SHORT COMMUNICATION

Effect of Growth Temperature on the Lipid Composition of *Mycobacterium smegmatis* ATCC 607

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(Received 2 April 1979)

The total lipid content of *Mycobacterium smegmatis* ATCC 607 was the same whether it was grown at 27 or 37 °C. The total phospholipid content, however, increased significantly at 27 °C. Phosphatidylethanolamine increased most markedly with a simultaneous decrease in phosphatidylinositol mannosides. Among individual phosphatidylinositol mannosides, tri- and tetra-acylated dimannophosphoinositides and tetra-acylated hexamannophosphoinositides all decreased at the lower growth temperature. Triacylglycerols and monoacylglycerols increased at the lower temperature but diacylglycerols were unaffected. Unsaturated fatty acids of total phospholipids increased as the temperature was lowered.

INTRODUCTION

The lipids of micro-organisms are sensitive to physical and chemical changes in the environment. Lipid composition in bacteria is affected by growth rate, media composition, pH, oxygen tension and growth temperature (Cronan & Vagelos, 1972; Goldfine, 1972; McElhaney, 1976). Since most bacterial lipids are present in membranes, these environmentally induced changes have a major physiological significance. The majority of reports concerning the effects of environmental factors on the lipid composition of bacteria have dealt only with one class of lipid, usually fatty acid composition (Farrell & Rose, 1967; Raziuddin, 1976; Okuyama *et al.*, 1977). Comparatively little work has been done on the effect of single environmental factors on the overall lipid composition of bacteria including mycobacteria (Dhariwal *et al.*, 1977). The present study deals with the changes produced in the various constituents of different lipid classes of *Mycobacterium smegmatis* ATCC 607.

METHODS

Mycobacterium smegmatis ATCC 607 was maintained on Lowenstein–Jensen medium. The organism was grown in modified Youman's medium (Subrahmanyam, 1964) as a surface culture at 27 and 37 °C. Bacteria grown at 37 °C were used as inocula for cultures grown at 27 °C. All cultures were harvested in the early-stationary phase (i.e. after 7 d at 27 °C or 6 d at 37 °C).

Cells dried at 60 °C were used only for the quantification of total lipids and phospholipids whereas wet cells were used to determine phospholipid composition. All of the reagents and solvents used were of analytical grade. Fatty acid standards were obtained from Applied Science Laboratories, Pennsylvania, U.S.A.; tuberculostearic acid was a gift from Professor E. Lederer, CNRS, Gif sur Yvette, France.

Extraction of lipids. Both wet cells and those dried at 60 °C were suspended in chloroform/methanol (2:1, by vol.), stirred for 3 h and filtered; the process was repeated with fresh solvents and the filtrates were combined. Lipids were extracted and purified by the procedure of Folch *et al.* (1957).

Analytical procedures. Total lipids were quantified gravimetrically per g dry wt of bacteria dried at 60 °C. Individual components of phospholipids and phosphatidylinositol mannosides were separated and quantified

Table 1. *Effect of growth temperature on the lipid composition of Mycobacterium smegmatis* ATCC 607

Lipid class	Growth temperature	
	37 °C	27 °C
Total lipids [mg (g dry wt) ⁻¹]	177.0 ± 14.7	188.9 ± 14.6
Total phospholipids [mg (g dry wt) ⁻¹]	26.5 ± 0.8	38.5 ± 2.2***
Phospholipids (relative %)		
Phosphatidylinositol mannosides	47.1 ± 2.2	32.7 ± 1.8***
M ₂ F ₃	21.7 ± 2.1	18.0 ± 1.0*
M ₂ F ₄	15.0 ± 2.4	5.7 ± 0.5***
M ₃ +M ₄	4.6 ± 0.9	3.7 ± 0.9
M ₆ F ₃	3.3 ± 1.1	3.8 ± 0.9
M ₆ F ₄	4.4 ± 0.8	2.7 ± 0.8*
Phosphatidylethanolamine	12.3 ± 2.5	23.4 ± 0.7***
Cardiolipin	39.8 ± 3.4	41.0 ± 1.8
Total acylglycerols [mg (g dry wt) ⁻¹]	41.3 ± 1.5	49.1 ± 3.0**
Acylglycerols [mg (g dry wt) ⁻¹]		
Monoacylglycerols	3.1 ± 0.4	4.9 ± 0.3**
Diacylglycerols	7.1 ± 0.8	7.3 ± 1.2
Triacylglycerols	31.0 ± 1.5	36.9 ± 2.7*
Fatty acids (relative %)		
Myristic acid	2	2
Palmitic acid	44	44
Palmitoleic acid	3	3
Stearic acid	4	6
Oleic acid	11	11
Tuberculostearic acid	33	26
Unknown component†	3	8

Values are means ± s.d. of four different batches, except for fatty acids where the averages of three batches are given. The results were considered to be significant with $P < 0.05$. The levels of significance are denoted by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Abbreviations: M₂F₃, triacylated dimannophosphoinositide; M₂F₄, tetra-acylated dimannophosphoinositide; M₃+M₄, tri- and tetra-acylated mannosides; M₆F₃, triacylated hexamannophosphoinositide; M₆F₄, tetra-acylated hexamannophosphoinositide.

† Unknown component which has the same retention time as methyl linoleate.

as described by Talwar & Khuller (1977). Neutral lipids were separated by thin-layer chromatography as described by Dhariwal *et al.* (1977). Fatty acid methyl esters, prepared from phospholipids (acetone-insoluble fraction) by heating with anhydrous methanol acidified with conc. H₂SO₄ (Gander *et al.*, 1972), were resolved by gas-liquid chromatography on a 1.5 m column of 10 % diethylene glycol succinate on 100 to 200 mesh diatomite C-AW (packed column obtained from Pye Unicam) at 180 °C using N₂ as carrier gas at 45 ml min⁻¹. Since oleic and tuberculostearic acid have the same retention time on this column, the fatty acid methyl esters were brominated (Kates, 1972) and then rechromatographed. Fatty acids were identified by comparing their retention times with those of standards. Relative fatty acid composition was determined by triangulation of peak areas.

RESULTS AND DISCUSSION

There were considerable differences in the relative amounts of the major phospholipids, phosphatidylinositol mannoside components, acylglycerols and in the fatty acid composition of phospholipids at the two growth temperatures (Table 1). The total lipid content of the bacteria was not significantly affected by the growth temperature but phospholipids were 45 % higher at 27 °C than at 37 °C. The lipid phosphorus content in both thermophilic and mesophilic bacteria has previously been observed to increase at higher temperatures (Ray *et al.*, 1971; Lee & Oo, 1971; Veerkamp, 1977), although in *Escherichia coli* (De Siervo, 1969; Okuyama, 1969; Bright-Gaertner & Proulx, 1972) and *Staphylococcus aureus* (Joyce

et al., 1970) it has been found to increase at lower temperatures. It appears from our results, and the earlier work of De Siervo (1969), that at lower temperatures the synthesis of phospholipids is favoured, or alternatively the breakdown of phospholipids is slower.

Phosphatidylinositol mannosides were decreased by 30 % at the lower temperature, i.e. similar to the decrease observed in *Mycobacterium phlei* (Dhariwal *et al.*, 1977). However, there was a 90 % increase in phosphatidylethanolamine at 27 °C (Table 1). Others (Raziuddin, 1976; Dhariwal *et al.*, 1977; Okuyama *et al.*, 1977; Khuller, 1977) have noted a decrease in phosphatidylethanolamine at lower temperature, although De Siervo (1969) observed an increase in *E. coli*. The decrease in phosphatidylinositol mannosides and simultaneous increase in phosphatidylethanolamine may have a protective role in maintaining membrane fluidity. Cardiolipin content has previously been found to be higher (Raziuddin, 1976; Dhariwal *et al.*, 1977) or lower (De Siervo, 1969; Okuyama *et al.*, 1977) in cells grown at 27 °C compared with those grown at 37 °C; no change in cardiolipin content was noted in this investigation with *M. smegmatis*. There were significant changes in the individual components of the phosphatidylinositol mannosides at the different growth temperatures: at 27 °C, the dimannoside containing four fatty acid residues (M_2F_4) was 62 % lower than at 37 °C, the dimannoside containing three fatty acids (M_2F_3) was 17 % lower and the hexamannoside with four fatty acids (M_6F_4) was 39 % lower. The tri- and tetramannosides ($M_3 + M_4$) and the hexamannoside containing three fatty acids (M_6F_3) did not change significantly (Table 1). The marked decrease in the M_2F_4 component at 27 °C is similar to that found in *M. phlei* (Dhariwal *et al.*, 1977). This may be due to a block in the biosynthetic pathway of higher mannosides.

The total acylglycerol content was significantly higher in cells grown at 27 °C than at 37 °C. The triacylglycerols showed a smaller increase (19 %) than the monoacylglycerols (56 %), while the diacylglycerols were unaffected (Table 1). These results are in agreement with the findings of Hunter & Rose (1972). However, such changes have not been observed in some other micro-organisms (Haest *et al.*, 1969; Cullen *et al.*, 1971; Gill, 1975).

The relative proportion of unsaturated fatty acids increased markedly at the lower growth temperature. The increase in unsaturation was mainly due to the significant decrease in tuberculostearic acid and increase in an unknown component (Table 1). These results are in accord with earlier findings (Farrell & Rose, 1967; Barran & Miller, 1976; Raziuddin, 1976; Okuyama *et al.*, 1977; Dhariwal *et al.*, 1977). The increase in unsaturation may be due to an increase in the dehydrase/desaturase enzyme (Cronan, 1975) or to the altered activity of enzymes at the lower temperature (Sinensky, 1971).

The exact influence of temperature on physical properties of the membrane cannot be deduced from these present results since compositional effects may partly counteract one another (Barran & Miller, 1976), and also the change in growth rate with change in temperature may contribute to the changes in overall lipid composition (Brown & Rose, 1969). The compositional adaptation may serve to maintain a constant membrane fluidity in response to temperature changes.

This investigation was supported, in part, by a grant from the Indian Council of Medical Research.

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