UNUSUAL POSITIONAL DISTRIBUTION OF FATTY ACIDS IN PHOSPHATIDYLGLYCEROL OF STEROL-REQUIRING MYCOPLASMAS

S. ROTTEM and O. MARKOWITZ,
Biomembrane Research Laboratory, The Hebrew University – Hadassah Medical School, Jerusalem, Israel

Received 10 September 1979

1. Introduction

Phospholipids derived from a variety of natural sources commonly show a nonrandom distribution of fatty acids. Fatty acids having higher melting points, e.g., saturated fatty acids, are located at position 1 of the glycerol while fatty acids with lower melting points, e.g., unsaturated, branched-chain or cyclopropane-containing fatty acids, are usually found at position 2 [1,2]. Until recently, very little was known about the positional distribution of fatty acids in membrane lipids of mycoplasmas, the smallest self-replicating procaryotes. Previous studies using *Acholeplasma laidlawii* B [3,4] showed that the fatty acid positional distribution in phospho- and glycolipids of this organism is in accord with that found elsewhere in nature. On the other hand, in *Mycoplasma gallisepticum*, it was recently found that unsaturated fatty acids were present more abundantly in position 1 of membrane phospholipids, while saturated fatty acids were present in position 2 [5]. This communication demonstrates that the phosphatidylglycerol (PG) of all the cholesterol-requiring *Mycoplasma* species tested has an unusual positional distribution of fatty acids, with unsaturated fatty acids located primarily at position 1 and saturated fatty acids at position 2 of the *sn*-glycerol 3-phosphate.

2. Materials and methods

Mycoplasma or *Acholeplasma* species were grown in Edward medium [6] containing either 5% horse serum, 20 μM palmitic and 20 μM oleic acids, or 0.5% bovine serum albumin, cholesterol (15 μg/ml), 20 μM palmitic acid and 40 μM oleic acid. To label membrane lipids, the medium was supplemented with 0.005 μCi/ml [9,10-^{3}H]palmitic acid (500 Ci/mol) and 0.002 μCi/ml [1-^{14}C]oleic acid (56 Ci/mol), both products of the Radiochemical Centre, Amersham. The cultures were incubated at 37°C for 14–26 h and growth was followed by measuring A_{460} of the culture. Most experiments were performed with cultures at the midexponential phase of growth (A_{640} = 0.15–0.20) containing 70–90 μg cell protein/ml. The cells were harvested by centrifugation at 12 000 × *g* for 20 min and washed once in a cold 0.25 M NaCl solution. Lipids were extracted from the cells by the procedure in [7]. Protein, phospholipids and total cholesterol content of cells was determined as in [5]. PG was separated from total membrane lipids by thin-layer chromatography on silica gel H plates developed with chloroform–methanol–water (65:25:4, v/v/v). The PG spots (containing ~0.5 μmol) were scraped off the plates into test tubes containing in 1 ml total volume: 50 mM Tris–HCl (pH 7.4); 25 mM $CaCl_2$; 1.25 mg bovine serum albumin. Aliquots (0.1 ml) of a solution (1 mg/ml) of purified phospholipase A_2 (Boehringer) were then added and the test tubes were incubated at 37°C for 2 h. The reaction was stopped by adding 1 ml 0.1 M ethylenediaminetetraacetic acid. Lipids were extracted from the reaction mixture [7] and chromatographed on silica gel H plates. Radioactivity (dpm) in the lysophosphatidylglycerol (Lyso PG) and free fatty acid spots were determined using a Packard Model 2650 Tricarb scintillation spectrometer. The fatty acid composition in the Lyso PG and free fatty acid spots were

determined by gas liquid chromatography [8] of the fatty acid methyl esters prepared by heating the lipid samples in 14% boron trifluoride in methanol (Sigma St Louis, MO) at 72°C for 15 min.

3. Results and discussion

The positional distribution studies were performed with PG preparations from various mycoplasmas. This is the most common phospholipid in mycoplasmas [9]. In the representative species tested, grown in horse serum-supplemented Edward medium [6], PG forms between 15–60% (by wt) of the total membrane lipids, with hexadecanoic (C16:0) and octadecenoic (C18:1) acids comprising together 80–90 mol% of its fatty acids. The *Mycoplasma* species are unable to synthesize or change either saturated or unsaturated fatty acids, while the capability of the *Acholeplasma* species to synthesize saturated fatty acids is highly suppressed when exogenous fatty acids are added to the growth medium [10]. Hence, the hexadecanoic and octadecenoic acids found in the PG preparations from *Mycoplasma* or *Acholeplasma* species are mostly fatty acids of exogenous origin. Therefore, when the organisms were grown in a medium containing [^{3}H]palmitic and [^{14}C]oleic acid, the ^{3}H/^{14}C ratio in PG and its breakdown products obtained after phospholipase A_2 treatment was taken as a measure of the positional distribution of palmitic and oleic acids in the PG. Since phospholipase A_2 catalyzes the hydrolysis of fatty acid ester linkages exclusively at position 2, the radioactivity in the Lyso PG represents the radioactive fatty acids located at position 1. Radioactivity in the free fatty acids liberated by the enzyme represents fatty acids located in position 2. This is in accordance with the observations [3,4] and with the normal distribution of fatty acids found in almost all natural phospholipids [1,2]. However, in PG preparations from all the cholesterol-requiring mycoplasmas tested, the ^{3}H/^{14}C ratio was low in Lyso PG and high in the free fatty acid fraction (table 1), suggesting that oleic acid is located mostly in position 1 and palmitic acid in position 2.

The notable exception encountered in PG preparations from the cholesterol-requiring *Mycoplasma* species is further demonstrated in table 2, in which the fatty acid composition at position 1 and at position 2 of PG from *A. laidlawii* and *M. capricolum* cells are presented. The unusual positional distribution of fatty acids in the PG preparation of *M. capricolum* is clearly indicated when the positional specificity of palmitic and oleic acids were calculated as the ratio of each of the fatty acids present at position 1 to that at position 2. So far only very few systems with phospholipids possessing an unusual positional distribution

Table 1
Positional distribution of [^{3}H]palmitate and [^{14}C]oleate in phosphatidylglycerol preparations from various *Acholeplasma* and *Mycoplasma* species

Organism	Distribution of label (^{3}H/^{14}C ratio)		
	Phosphatidyl-glycerol	Lysophosphati-dylglycerol	Free fatty acids
Acholeplasma laidlawii strain A	0.9	2.8	0.6
A. granularum	0.8	2.1	0.7
Mycoplasma fermentans	2.5	0.4	10.2
M. mycoides subsp. *mycoides*	1.7	0.3	10.5
M. pneumoniae	1.4	0.2	4.9
M. capricolum	1.4	0.7	3.7
M. gallinarum	1.3	0.6	3.9
M. gallisepticum	1.1	0.2	7.9

The organisms were grown in a medium containing 5% horse serum and supplemented with [1-^{14}C]oleate and [9,10-^{3}H]palmitate as in section 2. The distribution of label was determined after phospholipase A_2 treatment of PG preparations as in section 2. The labeling ratios in Lyso PG and in the free fatty acid fraction represent the labeling ratios at positions 1 and 2 of the PG

Table 2
Positional distribution of fatty acyl chains in phosphatidylglycerol from *M. capricolum* and *A. laidlawii*

Fatty acid	*M. capricolum* Fatty acid content (mol%)			*A. laidlawii* Fatty acid content (mol%)		
	Position 1	Position 2	Position 1/ position 2	Position 1	Position 2	Position 1/ position 2
14:0	9.7	2.3	4.2	11.2	5.3	2.1
16:0	31.6	86.3	0.4	67.9	10.0	6.8
18:0	1.2	3.9	0.3	11.5	1.2	9.6
18:1	53.6	7.5	7.1	9.3	79.8	0.1

The organisms were grown in a medium containing 0.5% bovine serum albumin, cholesterol, and palmitic and oleic acids as in section 2. The fatty acid content at position 1 and position 2 was calculated from the fatty acid composition of lysophosphatidylglycerol and the free fatty acid fractions obtained after phospholipase A_2 treatment of the phosphatidylglycerol preparations as in section 2

of fatty acids have been described [11,12]. Among them is the phosphatidylethanolamine of *Clostridium butyricum* [11]. It is interesting to note the recent finding of common oligonucleotide sequences in the 16 S ribosomal RNAs from mycoplasmas and clostridia, taken to suggest a common ancestry to these groups of microorganisms [13].

The *Mycoplasma* species differ from *Acholeplasma* species in their growth requirement for cholesterol [14]. Cholesterol reaches levels of ~50 mol% of total membrane lipids in *Mycoplasma* species as opposed to low cholesterol levels ($\leqslant$ 10 mol%) in the cholesterol-nonrequiring *Acholeplasma* species [14]. It can be suggested that the requirement for cholesterol and its high content in the membrane of *Mycoplasma* species is associated with the presence of phospholipids with unusual positional distribution. One possibility is that the requirement for cholesterol is associated with different physical properties of the membrane imposed by the differences in the positional distribution of the fatty acids in membrane phospholipids. However, data obtained with artificial membrane systems [15,16] do not appear to support this suggestion. In these studies force–area curves at the air–water interface of structural isomers of phosphatidylcholine with monounsaturated chains at the 1 or 2 position were practically identical. The permeability of liposomes derived from these phosphatidylcholine preparations to glucose, erythritol and glycerol was also the same [15,16]. Moreover, after mixing with cholesterol, the mean molecular area at the air–water interface, the permeability of the liposomes to nonionic substances and the energy content of the gel–liquid crystalline phase transition were decreased to about the same degree with both structural isomers [15,16]. Another possibility is that the high cholesterol content in the membrane of *Mycoplasma* species affects the specificity of the acyltransferases located in the membrane [10]. This possibility was tested with *M. capricolum* cells adapted to grow in a cholesterol-poor medium [17], by serial passages of the organism in a medium containing 0.5% bovine serum albumin, 20 μM palmitic acid, 80 μM oleic acid and decreasing concentrations of horse serum. The horse serum was decreased from 5–0.15% by growing the cells in media containing one-half of the serum concentration of the previous medium. Cells adapted to the cholesterol-poor medium grew more slowly than cells maintained in the cholesterol-rich medium and their cultures reached the stationary phase of growth at a lower turbidity than cultures of the native strain. The adapted cells contained a much lower cholesterol concentration than that of the native cells resulting in a lower cholesterol to phospholipid molar ratio (table 3). On the other hand, the PG of the adapted strain was more saturated than that of the native strain, an increased saturation that may compensate at least in part for the low cholesterol content [18]. The positional distribution of palmitic and oleic acids in the PG preparations of the native and adapted strains were, however, very similar. After phospholipase A_2 treatment a high $^3H/^{14}C$ ratio was

Table 3
Positional distribution of radioactive palmitic and oleic acids in phosphatidylglycerol of native and adapted *M. capricolum* strains

Strain	Cholesterol/ phospholipid (molar ratio)	Distribution of label in PG ([^{3}H]palmitate/[^{14}C]oleate ratio)		
		Total	Position 1	Position 2
Native	1.10	1.15	0.79	9.40
Adapted	0.20	2.78	1.20	17.85

The organisms were grown in media containing [1-^{14}C]oleate and [9,10-^{3}H]palmitate. The distribution of the label was determined after phospholipase A_2 treatment of PG preparations as in section 2. The labeling ratios at positions 1 and 2 were obtained from the labeling ratios in lyso PG and in the free fatty acid fraction

found in the free fatty acids and a much lower ratio in Lyso PG from both native and adapted strains, suggesting that in *M. capricolum*, regardless of the cholesterol content of the membrane, [^{3}H]palmitate is preferentially incorporated at position 2 and [^{14}C]oleate at position 1.

Acknowledgement

This work was supported by grant no. 1746/78 from the United States-Israel Binational Science Foundation.

References

[1] Van Deenen, L. L. M. (1965) in: Progress of Chemistry of Fats and Other Lipids (Hollman, R. T. ed) vol. 1, pp. 1–47, Pergamon, New York.
[2] Cronan, J. E., jr and Gelman, E. P. (1975) Bacteriol. Rev. 39, 232–256.
[3] Saito, Y., Silvius, J. R. and McElhaney, R. N. (1977) Arch. Biochem. Biophys. 82, 443–454.
[4] Saito, Y. and McElhaney, R. N. (1978) Biochim. Biophys. Acta 529, 224–230.
[5] Rottem, S. and Markowitz, O. (1979) Biochemistry 18, 2930–2935.
[6] Razin, S. and Rottem, S. (1976) in: Biochemical Analysis of Membranes (Maddy, A. H. ed) pp. 3–26, Chapman and Hall, London.
[7] Bligh, E. G. and Dyer, W. J. (1969) Can. J. Physiol. 37, 911–917.
[8] Rottem, S. and Greenberg, A. S. (1975) J. Bacteriol. 121, 631–639.
[9] Smith, P. F. (1979) in: The Mycoplasmas (Barile, M. F. and Razin, S. eds) vol. 1, pp. 231–258.
[10] Saito, J. and McElhaney, R. N. (1977) Arch. Biochem. Biophys. 182, 455–464.
[11] Hildebrand, J. G. and Law, J. H. (1977) Biochemistry 3, 1304–1308.
[12] Okuyama, H., Kankura, T. and Nojima, S. (1967) J. Biochem. (Tokyo) 61, 732–737.
[13] Maniloff, J., Magrum, L., Zablen, L. B. and Woese, C. R. (1978) Zentralbl. Bakteriol. 241, 171.
[14] Razin, S. and Rottem, S. (1978) Trends Biochem. Sci. 3, 51–55.
[15] Demel, R. A., Geurts Van Kessel, W. S. M. and Van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 266, 29–40.
[16] De Kruyff, B., Demel, R. A., Slotboom, A. J., Van Deenen, L. L. M. and Rosenthal, A. F. (1973) Biochim. Biophys. Acta 307, 1–19.
[17] Clejan, S., Bittman, R. and Rottem, S. (1978) Biochemistry 17, 4579–4583.
[18] Rottem, S., Yashouv, J., Ne'eman, Z. and Razin, S. (1973) Biochim. Biophys. Acta 323, 495–508.