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On the Ornithinyl Ester of Phosphatidylglycerol of *Mycobacterium* 607

G. K. KHULLER AND D. SUBRAHMANYAM
Biochemistry Division, National Institute of Communicable Diseases, Delhi, India

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Ornithinyl ester of phosphatidyl glycerol was found to accumulate in *Mycobacterium* 607 under acidic conditions (*p*H 5.6) of growth or in cultures of ultraviolet-irradiated (320 to 420 nm) bacilli. There was a corresponding decrease in cardiolipin content of the organisms under these conditions.

Stancev et al. (14) reported that phosphatidylglycerol (PG) is an intermediate in the biosynthesis of cardiolipin in *Escherichia coli*. Although cardiolipin occurs in highest amounts among the phospholipids of mycobacteria, PG has not been found to be present at a significant level in this organism. However, the presence of traces of glyceryl phosphorylglycerol among the products obtained on mild alkaline hydrolysis of ^{32}P-labeled phospholipids of mycobacteria (D. Subrahmanyam, *unpublished observations*) suggested the occurrence of PG in detectable amounts. PG and amino acid esters of PG have been reported to be present in bacteria, and their relative concentration appears to depend on the *p*H of the growth media (4, 11). Further, PG content of particulate fractions of rat liver was shown by Schwarz et al. (13) to increase on whole-body X-ray irradiation. The present communication reports that, under acidic conditions of growth or in cultures of the bacilli exposed to ultraviolet irradiation, an amino acid ester of phosphatidylglycerol accumulated in *Mycobacterium* 607 to a considerable extent.

Large quantities of *Mycobacterium* 607 and *M. phlei* were grown in a modified Youman medium described elsewhere (15). The *p*H of the medium was adjusted daily to 5.6 when the bacilli were grown under acidic conditions. The *p*H of the media at the time of harvest was 5.8 to 6.0. For irradiation, 10 ml of the medium of *p*H 7.2 containing bacilli grown at the same *p*H were taken in a sterile screw-cap vial and exposed at a distance of 21.6 cm (8.5 inches) to a Black-Ray floodlight ultraviolet lamp with an intensity of 46.1 mw per cm^2 of radiation (320 to 420 nm) for 2 hr. The ultraviolet-irradiated bacilli were used for inoculation into Haffkine flasks containing the *p*H 7.2 medium. The final *p*H of the media prior to harvest of the irradiated cultures was 7.4. *Mycobacterium* 607 and *M. phlei* were grown for a period of 2 and 10 days, respectively. At the end of the growth period the lipids of the bacilli were extracted, purified, and examined by thin-layer chromatography (TLC) and by chemical analyses (5). Amino nitrogen and nitrogen were estimated by the methods of Lea and Rhodes (8) and Lang (7), respectively. Amino lipids were identified by spraying the plates with 0.2% ninhydrin in acetone-water (1:1) and heating at 110 C for 5 min. Phosphorus and glycerol in the deacylates and in sealed- tube hydrolysates of the lipid were estimated as detailed in a previous publication (15). PG from *E. coli* was a generous gift from S. Nojima.

A typical chromatogram of phospholipids of *Mycobacterium* 607 grown in acidic medium and from irradiated bacilli cultured at *p*H 7.2 developed with chloroform-methanol-water (65:25:4) is presented in Fig. 1. With this solvent system using silica gel G, it has been found that marker phosphatidylethanolamine (PE) and PG move as a single spot. Both in the *Mycobacterium* 607 grown at *p*H 5.6 and in irradiated organisms, a new ninhydrin-positive phospholipid, which moved behind PE as a distinct spot, was found to accumulate. This particular lipid was, however, absent in *M. phlei* treated similarly. The new lipid from *Mycobacterium* 607 was isolated by preparative-TLC in large amounts. On deacylation with mild alkali, the amino acid was released and the water-soluble phosphate ester on paper chromatography was found to cochromatograph with authentic glyceryl phosphorylglycerol in 1 M ammonium acetate (*p*H 7.5)-ethyl alcohol (35:65) and in phenol saturated with 0.1% ammonia. The ester had a glycerol-phosphorus ratio of 2. The amino acid released was found to be ornithine, which cochromatographed with the authentic amino acid on paper chromatography in pyridine-acetic acid-water (50:35:15), phenol-water (100:38), and on papers impregnated with

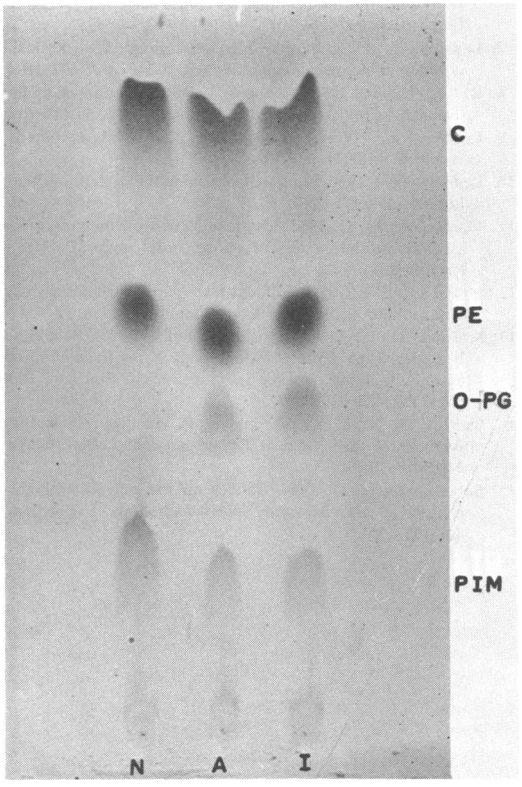

FIG. 1. *Thin-layer chromatogram of the phospholipids of Mycobacterium 607 grown under normal conditions (N); in acidic medium (A); the irradiated bacilli (I). Developing solvent: chloroform-methanol-water (65:25:4). The spots were developed by molybdenum spray. O-PG, ornithinyl ester of phosphatidylglycerol. Other major phospholipids in the chromatogram are: C, cardiolipin; PE, phosphatidylethanolamine; and PIM, phosphatidylinositomannosides.*

either 0.067 M phosphate buffer (*p*H 6.2) or 0.067 M boric acid-KCl buffer (*p*H 8.4) with phenol saturated with the same buffer (12) as developing solvent. The lipid had a phosphorus-amino nitrogen-nitrogen ratio of 1:1.7:2. Further, the lipid was emulsified in an MSE ultrasonic disintegrator (Measuring and Scientific Equipment, Ltd., London) at 20 kc/sec for 5 min in the cold in 0.2 M borate buffer, *p*H 9.0 (4), and incubated at room temperature (25 C) for 30 min. Suitable portions of chloroform and methanol were then added to form a Folch mixture (1), and the mixture was centrifuged to separate the aqueous and organic layers. This treatment resulted in release of ornithine which was identified in the aqueous layer by paper chromatography, and PG was identified by TLC as the sole lipid found in the organic layer. Emulsification by sonic treatment of the lipid under nonalkaline conditions did not result in release of the amino acid. The above data, therefore, led to the conclusion that the phospholipid was ornithinyl ester of PG.

Macfarlane (11) first discovered the presence of amino acyl esters of PG in bacteria. Houtsmuller and Van Deenen (3) identified ornithinyl PG among the phospholipids of *Bacillus cereus*. Ornithinyl PG was, however, not present in *Mycobacterium* 607 under normal conditions but was formed at a level comprising 10 to 13% of the total phosphatides when the bacilli were grown in acidic medium or in cultures of irradiated organisms. In confirmation with the observations of Lederer (9) and Lanéelle et al. (6), ornithinyl PG was not found to be a normal constituent of *Mycobacterium phlei*, and the present studies revealed that, unlike *Mycobacterium* 607, no such lipid accumulated in *M. phlei* grown in acidic medium or in irradiated organisms. Houtsmuller and Van Deenen (4) observed that in *Staphylococcus aureus* the relative amounts of PG and lysine ester of PG depended on the *p*H of the medium, acidic medium favoring accumulation of the amino acid ester. The amino acyl esters of PG were found to accumulate in certain other bacteria when the cells were under nongrowing conditions (10), but in *Mycobacterium* 607 it was formed in growing cells.

A decrease in cardiolipin was observed in *Mycobacterium* 607 to the same extent as the amino lipid formed with no significant change in the other phospholipids (Table 1). If PG is an intermediate in *Mycobacterium* 607 in the biosynthesis of the amino acyl PG and cardiolipin as in other bacteria (2, 14), the formation of ornithinyl PG might be expected to result in a corresponding decrease in cardiolipin content. Reasons for the accumulation of ornithinyl PG in irradiated bacilli are unknown. In *Mycobacterium* 607 the conversion of PG to cardiolipin may be affected under the present experimental conditions, and the available PG might be diverted in favor of ornithinyl PG synthesis. More

TABLE 1. *Cardiolipin and ornithinyl-phosphatidyl glycerol content of Mycobacterium 607 grown in acidic medium and in ultraviolet (UV)-irradiated cultures*

Phospholipid	Percentage of total phospholipids		
	Normal cultures	Acidic cultures	UV-exposed cultures
Cardiolipin	48–50	34–38	36–40
Ornithinyl-phosphatidyl glycerol	0	11–13	10–11

evidence is needed at the enzyme level to interpret these changes in lipid metabolism.

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