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Effects of Halo Analogs of Glycerol 3-Phosphate and Dihydroxyacetone Phosphate upon *Escherichia coli*

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The fluoro and chloro analogs of glycerol 3-phosphate inhibit the growth of *Escherichia coli* and affect bacterial enzymes involved in lipid synthesis.

We have been interested in structural analogs of glycerol 3-phosphate that interfere with bacterial growth and perturb phosphoglyceride metabolism. The analogs L-glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate inhibit the growth of *Escherichia coli* and are competitive inhibitors of acyl coenzyme A:*sn*-glycerol 3-phosphate acyltransferase and phosphatidylglycerol phosphate synthetase (18, 19). The former analog is bactericidal, and the latter is bacteriostatic (18, 19). A third analog, 3,4-dihydroxybutyl-1-phosphonate, is bacteriostatic and a competitive substrate for phosphatidylglycerol phosphate synthetase, and it does not affect acyl coenzyme A:*sn*-glycerol 3-phosphate acyltransferase (3, 15, 16, 20). The halo analogs of glycerol 3-phosphate and dihydroxyacetone phosphate have been used as tools to investigate several different enzymes. The interactions of these analogs with glycerol 3-phosphate dehydrogenase (4, 6, 8, 9, 17), fructose-bisphosphate aldolase (8, 9, 14), triosephosphate isomerase (8-11), and methylglyoxal synthase (21) have been studied. This report describes the interactions of 1-chloro-1-deoxyglycerol 3-phosphate, 1-fluoro-1-deoxyglycerol 3-phosphate, 1-chloro-3-hydroxyacetone phosphate, and 1-fluoro-3-hydroxyacetone phosphate with three different *E. coli* transport systems capable of recognizing glycerol 3-phosphate (7). It also evaluates the interactions of these halo analogs with bacterial acyl coenzyme A:*sn*-glycerol 3-phosphate acyltransferase and phosphatidylglycerol phosphate synthetase.

The bacterial strains used in these studies are described in Table 1. At a concentration of 2 mM, 1-fluoro- or 1-chloro-1-deoxyglycerol 3-phosphate inhibited the growth of *E. coli* strain 8 (Fig. 1). The cells seemed to be more susceptible to the chloro analog, as evidenced by the fact that 1-chloro-1-deoxyglycerol 3-phosphate inhibited growth at 100 μM, whereas this concentration of 1-fluoro-1-deoxyglycerol 3-phosphate had only a slight growth-inhibitory effect (Fig. 1). 1-Fluoro- or 1-chloro-3-hydroxyacetone phosphate also inhibited the growth of strain 8 at 2 mM (Fig. 2). Neither halo hydroxyacetone phosphate analog affected cell growth when present at 100 μM (data not shown). At 2 mM, each of the halo analogs was bacteriostatic for strain 8 as determined by standard plating techniques after 8 h of exposure (18). The growth of *E. coli* 5-6, a mutant of strain 8 lacking the glycerol 3-phosphate transport system, was not affected by the halo analogs (data not shown). Thus, the halo analogs entered strain 8 via the glycerol 3-phosphate transport system. Glycerol 3-phosphate is also able to enter cells via the hexose phosphate transport system and a transport system determined by the *ugp* genetic locus (7). At 2 mM, 1-fluoro- or 1-chloro-3-hydroxyacetone phosphate inhibited the growth of *E. coli* T5-6 (Fig. 3). 1-Fluoro-or 1-chloro-1-deoxyglycerol 3-phosphate did not affect the growth of this strain when present at 2 mM (data not shown). Therefore, the hexose phosphate transport system was able to distinguish between the hydroxyl and keto groups at C-2 of the halo analogs. The transport system determined by the *ugp* locus made a different type of distinction among the halo analogs. At 2 mM, 1-fluoro-3-hydroxyacetone phosphate and 1-fluoro-1-deoxyglycerol 3-phosphate were inhibitory to a strain with the *ugp*$^+$ genotype (Fig. 4), but not to an

TABLE 1. *Bacterial strains*

Strain	Genotype	Source reference
8	HfrC *glpR*^{c}*2 phoA8 tonA22* T2^r *rel-1 ugp* (λ)	E. C. C. Lin, Harvard Medical School, Boston, Mass. (12)
5-6	Same as 8, *glpT*	This laboratory (13)
T5-6	Same as 5-6, *uhp-35*[a]	This laboratory (7)
LA3432	*rpsL nalA glpT ugp*$^+$	W. Boos, University of Konstanz, Konstanz, West Germany (1, 2)
LA3433	*rpsL nalA glpT ugp*	W. Boos, Univ. of Konstanz (2)

[a] *uhp-35* is a regulatory mutation that makes the cells constitutive for hexose phosphate transport.

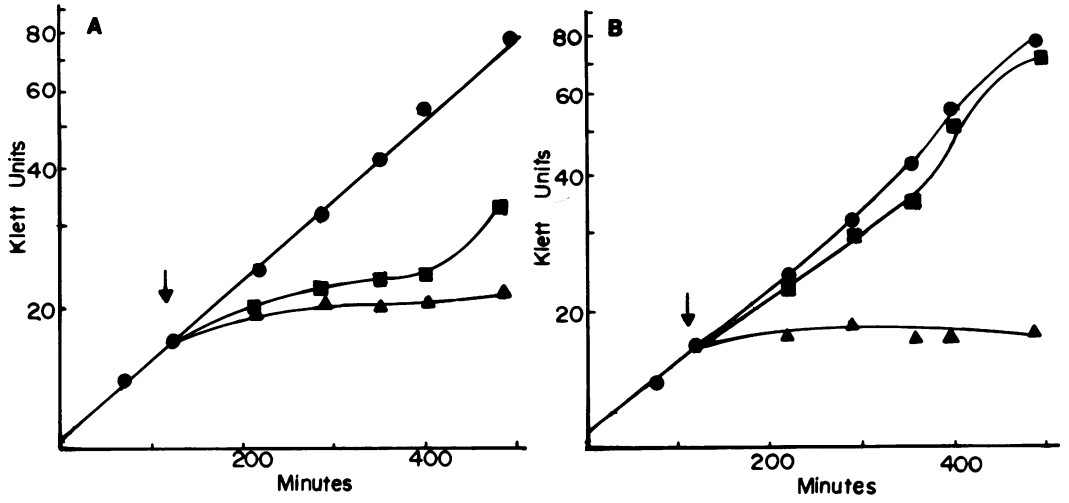

FIG. 1. *Effects of 1-chloro-1-deoxyglycerol 3-phosphate (A) and 1-fluoro-1-deoxyglycerol 3-phosphate (B) upon the growth of E. coli 8. The cells were cultured at 37°C in 10 ml of Garen-Levinthal minimal medium (5) with 0.5% potassium succinate as the sole carbon source. Growth was monitored as previously described (15). Halo analogs were added at the indicated turbidity (arrow). Symbols: ●, untreated culture; ■, 0.1 mM halo analog; ▲, 2 mM halo analog.*

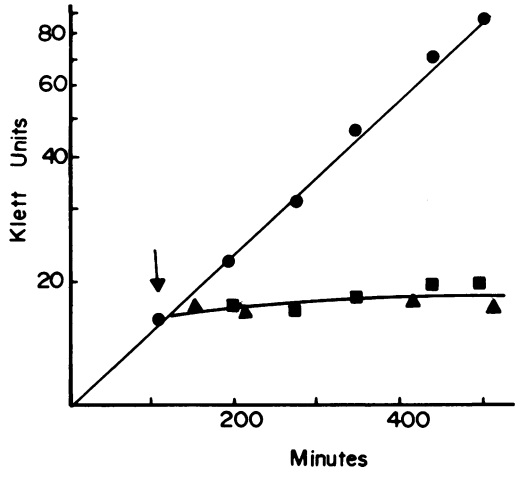

FIG. 2. *Effects of 1-chloro-3-hydroxyacetone phosphate and 1-fluoro-3-hydroxyacetone phosphate upon the growth of E. coli 8. The cells were cultured and growth was monitored as described in the legend to Fig. 1. Halo analogs were added at the indicated turbidity (arrow). Symbols: ●, untreated culture; ■, 2 mM 1-chloro-3-hydroxyacetone phosphate; ▲, 2 mM 1-fluoro 3-hydroxyacetone phosphate.*

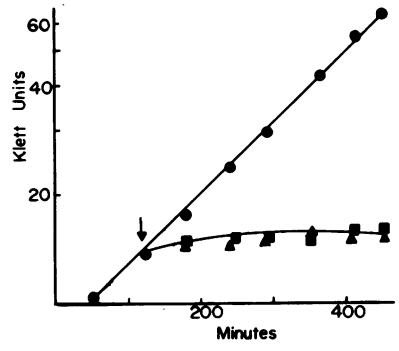

FIG. 3. *Effects of 1-chloro-3-hydroxyacetone phosphate and 1-fluoro-3-hydroxyacetone phosphate upon the growth of E. coli T5-6. The cells were cultured and growth was monitored as described in the legend to Fig. 1. The halo analogs were added at the indicated turbidity (arrow). Symbols: ●, untreated culture; ■, 2 mM 1-chloro-3-hydroxyacetone phosphate; ▲, 2 mM 1-fluoro-3-hydroxyacetone phosphate.*

isogenic strain with a *ugp* genotype. Furthermore, the chloro analogs failed to affect either strain when present at 2 mM (data not shown). It therefore appears that the transport system determined by the *ugp* locus is able to discriminate between chloro and fluoro analogs.

1-Chloro-1-deoxyglycerol 3-phosphate was a competitive inhibitor of the acyl coenzyme A:*sn*-glycerol 3-phosphate acyltransferase of *E. coli* (Fig. 5). None of the other halo analogs tested affected this enzyme (data not shown). The apparent K_i of a racemic mixture of 1-chloro-1-deoxyglycerol 3-phosphate was 2 mM, placing this analog between L-glyceraldehyde 3-phosphate (apparent K_i of 0.55 mM) and (*RS*)-3-hydroxy-4-oxobutyl-1-phosphonate (apparent K_i of 2.5 mM) (19). Each of the halo analogs was

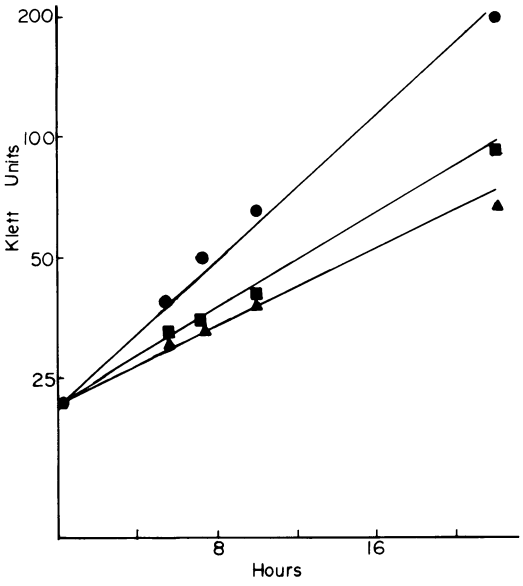

FIG. 4. *Effects of 1-fluoro-1-deoxyglycerol 3-phosphate and 1-fluoro-3-hydroxyacetone phosphate upon the growth of E. coli LA3432. The cells were cultured at 30°C but otherwise as described in the legend to Fig. 1. Growth was monitored as described in the legend to Fig. 1. The halo analogs were added to cells when the culture achieved a turbidity of 23 Klett units. Symbols: ●, untreated culture; ■, 2 mM 1-fluoro-3-hydroxyacetone phosphate; ▲, 2 mM 1-fluoro-1-deoxyglycerol 3-phosphate.*

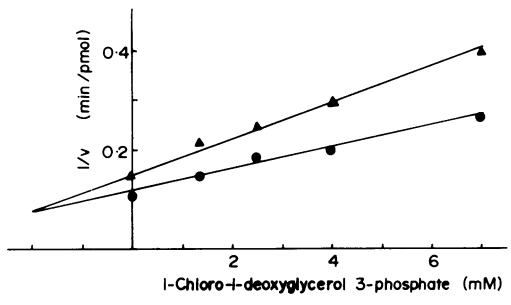

FIG. 5. *Effect of 1-chloro-1-deoxyglycerol 3-phosphate upon the acyl coenzyme A:sn-glycerol 3-phosphate acyltransferase of E. coli. Membranes were prepared from E. coli 8 as described by Cheng et al. (3). The activity of the acyl coenzyme A:sn-glycerol 3-phosphate acyltransferase of the membrane preparation was assayed by determining the incorporation of sn-[^{14}C]glycerol 3-phosphate (specific activity, 20 Ci/mol) into lipid as described by Tang et al. (18, 19). sn-Glycerol 3-phosphate concentrations: ▲, 40 μM; ●, 60 μM.*

a competitive inhibitor of phosphatidylglycerol phosphate synthetase (Table 2). The chloro analogs followed the expected trend, with the hydroxy analog exhibiting a lower K_i than the keto analog. The fluoro analogs were reversed. We have no explanation for this apparently anomalous behavior.

TABLE 2. *Apparent K_i values of the halo analogs for phosphatidylglycerol phosphate synthetase*[a]

Halo analog	Apparent K_i (mM)
rac-1-Chloro-1-deoxyglycerol 3-phosphate	2
1-Fluoro-3-hydroxyacetone phosphate	2.7
1-Chloro-3-hydroxyacetone phosphate	5.7
rac-1-Fluoro-1-deoxyglycerol 3-phosphate	7.5

[a] Membranes were prepared from *E. coli* 8 as described by Cheng et al. (3). The phosphatidylglycerol phosphate synthetase activity of the membrane preparation was assayed by determining the incorporation of *sn*-[^{14}C]glycerol 3-phosphate (specific activity, 20 Ci/mol) into lipid, and the apparent K_i of each of the halo analogs was obtained as described by Tang et al. (18, 19).

The data presented indicate that the halo analogs of glycerol 3-phosphate and dihydroxyacetone phosphate affect the enzymes of lipid metabolism in *E. coli*. The broad target specificity of the halo analogs may limit their usefulness as specific metabolic inhibitors. Nevertheless, they may prove to be useful tools for distinguishing among enzymes or transport systems performing similar functions. Clearly, the three transport systems for glycerol 3-phosphate each exhibit a characteristic recognition pattern for the halo analogs.

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