SHORT COMMUNICATION

Effect of Glycine on Phospholipids of *Mycobacterium smegmatis* ATCC 607

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Addition of glycine in low concentrations to modified Youman's medium led to a slight increase in both the total lipid and phospholipid content of *Mycobacterium smegmatis* ATCC 607 without a concomitant alteration in either the ratio of the individual phospholipids or the amount and characteristics of growth. However, high concentrations of glycine led to a decrease in both the total lipid and phospholipid content, a change in the ratio of the individual phospholipids, a decrease in the amount of growth and a change in its characteristics. The association of a decrease in the amount of growth and a change in its characteristic with a decrease in the content of triacylated mannophosphoinositides points to the biological importance of these phosphoglycolipids.

INTRODUCTION

L-forms of bacteria are induced by high concentrations of certain amino acids of which glycine is the most efficient (Dienes & Zamecnik, 1952). In the L-form the ratio of the various lipid components differs from that associated with the cell membrane of the parent bacterium (Ward & Perkins, 1968). For example, in the L-form of *Streptococcus pyogenes* the amount of glycolipid increases whereas that of the phospholipids decreases. In addition, the total lipid content of the L-form membrane of this organism is greater than that of the protoplast membrane (Cohen & Panos, 1966; Panos, 1967). As Panos (1967) has pointed out, the altered phospholipid content of the L-form membrane may have a significant effect on the organism, as lipids may orientate enzymes into a favourable configuration for activity.

The addition to the basal growth medium of those amino acids (at 1 M) capable of inducing the development of L-forms reduces the rate of growth of *Mycobacterium tuberculosis* (David, 1972). In view of these findings, the present study was undertaken to examine whether there is any correlation between the phospholipid content and composition and the growth of mycobacteria in a medium supplemented with various concentrations of glycine.

METHODS

Organism and culture conditions. *Mycobacterium smegmatis* ATCC 607 was grown at 37 °C for 7 d in 100 ml modified Youman's medium (Subrahmanyam, 1964), alone or supplemented with 1, 2, 5 or 10 g glycine l⁻¹, contained in 250 ml conical flasks. Triplicate cultures were grown in each medium, harvested by centrifugation at 3000 g for 30 min, and their lipids were extracted and analysed separately. Cells were dried at 60 °C to determine dry weight.

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Extraction and analysis of lipids. The lipids were extracted as described by Subrahmanyam (1964) and quantified gravimetrically. Phospholipid was estimated as phosphorus by the method of Bartlett (1959) as modified by Marinetti (1962). Individual phospholipids were separated from the extract of total lipids by preparative thin-layer chromatography on silica gel G plates using chloroform/methanol/water (65:25:4, by vol.) as developing solvent. The phospholipids were visualized by spraying with a molybdenum spray (Dittmer & Lester, 1964) and identified using standard marker lipids. To quantify individual phospholipids, spots were made visible by exposing the plates to iodine and then, when most of the iodine had disappeared, the phospholipid-containing areas of the silica gel were scraped off for analysis. The phosphoinositide components were separated by preparative thin-layer chromatography on sodium carbonate-impregnated silica gel H plates by the method of Banerjee *et al.* (1974), and the mannophosphoinositides were detected with an α -naphthol/sulphuric acid spray (Jacin & Mishkin, 1965). As before, those areas of silica gel containing phosphoinositides were scraped off separately for analysis. The amounts of individual phospholipids and phosphoinositides were determined by estimating the amount of phosphorus in each silica gel scraping. To identify individual phosphoinositides, the molar ratio of mannose, fatty acids and glycerol was determined after acid hydrolysis of the mannophosphoinositides. Mannose was estimated in the hydrolysate as described by Subrahmanyam & Singhvi (1965), fatty acid was extracted from the hydrolysate with ether and estimated by the method of Dole (1956) and glycerol was estimated by the method of Bok & Demain (1977).

RESULTS AND DISCUSSION

No effect was observed on growth and bacterial characteristics when the concentration of glycine in the basal medium was less than 5 g l^{-1} . At this concentration or above, the lag phase was prolonged although, as in the control culture, the stationary phase was reached after 7 d incubation. However, the thick surface growth normally seen was replaced by a thin less-pigmented film of growth and hence the yield of cells was greatly reduced. The water content of the bacteria was also slightly reduced (Table 1).

Only in those bacteria grown in basal medium supplemented with the highest concentration of glycine tested (10 g l^{-1}) was there a weakening of the acid-fast reaction. The weakening of the reaction indicates an alteration in the chemical composition of the outer part of the cell wall. No L-forms were observed.

Supplementation of the basal medium with 1 or $2 \text{ g glycine l}^{-1}$ led to slight increases in the amounts of total lipids and total phospholipids per g dry cells, whereas with 5 or $10 \text{ g glycine l}^{-1}$ there was a marked reduction, this being greater in those organisms grown in the presence of $10 \text{ g glycine l}^{-1}$. Except for organisms grown in basal medium supplemented with $1 \text{ g glycine l}^{-1}$ all the values recorded were significantly different from those for organisms grown in basal medium alone (Table 1). The changes in the amount of the major individual phospholipids mirrored those of total phospholipids with the exception of phosphatidylethanolamine which was not significantly different for organisms grown in the presence of $5 \text{ g glycine l}^{-1}$ from those grown in basal medium alone (Table 1).

The increase in the content of individual phosphoinositides in organisms grown in the presence of 1 or $2 \text{ g glycine l}^{-1}$ was almost the same (Table 1). Those grown in basal medium supplemented with $5 \text{ g glycine l}^{-1}$ contained less phosphatidylinositol, triacylated dimannophosphoinositide and triacylated hexamannophosphoinositide, whereas their content of the comparable tetra-acylated mannophosphoinositides was little different from that of organisms grown in basal medium alone. Although there was a significant decrease in all mannophosphoinositides in organisms grown in the presence of $10 \text{ g glycine l}^{-1}$, this was least with the tetra-acylated forms (Table 1). The decrease in tetra-acylated forms is most likely a result of the very small amount of triacylated mannophosphoinositides, which are the presumed precursors of tetra-acylated mannophosphoinositides. These changes in the content of individual mannophosphoinositides altered their ratio with respect to one another. In contrast, there was little change in the ratio of the major phospholipids (Table 1).

With glycine at 5 or 10 g l^{-1} in the basal medium, the amounts of the two triacylated mannophosphoinositides were decreased to a greater extent than those of the two tetra-acylated forms and, at 10 g l^{-1} , than that of phosphatidylinositol. It is therefore

Table 1. *Effect of glycine on the cell yield and lipid composition of M. smegmatis ATCC 607*

Cultures, grown in triplicate, were harvested after 7 d incubation at 37 °C. Values are the means $\pm$ s.e.m. The results were considered to be significant with $P < 0.05$. The levels of significance are denoted by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values in round brackets are percentages of control values (without glycine). Values in square brackets are percentages of total phospholipids or total phosphoinositides, as appropriate.

	Glycine concn (g l ⁻¹)				
	0	1	2	5	10
Cells					
Wet weight [g (100 ml culture) ⁻¹]	5.8 $\pm$ 0.3	5.6 $\pm$ 0.2	5.7 $\pm$ 0.3	3.4 $\pm$ 0.2*** (59)	1.8 $\pm$ 0.1*** (31)
Dry weight (% wet weight)	10.4 $\pm$ 0.2	10.6 $\pm$ 0.2	10.4 $\pm$ 0.2	11.1 $\pm$ 0.2* (107)	11.2 $\pm$ 0.2* (108)
Total lipids [mg (g dry wt)⁻¹]	235.0 $\pm$ 10.0	254.0 $\pm$ 8.0 (108)	286.0 $\pm$ 11.0** (122)	151.0 $\pm$ 6.0*** (64)	80.0 $\pm$ 4.0*** (34)
Total phospholipids [mg (g dry wt)⁻¹]	38.2 $\pm$ 3.6	46.4 $\pm$ 4.1 (121)	48.6 $\pm$ 3.8* (127)	28.0 $\pm$ 2.4* (73)	19.0 $\pm$ 1.8*** (50)
Phospholipids [mg (g dry wt)⁻¹]					
Cardiolipin	14.9 $\pm$ 0.5 (39)	18.5 $\pm$ 1.7 (124) [40]	19.4 $\pm$ 2.0* (130) [40]	10.8 $\pm$ 1.0* (72) [39]	7.1 $\pm$ 0.8*** (48) [37]
Phosphatidylethanolamine	6.1 $\pm$ 0.1 (16)	7.4 $\pm$ 0.9 (121) [16]	8.1 $\pm$ 0.8* (133) [17]	5.0 $\pm$ 0.1 (82) [18]	3.7 $\pm$ 0.3** (61) [20]
Total phosphoinositides	16.1 $\pm$ 1.6 (42)	19.8 $\pm$ 1.1 (123) [43]	20.2 $\pm$ 2.0* (125) [41]	11.1 $\pm$ 0.8* (69) [40]	7.6 $\pm$ 0.8*** (47) [40]
Phosphoinositides† [mg (g dry wt)⁻¹]					
Phosphatidylinositol	3.6 $\pm$ 0.4 (23)	4.8 $\pm$ 0.4* (131) [24]	4.7 $\pm$ 0.3* (130) [23]	1.9 $\pm$ 0.1*** (53) [17]	2.0 $\pm$ 0.1** (55) [26]
M ₂ F ₃	5.2 $\pm$ 0.3 (33)	6.3 $\pm$ 0.4 (121) [32]	6.4 $\pm$ 0.4 (123) [32]	3.2 $\pm$ 0.2** (62) [29]	1.5 $\pm$ 0.1*** (28) [19]
M ₂ F ₄	3.0 $\pm$ 0.3 (19)	3.6 $\pm$ 0.3 (120) [18]	3.7 $\pm$ 0.2 (122) [18]	3.1 $\pm$ 0.1 (102) [28]	2.3 $\pm$ 0.1* (77) [31]
M ₆ F ₃	1.9 $\pm$ 0.1 (12)	2.2 $\pm$ 0.1 (114) [11]	2.3 $\pm$ 0.1* (119) [11]	0.8 $\pm$ 0.1*** (41) [7]	0.2 $\pm$ 0.03*** (9) [2]
M ₆ F ₄	2.3 $\pm$ 0.2 (14)	3.0 $\pm$ 0.3 (128) [15]	3.0 $\pm$ 0.2* (127) [15]	2.2 $\pm$ 0.2 (93) [19]	1.6 $\pm$ 0.1* (71) [21]

† M₂F₃, triacylated dimannophosphoinositide; M₂F₄, tetra-acylated dimannophosphoinositide; M₆F₃, triacylated hexamannophosphoinositide; M₆F₄, tetra-acylated hexamannophosphoinositide.

conceivable that at these concentrations of glycine an enzymic step for the biosynthesis of triacylated mannophosphoinositides is blocked.

Small molecules may act as regulatory signals for enzymes involved in different metabolic pathways, e.g. one amino acid may influence enzymes on the biosynthetic pathway of one or more other amino acids (Jensen, 1969). Hence it is possible that high concentrations of glycine influence either directly or indirectly enzymes involved in the biosynthesis or degradation of lipids.

Besides a decrease in the content of triacylated mannophosphoinositides there was a concomitant reduction in the yield of bacteria coupled with an alteration in their characteristics. Taking these facts into account and in view of the possible vital role of glycophospholipids in cell-wall and membrane function (Toon *et al.*, 1972; Barksdale & Kim, 1977) it may well be that the triacylated di- and/or hexamannophosphoinositides are essential for metabolic and, hence, growth regulation. Another possibility is that the alteration in the ratio of the triacylated and tetra-acylated mannophosphoinositides is detrimental to growth. Whatever the reason, the present study emphasizes the importance of the glycine concentration on the growth and lipid metabolism of mycobacteria as exemplified by *M. smegmatis*.

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