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THE INTERACTION OF POLYAMINO ACIDS WITH LIPID MONOLAYERS

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SUMMARY

Polyamino acids were utilized to study the role of specific amino acid side chains in polypeptide binding to lipid monolayers. Surface pressure changes and binding of labeled polyamino acids to lipid films were studied for combinations of 16 different polyamino acids and 11 different lipid films. The effects of helicalization, optical isomerism and charge interaction were investigated. Evidence is provided which suggests that helicalization does not contribute to surface pressure changes. The D and L optical isomers of polylysine were found to have differential interaction with phosphatidylserine films, leading to the conclusion that polylysine is bound with its optically active carbon juxtaposed to the optically active carbon of phosphatidylserine. In general, surface pressure changes occurred only when basic polyamino acids interacted with negatively charged lipid films. However, significant binding of labeled polylysine to lipid films with no net charge could be measured in the absence of an accompanying surface pressure change. It was concluded that polylysine could bind to phosphatidylcholine films by intertwining among the phosphorylcholine groups without distorting overall film properties. This finding agrees with previous work on lipid multilayers, and has significance in regard to lipid–protein interactions in natural membranes.

INTRODUCTION

The complexity of lipid-protein bonding in natural membrane structures has prompted many efforts toward elucidation of its nature by the investigation of simpler model systems. A lipid monolayer oriented at an air–water interface is a well defined lipid model. Interaction of protein with a lipid monolayer increases the surface pressure of the monolayer if all or part of the protein molecule penetrates the lipid film [1–5]. If binding takes place without penetration (adsorption), no surface pressure change is observed [3]. Penetration and adsorption can have both electrostatic and hydrophobic components, and it is likely that a number of amino acids in a complex native protein act in concert to form multiple [3–6] bonds with the lipid monolayer. Such simultaneous interaction is likely to obscure determination of individual contributions of different amino acids. In the work reported here, this problem was avoided by utilizing polyamino acids for the protein component.

Polylysine is a commonly studied polyamino acid because of its high solubility in water. Demel et al. [7] added polylysine to subsolutions of phosphatidylserine and cerebrocide sulphate monolayers, and found that the surface pressure decreased slightly, then slowly increased to the initial value. They concluded that no penetration occurred, and that polylysine was electrostatically adsorbed. Hammes and Schullery [8] used several methods to investigate the interaction between sonicated aqueous dispersions of phosphatidylserine and poly-L-lysine. They found that poly-L-lysine upon binding underwent a conformational change from random coil to helix. Poly-L-lysine was also shown to penetrate monolayers of stearic acid and cause an increase in surface pressure [9, 10]. Hammermeister and Barnett [11] concluded that this interaction involved interrelated electrostatic and penetration effects.

The present work investigates factors governing the interaction of different polyamino acids with specific lipid monolayers. While this model system is only distantly related to lipid–protein organization in biomembranes, it does offer a convenient method for characterizing interactions occurring between lipid and protein components in biological structures. Together with a general overview of polypeptide-lipid monolayer interactions an attempt is made to answer several specific questions: (1) Do the structural differences and resulting physical properties of the lipids in the monolayer have an effect on interaction? (2) What is the effect of optical isomerism of amino acid residues on their ability to interact with lipid monolayers? (3) Is the coil-to-helix transition observable as a surface pressure change upon interaction of poly-L-lysine with phosphatidylserine monolayers?

MATERIALS AND METHODS

Polyamine acids

Homopolymers of L-lysine (4000, 30 000 and 150 000 molecular weight), L-arginine, L-serine, L-aspartic acid, L-methionine, L-tyrosine, L-valine, L-tryptophan, L-phenylalanine, L-leucine, L-alanine, glycine, L-histidine and L-ornithine were obtained from Sigma (Saint Louis, Mo.). Poly-D-lysine and poly-L-[^{14}C]lysine (specific activity 0.293 mCi/g) were obtained from New England Nuclear (Boston, Mass.). Poly-L-glutamic acid was obtained from Pilot (Santa Fe Springs, Calif.), and poly-D,L-lysine was obtained from Miles (Elkhart, Ind.). All were used without further purification; repeated dialysis against subphase solution did not affect the results.

Lipids

Distearoyl-L-α-phosphatidylcholine, dipalmitoyl-L-α-phosphatidylcholine, dioleolyl-L-α-phosphatidylcholine, cardiolipin (bovine), phosphatidylserine (bovine), phosphatidylinositol (plant), and sphingomyelin (bovine) were obtained from Supelco (Bellefonte, Pa.). The dicetyl phosphate was obtained from Sigma, dipalmitoylphosphatidylethanolamine from Sedary (London), and octadecyl phosphate and stearic acid were donated by Dr David Deamer. All lipids were used without further purification.

Determination of interaction

Films of the lipids were cast from 2.5 mg/ml chloroform solutions on an 80 ml subphase contained in a 9 cm × 12 cm rectangular trough milled from a block of Teflon fixed to a stainless steel base. A pair of motor-driven barriers, pressed by

springs against the rim of the trough, extended into the subphase and formed a seal at the sides of the trough. The barriers were initially set 7 cm apart giving a monolayer area of 63 cm^2.

The subphase solution employed was 0.1 M NaCl adjusted to pH 7 with 0.1 M NaOH and 0.1 M HCl prepared from reagent grade materials. The subsolution was found to vary by less than $\pm$0.25 pH unit during the experiments and was, therefore, used without buffer. Doubly distilled water was used for all solutions. Addition of 1 ml 0.1 M EDTA to the subsolution was found to have no effect on the results, and its use was discontinued.

The surface pressure was continuously recorded using a 2-cm periphery platinum Wilhelmy plate suspended from the beam of a Cahn RG electrobalance. A chart recorder calibrated to read 5 dynes full scale allowed continuous monitoring of the surface pressure.

A 1-ml Hamilton syringe was loaded with 1 mg of polyamino acid either dissolved or sonicated into 0.5 ml of subphase solution. The syringe was then placed in a remotely operated mechanical device for depressing the plunger. Its needle rested in the subphase of the trough. A second 1-ml syringe was set in the device to withdraw subsolution at a rate equal to that of the addition of the polyamino acid solution.

The trough and balance were placed inside an incubator set to control at 35 °C. After 30 min of temperature equilibration, the polyamino acid solution was added to the subsolution by remote actuation of the delivery device. Addition of the polyamino acid sample took 59 s. Stirring was accomplished with a Teflon-coated magnetic bar driven by a motor mounted magnet below the trough. Interaction was allowed to take place for 10 min when surface pressure change was being observed and 30 min when the monolayer was isolated for radioassay, as described below. After completion of each run, the trough was removed from the incubator and cleaned with Alconox. It was then rinsed for 5 min with tap water and finally rinsed with doubly distilled water.

When the interaction of lipid monolayers with a radioactive polyamino acid was measured, a small area of the monolayer was moved along a channel onto a subphase free of polyamino acid. The barriers were moved to maintain constant surface pressure. Since the initially rapid movement of the monolayer may have caused a local loss of surface pressure, the sample collection area was cleaned by drawing a clean glass slide cover through the surface allowing fresh monolayer to enter. The channel was closed, and all but the top 0.5 ml of the subphase in the collection area was drained from the bottom. 10 ml of Aquasol (New England Nuclear) scintillation counting fluor were added to the top 0.5 ml containing the sample monolayer. A background sample was prepared using 0.5 ml of the bottom subphase. Counting was carried out on a Beckman CPM-100 liquid scintillation system for 10-min periods. Selected samples were internally standardized with [^{14}C]toluene of known activity, and counting was repeated as before.

RESULTS

The affinity of polyamino acids for different lipids was investigated by observing their interaction with lipid monolayers on a 0.1 M NaCl (pH 7) subphase at 35 °C. Table I presents the positive and negative surface pressure changes recorded in a 10-

TABLE I

SURFACE PRESSURE CHANGES IN dynes/cm OBSERVED IN A 10-min PERIOD FOLLOWING THE INJECTION OF 1 mg OF EACH POLYAMINO ACID INTO THE 0.1 M NaCl (pH 7) SUBPHASE BELOW EACH LIPID MONOLAYER AT AN INITIAL SURFACE PRESSURE OF 5 TO 9 dynes/cm

The temperature of the 80-ml subphase was 35 °C, and the area of the monolayer remained constant. The known molecular weights ($\times 10^{-3}$) of the polyamino acids are: ornithine, 90; lysine, 4, 30, and 150; arginine, 65; histidine, 6; asparagine, 22; glutamic acid, 80; leucine, 50; glycine, 10; alanine, 25; tyrosine, 78; asparagine, 5; methionine, 40; and serine, 7. Surface pressure change was not affected by the molecular weight of poly-L-lysine. In cases where the surface pressure change reversed direction during the observation, the total change for each direction is listed in descending order of occurrence. Within the initial surface pressure range, the results are within 25 % variation.

Lipid	Polyamino acid															
	Basic [12, 13]				Acidic [13]		Aliphatic		Aromatic							
	Ornithine	Lysine	Arginine	Histidine	Aspartic acid	Glutamic acid	Valine	Leucine	Glycine	Alanine	Tryptophan	Phenylalanine	Tyronine	Asparagine	Methionine	Serine
No net charge																
Dipalmitoyl-L-α-phosphatidylcholine	★	★	0.6	★	0.6	★	★	★	★	★	★	★	★	★	★	★
Distearoyl-L-α-phosphatidylcholine	★	★	★	★	★	★	★	★	★	★	★	★	★	★	★	★
Dioleoyl-L-α-phosphatidylcholine	★	0.8	1.2	★	★	★	★	★	★	★	★	★	★	★	★	★
Sphingomyelin (bovine)	★	★	0.8	★	★	★	★	★	★	★	★	★	3.2	★	★	★
Dipalmitoylphosphatidylethanolamine	0.5	★	0.9	★	★	★	★	★	★	★	★	★	★	★	★	0.6
Negatively charged [14–17]																
Phosphatidylserine (bovine)	−3.9 0.9	−2.8 2.3	−3.1 3.1	1.3	★	−1.2	−1.1	−0.7	★	★	★	★	1.1	★	★	0.6
Cardiolipin (bovine)	−2.3	−3.3 1.0	−2.9	−0.6	★	★	★	★	★	★	★	★	0.5	★	★	★
Phosphatidylinositol (plant)	−3.4 1.1	−3.0 2.1	−1.6 2.2	2.3	★	★	★	−0.7	★	★	★	★	0.8	★	★	★
Dicetyl phosphate	−1.6 1.0	−2.6 1.5	6.5	2.3	0.7	★	★	−0.6	★	★	★	★	1.5	0.7	★	★
Stearic acid	−1.4 5.0	3.4	18.3	3.5	1.1	★	★	★	★	★	★	★	1.5	★	★	0.8
Octadecyl phosphate	−1.8 23.9	15.8 −1.1	27.2 −0.8	9.2	1.7	−1.1	★	−0.5	★	★	★	★	1.5	1.0	★	1.1

★ Surface pressure changes less than ±0.5 dyne/cm were omitted.

min experimental period. In all cases 1 mg of polyamino acid was used, and the monolayer was maintained at constant area. The initial surface pressure was 7 ± 2 dynes/cm. Surface pressure changes of less than 0.5 dyne/cm were generally not repeatable and are omitted from the table. In some cases the surface pressure change reversed direction during the experimental period; such results are presented in descending order.

The data of Table I show that surface pressure changes take place when positively charged polyamino acids interact with negatively charged lipids. Aliphatic polyamino acids interacting with films having no net charge do not produce significant surface pressure changes. Consistent but small surface pressure changes are apparent for combinations of the negatively charged lipids and poly-L-tyrosine which is capable of forming side chains hydrogen bonds with the polar groups of the lipids. With the exception of the sphingomyelin–poly-L-tyrosine combination, all other combinations with lipids having no net charge caused little or no change in surface pressure. On repetition, this experiment gave results that varied by less than 5 %.

Three different categories of surface pressure change were observed (see Fig. 1). The uncharged lipids produced positive curves with a diminishing rate of change; similar curves were obtained for combinations of poly-L-serine, poly-L-tyrosine, and poly-L-asparagine with negatively charged lipids. Simple negative curves of decreasing

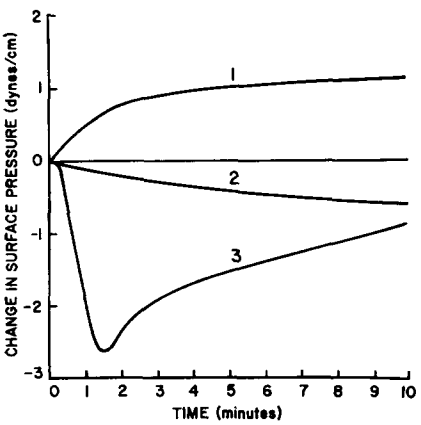

Fig. 1. Representative types of surface pressure change presented in Table I. (1) Poly-L-arginine and dioleoyl-L-α-phosphatidylcholine, (2) poly-L-leucine and dicetyl phosphate, (3) poly-L-lysine and phosphatidylserine.

rate of change were obtained with aliphatic polyamino acids. Where the lipids and polyamino acids were of opposite charge, the change in surface pressure was often found to reverse its direction during the observation period. The interaction of poly-L-lysine and phosphatidylserine gave an initial decrease in surface pressure, then a slow increase to approximately the initial surface pressure, corroborating the results of Demel et al. [7]. This type of change is of particular interest since studies of the poly-L-lysine–phosphatidylserine and poly-L-ornithine–phosphatidylserine interaction in bulk solution indicated conformational changes in the poly-L-lysine and poly-L-ornithine [8]. Both show initial negative surface pressure change followed by a positive increment as indicated in Table I.

To further evaluate whether helicalization of the polyamino acid contributed to the observed reversals in surface pressure change, interaction of phosphatidylserine monolayers with poly-L-lysine, poly-D-lysine and poly-D,L-lysine were compared. Poly-L-lysine and poly-D-lysine form helices with opposite screw sense, while the random copolymer poly-D,L-lysine is to a large extent prevented from forming helices. In Fig. 2 it is clear that surface pressure changes resulting from the subphase injection of 1 mg of each of these polyamino acids are the same for the initial negative change and only slightly different in the region of positive change. Since the results for poly-L-lysine and poly-D,L-lysine are similar, it is unlikely that helicalization is responsible for the observed surface pressure change.

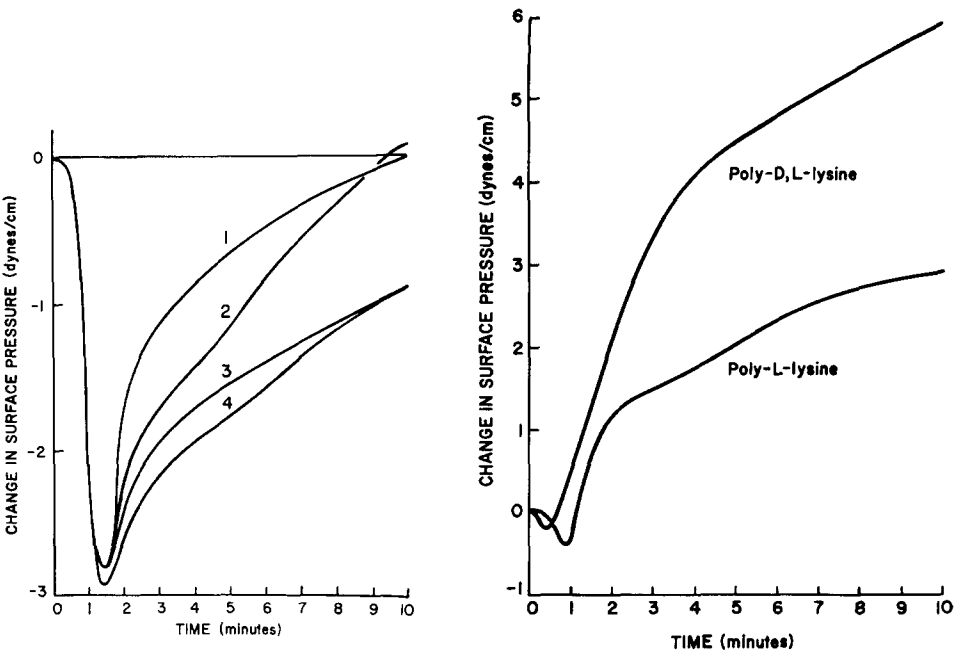

Fig. 2. Change in surface pressure following injection of 1 mg of polyamino acid into the subphase below monolayers of phosphatidylserine at initial surface pressures of 7 dynes/cm. The 0.1 M NaCl (pH 7) subphase was maintained at 35 °C. Where poly-D-lysine and poly-L-lysine were injected together, 0.5 mg of each were used. (1) Poly-D-lysine, (2) poly-L-lysine and poly-D-lysine, (3) poly-L-lysine, (4) poly-D,L-lysine. The molecular weight of poly-L-lysine was 150 000, that of poly-D-lysine was 120 000, and that of poly-D,L-lysine was 20 000.

Fig. 3. Change in surface pressure following injection of 1 mg of poly-L-lysine and poly-D,L-lysine into the subphase below monolayers of stearic acid. The conditions were the same as in Fig. 2. The molecular weight of poly-L-lysine was 30 000 and that of poly-D,L-lysine was 20 000.

In Fig. 3 the surface pressure changes observed following interaction of poly-L-lysine and poly-D,L-lysine with monolayers of stearic acid are compared. Poly-D,L-lysine, with its impaired helix-forming capacity, produced a larger elevation in the surface pressure. It is possible that once sufficient positive charges of the poly-L-lysine are cancelled by binding to negative carboxyl groups of the stearic acid, a transition

from random coil to helix occurs. The resulting bulky helical structure would be less capable than the non-helical structure of poly-D,L-lysine of entering the monolayer and causing a change in surface pressure.

Further inquiry into the relationship between binding of poly-L-lysine and the change in surface pressure was carried out using poly-L-[^{14}C]lysine under the same conditions as used for the work presented in Table I. A 30-min experimental period was used to insure complete interaction. Following interaction the monolayer was moved across a clean subphase to remove weakly associated polylysine. It was collected on a clean subsolution of known area, and any poly-L-[^{14}C] lysine remaining with the sample monolayer was considered to be bound to it.

The results obtained for the charged lipids are presented in Fig. 4. The corrected count of ^{14}C disintegrations has been converted to residues of poly-L-[^{14}C] lysine bound per cm^2 of monolayer sample. The results indicate an optimum binding at low initial surface pressures for stearic acid, phosphatidylinositol, octadecyl phosphate and dicetyl phosphate. Phosphatidylserine, on the other hand, shows no optimum but an increase in binding with an increase in initial surface pressure.

If the bound polylysine is assumed to be in the fully extended conformation at the interface, the residue area can be approximated by 35 Å^2. The areas represented by the amounts of poly-L-[^{14}C]lysine bound would range, at moderate surface pres-

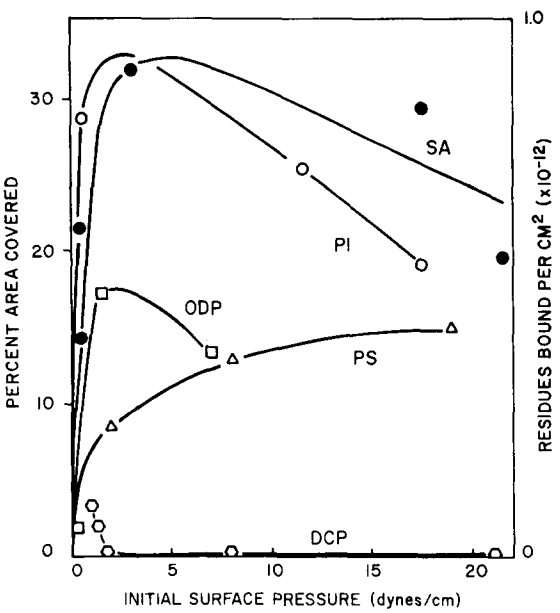

Fig. 4. Effect of initial surface pressure on the recovery of poly-L-[^{14}C]lysine with 8.1-cm^2 samples of charged lipid monolayers with which it has been allowed to interact for 30 min. In each case the temperature was 35 °C and 1 mg of poly-L-lysine was added to the subphase. Percent area covered represents the percent of the 8.1-cm^2 interfacial area covered by the bound poly-L-lysine. It has been calculated from the number of residues bound, assuming the poly-L-lysine to be fully extended to 35 Å^2 per residue at the interface. The molecular weight of the poly-L-[^{14}C]lysine was about 95 000 and its specific activity was 0.293 mCi/g. SA, stearic acid; PI, phosphatidyl inositol; ODP, octadecyl phosphate; PS, phosphatidylserine; and DCP, dicetyl phosphate.

sures, from near zero for dicetyl phosphate to over 30 % for stearic acid and phosphatidylinositol. The negligible values obtained for dicetyl phosphate serve to indicate that the poly-L-[^{14}C]lysine is not carried along with subphase solution onto the collection area.

Fig. 5 presents the comparative results for lipids with no net charge. The amounts of poly-L-[^{14}C]lysine bound by the uncharged lipids are generally less than those for charged lipids, but are surprisingly large when one considers the lack of surface pressure change during interaction. Most of the uncharged lipids have an optimum initial surface pressure for binding polylysine. Distearoylphosphatidylcholine has a higher optimum initial surface pressure than dioleolylphosphatidylcholine. It appears that dipalmitoylphosphatidylcholine might have an optimum at a much higher initial surface pressure. This would indicate that both saturation and shorter chain length have the same effect on the binding of poly-L-[^{14}C]lysine. There is no apparent correlation of the amount of poly-L-[^{14}C]lysine bound and the packing densities of the lipids in the monolayer [14, 18].

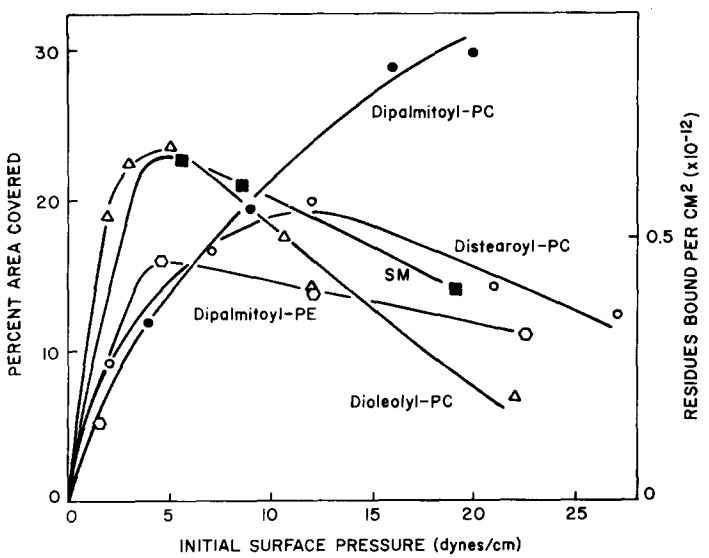

Fig. 5. Effect of initial surface pressure on the recovery of poly-L-[^{14}C]lysine with 8.1-cm^2 samples of uncharged lipid monolayers with which it has been allowed to interact for 30 min. The conditions were the same as in Fig. 4. PC, phosphatidylcholine; PE, phosphatidylethanolamine; and SM, sphingomyelin.

DISCUSSION

In most studies of lipid-protein bonding, the contributions of individual lipids of the natural mixture or individual amino acids of a protein cannot be readily distinguished. In this study, simplification has been achieved by using monolayers of pure lipids to represent the lipids in natural structures and polyamino acids to represent the amino acids in proteins. The employment of monolayers of pure lipid may well represent lipids in natural structures which have concentrated by lateral [19, 20]

and ion-induced [21, 22] phase separation. The polyamino acids may also represent proteins with high percentages of one amino acid [23–26], long sequences of a single amino acid [27] or large numbers of one amino acid juxtaposed in their tertiary structures [28].

Surface pressure changes observed in the present work arise from what appears to be the electrostatic binding of the argenyl, lysyl, ornithyl and histidyl side chains, and the hydrogen bonding of the tyrosyl side chains to monolayers of negatively charged lipids. This confirms the conclusions of Zwaal and van Deenen [29] who studied the interaction of positively charged red blood cell membrane proteins with sonicates of negatively charged lipids. They indicated that the initial interaction probably involved ion-pairing or hydrogen bridges between the phosphate groups of the phospholipids and the positively charged amino acids of the protein.

Poly-L-lysine becomes increasingly helical with charge cancellation above about 50 % [30]. Hammes and Schullery [8] have suggested that the helicity of poly-L-lysine when complexed with phosphatidylserine may exceed 85 %. Such conformational change could be responsible for the disruption of phosphatidylserine structure as seen by electron microscopy [31] and transmembrane leakage of glucose [31] or $^{22}Na^{+}$ [32, 33]. Structure disruption by poly-L-lysine was found to be a surface charge-dependent phenomenon not observed for the neutral phosphatidylcholine [34]. However, the nearly identical results for the interaction of poly-L-lysine and poly-D,L-lysine with phosphatidylserine exclude helicalization as the cause of negative or positive surface pressure change or the reversal of surface pressure change.

Electrostatic binding has frequently been reported to be coupled with hydrophobic effects in lipid–protein bonding [8, 29, 32, 33, 35–40] with some indications that the electrostatic interaction precedes the hydrophobic interaction [3, 41]. The presence of salt in high concentration was previously found to prevent the binding of poly-L-lysine to phosphatidylcholine sonicates but was found not to disrupt the bond once it was formed [42]. This indicates the likelihood of the hydrophobic region of the lysyl side chain* participating in the formation of lipid–protein bonds and, as well, its involvement in the subsequent hydrophobic bonding.

The use of poly-L-lysine and poly-D,L-lysine binding with phosphatidylserine monolayers as a test for the contribution of helicalization to the surface pressure change gives results which are compatible with the explanation that the initial negative surface pressure change corresponds to closer packing of the lipids in the monolayer as their mutually repelling negative charges are cancelled by the binding of positively charged polyamino acid side chains. The subsequent positive changes in surface pressure could correspond to the entry of the more hydrophobic charge-cancelled helix or coil polylysine into the lipid monolayer.

Repetition of this test using stearic acid resulted in a larger positive surface pressure change on addition of poly-D,L-lysine. This result suggests that the helical conformation may, in some cases, impede penetration.

Natural phosphatidylserine, which is the L-isomer [44], shows a differing surface pressure change when binding to poly-L-lysine and poly-D-lysine. Such discrimination, if it is the result of the isomerism, is most likely to occur if the isomeric carbon atoms of the phospholipid and polyamino acid molecules are localized in close proximity

* The hydrophobicity of the lysyl side chain is 1.50 kcal/mole [43].

to each other. These results suggest that the binding of the polypeptide chains takes place in the polar group region of the phosphatidylserine monolayer.

This conclusion is further verified by the results of Hammes and Schullery [8] who found that the presence of cholesterol in sonicated dispersions of phosphatidylserine had no effect on the binding of poly-L-lysine. They suggested that binding of poly-L-lysine occurs only in the headgroup region of phosphatidylserine.

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