Biochimica et Biophysica Acta, 426 (1976) 258–270

BBA 77212

CHARACTERIZATION OF THE MYCOPLASMA MEMBRANE PROTEINS

VI. COMPOSITION AND DISPOSITION OF PROTEINS IN MEMBRANES FROM AGING *MYCOPLASMA HOMINIS* CULTURES

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(Received July 9th, 1975)

SUMMARY

Membranes of *Mycoplasma hominis* cells from cultures progressing from the mid to the end of the logarithmic phase of growth became richer in protein, poorer in phospholipids and cholesterol, heavier in density, and more viscous as determined by EPR. The membrane-bound ATPase activity declined steeply. Electrophoretic analysis failed to show marked changes in membrane protein composition on aging, apart from an increase in the staining intensity of one protein band ($M_r \approx 130\,000$) concomitant with a decrease in the staining intensity of several minor protein bands of high molecular weight.

To test for possible changes in the disposition of the various membrane proteins on aging of cultures, a comparison was made of the susceptibility of membrane proteins of intact cells and isolated membranes to trypsinization and lactoperoxidase-mediated iodination. The iodination values and the percent of membrane protein released by trypsinization of intact cells were similar in cells from cultures of different ages, indicating no significant changes in the organization of the proteins on the outer membrane surface. On the other hand, trypsinization and iodination of isolated membranes were found to be most markedly affected by the culture age, indicating significant changes in the organization of the proteins on the inner membrane surface. Thus, the iodination values of isolated membranes decreased by almost two fold, while the percentage of protein released from the membrane by trypsin increased from 28 % to 50 % during the experimental period. It is suggested that aging in *M. hominis* cultures is accompanied by a continuous increase in the packing density of the protein molecules on the inner surface of the cell membrane.

INTRODUCTION

The process of aging in mycoplasma cultures is accompanied by a marked decrease in the activity of membrane-associated enzymes [1, 2] and transport systems [3, 4] and quite frequently culminates in lysis of the wall-less organisms. There is growing evidence to indicate that these manifestations of aging are associated with

alterations in the composition and physical properties of the cell membrane. Thus, the phospholipid [5, 6] and cholesterol content [5] of membranes from different mycoplasma species were found to decrease most markedly on aging of cultures, resulting in a significantly higher ratio of protein to lipid, a higher density and reduced fluidity [5, 6] of the membranes. The steep decline in the rate of phospholipid synthesis in aging *Mycoplasma hominis* cultures [6] and the consequent decrease in uptake of exogenous cholesterol [5] may explain the increased membrane protein-to-lipid ratio, presuming that membrane protein synthesis declines at a lower rate than that of membrane phospholipids.

The finding that membranes from aging mycoplasma cultures become richer in protein raises two questions: (a) does the membrane protein composition remain constant during aging, or is there some enrichment in specific membrane proteins? and, (b) are there any changes in the disposition of the various membrane proteins during aging? The experiments reported in the present communication were aimed at answering these questions.

MATERIALS AND METHODS

Organisms and growth conditions

M. hominis (ATCC 15056) was grown statically at 37 °C in a modified Edward medium [7] supplemented with 20 mM L-arginine and adjusted to pH 6.5. To label membrane lipids, 1 μCi of [1-^{14}C]palmitic acid was added to each liter of the growth medium. Growth was followed by absorbance measurements at 640 nm. When the absorbance of the culture reached the values of about 0.1, 0.2, 0.3, and 0.4, samples were withdrawn and the organisms were harvested, washed once and resuspended in 0.25 M NaCl.

Assessment of osmotic fragility of cells

Osmotic fragility of the washed cells was determined as previously described [8]. Results were expressed as per cent lysis in 0.015 M NaCl, calculated according to the formula: [(absorbance in 0.25 M NaCl−absorbance in 0.015 M NaCl)/absorbance in 0.25 M NaCl]$\times$100.

Isolation of cell membranes

Cell membranes were isolated by osmotic lysis of the washed organisms [7]. The membranes were washed with deionized water, then with 0.05 M NaCl in 0.01 M phosphate buffer, pH 7.5, and again with deionized water. The washed membranes were resuspended in 0.25 M NaCl and the amount of protein in the suspension was determined according to Lowry et al. [9].

Iodination procedure

Lactoperoxidase-mediated iodination of membrane proteins [10] was carried out by a modification of the procedure of Hubbard and Cohn [11]. Glucose oxidase (EC 1.1.3.4) and glucose were used to generate H_2O_2 for the catalysis of iodination by lactoperoxidase (EC 1.11.1.7). Commercially available lactoperoxidase from milk (Calbiochem) was further purified on a Sephadex G-150 column according to Morrison and Hultquist [12]. Fractions showing an $A_{412} : A_{280}$ ratio of 0.85 to

0.90 were used. The reaction mixture (2 ml) consisted of 0.05 M phosphate buffer, pH 7.5 in 0.15 M NaCl, washed cells or isolated membranes (1 mg membrane protein), 50 μg of purified lactoperoxidase, 10 μCi of $K^{125}I$ in 10 μM unlabeled KI, 10 units of glucose oxidase (Miles Laboratories, Kankakee, Ill.). Glucose (100 μg) was added to start the reaction and another portion of 100 μg glucose was added 2 min later. After 10 min incubation at room temperature with stirring, the reaction was terminated by the addition of 0.1 ml of 0.1 M sodium azide. After thorough mixing, 8 ml of 0.05 M phosphate buffer, pH 7.5 in 0.15 M NaCl, were added and the test tubes were immediately centrifuged. The iodinated cells or membranes were washed twice with 10^{-5} M KI in 0.05 M phosphate buffer containing 0.15 M NaCl. The cells were then osmotically lysed and the membranes were separated from the cytoplasmic fluid, washed as described above, and resuspended in deionized water. Aliquots of the membrane preparations were taken for radioactivity measurements in a Packard Auto Gamma spectrometer.

Release of peripheral membrane proteins

The loosely attached, peripheral membrane proteins were released from the washed membranes by a modification of the procedure of Muñoz et al. [13]. The washed membranes were labeled with ^{125}I as described above and were washed once in 100 mM Tris-HCl buffer, pH 7.4, and then in 3 mM Tris-HCl buffer with or without 10 mM EDTA. The wash fluids were collected and the pellets were resuspended in deionized water. Radioactivity and protein measurements were determined on aliquots from the wash fluids and the pellets.

Proteolytic digestion

Suspensions of whole cells or isolated membranes prepared in 0.4 M sucrose (each containing 1 mg membrane protein/ml) were treated with 50 μg/ml trypsin (2 times crystallized, Sigma) for 2 h at 37 °C. Digestion was stopped by the addition of the trypsin inhibitor *N*-α-*p*-tosyl-L-lysine chloromethyl ketone · HCl (Sigma) to a final concentration of 40 μg/ml. Twelve volumes of cold 0.25 M NaCl were added and the test tubes were centrifuged in the cold. The pellets were washed in 0.25 M NaCl and, in the case of cells, osmotic lysis was carried out at this stage to isolate the cell membranes. The degree of proteolytic digestion was assessed by comparing the Lowry-reactive material in the treated and the native membranes.

Gel electrophoresis

The sodium dodecyl sulfate gel electrophoresis system of Fairbanks et al. [14] was employed with some variations. Membrane proteins were solubilized by boiling the membranes for 3 min in 10 mM Tris-HCl buffer, pH 8.0, containing 1 % sodium dodecyl sulfate, 1 mM EDTA, 2 % 2-mercaptoethanol, and 10 μg/ml bromophenol blue. After cooling, urea was added to the solubilized mixture to a final concentration of 4 M. The solution was then centrifuged at 27 000 × *g* for 15 min to remove non-solubilized material, the amount of which was usually negligible. Samples (50–150 μg protein) were put on 5.6 % polyacrylamide gels (6 mm × 90 mm) containing 1 % sodium dodecyl sulfate. Electrophoresis was carried out for about 5 h at room temperature at a constant current of 4 mA per tube. The electrophoresis buffer contained 1 % sodium dodecyl sulfate according to Fairbanks et al. [14].

After the run, the gels were fixed with 25 % isopropyl alcohol in 10 % acetic acid solution and were left in this solution overnight at 37 °C with shaking. The gels were then stained for 1 h with a 0.25 % coomassie blue solution in 9 vol. of glacial acetic acid+91 vol. of 50 % methanol. For destaining, the gels were incubated for 6–8 h at 37 °C with shaking in a solution of 15 % methanol and 10 % acetic acid, and then transferred to a 10 % acetic acid solution for 12–15 h at 37 °C. When radioactive iodine-labeled membrane proteins were analyzed, pairs of identical gels were prepared; one was stained and the other was sliced laterally into 2-mm sections which were counted in a Packard Auto Gamma spectrometer.

RESULTS

Changes in membrane composition and properties on aging of cultures

Table I summarizes some of the changes which take place in membranes of *M. hominis* cells harvested from cultures showing absorbance values from 0.1 to 0.4 at 640 nm. By maintaining the growth conditions and size of inoculum as constant as possible, the experiments could usually be started with an 18 h culture and ended with a 23 h culture. The results of viable counts, given in Table I, indicate that this experimental period falls within the late logarithmic phase of growth. During the experimental period, the number of viable organisms increased approximately 2.3 fold, while the absorbance of the culture increased 4 fold, suggesting that the number of nonviable organisms in the culture is already on the rise, as would be expected at the late logarithmic phase of growth. Younger cultures with absorbance values lower than 0.1 were not tested because of the difficulty in collecting sufficient membrane material for analysis, while cultures with absorbance values higher than 0.4 were excluded because of the steep decline in the number of viable organisms and the pronounced cell lysis occurring at this stage. Therefore, in all experiments throughout this study, cultures showing absorbance values of 0.1, 0.2, 0.3, and 0.4 were selected for membrane analysis.

Table I shows the marked decrease in the ratio of membrane lipid to protein on aging of the culture, resulting in a significant increase in membrane density. The changes in membrane composition were accompanied by a decrease in the specific activity of the membrane-bound ATPase activity and in membrane fluidity as indicated by the decreased freedom of motion of the *N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-ketostearic acid incorporated into the membrane (Table I). The freedom of motion of the spin-labeled probe in the membrane is inversely proportional to the hyperfine splitting value [15].

Changes in composition and disposition of membrane proteins on aging

No major changes in membrane protein composition were revealed by electrophoretic analysis of organisms harvested during the progress of the culture from an absorbance of 0.1 to 0.4 (Fig. 1). However, careful inspection of the electrophoretic patterns revealed some quantitative differences in the staining intensity of some of the high-molecular weight proteins located in the upper part of the gels. While some of these protein bands became weaker on aging of the cultures, one band (D_1) became more accentuated. The percentage of peripheral membrane proteins, as measured by the amount of protein released by washing of the membranes in low-ionic strength

TABLE I

CHANGES IN MEMBRANE COMPOSITION AND PROPERTIES IN AGING *M. HOMINIS* CULTURES

Age of culture	Absorbance of culture at 640 nm	Viable organisms (colony-forming units per ml)	Membrane lipid to protein ratio			Membrane density (g/cm^3)	Membrane fluidity (hyperfine splitting in gauss)**	ATPase activity†
			[^{14}C]Palmitate-labeled lipids (cpm/mg protein)	μg lipid Pi per mg protein*	μg cholesterol per mg protein*			
18 h 15 min	0.10	$7.5 \cdot 10^8$	30 280	9.5	226	1.162	53.9	3.07
19 h 25 min	0.20	$1.0 \cdot 10^9$	26 460	ND	ND	ND	ND	2.72
21 h 15 min	0.30	$1.2 \cdot 10^9$	21 570	7.5	158	1.172	55.2	2.00
23 h 00 min	0.40	$1.7 \cdot 10^9$	18 320	6.3	121	1.183	56.0	1.40

* From Razin [5].

** From Rottem and Greenberg [6].

† μMoles of inorganic phosphate released from ATP per mg protein in 30 min, tested according to Pollack et al. [1].
ND = not done.

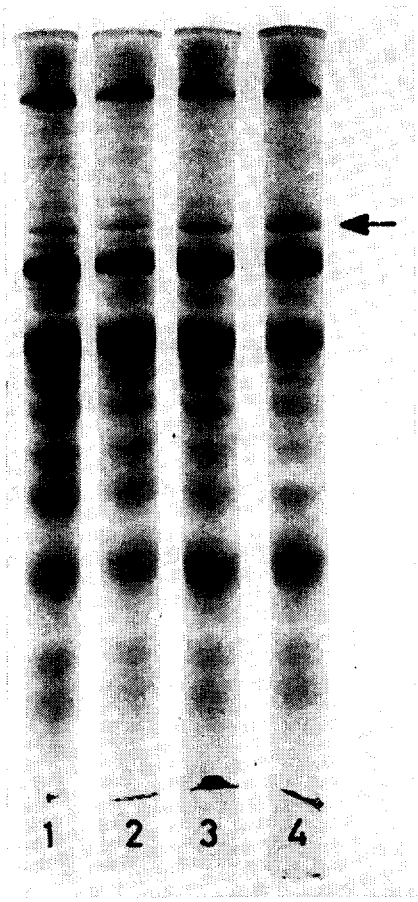

Fig. 1. Electrophoretic patterns of membrane proteins of *M. hominis* harvested at culture absorbances of: 0.10 (1), 0.20 (2), 0.30 (3) and 0.40 (4). Arrow points to Band D_1 which becomes more accentuated on aging of the culture.

buffers, with or without EDTA, was not affected by the aging of cultures, and was about 12 % throughout the tested growth period.

To test for possible changes in the disposition of membrane proteins on aging of *M. hominis* cultures, the lactoperoxidase-mediated iodination procedure was applied. Table II compares the iodination values of isolated membranes and membranes of intact cells harvested at different culture turbidities. The iodination values of isolated membranes always exceeded those of membranes of intact cells. Yet, the ratio of label in isolated membranes to that in membranes of intact cells decreased markedly on aging due to a most significant decrease in the specific labeling values of isolated membranes as against nearly constant labeling values in membranes of intact cells. Fig. 2 shows that only part of the membrane proteins of intact cells were labeled, whereas with isolated membranes essentially all the protein bands detected on the gels were labeled. However, the culture age did not affect the labeling intensity of the membrane proteins of treated intact cells, as opposed to a most pronounced

TABLE II

EFFECT OF THE CULTURE AGE ON THE LACTOPEROXIDASE-MEDIATED ^{125}I LABELING OF MEMBRANES OF INTACT CELLS AND ISOLATED MEMBRANES

Absorbance of culture at 640 nm	Radioactivity (cpm/mg membrane protein)		Labeling ratio (membranes/cells)
	Cells	Membranes	
0.10	125 590	352 810	2.7
0.21	115 760	264 940	2.1
0.30	128 500	230 250	1.7
0.40	120 520	190 370	1.5

decrease in the labeling intensity of essentially all the proteins of isolated membranes with progressing age (Fig. 2), a finding which is in accord with the data presented in Table II. Another point which can be seen in Fig. 2 is the increase in labeling of Band D_1 in treated cells, indicating that this protein, which becomes more prominant as the culture ages, is located on the outer membrane surface.

To test whether the decrease in the labeling intensity of isolated membranes is a result of defective membrane isolation from the older cultures and contamination

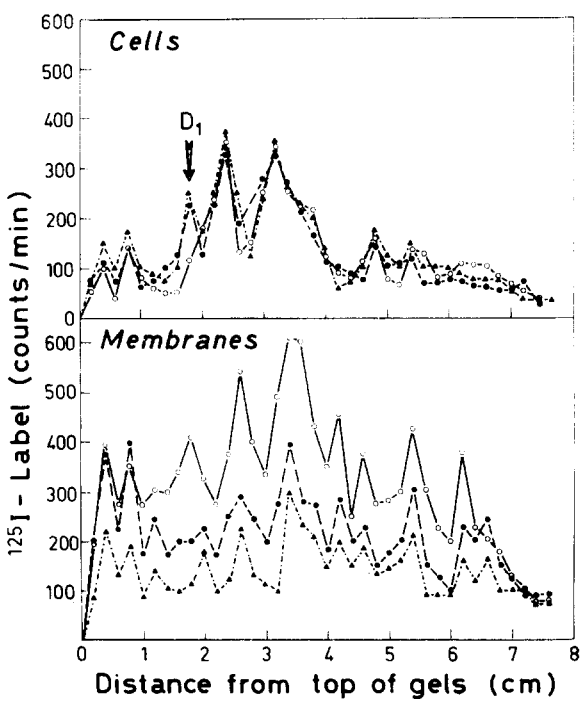

Fig. 2. Distribution of iodine label in *M. hominis* membrane proteins. Isolated membranes or intact cells from cultures reaching an absorbance at 640 nm of 0.10 (○–○), 0.30 (●–●), or 0.40 (▲–▲) were subjected to lactoperoxidase-catalyzed iodination. Membranes were isolated by osmotic lysis of the iodinated cells. The labeled membranes were electrophoresed in polyacrylamide gels containing sodium dodecyl sulfate. The gels were sliced and the iodine label was determined in the slices.

by unbroken cells, osmotic fragility of the organisms was assessed. The value obtained, about 70 % lysis, was essentially the same for organisms harvested from cultures showing absorbance values from 0.1 to 0.4. Moreover, in all experiments only a minor fraction of the iodine label (about 6 %) was detected in the cytoplasmic fraction of the treated cells, indicating that no significant changes in the permeability of the cells to the labeling agent took place during aging of the cultures.

Effect of culture age on susceptibility of membrane proteins to proteolytic digestion

Trypsin was chosen as the proteolytic agent because of its more selective activity [16]. Cells or isolated membranes from aging cultures were first subjected to lactoperoxidase-mediated iodination and then were treated with trypsin. As can be seen in Table III, the exposure of intact cells to trypsin resulted in the removal of between 13 and 16 % of the Lowry-reactive material from the membrane, the culture age having no significant influence. On the other hand, with isolated membranes the percentage of the Lowry-reactive material released by trypsin treatment increased most significantly with the aging of cultures. Nevertheless, the percentage of the iodine label removed by trypsin was about the same ($\approx$ 45 %) for intact cells and isolated membranes, and was not influenced to any marked degree by the age of culture (Table III).

Electrophoretic analysis of trypsin-treated membranes and membranes from trypsin-treated cells failed to show any significant influence of the culture age on the susceptibility of the membrane proteins to trypsin treatment, though, as was already shown by Amar et al. [16], treatment of isolated membranes caused more pronounced changes in the protein profiles than treatment of membranes of intact cells (Fig. 3). The digestion and removal of the high-molecular weight membrane proteins by trypsin treatment of either intact cells or isolated membranes shown in the stained gels (Fig. 3) is also evident in Fig. 4A where the distribution of the iodine label in the gels is given. Among the protein bands removed by trypsin is D_1, the band which increased in intensity of staining and labeling on aging of the culture (Figs. 1 and 2).

When isolated membranes and intact cells from cultures of progressing age were first trypsinized and then subjected to iodination, the labeling intensity of the membranes was almost the same as that obtained by labeling of untreated cells or

TABLE III

DIGESTION OF MEMBRANE PROTEINS AND REMOVAL OF IODINE LABEL BY TRYPSIN

Cells or isolated membranes were subjected to lactoperoxidase-mediated iodination and then were treated with 50 μg trypsin/ml for 2 h at 37 °C.

Absorbance of culture at 640 nm	Membranes of intact cells		Isolated membranes	
	% Protein released	% Radioactivity released	% Protein released	% Radioactivity released
0.10	13.6	45.5	28.0	43.5
0.21	15.7	49.8	32.5	47.5
0.32	15.7	45.7	40.1	45.7
0.41	16.2	41.3	49.6	37.2

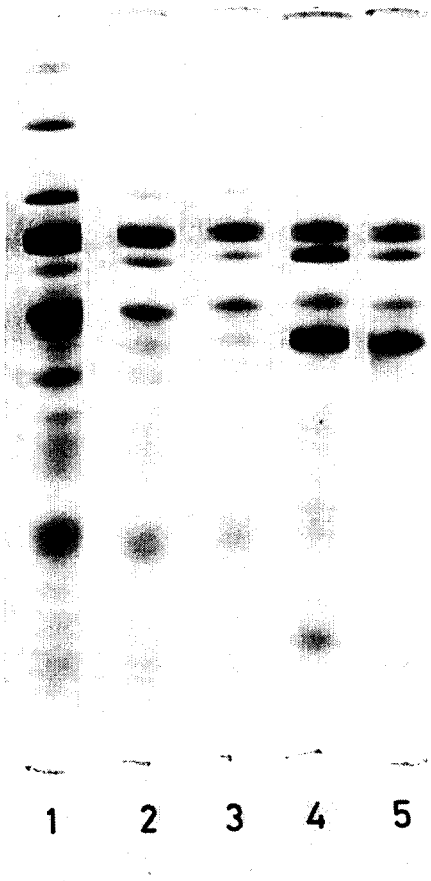

Fig. 3. Electrophoretic patterns of membrane proteins of *M. hominis* digested by trypsin. 1, untreated membranes from cells harvested at culture absorbance of 0.40; 2 and 3, digestion carried out on intact cells harvested at culture absorbances of 0.10 and 0.40, respectively; 4 and 5, digestion carried out on isolated membranes from cells harvested at culture absorbances of 0.10 and 0.40, respectively.

membranes. However, the distribution of label in the gels was quite different, as can be seen on comparison of Fig. 4B with Fig. 2. A significant portion of the label in trypsin-treated cells and membranes is found in the lower part of the gels, indicating that the hydrolysis of the high-molecular weight membrane proteins by trypsin resulted in their fragmentation to smaller polypeptides, some of which were still associated with the membrane in a way enabling their interaction with lactoperoxidase.

DISCUSSION

The progressive increase in the ratio of membrane protein to lipid in aging mycoplasma cultures with the accompanying decrease in membrane fluidity and in activity of membrane-bound enzymes and transport systems have been discussed in our previous publications [5, 6]. In this study we concentrated primarily on the pro-

tein component of the membrane, with the aim of determining whether or not aging of mycoplasma cultures resulted in changes in the composition and disposition of the membrane proteins. Our data, gathered from experiments on aging *M. hominis*

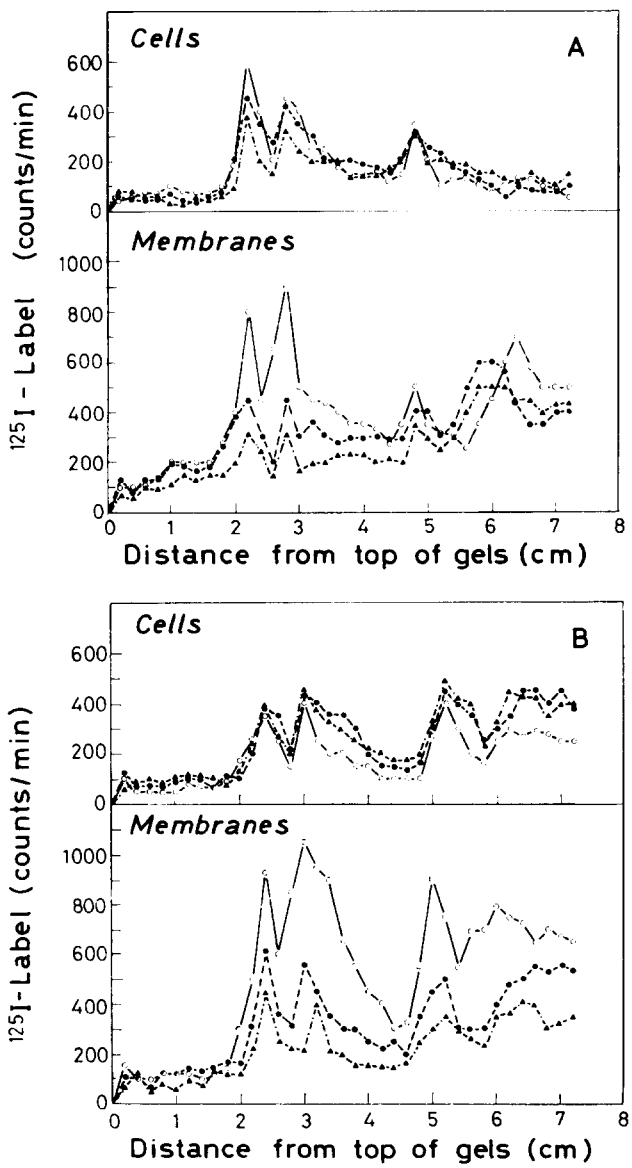

Fig. 4. Distribution of iodine label in *M. hominis* membrane proteins before and after trypsinization. (A) Isolated membranes or intact cells from cultures reaching an absorbance at 640 nm of 0.10 (○–○), 0.30 (●–●), or 0.40 (▲–·–▲) were subjected to lactoperoxidase-mediated iodination followed by digestion with 50 μg trypsin/ml for 2 h at 37 °C. All other details are as described in the legend to Fig. 2. (B) The same legend as for A, but the cells and isolated membranes were first treated with trypsin and then subjected to lactoperoxidase-mediated iodination.

cultures, indicate the occurrence of some changes, but the overall profile of the major membrane proteins, detectable by polyacrylamide-gel electrophoresis, remained the same throughout the late logarithmic phase of growth. The only visible changes were a reduction in the staining intensity of some minor proteins in the high-molecular weight region, and a consistent and progressive intensification of one protein band, Band D_1. Why this protein becomes more prominant on aging of the culture is unclear. It could be the result of its preferential synthesis or its lower rate of turnover, as compared to other membrane proteins. It could also represent the accumulation of a very large polypeptide fragment split by an endogenous peptidase from those high molecular weight proteins which diminish in quantity on aging (Fig. 1). The possibility that this protein is a cytoplasmic protein which gradually became adsorbed onto the inner surface of the membrane [17] is unlikely since protein D_1 is apparently localized on the outer rather than on the inner surface of the cell membrane (see Fig. 2). It could be argued, however, that this protein originates from the growth medium.

Lactoperoxidase-mediated iodination and trypsinization of intact mycoplasma cells can provide information on proteins exposed on the outer membrane surface [16]. We found that the iodination values of membranes from treated cells, and the percentage of membrane protein lost by trypsinization of intact cells were not significantly altered during aging of the cultures. Thus, there is no indication of major changes in these externally located proteins during aging of cultures, apart from a clear increase in the quantity of Band D_1 (Fig. 2). Iodination and trypsinization of isolated membranes provides information also on the proteins localized on the inner membrane surface, facing the cytoplasm [16]. However, the interpretation of this data is more difficult since the labeling intensity of isolated membranes was consistently found to decrease on aging (Table II, Fig. 2), whereas the percentage of protein released from the membranes by trypsin was found to increase (Table III). To explain these seemingly conflicting results two alternative hypotheses are proposed. The concept underlying these hypotheses is schematically shown in Fig. 5.

Both hypotheses are based on the assumption that on aging more proteins have to be accommodated within or on the same area of lipid bilayer, in accordance with the progressive increase in membrane protein-to-lipid ratio. As was discussed

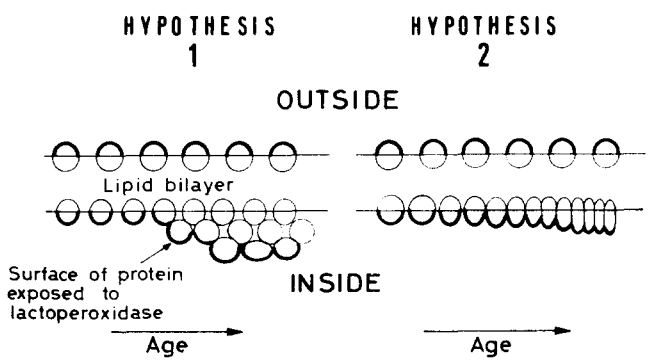

Fig. 5. Possible changes in the arrangement of protein components in membranes of *M. hominis* during aging of culture.

above, the iodination and trypsinization data indicate that during aging no major changes occur in the amount and disposition of the proteins on the outer membrane surface. Accordingly, the progressive accumulation of the proteins during aging must take place on the inner membrane surface. The first hypothesis is that the newly synthesized membrane proteins pile up on top of each other on the inner membrane surface. As a result of this, the percentage of membrane proteins available to interaction with lactoperoxidase decreases, explaining the progressive decline in the specific labeling values of isolated membranes. This hypothesis also explains quite adequately the increasing percentage of membrane protein released by trypsin treatment of isolated membranes since, unlike lactoperoxidase, trypsin will act progressively, "chewing up" the proteins accumulating outside the lipid bilayer. However, if this hypothesis is correct then the proteins accumulating outside the lipid bilayer should resemble peripheral membrane proteins in being susceptible to detachment from the membrane by washing in dilute buffer with EDTA. The experimental evidence fails to support this assumption. Furthermore, the rather constant protein profile of membranes on aging speaks against a selective increase in the synthesis of peripheral membrane proteins, and it seems implausible to assume that the amphipathic integral membrane proteins could accumulate outside the lipid bilayer.

The alternative hypothesis, also shown schematically in Fig. 5, suggests that the newly synthesized proteins added to the inner membrane surface on aging become more densely packed since they do not detach from the lipid bilayer. The dense packing will act to reduce the surface area on the protein molecules available for interaction with lactoperoxidase, explaining the progressive decrease in iodination values on aging. On the other hand, the dense packing would not be expected to affect the susceptibility of the proteins to proteolytic digestion and thus the increasing percentage of the digestable protein on aging has still to be explained. It could be suggested that the dense packing of the protein molecules is a result of the decrease in membrane fluidity on aging [6] and may cause the extrusion of a larger portion of the molecules outside the lipid bilayer (Fig. 5) better exposing them to trypsin action.

It is much easier to explain why trypsin did not remove all the iodine label from lactoperoxidase-treated membranes, although all the membrane proteins exposed to lactoperoxidase would be expected to be also exposed to trypsin. The answer lies in the finding that a significant part of the labeled peptide fragments produced by trypsin remains attached to the membrane, as can be seen by comparing Fig. 2 with Fig. 4A. These figures show that, following trypsinization of iodinated membranes, a higher percentage of the residual label is associated with low-molecular weight polypeptides.

In conclusion, our results indicate that the process of aging in *M. hominis* cultures is accompanied by significant changes in the organization of membrane proteins, in particular on the inner membrane surface. However, the details of these changes are as yet unclear. Although, for obvious reasons, the process of aging in cultures of procaryotes may be very different from that of eucaryotic cells, even when the latter are cultured in vitro, some of the basic principles associated with the effects of aging on the cell membrane may be common to procaryotes and eucaryotes. If true, the advantages of mycoplasmas as convenient tools in membrane studies may also be exploited in studies on membrane phenomena associated with aging.

ACKNOWLEDGEMENT

This work was supported by a grant from the Joint Research Fund of The Hebrew University and Hadassah.

REFERENCES

1 Pollack, J. D., Razin, S. and Cleverdon, R. C. (1965) J. Bacteriol. 90, 617–622
2 Rottem, S. and Razin, S. (1966) J. Bacteriol. 92, 714–722
3 Razin, S., Gottfried, L. and Rottem, S. (1968) J. Bacteriol. 95, 1685–1691
4 Rottem, S. and Razin, S. (1969) J. Bacteriol. 97, 787–792
5 Razin, S. (1974) FEBS Lett. 47, 81–85
6 Rottem, S. and Greenberg, A. (1975) J. Bacteriol. 121, 631–639
7 Razin, S. and Rottem, S. (1974) in Methods in Enzymology (Fleischer, S., Packer, L. and Estabrook, R., eds.), Vol. 32B, pp. 459–468, Academic Press, New York
8 Razin, S., Tourtellotte, M. E., McElhaney, R. N. and Pollack, J. D. (1966) J. Bacteriol. 91, 609–616
9 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
10 Phillips, D. R. and Morrison, M. (1970) Biochem. Biophys. Res. Commun. 40, 284–289
11 Hubbard, A. L. and Cohn, Z. A. (1972) J. Cell Biol. 55, 390–405
12 Morrison, M. and Hultquist, D. E. (1963) J. Biol. Chem. 238, 2847–2849
13 Muñoz, E., Nachbar, M. S., Schor, M. T. and Salton, M. R. J. (1968) Biochem. Biophys. Res. Commun. 32, 539–546
14 Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617
15 Hubbell, W. L. and McConnell, H. M. (1969) Proc. Natl. Acad. Sci. U.S. 64, 20–27
16 Amar, A., Rottem, S. and Razin, S. (1974) Biochim. Biophys. Acta 352, 228–244
17 Kadlubowski, M. and Harris, J. R. (1974) FEBS Lett. 47, 252–254