NOTES

ALPHA-HYDROXYGLUTARIC ACID SYNTHETASE

HENRY C. REEVES AND SAMUEL J. AJL

Research Laboratories, Department of Biochemistry, Albert Einstein Medical Center, Philadelphia, Pennsylvania

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In studying the specificity of highly purified malate synthetase (Wong and Ajl, J. Am. Chem. Soc. **78**:3220, 1956), it was found that there was no reaction with either propionyl-coenzyme A (CoA) or butyryl-CoA in the presence of glyoxylate. It occurred to us, however, that the synthesis of organic molecules larger than malic acid may be possible by mechanisms similar to that of malate synthetase, provided the cells are adapted to grow on compounds other than acetate. Thus, propionate-grown *Escherichia coli* was shown to contain an enzyme which catalyzes the formation of alpha-hydroxyglutaric acid from propionyl-CoA and glyoxylate. This new enzyme has tentatively been named alpha-hydroxyglutarate synthetase.

E. coli E-26 was grown with vigorous aeration for 48 hr at 37 C in the mineral salts medium previously described (Reeves and Ajl, J. Bacteriol. **79**:341, 1960), with 0.2% sodium propionate as the sole carbon source. The cells were harvested, disrupted in a sonic oscillator, and the debris removed by centrifugation for 30 min at 15,000 × *g*. The supernatant fluid constituted the enzyme preparation.

An equimolar relationship exists between the disappearance of glyoxylate and propionyl-CoA (Fig. 1). That the reaction is enzymatic has been shown by the fact that no disappearance of either of the substrates takes place in the presence of boiled extract. In the presence of both glyoxylate and propionyl-CoA, alpha-hydroxyglutarate is formed (Fig. 2). No formation of the latter is apparent when either glyoxylate or propionyl-CoA is omitted or when a boiled extract is used We are now in the process of developing a quantitative assay for the product.

Alpha-hydroxyglutaric acid was identified qualitatively as a product of the enzymatic reaction by paper chromatography, utilizing two solvent systems. A sample of an ether extract from the reaction mixture gave a spot (Fig. 2, A_3 and B_3) with the same R_F value as authentic alpha-hydroxyglutarate (Fig. 2, A_1 and B_1) in both solvent systems. Further, a mixture of

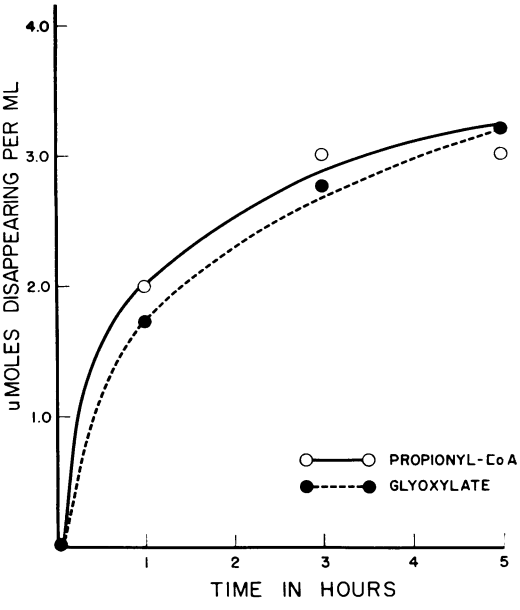

FIG. 1. *Enzymatic disappearance of glyoxylate and propionyl-CoA. The complete reaction mixture contained the following, expressed as μmoles: tris·HCl buffer (pH 8.0), 100; $MgCl_2$, 5.0; sodium glyoxylate, 13.0; propionyl-CoA, 12.0; and 0.5 ml of either crude extract (2.8 mg protein) in the experimental, or 0.5 ml of boiled extract in the control. The total volume was 1.5 ml; incubation was at 37 C. Propionyl-CoA was prepared according to the method of Simon and Shemin (J. Am. Chem. Soc.* **75**:*2520, 1953). The reaction was stopped by the addition of 7% sodium tungstate in 25% H_2SO_4, and glyoxylate was determined according to McFadden and Homes (Anal. Biochem.* **1**:*240, 1959); propionyl-CoA according to Lipmann and Tuttle (J. Biol. Chem.* **159**:*21, 1945), and protein by the method of Warburg and Christian (Biochem. Z.* **310**:*384, 1945). All values are corrected for non-enzymatic disappearance.*

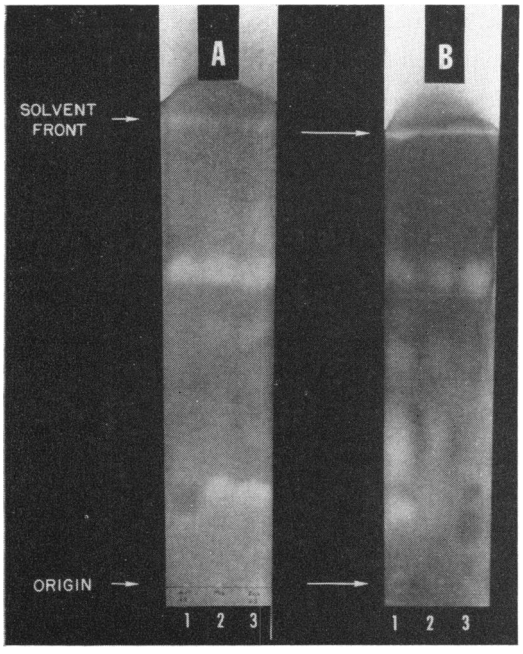

FIG. 2. *Enzymatic formation of alpha-hydroxyglutaric acid. The complete reaction mixture is the same as described in Fig. 1. No formation of alpha-hydroxyglutarate was observed in control tubes which contained boiled extract, or in which either glyoxylate or propionyl-CoA were omitted. After incubation for 6 hr at 37 C, the reaction was stopped by the addition of 7% sodium tungstate in 25% H_2SO_4. Protein was removed by centrifugation and the supernatant extracted continuously with ether for 18 hr in a Kutscher-Steudel type extractor (Umbreit et al., Manometric techniques, 3rd ed., Burgess Publishing Co., Minneapolis, 1957). The ether was then evaporated off on a steam bath and the residue dissolved in 2.0 ml of water. Authentic alpha-hydroxyglutaric acid was prepared according to the method of Fischer and Moreschi (Ber. Deut. Chem. Ges. 45:2447, 1912). Solvent system A was n-butanol: formic acid: water (4:1.5:1). Solvent system B was ether: formic acid: water (5:2:1). Chromatography was ascending at 26 C. Acids were located by spraying with 0.04% bromcresol green in 95% ethanol.*

authentic alpha-hydroxyglutarate and the experimental product produced only one spot (Fig. 2, A_2 and B_2). The R_F of 0.61 in the *n*-butanol–formic acid–water system is identical with the value reported by Beppu and Arima (J. Biochem. (Tokyo) **48**:557, 1960) for alpha-hydroxyglutarate in this solvent system.

The function of malate synthetase in cell metabolism has recently been explained on the basis of fulfilling an essential gap in our knowledge of C_2 metabolism in bacteria (Wong and Ajl, Science **126**:3281, 1957; Kornberg, Ann. Rev. Microbiol. **13**:102, 1959; Reeves and Ajl, J. Bacteriol. **79**:341, 1960). Since alpha-hydroxyglutarate synthetase also catalyzes the *de novo* formation of carbon-carbon bonds, it too may play an important role in the biosynthesis of cell constituents. The precise function of this new enzyme in the growth of bacteria on propionate as the sole source of carbon is now being investigated.

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RESISTANCE OF RHIZOBIA TO ANTIMICROBIAL AGENTS

R. J. DAVIS

Soil and Water Conservation Research Division, Soils Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

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Reports on the reaction of the rhizobia toward various antimicrobial agents are minimal. Recent interest in the genetics of these organisms made such knowledge desirable. A total of 21 rhizobial strains from various sources, representing seven cross-inoculation groups, was checked against 34 agents. These strains were selected at random from stock cultures. Resistance, or sensitivity, was determined, using Bacto sensitivity discs on spread plates. Standard yeast-mannitol agar with tris(hydroxymethyl)aminomethane buffer was employed. All 21 strains were resistant to the following agents: sulfamethoxypyridazine, 300 μg; sulfisomidine, 300 μg; sulfadiazine, 300 μg; sulfamerazine, 300 μg; sulfathiazole, 300 μg; sulfamethylthiadiazole, 300 μg; Triple sulfa