SHORT COMMUNICATION

Light-induced Changes in the Phospholipid Composition of *Mycobacterium smegmatis* ATCC 607

By A. SUBRAMONIAM*† AND D. SUBRAHMANYAM

Department of Biochemistry, Postgraduate Institute of Medical Education and Research,
Chandigarh-160012, India

(Received 22 January 1981; revised 28 August 1981)

The growth of *Mycobacterium smegmatis* ATCC 607 with intermittent exposure to light for periods of 4 h d⁻¹ led to changes in the amounts of the lipid components compared with those present in cells grown in the dark. When grown in continuous light the changes were less marked. Pigmentation was absent in cells grown in the dark.

INTRODUCTION

Many species of mycobacteria are photochromogenic (Barksdale & Kim, 1977) and contain carotenoid pigments. These pigments partially protect the bacteria from the adverse effects of light (Harrison, 1967). Light influences certain enzymes and non-enzymic proteins. Prephytone pyrophosphate synthetase was found to be totally photo-induced in a *Mycobacterium* species and the constitutive activity of geranyl pyrophosphate synthetase, present in dark-grown cells, was increased after exposure to light (Johnson *et al.*, 1974). Stimulation of the synthesis of a cell surface polypeptide of a species of *Arthrobacter* also required light (Hoover, 1977). Several of the active transport systems for nutrients present in *Escherichia coli* were inactivated by intense visible light (Barran *et al.*, 1974). Since light plays a role in the induction of certain enzymes and in the transport of certain nutrients across the cell membrane in bacteria, the present study was undertaken to examine the influence of light on membrane and cell wall lipids of mycobacteria.

METHODS

Organism and culture conditions. *Mycobacterium smegmatis* ATCC 607 was grown at 37 °C for 6 d in 100 ml modified Youman's medium (Subrahmanyam, 1964), contained in 250 ml conical flasks. Triplicate cultures were exposed to the light from a 100 W lamp, placed at a distance of 1 m, throughout the period of growth or for 4 h d⁻¹. Control cultures were kept in the dark throughout. Organisms were harvested by centrifugation at 3000 g for 30 min and their lipids were extracted and analysed separately. To determine dry weight, cells were dried at 60 °C.

Extraction and analysis of lipids. The lipids were extracted, analysed and quantified as described previously (Subramoniam & Subrahmanyam, 1981).

RESULTS AND DISCUSSION

The yield of organisms from a culture exposed to light continuously or intermittently was no different from that of a culture kept in the dark. Similarly, neither the water content nor the

† Present address: Molecular Genetics Laboratory, Department of Botany, Howard University, Washington, D.C. 20059, U.S.A.

Table 1. *Effect of light on phospholipids of M. smegmatis ATCC 607*

Cultures, grown in triplicate, were harvested after 6 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 (dark-grown). Values in square brackets are percentages of total phospholipids or total phosphoinositides, as appropriate.

	Dark (control)	Continuous light	Intermittent light
Total lipids [mg (g dry wt) ⁻¹]	273 $\pm$ 12	229 $\pm$ 10* (84)	185 $\pm$ 8*** (68)
Total phospholipids [mg (g dry wt) ⁻¹]	34.5 $\pm$ 2.0	33.8 $\pm$ 1.2 (98)	28.5 $\pm$ 1.6* (83)
Phospholipids [mg (g dry wt) ⁻¹]			
Cardiolipin	15 $\pm$ 1.1 [44]	12.2 $\pm$ 1.0 (81) [36]	9.1 $\pm$ 0.7** (61) [32]
Phosphatidylethanolamine	5.8 $\pm$ 0.6 [17]	10.5 $\pm$ 0.7*** (181) [31]	8.9 $\pm$ 0.5** (153) [31]
Total phosphoinositides	13.1 $\pm$ 0.9 [39]	11.4 $\pm$ 0.5 (87) [33]	10.3 $\pm$ 0.4* (77) [36]
Phosphoinositides† [μ g lipid P (g dry wt) ⁻¹]			
Phosphatidylinositol	130 $\pm$ 8.0 [25]	115 $\pm$ 7.0 (88) [26]	88 $\pm$ 6.0** (68) [22]
M ₂ F ₃	188 $\pm$ 7.5 [36]	208 $\pm$ 8.6 (111) [46]	186 $\pm$ 7.0 (99) [46]
M ₂ F ₄	84 $\pm$ 4.0 [16]	39 $\pm$ 3.0*** (46) [9]	41 $\pm$ 2.0*** (49) [10]
M ₆ F ₃	51 $\pm$ 3.0 [10]	45 $\pm$ 2.4 (88) [10]	46 $\pm$ 2.2 (90) [11]
M ₆ F ₄	65 $\pm$ 2.5 [13]	40 $\pm$ 1.8*** (62) [9]	44 $\pm$ 1.8*** (68) [11]

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

acid-fast reaction of the bacteria was affected (data not given). However, the degree of pigmentation reflected the extent of their exposure to light; it was greatest in cultures exposed continuously, less in those exposed intermittently and absent in those kept in the dark.

The amounts of total lipids and total phospholipids per g dry cells were less in cultures exposed to light for 4 h d⁻¹ than in those grown in the dark (Table 1). Quite unexpectedly, the amount of total phospholipids in cells exposed to light continuously was the same as that in the cells grown in the dark, and the reduction in total lipids was less than that in cultures exposed to light intermittently (Table 1). It is known that the adverse effects of light are reduced by the presence of carotenoid pigments (Harrison, 1967; Krinsky, 1976). As organisms grown in continuous light were more pigmented, these pigments could have counteracted some of the effects of light or the organisms might have developed other protective mechanism(s) to reduce the adverse effects of light.

Among the individual phospholipids, the changes in the amounts of cardiolipin and total phosphoinositides mirrored those of total lipids. However, the observed decrease in these phospholipids in cultures continuously exposed to light fell short of statistical significance (Table 1). In contrast to cardiolipin and total phosphoinositides, the amount of phosphatidylethanolamine increased in cultures exposed to light; this phospholipid was present in greater amounts in cultures exposed to light continuously than in those exposed intermittently. It is possible that light could have increased the activity and/or biosynthesis of one or more of the enzymes involved in the biosynthesis of phosphatidylethanolamine. Alternatively, the degradative enzymes of phosphatidylethanolamine could have been affected by light. The

changes in the amounts of individual phospholipids in cultures grown in light altered their ratio with respect to one another (Table 1).

The changes in the amount of phosphatidylinositol were similar to those of total phosphoinositides which decreased significantly only in cultures exposed to light intermittently. The amounts of triacylated di- and hexa-mannophosphoinositides were not altered significantly in cultures exposed to light either continuously or intermittently. By contrast, the amounts of comparable tetra-acylated lipids were reduced; the percentage reduction observed with continuous and intermittent exposure to light was almost identical (Table 1). It is possible that the enzymes involved in the presumed conversion of triacylated mannophosphoinositides into tetra-acylated forms could have been affected by light. The changes in the amounts of individual mannophosphoinositides changed their ratio to one another (Table 1).

A reduction in the amount of triacylated mannophosphoinositides is associated with a reduction in the yield of organisms and an alteration in their characteristics (Subramoniam & Subrahmanyam, 1981). In the present study, though the amounts of tetra-acylated mannophosphoinositides were reduced, the yield of cells and their staining properties were not altered. This suggests that the tetra-acylated mannophosphoinositides do not play a significant role in growth regulation. Further, the present observations show that certain alterations in the phospholipid composition are not necessarily detrimental to the organism. Finally, it is suggested that light, besides having an influence on the enzymes involved in the metabolism of lipids, also plays a role in the development of possible protective mechanism(s) in cells exposed to light, thereby shielding their lipid metabolism from the adverse effects of light.

REFERENCES

- BARKSDALE, L. & KIM, K. (1977). *Mycobacterium*. *Bacteriological Reviews* **41**, 217–372.
- BARRAN, L. R., DAOUST, J. Y., LABELLE, J. L., MARTIN, W. G. & SCHNEIDER, H. (1974). Differential effects of visible light on active transport in *E. coli*. *Biochemical and Biophysical Research Communications* **56**, 522–528.
- HARRISON, A. L. (1967). Survival of bacteria. Harmful effects of light with some comparisons with other adverse physical agents. *Annual Review of Microbiology* **21**, 143–156.
- HOOPER, J. K. (1977). Photodynamic induction of a bacterial cell surface polypeptide. *Journal of Bacteriology* **131**, 650–656.
- JOHNSON, J. H., REED, B. C. & RILLING, H. C. (1974). Early photoinduced enzymes of photoinduced carotenogenesis in a *Mycobacterium* species. *Journal of Biological Chemistry* **249**, 402–406.
- KRINSKY, N. I. (1976). Cellular damage initiated by visible light. *Symposia of the Society for General Microbiology* **26**, 209–239.
- SUBRAHMANYAM, D. (1964). Studies on the polyglycerol phosphatide of *Mycobacterium tuberculosis*. *Canadian Journal of Biochemistry* **42**, 1195–1199.
- SUBRAMONIAM, A. & SUBRAHMANYAM, D. (1981). Effect of glycine on phospholipids of *Mycobacterium smegmatis* ATCC 607. *Journal of General Microbiology* **124**, 203–206.