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INTERACTION OF POLY(L-LYSINE) AND Ca^{2+} WITH STEARIC ACID MONOLAYERS

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SUMMARY

Interaction of poly(L-lysine) and Ca^{2+} with stearic acid monolayers is studied at pH 9.1, 9.9 and 10.7. The competition between the condensation effect of Ca^{2+} and the expansion effect of the protein on the monolayer is seen to depend on surface pressure as well as pH. Ca^{2+} is much less effective in the competition when the poly(L-lysine) penetration into the monolayer is stabilized by electrostatic interactions.

INTRODUCTION

The nature of lipid–protein interactions and their role in the structure of biological membranes is an area of great interest and complexity [1]. One technique used in studying this problem is the determination of properties of lipid monolayers placed over solutions containing the protein as a solute [2]. We report a study on the interaction of poly(L-lysine) and Ca^{2+} with stearic acid monolayers. While this system is only indirectly related to the question of biomembrane stability, it offers the advantage of a clearly defined model system for which the nature of the protein interaction and its change due to the presence of a physiologically important cation can be studied. The stearic acid monolayer has been extensively studied [3]. Poly(L-lysine) interacts to expand the monolayer at all pH values and surface pressures while Ca^{2+} has a condensing effect [4]. Thus we have an experiment that allows a comparison of competing effects on a simple lipid monolayer.

MATERIALS AND METHODS

Stearic acid of 99+% purity and poly(L-lysine) hydrobromide with an average molecular weight of 80 000 were purchased from Sigma. The spreading solvent was 99+% pure *n*-hexane from Fischer, all other chemicals were reagent grade and deionized water was distilled from alkaline permanganate in an all-glass system. A Langmuir film balance was constructed following the discussion of

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Gaines [5]. The buffer chosen for this work was Na_2CO_3–Na_2HCO_3 and the pH, with an ionic strength of 0.05 at all times [6], was constantly monitored and found to hold constant at ± 0.1 unit for all experiments. The temperature was held constant at 25.0 ± 0.2 °C and the room temperature varied at 22–25 °C. We used a compression rate of 8 mm/min in steps of 2 mm/(5 s) plus 10 s rest while restoring the float to normal position. The variation of π–A isotherms as a function of monolayer compression rate is considerable, as has been noted [7]. Our system and techniques were checked by reproducing to within experimental error a number of studies [3,8]. Our estimated maximum experimental error is $\pm 2.2\%$ in area/molecule and $\pm 2.3\%$ in dynes/cm. The protein concentration was 0.06 mg/ml unless stated otherwise and the Ca^{2+} concentration was 2 mM.

RESULTS AND DISCUSSION

In Figs 1, 2 and 3 we present the data as surface pressure versus area per stearic acid molecule (π–A) isotherms. Four different systems were studied: the stearic acid monolayer over the buffered aqueous subsolution, the monolayer over the subsolution containing the Ca^{2+} solute, the monolayer over the subsolution containing the poly(L-lysine) solute, and the monolayer over the subsolution containing both the Ca^{2+} and protein solutes.

A comparison of our isotherms for stearic acid over a subsolution containing poly(L-lysine) with those of Shan [9] shows a striking difference in the nature of the curves as Shan has sharp discontinuities at pH 9.0 and 9.9*. In an effort

* Since we have the possibility of poly(L-lysine) absorption to the clean reference surface, which results in our observing only a difference in surface pressures between the monolayer and contaminated reference surface, we make only a qualitative comparison of the data. Poly(L-lysine) at a concentration of 0.12 mg/ml has a reported maximum surface pressure of 3 dynes/cm [9]. By always sweeping the reference surface clean before compressing the monolayer (Matalon, R. and Schulman, J. H. (1949) J. Interface Sci. 4, 89–90), we believe this error has been reduced to approximately the limits of our estimated experimental error.

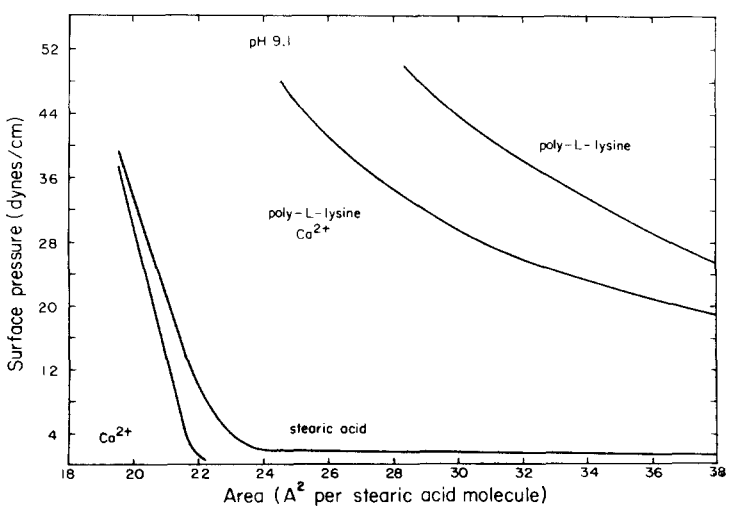
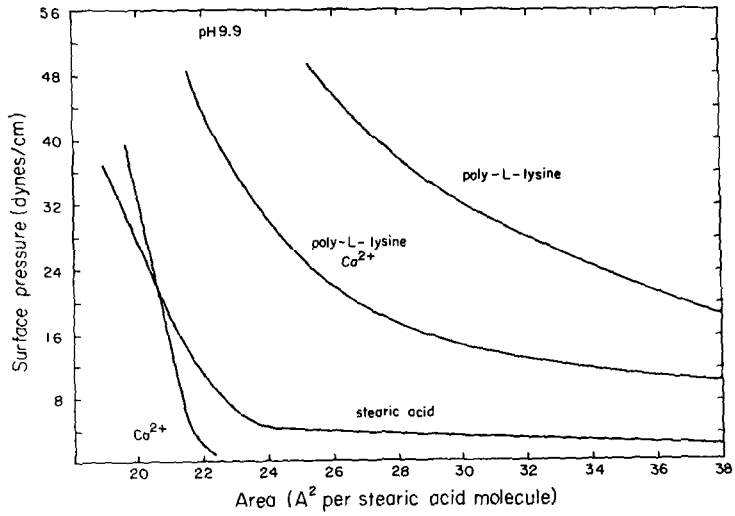


Fig. 1. π–A isotherms at pH 9.1.



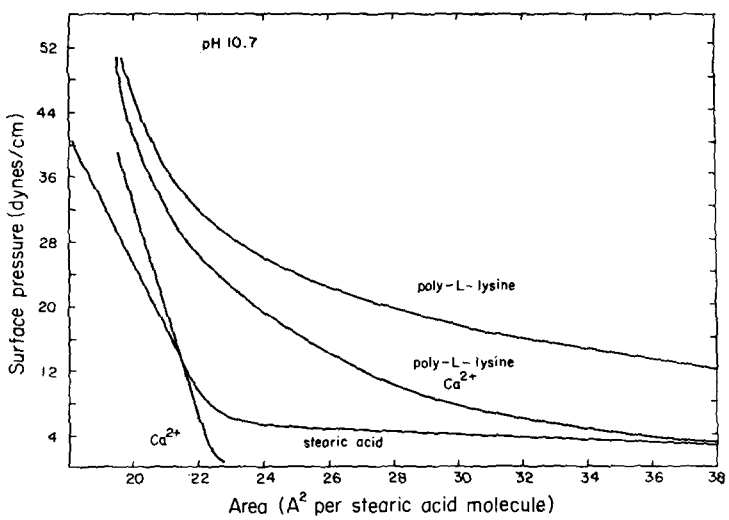


Fig. 2. π–A isotherms at pH 9.9.

Fig. 3. π–A isotherms at pH 10.7.

to reproduce the discontinuous curves we ran a series of measurements with Shan's protein concentration, 0.12 mg/ml, and buffer system, glycine–NaOH, at pH 9.9. Our π–A isotherms exhibited no discontinuities. Trying to reproduce his data at pH 9.1 we found the Tris–HCl buffer lost its effect allowing a pH change of 1 unit. The irregular curves Shan obtained might be due to the fact that he used the Wilhelmy method while we used the Langmuir method. Both Rogness [10] and Shan [11] have noted the possible error of using the Wilhelmy technique with a platinum plate for monolayers that are positively changed. Hittmeir et al. [12]* have made

* See also Munden et al. [7] for related problems with the Cahn automated Wilhelmy method.

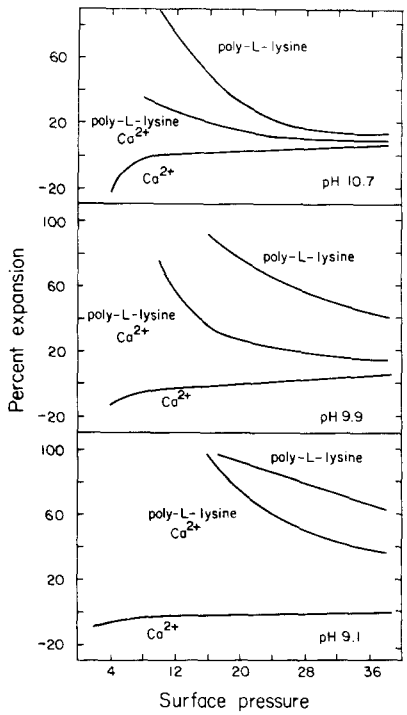


Fig. 4. Percent expansion as a function of monolayer surface pressure.

comparative studies of a given system with the two types of balances and report smooth curves for the Langmuir balance and discontinuous curves for the Wilhelmy balance.

The effects of poly(L-lysine) and Ca^{2+} on the monolayers are perhaps most compactly illustrated and easily analyzed when the data is presented as graphs of percent expansion versus surface pressure, see Fig. 4, with the reference system being the monolayer over the buffered subsolution containing neither solute. An example of this function from the π–A isotherms at $\pi = 20$ dynes/cm is:

(% expansion of Ca^{2+} at $\pi = 20$) =

$$[(A \text{ of Ca at } \pi = 20) - (A \text{ of reference at } \pi = 20)]/(A \text{ of reference at } \pi = 20).$$

For the Ca^{2+} system the 3 curves change very little above 10 dynes/cm. From the π–A isotherms this corresponds to an area of 21.5 Å^2 per monolayer molecule which is compared to the limiting area of 20 Å^2 at the stearic acid collapse pressure. The qualitative difference of the curves can be readily accounted for by the increased ionization of the monolayer [13] with increasing pH. This allows increasing interaction of Ca^{2+} with carboxylate anions giving more extensive complex formation, which has been described via a copolymeric lattice model by Deamer and Cornwell [14]. The gradual decrease in the percent condensation of this system with increasing surface pressure is a clear indication of the rigidity of the complex compared to the stearic acid monolayer.

Considering the poly(L-lysine) system, the data of Applequist and Doty [15] shows that the protein undergoes a transition from coil to helical form in the pH range 9–11. However, findings of Hammes and Schullery [16] demonstrate that poly(L-lysine) can exist in the α-helix conformation even at pH 7 when it interacts with negatively charged phospholipid liposomes. Since our monolayers are always highly ionized [13] it seems likely that electrostatic interactions between the monolayer and poly(L-lysine) will stabilize the helical conformation in this case as well. Further data on poly(L-lysine) [15] in a solution that is 1 M NaBr and 0.2 M $NaHCO_3$, establishes that the protein is approximately 90, 75, and 30% in the cationic form for the pH value 9.1, 9.9 and 10.7 respectively. In our experiments then there should be at least some qualitatively distinct differences based on electrostatic effects. From Fig. 4 we see that the percent expansion decreases steadily with increasing surface pressure which is characteristic of a system where solute penetration into the monolayer has occurred. Also, the observation that the percent expansion decreases markedly with increasing pH indicates that solute–monolayer interactions are at least partly electrostatic in nature. Since the slopes of the curves become much steeper with increased pH it seems that the two effects, penetration and electrostatic interaction, are interrelated. The high-pressure limits demonstrate that poly-(L-lysine) behaves very much like Ca^{2+} at pH 10.7 while the two systems are distinctly different at pH 9.1. One possible mechanism for the poly(L-lysine) interaction is solute penetration into the monolayer which is then stabilized by electrostatic attraction between the cationic protein and the negatively charged monolayer. At pH 10.7 the interaction changes from largely a penetration effect at low pressure to an almost purely electrostatic interaction for pressures above 28 dynes/cm. At pH 9.1, where the protein is about 3 times more cationic, the electrostatically facilitated penetration is stable even at high surface pressures up to the collapse pressure of the monolayer. In the situation when the penetrated protein is not electrostatically stabilized it is not surprising that it can be rather easily forced out of the monolayer by increasing the surface pressure since the protein side chain that can undergo penetration is *n*-butylamino, $(CH_2)_4NH_2$. This is a rather small group which in addition to overcoming all the usual energy requirements for moving from the solution into the monolayer [17] would probably need to force some rearrangement of the polypeptide backbone for full penetration to occur. Even then the stabilization due to hydrophobic interactions would be rather small*.

The competition between the tendency of Ca^{2+} to form a rigid monolayer and the protein expansion, illustrated in Fig. 4, shows Ca^{2+} becomes increasing effective with increasing surface pressure. The mixed solute system is largely protein-like at pH 9.1 but is essentially like the Ca^{2+} system for high pressure at pH 10.7 which is consistent with the geometric requirements of the lattice model [14]. The area/molecule at the collapse pressure for pH 9.1 is 24 $Å^2$ which is too large for extensive complex formation to occur. At pH 10.7 the area decreases from 24 $Å^2$ at 28 dynes/cm, where the system starts to appear similar to the Ca^{2+} system, to an area of 20 $Å^2$ at the collapse pressure. The area/molecule at the collapse pressures

* A comparison of percent cation form of the protein [15] with the $\triangle(\triangle V)$ data of Shan [9] is in qualitative agreement with this mechanism if we correct the pH for his Tris–HCl buffer as noted above.

for all the systems are very similar at pH 10.7 and quite different at pH 9.1 which indicates that when the poly(L-lysine) penetration is further stabilized by electrostatic interaction the Ca^{2+} is much less effective in the competition than when the penetration is not electrostatically facilitated.

CONCLUSIONS

The description of poly(L-lysine) interaction as being both electrostatic and hydrophobic in nature is in agreement with the conclusions of a number of earlier studies [16,18,19]. The hydrophobic interactions are most important at pH closer to physiological values where it is stabilized by electrostatic forces. It is clear that the protein effects a definite structural change on the stearic acid monolayer which is strongly dependent on the surface tension of the monolayer thus it is important to determine the pressure dependence in model systems as the question of the tension existent in a biomembrane is still not settled.

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