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DIFFERENCES IN SUSCEPTIBILITY TO PHOSPHOLIPASE C OF FREE AND MEMBRANE-BOUND PHOSPHOLIPIDS OF *MYCOPLASMA HOMINIS*

SHLOMO ROTTEM, MIRIAM HASIN and SHMUEL RAZIN

Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem (Israel)

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SUMMARY

Phospholipase C from *Bacillus cereus* failed to attack the phospholipids of intact cells or isolated membranes of *Mycoplasma hominis*, but readily hydrolyzed the extracted phospholipids dispersed in water. Subjection of the membranes to prolonged ultrasonic irradiation, alternate freezing and thawing, or low concentrations of detergents did not render the phospholipids susceptible to the enzyme action.

2. The removal of part of the membrane proteins by proteolytic enzymes enabled phospholipase C to hydrolyze membrane phospholipids. The binding of lysozyme to the protein-depleted membranes interfered with phospholipase C action.

3. A significant part of the phospholipids of reaggregated *M. hominis* membranes could be hydrolyzed by phospholipase C, indicating that the reaggregated membranes differ from the native membranes in organization.

4. An endogenous phospholipase A activity, hydrolyzing both acyl ester bonds in phosphatidylcholine, was found in *M. hominis* membranes. The enzyme(s) was (were) neither activated by Ca^{2+} nor inhibited by EDTA. It was markedly inhibited by *p*-chloromercuribenzoate and detergents and was completely destroyed by heating at 70 °C for 10 min.

5. It is suggested that the phospholipids in native *M. hominis* membranes are masked, probably by membrane proteins, and are thus protected from hydrolysis by exogenous phospholipase C. The membrane phospholipids may, however, be degraded by an endogenous, membrane-bound phospholipase A, which can exert its activity even at temperatures as low as −20 °C.

INTRODUCTION

The use of proteolytic and lipolytic enzymes as tools for studying the molecular organization and disposition of membrane proteins and lipids has gained many advocates in recent years[1–3]. Phospholipase C from various sources has been widely used for this purpose. Early studies carried out on erythrocyte ghosts showed that a major portion of their phospholipids were hydrolyzed by phospholipase C resulting in the accumulation of the diglycerides as droplets accompanied by a marked reduction in the surface area of the ghosts[4,5]. These observations were taken to indicate that the major part of membrane phospholipids is organized as a bilayer[5] and that at

least some of the phospholipid polar groups are not covered by protein and are thus in direct contact with the outside milieu[4]. The picture became more complicated by later findings which showed that phospholipase C or A failed to attack the phospholipids of intact erythrocytes, unless the cells were first lysed or swollen in a hypotonic solution[6–8] or treated with sublytic concentrations of detergents[9]. These observations suggested that the polar head groups of the phospholipids on the outer-membrane surface of intact erythrocytes are masked, possibly by glycoproteins[7].

In spite of the fact that mycoplasma membranes have become very popular objects in membrane studies, no published data is available on their susceptibility to phospholipase action. Preliminary studies (S. Razin and V. Barash, unpublished data) showed the accumulation of free fatty acids on treatment of isolated *Acholeplasma laidlawii* membranes with pancreatic phospholipase A. Treatment of *A. laidlawii* cells with the same enzyme preparation caused their lysis, apparently by the surface-active fatty acids. The cell membrane of *Mycoplasma hominis* was chosen for the present study because the disposition of its proteins is currently under investigation in our laboratory and because its major phospholipid, phosphatidylglycerol, is susceptible to hydrolysis by phospholipase C. This communication will show that the phospholipids of membranes of intact *M. hominis* cells or of isolated membrane preparations are resistant to attack by phospholipase C unless part of the membrane protein is removed by proteolytic enzymes. In addition, the presence of a membrane-bound, endogenous phospholipase A activity in *M. hominis* is reported.

MATERIALS AND METHODS

Organism and growth conditions

M. hominis (ATCC 15056) was grown in 2–5-liter vol. of Edward medium[10] adjusted to pH 6.5 and supplemented with 2% PPLO serum fraction (Difco) and 20 mM L-arginine. For labeling the membrane lipids 1.0 μCi [1-^{14}C]palmitic acid (55 mCi/mole, The Radiochemical Centre, Amersham, England) was added to each liter of growth medium. The organisms were harvested after a 16–20-h incubation at 37 °C and were washed twice in 0.25 M NaCl.

Isolation of cell membranes and lipid extraction

Cell membranes were obtained by osmotic lysis of the organisms[11]. The membranes were washed with deionized water, then with 0.05 M NaCl in 0.01 M phosphate buffer, pH 7.5, and again in deionized water. The washed membranes were resuspended in a buffer solution containing 0.15 M NaCl, 0.05 M Tris, and 0.01 M 2-mercaptoethanol adjusted to pH 7.4 with HCl (referred to as β-buffer, ref. 12), diluted 1:20 with deionized water (dilute β-buffer). Protein in the membrane suspensions was determined by the Folin-phenol method of Lowry *et al.*[13]. Lipids were extracted from freeze-dried cells or membrane preparations with chloroform-methanol (2:1; v/v) at 45 °C for 2 h and the extracts were dried under a stream of N_2. Lipid extracts to be used as substrates for phospholipase C were dispersed in dilute β-buffer by sonication for 15 min in an M.S.E. Ultrasonic disintegrator (60 W, 20 kcycles) at 1.5 A.

Measurement of phospholipase C activity

Hydrolysis of *M. hominis* phospholipids by a partially purified preparation of

phospholipase C (EC 3.1.4.3) from *Bacillus cereus* (kindly provided by A. Loyter, The Hebrew University, Jerusalem) was measured by the formation of diglycerides. The reaction mixtures contained 5 mg bovine serum albumin, 0.1 mM $ZnCl_2$, 25 μg of the enzyme preparation and either whole cells, isolated membranes or dispersed membrane lipids (each containing the equivalent of 1–1.5 mg radioactive membrane lipids) in 1 ml of dilute β-buffer. Controls with no phospholipase C were included. After incubation at 37 °C for 2 h the reaction was stopped by boiling for 3 min and the material was freeze-dried. The lipids were extracted from the freeze-dried material with chloroform–methanol (2:1; v/v) at 45 °C for 2 h. The extract was dried under N_2, redissolved in 0.2 ml of chloroform and subjected to chromatography on silica gel G plates (0.5 mm thick) using the developing solvent system described by Freeman and West[14] for the separation of neutral lipids. The lipid spots were detected by iodine vapor, then scraped off the plate into scintillation vials containing 10 ml of a dioxane–toluene scintillation liquor[11] and radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. Phospholipase C was expressed as the percentage of radioactivity in the diglyceride fraction of the total radioactivity in membrane lipids.

Measurement of endogenous phospholipase A activity

The endogenous phospholipase activity of *M. hominis* membranes was determined by measuring the release of radioactive fatty acids from membrane-polar lipids or from radioactive phosphatidylcholine preparations (kindly provided by Z. Ben Gershon, The Hebrew University, Jerusalem). The reaction mixture for measuring fatty acid release from membrane-polar lipids contained washed radioactive membranes (1.5 mg membrane protein) in 1 ml of dilute β-buffer. The reaction mixture for measuring fatty acid release from phosphatidylcholine contained washed membranes (1.5 mg membrane protein) and 0.1 mg of either 1-[9,10-$^3H_2$]palmitoylphosphatidylcholine or 2-[1-^{14}C]oleoylphosphatidylcholine. The reaction was stopped after incubation at 37 °C for 2 h by boiling for 3 min and the material was freeze-dried. The lipids were extracted from the freeze-dried material, chromatographed on silica gel G plates and radioactivity in the various lipid spots was determined as described above. Endogenous phospholipase A activity was expressed as the percentage of radioactivity in the free fatty acid fraction of the total radioactivity in membrane lipids, after subtraction of radioactivity values of free acids at zero time.

Solubilization of membranes

Labeled membranes (1.5 mg protein/ml) were treated for 15 min at 37 °C with 0.25–10 mg/ml of sodium deoxycholate, Triton X-100, sodium dodecylsulfate or Brij 58. The solubilized membrane material was separated from the nonsoluble residue by centrifugation at $100000 \times g$ for 1 h at 4 °C. Both fractions were assayed for protein, radioactivity, phospholipase A activity and for their susceptibility to phospholipase C.

Reaggregation of solubilized membrane material

Labeled membranes (4 mg membrane protein/ml) were solubilized by incubation in 20 mM sodium dodecylsulfate for 15 min at 37 °C. The solubilized material was dialysed for 4 days at 4 °C against 1000 vol. of dilute β-buffer containing various

concentrations of $MgCl_2$. The reaggregated material was collected by centrifugation at $37000 \times g$ for 45 min, resuspended in dilute β-buffer and assayed for protein, radioactivity and susceptibility to phospholipase C. Density-gradient analysis of the reaggregated membrane material was carried out as described before[11], using 11.5 ml of a linear sucrose gradient of 30–60%.

Proteolytic digestion of membranes

Digestion of labeled *M. hominis* membranes (3 mg membrane protein/ml) by pronase (50–200 μg/ml), trypsin (50 μg/ml) or papain (50 μg/ml) was carried out at 37 °C for 2 h in a final volume of 1 ml. To stop digestion the samples were diluted with 9 vol. of ice-cold dilute β-buffer and centrifuged in the cold at $37000 \times g$ for 45 min. The supernatant fluid was separated and the pellet was washed with cold 10-ml portions of dilute β-buffer and resuspended in 1 ml of the same buffer. The digested membranes were assayed for protein, radioactivity and susceptibility to phospholipase C.

Binding of lysozyme to digested membranes

Equal volumes of a washed digested membrane suspension (digested by 200 μg pronase/ml) and a solution of lysozyme (2–16 mg/ml) were mixed and incubated at 37 °C for 30 min. The resulting membrane–lysozyme complex was centrifuged at $37000 \times g$ for 45 min, washed once with 10 ml of dilute β-buffer and suspended in the same buffer. The membrane–lysozyme complex was assayed for protein, radioactivity and susceptibility to phospholipase C.

RESULTS

Effect of phospholipase C on M. hominis cells and isolated membranes

Phospholipase C of *B. cereus* had no effect on whole cells or isolated membranes of *M. hominis*. The enzyme was, however, capable of hydrolyzing sonicated dispersions of *M. hominis* polar lipids as was shown by the increase in radioactivity in the diglyceride fraction and the concomitant decrease in radioactivity in the polar lipid fraction (Table I). Phosphatidylglycerol which comprises up to 85% of the total

TABLE I

PHOSPHOLIPASE C ACTION ON *M. HOMINIS* CELLS, MEMBRANES AND ISOLATED LIPID DISPERSIONS

Preparation	*Phospho-lipase C*	*Radioactivity in diglyceride fraction (% of total)*
Whole cells	+	0.9
	−	0.8
Isolated membranes	+	1.3
	−	0.8
Membrane lipid dispersions	+	26.4
	−	1.4

polar lipid fraction of *M. hominis*[15] was practically the only phospholipid hydrolyzed by the enzyme and 98% of the radioactivity found in the diglyceride fraction was derived from it. The percentage of phosphatidylglycerol hydrolyzed varied from 8–35%, probably depending on the liposome size in different lipid dispersions.

Fragmentation of the isolated membranes by prolonged sonication (up to 10 min) or 5 times alternate freezing and thawing procedures did not render them susceptible to the phospholipase C action. Some hydrolysis of membrane polar lipids was, however, noticed when membranes preheated at temperatures above 60 °C were subjected to the enzyme (Fig. 1).

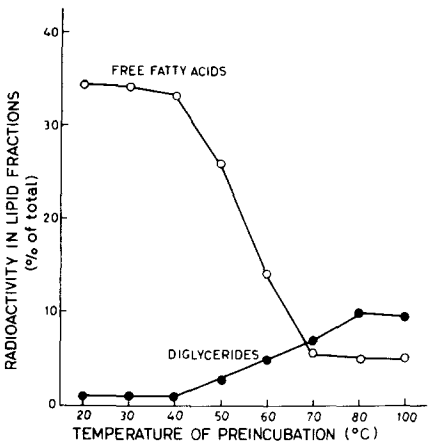

Fig. 1. The effect of heating on the endogenous phospholipase A activity of *M. hominis* membranes (○—○) and on their susceptibility to phospholipase C (●—●). Membrane suspensions (2 mg membrane protein/ml) were heated at the indicated temperatures for 10 min, cooled down and phospholipase A and C activities were measured as described under Materials and Methods.

TABLE II

THE EFFECT OF PHOSPHOLIPASE C ON NATIVE AND DIGESTED *M. HOMINIS* MEMBRANES

Preparation	*% Protein digested*	*Radioactivity in diglycerides (% of total)*
Native membranes	0	1.4
Membranes digested by 50 μg/ml pronase	25.1	14.9
Membranes digested by 200 μg/ml pronase	43.2	38.0
Membranes digested by 50 μg/ml trypsin	15.9	6.0
Membranes digested by 50 μg/ml papain	16.0	1.0

Table II shows that the partial removal of membrane proteins by treatment with proteolytic enzymes rendered the residual membrane lipid susceptible to phospholipase C. Pronase was the most efficient of the proteoytic enzymes, and the removal of about 40% of the membrane proteins by it led to the hydrolysis of up to

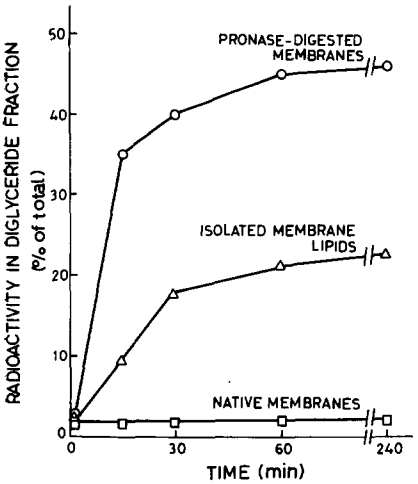

Fig. 2. Phospholipase C activity on native and pronase-digested *M. hominis* membranes and on aqueous dispersions of isolated membrane lipids.

45% of the membrane polar lipids by phospholipase C. Comparison of the rate of phospholipase C activity on native membranes, pronase-digested membranes, and a dispersion of membrane polar lipids is shown in Fig. 2. Maximal hydrolysis was always obtained within 30 min of incubation and the radioactivity in the formed diglycerides never exceeded 50% of the radioactivity in the total lipid fraction. Table III shows that the binding of lysozyme to the pronase-digested membranes markedly inhibited the hydrolysis of the membrane polar lipids by phospholipase C.

Fig. 3 compares the solubilization of *M. hominis* membranes by several ionic and non-ionic detergents. Sodium dodecylsulfate and sodium deoxycholate solubilized membrane proteins more effectively than membrane lipids, the reverse being true for Triton X-100 and Brij 58. The susceptibility of the solubilized membrane material and insoluble residue to phospholipase C is shown in Fig. 4. The susceptibility of membrane polar lipids to the enzyme was closely associated with the solubilization

TABLE III

THE EFFECT OF LYSOZYME BINDING TO PRONASE-DIGESTED *M. HOMINIS* MEMBRANES ON THE SUSCEPTIBILITY OF MEMBRANE PHOSPHOLIPIDS TO HYDROLYSIS BY PHOSPHOLIPASE C

Membranes were digested with pronase (200 μg/ml) for 2 h at 45 °C. Binding of lysozyme was performed as described in Materials and Methods.

Lysozyme added to binding mixture (mg/ml)	*Lysozyme bound (mg/mg membrane protein)*	*Radioactivity in diglycerides (% of total)*
None	0	43.2
1	1.3	30.5
4	1.8	25.8
8	2.1	19.3

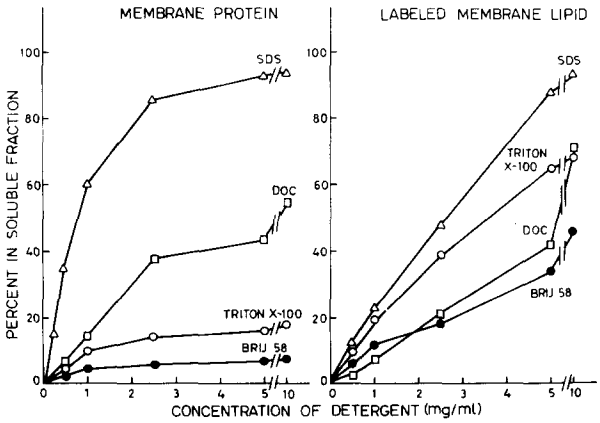

Fig. 3. Solubilization of the protein and lipid components of *M. hominis* membranes by detergents. Membrane lipids were labeled during growth with [1-^{14}C]palmitic acid. SDS, sodium dodecylsulfate; DOC, sodium deoxycholate.

process, except for Brij 58 where no degradation of the solubilized membrane polar lipids could be demonstrated. Sodium dodecylsulfate, at concentrations above 2 mg/ml, exerted an inhibitory effect on phospholipase C activity. Hydrolysis of the polar lipids of the insoluble membrane residue could not be demonstrated after treatment of the membranes with Triton X-100 and Brij 58 and it was very poor after treatment with sodium dodecylsulfate or deoxycholate.

Reaggregation of sodium dodecylsulfate-solubilized *M. hominis* membrane material by prolonged dialysis against dilute β-buffer containing Mg^{2+} resulted in the formation of membrane-like structures. The reaggregated membrane material contained protein and lipid, banding together in a sucrose density gradient. The effect

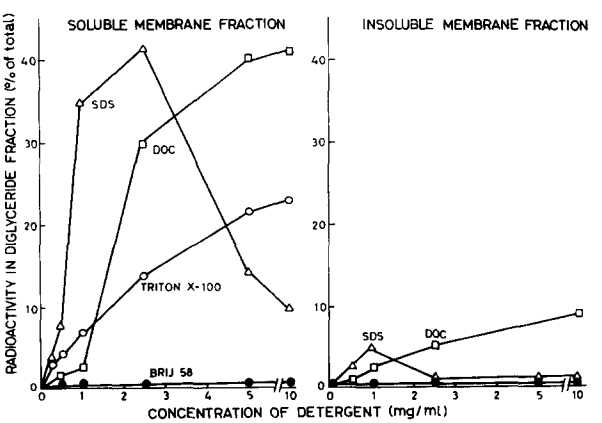

Fig. 4. The effect of detergents on the susceptibility of *M. hominis* membranes to phospholipase C. Membrane suspensions (2 mg membrane protein/ml) were incubated with the indicated concentrations of detergents for 15 min at 37 °C. The solubilized material was separated from the insoluble residue by centrifugation and the susceptibility of both fractions to phospholipase C was tested as described in Materials and Methods. SDS, sodium dodecylsulfate; DOC, sodium deoxycholate.

TABLE IV

THE EFFECT OF PHOSPHOLIPASE C ON NATIVE AND REAGGREGATED MEMBRANES OF *M. HOMINIS*

M. hominis membranes containing labeled lipid were solubilized by 20 mM sodium dodecylsulfate and dialysed against dilute β-buffer containing 5 or 20 mM Mg^{2+}.

Preparation	*Mg^{2+} in dialysis buffer (mM)*	*Lipid/protein (counts/min per mg protein)*	*Radioactivity in diglyceride fraction (% of total)*
Native membranes		96 000	1.3
Reaggregated membranes	5	190 000	11.4
	20	90 000	23.0

of phospholipase C on the reaggregated membranes is shown in Table IV. Although the reaggregated membranes obtained in the presence of 20 mM Mg^{2+} exhibited a lipid–protein ratio resembling that of the native membranes, a significant part of the polar lipids of the reaggregated membranes were hydrolyzed by phospholipase C, while those of the native membranes were not.

Endogenous phospholipase activity in M. hominis membranes

Table V shows that the incubation at 37 °C of isolated *M. hominis* membrane suspensions in dilute β-buffer resulted in the breakdown of a significant portion of the membrane polar lipids. Since the breakdown products were mainly free fatty acids, the presence of an endogenous phospholipase A activity in the membranes was indicated. Table V shows also that the amount of free fatty acids released was much lower when whole cells were used instead of isolated membranes. The endogenous phospholipase A activity was found to be located exclusively in the cell membrane. No activity was relased into the medium during the process of membrane isolation, or by four additional washings of the membrane preparation with dilute β-buffer. The effect of prolonged storage at low temperatures of whole cells or membrane preparations on the endogenous phospholipase A activity is shown in Fig. 5. Whereas no phospholipase activity could be demonstrated during storage at −70 °C, rapid hydrolysis of membrane polar lipids took place at 4 °C, and a low but constant rate of hydrolysis was detected at −20 °C. The membrane-bound phos-

TABLE V

THE HYDROLYSIS OF *M. HOMINIS* PHOSPHOLIPIDS BY AN ENDOGENOUS PHOSPHOLIPASE A

Preparation	*Incubation time (h)*	*Radioactivity in lipid fractions (% of total)*		
		Polar lipids	*Diglycerides*	*Free fatty acids*
Whole cells	0	94.3	0.7	1.6
	2	90.5	0.8	6.6
Isolated membranes	0	91.8	1.1	4.6
	2	71.0	1.8	26.0

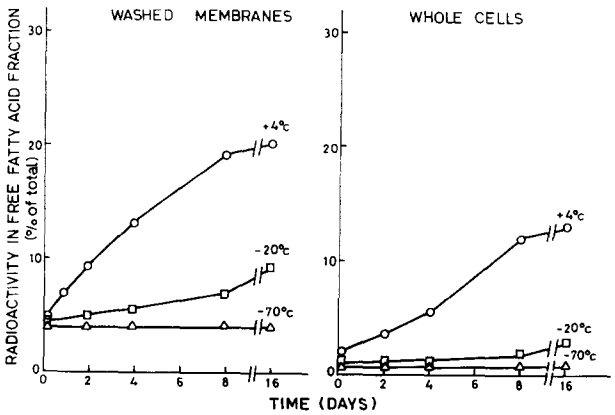

Fig. 5. Endogenous phospholipase A activity of *M. hominis* membranes and cells kept at low temperature.

pholipase A activity exhibited a very broad pH-optimum. Thus, the rate of hydrolysis of the polar membrane lipids was almost the same between pH 6.0 and 8.5. At pH 5.0 the rate was about 50% less and at pH 9.0 about 70% less than that at pH 7.5.

The addition of Ca^{2+} (10–100 mM) or EDTA (10 mM) to the membrane suspension did not affect the endogenous phospholipase A activity, indicating either that this enzyme does not require divalent cations for activity or that the amount of Ca^{2+} in the membrane suffices for its activity. *p*-Chloromercuribenzoate (10^{-3} M) almost completely inhibited the endogenous phospholipase activity. As is apparent from Fig. 1 the enzyme is heat-labile, losing its activity completely by heating the membrane preparations at 70 °C for 10 min. The effect of detergents on the degradation of membrane polar lipids by the endogenous phospholipase A is shown in Fig. 6. Both

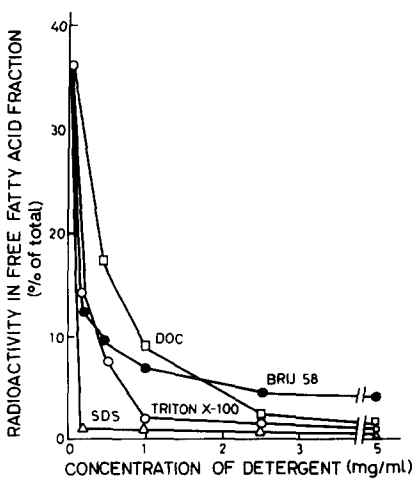

Fig. 6. The effect of detergents on the endogenous phospholipase A activity of isolated *M. hominis* membranes. Labeled membranes (1.5 mg membrane protein/ml) were treated for 15 min at 37 °C with various concentrations of detergents and assayed for phospholipase A activity as described in Materials and Methods. SDS, sodium dodecylsulfate; DOC, sodium deoxycholate.

TABLE VI

THE HYDROLYSIS OF 1-[9,10-$^3H_2$]PALMITOYLPHOSPHATIDYLCHOLINE AND 2-[1-^{14}C]OLEOYLPHOSPHATIDYLCHOLINE BY *M. HOMINIS* MEMBRANES

Substrate	*Radioactivity in fatty acids released (% of total)*
1-[9,10-$^3H_2$]Palmitoylphosphatidylcholine	24.9
2-[1-^{14}C]Oleoylphosphatidylcholine	21.5

ionic and nonionic detergents were potent inhibitors of the phospholipase activity, sodium dodecylsulfate being the strongest inhibitor, causing complete inhibition at concentrations as low as 0.25 mg detergent/mg membrane protein.

The hydrolysis of exogenous phosphatidylcholine by *M. hominis* membranes is shown in Table VI. It can be seen that the radioactivity released from both 1-[9,10-$^3H_2$]palmitoylphosphatidylcholine or 2-[1-^{14}C]oleoylphosphatidylcholine was about the same. This radioactivity was fully recovered in the free-fatty acid fraction and not in lyso compounds.

DISCUSSION

Our study shows that phospholipase C from *B. cereus* is capable of degrading isolated *M. hominis* phospholipids, which consist almost entirely of phosphatidylglycerol[15]. However, the same phospholipids when present in membranes of intact *M. hominis* cells resist attack by the enzyme. Furthermore, in contrast to the results obtained with erythrocyte ghosts[6,7] phospholipase C also failed to hydrolyze the phospholipids of isolated *M. hominis* membranes, suggesting that the phospholipids are masked and inaccessible to the enzyme on both membrane surfaces and, unlike the phospholipids of erythrocytes[6,7], are not exposed to the enzyme by cell lysis. Unmasking of *M. hominis* membrane phospholipids could not be achieved by various physical treatments of the isolated membranes, such as prolonged ultrasonic irradiation and alternate freezing and thawing, or by treatment with low concentrations of detergents, found to increase the susceptibility of the phospholipids of the intact erythrocyte membrane to phospholipase A[9].

Membrane proteins appear to be at least partially responsible for the masking of the phospholipids in *M. hominis* membranes. The partial digestion of membrane proteins by proteolytic enzymes enabled the hydrolysis of membrane phospholipids by phospholipase C. Of the enzymes tested, pronase was the most active one, apparently because of its ability to degrade practically all the membrane proteins to small peptides, part of which are released into the medium[16]. The removal by pronase of about 40% of the polypeptide chains attached to the membrane enabled the hydrolysis of up to 45% of the membrane phospholipids by phospholipase C. This value is lower than that obtained with isolated erythrocyte membranes[17,18] or with the nerve axon membrane[19] in which up to 70% of the total phospholipids are degraded by the same enzyme. This difference might be the result of insufficient removal

of the masking polypeptide chains from *M. hominis* membranes or might be due to a different lipid composition since erythrocyte and nerve membranes contain mainly the neutral zwitterionic phospholipids which are very sensitive to hydrolysis by phospholipase C[20]. The finding that the binding of lysozyme to the pronase-digested membranes interferes with the hydrolysis of membrane phospholipids by phospholipase C supports our suggestion that membrane proteins are responsible for masking the phospholipids in *M. hominis* membranes. Nevertheless, it is evident that the binding of lysozyme to the protein-depleted membranes does not restore the native structure of the membrane, since lysozyme is bound to the membrane mainly by electrostatic bonds which are easily disrupted by high salt concentrations[21] whereas membrane proteins are nor released under these conditions.

Membrane proteins of *M. hominis* were more effectively solubilized than membrane lipids by the ionic detergents sodium dodecylsulfate and sodium deoxycholate, as was also found for *A. laidlawii* membranes[22]. However, the nonionic detergents, Triton X-100 and Brij 58, reacted differently on *M. hominis* and on *A. laidlawii* membranes. As against the findings with *A. laidlawii*[22] these detergents failed almost completely to solubilize the membrane proteins of *M. hominis*, while solubilizing a significant portion of membrane lipids. As could be expected, the detergent-solubilized membrane lipids were susceptible to hydrolysis by phospholipase C, with the notable exception of the soluble material obtained with Brij 58. Nevertheless, the lipids in the insoluble membrane still showed little susceptibility to phospholipase C.

The solubilized membrane material obtained with sodium dodecylsulfate reaggregated to lipoprotein complexes on dialysis against Mg^{2+}. As with *A. laidlawii*[11], the lipid–protein ratio in the reaggregated material depends on the Mg^{2+}-concentration in the dialysis buffer, being higher at low Mg^{2+}. The susceptibility of the phospholipids in the reaggregated membrane material to phospholipase C supports a different mode of organization of the protein and lipids in native and reaggregated membranes, already indicated by freeze-etch studies of *A. laidlawii* membranes[23].

Phospholipases are widely distributed in animal and plant tissues and in microorganisms and apparently fulfill an essential role in the turnover of membrane phospholipids[24]. Mycoplasmas do not appear to form an exception to this rule since a membrane-bound lysophospholipase has recently been described in *A. laidlawii*[25] and our present study demonstrates the presence of a potent phospholipase A activity in *M. hominis* membranes. This enzyme(s) is apparently responsible for the self-digestion of membrane phospholipids during storage. The fact that the enzyme(s) exerts its activity even at -20 °C should be borne in mind when selecting the storage temperature of isolated membrane preparations. It seems that storage at -70 °C will ensure that no degradation of membrane phospholipids will take place during prolonged storage periods.

Resembling the lysophospholipase of *A. laidlawii*[25], the phospholipase A of *M. hominis* was found to be relatively heat-labile, not inhibited by EDTA but inhibited by detergents (Fig. 6). Incubation of *M. hominis* membranes with phosphadityl-choline preparations containing different labeled fatty acids at positions 1 and 2 showed that the membrane-associated enzyme is capable of hydrolyzing the acyl ester bonds at both positions with equal efficiency. This might indicate an enzyme with low specificity or the presence of both phospholipase A_1 and A_2 in the membranes.

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