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# Uptake of Glycerol 3-Phosphate and Some of Its Analogs by the Hexose Phosphate Transport System of *Escherichia coli*

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The hexose phosphate transport system transported glycerol 3-phosphate and its analogs 3,4-dihydroxybutyl-1-phosphonate, glyceraldehyde 3-phosphate, and 3-hydroxy-4-oxobutyl-1-phosphonate.

3,4-Dihydroxybutyl-1-phosphonate, 3-hydroxy-4-oxobutyl-1-phosphonate, and glyceraldehyde 3-phosphate are analogs of glycerol 3-phosphate that inhibit the growth of *Escherichia coli*. The first two drugs are bacteriostatic (10, 13), and the third is bactericidal (12). All three analogs are substrates for the *sn*-glycerol 3-phosphate transport system of *E. coli* (10, 12, 13). Glycerol 3-phosphate is itself bacteriostatic to cells lacking the catabolic glycerol 3-phosphate dehydrogenase (3). Attempts to isolate analog-resistant mutants have been seriously hampered by the high mutation rate of the glycerol 3-phosphate transport system. For this reason, we have searched for alternate uptake pathways. The hexose phosphate transport system appeared to be a reasonable possibility because of its broad specificity (4, 16).

The ability of the hexose phosphate transport system to recognize glycerol 3-phosphate and its analogs was tested by comparing the sensitivities of strain 5-6 (*glpD glpR*$^c$ *glpT*) (7) and strain T5-6 (*glpD glpR*$^c$ *glpT uhp-35*) to these compounds. Strain T5-6 was constructed by transduction (9) with bacteriophage P1 as the vector, strain RK1042·*uhp-35* (F$^-$ *ilv argH his metB pyrE60 bgl*$^+$ *mtl rpsL uhp-35*) (6) as the *uhpR*$^c$ donor, and strain 5-6 as the recipient. Transductants were selected for the ability to use fructose 1-phosphate as the sole carbon source. The growth of strain T5-6 was inhibited by glycerol 3-phosphate and its analogs (Fig. 1), whereas that of strain 5-6 was not affected (data not shown). Several other hexose phosphate transport constitutive transductants of strain 5-6 were isolated and tested. All produced results identical to those presented for T5-6. Strain T5-6 is also sensitive to 1-deoxy-1-dihydroxyphosphonylmethylfructose, an analog of fructose 1-phosphate (14). This sensitivity forms the basis for selecting for spontaneous fructose 1-phosphate transport-negative mutants of T5-6. Such mutants are resistant to the fructose 1-phosphate analog, can no longer use fructose 1-phosphate as the sole carbon source, and exhibit the same resistance pattern to glycerol 3-phosphate and its analogs as strain 5-6 (data not shown).

The uptake of either [$^{14}$C]glycerol 3-phosphate or 3,4-dihydroxy[$^3$H]butyl-1-phosphonate by *E. coli* is reflected in the synthesis of labeled lipids (11, 15). The incorporation of label into the phosphoglyceride fraction was monitored by procedures that have been described (11). [$^{14}$C]glycerol 3-phosphate was incorporated into the phosphoglycerides of strain T5-6 but not into the lipids of either strain 5-6 or the fructose

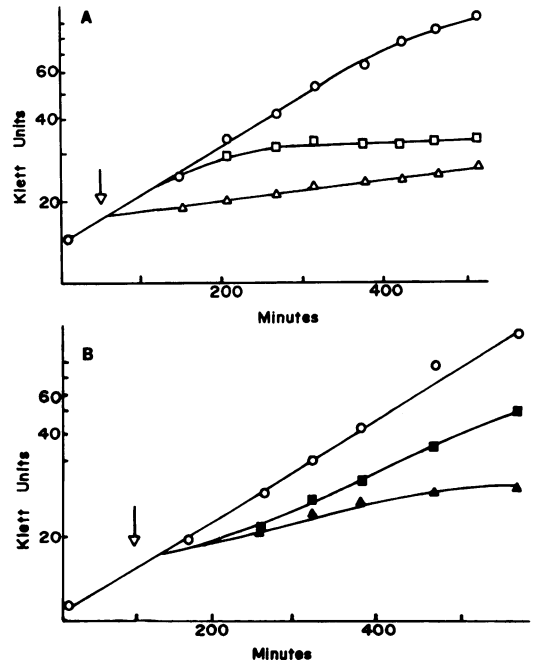


FIG. 1. *Effect of glycerol 3-phosphate and its analogs upon the growth of strain T5-6. The culture medium consisted of 10 ml of Garen and Levinthal minimal medium (5) with 0.5% potassium succinate as the sole carbon source. Growth was monitored as previously described (10). Drugs (2.5 mM) were added at the indicated turbidity (arrow). Symbols: ▲, glycerol 3-phosphate; □, 3,4-dihydroxybutyl-1-phosphonate; △, glyceraldehyde 3-phosphate; ■, 3-hydroxy-4-oxobutyl-1-phosphonate; ○, untreated cells.*

1-phosphate transport-negative revertants of T5-6 (Fig. 2A). Similar results were obtained for 3,4-dihydroxy[$^3$H]butyl-1-phosphonate incorporation (Fig. 2B).

Thus, there are three transport systems that recognize glycerol 3-phosphate. The first of these is the glycerol 3-phosphate transport system, genotype *glpT*, that has been studied extensively in Lin's laboratory (8). This transport system has a relatively broad specificity (8, 10, 12, 13). A second transport system that appears to have a relatively narrow specificity was recently described (1, 2). This system, genotype *ugp*$^+$, was detected by examining the phenotypic revertants of cells with a *glpT* genotype (1). The cells used in the current study lacked the *ugp*$^+$ locus. However, cells with the genotype *ugp*$^+$ *glpT* were inhibited by 3,4-dihydroxybutyl-1-phosphonate (unpublished data of this laboratory). The third transport system, the hexose phosphate transport system, appears to have the broadest specificity. The availability of three alternative transport systems should prove to be of considerable help in the search for drug-resistant mutants.

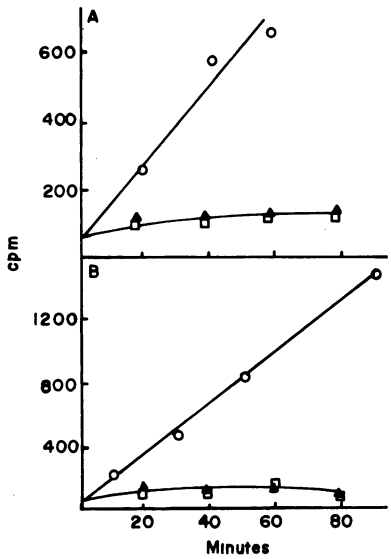


FIG. 2. *Incorporation of sn-glycerol 3-phosphate and* DL*-3,4-dihydroxybutyl-1-phosphonate into the phospholipid fraction of strain 5-6 (glpD glpR*$^c$ *glpT), strain T5-6 (glpD glpR*$^c$ *glpT uhp-35) and a spontaneous fructose 1-phosphate transport-negative revertant of strain T5-6. The revertant was isolated by the procedure described in the text. Culture conditions were as described in the legend to Fig. 1. To early-log-phase cells was added either* DL*-3,4-dihydroxy[$^3$H]butyl-1-phosphonate (180 μM; specific activity, 30 μCi/μmole) (15) or sn-[$^{14}$C]glycerol 3-phosphate (20 μM; specific activity, 38 μCi/μmol). The incorporation of label into the lipid fraction was monitored as previously described (11). (A) [$^{14}$C]glycerol 3-phosphate. (B) 3,4-Dihydroxy[$^3$H]butyl-1-phosphonate. Symbols: ▲, strain 5-6; ○, strain T5-6; □, a fructose 1-phosphate transport-negative revertant of strain T5-6.*

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