ml), of the extracted acids obtained as previously described but dissolved in 0.5 ml of CB-10, were applied to the column and eluted using CB-10. Four ml fractions were collected and titrated with 0.002 M KOH in methanol after the addition of 1 drop of 0.04% (w/v) cresol red in methanol as indicator.

As can be seen in Fig. 1, this system affords excellent separation of acetate and lactate. From the titration data, it can be calculated that 119 μM of acetate and 59 μM of lactate were recovered from the 260 μM of α-hydroxyglutarate present in the original reaction mixture. The low yields may be due to the omission of necessary co-factors; we are presently investigating this possibility. The lack of stoichiometry between the acetate and lactate recovered may indicate that the lactate is further metabolized by the crude extracts.

Since E. coli grown under the conditions described contain the enzyme of the TCA cycle and isocitrate lyase (EC 4.1.3.1) activity in addition to the enzymes catalyzing both the synthesis and cleavage

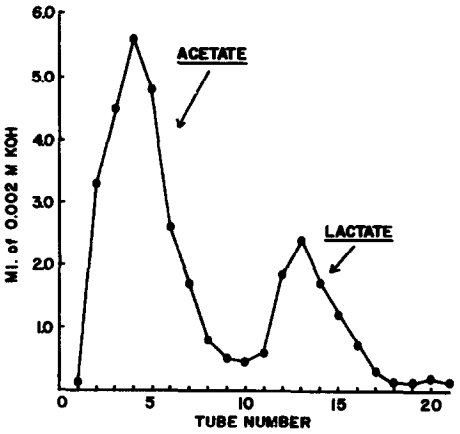

Fig. 1. Chromatographic resolution and identification of lactate and acetate. (See text for details)

of α-hydroxyglutarate, the series of reactions shown in Fig. 2 may play a role in the growth of these cells that have been adapted to utilize propionate as sole carbon source.

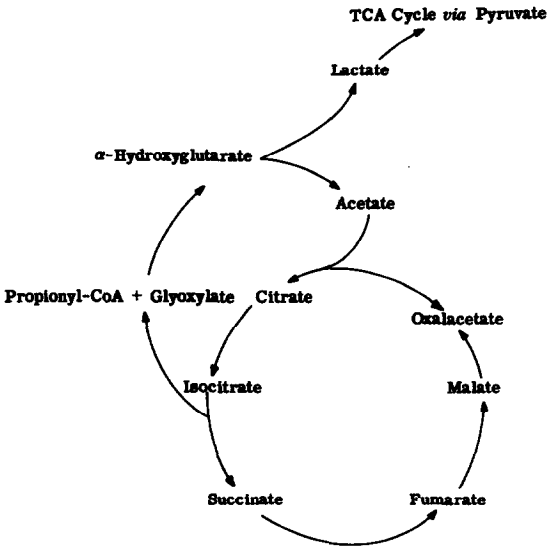

Fig. 2. Suggested pathway of propionate metabolism in Escherichia coli.

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