Carboxylgruppen an Peptidseitenketten beteiligt sind, die von verschiedenen Polysaccharidketten ausgehen, dann kann eine Kontraktion des Gesamtsacculus resultieren.

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## Lipid–protein interaction in monolayers. Effect of conformation of poly-L-lysine on stearic acid monolayers*

Lipid–protein interactions are of great interest for understanding the structure and function of biological membranes. The monolayer approach has been found very useful for understanding molecular aspects of lipid–protein and protein–protein interactions presumably occurring in biomembranes[1–5].

To investigate the interaction of water with films, TRAPEZNIKOV[6] and GARRETT[7] 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.

A simple model system of stearic acid and poly-L-lysine was selected to investigate various aspects of the ionic interaction in the lipid–protein association, since the ionic properties of stearic acid monolayers[8–10] and of poly-L-lysine solutions[11] have been well 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 the ionic interaction at the interface.

Poly-L-lysine hydrochloride (mol. wt. 100000–200000) was bought from Mann Res. Labs. (New York). Highly purified (>99%) stearic acid was purchased from Applied Sci. Labs. (State College). For pH close to 2, the solutions of 0.05 M HCl were used; for pH 3–6, 0.05 M buffer solutions of citric acid–sodium citrate were used; for pH 7–9, 0.05 M buffer solutions of Tris–HCl were used; for pH 10–11,

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0.05 M buffer solutions of glycine–NaOH were used; for pH 12–13, 0.05 and 0.1 M solutions of NaOH were used. The buffer solutions were prepared according to *Biochemists' Handbook*[12]. 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 were 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.

The surface pressure was measured by a modified Wilhelmy plate method and the surface potential by a radioactive electrode, as described previously[13,14]. The monolayers of stearic acid were spread on buffered subsolutions in the presence and absence of poly-L-lysine (12 mg/100 ml subsolution) in the subsolution.

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 *plus* 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.

Figs. 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. As shown in Fig. 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 2–6.

It has been shown[15,16] that the binding of $Ca^{2+}$ to stearic acid monolayers begins to occur at about pH 5; this causes condensation of the monolayers. In contrast,

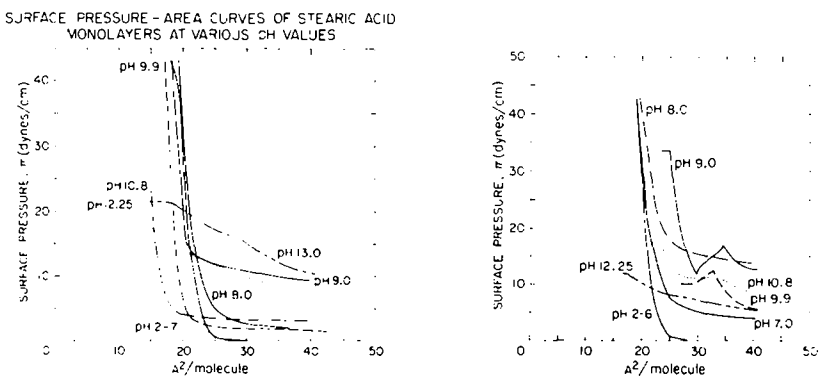


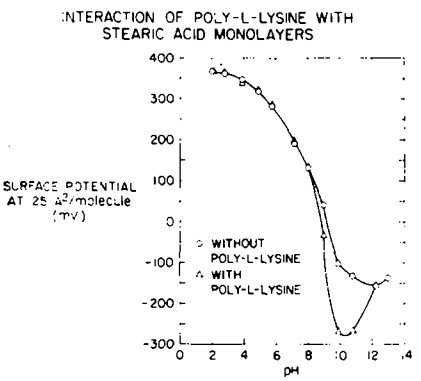

Fig. 1. Surface pressure–area curves of stearic acid monolayers on buffered subsolutions at various pH values at 22°.

Fig. 2. Surface pressure–area curves of stearic acid monolayers on buffered subsolutions containing 0.12 mg/ml of poly-L-lysine at various pH values at 22°.

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).

Fig. 3 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 10–11, in which surface potential decreases by about 175–185 mV. It should be pointed out that the presence of $Ca^{2+}$ in the subsolution also decreases the surface potential of stearic acid monolayers by about 200 mV (ref. 17). In contrast, the interaction of $Ca^{2+}$ with lecithin, sphingomyelin, cardiolipin and dicetyl phosphate monolayers increases the surface potentials[13,18,19].

Fig. 4A shows the data of APPLEQUIST AND DOTY[11], which indicate that random coil-to-helix transition in poly-L-lysine solutions occurs in the pH range 10–11. Fig. 4B 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 Fig. 4, did not exceed 10–15 sec over the whole pH range. It is evident from Fig. 4 that at pH 11, the conformation of poly-L-lysine molecules, which is nearly helical, presumably affords maximum stability to bubble lamellae. Fig. 4 shows that poly-L-lysine solutions exhibit surface activity (or surface pressure) in the pH range 10–11. Although the surface pressure of poly-L-lysine is very low (approx. 3 dynes/cm), it strikingly influences the bubble stability. It should be pointed out that on the basis of degree of ionization of poly-L-lysine, one would expect less interaction at pH 11, since some of the $NH_3^+$ groups on the polymer are dissociated at this pH. However, a change in the conformation of the



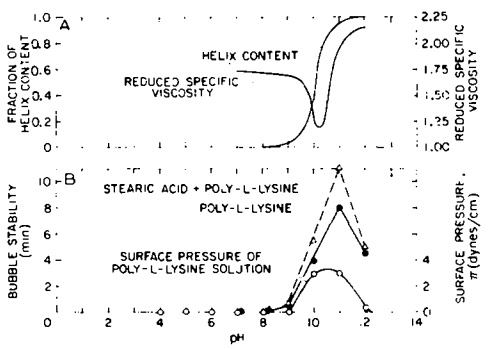




Fig. 3. Surface potentials of stearic acid monolayers at 25 $A^2$/molecule on buffered subsolutions in the absence (⊙) and presence (△) of poly-L-lysine (0.12 mg/ml) at various pH values at 22°.

Fig. 4. Data of APPLEQUIST AND DOTY on helix content and reduced specific viscosity of poly-L-lysine solutions (A). Surface pressure (or surface activity), and bubble stability of poly-L-lysine solutions with or without stearic acid monolayers (B). 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 (not shown in the diagram) was 10–15 sec in the whole pH range.

polymer will bring about a change in spatial orientation of remaining charged groups which may influence the interaction of poly-L-lysine with stearic acid monolayers.

The data presented in this paper suggest that helical conformation and surface activity of poly-L-lysine at pH 11 decrease the rate of drainage of water in the bubble lamellae and hence, increase the bubble stability. The interaction between stearic acid and poly-L-lysine causes further increase in the bubble stability (Fig. 4).

It appears that the helical conformation of poly-L-lysine 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 10–11. Moreover, using $^2H$ exchange, infrared spectroscopy and electron diffraction methods to study skimmed monolayers, MALCOLM[20,21] has shown that helical conformation of polypeptides is retained in the monolayer at the air–water interface. In summary, the results presented in this paper indicate that the ionic interaction between stearic acid and poly-L-lysine in monolayers is strikingly influenced by the conformation of the polymer, which also influences the hydration and rate of drainage in monolayers.

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