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Penetration of Lysine Hydrochloride into Lecithin Monolayers from Underlying Aqueous Solutions

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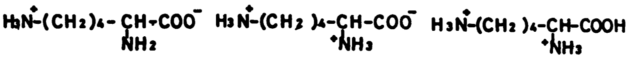
The surface pressure of the monolayers of dimyristoyl phosphatidylcholine (DMPC) on lysine mono- and dihydrochloride solutions was measured and the amount of positively charged lysine which had penetrated into a DMPC monolayer was calculated by successive approximation. The amount of penetration was smaller for lysine monohydrochloride than for the dihydrochloride. Moreover, both were smaller than that of uncharged lysine because mono- and dihydrochloride are more hydrophilic than uncharged lysine. The average area per molecule in the monolayer consisting of lysine and DMPC, A_{23}, was obtained. In the case of charged lysine, A_{23} increased with the mole fraction of lysine in a monolayer, X_2, in contrast it decreased with X_2 in the case of uncharged lysine. The partial molecular area of positively charged lysine was considerably larger than that of uncharged lysine. This indicates that the remarkable expansion of a monolayer is caused by the penetration of positively charged lysine because of electrostatic repulsion in the monolayer. The effect of sodium chloride on the adsorption of lysine was also investigated, but the amount adsorbed was independent of the presence of 0.5 mol dm^{-3} sodium chloride.

It has been widely accepted, according to the fluid mosaic model of Singer and Nicolson,[1] that biomembranes consist essentially of bimolecular lipid layers in which some proteins are dissolved. Many investigations of the interaction between lipids and proteins at the interfaces, have been made in order to elucidate the structure and the functions of the biomembranes.[2–7] From this point of view, the interaction between lecithin monolayers as a model of lipid membranes and lysine,[8] leucine and norleucine[9] as the components of proteins at the air/water interface was discussed in our previous papers.

Three typical patterns of the effects of different proteins on the properties of lipid bilayers and monolayers have been proposed by Papahadjopoulos *et al.*[10] 1) Simple adsorption, 2) adsorption and deformation, and 3) penetration. Which of the above three patterns is preferable depends on the kind of lipids or proteins that interact with each other, but, even for the same substances, the pattern or the intensity of interaction depends on the state and conditions of their existence. In the case of a basic amino acid, such as lysine, which was used in our previous work, the interaction with a lecithin monolayer should change with the pH of the solution, since the dissociation states of the amino and carboxyl groups of the amino acid changes. For lysine the pK values are: pK_1(COOH)$=2.18$, pK_2(α-NH$_3^+$)$=8.95$, and pK_3(ε-NH$_3^+$)$=10.53$,[11] and the possible charged forms of lysine, *i.e.*, free base (Lys), lysine monohydrochloride (Lys·HCl), and lysine dihydrochloride (Lys·2HCl) are (a), (b), and (c), in Fig. 1, respectively. The net charge is 0, +1, +2, in order of (a), (b), (c). Some appreciable changes in the interaction with lipids are expected to be caused by the difference between the charged forms. In this paper, Lys·HCl and Lys·2HCl were used, the amounts adsorbed onto lecithin monolayers were obtained, and discussed in comparison with the case of Lys.[8] Moreover, sodium chloride (NaCl) was used as an added electrolyte, and the effect of chloride ion (Cl$^-$) and that of the ionic strength were also investigated.



$H_3\overset{+}{N}-(CH_2)_4-\underset{NH_2}{CH}-COO^-$ $H_3\overset{+}{N}-(CH_2)_4-\underset{^+NH_3}{CH}-COO^-$ $H_3\overset{+}{N}-(CH_2)_4-\underset{^+NH_3}{CH}-COOH$

(a) (b) (c)

Fig. 1. Three forms of uncharged and charged lysine. (a) L-Lysine(free base) [Lys], (b) L-lysine monohydrochloride [Lys·HCl], and (c) L-lysine dihydrochloride [Lys·2HCl].

Experimental

Materials. Lys·HCl and Lys·2HCl, purchased from Wako Chemical Industries, Ltd, were dissolved in redistilled water. L-α-Dimyristoyl phosphatidylcholine (DMPC) from Sigma Company was used as lecithin, dissolved in distilled chloroform and preserved in a refrigerator until use. The stock solution was prepared every few days in order to avoid disintegration. NaCl from Merck and Co., Inc. was used without further purification.

Surface-tension Measurement. The surface tension of lysine hydrochloride solution, γ_{lys}, was measured by the capillary-height method, and the surface pressure of lysine hydrochloride, F_{lys}, was obtained by Eq. 1:

$$F_{\mathrm{lys}} = \gamma_0 - \gamma_{\mathrm{lys}}, \tag{1}$$

where γ_0 is the surface tension of pure water. The solution temperature was kept at 25±0.5 °C.

Surface-pressure Measurement. The surface pressure of the DMPC monolayer was measured by Wilhelmy's plate method. The details of this method were the same as described before.[8] The experimental error of the measurement was within ±0.1 mN m^{-1}. The temperature of the solution was kept at 25±0.5 °C.

Results

Surface Tension of Lysine Hydrochloride Solution. Figure 2 shows the surface tension of Lys·HCl or Lys·2HCl solution. The surface tension of Lys solution as already reported in Fig. 1 of Ref. 8 is also shown by open circles(○) in Fig. 2. It is seen that the surface tension decreases slightly with the increase in the concentration of solution, C_2, and the extent of the decrease is almost independent of the charged forms of the lysine.

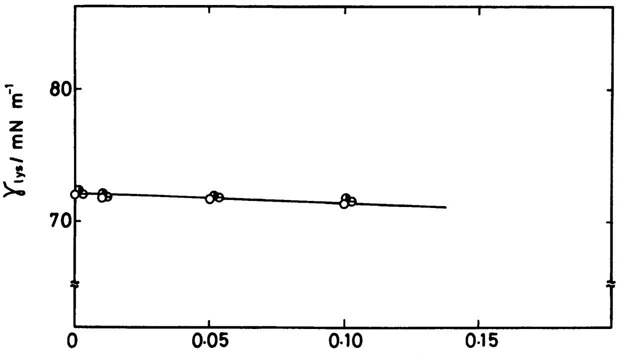


Fig. 2. The relation of surface tension, γ_{lys}, and the concentration of solution, C_2. (◑) Lys·HCl, ⊖Lys·2HCl. Symbols (○) designate the values in the case of Lys.

Surface Pressure-Area Curves of the DMPC Monolayers. The surface pressure-area curves of the DMPC monolayers spread on Lys·HCl and Lys·2HCl solutions are shown in Figs. 3(a) and (b), respectively. Although the curve shifted to the higher surface pressure region with the increase in concentration of lysine, C_2, in both cases, the extent of the shifts was larger in the case of Lys·2HCl than that of Lys·HCl.

The Amount Adsorbed onto a DMPC Monolayer. The relation between the surface pressure, F, and the concentration, C_2, at various constant areas per molecule of lecithin, A_3, was obtained from Figs. 3(a) and (b) and the amount of lysine adsorbed onto a DMPC monolayer was calculated, as described before,[8] by the following equation:

$$\Gamma_2 = \frac{1}{iRT}\left(1-\frac{\bar{A}_3}{A_3}\right)\left(\frac{\partial F}{\partial \ln C_2}\right)_{A_3}, \qquad (2)$$

where Γ_2 is the adsorbed amount of solute, R is the gas constant, T is the absolute temperature, and $\bar{A}_3$ is the partial molecular area of DMPC. $\bar{A}_3$ was approximated by the molecular area of DMPC on pure water, A_3°, by Pethica.[12] Hereafter, this will be called the first approximation. The values of the coefficient, i, to be discussed later in detail, are $i=2$ and 3 for Lys·HCl and Lys·2HCl, respectively, since no added electrolytes coexist in this case. Values of Γ_2 thus calculated in the first approximation are shown in Fig. 4. The values of Γ_2 in the case of Lys[8] are also shown in this figure. Besides the fact that the amount adsorbed Γ_2 increases with the concentration, C_2, it is seen from Fig. 4 that the value of Γ_2 is smaller for Lys·HCl(b) and Lys·2HCl(c) than for Lys(a). This indicates that the positively charged lysine(Lys·HCl or Lys·2HCl) is less adsorbed onto an interface than the uncharged lysine (Lys) because the hydrophobicity of the former two is stronger than that of the latter.

The Effect of Added Electrolytes. As an electrolyte, 0.5 mol dm^{-3} NaCl was added into the lysine solution and the effect of the ionic strength on the adsorption of lysine was investigated. The $\gamma_{lys}-C_2$ curves for Lys·HCl and Lys·2HCl are shown in Figs. 5(a) and (b), respectively. It was found that, in both cases, the surface tension decreased slightly with the increase in C_2, irrespective of whether or not a NaCl was added. The surface pressure-area curves of the DMPC monolayers spread on Lys·HCl and Lys·2HCl solutions with 0.5 mol dm^{-3} NaCl are shown in Figs. 6(a) and (b), respectively. The calculation of the amount of lysine adsorbed in the presence of added electrolytes is discussed later in detail.

Discussion

The Mixed Monolayers of DMPC and Lysine. It

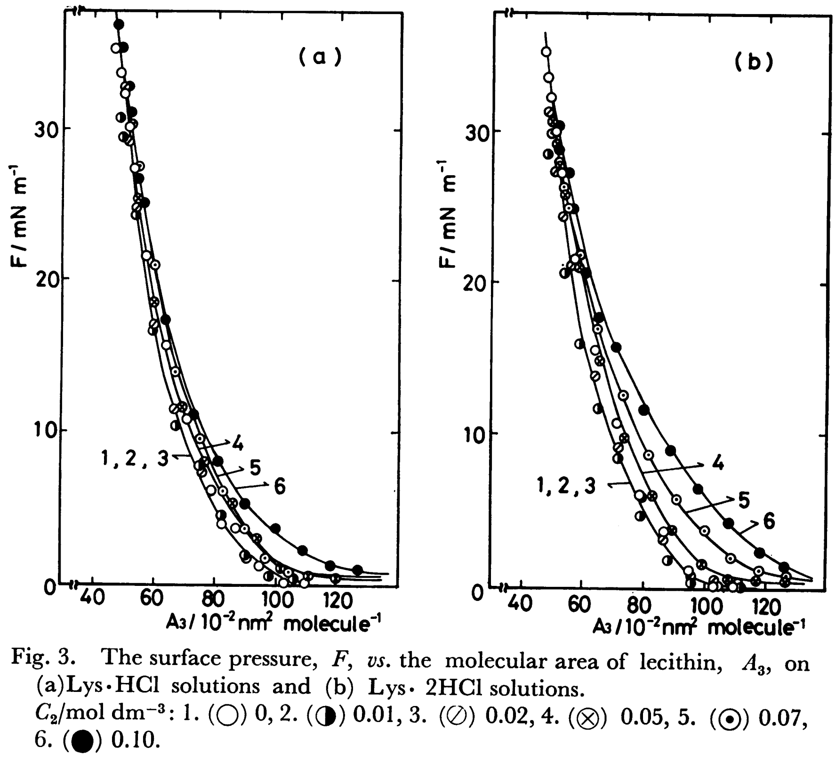


Fig. 3. The surface pressure, F, *vs.* the molecular area of lecithin, A_3, on (a)Lys·HCl solutions and (b) Lys· 2HCl solutions.
C_2/mol dm^{-3}: 1. (○) 0, 2. (◑) 0.01, 3. (⊘) 0.02, 4. (⊗) 0.05, 5. (⊙) 0.07, 6. (●) 0.10.

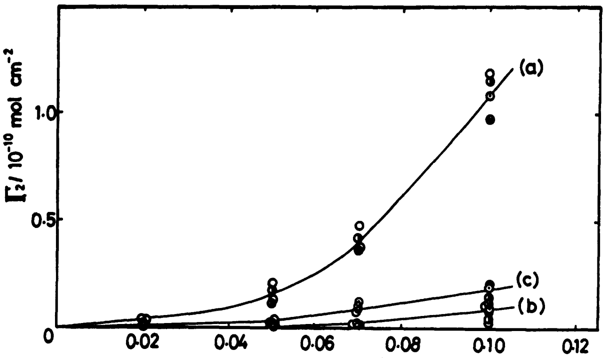


Fig. 4. The amounts of three differently charged lysine adsorbed onto DMPC monolayer, Γ_2, *vs.* C_2. (a) Lys, (b) Lys·HCl, (c) Lys·2HCl.
$A_3/10^{-2}$ nm^2 molecule^{-1}: ⊗ 100, ⊙90, ◑80, ○ 75.

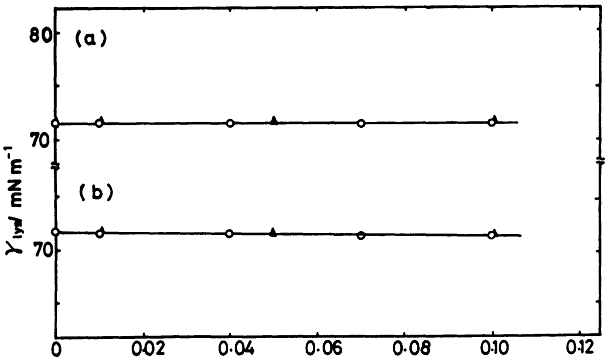


Fig. 5. Surface tension of various lysine solutions. (a) Lys·HCl, (b) Lys·2HCl. ▲ In salt-free water, ○ with 0.5 mol dm^{-3} NaCl.

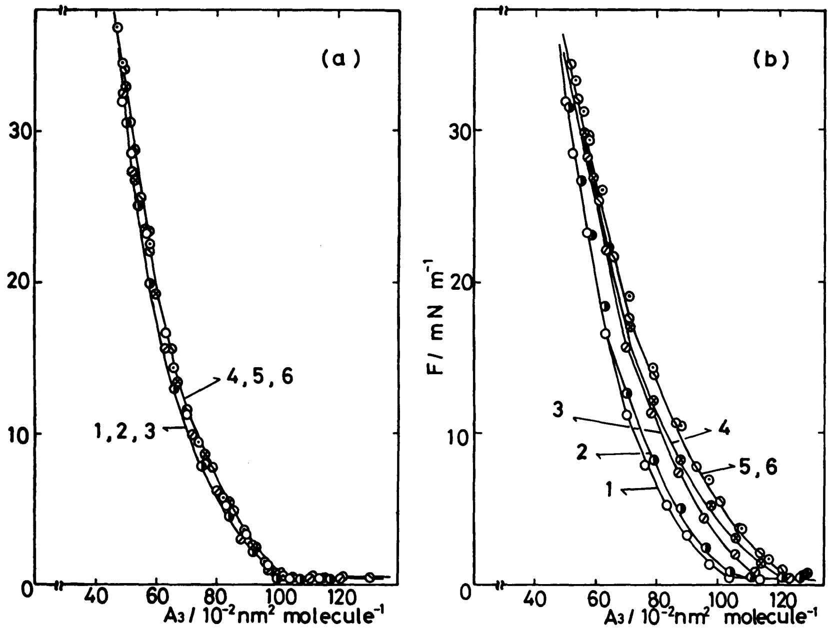


Fig. 6. F–A_3 curves of the DMPC monolayers in the presence of 0.5 mol dm^{-3} NaCl. (a) On Lys·HCl solutions, (b) on Lys·2HCl solutions.
C_2/mol dm^{-3}: 1. (○) 0, 2. (◑) 0.01, 3. (⊘) 0.02, 4. (⊗) 0.05, 5. (⊙) 0.07, 6. (⦸) 0.10.

may be considered that at an interface, a mixed monolayer of DMPC and lysine is formed as the result of the penetration of lysine into a DMPC monolayer. The mole fraction of lysine, in this mixed monolayer, X_2, and the average area per molecule of the monolayer, A_{23}, are given by:

$$X_2 = \frac{\Gamma_2}{\Gamma_2+\Gamma_3}, \tag{4}$$

and

$$A_{23} = \frac{1}{(\Gamma_2+\Gamma_3)N_A}, \tag{5}$$

respectively, where Γ_3 is the moles of DMPC per unit area $(=1/(A_3 \cdot N_A))$, and N_A is the Avogadro number. The relation between X_2 and A_{23} at a given surface pressure is shown in Figs. 7(a) and (b) in the cases of Lys·HCl and Lys·2HCl, respectively. It was found that, in each case, the value of A_{23} increased with the increase in the mole fraction of lysine, X_2. Because of the relatively small amount of penetration, the value of X_2 was smaller than 0.1 or 0.15 in the case of Lys·HCl or Lys·2HCl, respectively, even at a low pressure region, in spite of that the concentration of lysine solution, C_2, was as high as 0.1 mol dm^{-3}.

It can be seen in Fig. 7 that the partial molecular area of DMPC on the solution, $\bar{A}_3$, is almost constant in the region other than at very small X_2-value, but the value of $\bar{A}_3$ is not equal to that on pure water $(X_2=0)$, $A_3{}^\circ$. The amount of lysine hydrochloride adsorbed onto a DMPC monolayer, Γ_2, was therefore, recalculated by substituting in Eq. 2 the value of $\bar{A}_3$ obtained from Fig. 7, instead of A_3°, as described in the previous paper.[8] This will be called the second approximation. The successive approximation was allowed to proceeded three times by which the value of Γ_2 had converged to a defined value. The values, thus obtained, are shown in

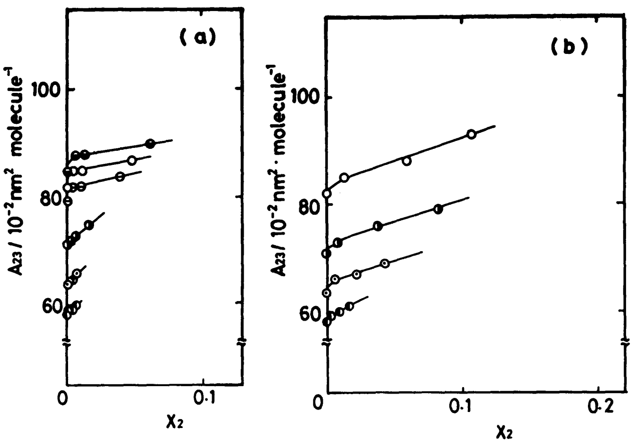


Fig. 7. The relation of the average area per molecule of a lysine–DMPC mixed monolayer, A_{23}, and the mole fraction of lysine, X_2. (a) Lys·HCl, (b) Lys·2HCl. F/mN m^{-1}: ◓ 4, ○ 5, ⊖ 6, ◑ 10, ⊙ 15, ○ 20.

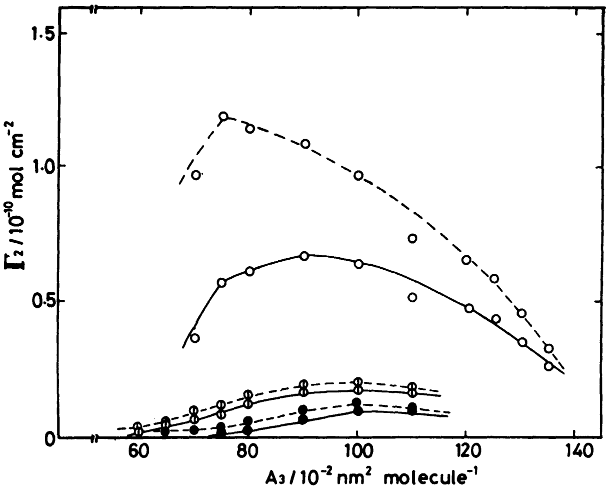


Fig. 8. The converged values of the amount of lysine adsorbed onto DMPC monolayer, when the concentration of solution, C_2, is 0.10 mol dm^{-3}. ● Lys·HCl, ⦶ Lys·2HCl. Broken lines show the values obtained on the assumption that $\bar{A}_3 = A_3^\circ$. Symbols (○) designate the values in the case of Lys.[8]

Fig. 8 by solid lines for example where the concentration of each lysine solution, C_2, is 0.10 mol dm^{-3}. Broken lines show the values calculated on the assumption that $\bar{A}_3 = A_3^\circ$. The data of lysine (free base)[8] is also shown in open circles(○). Although the converged values are smaller than the values obtained on the assumption that $\bar{A}_3 = A_3^\circ$, the sequence of the larger adsorbed amount was not changed, *i.e.*, it was Lys>Lys·2HCl>Lys·HCl, in each approximation, and this sequence was independent of the value of C_2. This order also coincided with the extent of shifts in surface pressure-area curves (Fig. 3). The result that Lys$^+$ has three units of electric charge (two + and one −) and is more hydrophilic than Lys^{2+} which has only two units of electric charges. Positive and negative charges in Lys$^+$ can interact with water molecules separately, in contrast to the case of macromolecules, such as proteins at the isoelectric point. In the latter case, the effect of the positive and negative electric charges would fairly compensate with each other since the distance to the water molecule at the surface of the macromolecule may be larger than the distance between the positive and negative charges.

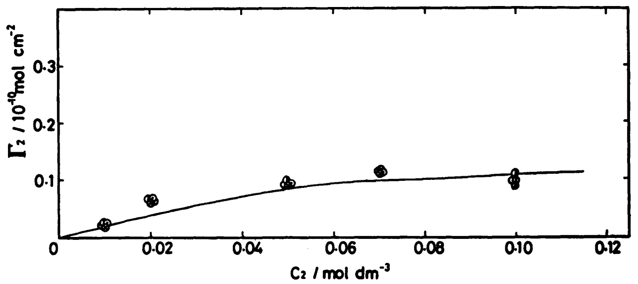


Fig. 9. The converged values of the amount of Lys·2HCl adsorbed onto DMPC monolayer, Γ_2, *vs.* C_2, in the presence of 0.5 mol dm^{-3} NaCl. $A_3/10^{-2}$ nm^2 molecule^{-1}: ⊗ 100, ⊙ 90, ◑ 80, ○75.

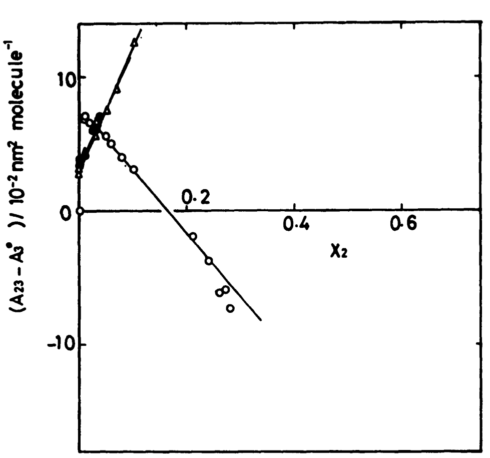


Fig. 10. $(A_{23} - A_3^\circ)$ *vs.* X_2, obtained by the last approximation. ● Lys·HCl, △ Lys·2HCl. Symbols (○) designate the values in the case of Lys.[8]

The Effect of Added Electrolytes. The adsorbed amount of lysine, Γ_2, in the presence of electrolytes was calculated from Figs. 6(a) and (b), by using Eq. 2. When lysine hydrochloride $[(\mathrm{Lys}^{+z_+})(\mathrm{Cl}^-)_{\nu_-}]$ (its concentration, C_2) and NaCl(C_3) are contained in the solution, the coefficient, i, in Eq. 2 is given by

$$i = 1 + \nu_- \frac{\nu_- C_2}{\nu_- C_2 + C_3}. \qquad (3)$$

In this experiment, ν_- is equal to 1 or 2 in the case of Lys·HCl or Lys·2HCl, respectively, C_2 is 0—0.1 mol dm^{-3}, and C_3 is 0.5 mol dm^{-3}. Figure 9 shows the results thus obtained in the case of Lys·2HCl. The converged Γ_2-values are shown in this figure. It is seen that the values of Γ_2 in the presence of 0.5 mol dm^{-3} NaCl did not differ much from those in the absence of NaCl shown in Fig. 8. The same was also seen for Lys·HCl, though it is not shown in the figure. These facts indicate that the adsorption of lysine is not appreciably affected by the added NaCl electrolyte.

The Interaction between Charged Lysine and DMPC.
The relation between the mole fraction of lysine, X_2, and the average area per molecule of the mixed monolayer, A_{23}, was obtained again, by using recalculated

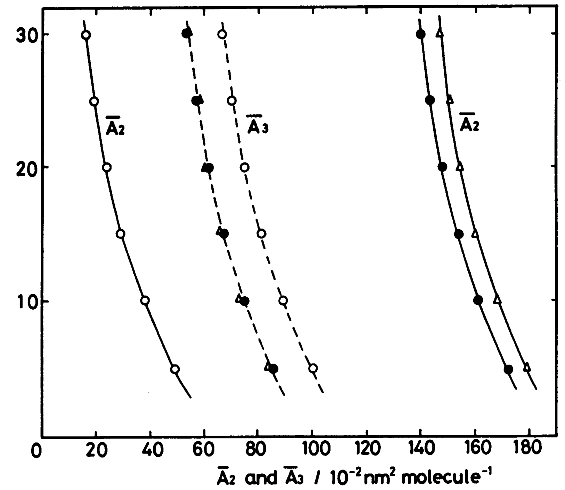


Fig. 11. The relation of the surface pressure, F, and the partial molecular areas of lysine, $\bar{A}_2$, and DMPC, $\bar{A}_3$. Solid lines show the values of $\bar{A}_2$, and broken lines show those of $\bar{A}_3$. ● Lys·HCl, △ Lys·2HCl. Symbols (○) designate the values in the case of Lys.[8]

values of Γ_2, in the case of lysine hydrochloride, and the values of $(A_{23}-A_3^\circ)$ were plotted against X_2 in Fig. 10. The open circles designate in the case of Lys. As the result of subtracting A_3° from A_{23}, the values of $(A_{23}-A_3^\circ)$ came on to one curve, independent of the surface pressure, F. It has also been found that $(A_{23}-A_3^\circ)$ increased with X_2 in the case of Lys·HCl of Lys·2HCl, on the contrary to the decrease in the case of Lys. This indicates that the positively charged lysine molecule which had penetrated into a DMPC monolayer occupied a larger area than lecithin. From Fig. 10, the relation between X_2 and A_{23} was obtained by the least-square method, and described in the cases of Lys·HCl and Lys·2HCl as follows:

$$A_{23} = A_3^\circ + 3.4 + 86.3X_2 \qquad \text{(for Lys·HCl)} \qquad (6)$$

$$A_{23} = A_3^\circ + 3.2 + 93.6X_2 \qquad \text{(for Lys·2HCl)}, \qquad (7)$$

where Eq. 6 is, for the X_2 value smaller than 0.1, and Eq. 7 is for X_2 smaller than 0.15. The experimental errors of the constant in Eqs. 6 and 7 are 3.4±0.5, 86.3∓3.5, 3.2±0.5, and 93.6∓3.0, respectively. From these equations, the partial molecular area of lysine, $\bar{A}_2$, and that of DMPC, $\bar{A}_3$, are calculated respectively as follows:

$$\begin{cases} \bar{A}_2 = A_3^\circ + 89.7(\pm 3.0) \\ \bar{A}_3 = A_3^\circ + 3.4(\mp 0.5) \end{cases} \qquad \text{(for Lys HCl)} \qquad (6a)$$

$$\begin{cases} \bar{A}_2 = A_3^\circ + 96.8(\pm 2.5) \\ \bar{A}_3 = A_3^\circ + 3.2(\mp 0.5) \end{cases} \qquad \text{(for Lys·2HCl)} \qquad (7a)$$

These are shown in Fig. 11. The values of A_2 and $\bar{A}_3$ in the case of Lys are also shown with open circles. Solid lines and broken lines show the values of $\bar{A}_2$ and $\bar{A}_3$, respectively. The partial molecular area of DMPC, $\bar{A}_3$, was almost the same for both Lys·HCl and Lys·2HCl, although it was a little smaller than for Lys. On the other hand, the partial molecular area of lysine, $\bar{A}_2$, for Lys·HCl and Lys·2HCl, was remarkably larger than that for Lys, and the value of Lys·2HCl, which has Lys^{2+}, was a little larger than that of Lys·HCl, which has Lys^{+}. From this experiment on lysine hydrochloride, the following conclusions were deduced: 1) the amount of positively charged lysine adsorbed onto a DMPC monolayer is small because of its stronger hydrophilicity, and 2) the remarkable expansion of a monolayer is caused by electrostatic repulsion in the monolayer, at the time as the penetration of the charged lysine.

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