J. Membrane Biol. 11, 237–254 (1973)

Interaction of Bilayers with Basic Polypeptides

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Received 7 July 1972

Summary. The interaction of bilayers formed from glyceryl monooleate + oleic acid with basic polypeptides was studied. Copolymers of lysine with different amino acids interact with the bilayers reducing their resistance and increasing their capacitance.

The largest effect was observed with the copolymer L-lysine/L-phenylalanine (1.4:1) which decreases the specific resistance by more than 3 orders of magnitude. The smallest effects on the resistance were obtained with the very hydrophilic copolymer of L-lysine/L-serine (1.1:1). The contribution of the double-layer potential to the measured transmembrane potentials of the charged bilayers is considered and calculated for the case of the membrane containing dissociable components in the absence of interacting copolymers.

The question of whether a bimolecular layer of phospholipids is a basic structure of the biological membranes was an open one for many years. Recently, calorimetric measurements [16] and X-ray diffraction data [22] have indicated that the phospholipid bilayer is indeed a structural component of at least some of the biological membranes.

The model membranes have many properties similar to biological ones [20] but differ in others, especially those involving interactions with proteins.

To achieve more accurate simulation of biological membranes, using phospholipid bilayers, the latter have been interacted with various other materials [20].

As biological membranes contain ~20 to 80% of protein [17] the study of interaction of bilayers with proteins is an important factor for understanding the detailed structure and function of biological membranes.

The interaction of bilayers with proteins leading to changes in electrical properties was reported by several groups [1, 9, 15]. Recently Montal [13] described the interaction of bilayers with poly-L-lysine.

Despite the above-described investigations into the properties of the protein complexes, different factors governing the lipid-protein interactions

and the mechanism by which the electrical properties of bilayers are modified are unclear.

To understand the nature of the interaction between bilayer membranes and proteins, we attempted in this work a detailed study of the interaction between basic polypeptides of different compositions and bilayers of glyceryl monooleate containing oleic acid. Since polypeptides have a known composition and structure, they offer a wide possibility of chemical and structural modifications.

The influence of polypeptides on the electrical properties of the bilayers was studied and correlated with the degree of hydrophobicity of the different amino acids.

Materials and Methods

Glyceryl monooleate (Monoolein Sigma grade), oleic acid (Sigma grade) and Trizma Base (reagent grade) were purchased from Sigma, St. Louis, Mo. The purity of the first two materials was checked in the laboratory by thin-layer chromatography. Decane was obtained from BDH (Poole, England) and was distilled under vacuum.

The polypeptides used were: poly-L-lysine, HBr (mol wt 23,000); copolymers: L-lysine/L-phenylalanine, HBr 1.4:1 (mol wt 30,000), L-lysine/L-serine, HBr 1.1:1 (mol wt 35,000), L-lysine/L-tyrosine, HBr 1:1 (mol wt 41,000) – all random copolymers. These polypeptides were purchased from Miles Yeda, Kiryat Weizmann, Rehovot, Israel. A block copolymer L-lysine/L-leucine, HBr 3:1 and a random copolymer L-ornithine/L-leucine, HBr 1:1 were a gift from Dr. K. Rosenheck (Polymer Dept., The Weizmann Institute of Science).

The purity of the peptides, ratio of the components and the concentration of the stock solutions (10 to 20 mg/ml) were checked by an amino acid analyzer.

All other reagents were of analytical grade. The water was first deionized by passage through ion exchange columns and then distilled over permanganate in a Pyrex glass still.

The films were prepared by a brush technique [14] in a cell similar to that described by Hanai, Haydon and Taylor [7]. The volume of the inner cell was ~ 5 ml and of the outer one ~ 80 ml. The cell was placed in a perspex box through which water circulated from a thermostated bath. An arrangement for stirring was provided. The temperature of the experiments was 25 ± 1 °C.

The membranes were formed either from 1 % glyceryl monooleate (gmo) in *n*-decane or 0.9 % glyceryl monooleate + 0.1 % oleic acid (ol) in *n*-decane (molar ratio of gmo to ol 7:1).

The aqueous solution was either NaCl (10^{-3} to 10^{-1} N), pH 6.7 (unbuffered solution) or NaCl (10^{-3} to 10^{-1} N) buffered with Tris HCl (10^{-3} N) either at pH 7.2 or 8.5. The pH was checked at the beginning and at the end of the experiments, and in the case of pH 8.5 no change with time was detected. The film was viewed through a low-power microscope equipped with calibrated reticule for area determinations.

The apparatus used in measuring electrical properties of the bilayers was similar to that described by Redwood, Müldner and Thompson [15] and consisted of two Ag/AgCl electrodes of large area, a switch providing measurement of d-c current, capacity and membrane potential, two Keithley electrometers 602 for measuring the applied potential and 610C for measuring the current. The current was monitored on a 5-inch Varian

G10 Graphic recorder. The current-voltage measurements were carried out over a range from 5 mV up to the breakdown potential. In all the cases, the I–V curves were linear up to 50 mV. The d-c resistance measurements were routinely performed in the linear region.

The capacitance of the films was measured with a capacitance bridge built at the Weizmann Institute. It was equipped with only two frequencies, 440 Hz and 1,400 Hz, limiting the measurements to these two frequencies. The bridge was connected to a Hewlett-Packard Wide Range 200 CD oscillator and the amplitude of the applied voltage was less than 20 mV. As a null instrument, a Cathode Ray Oscillograph was used. The bridge performance was checked with standard resistors and capacitors and glyceryl monooleate films in 0.1 N NaCl [3]. The results agreed with the literature values to within experimental error.

Generally, the polypeptides from the stock solutions were added with a Hamilton syringe to the outer stirred compartment, but in some cases the addition was made to the inner stirred compartment. In both cases similar results were obtained.

The polypeptides were either added after the film drained to the "black" state or films were formed in the peptide-containing solutions. In the second case the draining was sometimes very slow; it was speeded by application of 20 mV to the electrodes. In all cases the draining did not take more than 10 to 15 min. Occasionally, the stirring caused appearance of colors in the film. In those cases the stirring was discontinued until the film became black again.

In the transference number experiments the membrane potential due to salt gradient was measured. The grounded electrode (0 electrode) was always in the outer compartment. The salt gradient was produced by addition of 4 N NaCl to the outer compartment containing 10^{-3} N NaCl + 10^{-3} N Tris HCl until the ratio of NaCl from the outer to inner compartments was 4.1:1. The transmembrane potential of pure films or of low-resistance films modified by the copolymer L-lysine/L-phenylalanine was obtained. In the second case the salt was added only after the bilayer reached a steady value of resistance, lower than 10^5 Ω cm^2. In each case the film was broken at the end of the experiment and the potential difference measured for calculating the bulk transference numbers.

Results

For bilayers formed from 1% glyceryl monooleate in *n*-decane (the aqueous phase was NaCl solution either unbuffered or buffered) the following results were obtained.

At pH 6.1 to 6.3 (10^{-3} N NaCl) the unmodified films were stable for more than 2 hr but addition of as little as 0.3 μg/ml ($\sim 10^{-8}$ M) copolymer L-lysine/L-phenylalanine to the outer compartment in the presence of black film broke the bilayer immediately and no black films could be generated in this solution. The films broke immediately after painting the lipid on the hole.

These experiments were repeated with poly-L-lysine at concentrations of 4 to 8 μg/ml [(1.5 to 3) 10^{-7} M] in 10^{-2} M NaCl.

The films were more stable than in the previous case; asymmetric films (addition of poly-L-lysine to one side) were stable for a few minutes and

films could be generated in the polypeptide-containing aqueous solutions, but no change in resistance was found.

The experiments with L-lysine/L-phenylalanine copolymer were repeated at pH 8.5. (The aqueous phase was 10^{-3} N NaCl or 10^{-2} N NaCl buffered with 10^{-3} N Tris HCl.) The copolymer over the range of the concentrations 0.3 to 2 μg/ml (1×10^{-8} to 6×10^{-8}), was added in each case after formation of the film, causing immediate rupture of the bilayer and no black films could be generated in the peptide solutions. Similar results were obtained with poly-L-lysine at the concentration of 0.8 μg/ml (3×10^{-8} M).

In the following experiments the bilayers were formed from 0.9% glyceryl monooleate + 0.1% oleic acid in *n*-decane. Initially, the experiments were performed in unbuffered 10^{-2} N NaCl, pH 6.5. After addition of 0.1 μg/ml (3×10^{-9} M) copolymer lysine/phenylalanine, the bilayer broke and stable films could no longer be obtained. The same effect of addition of the copolymer was found at pH 7. At pH 7.2 (10^{-3} N NaCl + 10^{-3} N Tris) bilayers could be generated in a solution containing 0.3 to 0.6 μg/ml (10^{-8} to 2×10^{-8} M) copolymer of lysine/phenylalanine. In this instance, an immediate decrease in resistance after drainage of the films was observed, but the bilayers broke after reaching the value of 1 to 2×10^5 Ω cm^2. By raising the pH to 8, the bilayers became stable and the decrease in resistance was about 3 orders of magnitude.

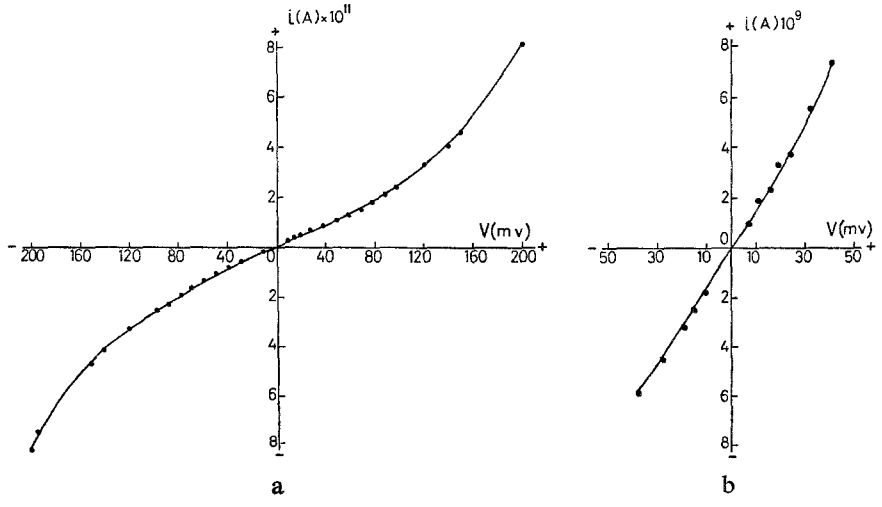

Fig. 1. The current-voltage relation for a bilayer formed from 0.9% glyceryl monooleate + 0.1% oleic acid in *n*-decane. (*a*) Unmodified bilayer. Aqueous phase: NaCl 10^{-2} N, Tris HCl 10^{-3} N, pH 8.5; Film area: 1.3×10^{-2} cm^2. (*b*) Bilayer modified by addition of 0.5 μg/ml of the copolymer L-lysine/L-phenylalanine to one side of the bilayer. Aqueous phase: NaCl 10^{-3} N, Tris HCl 10^{-3} N, pH 8.5; Film area: 1.66×10^{-2} cm^2

The most stable bilayers were obtained at pH 8.5, so it was decided to work at this pH. All the subsequent experiments therefore were done in NaCl solutions buffered with 10^{-3} N Tris HCl to pH 8.5.

Fig. 1*a* shows the current-voltage relation for a bilayer prepared from 0.9% glyceryl monooleate + 0.1% oleic acid in *n*-decane. As seen in the figure, the curve has a usual shape. The electric resistance of the bilayers is high, 5×10^7 Ω cm^2. The dielectric breakdown occurs at 190 ± 40 mV imposed voltage. The bilayers were stable for at least 2 hr and the change of the electrical resistance with time was very small.

Modification of the Electrical Properties of the Bilayers by the Copolymer L-*Lysine*/L-*Phenylalanine*

Addition of 0.6 to 3.2 μg/ml (2×10^{-8} to 10^{-7} M) polypeptide to the outer stirred solution causes a decrease of the electrical resistance by ~3 orders of magnitude. The decrease is gradual and the final low value of resistance for a polypeptide concentration of 0.5 μg/ml is reached in about 40 min (Fig. 2). When the concentration of the copolymer was not higher than 1.5 μg/ml (5×10^{-8} M) the bilayers were stable for at least half an hour. Above this concentration of copolymer the low resistance value was reached in shorter time, but the films did not last for more than 5 min.

The decrease in resistance was the same if the polypeptide was added to only one side of the black membrane (inner or outer) in this case called an asymmetric membrane, or the films were formed in aqueous solution already containing the polypeptide in this case called a symmetric membrane.

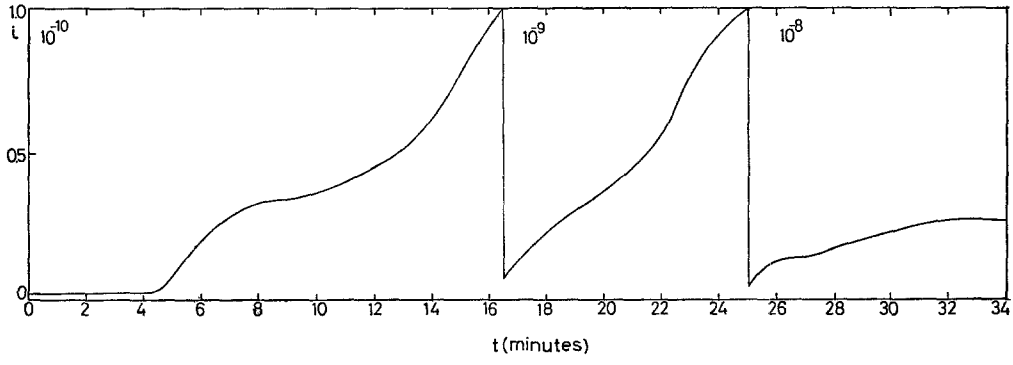

Fig. 2. Recorder retracing of the current as a function of time after addition at zero time of 0.5 μg/ml copolymer L-lysine/L-phenylalanine to the outer compartment. Bilayer formed from 0.9% glyceryl monooleate + 0.1% oleic acid in *n*-decane. Film area: 1.66×10^{-2} cm^2; Aqueous phase: NaCl 10^{-3} N, Tris HCl 10^{-3} N, pH 8.5. Imposed voltage + 15 mV

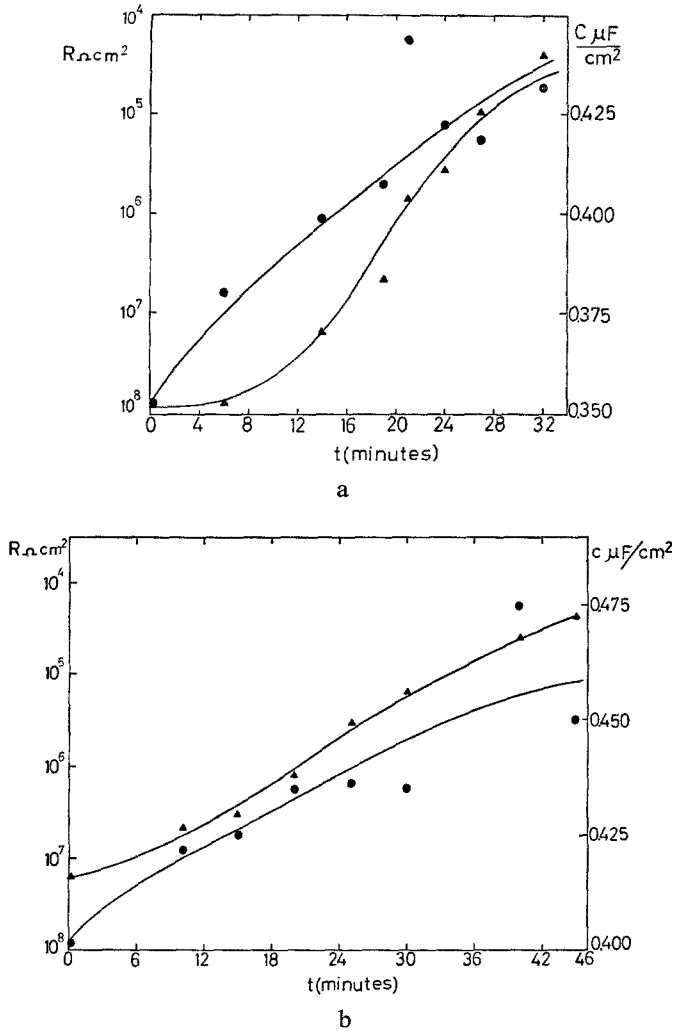

Fig. 3. The specific resistance $R(\Omega\,\mathrm{cm}^2)$ ▲▲, and the specific capacitance $C(\mu\mathrm{F/cm}^2)$ •••, as a function of time. The film was formed from 0.9% glyceryl monooleate + 0.1% oleic acid in *n*-decane. (*a*) 1.2 μg/ml copolymer L-lysine/L-phenylalanine was added to one side at zero time. Aqueous phase: NaCl 10^{-2} N, Tris HCl 10^{-3} N, pH 8.5. (*b*) 0.6 μg/ml copolymer L-lysine/L-phenylalanine was added at zero time. Aqueous phase: NaCl 10^{-1} N, Tris HCl 10^{-3} N, pH 8.5

In the second case, the initial resistance (immediately after the film became black) was lower by at least one order of magnitude than that of an unmodified film.

At the lowest salt concentration employed (10^{-3} N NaCl + 10^{-3} N Tris HCl) the asymmetric films in the low-resistance state were stable for about

half an hour. Increasing the salt concentration causes a decrease of the stability of the bilayers and at 10^{-1} N NaCl the bilayers in the low resistance state broke within 5 min. In all the salt concentrations employed, the symmetric films are longer-lived than the asymmetric ones.

The current-voltage curves in the presence of the polypeptide are linear (Fig. 1*b*); the dielectric breakdown is shifted to much lower imposed potentials (50 ± 10 mV).

Interaction with the polypeptide produces an increase in the capacitance of the membranes. The increase is gradual with time and parallels the decrease in resistance. Fig. 3*a* and *b* present the changes in the specific capacitance and in the specific resistance as functions of time. In the lowest salt concentration employed (10^{-3} N) at 440 Hz (the lowest bridge frequency employed) the system containing the bilayer still shows dielectric dispersion [7] so it cannot represent the zero frequency values, and therefore no capacitance values for this salt concentration are reported.

In the case of asymmetric films, the membrane potential (at zero applied potential and without salt gradient) was measured. No change of potential caused by adsorption of the peptide was found; the measured potential was smaller than 4 mV.

The values of specific resistance and capacitance of the unmodified and modified films are presented in Table 1.

Modification of the Properties of Bilayers with Other Basic Polypeptides

The influence of different polypeptides on the electrical properties of the bilayers was compared. All the experiments were done at pH 8.5. The block copolymer L-lysine/L-leucine at concentrations of 0.8 to 2.7 μg/ml have similar effects on the electrical resistance of the glyceryl monooleate-oleic acid films, as does the previously reported L-lysine/L-phenylalanine copolymer. But in this case it was more difficult to drain the films formed in the peptide-containing solution. No random copolymer of L-lysine/L-leucine was available, so the effects of copolymer could be compared only with a random copolymer of L-ornithine/L-leucine, which also had a higher molecular weight. It was found that in 10^{-2} N NaCl + 10^{-3} N Tris HCl at the polypeptide concentration of 0.8 to 1.6 μg/ml no stable asymmetric films were obtained. At 10^{-3} N NaCl + 10^{-3} N Tris HCl symmetric and asymmetric films (polypeptide adsorbed from one compartment only) were stable. At this salt concentration the two copolymers behaved similarly with respect to their ability to modify the electrical properties of the bilayers, so no effect of structure was detected.

Table 1. Effect of addition of L-lysine/L-phenylalanine copolymer on the resistance and specific capacitance of the bilayers

Salt concentration	Resistance (Ω cm^2)		No. of exps.	Resistance (Ω cm^2)	No. of exps.	Capacitance (μF/cm^2)	No. of exps.	Capacitance (μF/cm^2)	No. of exps.
	Initial	Final (asymmetric films)		Final (symmetric films)		(Unmodified films)		(Modified [a] films)	
10^{-3} N NaCl + 10^{-3} N Tris HCl	$3\times10^7-7\times10^7$	$9\times10^3-1\times10^5$	12	$9\times10^3-2\times10^5$	12	—	—	—	—
10^{-2} N NaCl + 10^{-3} N Tris HCl	$2\times10^7-7\times10^7$	$2\times10^4-2\times10^5$	10	$2\times10^4-2\times10^5$	29	0.385 ± 0.022	37	0.461 ± 0.034	23
3×10^{-2}N NaCl + 10^{-3}N Tris HCl	5.7×10^7	4×10^4	1	$1\times10^4-3\times10^4$	4	0.364 ± 0.030	6	0.455 ± 0.019	5
10^{-1} N NaCl + 10^{-3} N Tris HCl	$2\times10^7-5\times10^7$	$2\times10^4-3\times10^5$	3	$5\times10^3-2\times10^5$	14	0.397 ± 0.014	11	0.431 ± 0.016	14

[a] In capacitance results the values for symmetric and asymmetric bilayers are reported together.

Table 2. Effect of addition of the copolymer L-lysine/L-leucine or L-ornithine/L-leucine on the specific resistance of the bilayers

Copolymer lysine/leucine						Copolymer ornithine/leucine				
Salt concentration	Resistance (Ω cm^2)		No. of exps.	Resistance (Ω cm^2)	No. of exps.	Resistance (Ω cm^2)		No. of exps.	Resistance (Ω cm^2)	No. of exps.
	Initial	Final (asymmetric films)		Final (symmetric films)		Initial	Final (asymmetric films)		Final (symmetric films)	
10^{-3}N NaCl + 10^{-3}N Tris	2×10^7 to 7×10^7	5×10^4 to 1×10^5	5	1×10^5 to 2×10^5	4	2×10^7 & 7×10^7	1×10^4 & 4×10^4	2	2×10^4 & 2×10^5	2
10^{-2}N NaCl + 10^{-3}N Tris	2×10^7 to 7×10^7	2×10^5	2	8×10^4 & 5×10^5	2	2×10^7 to 7×10^7	—	—	2×10^4 & 4×10^5	2
10^{-1}N NaCl + 10^{-3}N Tris	2×10^7 to 7×10^7	3×10^5 & 6×10^5	2	1×10^5 to 2×10^5	3	—	—	—	—	—

The results obtained with the copolymers L-lysine/L-leucine and L-ornithine/L-leucine are presented in Table 2.

The effects produced by a copolymer L-lysine/L-serine and polylysine alone on the electrical properties of the bilayers were relatively small, but they did affect the stability of the films. The copolymer had a very small influence on the specific resistance of the glyceryl monooleate-oleic acid films (no capacitance was measured). When the copolymer lysine/serine or polylysine was added to the aqueous phase in the presence of the black film, the bilayer ruptured in 15 to 30 min. Films formed in solutions containing polypeptide were stable only up to 45 min. The concentrations of the polypeptides used were: 1 to 3.5 μg/ml (4×10^{-8} to 10^{-7} M for polylysine; 2×10^{-8} to 10^{-7} M for lysine/serine copolymer). No higher concentrations could be used because they caused immediate breakage of the films.

The polypeptides at the appropriate concentration were added either to the outer or inner compartment and both compartments were stirred. Without salt gradient no membrane potentials were found, even for asymmetric films.

The measured resistances in the presence of these two polypeptides and capacitances, measured only in the presence of polylysine were rather erratic. In most of the cases only very small increases in conductances and capacitances were observed but in some cases sudden decreases in resistances up to $10^5\ \Omega\,cm^2$ and increases of capacitances up to $0.430\ \mu F/cm^2$ were detected in the case of films generated from polylysine-containing solution.

Only preliminary experiments were performed with the copolymer L-lysine/L-tyrosine. It was found that this peptide causes a decrease in the electrical resistance of the glyceryl monooleate-oleic acid films by 2.5 orders of magnitude only at concentrations higher than 3.5 μg/ml ($\sim 7 \times 10^{-9}$ M) and only when the films are formed in the polypeptide-containing solution.

Discussion

The results presented in this paper show that interaction with basic polypeptides modifies the electrical properties of the bilayers. The presence of an electrical charge on the membranes is an important factor, because it influences the stability of the membranes interacting with the polypeptides. Bilayers formed from pure glyceryl monooleate became unstable after addition of the basic polypeptides. Only after addition of oleic acid to the film-forming solution and at high pH the bilayers became stable. The

stabilizing effect of the charges can be explained by their reduction of the solubility of the nonpolar residues in the polar region of the membrane by the electrical field formed which thus prevents fluctuations from the stable structure of the inner hydrophobic layer.

The importance of the presence of the negative charge in the interaction of proteins and polylysine with liposomes was stressed by several workers [11, 19]. Montal [13] found that polylysine changes the electrical properties of negatively charged bilayers only, Steinemann and Läuger [18] detected higher adsorption of cytochrome *c* on phosphatidyl inositol bilayers (negatively charged) than on those formed from zwitter-ionic phospholipid (dioleyl lecithin).

In this work the negative charge was introduced by adding oleic acid to the membrane-forming solution. The pK of the carboxylic acids in monolayers is about 7.5 [2] and is dependent on the surface concentration and ionic strength.

It is not known as yet how much of the oleic acid is incorporated into the bilayers, but an appreciable charge density is probably required for stabilization of the interacting bilayer as the films interacting with the polypeptides become stable only above pH 8, when the degree of ionization of oleic acid is quite high. The initial interaction is probably electrostatic because at this pH the polypeptides employed in this work are still positively charged (pK_0 of polylysine is 10.44) [10] but the hydrophobic interactions are also very important. The drop in resistance induced by the polypeptides is independent of salt concentration, but at higher ionic strengths the modified bilayers become less stable. In an attempt to evaluate the influence of hydrophobic interactions in modifying the properties of the films, the interaction with polylysine and its copolymers with amino acids of different degrees of hydrophobicity was studied. It was found that hydrophobic interactions play a very important role in modification of the electrical properties of the bilayers. Poly-L-lysine itself gave irreproducible and usually small changes in the specific resistance of the bilayers. These results differ from the data of Montal [13], who found a decrease in specific resistance by 10^3 upon interaction of cholesterol-dodecylphosphate bilayers with polylysine.

Introduction of neutral hydrophobic amino acids into the polymer enhances adherence to the bilayer despite the decrease in the positive charge density of the copolypeptide.

The most hydrophilic copolymer studied was the copolymer of L-lysine/L-serine. This copolymer did not influence the resistance of the films and caused only a small decrease in stability. On the other hand, the more

hydrophobic copolymers, lysine/phenylalanine, lysine/leucine or ornithine/leucine lowered the specific resistance by more than 1,000-fold and raised somewhat the specific capacitance (measured only in the case of the interaction with the copolymer lysine/phenylalanine) (Tables 1 and 2). It is possible that the polypeptides, while interacting with the bilayers, undergo conformational changes as described by Hammes and Schullery [6] who studied the complexes of phosphatidyl serine liposomes with polylysine stabilized by both electrostatic and hydrophobic forces. The conformational change of a polypeptide in a bilayer may be a slow process, which could explain the instability of the bilayer during rapid penetration of the polypeptide from a concentrated solution.

Giannoni, Padden and Roe [4] obtained lamellar complexes of lecithin and poly-L-tyrosine by precipitation from methanol water solution in spite of the fact that both components have no charge. They assumed that the poly-L-tyrosine may be partly layered on the membrane and may also partly penetrate it. Nevertheless, a similar picture may prevail during interaction of the polypeptides with bilayers. The detailed mechanism of the modifying action of the basic and to some extent hydrophobic copolypeptides is not clear. However, the importance of the hydrophobic groups indicates that they penetrate and facilitate penetration of some more hydrophylic residues into the hydrocarbon core, introducing regions of higher dielectric constant and higher ionic conductance.

In the presence of the copolymer lysine/phenylalanine, at a concentration of ~ 1 μg/ml, a low-resistance value is reached in about 40 min. It is difficult to estimate to what surface coverage by polypeptide this low resistance corresponds. If the increment in the conductance of the bilayer is through holes of specific conductance equal to that of the aqueous solution, the total required area of these holes would be only 10^{-7} of the total area of the bilayer. Assuming, on the other hand, diffusion-controlled adsorption of the polypeptide on the bilayer, 20 and 25% complete coverage of the surface by the polypeptide is reached within 40 min if the solution is still or stirred, respectively.

It is possible that the penetration is a slow process and only a small fraction of the adsorbed polypeptide penetrates the bilayer to cause modification of its electrical properties. By addition of more than 2 μg/ml copolymer lysine/phenylalanine the decrease in resistance can be reached in shorter time, but films become unstable. It seems that the abrupt accumulation of the material on the surface and penetration of the bilayer without conformational accommodation of the polypeptide chains, introduces instabilities [6]. The right accommodation is possibly achieved during slow

incorporation of the polypeptide into the bilayer. Supporting evidence for this hypothesis might be obtained from the time dependence of the optical properties of the incorporated polypeptides.

Some direct evidence for the penetration comes from the fact that there is no difference in electrical properties (at least with the more hydrophobic polypeptides) whether the polypeptide is added on one side of the film only or if the bilayers are formed in polypeptide-containing solution. No membrane potential of the type reported by Montal [13] was found in the case of asymmetric films. This is not surprising since for a reversible system containing electrodes without liquid junctions, thermodynamics does not allow for this kind of transmembrane potential.

The interaction of the bilayers with the lysine/phenylalanine copolymer causes an increase in the specific capacitance. The change in capacitance may be caused by two factors: an increase in dielectric constant of the core and an increase in the overall thickness of the membrane. The two factors have an opposite effect on the magnitude of the capacitance values. Cherry, Berger and Chapman [1] found an increase in thickness caused by adsorption of apoprotein on bilayers. It is likely that also in our case the interacting polypeptides increase the thickness of the membrane. The adsorbed polypeptide layer has a considerably higher dielectric constant than the hydrocarbon, but its contribution to the measured capacitance will be relatively small. A 13-Å thick adsorbed layer of a dielectric constant ($\varepsilon = 10$) would lower the specific capacitance by about 5%. Penetration of the bilayer may have a larger effect in the opposite direction. Assuming that the domains penetrated by the polypeptide have a dielectric constant ($\varepsilon = 10$), modification of only 1% of the membrane area by penetration would increase the capacitance by 5%.

Increasing ionic strength may influence the effect of the interacting polypeptide on the capacitance in three ways. (1) It lowers the electrostatic interaction. (2) Through the salting out effect it reduces water solubility of the hydrophobic groups and enhances their penetration into the membrane. (3) The same effect enhances interaction between the hydrophobic groups and thus may lower the dielectric constant of the adsorbed layers of polypeptides as well as of the penetrated membrane domains.

The second effect increases the overall capacitance while the third effect decreases it, which may explain the maximal capacitance observed at some intermediate ionic strengths (Fig. 4). A more profound analysis of the capacitance changes has to wait until more data at different frequencies are obtained.

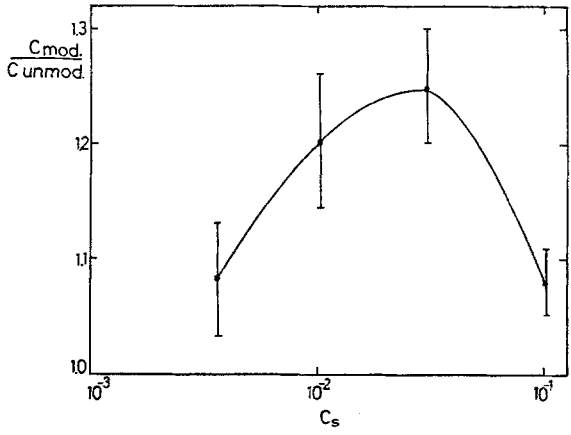

Fig. 4. The ratio of the capacities of modified and unmodified bilayer (C_{mod}/C_{unmod}) as a function of salt concentration C_s. The bars indicate the error

The Transmembrane Potential

The measured potential is composed of three terms, the polarization potentials of the reversible electrodes E_p, the diffusion potential E_D [21] and the electrical double-layer potential difference E_{dl}

$$E_{\text{measured}} = E_p + E_D + E_{dl} \tag{1}$$

$$E_p = \frac{2.3RT}{F} \log \frac{[Cl^-]_2}{[Cl^-]_1}, \tag{2}$$

$$E_D = \frac{U_{Na} - U_{Cl}}{U_{Na} + U_{Cl}} \frac{2.3RT}{F} \log \frac{U_{Na}[Na^+]_2 + U_{tr}[tr^+] + U_{Cl}[Cl^-]_2}{U_{Na}[Na^+]_1 + U_{tr}[tr^+] + U_{Cl}[Cl^-]_1}. \tag{3}$$

U are the apparent mobilities of ions across the membrane comprising the electrical double-layer dependent distribution coefficients between the aqueous solution and the membrane. $[tr^+]$ = concentration of Tris ion.

Since $U_{tr}[tr^+]$ is small as compared with $U_{Na}[Na^+]$ and $U_{Cl}[Cl^-]$, $(U_{Na}/U_{Na} + U_{Cl})$ can be identified with the cationic transference number t^+_{Na} given by

$$t^+_{Na} \simeq \frac{U_{Na}}{U_{Na} + U_{Cl}} \tag{4}$$

$t^+_{Na} + t^-_{Cl} \simeq 1$.

Unfortunately, we do not know the content of the oleic acid in the bilayer nor the surface charge density on the two sides of the membrane which is essential for calculation of the contribution of the electrical double layers to the potential. Independent determination of the electrical double-layer potential is being planned. In case of bulk measurements, the potential

is built only of the two terms since the double-layer potential is zero. Indeed, the transference numbers calculated from the measured potentials in the absence of the bilayers ($t_+ = 0.400 \pm 0.02$) under the assumption that activity coefficients = 1 coincide within experimental error with the literature values [8].

In the presence of a charged bilayer, the transmembrane potential contains a contribution to the electrical double layer which cannot always be readily evaluated. As will be shown further on, the contribution of the electrical double layer to the transmembrane potential can be calculated for the case of a membrane constituent ionized on the membrane surface. To demonstrate this we shall consider our unmodified bilayer, where the undissociated oleic acid on either surface of the membrane is in equilibrium. Since the standard chemical potentials for the uncharged oleic acid are the same on the two sides of the membrane, the surface concentrations are also equal $\Gamma_1 = \Gamma_2$. The dissociation of the carboxylic groups is controlled on either side of the membrane by equilibrium with the adjacent bulk solution

$$\mathrm{pH}_1 = \mathrm{pK} + \ln \frac{\Gamma_1^-}{\Gamma_1} + e\psi_1/kT, \tag{5}$$

$$\mathrm{pH}_2 = \mathrm{pK} + \ln \frac{\Gamma_2^-}{\Gamma_2} + e\psi_2/kT \tag{6}$$

where pK is that of the oleic acid in the bilayer, pH_1 and pH_2 are the pH's on both sides of the membrane, Γ is the surface concentration of unionized oleic acid and Γ_1^-, Γ_2^- are the surface concentrations of ionized oleic acid on both sides, ψ_1, ψ_2 are the respective double-layer potentials, namely the potential differences between the plane of charges and the interior of the solution on either side of the bilayer and e is the charge of an electron. As the pH on both sides of the membrane is the same and since $\Gamma_1 = \Gamma_2$, we obtain

$$eE_{dl} = e(\psi_1 - \psi_2) = kT \ln(\Gamma_2^-/\Gamma_1^-). \tag{7}$$

As we are not interested in the absolute values of the potentials but only in their difference E_{dl}, we need only one other relation between ψ and Γ^- and this is obtained from the Gouy-Chapman theory since

$$e\Gamma^- = \sigma \tag{8}$$

where σ is the surface charge density. For a uni-univalent salt [5] of concentration C_s

$$\sigma = BC_s^{1/2} \sinh \frac{e\psi}{2kT} \tag{9}$$

where

$$B=\left(\frac{2\varepsilon RT}{\pi}\right)^{1/2}. \tag{10}$$

Combining Eqs. (7), (8) and (9)

$$e(\psi_1-\psi_2)+kT\ln\frac{\sinh(e\psi_1/2kT)}{\sinh(e\psi_2/2kT)}=\frac{1}{2}kT\ln(C_s^{(2)}/C_s^{(1)}). \tag{11}$$

Let us take the case when $e\psi/2kT$ is large enough to be able to neglect exp-$(e\psi/2kT)$; then from Eq. (11)

$$E_{dl}=(\psi_1-\psi_2)=\frac{kT}{3e}\ln(C_2^{(2)}/C_s^{(1)}). \tag{12}$$

In the alternative case, when $e\psi/2kT\ll 1$ the $\sinh e\psi/2kT$ can be expanded and the first two terms being taken and then Eq. (11) assumes the following form

$$\frac{e(\psi_1-\psi_2)}{kT}+\ln\frac{\psi_1}{\psi_2}=\frac{1}{2}\ln\frac{C_s^{(2)}}{C_s^{(1)}}. \tag{13}$$

In the case of ionized oleic acid, the surface charge density is probably high enough enabling the use of Eq. (12) for calculating the difference in double-layer potentials.

Using Eq. (12), E_{dl} was calculated. The data are presented in Fig. 5. In Fig. 5 we show also the transmembrane potential of unmodified bilayer as a function of salt concentrations. Using Eqs. (1)–(4) and data from Fig. 5 the cationic transference number t^+ of the unmodified bilayer was calculated. As it is possible to see from the same figure, t^+ is between 0.545 and 0.479, being higher than the calculated bulk transference numbers. The decrease in t^+ with increase in salt concentration can come from overestimation of the double-layer potential difference at the higher salt concentrations. ψ decreases with increase in salt concentration and the approximations assumed in Eq. (12) may no longer be valid so that Eq. (11) has to be used for calculating the double-layer potential. We were unable to calculate the transference numbers of the modified bilayers, but the measured potentials were ~20% higher than those of bulk at the same salt concentration.

Even though MacDonald and Bangham [12] considered the effect of the electrical double layer on the transmembrane potential, the interrelation between the ionic strength in the adjacent bulk phase, the double-

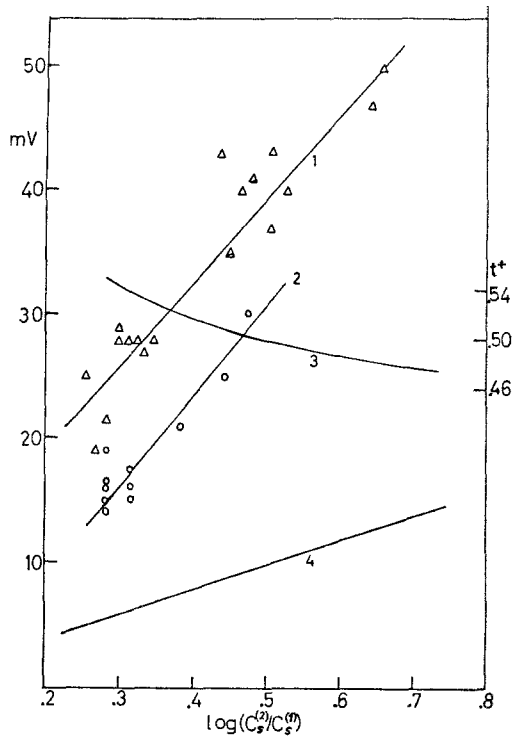

Fig. 5. (1) Transmembrane potential across unmodified bilayer. (2) Transmembrane potential across modified bilayer. (3) Cationic transference number t^+. (4) Double-layer potential difference, as a function of log salt concentration in the outer and inner compartments

layer potential and surface charge density were not considered in detail. We suggest that the interrelation between the structure of the membrane surface and the composition of the adjacent bulk phase is of general validity. Different compositions of bulk solutions on the two sides of the membrane can impose asymmetry on the membrane not only by forcing a different charge density on the two sides of the bilayer, but also by asymmetric accumulation of various functional groups through specific interactions with different bulk constituents on the two boundaries.

By the same reasoning, interaction with polypeptides modifies not only the final membrane surface but also the structure of the lipid bilayer at the boundary with the interacting layer of polypeptide. However, the interaction and penetration are too complicated a phenomenon to enable, at present, prediction of the final structure, surface charge density or surface potential of the final membrane complex.

The authors would like to thank Dr. R. Pagano for many helpful discussions during performance of this work and Dr. K Rosenheck for the gift of the polypeptides. One of the authors (D.B.) would like to thank Prof. T. E. Thompson, Dept. of Biochemistry, University of Virginia, Charlottesville for enabling her to study the bilayer techniques in his laboratory.

The work was supported by NIH Grant No. 06-034-1.

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