THE BIOSYNTHETIC L-α-GLYCEROL PHOSPHATE DEHYDROGENASE OF E. COLI

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Received June 19, 1968

L-α glycerol phosphate* is required by E. coli for the synthesis of phosphatidic acid, (Pieringer; Ailhaud and Vagelos) but the reactions that produce this compound have not been well defined. When the organism is growing with glycerol as the carbon source glycerol-P can be directly removed from the catabolic pathway for biosynthetic purposes, but growth on other carbon sources necessitates that glycerol-P be produced by a discreet biosynthetic reaction. This report describes the partial purification of a glycerol-P dehydrogenase from glucose grown E. coli which analogous to the enzyme from mammalian tissue utilizes pyridine nucleotides to produce glycerol-P from dihydroxyacetone-P (Baranowski). On the basis of its cofactor requirement and its presence in a mutant strain lacking the catabolic enzyme, this enzyme is distinguishable from the enzyme described by Lin et al. Its inhibition by long chain acyl-CoA derivatives affords a means for adjusting glycerol-P production to the concentration of the other substrate used for phosphatidic acid synthesis.

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*Abbreviations used are: glycerol-P, L-α-glycerol phosphate, Pal-CoA, palmitoyl-CoA, Ste-CoA, stearoyl-CoA.

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Materials and Methods — Growth of bacteria and preparation of enzymes.

One liter batches of E. coli strain B were grown at 37° C with airation in a minimal medium (Pizer and Potochny) supplemented with 0.1% casamino acids and 0.2% glucose. When the Klett reading reached 160 (No. 43 filter), the cells were harvested by centrifugation, resuspended in 15 ml of 10 mM triethanolamine-HCl buffer, pH 7.5 and disrupted by sonication. Cell debris was removed by centrifugation and nucleic acids were precipitated by the addition of 5 ml of 5% streptomycin sulfate. The precipitate was removed by centrifugation and the supernatant fluid fractionated with ammonium sulfate. Glycerol-P dehydrogenase was present in the fraction obtained between 45 and 55% saturation. All centrifugations were performed at 0-4° C in a Servall RC2 centrifuge. Ammonium sulfate was added in the form of a saturated solution previously adjusted to pH 7.0 with NH_4OH. All buffers contain 10 mM dithiothreitol.

Chemicals and chemical procedures - Pal-CoA, Ste-CoA, DL-α-glycerol-P, DPNH, and TPNH were purchased from Sigma. Dihydroxyacetone phosphate demethylketal was synthesized by the procedure of Ballou and Fischer. Glycerol-P was determined enzymatically (Hohorst). Protein was determined by the procedure of Lowry et al.

Enzyme assay — Glycerol-P dehydrogenase was assayed by following the dihydroxyacetone-P dependent oxidation of TPNH in a Zeiss spectrophotometer. The standard assay contained in 0.5 ml, 30 μmoles triethanolamine-HCl buffer, pH 7.5, 5 μmoles dithiotreitol, 0.05 μmoles TPNH, 0.4 μmoles dihydroxyacetone-P and enzyme. The reaction which was run at 25° C was initiated by the addition of substrate. The absorbancy changes were measured at 15 second intervals for 1.5 minutes. One unit of enzyme activity equals 1 mμ mole of TPNH oxidized per minute.

Results. Properties of glycerol-P dehydrogenase — The dehydrogenase preparation obtained by dissolving the amonium sulfate precipitate in 10 mM phosphate buffer, pH 7.5 was stable for at least two weeks when stored at -20° C. Prior to kinetic studies the phosphate buffer was exchanged for triethanolamine-HCl buffer, pH 7.5 by passage through a Sephadex G-25 column. The column effluent had 28 units of enzyme activity per mg of protein. Preparations with comparable activity were obtained from E. coli B and E. coli K10 strain 8, which lacks the catabolic glycerol-P dehydrogenase (Hayashi and Lin). The pH optimum was 7.5 and enzyme activity was reduced to 46% when TPNH was replaced by DPNH. To establish that the product of the reaction was L-α-glycerol-P the volume of the assay was increased and after incubation the enzyme was precipitated with $HClO_4$. The recovery of L-α-glycerol-P in samples of the supernatant fluid was 75% of that expected from the observed TPNH oxidation. Glycerol-P was not detected in control incubations that lacked with TPNH or dihydroxyacetone-P.

Inhibition of glycerol-P dehydrogenase. Glycerol-P dehydrogenase activity was strongly inhibited when either Pal-CoA or Ste-CoA was added to the enzyme assay. This inhibition was specific since acetyl-CoA had no effect. The relationship between long chain acyl-CoA's concentration and enzyme activity (Fig. 1A) shows that for both compounds maximum inhibition was 90% and 25 μM produced about 50% inhibition. The sigmoidicity of the Pal-CoA curve and the stepwise character of the Ste-CoA curve indicate that the enzyme possesses multiple binding sites for acyl-CoAs. The modified Hill plot, (Fig. 1B), had a slope of 3.1 for Pal-CoA suggesting that cooperative binding of this compound occurred at a minimum of 4 binding sites (Monod, et al.). The binding of Ste-CoA appeared to involve two classes of noninteracting binding sites with distinguishable

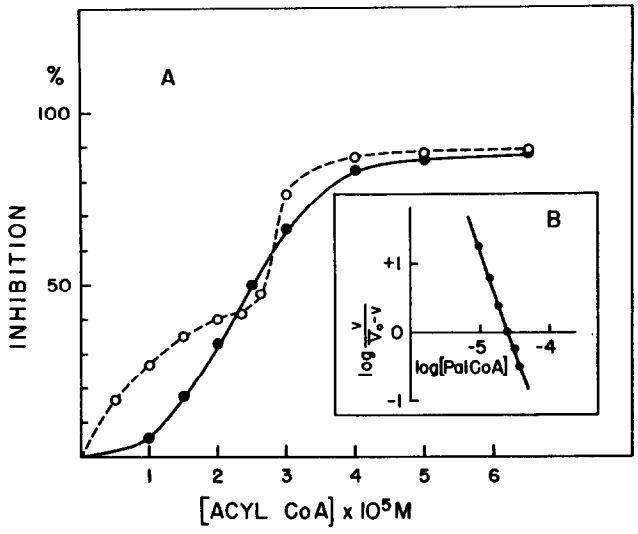

Fig. 1: Inhibition of glycerol-P dehydrogenase by Pal-CoA and Ste-CoA. A. Pal-CoA ● - ●. Ste-CoA 0 - 0. B. Hill plot of data for Pal-CoA.

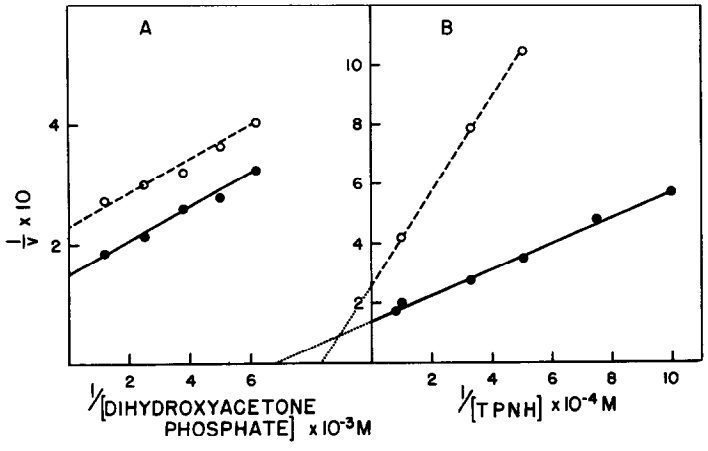

Fig. 2: Kinetics of glycerol-P dehydrogenase. A. The dihydroxyacetone-P concentration was varied as shown. B. The TPNH concentration was varied as shown. No Pal-CoA ● - ●. Plus 20 μM Pal-CoA 0 -- 0.

affinities. The results of kinetic studies of Pal-CoA inhibition (Fig. 2A and B) show that inhibition was uncompetitive in respect to dihydroxyacetone-P and of the "mixed" type with respect to TPNH (Dixon and Webb). The apparent

Km's for dihydroxyacetone-P and TPNH were 180 μM and 31 μM respectively. No suggestion of cooperation between binding sites for substrate or co-enzyme was observed.

Discussion — That the enzyme described in this paper is the biosynthetic glycerol-P dehydrogenase was inferred from the fact that it was present in E. coli K10 strain 8 which lacks the catabolic dehydrogenase.

In E. coli, as has been described by Bortz and Lynen for rat liver, Pal-CoA regulates fatty acid synthesis by inhibiting acetyl-CoA carboxylase (Kito and Pizer, unpublished data). The Pal-CoA concentrations required to inhibit the glycerol-P dehydrogenase and acetyl-CoA carboxylase were similar and higher than those needed for phosphatidic acid synthesis (Kito and Pizer, unpublished data). The inhibition by Pal-CoA of these two enzymes could provide an effective means for balancing the supply of substrates for phosphatidic acid synthesis.

Acknowledgements — We wish to thank Dr. E. Lin for sending us E. coli strain K10-8. Supported by grant AI-03957 from the National Institutes of Health.

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