pH-DEPENDENT CHANGES IN DENSITY OF PLASMA MEMBRANES OF GROWING *MYCOPLASMA LAIDLAWII* CELLS

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Received 24 August 1970

1. Introduction

Small variations in the density of different plasma membrane preparations isolated from *M. laidlawii* have frequently been observed in our laboratory. Engelman and Morowitz [1] reported that their preparations of *M. laidlawii* membranes had a density of 1.18 ± 0.005 g/cm^3 while ours had a density of 1.17 ± 0.002 g/cm^3 [2, 3]. The conjecture that these variations were due to varying amounts of nucleic acid contaminants, could, however, not be substantiated experimentally. Another unexplained finding was the frequent appearance of a minor band next to the major band in density gradient analyses of *M. laidlawii* membrane preparations, despite extensive morphological evidence that Mycoplasma has no other membrane besides the single plasma membrane [4]. The present report may provide at least a partial explanation for the two puzzling observations by showing that the pH of the growth medium has a marked influence on the density of *M. laidlawii* membranes. The membrane density varied from 1.140 g/cm^3 at pH 7.0 to 1.184 g/cm^3 at an initial pH of 8.5. The differences were shown to reflect changes in the ratio of membrane lipid to protein. The variability in the composition of the membranes in growing cells qualifies the use of density as a criterion for distinguishing different types of cell membranes.

2. Materials and methods

Mycoplasma laidlawii (oral strain) was grown statically in a modified Edward medium [5]. To label the membrane lipids, 25 μCi of 9, 10-^{3}H-oleic acid (Radiochemical Centre, Amersham, England) were added to each liter of the growth medium. The organisms were harvested after various periods of incubation at 37° and washed twice in the cold with 0.25 M NaCl. Osmotic fragility of the organisms was tested according to Razin [5] and expressed as percentage lysis in 0.06 M NaCl, calculated according to the formula:

$$\frac{\text{(absorbance in 0.25 M NaCl}-\text{absorbance in 0.06 M NaCl)}}{\text{(absorbance in 0.25 M NaCl)}} \times 100.$$

Cell membranes were isolated by osmotic lysis of the organisms [2]. The membrane preparations were tested for nucleic acid content [6]. Protein was determined by the Folin-phenol method of Lowry et al. [7], using crystalline bovine plasma albumin as standard. Membrane proteins were chromatographically analyzed in polyacrylamide gels containing 7.5% acrylamide, 35% acetic acid and 5 M urea [3]. Membrane lipids were extracted with chloroform-methanol (2:1, v/v) according to Folch et al. [8]. Total membrane lipid was estimated by the colorimetric method of Saito and Sato [9], using the bichromate reagent and cholesterol as standard. Polar lipids were separated from the nonpolar lipids by chromatography on silicic acid columns [10]. Thin-layer chromatography of the polar lipid fraction was carried out on Silica Gel G (0.25 mm thick) chromatoplates, using chloroform-methanol-water (65:25:4, by vol.) as the developing solvent. Lipid spots were detected with

iodine vapor, phospholipid spots with the molybdate spray reagent and glycolipids with the $AgNO_3$ reagent [11]. The lipid spots were scraped off and their radioactivity was determined. Radioactivity in membranes was estimated as described by Kahane and Razin [11], using a Packard Tri-Carb liquid scintillation spectrometer. Radioactivity in lipids was determined in vials containing 10 ml of toluene scintillation liquor (4 g 2.5 diphenyloxazole, 100 mg 1,4-bis-(5-phenyloxazolyl-2)benzene in 1 l of toluene). NADH oxidase (EC 1.6.99.3) and ATPase (EC 3.6.1.3) activities were measured as described by Pollack et al. [12].

For isopycnic density gradient analysis samples (0.15 ml, containing 200 to 300 μg protein) of membranes were layered over 4.8 ml of linear sucrose gradients. Each gradient had a density difference of 0.05 g/cm^3 from top to bottom. The total density range covered by the different gradients was between 1.11 to 1.20 g/cm^3. The gradients were centrifuged at 39,000 rpm in the SW 39 rotor of a Spinco model L-2 ultracentrifuge for 2 hr at 10°. The density of the bands was estimated according to their location on the gradient. To minimize edge effect errors, appropriate gradient ranges were chosen so that the membrane bands would sediment to about the centre of the gradient tube.

3. Results

Adjustment of the Edward medium to different pH values resulted in marked changes in the density of the *M. laidlawii* membranes isolated (table 1). The differences in membrane density could not be accounted for by nucleic acid contamination as the nucleic acid content of the various membrane preparations was very low (0.3 to 0.5% of the membrane dry weight). They could, however, be attributed to marked changes in the lipid:protein ratio of the membranes (table 1). Nevertheless, the membranes exhibited essentially the same electrophoretic protein pattern in polyacrylamide gels.

Analysis by thin-layer chromatography of the polar lipid fraction (constituting about 85% by weight and over 95% of the radioactivity of the total membrane lipid) showed no qualitative differences in membranes of varying densities (table 2). Some quantitative differences could, however, be detected: low density membranes had a lower percentage of lipid having an R_f of 0.22.

When the initial pH of the medium was raised the growth of *M. laidlawii* was improved. Growth was best at pH 9.15, though the highest membrane density was obtained when the initial pH of the medium was 8.50 (table 3). Except at pH 9.15, the osmotic fragility of the cells grown at different initial pH values

Table 1
The density and composition of membranes of *M. laidlawii* cells grown at different pH values.

Initial pH of medium	Density of membranes (g/cm^3)	Membrane lipid to protein ratio	
		(mg lipid/ mg protein)	(cpm/mg protein)
7.00	1.140	0.82	121,000
7.50	1.167	0.59	84,500
8.50	1.181	0.47	51,100

The organisms were grown for 20 hr in Edward broth supplemented with ^{3}H-oleic acid and adjusted to different pH values.

Table 2
Distribution of radioactivity in lipid components of *M. laidlawii* membranes of various densities.

Lipid component (R_f)	Tentative identification	Density of membranes (g/cm^3)		
		1.140	1.167	1.181
		% of total radioactivity		
Origin	not identified	0.7	0.7	1.2
0.12	phospholipid	5.8	14.4	13.4
0.22	not identified	3.6	5.3	14.0
0.40	phospholipid	9.1	3.0	4.3
0.50	phospholipid	3.5	6.7	2.3
0.62	glycolipid	26.2	22.1	21.3
0.80	glycolipid and phospholipid	46.5	46.5	41.7
0.90	not identified	4.1	0.7	1.2
Front	not identified	0.5	0.6	0.6

Membranes were derived from cells grown with ^{3}H-oleic acid under the conditions described in table 1. Lipids were extracted and separated by thin-layer chromatography and their radioactivity was determined as described in Materials and methods.

Table 3
Some properties of cells and membranes of *M. laidlawii* grown at different pH values.

Initial pH of growth medium	Cell yield (mg protein/l medium)	Osmotic fragility of cells (% lysis in 0.06 M NaCl)	Density of membranes (g/cm^3)		Enzymic activities of membranes	
			Major band	Minor band	NADH oxidase	ATPase
7.00	20	34	1.140	no band	not tested	not tested
7.75	186	40	1.162	1.171	0.28	0.8
8.50	256	80	1.184	1.171	0.46	1.0
9.15	302	73	1.181	1.171	0.47	1.0

The organisms were grown in Edward medium adjusted to different pH values for 21 hr at 37°. The inoculum consisted of logarithmic-phase cells grown in a medium of the same initial pH as that of the test medium. NADH oxidase activity is expressed as the decrease in absorbance at 340 nm per mg protein per min, and ATPase activity as μmoles of inorganic phosphate released per mg protein in 30 min.

increased with the increase in the pH of the medium, corresponding with the changes in membrane density. A minor band, which differed in density from the major band, constituted about 10 to 30% of the total membrane protein, frequently accompanied the major membrane band (table 3). It could be detected only when the amount of membrane material layered on the sucrose gradient exceeded 200 μg of protein. In lower density membranes NADH oxidase and ATPase activities appeared to be lower (table 3).

The experiment summarized in table 4 indicates that the changes in membrane density may be reversible: membrane density increased upon the cells being transferred to a growth medium of a higher pH, but not to the same extent as in cells grown at the high pH medium throughout.

4. Discussion

Changes in the lipid to protein ratio have been recorded in several microbial and animal cell membranes during various physiological phases of growth [13–15] or after selective inhibition of membrane protein or lipid synthesis [11, 16]. The present results show that the pH of the growth medium has a pronounced effect on the lipid to protein ratio in *M. laidlawii* membranes. Hence the difference between the density of *M. laidlawii* membranes reported by Engelman and Morowitz (1.18 ± 0.005 g/cm^3) and by us (1.170 ± 0.002 g/cm^3) (ref. 1) may be due to the higher pH of their growth medium (pH 8.3 as against pH 7.8).

There is little doubt that the pH of the medium influences the intracellular pH. Thus, the intracellular pH of *Escherichia coli* was found to increase proportionally to the pH of the medium [17]. Changes in the intracellular pH may affect the rate of biosynthesis or turnover of membrane proteins and lipids leading to changes in membrane density.

The flexible composition of the mycoplasma membrane seems to be indicated by the modification of its density upon transfer of the cells from low to high pH media (table 4), although probably not all the cells of the culture are so affected. This partial modification may be responsible for more than one band appearing in the density gradient analysis of the membranes. Similarly, Salton [18] observed 3 bands in the density gradient analysis of plasma membranes isolated from *Micrococcus lysodeikticus* protoplasts.

Density gradient analysis has been widely used as

Table 4
Induction of changes in membrane density by transfer of cells to medium of a higher pH value.

Growth conditions (incubation period and initial pH of Edward medium)	Density of membranes (g/cm^3)
15 hr at pH 7.75	1.153
15 hr at pH 7.75 and transfer to pH 8.50 for 15 hr	1.174
15 hr at pH 8.50 and transfer to pH 8.50 for 15 hr	1.181

a preparative tool for the separation of various membrane types of the eucaryotic cell [19, 20] and the separation of mesosomes from the plasma membrane of bacteria [21]. In showing that the density of a certain type of membrane may vary quite considerably according to its growth conditions our findings impose some restrictions on the use of this technique. This also accounts for the fact that a single type of membrane may form more than one band in the density gradient.

Acknowledgments

This investigation was supported by grant 6 D-IV from the Ford Foundation. The valuable technical assistance of M.Wormser is gratefully acknowledged.

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