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The Penetration of Lysine into the Monolayers of Lecithin from the Underlying Aqueous Solutions

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The surface pressure of the monolayers of dimyristoyl phosphatidylcholine (DMPC) on the lysine solutions was measured. The monolayer was more expanded on the solution than on pure water. The amount of lysine penetrating into the DMPC monolayer was calculated by means of a successive approximation. It was found that the amount of lysine penetrating increased with the increase in the concentration of the solution and with the decrease in the area per molecule of a DMPC monolayer, but it decreased with the further decrease in the area per molecule of DMPC. The average area per molecule of the monolayer consisting of lysine and DMPC was linearly related to the mole fraction of lysine. This linear relation indicates that partial molecular areas of both DMPC and lysine are independent of the amount of lysine in the monolayer. It is concluded that lysine and DMPC do not interact in the monolayer, although the monolayer of lysine and DMPC is formed as a result of the interaction between DMPC and lysine in a subsolution.

It has been widely believed, according to the fluid mosaic model of Singer and Nicolson,[1)] that the biomembranes consist essentially of bimolecular lipid layers in which some proteins are dissolved. Much attention has been paid to the studies of the penetration of proteins into phospholipid monolayers, since lipid molecules in monolayers are oriented just as in the bimolecular layers. In these studies, the increase in either the area per molecule at a constant surface pressure or the surface pressure at a constant area per molecule has been measured for a phospholipid monolayer spread on an aqueous solution,[2,3)] and the formation of mixed monolayers of phospholipid and protein has been discussed.[4)] The monolayer method has thus been proved to be useful for investigating the lipid-protein interaction at the liquid-air interface.

In this work, the change in the surface pressure, caused by the penetration of L-lysine into the monolayer of lecithin, was measured. L-Lysine is an essential amino acid which is indispensable to growth and which plays various other important roles, for example, in the regulation of emzyme reactions[5)] and in inactivating viruses,[6)] and lecithin is the most typical component of biomembranes. As the lecithin, L-α-dimyristoyl phosphatidylcholine(DMPC) was used in this work; the amount of lysine penetrating into the monolayer of DMPC was calculated using the data obtained. The features of the interaction between lysine and DMPC were discussed.

Experimental

Materials. L-Lysine(free base) from Nakarai Chemicals, Ltd, was dissolved in redistilled water. DMPC from the Sigma Company was dissolved in distilled chloroform and the solution preserved in a refrigerator for several days before use.

Surface-tension Measurement. The surface tension of the lysine solution, $\gamma_{\rm lys}$, was measured by the capillary-height method, while the surface pressure of lysine, $F_{\rm lys}$, was obtained by means of Eq. 1:

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

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

Surface-pressure Measurement. The surface pressure of the DMPC monolayer was measured by Wilhelmy's plate method. Since the surface tension of a subsolution is not always equal to that of pure water, the surface pressure, F, was calculated by means of Eq. 2:[7)]

$$F = \gamma_0 - \gamma = F' + F_{\rm lys}, \tag{2}$$

where γ is the surface tension of a lysine solution covered with a lipid monolayer, while F' is the practical surface pressure defined by:

$$F' = \gamma_{\rm lys} - \gamma, \tag{3}$$

and is measured directly by Wilhelmy's plate method as the decrease in the surface tension of a lysine solution upon the spreading of a lecithin monolayer. That is:

$$F' = (T_{\rm lys} - T')/l, \tag{4}$$

where l is the length around the horizontal cross section of the plate. The values of $T_{\rm lys}$ and T' were measured as follows. A lysine solution of 57 cm^3 was poured into a trough (5.0 cm×20.0 cm×0.4 cm) coated with Teflon: the temperature of the solution was kept at 25±0.5 °C by circulating thermostatted air. After the surface of lysine solution had been swept by a barrier covered with a Teflon sheet, a ground glass plate was suspended vertically on the liquid surface and the force acting on the plate, $T_{\rm lys}$, was measured by means of a torsion balance, Shimadzu T-NR. The value of $T_{\rm lys}$ was measured several times until the equilibrium value was obtained. Then a lipid monolayer was spread from an AGLA micrometer syringe(accuracy ±0.0002 cm^3) and left for 5 min. Then, the force acting on the plate, T', was measured by compressing the monolayer with a barrier. The equilibrium value of T' was obtained within 10 min.

The trough was used after any contaminants had been wiped off with ethanol and it had been rinsed well with redistilled water. The Teflon sheet and the ground glass plate were used after they had been immersed in heavy chromic acid and in fuming nitric acid respectively for more than one night and then rinsed well with redistilled water.

Results

Surface Tension of the Lysine Solution. Figure 1 shows the surface tension of a lysine solution. It is seen that the surface tension decreases slightly with the increase in the concentration of lysine, C_2. There-

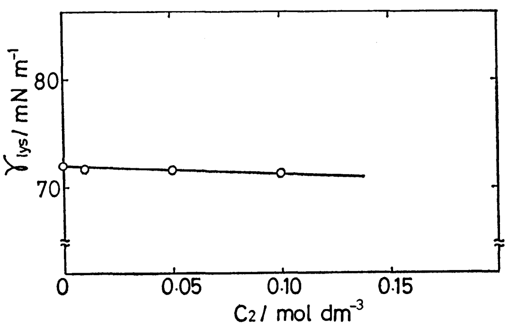

Fig. 1. The relation of surface tension, γ_{lys}, and the concentration of lysine, C_2.
Temp 25±0.5 °C.

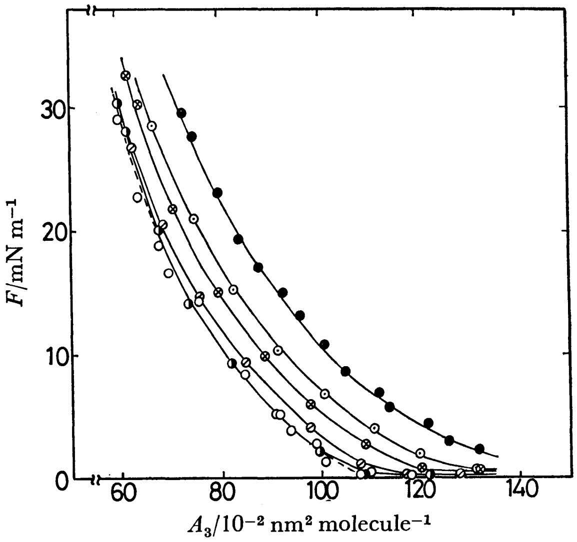

Fig. 2. F-A_3 curves of the DMPC monolayers on the lysine solutions.
C_2/mol dm^{-3}: ○ 0, ◑ 0.01, ⊘ 0.02, ⊗ 0.05, ⊙ 0.07, ● 0.10.

fore, lysine is not adsorbed appreciably onto the liquid-air interface.

Surface Pressure-area Curves of the DMPC Monolayers. Figure 2 shows the relation between the surface pressure of the DMPC monolayer on an L-lysine solution, F, and the area per molecule of DMPC, A_3. When the concentration of lysine, C_2, is lower than 0.01 mol dm^{-3}, the F-A_3 curve of the DMPC monolayer on a lysine solution nearly coincides with that on pure water. This is because the amount of lysine penetrating into or adsorbed onto the monolayer is not so large as to change the surface pressure. When C_2 is higher than 0.01 mol dm^{-3}, the F-A_3 curve of the higher C_2 shifts to the right with an increase in C_2. This is attributable to the fact that the monolayer is expanded by the adsorption or penetration of lysine from the subsolution.

The Amount of Lysine Adsorbed. The relation between the surface pressure, F, and the concentration of lysine, C_2, at a constant area per molecule of lecithin, A_3, is shown in Fig. 3, from which the amount adsorbed may be calculated by use of Gibbs' adsorption equation:

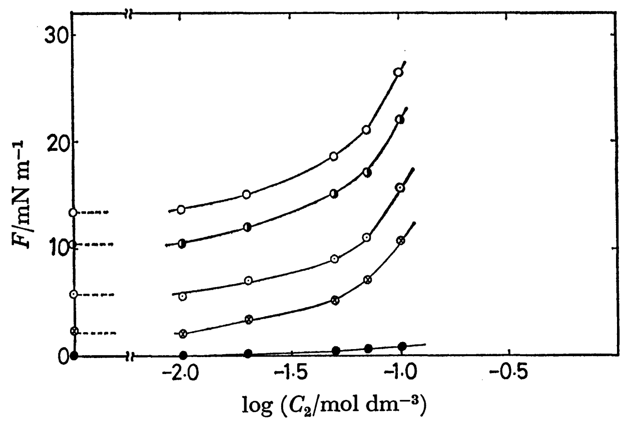

Fig. 3. The relation of the surface pressure, F, and the concentration of lysine, log C_2, at various areas per molecule of DMPC, A_3.
A_3/10^{-2} nm^2 molecule^{-1}: ● No insoluble monolayer, ⊗ 100, ⊙ 90, ◑ 80, ○ 75.

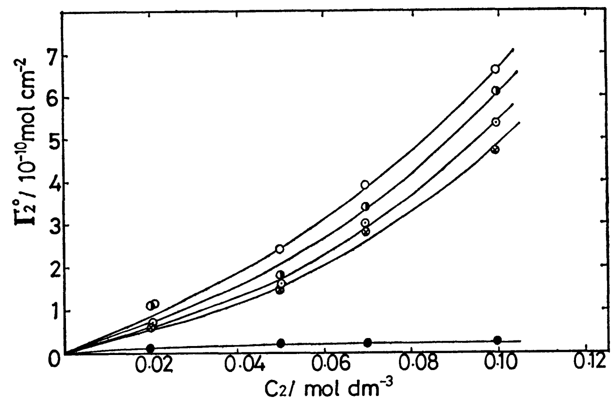

Fig. 4. The amount of lysine adsorbed onto DMPC monolayer, Γ_2°, *vs.* C_2.
Calculated from Eq. 5. A_3/10^{-2} nm^2 molecule^{-1}: ● No insoluble monolayer, ⊗ 100, ⊙ 90, ◑ 80, ○ 75.

$$\Gamma_2^\circ = \frac{1}{RT}\left(\frac{\partial F}{\partial \ln C_2}\right)_{A_3}, \tag{5}$$

where R is the gas constant, T is the absolute temperature, and Γ_2° is the amount of lysine adsorbed onto the portion of the interface where lecithin does not exist. The value of Γ_2° thus obtained is shown in Fig. 4 as a function of the concentration of lysine, C_2. It seems that lysine is not adsorbed appreciably onto the interface without a lecithin monolayer, but the amount of lysine adsorbed increases with the increase in C_2 when a lecithin monolayer exists at the interface.

In Eq. 5, the existence of an insoluble component, DMPC, at the interface is disregarded. Actually, however, both adsorbed lysine and the DMPC monolayer coexist at the interface, and the part of the interface already occupied by DMPC is not available for the adsorption of lysine. Gibbs' adsorption equation 5 is, therefore, modified by taking the partial molecular area of DMPC, $\bar{A}_3$, into account. The designed modification of Gibbs' adsorption equation is given by:

$$\Gamma_2 = (1-\bar{A}_3/A_3)\Gamma_2^\circ, \tag{6}$$

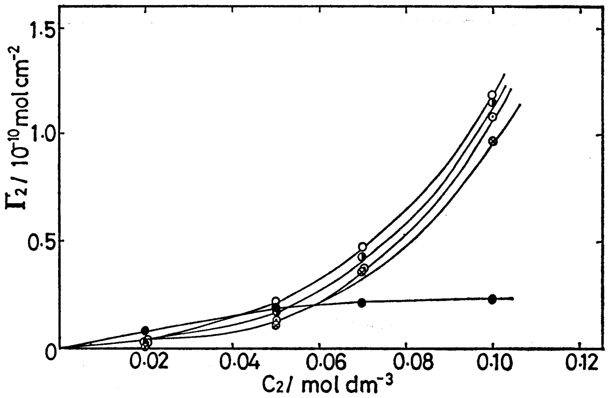

Fig. 5. The amount of lysine adsorbed onto DMPC monolayer, Γ_2, *vs.* C_2.
Calculated from Eq. 7. $A_3/10^{-2}$ nm^2 molecule^{-1}: ● No insoluble monolayer, ⊗ 100, ⊙ 90, ◑ 80, ○ 75.

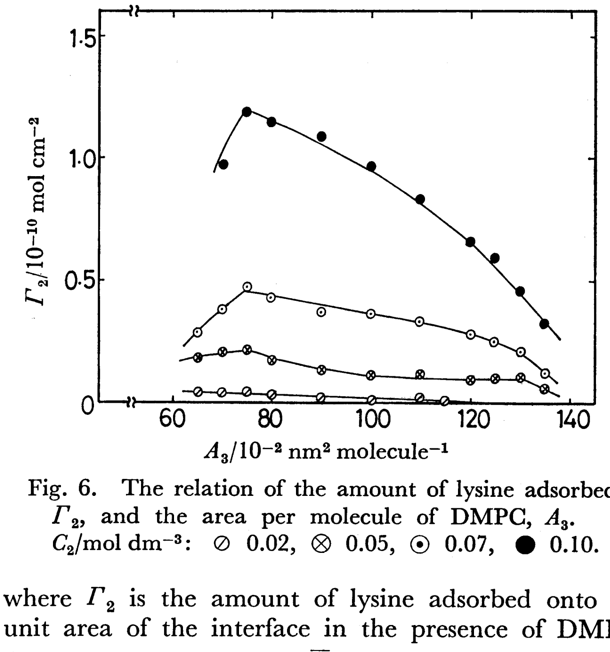

Fig. 6. The relation of the amount of lysine adsorbed, Γ_2, and the area per molecule of DMPC, A_3.
C_2/mol dm^{-3}: ⊘ 0.02, ⊗ 0.05, ⊙ 0.07, ● 0.10.

where Γ_2 is the amount of lysine adsorbed onto the unit area of the interface in the presence of DMPC. According to Pethica,[8] $\bar{A}_3$ is approximated by the molecular area of DMPC on pure water, A_3°; Eq. 6 is, therefore, rewritten as follows:

$$\Gamma_2 = (1 - A_3^\circ/A_3)\Gamma_2^\circ. \tag{7}$$

Hereafter, this will be called the first approximation. This equation has been used to calculate the amount of solute molecules adsorbed onto the monolayer on subsolutions.[9,10]

The relation between the amount of lysine adsorbed, Γ_2, as calculated from Eq. 7, and the concentration of lysine, C_2, is shown in Fig. 5. The amount of lysine adsorbed decreased in the presence of an insoluble component at the interface, but it is still larger than the value in the case of no insoluble component, at least in the high-C_2 region. This would indicate that there exists a strong affinity between lysine and lecithin.

If the concentration of lysine, C_2, is kept constant, the amount of lysine adsorbed, Γ_2, changes with the area per molecule of DMPC, A_3, as is shown in Fig. 6. The amount adsorbed is higher at a higher concentration of lysine, and the curve has a maximum at high values of C_2. This would indicate the following points. As has already been shown in Fig. 1, lysine is not adsorbed appreciably onto a clean interface. The value of Γ_2 is, therefore, small when A_3 is large. Lysine is, however, adsorbed more onto a lecithin monolayer than onto a clean interface, and the amount adsorbed increases with the density of lecithin, that is, with the decrease in A_3. However, lysine is excluded from the monolayer, and the amount adsorbed decreases with the further decrease in the area per molecule of lecithin.

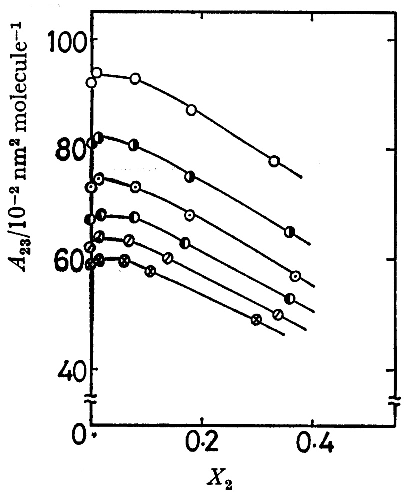

Fig. 7. The relation of the average area per molecule, A_{23}, of a lysine-DMPC mixed monolayer, and the mole fraction of lysine, X_2.
F/mN m^{-1}: ○ 5, ◑ 10, ⊙ 15, ◐ 20, ⊘ 25, ⊗ 30.

Discussion

The Mixed Monolayers Formed by the Penetration of Lysine. It may be stated that a mixed monolayer of lysine and DMPC is formed as a result of the penetration of lysine into a DMPC monolayer when a lysine solution is used as a subphase. The mole fraction of lysine in this mixed monolayer, X_2, is given by:

$$X_2 = \frac{\Gamma_2}{\Gamma_2 + \Gamma_3}. \tag{8}$$

Here, Γ_3 is the moles of DMPC per unit of area defined by:

$$\Gamma_3 = \frac{1}{A_3 \cdot N_A}, \tag{9}$$

where N_A is the Avogadro number. The average area per molecule of a lysine–DMPC mixed monolayer, A_{23}, is defined by:

$$A_{23} = \frac{1}{(\Gamma_2 + \Gamma_3)N_A}. \tag{10}$$

The relation between the average area per molecule and the mole fraction of lysine at a constant surface pressure is shown in Fig. 7. It may be seen from this figure that the partial molecular areas of both DMPC and lysine are dependent on X_2, but they

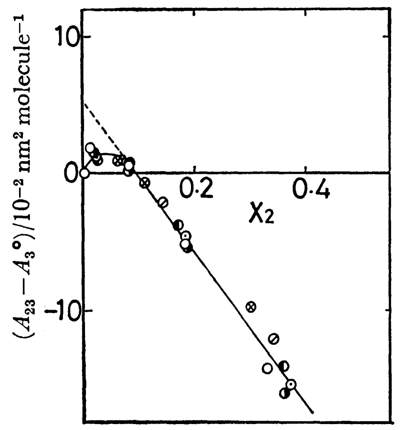

Fig. 8. $(A_{23}-A_3{}^\circ)$ *vs.* X_2.
F/mN m^{-1}: ○ 5, ◑ 10, ⊙ 15, ◐ 20, ⊘ 25, ⊗ 30.

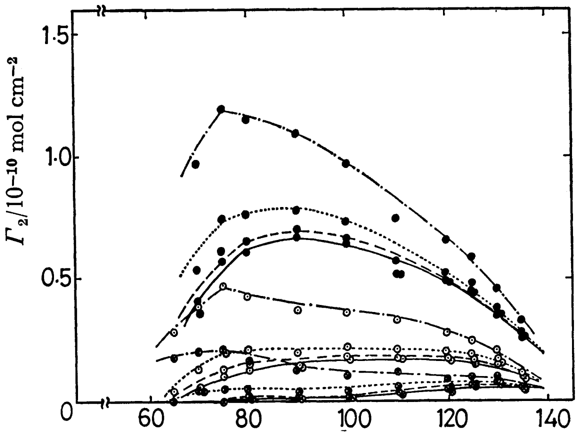

Fig. 9. The amount of lysine adsorbed onto DMPC monolayer, Γ_2, *vs.* A_3.
—·—·: By the 1st approximation, ······: by the 2nd approximation, ----: by the 3rd approximation, ——: by the 4th approximation.
C_2/mol dm^{-3}: ⊗ 0.05, ⊙ 0.07, ● 0.10.

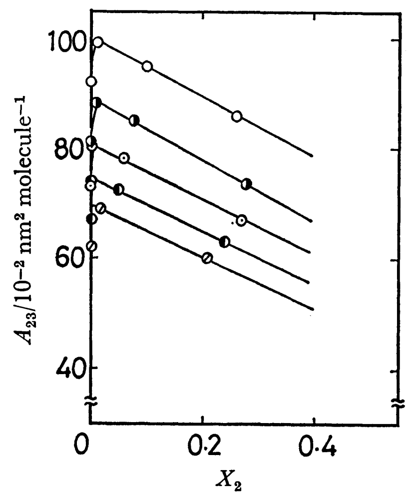

Fig.10. The relation of the average area per molecule, A_{23}, and the mole fraction of lysine, X_2, by the 4th approximation.
F/mN m^{-1}: ○ 5, ◑ 10, ⊙ 15, ◐ 20, ⊘ 25.

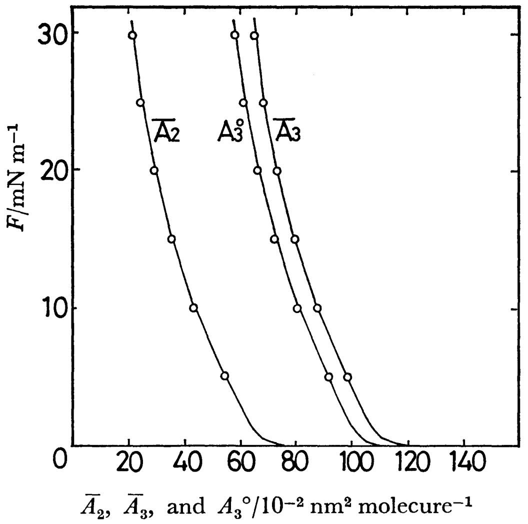

Fig.11. The relation of the surface pressure, F, and the partial molecular areas of lysine, $\bar{A}_2$, and DMPC, $\bar{A}_3$.
The F-A_3 curve of DMPC on pure water was designated as $A_3{}^\circ$.

are practically independent of X_2 and are nearly constant in the region where X_2 is larger than 0.07. By extrapolating the linear portion of X_2, it is found that the partial molecular area of DMPC on a lysine solution, $\bar{A}_3$, is not equal to that on pure water, $A_3{}^\circ$, at each surface pressure. This indicates that the approximation, $\bar{A}_3=A_3{}^\circ$, used in Eq. 7 was not appropriate.

Since all the curves on Fig. 7 had the same shape, $(A_{23}-A_3{}^\circ)$ was plotted against X_2 in Fig. 8. It was found that all the plots come on one curve and that $(A_{23}-A_3{}^\circ)$ is linearly related to X_2 when X_2 is larger than 0.07. This linear portion is written by:

$$A_{23}-A_3{}^\circ = 5.1-55.0X_2, \qquad (11)$$

and when X_2 is equal to zero, Eq. 11 is rewritten as follows:

$$\bar{A}_3 = A_3{}^\circ + 5.1. \qquad (12)$$

The amount adsorbed, Γ_2, was, therefore, recalculated by Eqs. 6 and 12; this will be called the second approximation. The successive approximation was repeated until the value of Γ_2 converged to a defined value.

The approximation was repeated in this way, by using $\bar{A}_3-A_3{}^\circ=6.5$ for the third and 7.0 for the fourth approximation. The values of Γ_2 thus obtained are plotted against A_3 in Fig. 9. Although the value of Γ_2 decreased with higher approximations, the general features did not change; *i.e.*, (1) Γ_2 increases with the increase in C_2 and (2) Γ_2 increases with the decrease in A_3 at a constant C_2, but decreases at a small value of A_3.

The Interaction between Lysine and DMPC. The relation between the average area per molecule, A_{23}, and the mole fraction of lysine, X_2, obtained by the fourth approximation is shown in Fig. 10. By comparing it with Fig. 7, we may conclude that A_{23} is linearly related to X_2, in the higher approximations at each surface pressure and that the relation may be expressed by the following empirical equation:

$$A_{23} = A_3^\circ + 7.0 - 44.0X_2. \tag{13}$$

This yields the following equations for the partial molecular areas of lysine and DMPC, $\bar{A}_2$ and $\bar{A}_3$, respectively:

$$\begin{aligned} \bar{A}_2 &= A_3^\circ - 37.0, \\ \bar{A}_3 &= A_3^\circ + 7.0. \end{aligned} \tag{13a}$$

The dependence of these quantities on the surface pressure is shown in Fig. 11.

The linear relation between A_{23} and X_2 shown in Fig. 10 thus shows that the partial molecular areas of both DMPC and lysine are independent of the extent of mixing as long as X_2 is smaller than about 0.3. This means that lysine and DMPC do not interact in the monolayer, although a mixed monolayer is formed by the interaction between lysine and DMPC. It can, therefore, be concluded, with regard to the intermolecular interaction between two components of a mixed monolayer formed by the penetration of one component, that the lateral interaction in the monolayer is quite different from the longitudinal interaction effective for the formation of a mixed monolayer, as is naturally to be expected from the high degree of orientation of molecules in the monolayer.

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