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LIPID-POLYMER INTERACTION IN MONOLAYERS: EFFECT OF CONFORMATION OF POLY-L-LYSINE ON STEARIC ACID MONOLAYERS

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ABSTRACT

Surface pressures and surface potentials of stearic acid monolayers were measured at various pH values in the presence and absence of poly-L-lysine in the subsolution. The presence of poly-L-lysine strikingly influences the state of stearic acid monolayers. Surface potential measurements indicated that the maximum interaction between poly-L-lysine and stearic acid monolayers occurred between pH 10 and 11. Poly-L-lysine solutions exhibited surface activity in the same pH range. Air bubbles covered with poly-L-lysine films showed maximum stability at pH 11. These results indicate that in the pH range 10-11, where coil-to-helix transition occurs in solution, poly-L-lysine has partial or complete helical conformation which causes the slowest rate of drainage of water in bubble lamellae, and which also exhibits surface activity and hence, increases the interaction of poly-L-lysine with stearic acid monolayers. The implications of these findings for lipid-protein associations in biomembranes are discussed.

INTRODUCTION

Lipid-protein interactions are of great interest to understand the structure and function of biological membranes. Various approaches have been taken to elucidate the interaction of lipids and proteins in biological membranes. In recent years, nuclear magnetic resonance and electron spin resonance spectroscopy have been used profitably to investigate these interactions (1-4). Phospholipid bilayers and monolayers have served as useful models for these studies (5-6). The monolayer

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approach has been found very useful to understand molecular mechanisms presumably occurring at the cell surface (7-10).

Earlier studies on lipid-protein interaction in monolayers were reported by Schulman and his co-workers (11-12) who investigated the interaction of albumin, globulin and haemoglobin with cholesterol, cephalin, cardiolipin, alkyl sulfate and alkyl trimethylammonium monolayers. Eley and Hedge (13,14) studied protein-protein and protein-lipid interactions in fibrinogen, thrombin, albumin, lecithin and cephalin monolayers. The interaction of synthetic dihydroceramide lactoside monolayers with globulin, albumin, and ribonuclease was investigated by Colacicco, Rapport and Shapiro (15). These workers (16) have also shown from their studies on the interaction of apoprotein with various lipid monolayers, that the unusual surface activity of apoprotein may be intimately related to the mechanism of formation of the lipo-protein. Recently, Arnold and Pak (17) have investigated protein-protein interaction in monolayers.

To investigate the interaction of water with films, Trapeznikov (18) and Garrett (19) have studied the stability of bubbles covered with a monolayer of surface-active materials. In general, the stability of such bubbles is related to the rate of drainage of water in the bubble lamellae. The interaction of polar groups with water (i.e. hydration of polar groups) impedes the drainage of water in the lamellae, and, hence, increases the time required to reach a critical thickness where bubble lamellae break. Therefore, more strongly hydrated molecules increase the bubble stability. This method was used in the present study to investigate the hydration of stearic acid and poly-L-lysine films.

It has been recognized that both ionic and hydrophobic interactions play a role in the lipid-protein association. A simple model system of stearic acid and poly-L-lysine was selected to investigate various aspects of the ionic interaction in the present studies, since the ionic properties of stearic acid monolayers (20,23) and of poly-L-lysine solutions (24) have been established. The objective of the present studies was to investigate how the ionization of carboxyl groups in the monolayer and the conformation of poly-L-lysine in the subsolution influence interactions at the interface.

EXPERIMENTAL

Materials: Poly-L-lysine hydrochloride (molecular weight 100,000 - 200,000) was bought from Mann Research Laboratories Inc. (New York, N.Y. 10006). Highly purified (>99%) stearic acid was purchased from Applied Science Laboratories, Inc. (State College, Pa., 16801). Inorganic chemicals of reagent grade and distilled-deionized water were used in all experiments.

For pH close to 2, the solutions of 0.05 M HCl were used; for pH 3 to 6, 0.05 M buffer solutions of citric acid-sodium citrate were used; for pH 7 to 9, 0.05 M buffer solutions of tris-HCl were used; for pH 10 to 11, 0.05 M buffer solutions of glycine-NaOH were used; for pH 12 to 13, 0.05 M and 0.1 M solutions of NaOH were used. The buffer solutions were prepared according to Biochemists' handbook (25). A stock solution of 5 mg poly-L-lysine per ml of distilled water was prepared. 2.4 ml (containing 12 mg of poly-L-lysine) of this solution was added to 100 ml of the subsolution for surface measurements. The stearic acid was dissolved in chloroform-methanol-hexane (1:1:3 v/v/v) in a concentration of about 0.8 mg/ml.

Methods: The surface pressure was measured by a modified Wilhelmy plate method, and the surface potential by a radioactive electrode, as described previously (26). The state of the monolayers was determined qualitatively by the talc method (27). The monolayers of stearic acid were spread on buffered subsolutions in the presence and absence of poly-L-lysine (12 mg/100 ml subsolution).

Bubble stability: The survival time (i.e., the time interval between the formation and collapse) of bubbles was measured with a stopwatch after producing a small air bubble by a dropper (tip diameter 1 mm)under monolayers and subsolutions in the following manner. When a monolayer was compressed to its limiting area ($\approx$20 A^2/molecule), a bubble was produced on each side of the compression glass barrier. For subsolutions containing poly-L-lysine, the monolayer side of the compression barrier showed surface properties of stearic acid + poly-L-lysine, whereas the other side of the barrier showed those of adsorbed film of poly-L-lysine alone. At least ten measurements were made for bubble stability. It should be pointed out that since the collapse of a bubble produces considerable structural reorganization and rearrangement of molecules in the monolayer, a second bubble should not be produced in the same region of the monolayer. Therefore, all ten bubbles were produced in different parts of the monolayer and their average survival time was calculated.

The surface tension of buffered solutions of poly-L-lysine was measured with a Roller-Smith surface tensiometer. The surface pressure (π) of poly-L-lysine solutions is defined as $\pi = \gamma_o - \gamma_p$, where γ_o is the interfacial tension without poly-L-lysine and γ_p is that with poly-L-lysine in the subsolution; hence, π represents the lowering of the surface tension of buffer solutions by the presence of poly-L-lysine.

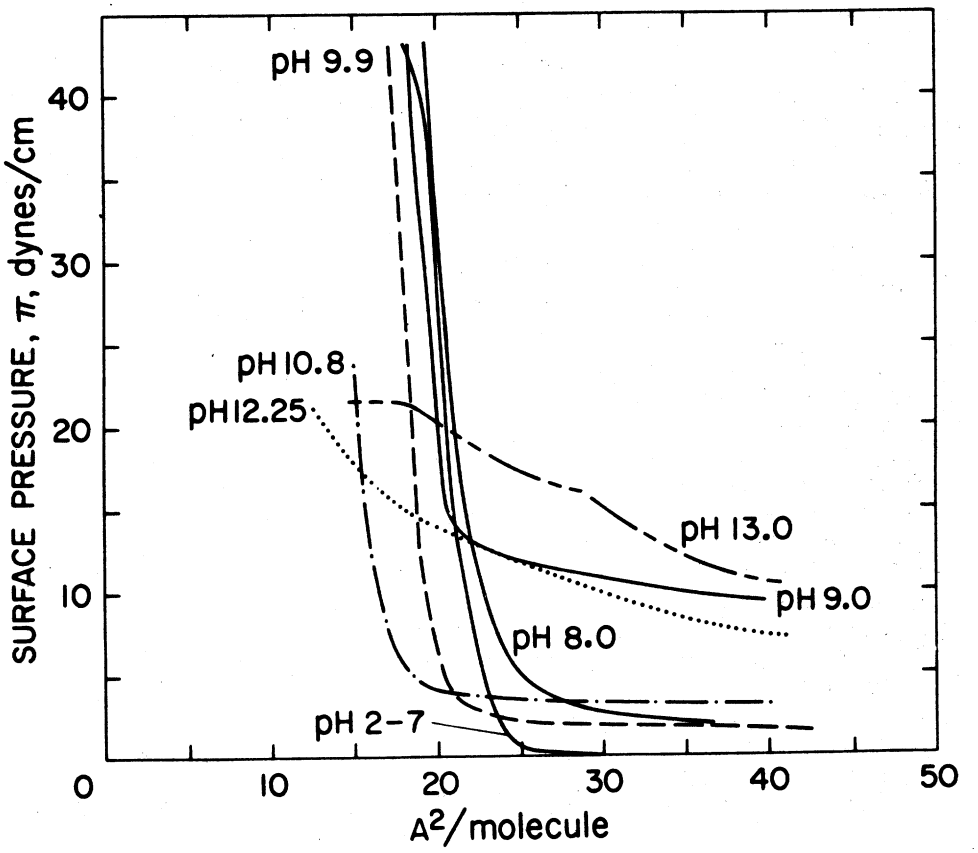

Fig. 1. Surface pressure-area curves of stearic acid monolayers on buffered subsolutions at various pH values at 22°C.

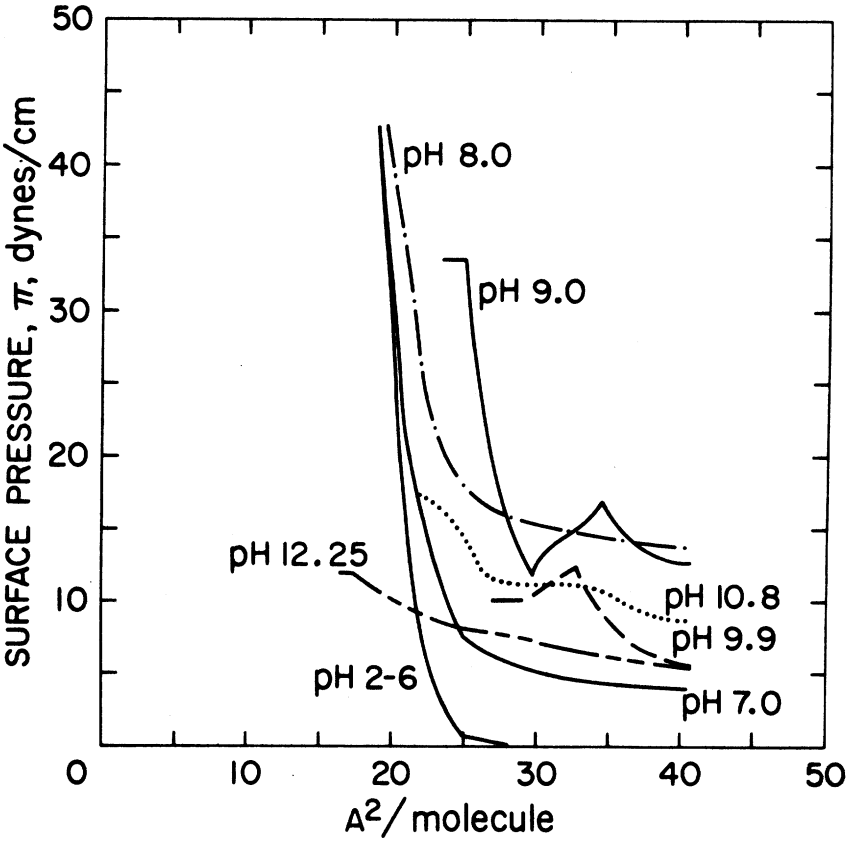

Fig. 2. Surface pressure-area curves of stearic acid monolayers on buffered subsolutions containing 0.02 mg/ml of poly-L-lysine at various pH values at 22°C.

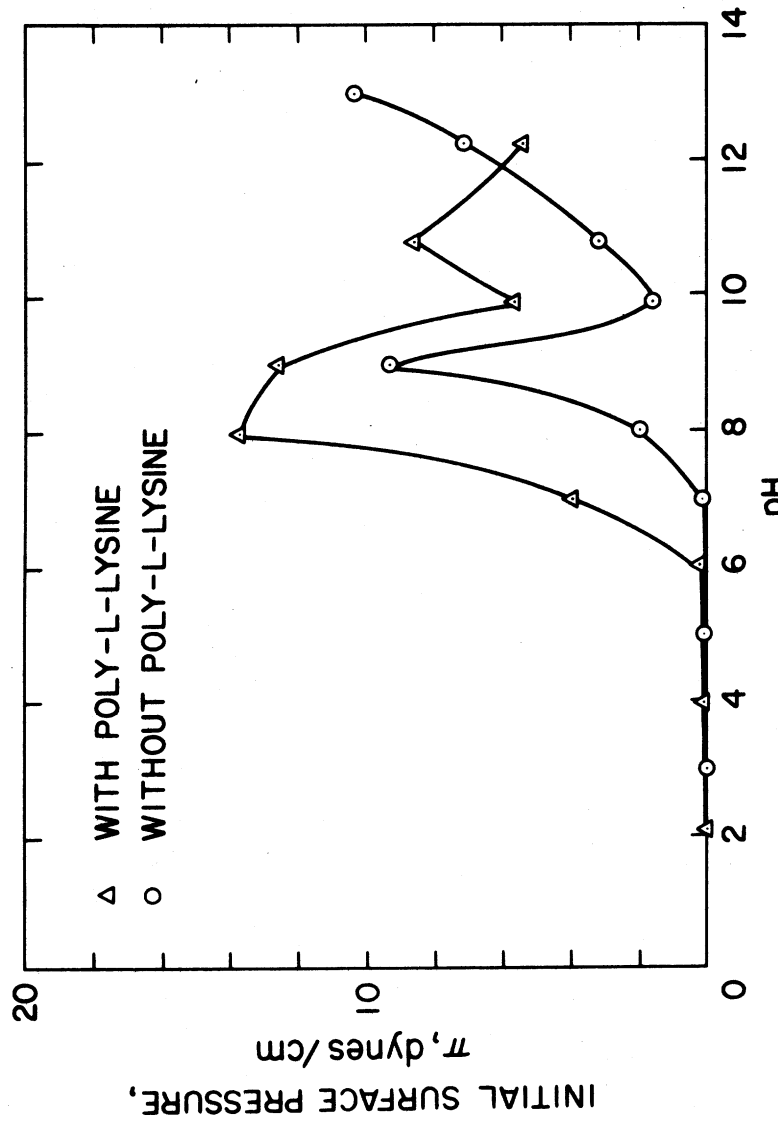

Fig. 3. Initial surface pressure values of stearic acid monolayers on buffered subsolutions in the absence (o) and presence (Δ) of poly-L-lysine (0.12 mg/ml) at various pH values at 22°C.

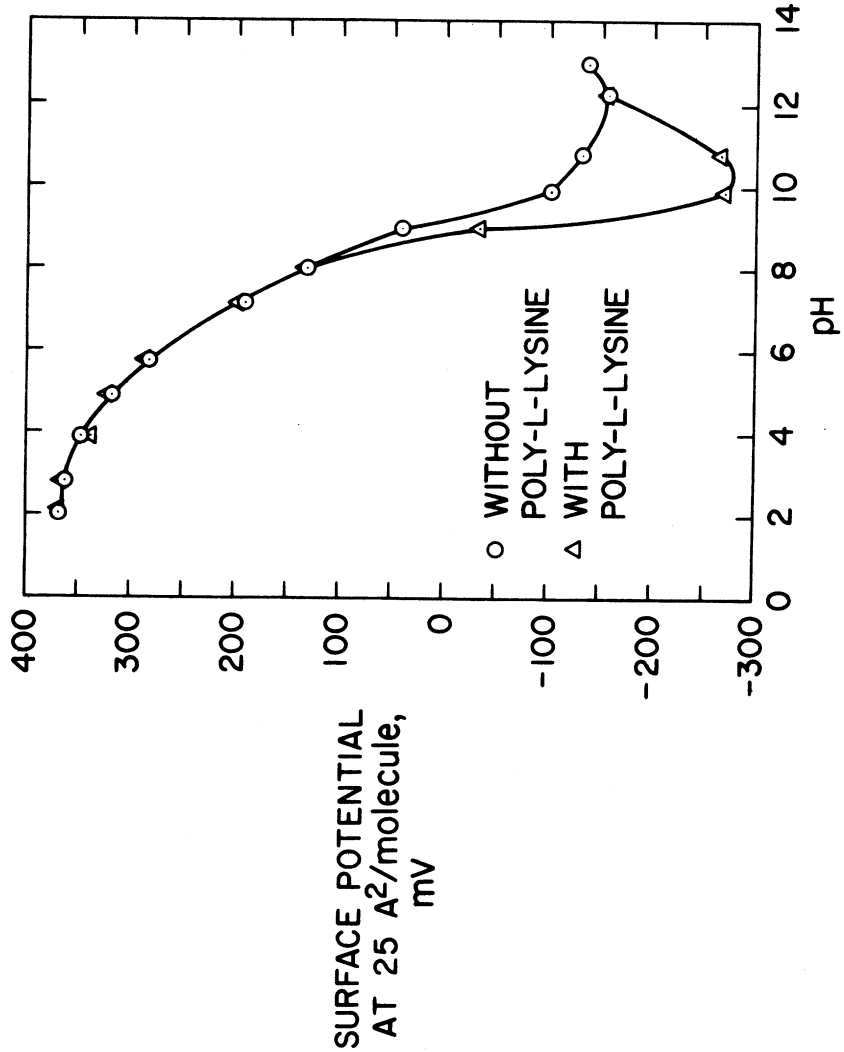

Fig. 4. Surface potentials of stearic acid monolayers at 25 A²/molecule on buffered subsolutions in the absence (o) and presence (Δ) of poly-L-lysine (0.12 mg/ml) at various pH values at 22°C.

RESULTS

Figures 1 and 2 show surface pressure-area curves of stearic acid monolayers in the absence and presence of poly-L-lysine in subsolutions of different pH values. It has been shown (28) that the plateau region in the surface pressure-area curves of stearic acid at large area per molecule is directly related to repulsion in the monolayers because of ionization of carboxyl groups. Figure 3 shows the surface pressure values where the plateau region begins for different pH values. In general, the presence of poly-L-lysine increases these initial surface pressure values and shifts the curve to the left (or acid side).

Figure 4 shows the surface potentials of stearic acid monolayers at 25 A^2/molecule in the presence and absence of poly-L-lysine at various pH values. It is evident that the interaction of poly-L-lysine with stearic acid monolayers lowers the surface potential and that the maximum interaction occurs in the pH range 10 to 11.

Table I summarizes the state of stearic acid monolayers near the collapse pressure in the presence and absence of poly-L-lysine in the subsolution at various pH values. It shows that the interaction between stearic acid monolayers and poly-L-lysine in the pH range 9 to 11 solidifies the monolayers.

The upper part of figure 5 shows the data of Applequist and Doty (24) on poly-L-lysine solutions. The lower part of figure 5 shows our data on the bubble stability of stearic acid monolayers in the presence of poly-L-lysine in the subsolution. The bubble stability for stearic acid monolayers alone, which is not shown in figure 5, did not exceed 10-15 seconds over the whole pH range. The surface activity (or surface pressure) of poly-L-lysine solutions and bubble stability at various pH values are also shown in figure 5. It is evident from figure 5 that at pH 11, the conformation of poly-L-lysine molecules, which is nearly helical and surface-active, affords maximum stability to bubble lamellae.

DISCUSSION

Figure 1 shows that for pH values from 2 to 9, the limiting area of stearic acid is approximately the same ($\approx$20 A^2/molecule), implying that the monolayers are insoluble in this pH range. At pH 9.9 and 10.8, the limiting areas are respectively 16 and 18 A^2/molecule, which may be due to slight solubility of ionized stearic acid molecules in the subsolution, or to rearrangement of molecules in the monolayers. The initial surface pressure values

TABLE I

pH	Subsolution	Subsolutions without Poly-L-lysine: The state of monolayers	Subsolutions with Poly-L-lysine: The state of monolayers
2.0	HCL solution	liquid	liquid
2.8	citric acid-sodium citrate buffer	liquid	liquid
3.9	citric acid-sodium citrate buffer	liquid	liquid
4.9	citric acid-sodium citrate buffer	liquid	liquid
5.75	citric acid-sodium citrate buffer	liquid	liquid
7.15	tris - HCl	solid	solid
8.0	tris - HCl	solid	solid
9.0	tris - HCl	gel (+)*	solid
9.9	glycine-NaOH buffer	gel (+)	solid
10.8	glycine-NaOH buffer	gel (++)	solid
12.25	NaOH solution	gel (+++)	gel

*The number of + signs indicates qualitatively the increasing surface viscosity of the monolayers.

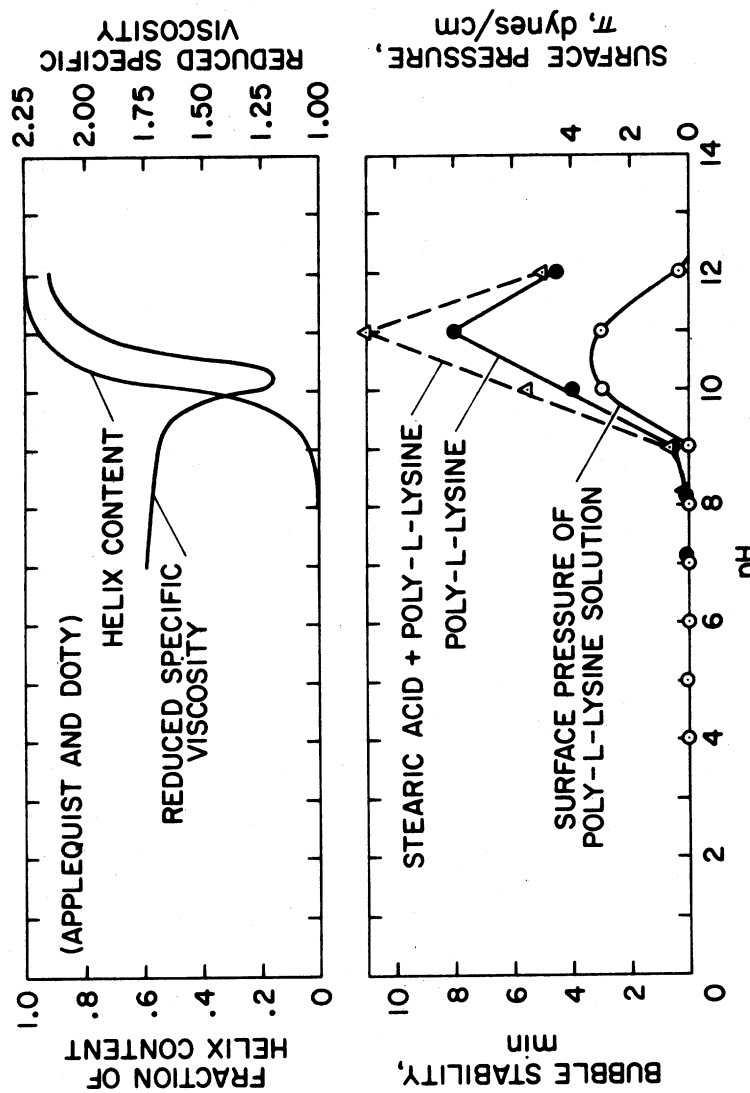

Fig. 5. Data of Applequist and Doty on helix content and reduced specific viscosity of poly-L-lysine solutions (upper part) surface pressure (or surface activity), and bubble stability of poly-L-lysine solutions (lower part). The bubble stability of stearic acid monolayers in the presence of poly-L-lysine is shown by a broken line, whereas that of stearic acid alone was 10-15 seconds in the whole pH range.

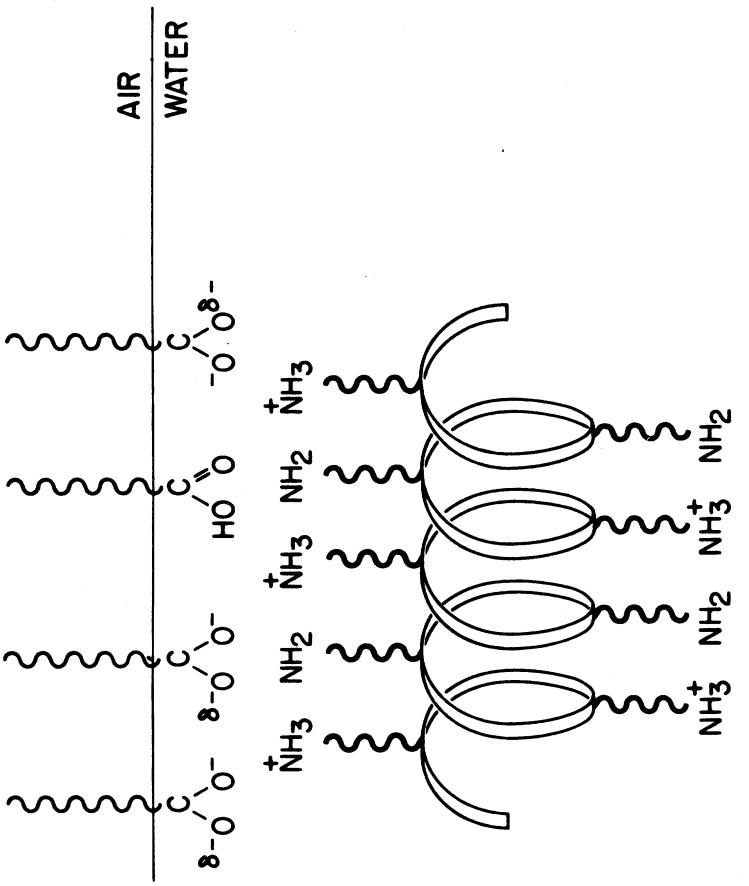

Fig. 6. Schematic representation of interaction between stearic acid and poly-L-lysine in monolayers at pH 11. δ- represents a partial ionic charge on oxygen atoms.

of the plateau region indicate the extent of ionic repulsion in monolayers (fig. 1). As shown in figure 2, the presence of poly-L-lysine strikingly alters the surface pressure-area curves of stearic acid monolayers above, but not below pH 6, indicating that poly-L-lysine does not interact with stearic acid monolayers in the pH range 2 to 6.

It is interesting to compare the effect of cations such as calcium with poly-L-lysine on stearic acid monolayers. It has been shown (29-31) that the binding of calcium ions to stearic acid monolayers begins to occur at about pH 5; this causes condensation of the monolayers. In contrast, the interaction between poly-L-lysine and stearic acid expands the monolayers presumably due to penetration of side chains of poly-L-lysine in the monolayers. The kinks in the surface pressure area curves at pH 9.0 and 9.9 at about 35 A^2/molecule indicate the areas at which presumably some of the penetrated side chains of poly-L-lysine are squeezed out of the monolayers (fig. 2).

Figure 3 shows the initial surface pressure values of the plateau region in the presence and absence of poly-L-lysine in subsolutions. Stearic acid monolayers without poly-L-lysine show a maximum at pH 9, whereas, the maximum occurs at pH 8.0 in the presence of poly-L-lysine in the subsolution.

It has been shown (28, 32) that at pH 9, where 50% of the molecules are ionized (i.e., pK = 9), there is maximum separation between the molecules in stearic acid monolayers. It is clear that in the presence of poly-L-lysine in subsolutions, the maximum separation occurs at pH 8.0, which suggests that the pK value has shifted by one pH unit. A similar decrease in the pK of oleic acid by the presence of calcium ions was reported by Benzonana and Desnuelle (33). A second maximum observed at pH 11 may be due to the penetration of poly-L-lysine into the monolayers. In general, the initial surface pressure values above pH 6 are higher in the presence of poly-L-lysine in the subsolution than in the absence of it. This can be explained as follows: because of coulombic attraction the cationic side chains of poly-L-lysine may penetrate the negatively charged stearic acid monolayers. This will increase the surface concentration of molecules and, hence, the surface pressure in monolayers.

Figure 4 shows the surface potentials of stearic acid monolayers in the presence and absence of poly-L-lysine in subsolutions. It shows that the maximum interaction occurs in the pH range 10-11 where the surface potential decreases by about 175-185 mv. It is interesting to note that the presence of calcium ions in the subsolution also decreases the surface potential of stearic acid monolayers by about 200 mv (31). Hence, the

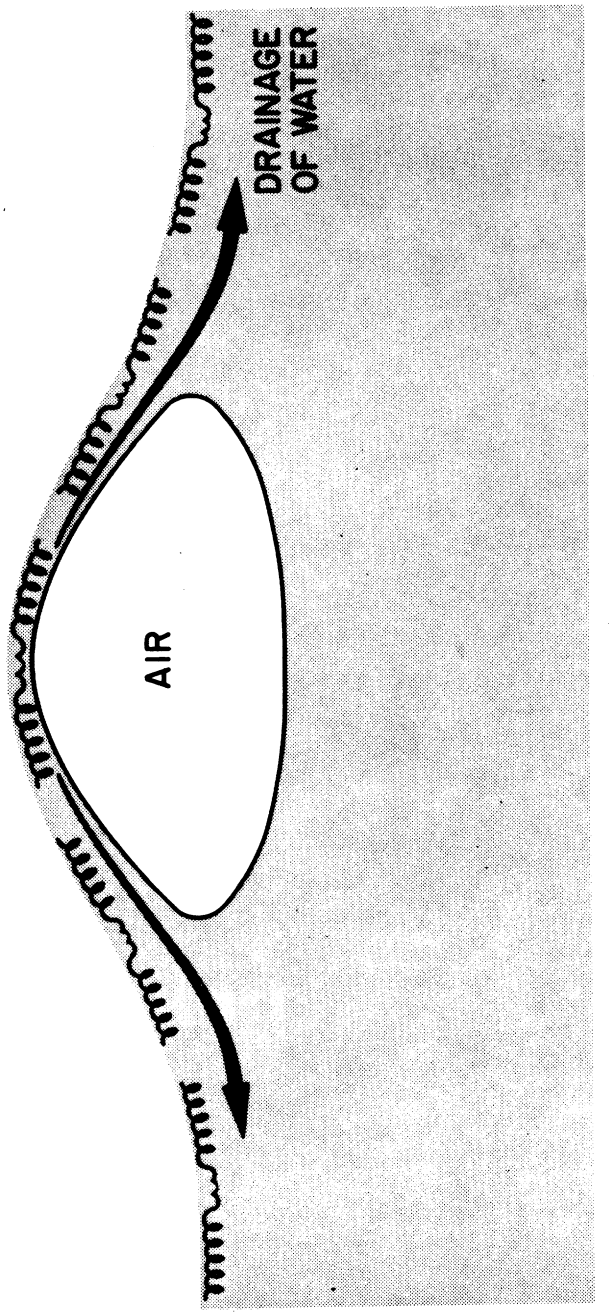

Fig. 7. Schematic representation of the mechanism governing the stability of an air bubble covered with a film. A faster rate of drainage of water in the lamellae decreases the bubble stability.

mechanism of action of poly-L-lysine on stearic acid monolayers is presumably the same as that of calcium ions. In contrast, the interaction of calcium ions with lecithin, sphingomyelin, cardiolipin and dicetyl phosphate monolayers increases the surface potentials (26, 34, 35).

Applequist and Doty (24), using birefringence, viscosity, sedimentation and optical rotatory dispersion measurements, have shown that random coil-to-helix transition in poly-L-lysine solutions occurs in the pH range 10 to 11. The dissociation of NH_3^+ groups also takes place in this pH range. Figure 6 schematically shows the interaction of poly-L-lysine with stearic acid monolayers at pH 11 where poly-L-lysine molecules have nearly helical conformation.

Figure 5 shows that poly-L-lysine solutions exhibit surface activity (or surface pressure) in the pH 10 to 11. Although the surface pressure of poly-L-lysine is very low (3 dynes/cm), it strikingly influences the bubble stability. Below pH 9, the bubble stability of poly-L-lysine solution is about 10 seconds, whereas at pH 11, its bubble stability is 8 minutes.

The bubble stability of stearic acid monolayers in the absence of poly-L-lysine is about 10-15 seconds over the whole pH range, whereas that of stearic acid in the presence of poly-L-lysine is about 11 minutes at pH 11. It is evident that this enhancement of bubble stability is due to the presence of poly-L-lysine since it also shows maximum bubble stability (8 mins.) without stearic acid monolayers. The upper part of figure 5 shows that at pH 11, poly-L-lysine has almost helical conformation in solution.

Figure 7 schematically shows the mechanism of bubble stability in the presence of a film. If the rate of drainage is rapid, the bubble has a shorter survival time. If the molecules in the film impede the drainage of water due to film-water interaction, the stability of the bubble increases. The data presented in this paper suggest that helical conformation of poly-L-lysine decreases the rate of drainage of water in the bubble lamellae and, hence, increases the bubble stability. The interaction between stearic acid and poly-L-lysine causes further increase in the bubble stability.

The question may be raised whether poly-L-lysine molecules at pH 11 retain their helical conformation at the interface or not. In this author's opinion, the helical conformation is preserved at the interface. If poly-L-lysine molecules are denatured at the interface, one would not observe the striking properties in the pH range 10 to 11. Moreover, using deuterium exchange, infra-red spectroscopy and electron diffraction methods

to study skimmed monolayers, Malcolm (36, 37) has shown that helical conformation of poly-peptides is retained in the monolayer at the air-water interface.

It is difficult to extrapolate the results of monolayer studies to interactions in biological membranes. However, the correlation of these studies with those of others on biological membranes should be mentioned. It has been established by various workers with different techniques that membrane proteins have partially helical conformation (38-40). From the results reported in this paper it appears that partially helical conformation of proteins in biological membranes may cause maximal interaction with water (i.e., maximum hydration) and enhance the interaction with lipids presumably because of the surface activity of the proteins in such conformation.

In summary, the results presented in this paper indicate that the lipid-polymer interaction in monolayers is strikingly influenced by the conformation of the polymer. Extensive studies on the interaction between various lipids and poly-peptides in monolayers are in progress in this author's laboratory.

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