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L-Glyceraldehyde 3-Phosphate, a Bactericidal Agent

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At a concentration of 2.5 mM, DL-glyceraldehyde 3-phosphate has a bactericidal effect upon *Escherichia coli*. The glycerol 3-phosphate transport system is required for the entry of the biologically active L-enantiomer. L-Glyceraldehyde must be phosphorylated by the cell to exert its full effect upon growth. The addition of DL-glyceraldehyde 3-phosphate to a culture of *E. coli* caused no preferential inhibition of the accumulation of deoxyribonucleic acid, ribonucleic acid, or phosphoglycerides, although protein accumulation was less affected. Studies with mutant strains ruled out catabolic glycerol 3-phosphate dehydrogenase, anabolic nicotinamide adenine dinucleotide (phosphate):*sn*-glycerol 3-phosphate oxidoreductase, and fructose 1,6-diphosphate aldolase as the primary sites of action. L-Glyceraldehyde 3-phosphate is a competitive inhibitor of *sn*-glycerol 3-phosphate in the reactions catalyzed by acyl coenzyme A:*sn*-glycerol 3-phosphate acyltransferase (K_i of 1.8 mM) and cytidine 5'-diphosphate-diglyceride:*sn*-glycerol 3-phosphate phosphatidyltransferase (K_i of 2.7 mM). A K_m mutant for the former enzyme was susceptible to the inhibitor. L-Glyceraldehyde 3-phosphate does not affect acyl coenzyme A:lysophosphatidate acyltransferase activity. In vivo, phosphatidylethanolamine and phosphatidylglycerol accumulation are inhibited to the same extent by the addition of DL-glyceraldehyde 3-phosphate to a culture of *E. coli*.

Previous studies with 3,4-dihydroxybutyl-1-phosphonate made us aware that analogues of glycerol 3-phosphate can have profound effects upon cellular metabolism (4, 15–17). The hydrated form of L-glyceraldehyde 3-phosphate may be considered to be an analogue of *sn*-glycerol 3-phosphate in which a hydroxyl group replaces a hydrogen at C1. In aqueous solution, glyceraldehyde 3-phosphate exists as an equilibrium mixture of the geminal diol (hydrated form) and free aldehyde in a molar ratio of 29:1 (19). The data in the present report indicate that L-glyceraldehyde 3-phosphate is bactericidal and interferes with two enzymes involved in phosphoglyceride synthesis.

MATERIALS AND METHODS

Chemicals. L-[^{3}H]isoleucine, *sn*-[^{14}C]glycerol 3-phosphate, and carrier-free [^{33}P]phosphate were purchased from the New England Nuclear Corp., Boston, Mass. The following materials were obtained from the Sigma Chemical Co., St. Louis, Mo.: reduced nicotinamide adenine dinucleotide phosphate, type III; dithiothreitol; DL-glyceraldehyde 3-phosphoric acid (diethyl acetal, monobarium salt); *N*,*N*-bis-(2-hydroxyethyl) glycine, Bicine; 5,5'-dithiobis-(2-nitrobenzoic acid), DTNB; tris(hydroxymethyl)aminomethane; D- and L-glyceraldehyde; and octylphenoxypolyethoxyethanol, the nonionic detergent Triton X-100; and bovine serum albumin. D-Glyceraldehyde 3-phosphoric acid (diethyl acetal, dicyclohexyl ammonium salt) was a product of Boehringer Mannheim Corp., New York, N.Y. Glyceraldehyde 3-phosphoric acid was generated from the corresponding acetals by mixing with an aqueous suspension of Dowex 50, hydrogen ion form, using the procedure recommended by the Sigma Chemical Co.

1-Palmitoyl glycerol 3-phosphate (lysophosphatidic acid) and cytidine diphosphate-dipalmitin (CDP-dipalmitin) were purchased from Serdary Research Laboratories Inc., London Ontario, Canada. Oleoyl coenzyme A and palmitoyl coenzyme A (CoA) were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. The bacterial phospholipid standards phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin were purchased from Supelco Inc., Bellefonte, Pa.

Silica Gel G thin-layer plates were obtained from Analabs, Inc., North Haven, Conn. Eugonagar was a product of Fisher Scientific, Fair Lawn, N.J. 2-Mercaptoethanol was a product of Matheson, Coleman and Bell, Norwood, Ohio. All other chemicals were of reagent grade.

Bacterial strains and culture conditions. The bacterial strains studied are described in Table 1. The glycerol 3-phosphate transport-negative mutants are derivatives of *E. coli* 8. A complete description of their isolation and characterization will be the subject of a future report. Briefly, strain 8 was mutagenized with *N*-methyl-*N*'-nitronitrosoguanidine. Cells resistant to 3,4-dihydroxybutyl-1-phosphonate were selected, and assayed for the glycerol 3-phosphate transport system.

The effect of glyceraldehyde 3-phosphate and related compounds upon growth was determined by monitoring growth turbidimetrically in a Klett-Summerson colorimeter with a 660-nm filter. Cells were cultured in 10 to 25 ml of medium in 250-ml Erlenmeyer flasks fitted with side arms. Incubations were in a New Brunswick Aquatherm G86 or Metabolyte G77 water bath shaker at 200 rpm. In all cases, several generations of exponential growth were allowed for the dilute starting cultures to attain the cell density at which the experiments were initiated. Viability studies were performed by diluting cultures and plating on Eugonagar. All incubations were at 37°C.

Three types of minimal media were employed in growth studies. *E. coli* CY 115 and 7 were cultured in Vogel and Bonner minimal medium (21) containing 0.5% glycerol as the sole carbon source and supplemented with thiamine-hydrochloride, 2 mg/liter; methionine, 50 mg/liter; and tryptophan, 50 mg/liter. *E. coli* strains 8, NP 315, and derivatives of strain 8 defective in the glycerol 3-phosphate transport system were cultured in Garen and Levinthal minimal medium containing 0.6 mM phosphate (8) with 0.5% potassium succinate as the sole carbon source. A Bicine-buffered medium was utilized in experiments involving the effects of glyceraldehyde on the growth of *E. coli* 8 and 9. This medium contained Bicine-hydrochloride, 100 mM (pH 7.4); NaCl, 90 mM; KCl, 40 mM; NH_4Cl, 200 mM; and all the other salts in concentrations required for the Garen and Levinthal synthetic medium (8). Cells used for the preparation of enzyme extracts were cultured in Garen and Levinthal minimal medium as described above and harvested in late-logarithmic phase.

Macromolecular and phospholipid synthesis. Cultures of *E. coli* 8 at a cell density of 15 to 20 Klett units (1 Klett unit is equivalent to approximately 5 $\times 10^6$ cells/ml) were simultaneously mixed with various concentrations of DL-glyceraldehyde 3-phosphate or water and 1 to 3 μCi of [^{33}P]phosphate per ml. Samples of 1 ml were removed at the indicated times and treated as previously described (16) to measure the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and phosphoglyceride. A portion of the chloroform-soluble material was retained for thin-layer chromatography of the phosphoglycerides (14).

Protein synthesis was followed by measuring the conversion of isolecucine into a trichloroacetic acid-insoluble material. One milliliter of culture medium was simultaneously mixed with 0.45 μCi of L-[^{3}H]isoleucine and 15 μg of L-isoleucine and DL-glyceraldehyde 3-phosphate. Samples of 0.1 ml were removed and assayed as previously described (15).

Assay of acyl CoA:*sn*-glycerol 3-phosphate acyltransferase. Membranes were prepared from *E. coli* 8 or BB26-36 R2 as described previously (4). The activity of acyl CoA:*sn*-glycerol 3-phosphate acyltransferase of the membrane preparation was assayed by determining the incorporation of [^{14}C]glycerol 3-phosphate (12.4 mCi/mmol) into lipid. The assays were performed at 30°C and initiated by the addition of palmitoyl CoA. The specific activity at maximum velocity of the strain 8 preparations was 2.4 nmol/min per mg. In some cases, the activity of acyl CoA:*sn*-glycerol 3-phosphate was also assayed by monitoring the release of CoA by measuring its chemical interaction with DTNB by a modification of the procedure of Cronan et al. (6). The assay mixture (total volume, 1 ml) contained 100 μmol of Bicine-hydrochloride, pH 8.5; 5 μmol of $MgCl_2$; 1 mg of bovine serum albumin; 50 nmol of palmitoyl CoA; 1.0 μmol of DTNB; 60 μg of enzyme; and 0.75 μmol of *sn*-glycerol 3-phosphate. The initial rates were monitored by measuring absorbance at 25°C in a Gilford model 250 spectrophotometer equipped with a multiple-sample absorbance recorder.

Assay of acyl CoA:lysophosphatidate acyltransferase. The preparation of this particulate enzyme was the same as that of acyl CoA:*sn*-glycerol 3-phosphate acyltransferase. The acyl CoA:lysophosphatidate acyltransferase was assayed by a colorimetric procedure that was essentially the same as described above for acyl CoA:glycerol 3-phosphate acyltransferase (6, 20). The reaction mixture (total volume of 1 ml) contained 100 μmol of Bicine-hydrochloride, pH 8.5; 0.5 μmol of $MgCl_2$; 1 mg of bovine

TABLE 1. *Bacterial strains studied*[a]

Strain	Mating type	Genotype	Source and reference
7	HfrC	*glpR*^{c}*2 phoA8 tonA22 T*$_2^R$ *rel-1* (λ)	E. C. C. Lin, Harvard Medical School (10)
8	HfrC	Same as 7, *glpD3*	J. Cronan, Jr., Yale University (9)
9	HfrC	Same as 8, *glpK*	E. C. C. Lin, Harvard Medical School (9)
BB26-36 R2	HfrC	Same as 8, *gpsA3*^{FR} *plsB26*	R. Bell, Duke University Medical Center (2)
NP 315 (formerly h8)	Hfr	*tonA22 thi*$^-$ *rel-1 T*$_2^R$ *fda*(Ts)	F. C. Neidhardt, University of Michigan (3)
CY 115	F$^-$	*gpsA20 metE70 trpE8 xyl-5 tsx-67 str-109*	J. Cronan, Jr., Yale University (5)

[a] Genetic nomenclature is that of Taylor and Trotter (18). The allele numbers are those of the Coli Genetic Stock Center at Yale University.

serum albumin; 60 nmol of oleoyl CoA; 1.0 μmol of DTNB; 60 μg of enzyme; and 25 nmol of 1-palmitoyl glycerol 3-phosphate.

Assay of CDP-diglyceride:*sn*-glycerol 3-phosphate phosphatidyltransferase. The particulate fraction was prepared from *E. coli* 8 as described previously (4). The activity of CDP-diglyceride:*sn*-glycerol 3-phosphate phosphatidyltransferase was assayed by monitoring the conversion of [^{14}C]glyerol 3-phosphate into chloroform-extractable material as described previously (4). The assay mixture (total volume of 0.25 ml) contained 62.5 μmol of Bicine-hydrochloride, pH 8.0; 20 μmol of CDP-dipalmitin; 2.5 μmol of $MgCl_2$; 1.25 μmol of 2-mercaptoethanol; 0.5 mg of Triton X-100; 55 μg of particulate enzyme; and the indicated concentrations of *sn*-[^{14}C]glycerol 3-phosphate (24.8 mCi/mmol). The enzyme assay was performed at 37°C and initiated by the addition of enzyme preparation.

All enzyme preparations were prepared at 1 to 4°C. Protein concentrations were determined by the method of Lowry et al. (12). The rates of reaction were linearly dependent upon enzyme concentration and time in the range reported.

RESULTS

DL-Glyceraldehyde 3-phosphate is a potent inhibitor of the growth of *E. coli* 8. Figure 1 shows the effects of various concentrations of this compound upon the growth of *E. coli* 8. The data presented in Fig. 2 reveal that 2.5 mM DL-glyceraldehyde 3-phosphate is bactericidal. The L enantiomer of the phosphate, not currently available for study, must account for the inhibition, since D-glyceraldehyde 3-phosphate does not influence cell growth. The *sn*-glycerol 3-phosphate transport system is required for inhibition of cell growth, since several independently isolated transport-negative mutants were resistant to DL-glyceraldehyde 3-phosphate. One such experiment is depicted in Fig. 3.

We examined the question of whether L-glyceraldehyde 3-phosphate or its nonphosphorylated derivative was the true inhibitor. As evident from Fig. 4A, L-glyceraldehyde has some inhibitory effect upon strain 9, a glycerokinase-deficient mutant of strain 8 (9). However, strain 8 was considerably more susceptible to L-glyceraldehyde (Fig. 4B). The ability of glycerokinase to catalyze the phosphorylation of L-glyceraldehyde (10) provides an explanation for the difference in susceptibility observed between the two strains. These results clearly indicate that the phosphorylated derivative is important in the bactericidal effects. The slight inhibition observed in strain 9 may be due to leakiness of the mutation, a second kinase activity, or an effect of the nonphosphorylated aldehyde. We do not have sufficient data to

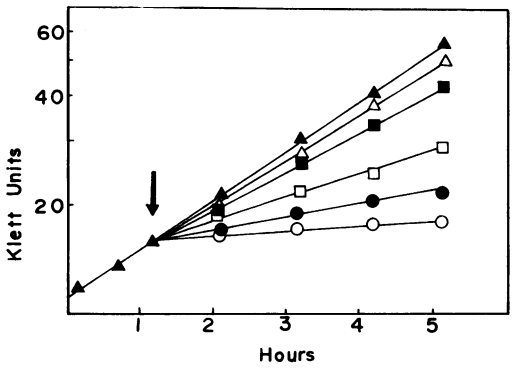

FIG. 1. *Effects of various concentrations of* DL-*glyceraldehyde 3-phosphate on the growth of E. coli 8 cultured in Garen and Levinthal medium (8) supplemented with 0.5% potassium succinate. At the time indicated by the arrow, the inhibitor was added to the following final concentrations:* ○, *2.5 mM;* ●, *1.0 mM;* □, *0.5 mM;* ■, *0.25 mM;* △, *0.10 mM; and* ▲, *untreated.*

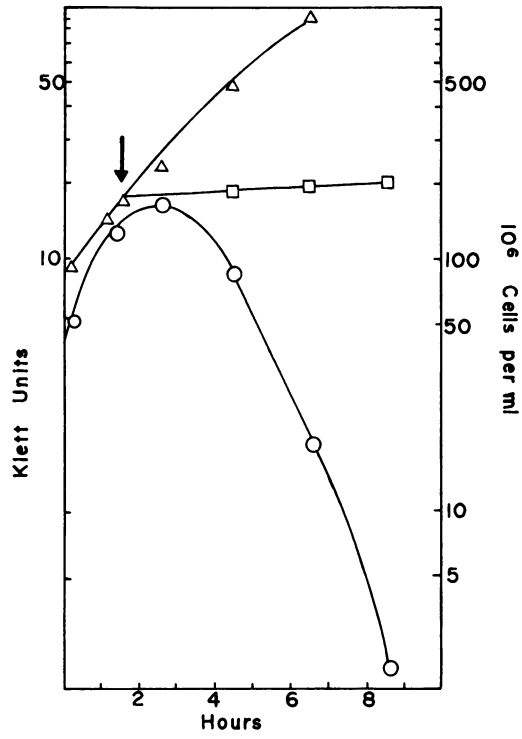

FIG. 2. *Effect of 2.5 mM* DL-*glyceraldehyde 3-phosphate on the viability of E. coli 8 cultured in Garen and Levinthal medium (8) supplemented with 0.5% potassium succinate. The inhibitor was added at the time indicated by the arrow. Cell viability was determined as described in Materials and Methods. Symbols:* △, *cell turbidity in Klett units of untreated cultures;* □, *cell turbidity in Klett units of inhibitor-treated cultures; and* ○, *viability of inhibitor-treated cultures. The bactericidal effects were also found in Bicine-hydrochloride-buffered medium.*

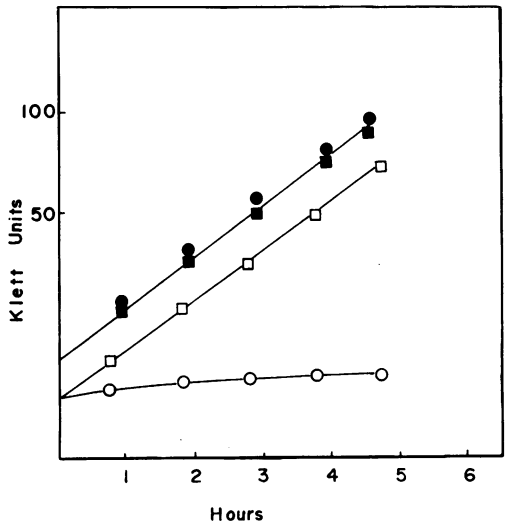

FIG. 3. *Effects of* DL-*glyceraldehyde 3-phosphate on the growth of E. coli 8 and a glycerol 3-phosphate transport-negative mutant of this strain. Cells were cultured in Garen and Levinthal medium (8) supplemented with 0.5% potassium succinate.* DL-*Glyceraldehyde was added to early-logarithmic-phase cultures at the start of the experiment. The additions were as follows: strain 8:* □, *untreated;* ○, *2 mM* DL-*glyceraldehyde 3-phosphate. Glycerol 3-phosphate transport negative-mutant of strain 8:* ■, *untreated;* ●, *2 mM* DL-*glyceraldehyde 3-phosphate.*

distinguish among the possibilities. D-Glyceraldehyde has an equally slight effect upon strains 8 and 9 (Fig. 4 A and B), which was not studied further.

One method for delineating the site of action of a drug is to examine the effects of various concentrations of the drug on macromolecular and lipid synthesis. This approach has been helpful in studies with another glycerol 3-phosphate analogue, 3,4-dihydroxybutyl-1-phosphonate (16, 17), and with phenethyl alcohol (14). However, as evident from Table 2, this method failed to reveal a single most sensitive site of action for DL-glyceraldehyde 3-phosphate. Experiments similar to those reported in Table 2 for shorter incubation periods and different glyceraldehyde 3-phosphate concentrations also failed to differentiate a site of action. Among the processes examined, protein synthesis appears to be the least affected by glyceraldehyde 3-phosphate. It is therefore unlikely that this agent exerts its primary effect upon amino acid synthesis or energy metabolism.

A second approach to the site of action involves a study of the effects of a drug on various mutants. The lack of catabolic glycerol 3-phosphate dehydrogenase activity in strain 8 is not related to the inhibitory effects observed, since strain 7 and other cells having this activity were quite susceptible to DL-glyceraldehyde 3-

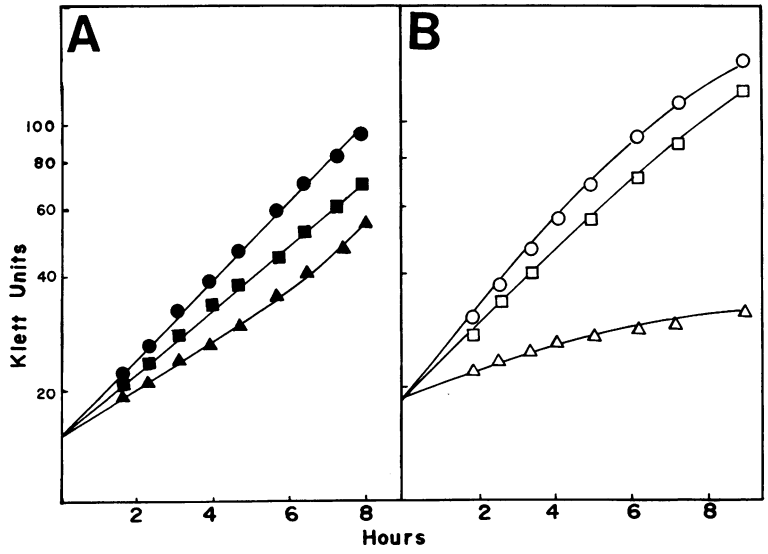

FIG. 4. *Effects of* L- *and* D-*glyceraldehyde on the growth of E. coli 8 and 9 in Bicine-buffered medium, as described in Materials and Methods, supplemented with 0.5% potassium succinate.* L- *or* D-*Glyceraldehyde was added to a final concentration of 2.5 mM to early-logarithmic-phase cultures at the start of the experiment. Symbols: (A) Strain 9:* ●, *untreated;* ■, D-*glyceraldehyde;* ▲, L-*glyceraldehyde. (B) Strain 8:* ○, *untreated;* □, D-*glyceraldehyde;* △, L-*glyceraldehyde.*

TABLE 2. *Effect of various concentrations of* DL-*glyceraldehyde 3-phosphate on macromolecular and lipid synthesis*[a]

Macromolecular synthesis	DL-Glyceraldehyde 3-phosphate			
	0.05 M		0.25 M	
	Total cpm per ml of cells	% of untreated cultures	Total cpm per ml of cells	% of untreated cultures
DNA	2,780	85	1,380	42
RNA	52,800	83	25,200	40
Protein	9,550	101	6,600	69
Phosphoglyceride	28,300	66	15,800	37

[a] DNA, RNA, protein, and phospholipid synthesis by *E. coli* 8 cultured in Garen and Levinthal medium (8) supplemented with 0.5% potassium succinate were monitored as a function of different concentrations of DL-glyceraldehyde 3-phosphate. Labeled precursors of DNA, RNA, and phospholipid, [^{33}P]phosphate, or the precursor of protein, [^{3}H]isoleucine, were added at the same time as the inhibitor and incubated for 60 min. The incorporation studies were performed as described in Materials and Methods.

phosphate. The doubling time of strain 7 increased from 1.8 h to greater than 19 h upon addition of inhibitor.

One possible site of activity for DL-glyceraldehyde 3-phosphate is the anabolic NAD(P):*sn*-glycerol 3-phosphate oxidoreductase (11). This enzyme is inhibited by 3,4-dihydroxybutyl-1-phosphonate (4). This possibility was eliminated by the observation that strain CY 115 (an anabolic dehydrogenase-deficient strain [5], cultured on synthetic medium containing glycerol as the sole carbon source, was quite susceptible to DL-glyceraldehyde 3-phosphate.

L-Glyceraldehyde 3-phosphate might also serve as a substrate of fructose 1,6-diphosphate aldolase. In this case, L-sorbose 1,6-diphosphate would be formed, which might then be the true inhibitor. This possibility was ruled out by the observation that *E. coli* NP 315, a temperature-sensitive fructose 1,6-diphosphate aldolase mutant (3), was quite susceptible to DL-glyceraldehyde 3-phosphate at either 30°C or the nonpermissive temperature, 42°C. At the higher temperature, the addition of 2.5 mM DL-glyceraldehyde 3-phosphate caused an increase in the doubling time from 2 h to greater than 20 h. The other glycolytic enzymes are not likely targets because of their greater sterospecificity for D-glyceraldehyde 3-phosphate.

A third approach to the site of action is to determine whether the drug inhibits a specific enzymatic activity in an in vitro assay. Acyl CoA:*sn*-glycerol 3-phosphate acyltransferase, the first enzyme of the phosphoglyceride biosynthetic pathway, is one of the potential target sites. When assayed by the radioactive assay method as shown in Fig. 5, DL-glyceraldehyde 3-phosphate is a competitive inhibitor of the acyltransferase. D-Glyceraldehyde 3-phosphate does not inhibit the acyltransferase. The apparent K_i for L-glyceraldehyde 3-phosphate is 1.8 mM. The results involving acyltransferase referred to thus far are for palmitoyl CoA as substrate. The incorporation of the oleoyl residue from oleoyl CoA was also inhibited by DL-glyceraldehyde 3-phosphate. The spectrophotometric assay confirmed the glyceraldehyde 3-phosphate inhibition of acyltransferase activity. Furthermore, this assay revealed that DL-glyceraldehyde 3-phosphate does not stimulate the hydrolysis of either palmitoyl CoA or oleoyl CoA and is therefore not a substrate for the acyltransferase.

It appeared possible that K_m mutants for the acyltransferase (2), by virture of an inability to recognize L-glyceraldehyde 3-phosphate, might be resistant to this compound. In agreement with the report of Bell and Cronan (2), we observed that the acyltransferase of BB26-36 R2 had a K_m of 577 μM for *sn*-glycerol 3-phosphate. In the presence of 400 μM *sn*-glycerol 3-phosphate, 7.1 mM DL-glyceraldehyde 3-phosphate inhibited mutant acyltransferase by greater than 60% when assayed at either 25 or 30°C. The acyltransferase of BB26-36 R2 therefore recognizes glyceraldehyde 3-phosphate. The drug is also bactericidal to strain BB26-36 R2. We have not examined other acyltransferase mutants.

Acyl CoA:lysophosphatidate acyltransferase, the second enzyme of phosphoglyceride biosynthesis, was not sensitive to DL-glyceraldehyde

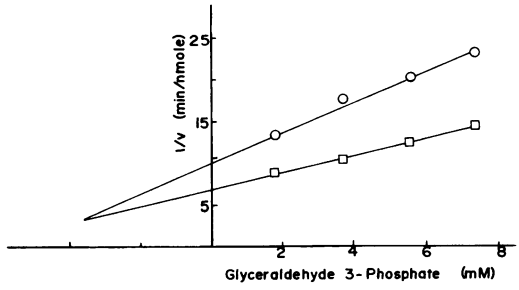

FIG. 5. *Determination of the apparent K_i of* DL-*glyceraldehyde 3-phosphate for acyl CoA:sn-glycerol 3-phosphate acyltransferase by the Dixon method (7). The assay conditions are described in Materials and Methods. The reaction mixtures were* □, *64.6 μM, and* ○, *32.3 μM, in sn-[^{14}C]glycerol 3-phosphate 4 and contained varying concentrations of* DL-*glyceraldehyde 3-phosphate.*

3-phosphate. This is significant, since it indicates that all the sensitivity observed in the *sn*-glycerol 3-phosphate:acyl CoA acyltransferase assay is specific for this enzyme.

DL-Glyceraldehyde 3-phosphate is a competitive inhibitor of CDP-diglyceride:*sn*-glycerol 3-phosphate phosphatidyltransferase (Fig. 6). The D enantiomer was without effect. The apparent K_i for L-glyceraldehyde 3-phosphate is 2.7 mM. The physiological significance of this result was examined by determining the effect of concentrations of DL-glyceraldehyde 3-phosphate to 0.50 mM on the distribution of phosphoglycerides accumulated by *E. coli* 8 for 1 h. At 0.05, 0.10, 0.25, and 0.50 mM DL-glyceraldehyde 3-phosphate, phosphatidylglycerol accumulation was 73, 60, 44, and 25% of untreated cultures, respectively. The percentage of control for phosphatidylethanolamine was 73, 66, 44, and 23%, respectively. Thus, there was no differential effect on the accumulation of these major phospholipids. Cardiolipin accumulation was not noticeably affected by glyceraldehyde 3-phosphate. Although the above results were obtained using [^{33}P]phosphate to monitor phospholipid accumulation, similar results were obtained when [^{14}C]acetate served as the radioactive tracer. These findings are in striking contrast to the effects of 3,4-dihydroxybutyl-1-phosphonate, which preferentially inhibits the accumulation of phosphatidylglycerol (16, 17). The phosphonate analogue also has a lower apparent K_i for the phosphatidyltransferase, 740 μM (4).

DISCUSSION

DL-Glyceraldehyde 3-phosphate, an analogue of glycerol 3-phosphate, enters the cell via the *sn*-glycerol 3-phosphate transport system (Fig. 3) and is bactericidal (Fig. 2). Several lines of evidence point to L-glyceraldehyde 3-phosphate being the active agent. The D enantiomer is without effect, whereas the racemic mixture is quite active. Hayashi et al. have demonstrated that DL-glyceraldehyde 3-phosphate is an inhibitor of *sn*-glycerol 3-phosphate transport (9). The stereospecificity of this system and its requirement for cell susceptibility strongly suggests that the L form is the active one. Finally, L-glyceraldehyde is quite an effective inhibitor of cell growth in strains capable of phosphorylating this compound. The D form is considerably less active.

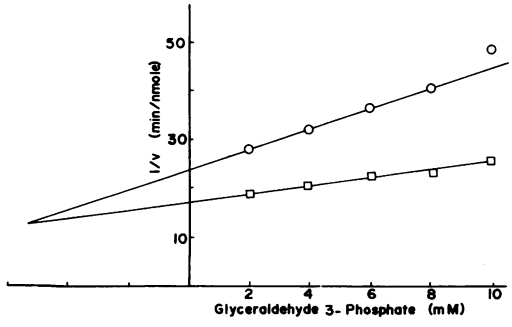

FIG. 6. *Determination of the apparent K_i of DL-glyceraldehyde 3-phosphate for CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase by the Dixon method (7). The assay conditions are described in Materials and Methods. The reaction mixtures were □, 64.6 μM, and ○, 32.3 μM, in sn-[^{14}C]glycerol 3-phosphate and contained varying amounts of DL-glyceraldehyde 3-phosphate.*

Comparison between the inhibitory effects of 3,4-dihydroxybutyl-1-phosphonate and glyceraldehyde 3-phosphate reveals several differences. Among these are the following: (i) 3,4-Dihydroxybutyl-1-phosphonate preferentially inhibits phosphatidylgycerol synthesis (16, 17), whereas the aldehyde does not. (ii) The phosphonate is bacteriostatic (15), whereas glyceraldehyde 3-phosphate is bactericidal (Fig. 2). (iii) L-Glyceraldehyde 3-phosphate is a competitive inhibitor for acyl CoA:*sn*-glycerol 3-phosphate acyltransferase (Fig. 5), whereas the phosphonate is not an inhibitor of this enzyme (4). (iv) Although both analogues are recognized by CDP-diglyceride:*sn*-glycerol 3-phosphate phosphatidyltransferase, the K_i for the phosphonate is 740 μM (4) and the K_i for L-glyceraldehyde 3-phosphate is 2.7 mM (Fig. 6).

The mechanism by which L-glyceraldehyde 3-phosphate kills cells is not known. Studies with mutants (1) and inhibitors (13, 14, 16, 17) indicate that a block in phosphoglyceride synthesis is frequently accompanied by an inhibition of macromolecular synthesis. If, as appears possible, the primary target of L-glyceraldehyde 3-phosphate is at the level of phosphoglyceride biosynthesis, this compound should prove to be an important research tool in the study of phosphoglyceride metabolism. This is particularly so, since the nonphosphorylated derivative should be quite permeable to many cell membranes and, therefore, not require a special transport system. Once inside the cell, glycerokinase can activate the L-glyceraldehyde as shown for *E. coli* (Fig. 4). Preliminary experiments with *Bacillus subtilis* and yeast revealed that the former is quite susceptible to L-glyceraldehyde and DL-glyceraldehyde 3-phosphate, whereas the latter is not (unpublished data of D. Klein, R. Engel, and B. Tropp). Further work is required to determine the primary target. We are currently examining this question.

It is of interest that, although D-glyceraldehyde 3-phosphate and *sn*-glycerol 3-phosphate

are ubiquitous metabolic intermediates, L-glyceraldehyde 3-phosphate is not a naturally occurring biological compound. The close structural relationship between glyceraldehyde 3-phosphate and glycerol 3-phosphate may be responsible for the evolution of optically unrelated intermediates for carbohydrate and phosphoglyceride metabolism.

ACKNOWLEDGMENTS

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