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# Second Pyridine Nucleotide-Independent L-α-Glycerophosphate Dehydrogenase in *Escherichia coli* K-12

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A soluble L-α-glycerophosphate dehydrogenase, which is stimulated by FAD and is independent of added pyridine nucleotides, functions during anaerobic catabolism of glycerol and L-α-glycerophosphate by *Escherichia coli*.

Aerobic catabolism of both glycerol and L-α-glycerophosphate (L-α-GP) by *Escherichia coli* requires an L-α-GP dehydrogenase (E. C. 1.1.2.1) that is particulate and independent of added pyridine nucleotides. Mutants lacking this enzyme fail to grow areobically on either glycerol or L-α-GP (4).

Glycerol or L-α-GP can also be dissimilated anaerobically by *E. coli* if a suitable electron acceptor such as nitrate (8) or fumarate (5) is available. We have recently found that mutants lacking the known L-α-GP dehydrogenase are unimpaired in their ability to metabolize either glycerol or L-α-GP anaerobically. This behavior is true for six independent mutants, including one with a partial deletion of the structural gene for the known dehydrogenase. Figure 1 depicts the anaerobic growth of one such mutant, strain 95, which lacks the known dehydrogenase and is totally incapable of growth aerobically on glycerol or L-α-GP (1). The medium contains L-α-GP as the principal carbon source and $KNO_3$ as oxidant. Except for a slight lag (possibly due to residual $O_2$ dissolved in the medium), the mutant grew as well as its wild-type parent, strain 1 [derived from *E. coli* K-12 Hfr Cavalli (4)]. Similar results were obtained when glycerol served as the principal carbon source.

While searching for an enzymatic basis for the anaerobic utilization of glycerol and L-α-GP by mutant strain 95, we found that cell-free extracts prepared from cultures grown anaerobically on glycerol or L-α-GP contain a second L-α-GP dehydrogenase hitherto unnoticed. For reasons described below, this enzyme will be termed the soluble L-α-GP dehydrogenase. (The known enzyme is characterized as particulate because centrifugation at 100,000 × *g* for 60 min sediments approximately 50% of its activity from cell-free extracts prepared by sonic disruption.) This second enzyme, like the particulate one, is independent of added pyridine nucleotides and can be assayed by the phenazine methosulfate-mediated reduction of a tetrazolium dye (q.v., Fig. 2). However, in contrast to the particulate enzyme, it is markedly stimulated (3- to 10-fold) when assayed in the combined presence of flavin adenine dinucleotide ($10^{-5}$ M) and the detergent Triton X-100 (0.2%). The activity of the soluble dehydrogenase in crude extracts prepared from anaerobically grown cells harvested in late log phase is 50 to 100 nmoles of L-α-GP oxidized per min per mg of protein.

This soluble enzyme was also found to be present in wild-type cells and could be partially separated from the particulate enzyme by ultracentrifugation through gradients of sucrose. A gradient profile obtained from an extract of wild-type cells (strain 1) grown anaerobically on medium supplemented with L-α-GP shows two broad peaks of L-α-GP dehydrogenase activity (Fig. 2a). The first is associated largely with material recovered from the bottom of the gradient and the second with more slowly sedimenting material. In the profile obtained from a similarly prepared extract of mutant strain 95 (Fig. 2b), the activity comprising the particulate peak is missing, but the activity in the soluble peak is undiminished.

Evidence that the slowly sedimenting enzyme is responsible for the anaerobic dissimilation of glycerol and L-α-GP in mutants that lack the particulate enzyme comes from the examination

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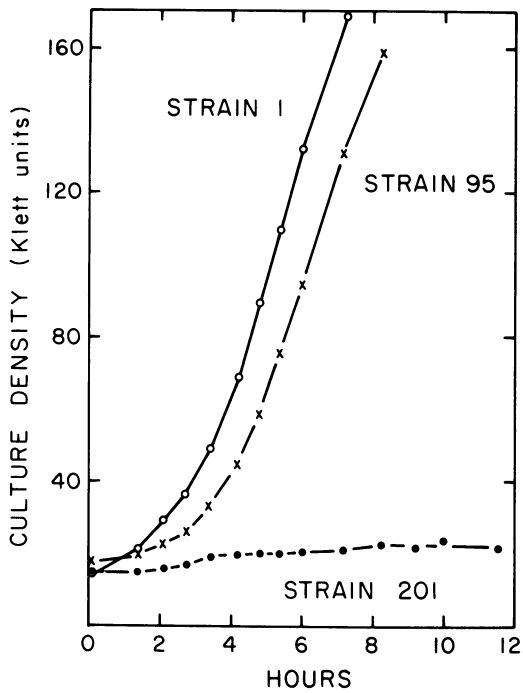


FIG. 1. *Anaerobic growth on L-α-GP. The medium contained inorganic components as previously described (9), 0.04 M DL-α-GP, and 0.025 M $KNO_3$. To provide vigorous anaerobic growth, a supplement of amino acids was added with the following concentration of individual components: lysine, leucine, arginine, 50 μg/ml; methionine, threonine, proline, serine, glycine, valine, phenylalanine, isoleucine, 30 μg/ml; cysteine, tryptophan, histidine, 10 μg/ml (all of the L configuration). The D-α-GP in the medium is probably physiologically inert since the transport system for L-α-GP has low affinity for the D isomer (2). Cultures were incubated at 37 C in fully filled and stoppered test tubes and were stirred magnetically. Growth was monitored turbidimetrically in a Klett colorimeter with no. 42 filter (one unit corresponds to approximately 4 × $10^6$ cells/ml).*

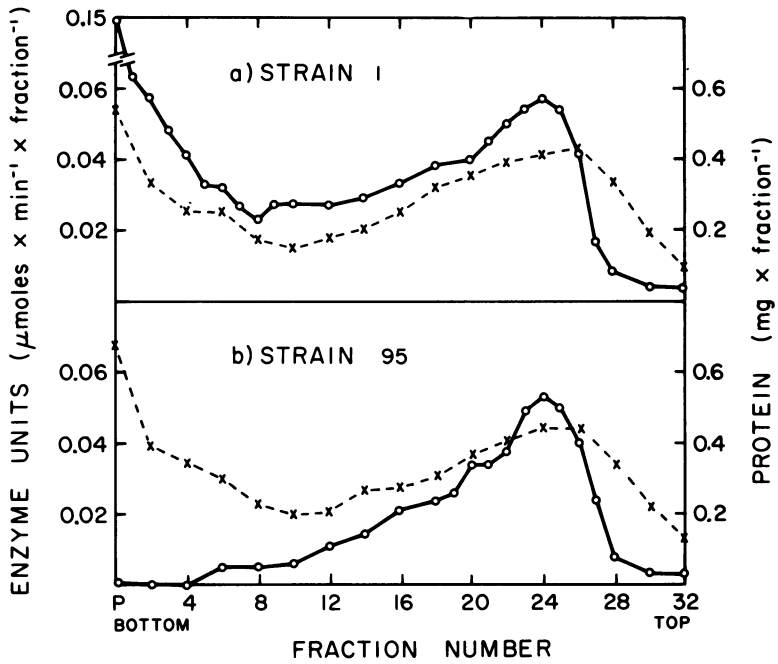


FIG. 2. *Sucrose gradient distribution of L-α-GP dehydrogenase activity. Cells were grown on mineral medium supplemented with 1% casein amino acids, 0.1% yeast extract, 0.04 M DL-α-GP, and 0.025 M $KNO_3$. Cultures were incubated on a rotary shaker at 37 C in 300-ml Erlenmyer flasks fully filled and stoppered and were harvested at a density of 100 Klett units. Extracts were prepared by sonic disruption of washed bacteria suspended in 1.0 ml of 0.1 M potassium phosphate buffer, pH 7.5. After centrifugation for 20 min at 35,000 × g, 0.4 ml of the supernatant fraction (containing 10 to 12 mg of protein) was layered on a linear 5 to 20% gradient of sucrose (4.3 ml) resting on a shelf of 60% sucrose (0.3 ml). The sucrose solutions contained 0.1 M potassium phosphate, pH 7.5, and $10^{-5}$ M flavin adenine dinucleotide. Tubes were centrifuged 10 hr at 100,000 × g at 4 C in a Spinco SW-39 rotor. Samples (0.16 ml) were removed by puncturing the bottom of the tube with a needle and were examined for L-α-GP dehydrogenase activity (○) and for protein (×) by the method of Lowry et al. (7). The pelleted material (P) was resuspended in buffer and similarly assayed. L-α-GP dehydrogenase assays were performed as described by Lin et al. (6), except that all mixtures contained a final concentration of $10^{-5}$ M flavin adenine dinucleotide, 0.2% Triton X-100, and 0.02% phenazine methosulfate. Routine assays were performed by using DL-α-GP, which gave the same activity as the pure L-isomer.*

of a double mutant (strain 201) which does not produce either type of L-α-GP dehydrogenase. This mutant was obtained by treatment of strain 95 with ethyl methanesulfonate followed by anaerobic penicillin selection in the combined presence of 0.01 M glycerol and 0.02 M DL-α-GP. Surviving cells were spread on agar plates containing 1% tryptone, 0.01 M glycerol, 0.02 M DL-α-GP, and 0.02 M $KNO_3$ and were incubated aerobically at 37 C until small colonies appeared. Incubation was then carried out under 95% $N_2$ and 5% $CO_2$ for two days. A range of colony sizes resulted, and among the smaller colonies were found some which lacked the soluble L-α-GP dehydrogenase. Extracts prepared from anaerobic cultures of strain 201 display less than 1% of the L-α-GP dehydrogenase activity of strain 95. When tested for growth, the double mutant exhibited no response to L-α-GP anaerobically (Fig. 1). Similarly, anaerobic growth on glycerol by strain 201 was strikingly reduced, the increase in turbidity being only about 20% that shown by its parent, strain 95.

A third L-α-GP dehydrogenase, which is linked to nicotinamide adenine dinucleotide phosphate, has been reported recently (3). This enzyme probably functions as a dihydroxyacetone phosphate reductase to provide L-α-GP for lipid synthesis when exogenous L-α-GP is not available. Strain 201 shows no requirement for added L-α-GP when growing on glucose as sole carbon and energy source either aerobically or anaerobically. Therefore, neither the soluble nor the particulate pyridine nucleotide-independent L-α-GP dehydrogenase is necessary for the anabolic generation of L-α-GP. Their function is apparently restricted to the oxidation of L-α-GP for catabolic dissimilation.

The reason the soluble L-α-GP dehydrogenase functions to support normal growth on glycerol or L-α-GP anaerobically but fails to support any growth on these compounds aerobically is obscure. This problem is currently under study and will be dealt with more fully in a subsequent report concerning the location of the structural gene for the soluble enzyme as well as the control of the expression of that gene.

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