Metabolism of L-Glyceraldehyde 3-Phosphate in Escherichia coli

METARAMBA K. G. S. KALYANANDA, ROBERT ENGEL, AND BURTON E. TROPP*

Doctoral Programs in Biochemistry and Chemistry, The City University of New York, Queens College, Flushing, New York 11367

Received 17 February 1987/Accepted 21 February 1987

When either 3H-labeled L-glyceraldehyde or 3H-labeled L-glyceraldehyde 3-phosphate (GAP) was added to cultures of Escherichia coli, the phosphoglycerides were labeled. More than 81% of the label appeared in the backbone of the phosphoglycerides. Chromatographic analyses of the labeled phosphoglycerides revealed that the label was normally distributed among phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin. These results suggest that L-glyceraldehyde is phosphorylated and the resultant L-GAP is converted into sn-glycerol 3-phosphate (G3P) before being incorporated into the bacterial phosphoglycerides. Cell-free bacterial extracts catalyzed an NADPH-dependent reduction of L-GAP to sn-G3P. The partially purified enzyme was specific for L-GAP and recognized neither d-GAP nor dihydroxyacetone phosphate as a substrate. NADH could not replace NADPH as a coenzyme. The L-GAP:NADPH oxidoreductase had an apparent Km of 28 and 35 μM for L-GAP and NADPH, respectively. The enzyme was insensitive to sulfhydryl reagents and had a pH optimum of approximately 6.6. The phosphonic acid analog of GAP, 3-hydroxy-4-oxobutyl-1-phosphonate, was a substrate for the reductase, with an apparent Km of 280 μM.

L-Glyceraldehyde 3-phosphate (GAP) is a bactericidal agent that enters Escherichia coli via either the sn-glycerol 3-phosphate (G3P) or hexose phosphate transport system (5, 12). Intracellular L-GAP can also be formed by a glycero kinase-catalyzed phosphorylation of L-glyceraldehyde that is taken up from the growth medium (12). The mechanism by which L-GAP causes E. coli to lose viability is not known.

One possibility is that L-GAP exerts its bactericidal effect by interfering with a critical biochemical reaction. In aqueous solution, L-GAP exists as an equilibrium mixture of the hydrated and free aldehyde in a molar ratio of 29:1 (14). Since the hydrated form may be considered an analog of sn-G3P, L-GAP has the potential to interfere with enzymes involved in sn-G3P metabolism. L-GAP is a competitive inhibitor of both sn-G3P acyltransferase and phosphatidylglycerol phosphate synthetase (12, 13). However, these inhibitory activities do not necessarily explain the bactericidal activity of L-GAP. The sn-G3P analogs 3-hydroxy-4-oxobutyl-1-phosphonate and 1,3,4-trihydroxybutyl-1-phosphonate are bacteriostatic rather than bactericidal, even though they also inhibit the two lipid enzymes (13; unpublished data).

A second possibility is that L-GAP is not itself bactericidal but is instead a precursor for the true bactericidal agent. L-[3-3H]glyceraldehyde and L-[3-3H]GAP were synthesized to investigate this possibility. While following the metabolic fate of the labeled compounds, it became evident that E. coli has the heretofore unreported ability to convert L-GAP into sn-G3P. This report describes the evidence for the in vivo conversion of L-GAP into sn-G3P as well as for the existence of an NADPH-dependent enzyme that catalyzes the reaction.

MATERIALS AND METHODS

Chemicals. D- and L-glyceraldehyde grade II, Dglyceraldehyde 3-phosphate (diethylacetal, monobarium salt), rac-glyceraldehyde 3-phosphate, dihydroxyacetone phosphate, l-mannose, ATP, NADP+, NADPH (type 1, chemically reduced), NADPH (type III, enzymatically reduced), N,N-bis-(2-hydroxyethyl) glycine (bicine), Tris, cacoacylic acid, casein hydrolysate, rabbit muscle sn-G3P dehydroglycerone, triose phosphate isomerase (type III), E. coli alkaline phosphatase (type III), and E. coli glycerokinase were purchased from Sigma Chemical Co., St. Louis, Mo. DEAE-Sephadex A50 was a product of Pharmacia Fine Chemicals, Piscataway, N.J. Dowex 50 and Dowex 1 were obtained from Bio-Rad Laboratories, Richmond, Calif. DEAE-cellulose (DE-52) and Whatman 3MM chromatography paper were purchased from Scientific Products, Edison, N.J. Silica Gel G thin-layer plates were obtained from Analabs Inc., North Haven, Conn. Membrane filters (type HAWP, pore size 0.45 μm) were purchased from Millipore Corp., Bedford Mass. Tritium-labeled sodium borohydride was purchased from ICN Corp., Irvine, Calif. Dl-3-Hydroxy-4-oxobutyl-1-phosphonate was prepared as described previously (3).

Preparation of L-[3-3H]GAP and tritium-labeled sn-G3P. L-Mannose (40 mg) was reduced with 2.7 mg of tritiated sodium borohydride (specific activity, 350 μCi/mmol) in 0.5 ml of water. Sodium borate was removed from the mixture by successive chromatography on microcolumns (1 by 0.5 cm) of Dowex 50, H+, and then Dowex 1, OH−. After each elution with distilled water, the radioactive material was concentrated by lyophilization. The L-[3-3H]mannitol thus obtained was incubated with 320 μl of zinc chloride (20% (wt/vol) in anhydrous acetone) for 2 h at 37°C to obtain the 1:2:5:6-diacetonide of L-mannitol. The mixture was treated with 1 ml of 33% potassium carbonate, and the resulting zinc carbonate was removed by centrifugation. The supernatant was repeatedly extracted with ether. The combined ether extracts were dried, suspended in 0.5 ml of a 7.4% (wt/vol) cold aqueous solution of sodium periodate, and permitted to stand for 10 min at 0°C. Salts were removed by ion-exchange resins as described for removal of sodium borate. The acetoxy-protecting groups were removed by a 30-min incubation with Dowex 50 H+ at 37°C. Chromatographic analysis of this material indicated that a small amount of unreacted L-[3-3H]mannitol was present as an impurity. L-[3-3H] glyceraldehyde was purified by paper chromatography with

* Corresponding author.
The labeled an-propanol-ethyl acetate–water (7:2:1) solvent system. The labeled glyceraldehyde was examined in several additional solvent systems. In each case, the labeled glyceraldehyde cochromatographed with authentic glyceraldehyde. The reaction mixture contained 2 μmol of L-[3-3H]glyceraldehyde (specific activity, 43.8 mCi/mmol) and 2.75 U, 55 nmol of phosphorolyzed substrate. The reaction mixture was incubated at 27°C for 1 h, and the labeled product was purified by chromatography on a DEAE-cellulose column. The activity of the labeled compound in the glycerokinase-catalyzed reaction confirmed that it was the L-enantiomer, because D-glyceraldehyde is not phosphorylated by this enzyme (7). It was not necessary to use chromatographically purified L-glyceraldehyde as starting material because the contaminating mannitol was readily separated from the labeled L-GAP during DEAE-cellulose chromatography. Because of its instability, a fresh sample of L-GAP had to be prepared before each experiment. The L-[3-3H]GAP did not react with NADH in the presence of triose phosphate isomerase and G3P dehydrogenase, eliminating the possibility of contamination with either D-GAP or dihydroxyacetone phosphate. The complete absence of the latter was also verified chromatographically. L-[3-3H]glycerol and sn-[3-3H]G3P were prepared by sodium borohydride reduction of L-[3-3H]glyceraldehyde and L-[3-3H]GAP, respectively. sn-[2-3H]G3P was synthesized by reduction of dihydroxyacetone with labeled sodium borohydride, followed by enzymatic phosphorylation of the purified [2-3H]glycerol, essentially as described for the production of L-[3-3H]GAP.

Enzyme assays. Three different procedures were used to assay L-GAP:NADPH oxidoreductase (L-GAP reductase). The method used depended on the degree of purity of the enzyme and the convenience of the assay. In the chromatographic method, an assay mixture containing 23.8 nmol of L-[3-3H]GAP (43.8 mCi/mmol), 0.6 μmol of NADPH (Sigma type III), 2.3 μmol of bicarbonate, pH 7.6, and 8 to 250 μg of protein in a final volume of 0.215 ml was incubated at 27°C. At the indicated time, 30 μl was pipetted into a tube containing 10 μl of 88% formic acid, and the tube was then placed in a boiling-water bath for 2 min. The soluble material was spotted onto Whatman 3MM chromatography paper and developed in ethyl acetate-formic acid-water (5:2:1). In the solvent extraction method, the assay mixture was identical to that described above. At the indicated times, 10-μl samples were withdrawn and pipetted into a screw-cap tube containing 30 μl of 2,4-dinitrophenylhydrazine reagent (10 mg of 2,4-dinitrophenylhydrazine and 5 μl of concentrated sulfuric acid dissolved in 2 ml of methanol) and 5 μl of water was added. Capped tubes were incubated for 10 min at 80°C, and then the caps were removed to permit complete solvent evaporation during a continued 20-min incubation. One-tenth milliliter of 10% sodium carbonate (wt/vol) was added to each tube, followed by 10 μl of unlabeled rac-G3P (50 mM) and 0.9 ml of water. The mixture was sequentially extracted with 1.5 ml of chloroform, 0.5 ml of liquefied phenol (88%, wt/vol), and twice with 1 ml of chloroform. A portion of the aqueous phase was transferred into a scintillation vial containing 0.5 ml of distilled water and 5 ml of Patterson-Greene scintillation fluid (9). Experiments with sn-[2-3H]G3P and l-[3-3H]GAP revealed that 62% of the G3P was recovered in the aqueous extract, whereas virtually none of the L-GAP was recovered in this fraction. Furthermore, there was an excellent correlation of results when the solvent extract assay was compared with the chromatographic assay. The solvent extraction method was particularly useful for assaying fractions during enzyme purification. After partial enzyme purification, a spectrophotometric assay was feasible because there were no interfering enzyme activities. In this assay, 1 ml of incubation mixture containing 50 μmol of triethanolamine hydrochloride, pH 7.4, 150 nmol of NADPH (Sigma type III), the indicated concentration of G3P, and partially purified enzyme. The absorption change at 340 nm was monitored at 27°C in a Gilford model 250 spectrophotometer fitted with a multisample absorbance recorder.

G3P synthetase was assayed as described by Spector and Pizer (11). Triose phosphate isomerase contamination of various enzyme fractions was detected by monitoring the absorption change at 340 nm of an assay mixture containing 50 μmol of triethanolamine hydrochloride, pH 7.5, 100 nmol of NADH, 0.7 U of rabbit muscle G3P dehydrogenase, 1.6 μmol of Dl-GAP, and enzyme in a final volume of 1 ml. One unit of L-GAP reductase and sn-G3P synthetase activity converts 1 nmol of substrate into product per min at 27°C, and 1 U of triose phosphate isomerase activity converts 1 μmol of substrate into product per min at 27°C.

Bacterial strains and culture conditions. Bicarbonate-buffered minimal medium (B-medium) has been described (12). The minimal media were supplemented with 0.2 to 0.5% of the indicated carbon source. CH medium contained 1% casein hydrolysate, pH 7.4, and 0.5% sodium chloride. Cell growth and viability were followed as described previously (12). The bacterial strains used were strain 7 (phoA8 glpR fhuA22 rel-1 T22 λ') (6), strain 8 (strain 7 but glpD) (6), strain 9 (strain 8 but glpK) (6), and strain BB20 (strain 8 but glpA20) (1). Strain 7 was generously provided by E. C. C. Lin (Harvard Medical School), strains 8 and 9 by B. Bachmann (E. coli Genetic Stock Center, Yale University), and BB20 by R. M. Bell (Duke University Medical School).

Phosphoglyceride synthesis and characterization. Cells were cultured in B-medium containing 0.5% potassium succinate as the sole carbon source. When the bacterial culture reached 7.5 × 10⁸ cells per ml, 3 ml was removed and treated with 1.9 μCi of either L-[1-3H]glycerol or L-[3-3H]glyceraldehyde or 6 ml was removed and treated with 3.8 μCi of L-[3-3H]GAP or sn-[3-3H]G3P. The specific activity of each radioactive reagent was 15 μCi/μmol. Samples were removed at the times indicated, and phospholipids were extracted and analyzed as described previously (10). Labeled lipids were deactivated by the procedure of Dittmer and Wells (2).

Purification of L-GAP reductase. A suspension containing 60 g of bacteria in 600 ml of 20 mM sodium phosphate, pH 7.6, was sonicated in three batches (2 s/ml) with 10-s bursts in a Bronson ultrasonic sonicator at setting 8. The crude extract was centrifuged at 5,000 × g for 20 min, the pellet was discarded, and the supernatant was centrifuged for another 30 min at 30,000 × g. The supernatant was treated with solid ammonium sulfate, and the 26 to 37% saturation cut was collected, suspended in 20 mM sodium phosphate buffer (pH 7.6), dialyzed against three changes (1.8 liters each) of the same buffer, and applied to a DEAE-Sephadex A-50 column (2.7 by 31 cm). The column was first eluted with 100 ml of 20 mM sodium phosphate buffer at pH 7.6 and
RESULTS AND DISCUSSION

The possibility that L-glyceraldehyde serves as a precursor for an abnormal phosphoglyceride was investigated by incubating early-log-phase strain 8 (glpR glpD) with either L-[3-3H]glyceraldehyde or [1-3H]glycerol for 40 min and comparing the isolated phosphoglyceride fractions by thin-layer chromatography. The distribution of label into phosphatidylyethanolamine, phosphatidylglycerol, and cardiolipin was the same whether the cultures were incubated with L-[3-3H]glyceraldehyde or [1-3H]glycerol (data not shown). Furthermore, no abnormal lipids were detected when the cells were labeled with L-[3-3H]glyceraldehyde. L-[3-3H]glyceraldehyde labeled phosphoglycerides to approximately one-third the extent observed when L-[1-3H]glycerol was used (Fig. 1).

E. coli glyceralokinase is known to catalyze the phosphorylation of L-glyceraldehyde to form L-GAP (7). It therefore seemed possible that L-glyceraldehyde must be converted into L-GAP before it can be incorporated into bacterial phosphoglycerides. This possibility was tested by comparing the incorporation of L-[3-3H]glyceraldehyde and [1-3H]glycerol into the phosphoglycerides of strains 8 and 9 (glpR glpD glpK). As expected, considerably more [1-3H]glycerol was incorporated into the lipids of strain 8 than into those of strain 9 (Fig. 1). The same was also true for L-[3-3H]glyceraldehyde (Fig. 1), suggesting that it must be converted into L-GAP before being incorporated into phosphoglycerides. In support of this hypothesis, the incorporation of L-[3-3H]GAP and sn-[3-3H]G3P into the phosphoglycerides of strain 9 was nearly identical to their incorporation into the phosphoglycerides of strain 8 (Fig. 2).

The phosphoglycerides isolated from strain 8, treated with either L-[3-3H]glyceraldehyde, [1-3H]glycerol, L-[3-3H]GAP, or sn-[3-3H]G3P, were subjected to mild alkaline hydrolysis. Between 10 and 19% of the label was associated with the fatty acid fraction and the remainder with the water-soluble fraction. When sn-[2-3H]G3P was used, approximately 99% of the label was in the aqueous fraction. These studies indicate that a small but significant proportion of sn-G3P was catabolized by strain 8 even though it lacks G3P dehydrogenase activity. Together, these results indicate that 81% or more of the label originally associated with L-glyceraldehyde or L-GAP was incorporated into the sn-G3P backbone of the phosphoglycerides. For phosphatidylglycerol and cardiolipin, this of necessity means that the label is present in the glycerol moiety.

The results described thus far can be explained most simply by postulating the following metabolic sequence: (i) glyceralokinase-catalyzed phosphorylation of L-glyceraldehyde into L-GAP (or direct uptake of L-GAP), (ii) conversion of L-GAP into sn-G3P, and (iii) incorporation of sn-G3P into phosphoglycerides. Several pathways for the conversion of L-GAP into sn-G3P may be envisioned. The most direct, aldehyde reduction, was the first examined. Soluble extracts prepared from E. coli catalyzed an NADPH-dependent reduction of L-GAP to sn-G3P (Fig. 3). NADH could not replace NADPH. The nature of the product was verified by chromatography in several solvent systems. Furthermore, after alkaline phosphatase hydrolysis, the labeled compound cochromatographed with glycerol. L-GAP dehydrogenase activity was found exclusively in the high-speed supernatant of crude extracts (data not shown). Extracts prepared from strain BB20 (gspa) catalyzed the reduction of L-GAP to sn-G3P (data not shown), indicating that G3P synthetase is not involved in the reduction.

FIG. 1. Incorporation of L-[3-3H]glyceraldehyde and [1-3H]glycerol into the phosphoglyceride fraction of E. coli. Strain 8 (gltR gltD) and strain 9 (gltR gltD gltK) were cultured in B medium (12) containing 0.5% potassium succinate as the sole carbon source at 37°C. At a turbidity of 0.5 Klett units, 3 ml of cells was removed and incubated with 1.9 μCi of either L-[3-3H]glyceraldehyde or [1-3H]glycerol. At the indicated times, 0.5-ml samples were removed, and the lipids were extracted as described previously (10). Symbols: △, strain 8; [1-3H]glycerol; △, strain 8, L-[3-3H]glyceraldehyde; ●, strain 9; [1-3H]glycerol; ○, strain 9, L-[3-3H]glyceraldehyde.

FIG. 2. Incorporation of L-[3-3H]GAP and sn-[3-3H]G3P into the phosphoglycerides of E. coli. Strains 8 and 9 were cultured as described in the legend to Fig. 1. At a turbidity of 0.5 Klett units, 6 ml of cells was incubated with 3.8 μCi of either L-[3-3H]GAP or sn-[3-3H]G3P. At the indicated times, 0.5-ml samples were removed, and lipids were extracted as described previously (10). Symbols: △, strain 8, L-[3-3H]GAP; ●, strain 8, sn-[3-3H]G3P; △, strain 9, L-[3-3H]GAP; ○, strain 9, sn-[3-3H]G3P.
FIG. 3. Conversion of l-GAP into G3P by E. coli extracts. A cell extract was prepared from E. coli strain 8 cultured to 70 Klett units in B-medium containing 0.5% potassium succinate as the sole carbon source. The assay mixture, containing 23.8 nmol of l-[3-3H]GAP (specific activity, 43.8 μCi/μmol), 600 nmol of NADPH, 2.3 μmol of bicine, pH 7.6, and 18 μg of soluble cell extract protein in a final volume of 0.215 ml, was incubated at 27°C for 0 (— — —) or 10 min (— —). The reaction was stopped as described in the text, and the product was spotted on Whatman 3MM chromatography paper. The chromatogram was developed in ethyl acetate-formic acid-water (5:2:1).

Before attempting enzyme purification, it was deemed desirable to develop a more convenient assay procedure. The solvent extraction assay described in Materials and Methods is based on the fact that GAP reacts with phenylhydrazine to form a water-insoluble product, whereas G3P does not react and remains water soluble. Identical kinetic results were obtained when the chromatographic and solvent extraction assays were used on the same incubation mixture. The solvent extraction assay was used in most subsequent experiments with crude extracts.

l-GAP reductase activity was extremely low in frozen E. coli paste purchased from a commercial source. This raised the possibility that culture conditions affect enzyme activity. Extracts prepared from cells cultured either in B-medium plus 0.5% potassium succinate or in CH medium had a much higher l-GAP reductase activity than an extract prepared from cells cultured in B-medium plus 0.5% glucose (Table 1). The reason for this is unclear. Since some increase in the l-GAP reductase activity was observed when extracts obtained from cells cultured in glucose were dialyzed (data not shown), the effect may be due in part to a low-molecular-weight metabolite. However, catabolite repression cannot be ruled out at this stage. The age of the culture also appeared to have some effect on l-GAP reductase activity (Table 1).

Enzyme was partially purified from strain 8 cultured in CH medium, harvested at 170 Klett units, and stored frozen until needed. The enzyme was partially purified by the process described in Materials and Methods and summarized in Table 2. The elution profile after chromatography on DEAE-Sephadex A-50 is shown in Fig. 4. Because activity could not be recovered from phosphocellulose or hydroxypatite columns, further attempts at purification were deferred. The partially purified enzyme was free of G3P synthetase activity (EC 1.1.1.94) but had some triose phosphate isomerase activity. Preliminary experiments indicated that the partially purified enzyme was stable after freezing. However, a considerable loss of activity was evident after a year's storage at −18°C. Although no loss in activity was observed after

<table>
<thead>
<tr>
<th>Table 1. Effect of culture conditions on l-GAP reductase activity in cell extracts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth medium</td>
</tr>
<tr>
<td>Bicine + succinate</td>
</tr>
<tr>
<td>Bicine + glucose</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
</tr>
</tbody>
</table>

*E. coli 8 was cultured in 125 ml of B-medium containing 0.5% potassium succinate or glucose as the sole carbon source in casein hydrolysate medium. The enzyme activity was measured by the solvent extraction assay (see Materials and Methods). One unit of enzyme activity is defined as the amount of enzyme that converts 1 nmol of l-GAP per min into sn-G3P.

FIG. 4. DEAE-Sephadex A-50 column chromatography for the purification of l-GAP reductase from the ammonium sulfate fraction. The details of the column chromatography procedure are presented in the text. The indicated column fractions were assayed for l-GAP reductase (●), triose phosphate isomerase (■), and protein concentration (□). Since sn-G3P synthetase activity was absent from the fractions containing l-GAP reductase, values for the synthetase are not presented. The spectrophotometric assay was used to determine l-GAP reductase activity, and the other assays were as described in the text. The potassium chloride concentrations were determined by comparison with standards by using a conductivity meter (□).

| TABLE 2. Purification procedure for l-GAP reductase* |
|----------------|----------------|----------------|----------------|----------------|
| Step | Total vol (ml) | Total protein (mg) | Total activity (U) | Sp act (U/mg of protein) | Yield (%) |
| Cell extract | 600 | 3,960 | 18,878 | 4.8 | 100 |
| Ammonium sulfate | 16 | 672 | 8,950 | 13.3 | 47 |
| DEAE-Sephadex fraction | 40 | 16.2 | 8,250 | 510 | 44 |
| A-50 pool | | | | | |

* Purification was carried out at 4°C. l-GAP reductase activity was measured by the solvent extraction method as described in Materials and Methods. See Table 1, footnote a, for definition of activity.
several freeze-thaw cycles, the enzyme was stored in small volumes and not reused.

L-GAP reductase has an absolute requirement for NADPH and cannot use NADH. Initial experiments with crude extracts indicated that enzymatically prepared NADPH (Sigma type III) was very active, while chemically prepared NADPH (Sigma type I) was inactive. Different batches of NADPH were tried with the same result. The chemically prepared coenzyme was quite active when assayed in a glutathione reductase-catalyzed reaction. Purification of the chemically prepared NADPH on a DEAE-cellulose column resulted in the isolation of an active coenzyme. Presumably, an inhibitor was removed by chromatography. Sodium bisulfite, a by-product of chemical reduction, was tested as an inhibitor of the partially purified L-GAP reductase. In a standard spectrophotometric assay mixture containing 124 μM DL-GAP, the addition of 5 or 19 μM sodium bisulfite resulted in 41 and 70% inhibition, respectively. Direct inactivation by adduct formation seems unlikely, because there was a large excess of L-GAP in relation to bisulfite. Instead, the substrate-bisulfite complex may be the true inhibitor, or bisulfite may react directly with the enzyme. In either case, bisulfite contamination could account for the inactivity of the chemically reduced coenzyme.

The partially purified enzyme had a pH optimum of approximately 6.6 (Fig. 5). Prior to this experiment, assays were performed at pH 7.6. All subsequent assays were run at pH 6.8 in triethanolamine hydrochloride buffer or at pH 6.7 in sodium cacodylate buffer. When L-GAP was present at 79 μM, the apparent Kₘ for NADPH was 35 μM, and when NADPH was present at 150 μM, the apparent Kₘ for L-GAP was 28 μM. Attempts to follow the reaction in the reverse direction have thus far been inconclusive. Neither D-GAP nor dihydroxyacetone phosphate was a substrate for the reductase. The GAP analog rac-3-hydroxy-4-oxobutyl-1-phosphonate was a substrate, with a relatively low apparent Kₘ (0.28 mM). Three other carbonyl compounds, methylglyoxal, L-glyceraldehyde, and D-glyceraldehyde, were poor substrates, with apparent Kₘ of 14, 28, and 100 mM, respectively. Because the enzyme was only partially purified, it is not clear whether recognition of these three carbonyl compounds is due to L-GAP reductase or contaminating activities.

L-GAP reductase was not inhibited by rac-G3P or by the sulfhydryl reagents iodoacetate (0.5 mM), N-ethylmaleimide (2.5 mM), or p-chloromercuribenzoate (0.06 mM). At 0.1 mM, MnCl₂ inhibited L-GAP reductase activity by 28%, while neither CaCl₂ nor MgCl₂ had any significant effect. At 0.1 and 1.0 mM, MnCl₂ stimulated the reductase activity by 28 and 91%, respectively. The manganese ion may interact with NADPH rather than with the enzyme itself.

L-GAP:NADPH oxireductase activity does not explain the toxicity of L-GAP. On the contrary, it seems to offer a method for detoxifying the compound, since it catalyzes the conversion of L-GAP into the normal metabolite sn-G3P. Therefore, the mechanism by which L-GAP kills E. coli still remains an open question.

Although an enzyme has been discovered that catalyzes the NADPH-dependent reduction of L-GAP into sn-G3P in vitro, its physiological role is not evident. L-GAP is not considered a natural metabolite. It was therefore surprising to discover a reductase that exhibited a rather low Kₘ for L-GAP and a fairly narrow range of substrate specificity. Neither dihydroxyacetone phosphate nor D-GAP, the two natural metabolites that most closely resemble L-GAP, could serve as substrate for the reductase. It is quite probable that the real substrate of the reductase is a natural metabolite that has not yet been tested. However, if L-GAP is the physiological substrate, then it is necessary to ask why E. coli would have evolved and maintained such an activity.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants GM-34688 and BSSG 07064 from the National Institutes of Health and by an award from the BHE/PSC grant program of the City University of New York. We thank Grace IlIanjian for technical assistance.

LITERATURE CITED