A MUTANT OF ESCHERICHIA COLI DEFECTIVE IN PHOSPHATIDIC ACID SYNTHESIS

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Most bacterial mutants with nutritional requirements have been found to be defective in a biosynthetic pathway. This report describes an auxotroph whose defect does not lie in the synthesis of the required nutrient, L-glycerol-3-phosphate (glycerol-P), but in an increased Km for utilization of glycerol-P as a precursor of phosphatidic acid. The pool of glycerol-P cannot be enlarged to compensate for this increased Km because glycerol-P inhibits its own biosynthetic enzyme, pyridine nucleotide linked glycerol-P dehydrogenase (EC 1.1.1.8).

Materials and Methods

L-glycerol-P and dithiothreitol were purchased from Calbiochem. TPNH, DL-glycerol-P and palmitoyl-CoA were purchased from Sigma Chemical Co. ^{14}C-glycerol-P was enzymatically synthesized and dihydroxyacetone phosphate was synthesized by the procedure of Ballou and Fischer.

Glycerol-P dehydrogenase was prepared and assayed as previously described (Kito and Pizer) except that the ammonium sulfate fraction contained the protein precipitated by 60% saturation. The glycerol-P transacylase was prepared as a particulate suspension from sonicated cells and was assayed by the modified procedure of Ailhaud and Vagelos described in the legend to Figure 1 (Kito and Pizer, J. Bacteriol., in press).

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Escherichia coli strain K951 has a deletion in the alkaline phosphatase structural gene (strain E15 of Garen and Levinthal); it lacks glycerol kinase and the catabolic glycerol-P dehydrogenase, it is constitutive for glycerol-P uptake (strain 9 of Hayashi, Koch, and Lin); and is auxotrophic for L-leucine. Strain K956 is additionally auxotrophic for glycerol-P, and was derived from K951 by ultraviolet mutagenesis followed by repeated enrichment by the penicillin selection method (Lubin). K956 does not respond to β-glycerophosphate, and the response to limiting amounts of DL-glycerol-P suggests that only the L-isomer promotes growth. Spontaneous phenotypic revertants of K956, no longer requiring glycerol-P for growth, were readily selected; one was chosen for further study (strain K956-R).

For the experiments described in this paper bacteria were grown at 37° in modified M-9 medium (Pizer and Potochny) supplemented with 2 mg/ml of glucose, 50 μg/ml of L-leucine and 0.1% casein hydrolysate (Nutritional Biochemicals). DL-glycerol-P, when present, was at 1 mM.

Results

Growth studies performed in liquid media showed that strains K951 and K956-R had a doubling time of approximately 70 minutes whether or not the medium was supplemented with glycerol-P. Strain K956 had doubling times of 100 and 300 minutes in the presence and absence of glycerol-P. Differences in the rates of growth were more pronounced when cells of strain K956 were spread on solid medium containing 0.01% casein hydrolysate. In the presence of glycerol-P colonies approximately 2 mm in diameter were observed in 24 hours while in the absence of glycerol-P five days were required to produce 0.5 mm colonies. The growth studies indicate that strain K956 has a "leaky" requirement for glycerol-P.

Although it had been initially expected that the requirement for glycerol-P was due to a defect in the presumptive biosynthetic enzyme, glycerol-P dehydrogenase, no significant difference in the activity of this enzyme in parent and mutant strains was found: the ammonium sulfate fractions obtained from K956 and K951 showed activities of 4.8 and 4.3 mμmoles/min/mg of protein. Moreover the enzyme from both organisms showed the same degree of sensitivity

Table I. Inhibition of Glycerol-P Dehydrogenase by Glycerol-P

Glycerol-P (mM)	Activity (%)	
	Strain K956	Strain K951
None	100	100
0.035	48	50
0.060	30	30
0.300	9	3

Activity was measured by following the dihydroxyacetone-phosphate dependent disappearance of TPNH. 100% activity corresponded to 4.8 mμmoles of TPNH oxidized per min per mg.

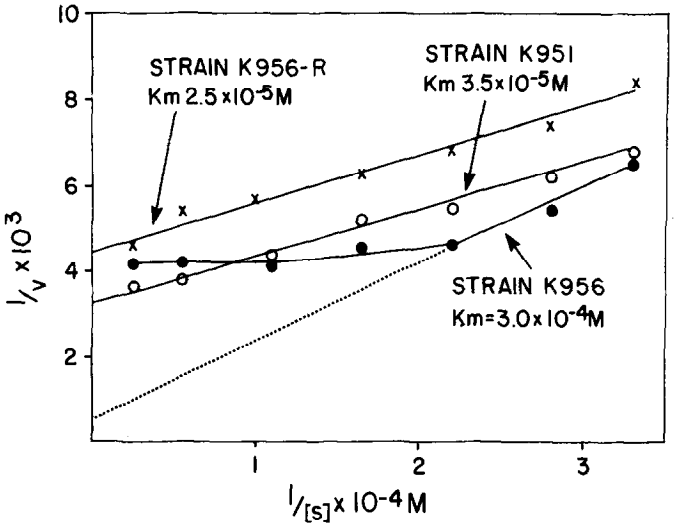

Figure Legend

Kinetics of glycerol-P, transacylase. A 0.15 ml incubation mixture contained: 10 μmoles 2-amino-methyl-propane-diol buffer (pH 8.3), 2 μmoles, $MgCl_2$, the indicated amounts of ^{14}C-glycerol-P (1,860 cpm mμ mole), 6.5 mμmoles palmitoyl-CoA, 50 mμmoles dithiothreitol and 50 μg of enzyme protein. The incubation was for 10 minutes at 10 C. 50 μl of the incubation was placed on the paper disk which was then immersed in trichloroacetic acid, washed, dried and counted in a scintillation counter. The data is presented as c.p.m. per disk.

to inhibition by glycerol-P (Table I). This inhibition was identical to that found with purified glycerol-P dehydrogenase (Kito and Pizer, manuscript in preparation). Since the biosynthetic enzyme appeared to be normal, the properties of glycerol-P transacylase, which catalyzes the

acylation of glycerol-P to form phosphatidic acid, were examined. The Km for glycerol-P was found to be about ten times higher for the enzyme preparation from strain K956 than that from the parent strain K951. The transacylase in the mutant also differed from the parental enzyme in that it showed substrate inhibition and relatively lower activity at pH values of 7.5 and 7.1 (Figure 1 and Table 2). The transacylase preparation obtained from K956-R was similar to that from K951, further indicating that the restricted growth of K956 was due to an altered glycerol-P transacylase.

Table 2. Effect of pH on Glycerol-P transacylase

	Activity (%)	
pH	Strain K956	Strain K951
8.3	100	100
7.5	78	88
7.1	29	36

The assay conditions described in the legend to Fig. 1 were used with 0.18 mM glycerol-P as substrate. Optimum activity is obtained at pH 8.3. Kito and Pizer, J. Bacteriol., in press.

Discussion

These results suggest that the nutritional requirement of strain K956 for glycerol-P is due to an increased Km for glycerol-P in the transacylase reaction responsible for phosphatidic acid synthesis. The rate of this reaction is presumably limited by the size of the intracellular glycerol-P pool, which in turn is dependent on the rate of conversion of dihydroxyacetone phosphate to glycerol-P. Because the enzyme that catalyzes this latter reaction is inhibited by high concentrations of glycerol-P, the intracellular concentration of glycerol-P cannot increase sufficiently to compensate for the increase in the Km of the mutant transacylase. For the mutant transacylase to function at half maximum velocity, 0.3 mM glycerol-P was required. At this concentration, biosynthesis of glycerol-P by the dehydrogenase is inhibited by greater than 90% (Table I). Recent studies with the purified enzyme show that inhibition

of dehydrogenase is accentuated by cooperative effects in the binding of glycerol-P, and that the activity of the dehydrogenase declines sharply as the glycerol-P concentration increases (Kito and Pizer, unpublished data). The auxotrophic phenotype of K956 appears to result, then, from the special circumstance of a failure of the mutant to compensate for the increased Km for glycerol-P because of the controls exerted by glycerol-P on its own rate of synthesis.

Based on this conclusion additional inferences can be made. In K956 the transacylase reaction presumably limits the overall rate of phospholipid; since a reduction in lipid synthesis reduced the growth rate, both processes must be interdependent. The quantity of transacylase does not appear to increase so as to compensate for the low rate of transacylation. Lastly, whether the acyl donor is acyl-CoA or acyl-carrier protein (Ailhaud and Vagelos), a single enzyme catalyzes the transacylation reaction.

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