FORMATION OF A NEW PHOSPHATIDYL GLYCEROL DERIVATIVE IN E. COLI

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Summary

A particulate fraction isolated from E. coli cells was found to convert ^{14}C-labelled phosphatidyl glycerol into an hitherto uncharacterized lipid. Mild alkaline hydrolysis of this lipid yielded glycerophosphorylglycerol as the only product. Detailed chromatographic analysis identified the intact lipid as either bisphosphatidic acid or a close analogue. These results suggest a new pathway for the turnover of phosphatidyl glycerol in E. coli.

Introduction

Lyso analogues of bisphosphatidic acid (BisPA) have been reported to occur in plant and animal tissues (1-3). Although such lipids have not yet been characterized in microorganisms, there is evidence suggesting their occurrence in bacteria. Working with Salmonella typhimurium, Ames (4) reported the presence of two lipids which yielded glycerophosphorylglycerol (GPG) after mild alkaline hydrolysis. One of these lipids was less polar than phosphatidyl glycerol (PG) or an aminoacyl derivative of PG. No further characterization of the unknown lipid was made. Likewise, Kanemasa et al (5) found that chromatography of E. coli B lipids separated two phosphatides which after deacylation yielded GPG. They concluded that both these components consisted of PG which had separated anomalously due to complex formation with phosphatidyl ethanolamine. No further characterization of the two designated PG components was made.

In experiments intended to further elucidate the pathways for the turnover of PG in E. coli, we found that cell-free preparations readily converted labelled PG to a lipid resembling cardiolipin. This same substance could be detected in cells growing on a medium containing glycerol-^{14}C. Further analysis of this component, described presently, revealed that it was neither PG nor cardiolipin (DPG) but bisphosphatidic acid or a close analogue.

Preparation of ^{14}C-labelled phosphatidyl glycerol

E. coli 015 cells cultured to the late log phase in a medium adjusted to pH 7.4. The composition of the medium was as previously described (6). The cells were sonicated in 25 ml of 0.06 M phosphate buffer, pH 7.4, containing 0.01 M cysteine hydrochloride. Labelled PG was prepared by incubating 2 ml of cell sonicate (40 mg protein) with 0.01 M $MgCl_2$, 0.44 mM CTP, 3.75 mM ATP, 0.1 mM CoA 0.13 mM sonicated palmitic acid and 10 μc of L-α-glycerophosphate all contained in 4 ml of buffer. L-α-glycerophosphate-U-^{14}C (Sp. Act. 16 μc/mMol) was purchased from International Chemical and Nuclear Corp. Lipids were extracted by the method of Bligh and Dyer (7) and separated on $(NH_4)_2SO_4$ - silica gel H plates (system E), prepared from a slurry of 45 g silica gel H in 120 ml of 3.75% (w/v) ammonium sulfate, activated 90 min and used immediately. The chromatograms were developed with chloroform-methanol-water 65:25:4 (by vol). Labelled PG separated as a distinct component (Rf. 0.75), it cochromatographed with synthetic PG (a generous gift of Dr. P. Bonsen, University of Utrecht) and was revealed by the periodate-Schiff reagent (8) and, or by scanning for radioactivity. Mild alkaline hydrolysis of this component yielded a single labelled product identified as GPG in systems A, 1M ammonium acetate

pH 7.5 - absolute ethanol (35:65 by vol) (9); B, phenol-water (100:38 w/v); C, phenol-water-acetic acid-ethanol (50:22:3:3 by vol) (10); D, isopropanol-water-28% ammonia (7:2:1 by vol) (11).

Formation of an uncharacterized lipid from ^{14}C-labelled phosphatidyl glycerol

A 1 liter culture of E. coli 015 was harvested as previously described. The cells were suspended in 25 ml of 0.06 M phosphate buffer pH 7.4 containing 0.01 M cysteine hydrochloride and sonicated for 15 one-minute periods in ice. Whole cells were removed by centrifugation at 5000 g for 10 min. and the supernatant obtained was centrifuged at 30,000 g for 30 minutes. The particulate fraction was washed once and resuspended in cysteine-phosphate buffer gave a protein concentration of 12 mg/ml as determined by the method of Lowry et al (12). ^{14}C-Labelled PG ($1.0-3.5 \times 10^5$ DPM) dissolved in 5 ml of ether was added to a mixture containing 0.6 ml of particulate suspension, 0.4 ml of 0.05 M $CaCl_2$ and 3.0 ml of buffer. Incubations were carried out for 3 h at 37^{o} with mechanical shaking. A control was prepared with autoclaved particulate fraction. After Bligh and Dyer extraction, the lipids were separated on silica gel G plates developed with chloroform-methanol-water 65:25:4 w/v (System F). Figure 1 reveals that active particulate fractions of E. coli readily converted labelled PG to a major product (lipid X) which was ninhydrin and periodate-Schiff negative but stained for phosphate (13). Two minor products were also obtained but were not further analysed.

Intact lipid X was eluted by Bligh and Dyer extraction of the appropriate area of the chromatoplate after separation in system F. Mild alkaline hydrolysis of the uncharacterized lipid

Table I

Chromatographic Analysis of the Deacylation Product of Lipid X

Substance	R_f value obtained in various systems			
	A	B	C	D
diglycerophosphorylglycerol (GPGPG)	0.58	0.15	-	-
glycerophosphorylglycerophosphate (GPGP)*	0.20	-	-	-
glycerophosphate (GP)	0.26	0.28	-	0.18
glycerol (G)	0.77	0.77	-	0.72
glycerol-1,3-diphosphate (GDP)	0.05	-	-	-
glycerophosphorylglycerol (GPG)	0.67	0.44	0.44	0.57
deacylated lipid X	0.64	0.41	0.47	0.56
deacylated lipid X further hydrolysed with 90% acetic acid (19)	0.26 0.77	-	-	-

* R_f value obtained by Chang and Kennedy (9)

yielded GPG as the only labelled product identified by co-chromatography with reference compounds. Furthermore, hydrolysis of the deacylated product in 90% acetic acid yielded glycerophosphate and glycerol (Table I).

Since lipid X did not react with periodate and formed only with active E. coli preparation, it was unlikely to be a PG fraction which had separated anomalously. To further eliminate this possibility, labelled PG and lipid X isolated in a pure form by sequential chromatography in systems F and G described below were rechromatographed in system F, either as a mixture or separately. Since both substances separated with unaltered R_f values salt effects were not responsible for the appearance of

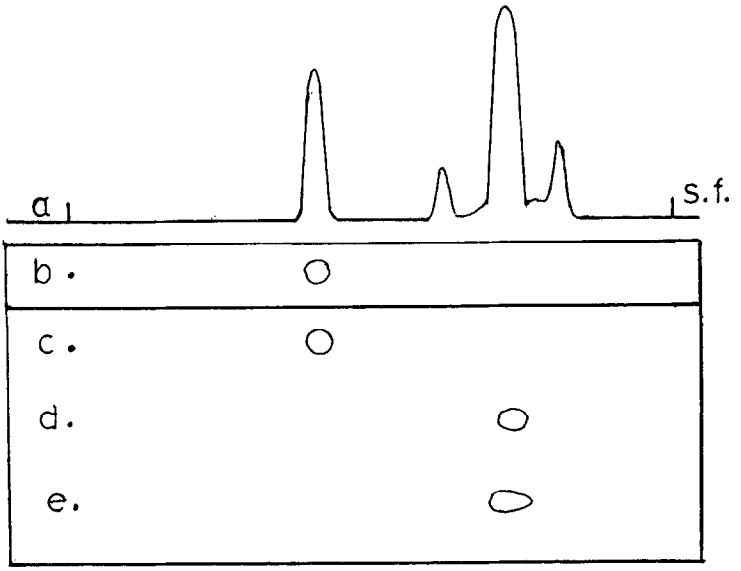

Fig. 1

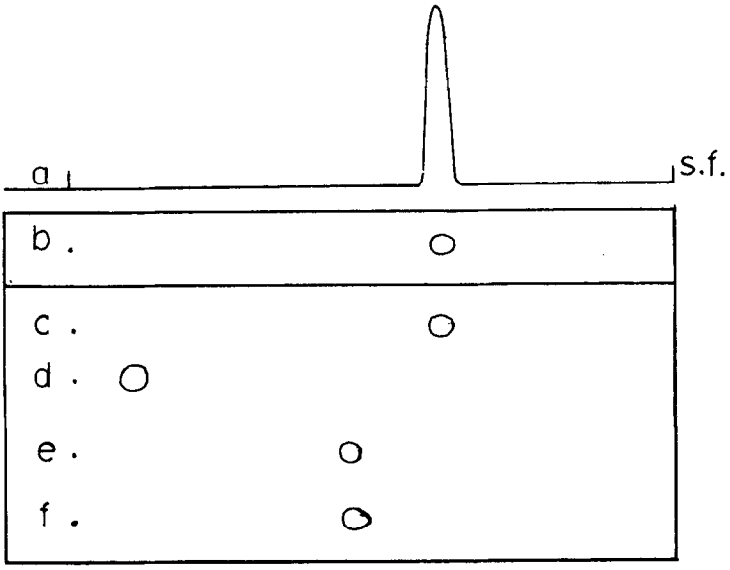

Fig. 2

Fig. 1 (a) Scan of chromatogram containing radioactive components produced by incubating E. coli preparation with ^{14}C-labelled phosphatidyl glycerol. The major, fast-running component is designated as lipid X in the text. (b) same chromatoplate stained with periodate-Schiff reagent (8) (c) phosphatidyl glycerol (d) ox-heart cardiolipin (e) tetra-palmityl-bisphosphatidic acid. Reference lipids were revealed with a phosphate spray (13). Separations were made on silica gel G plates with chloroform methanol-water 65:25:4 (v/v/v) as solvent (system F).

Fig. 2 (a) Scan of labelled lipid X isolated by chromatography in system F and rechromatographed in system G (silica gel G plates developed with chloroform-methanol-concentrated ammonia-water (70:30:4:2 by vol). (b) same chromatoplate as in (a) but stained for phosphate (4) (e) bisphosphatidic acid (d) phosphatidic acid (e) phosphatidyl glycerol (f) ox-heart cardiolipin.

fast-moving component containing GPG as basic structure. Whereas PG is known to be readily attacked by phospholipase D (14) lipid X was not degraded by prolonged incubations with this enzyme.

On silica gel G plates developed with chloroform-methanol-28% ammonia-water-70:30:4:2 (by vol.) (system G), lipid X cochromatographed with synthetic BisPA. Similar results were obtained when system F was used (Figs 1 and 2, lanes a and c).

On silica impregnated paper developed with the solvent system of Marinetti (15) (system H) lipid X ran as a single component and stained with tricomplex reagent (16) (Fig. 3). It cochromatographed with synthetic (tetrapalmityl)-Bis PA (purchased from Serdary Research Laboratories, London, Ontario).

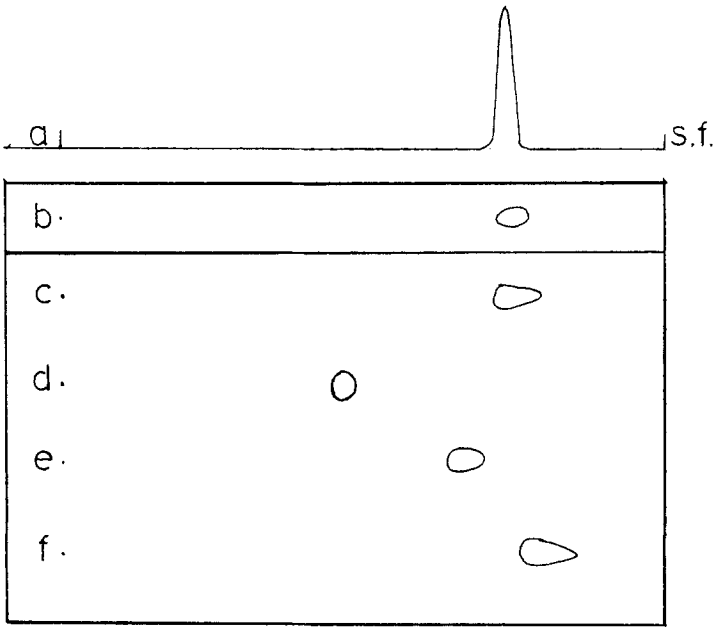

Fig. 3 (a) Scan of labelled lipid X isolated by chromatography in system F and rechromatographed in system H (15) (b) same chromatogram as in (a) but stained with tricomplex reagent (16) (c) bisphosphatidic acid (d) phosphatidyl glycerol (e) cardiolipin (f) phosphatidic acid.

Conclusion

It is established that PG serves as an intermediate for

the synthesis of cardiolipin when CDP-diglyceride is also present (17,18). Our results indicate that E. coli preparations can convert PG to a less polar lipid under other conditions. From our present data we conclude that the lipid is bisphosphatidic acid or a close analogue. Since the compound is formed with washed particulate fractions in the absence of added energy supply, a transacylation reaction may be involved. We are presently investigating this and other possibilities as well as the precise conditions which allow the formation of this lipid in various E. coli strains.

Acknowledgement

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