

# Phospholipids and Glycerides Composition during Spheroplasts Formation of *Mycobacterium smegmatis* ATCC 14468

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Z. Naturforsch. **39c**, 962–964 (1984); received April 18, 1984

Phospholipids, Glycerides, Spheroplasts, *Mycobacterium smegmatis*

The phospholipids and glycerides composition of spheroplasts of *Mycobacterium smegmatis* ATCC 14468 was examined. The percent total phospholipids in total lipids as well as cardiolipin were found to be higher in spheroplasts as compared to their parent forms. Increase in cardiolipin and free fatty acids content and decrease in triglycerides levels were observed during spheroplasts formation. The results suggest that increase in cardiolipin content in spheroplasts is an adaptational change concomitant with the loss of cell walls.

## Introduction

Lipids are abundantly present in the cell walls of bacteria. The loss of cell wall has been associated with the alterations in the lipid composition [1–5]. Experiments with *Bacillus cereus* [6] and *B. cereus*, *Streptococcus aureus* and *Micrococcus lysodeikticus* [3] indicated that phospholipids undergo rapid degradation with concomitant release of diglyceride rich vesicles during protoplast formation. Kusaka [3] attributed such an increase in diglyceride rich vesicles to the activation of phospholipase C. Among the individual phospholipids, cardiolipin content was found to be higher in *S. pyogenes* protoplasts [1], in *Staphylococcus aureus* autoplasts [5] and its derived L-forms [4]. In the present communication, we report the alterations in phospholipids and glycerides contents during spheroplasts formation in *Mycobacterium smegmatis* ATCC 14468.

## Materials and Methods

Culture conditions and preparation of spheroplasts from *M. smegmatis* ATCC 14468 were same as described earlier [7]. Total lipids were extracted, resolved into phospholipids and neutral lipids and individual fractions were estimated as reported

earlier [8]. Cell cultures were labelled for 1 h with [ $^{14}\text{C}$ ]palmitate (50.1 mCi/mmol) before harvesting adopting Dhariwal *et al.* method [9]. Spheroplasts were prepared from labelled cells using lysozyme method [7] and during incubation period, at indicated time intervals cells were collected, lipids were extracted. Radioactivity in the various lipid fractions was measured as described earlier [10].

## Results

**Lipid composition:** The phospholipid and glyceride composition of spheroplasts was examined and was compared with whole cells. Total lipids of spheroplasts were less abundant as compared to whole cells (Table I). But the percent total phospholipids content in total lipids of spheroplasts was higher as compared to its parent forms. Among the phospholipids, the increase in cardiolipin content was high in spheroplasts. Interestingly, cardiolipin content of total lipids was increased from 10% in whole cells to 19% in spheroplasts whereas the changes in phosphatidylethanolamine and total phosphatidylinositolmannosides were not marked. Since glycerides constitute the major fraction in neutral lipids of mycobacteria [8] and are also abundantly present in their cell walls, their contents were determined (Table II). Among the glycerides, triglycerides were reduced by 88% in spheroplasts as compared to whole cells. Mono- and diglycerides, were however not detectable in spheroplasts.

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0341-0382/84/0900-0962 \$ 01.30/0



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Table I. Major phospholipid composition of *M. smegmatis* spheroplasts and whole cells. Total lipids were extracted from spheroplasts and whole cells by chloroform:methanol (2:1) and resolved into individual fractions on thin layer chromatography. The phosphorus content of the individual fractions was then estimated as described in the text. The results are average of three separate experiments carried out in triplicate. Total lipid content for spheroplasts is 5.5 mg/100 mg · dry wt. of bacteria and for whole cells is 10.0 mg/100 mg · dry wt. of bacteria.

Phospholipid	% Phosphorus content in total lipids	
	Spheroplasts	Whole cells
Total phospholipids	31.0	22.0
Cardiolipin	19.0	10.8
Phosphatidylethanolamine	6.5	5.4
Total phosphatidyl-inositolmannosides	5.3	5.5

Table II. Glyceride content of *M. smegmatis* spheroplasts and whole cells. Glycerides were separated from total lipids by thin layer chromatography and the individual fractions were estimated as described in the text. Results expressed are average of three experiments carried out in triplicate.

Lipid component (mg/100 mg · dry wt.)	Spheroplasts	Whole Cells
Total lipids	5.50	10.00
Total glycerides	0.12	1.03
Monoglycerides	ND	0.07
Diglycerides	ND	0.08
Triglycerides	0.10	0.88

ND = Not detected.

Experiments with  $[1-^{14}\text{C}]$ palmitate: The time course of alterations in the lipid composition during the spheroplasts formation is shown in Figs. 1 and 2. The phospholipids and neutral lipids may not equally be labelled with  $[1-^{14}\text{C}]$ palmitate because of the differences in their pool size (manuscript under preparation) and as also been observed for whole cells [9]. But it did not affect the distribution of labelled lipid during the study. Of the phospholipids (Fig. 1), the radioactivity in cardiolipin and phosphatidylethanolamine increased considerably. Among the neutral lipids (Fig. 2), diglyceride content was maintained at a constant level without any change. The decrease in triglycerides and corresponding increase in free fatty acids were marked.

## Discussion

The results reported in the present investigation suggest that the lipid composition of bacterial membrane is altered during spheroplast formation. Unlike the earlier observations on the lipid composi-

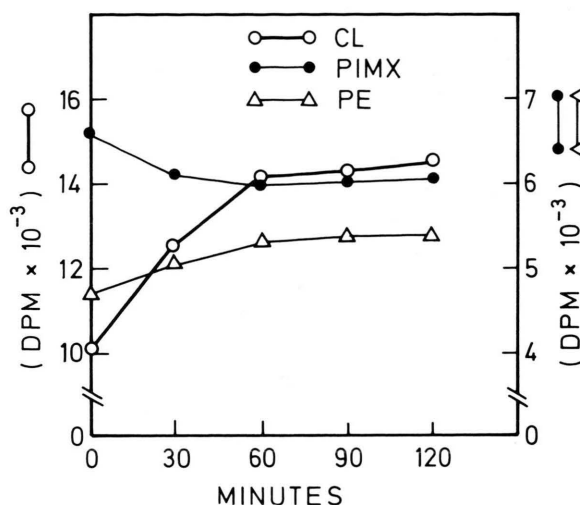


Fig. 1. Alterations in the phospholipid composition during spheroplasts formation in *M. smegmatis* ATCC 14468.  $[1-^{14}\text{C}]$ palmitate exposed cells were washed and incubated with lysozyme for the preparation of spheroplasts. During the process at indicated time intervals samples were removed for the extraction of total lipids. Phospholipids were then resolved into individual fractions and counted for radioactivity. Symbols: CL – Cardiolipin, PE – Phosphatidylethanolamine, PIMx – total Phosphatidylinositolmannosides.

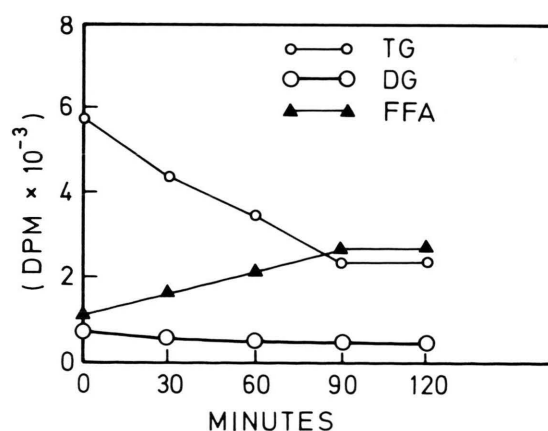


Fig. 2. Alterations in the diglyceride, triglyceride and free fatty acids during spheroplasts formation in *M. smegmatis* ATCC 14468. Experimental conditions were the same as described in Fig. 1, except that neutral lipids were separated and counted for radioactivity. Symbols: TG – triglyceride, DG – diglyceride, FFA – free fatty acids.

tion of some gram positive bacteria [6, 3], an increase in the percent of phospholipids in total lipids of spheroplasts were observed as compared to whole cells in the present study. Among the phospholipids, cardiolipin accounted for the major fraction in spheroplasts (Table I) and its content has been found to be increased rapidly during their formation (Fig. 1). The high levels of cardiolipin observed in phospholipids of spheroplasts of *M. smegmatis* could be interpreted as an adaptational change concomitant with the loss of cell walls. The considerable decrease in glycerides (Table II) could be attributed to the loss of cell walls.

The results, further, suggest that phospholipases are not getting activated during spheroplast formation (Fig. 2). Phospholipase C has been reported to be activated during the protoplasts formation in *S. aureus*, *B. cereus*, and *M. lysodeikticus* [3]. Activation of phospholipases A and C was ruled out in the present system due to the fact that no free fatty acids and diglyceride rich vesicles respectively were released into the spheroplasts inducing buffer during their formation (data not given). But decrease

in triglycerides content and increase in the free fatty acids (Fig. 2), in spheroplasts gives rise to the possibility that some lipases like triacylglycerol-lipase are getting activated under the conditions described. Activation of such lipase has been reported during protoplasts formation in *Candida lipolytica* [11].

The phospholipid composition reported in the present study is in agreement with the earlier reports on other species of bacteria [1, 4, 5]. The high levels of cardiolipin in spheroplasts may be involved in the process of preventing autolysis [5], may act as a barrier in sodium ion permeability [12] and increase the membrane stability. Increased level of cardiolipin observed during the loss of cell walls suggest that the membrane bound cardiolipin synthetase [13] and cell wall synthesis may be under mutual regulation in *M. smegmatis*. The present study offers a model system for investigating this relationship.

MVVSM is grateful to Council of Scientific and Industrial Research, New Delhi, India, for the award of a senior research fellowship.

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