J. Membrane Biol. 14, 57–62 (1973)

Interaction of Bilayers with Basic Polypeptides

II. Interaction of Phospholipid Bilayers with Copolymer L-Lysine/L-Phenylalanine

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Received 21 May 1973

Summary. Interaction of bilayers formed from phosphatidyl serine (PS) or phosphatidyl inositol (PI) with the copolymer L-lysine/L-phenylalanine (1.4:1) was investigated. The copolymer causes a decrease in electrical resistance of the PS bilayers by more than three orders of magnitude, the decrease being largest at the highest salt concentration (10^{-1} N). It also introduces rectification to the bilayers. The modifying effect on PI bilayers of the copolymer is much smaller, and the stability of the films is affected markedly.

Interaction of lipid bilayers with basic polypeptides was studied extensively in this laboratory [1]. The films were formed from glycerylmonooleate (gmo) + oleic acid (ol). As these are synthetic lipids, nonexistent in biological membranes, it was interesting to study the interaction of the polypeptide with natural acidic phospholipids which differ in fatty acid composition and polar groups, in order to evaluate to what extent the interaction with the polybase is a general phenomenon.

For this purpose the interaction of phosphatidyl serine or phosphatidyl inositol bilayers with the copolymer L-lysine/L-phenylalanine was studied. The results obtained are presented in this paper.

Materials and Methods

Bovine phosphatidyl serine (Grade I) was purchased from Lipid Products (Nutfield, England) and its purity checked in the laboratory by thin-layer chromatography. Phosphatidylinositol (plant) was purchased from Supelco Inc., Bellefonte, Pa. The membranes were formed either from 0.5% phosphatidyl serine or 0.6% phosphatidyl inositol in *n*-decane. The aqueous solution was either NaCl (10^{-3} to 10^{-1} N) buffered with Tris HCl (10^{-3} N) to pH 7.2, or NaCl at pH 4.1 or pH 6 (unbuffered). The electrical set-up and the membrane formation technique were as described previously [1]. In all the cases the polypeptide was added to the outer stirred compartment.

Results

Modification of the Phosphatidyl Serine (PS) Bilayers

The phosphatidyl serine bilayers formed at pH 7.2 are very stable and their electrical resistance higher than $10^8\ \Omega\ cm^2$.

Addition of the copolymer L-lysine L-phenylalanine to one side of the bilayer causes a decrease in electrical resistance, and dielectric breakdown drops to about 40 mV. The decrease in resistance is a function of salt and copolymer concentrations. Fig. 1 shows the final values of the specific resistance of bilayers modified by addition of 1.5 μg/ml (5×10^{-8} M) copolymer lysine/phenylalanine, as a function of salt concentration. The bars denote

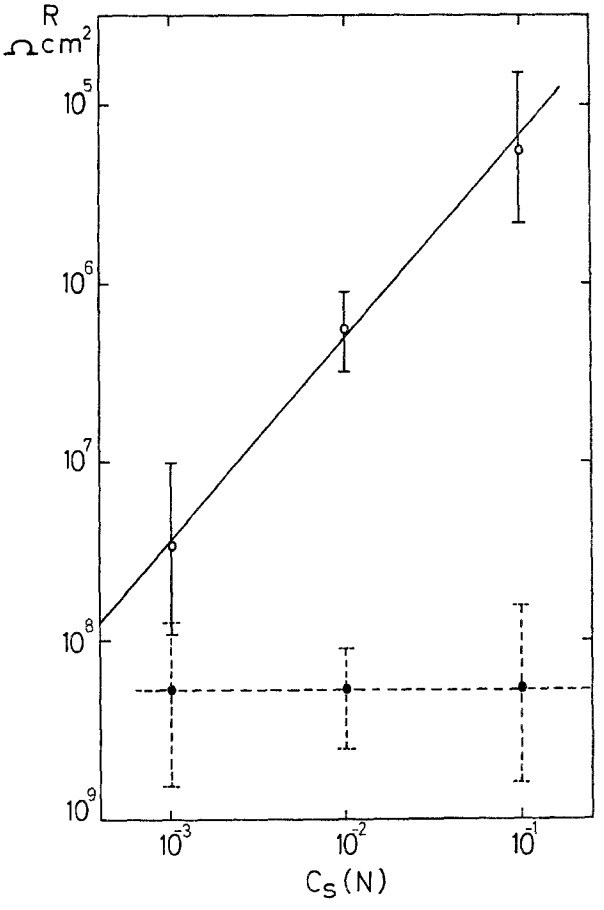

Fig. 1. The specific resistance — $R(\Omega\ cm^2)$ of phosphatidyl serine bilayers as a function of salt concentration. The full bars denote the range of the final resistance values of the modified bilayers. Broken bars indicate the specific resistance of unmodified bilayers. In addition to the salt concentration indicated in the Figure, 10^{-3} N Tris HCl, pH 7.2, was used in each experiment

Table 1. The specific capacitance of unmodified and modified bilayers

	Unmodified bilayers	No. exp.	Modified bilayers	No. exp.
10^{-1} N NaCl	0.342 ± 0.011 μF/cm²	28	0.364 ± 0.019 μF/cm²	8
10^{-2} N NaCl	0.350 ± 0.015 μF/cm²	19	0.362 ± 0.015 μF/cm²	6

the spread of the measured resistance values (5 to 7 experiments at each salt concentration employed). For comparison the broken bars give the specific resistance of unmodified bilayers. As can be seen from the Figure, the specific resistance of the modified films decreases with increase in salt concentration, while that of the unmodified films is practically independent of salt concentration.

The final resistance of the interacting bilayer is reached about half an hour after addition of 1.5 μg/ml polypeptide; the bilayers are stable at the low resistance value for about 5 min. Addition of lower concentrations of the copolymer produces a smaller lowering in resistance. The increase in resistance is paralleled by a small increase in capacitance (not larger than +10%). The results of the capacitance measurements are given in Table 1.

The asymmetric modification by the copolymer converts the bilayers into rectifying membranes as can be seen from the current-voltage curve shown in Fig. 2. The resistance is higher when the positive current flows from the polypeptide-containing compartment to the other compartment.

Contrary to the glycerylmonooleate + oleic acid films, it was impossible to obtain black (PS) films from the polypeptide-containing solution. In 10^{-1} N NaCl, the colored films remained stable for several hours, did not drain, but had very low resistance.

The influence of pH on the interaction was also investigated. In 10^{-1} N NaCl + HCl, pH 4.1, the decrease in resistance due to addition of the copolymer was much smaller; the final value was about 10^7 Ω cm², but the bilayers ruptured after shorter times than at pH 7.2.

Modification of the Phosphatidyl Inositol (PI) Bilayers

Preliminary experiments on the modification of PI bilayers by the copolymer were performed. The unmodified bilayers of phosphatidyl inositol have very high resistance (higher than 5×10^8 Ω cm²). Addition of as little as 0.1 μg/ml of the copolymer at pH 6 (unbuffered solution) or at pH 7.2 (Tris buffer) causes rupture of the film within 5 min after addition. When

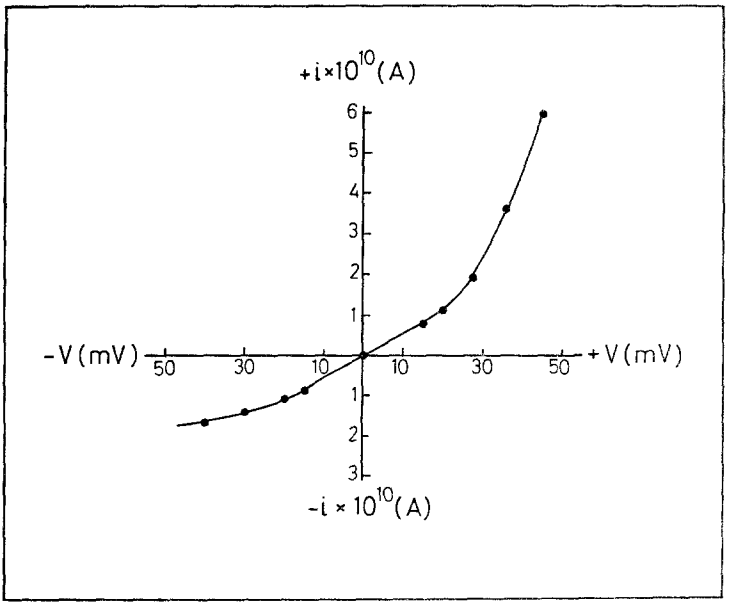

Fig. 2. The current-voltage relation for the phosphatidyl serine bilayer modified by addition of 1.5 μg/ml copolymer to one side of the film. Aqueous phase: NaCl 10^{-2} N, Tris HCl 10^{-3} N, pH 7.2; Film area: 7.6×10^{-3} cm^2

bilayers were stable for about half an hour, the resistance decreased ~1.5 to 2 orders of magnitude.

Contrary to phosphatidyl serine bilayers, black films could be generated in the copolymer-containing solution. The initial resistance of the films was lower than of the unmodified ones and the lowest value reached was 10^5 Ω cm^2. The bilayers remained alive for about 40 min. The experiments were performed in 10^{-2} or 10^{-3} N salt solution; no differences were observed within this range of salt concentrations.

Discussion

The results presented in this communication show the differences in the bahavior of bilayers formed from different lipids with respect to modification of electrical properties by a basic polypeptide.

As reported in the previous paper [1], addition of the copolymer L-lysine/L-phenylalanine to (gmo + ol) bilayers causes a decrease in resistance by about 2.5 orders of magnitude, independent of salt concentration, and a salt-dependent increase in capacitance (20%) with maximum at 3×10^{-2} N salt.

In the (gmo + ol) bilayers the negative charge was introduced by addition of oleic acid to the film-forming solution. As found from determination of

bilayer composition[1], there is about one negative charge for 200 to 250 Å^2 at pH 8.5. The charge density was much lower than in the case of phosphatidyl serine bilayers (about one negative charge/50 Å^2 [4] at pH 7). This difference in charge density probably affects the modifying properties of the copolymer, since the negative charge is a prerequisite for obtaining stable interacting films [1]. The salt concentration influences the degree of penetration of the polypeptide into the bilayer and the electrical interactions having opposite effects on both factors. The degree of penetration and the hydrophobic interactions increase with increase in salt concentration due to stronger adsorption and lower solubility of the polypeptide in water at higher salt concentration. Enhanced penetration favors pore formation. At higher salt concentrations these pores probably will be more conducting. All these effects are reflected by the decrease in resistance with increase in salt concentration. However, to explain the influence of salt concentration on the decrease in resistance, another possibility should also be taken into account. The linear relation between the conductance of the modified films and the salt concentration can be explained by the fact that even at the lowest salt concentration (10^{-3} N) pores completely filled with water are formed in the film, and the conductivity of these modified regions increases linearly with increase in salt concentration.

From the experimental data it is difficult to conclude which explanation for the decrease in resistance is the correct one.

The capacitance values for the unmodified phosphatidyl serine bilayers as obtained by us are lower than those reported by Ohki [2]. Addition of the copolymer causes very small increase in capacitance. As discussed previously [1], the change in capacitance is mainly due to two opposing factors. Increase in thickness due to adsorption of the polypeptide will probably cause a small decrease in capacitance as the adsorbed layer has a higher dielectric constant than the hydrocarbon core. On the other hand, the penetration of the polypeptide into the bilayer will increase the capacitance by introducing regions of higher dielectric constant. In the case of phosphatidyl serine films the penetration is probably much lower due to higher cohesiveness of the films as compared to those of (gmo + ol); the number of modified regions is low and this brings about a very small increase in capacitance in spite of the decrease in resistance by 10^3.

Phosphatidyl serine bilayers modified by the copolymer show rectification. This same effect was found by Ohki [3] after addition of Ca^{++} or protamine to one side of phosphatidyl serine bilayers.

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1 I. R. Miller and D. Bach. (*In preparation*)

This rectification is due to the difference in sign of the charge on both sides of the membrane. The unmodified bilayer is negatively charged. Addition of the copolymer to one side of the bilayer neutralizes the negative charges and introduces positive ones. Hence, the transference numbers of the respective ions on both sides of the bilayer become unequal, and the rectifying properties are introduced to the membrane.

This paper shows also the importance of the charge and the polar group on the interaction. The interaction of the polybase with the negative charge of the film causes stabilization of the bilayer. In the case of phosphatidyl serine bilayers at pH 4 (lower charge density) the stability is decreased as it is also in the case of phosphatidyl inositol bilayers where the bulky sugar probably interferes with the interaction.

I would like to thank Prof. I. R. Miller for the many helpful discussions. This research was supported by N.I.H. Grant No. 06-034-1.

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