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THE EFFECT OF A PROTEOLIPID FROM SARCOPLASMIC RETICULUM ON THE PHYSICAL PROPERTIES OF ARTIFICIAL PHOSPHOLIPID MEMBRANES

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SUMMARY

Sarcoplasmic reticulum membranes from skeletal muscle contain a proteolipid ($M_r \approx 12\,000$) which reduces both the nonspecific ion and water permeabilities of artificial planar phospholipid bilayers. The proteolipid does not show any ionophoric effect or specific pore formation for Ca^{2+}. The a.c. capacitance of the bilayers is unaffected whereas the refractive index is increased by the presence of proteolipid. The results support the view that the proteolipid interacts with the phospholipids in the bilayer interior and causes a condensation in the packing of the alkyl chains.

INTRODUCTION

Most of the animal and plant tissues so far investigated contain varying amounts of proteolipids, i.e. protein fractions insoluble in aqueous solvents and soluble in chloroform/methanol mixtures [1, 2]. It is generally assumed that these proteins are in some way associated with membrane structures. The first report of a proteolipid being part of sarcoplasmic reticulum membranes was given by MacLennan et al. [3, 4] who also described in detail its isolation and characterisation. As part of our recent investigations on the structural role of this proteolipid in sarcoplasmic reticulum membranes [5, 6], we have carried out a study on the effects of the proteolipid on some physical properties of artificial planar phospholipid bilayers which are convenient model systems for studies on transport across membranes [7–9]. Our experiments to be described here involved measurements of d.c. resistance, a.c. capacitance, optical refraction and water permeability of the bilayers. The results allow us to draw some conclusions on the nature of interaction between the proteolipid and the phospholipids.

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EXPERIMENTAL

Materials

The proteolipid was isolated from rabbit white skeletal muscle as described previously [6] following essentially the method originally designed by MacLennan et al. [3, 4]. In sodium dodecyl sulfate polyacrylamide gel electrophoresis [10] the material appeared as a single band corresponding in its velocity to a molecular weight of 12 000 [6]. The phosphorous content [11] of the preparation was found to be less than 1 %, by weight. Thin-layer chromatography [12] showed phosphatidylcholine as the main lipid contaminant, with traces of phosphatidylethanolamine being present.

Membrane phospholipids from sarcoplasmic reticulum were purified from the chloroform/methanol extract by passage over a silicic acid column [13]. The phospholipid composition estimated from thin-layer chromatography [12] and charring with chromic acid was phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine in the ratio of 80 : 15 : 5, respectively. The fatty acid composition of the phospholipids was determined by gas-liquid chromatography of the methyl esters as described in detail elsewhere [14]. The results are given in Table I.

Egg-yolk lecithin was purchased from Lipid Products South Nutfield, U.K., and *n*-decane (BDH, Laboratory Reagent) was purified over an alumina column. All other reagents were analytical grade, and the water was deionised and twice distilled in an all-glass still.

METHODS

Formation of bilayers. Unless otherwise stated bilayers were formed across a circular aperture of 0.20 cm diameter in a Teflon cell using the brush technique [7]. The diameter of the black area was 0.165 cm as measured under a microscope. Film forming solutions containing proteolipid were prepared by mixing phospholipid and proteolipid solutions in chloroform/methanol (2 : 1, v/v), and evaporation under N_2 gas and reduced pressure. The dry residue was dissolved in *n*-decane to give a phospholipid concentration of 10 mg/ml. The proportions of phospholipid and proteolipid used in these film forming solutions were within the range of 20 : 1 and 100 : 1 (lipid : protein, by weight) and resemble the proportions estimated from the yields in the preparation from the membranes. Within this range we were unable to detect any significant concentration dependence of the results.

Thick (coloured) films were obtained readily both from solutions with and without proteolipid. All results given here were obtained from films that were stable for at least 20 min after having reached the black state. The films containing proteolipid showed a greater stability against mechanical deformation and had a lifetime considerably longer than pure phospholipid bilayers.

Direct current resistance. The technique for determining the d.c. resistance of the bilayers bathed in 0.1 M NaCl has been described in detail elsewhere [15].

Alternating current capacitance. The a.c. capacitance was measured in an apparatus similar to that described by Hanai et al. [16]. Platinum electrodes were positioned into the 0.1 M NaCl bathing solutions, and were connected to a Wayne-Kerr Universal Bridge, B221 A. The bridge had an a.c. signal input from an Advance LF Signal Generator (Type SG 66). The capacitance of the bilayers was measured as

a function of frequency within the range of 500 and 5000 s^{-1}, and of applied voltage. The capacitance values of different bilayers are compared at 1000 s^{-1} and 46 mV.

Refractive index. Bilayers were formed in a 1×1 cm quartz cuvette using a technique by Van den Berg [17] across a 0.4 cm diameter circular aperture in a Teflon strip. The cuvette was placed in the central axis of a goniometer table and irradiated with plane polarised light of wavelength 6328 Å from a Bradley He-Ne laser source. The refractive index of the bilayers bathed in 0.1 M NaCl (pH 5.5) was calculated from the Brewster angle, i_B, using the equation,

$$\tan i_B = \frac{n_B}{n_0} \tag{1}$$

where n_B and n_0 are the refractive indices of the bilayer and aqueous phase, respectively. The laser source was oriented such that the beam was polarised in the plane of incidence with any stray component being eliminated by a polariser mounted on the objective. The Brewster angle was determined by rotating the goniometer table until the reflected light was visually assessed to be a minimum or zero intensity. The method of calculating the angle i_B at the zero reflection position involves the geometry of the optical system and has been described in full detail elsewhere [18].

Osmotic water permeability. The osmotic water permeability coefficient P_{os}, in $cm \cdot s^{-1}$, was determined using a previously described apparatus [19] and mode of operation [14]. The flux of water through the bilayer under an osmotic gradient (0.1 M/0.01 M NaCl) was measured by compensation using a 1.0 microlitre Hamilton syringe. The flux was monitored by readjusting the pattern of the light reflection from the horizontal bilayer at regular intervals for about 30 min.

RESULTS

Electrical measurements

The d.c. resistance of the pure phospholipid bilayers was $2.9 \cdot 10^7$ ohm $\cdot$ cm^2 and $1.2 \cdot 10^8$ ohm $\cdot$ cm^2, for egg-yolk lecithin and sarcoplasmic reticulum phospholipids, respectively. These values compare favourably with those reported by other authors [19, 20]. In the presence of proteolipid we found the values of $1.0 \cdot 10^8$ and $2.4 \cdot 10^8$ ohm $\cdot$ cm^2 for egg-yolk lecithin and sarcoplasmic reticulum phospholipids, respectively. Ohmic relationships were observed in all cases up to about 30 mV and the results were identical for reversed polarity. The experimental scatter from a minimum of five bilayers was smaller than 20 %.

In the presence of Ca^{2+} in the bathing solution (5 mM $CaCl_2$, 0.1 M NaCl) the resistance of sarcoplasmic reticulum phospholipid bilayers containing proteolipid was $1.0 \cdot 10^8$ ohm $\cdot$ cm^2, corresponding to a 60 % decrease of the value found in the absence of Ca^{2+}. A similar decrease was observed upon adjustment of the Ca^{2+} concentration to 5 mM at one side of the bilayer only.

In a series of experiments we injected small volumes of proteolipid solution in chloroform/methanol (1 mg/ml) into the inner compartment of the cell and monitored the changes in current while maintaining a constant voltage of 10 mV. A typical recorder tracing is shown in Fig. 1. The injection of proteolipid resulted in rapip decreases in current. However, it was essential to stir the aqueous solution con-

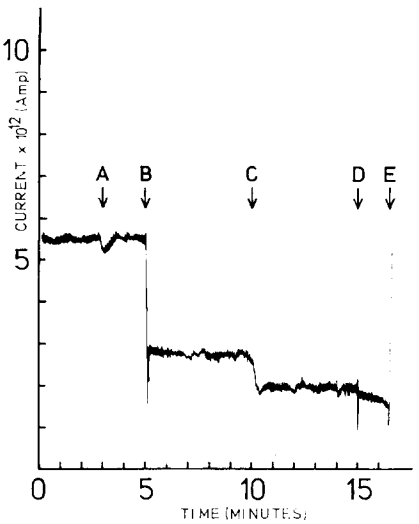

Fig. 1. Current across an egg-yolk lecithin bilayer at a constant voltage of 10 mV. (A) injection of 10 μl proteolipid in chloroform/methanol (2:1, v/v). (B) injection of 20 μl proteolipid solution under magnetic stirring. (C) and (D) injection of 20 μl proteolipid solution under continuous stirring. (E) Leakage.

tinuously in the inner compartment. Blank injections of pure chloroform/methanol and stirring showed no effect.

The a.c. capacitance values obtained for egg-yolk lecithin bilayers and those containing proteolipid failed to show any significant difference. The values were 0.35 ± 0.02 $\mu F \cdot cm^{-2}$ and 0.35 ± 0.01 $\mu F \cdot cm^{-2}$, respectively. The capacitance found for egg-yolk lecithin can be compared to those reported by Hanai et al. [16] of 0.38 ± 0.01 $\mu F \cdot cm^{-2}$, by Läuger et al. [21] of 0.33 ± 0.02 $\mu F \cdot cm^{-2}$, and by Cherry and Chapman [18] of 0.35 ± 0.03 $\mu F \cdot cm^{-2}$. Sarcoplasmic reticulum phospholipid bilayers showed a capacitance of 0.34 ± 0.01 $\mu F \cdot cm^{-2}$ which was also unchanged by the addition of proteolipid. By assuming the hydrocarbon interior of the bilayer to be 50 Å thick, and that the thickness is unchanged by the presence of proteolipid (Laggner, P. and Atkinson, D., unpublished), and by using the formula for a parallel plate capacitor we obtain a dielectric constant ε of 2.0 ± 0.1.

Osmotic water permeability. The osmotic water permeability coefficient, P_{os}, of sarcoplasmic reticulum phospholipids was found to be $(35 \pm 2.1) \cdot 10^{-4}$ cm $\cdot$ s^{-1}, which is somewhat higher than that of egg-yolk lecithin or phosphatidylserine bilayers [14, 22]. This can be rationalised on the basis of the higher unsaturation and shorter chain lengths [31] of the sarcoplasmic reticulum phospholipids (Table I). In the presence of proteolipid the permeability is reduced by about 50 % to a value of $(15.7 \pm 1.0) \cdot 10^{-4}$ cm $\cdot$ s^{-1}.

Refractive index. The refractive indices of six different egg-yolk lecithin bilayers as calculated from Eqn. 1, where n_0 is 1.334 for 0.1 M NaCl, give a mean value of 1.362 ± 0.013. This is in excellent agreement with the value of 1.37 ± 0.02 found for egg-yolk lecithin bilayers by a similar technique [23]. The mean value of six different egg-yolk lecithin bilayers containing proteolipid is 1.408 ± 0.006. This 3.5 % increase is significantly larger than the error limits of each measurement.

TABLE I

FATTY ACID COMPOSITION OF SARCOPLASMIC RETICULUM PHOSPHOLIPIDS (I), EGG-YOLK LECITHIN (II) AND PHOSPHATIDYLSERINE (III)

The data for II and III are taken from Ref. 14.

Carbon no.	I	II	III
12:0	—	—	0.8
12 unsaturated	—	—	0.6
14:0	8.6	0.4	0.5
14 unsaturated	—	0.4	0.4
16:0	30.8	29.0	3.3
16:1	t	2.4	0.4
16:2	—	t	0.2
18:0	11.1	17.1	48.3
18:1	17.7	37.0	29.4
18:2	30.4	12.3	2.4
18:3	1.4	0.4	1.4
20:0	t	t	2.7
20:1	—	t	3.1
20:2	—	t	2.1
22:0	—	—	3.0
22:1	—	—	1.1
20:3	—	1.0	t
(% unsaturated × no. of double bonds)	82.7	68.6	48.6
Shorter than C_{18}	39.4	32.2	6.2

t indicates a trace was seen.

DISCUSSION

Both the unspecific ion and water fluxes are generally considered to rely on the thermal mobility of the alkyl chains within the bilayer [22, 24, 25]. In the case of ion permeability additional parameters such as size and charge of the polar phospholipid headgroups play an important part. However, with one given phospholipid class, the ion permeability is also dependent on the frequency of imperfections ("kinks" [24]) in the packing of the alkyl chains.

It has been suggested [16] that the resistances of artificial phospholipid bilayers as measured in our experiments may reflect the number of conductance channels, or leaks, in the bilayers. The conclusions to be drawn here are, however, based upon a comparison of the bilayer resistances in the presence and absence of proteolipid. The absolute values, therefore, are here only relevant to assure that the bilayers are in the state normally observed in experiments with these artificial model systems.

We have observed a decrease of both ion and water permeability of the bilayers upon the incorporation of proteolipid. To us the most plausible interpretation of these effects is a hydrophobic interaction of the proteolipid with the phospholipids causing a condensation in the alkyl chain packing. This would lead to a reduced frequency of "kink" formation and thus to a reduced permeability. Such a mechanism would be also consistent with our previous spectroscopic results [6]. An interaction of the proteolipid with the polar interface of the bilayers is unlikely in view of the

results obtained with *n*-butanol extracted erythrocyte protein [26]. Our observation of a reduction in resistance by Ca^{2+} is contrary to previous experience with pure phosphatidylcholine and phosphatidylserine bilayers [20]. Although the possibility of some kind of specific calcium-proteolipid interaction cannot be excluded, our results clearly exclude any ionophoric mechanism, since the observed effect is by orders of magnitude smaller than with ionophoric peptides such as alamethicin [27, 28] and valinomycin [29, 30].

The proteolipid seems to retain its ability to interact with phospholipids for some time after injection into the aqueous phase. This does not necessarily indicate that it obtains a stable, water-soluble form readily upon injection, but more likely, that it keeps its original conformation for the short period required to reach the bilayer. This point is somewhat strengthened by the fact that stirring is required to speed up the diffusion to the bilayer and to obtain the observed effect.

Previous optical experiments [18, 23, 32] have shown that the ordered array of the phospholipid molecules confers optical anisotropy to the system. Therefore, it is necessary to assume different refractive indices $n_\perp$ and $n_\parallel$, in the directions perpendicular and parallel to the optical axis of the bilayer. The observed increase due to the proteolipid-phospholipid interaction in the overall refractive index coupled with an unchanged dielectric constant is at first somewhat surprising. However, the changes anticipated in the refractive indices are possibly too small to be observed by the less sensitive capacitance measurements. In addition, by measuring the capacitance in an a.c. field perpendicular to the plane of the bilayer the parameter obtained will be the the component parallel to the hydrocarbon chains, $\varepsilon_\parallel$, and thus $n_\parallel$ is likely to be also unchanged. It has been shown [18] that n_B, $n_\parallel$, and $n_\perp$ are related by the equation

$$n_B^2 = \frac{(n_\perp^2 - n_0^2)}{(n_\parallel^2 - n_0^2)} \cdot n_\parallel^2 \qquad (2)$$

Hence, we can deduce that for an increase in n_B, and for $n_\parallel$ to be unchanged, $n_\perp$ must increase from 1.463 (egg-yolk lecithin) to 1.471 (egg-yolk lecithin plus proteolipid). Here the condensation in alkyl chain packing would be seen as an increase in $n_\perp$ only.

In conclusion we suggest that one possible role of the proteolipid in sarcoplasmic reticulum membranes can be seen in reducing leakage and thus serving to to maintain the efficiency of the calcium pump system.

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