Penetration of Leucine and Norleucine into Lecithin Monolayers from Underlying Aqueous Solutions

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The surface pressure of the monolayers of dimyristoyl phosphatidylcholine(DMPC) on leucine and norleucine solutions was measured and the amounts of these amino acids which had penetrated into the DMPC monolayer were calculated by means of a successive approximation. Although the amounts of leucine and norleucine adsorbed onto the solution surface were nearly equal and were larger than that of lysine, the amount of leucine which had penetrated into the DMPC monolayer was remarkably small. That of norleucine was not so small, but was still smaller than that of lysine except for the region where the molecular area of DMPC is large. The partial molecular areas of amino acid, $\overline{A}_2$, and DMPC, $\overline{A}_3$, were calculated in each case. The value of $\overline{A}_2$, was the largest in the case of leucine and the smallest in the case of lysine, which means that the area occupied by lysine is smaller than that of norleucine because the interaction of ε-amino group with DMPC contracts the mixed monolayer. The change in the value of $\overline{A}_3$ due to the interaction with amino acid, however, was not large, which means that the change in the state of DMPC itself is not large and rather independent of the kind of amino acid.

It has been widely accepted that biomembranes such as protoplasmic membranes essentially consist of bimolecular lipid layers in which some proteins are dissolved.[1] The study of the penetration of proteins into lipid monolayers is, therefore, considered to be one of the useful techniques for the investigation of the structure and functions of biomembranes.[2,3] In order to discuss the interaction between lipids and proteins from this point of view, the penetration of L-lysine into lecithin monolayers was studied in our previous work.[4]

L-Leucine and L-norleucine have four carbon atoms in the side chains, which is the same as in the case of L-lysine. Leucine has a branched carbon chain, whereas norleucine does not. The side chain of lysine has an amino group at the end of the carbon side chain of norleucine. The effect of the ε-amino group of lysine and that of the branched carbon chain are, therefore, expected to become clear by the comparison of the features of the penetration of these amino acids into lecithin monolayers. In this work, the surface tension of aqueous solutions as well as the surface pressure of the solutions has been measured, and the amounts of these amino acids adsorbed onto the surface and penetrated into the monolayers, respectively, have been calculated. This will facilitate discussion of the interaction between these amino acids and lecithin.

Experimental

Materials. L-Leucine and L-norleucine from Nakarai Chemicals, Ltd., were dissolved in redistilled water. L-α-Dimyristoyl phosphatidylcholine (DMPC) from Sigma Company was used as lecithin; it was dissolved in distilled chloroform and preserved in a refrigerator until use. The stock solution was prepared every few days in order to avoid disintegration.

Surface-tension Measurement. Surface tension of amino acid solution, γ, was measured by the capillary-height method. The surface pressure of amino acid, F, was obtained by Eq. 1:

$$F = \gamma_0 - \gamma, \quad (1)$$

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. The details of this method were described in our previous paper.[4] The temperature of the solution was kept at 25±0.5 °C.

Results

Surface Tension of Amino Acid Solution. Figure 1 shows the surface tension of leucine or norleucine solution. The surface tension of lysine solution (Fig. 1 in Ref. 4) is also shown by a broken line. It is seen that the surface tension of the solution decreases slightly with the increase in the concentration of amino acid, C_2, both in the case of leucine and of norleucine. The extent of decrease was a little larger than that of lysine, but there were no differences between leucine and norleucine.

Surface Pressure-Area Curves of the DMPC Monolayers. Figure 2 shows the surface pressure-area curves of the DMPC monolayers spread on leucine solutions of various concentrations. The curve shifted only at the low surface pressure region when the concentration, C_2, was high. On the other hand, the surface pressure increased with increase in C_2, when norleucine solution was used, as shown in Fig. 3.

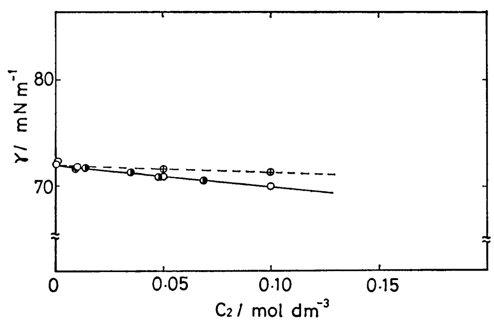

Fig. 1. The relation of surface tension, γ, and the concentration of solution, C_2.
○: L-Leucine, ◑: L-norleucine, ⊕: L-lysine. Temp: 25+0.5 °C.

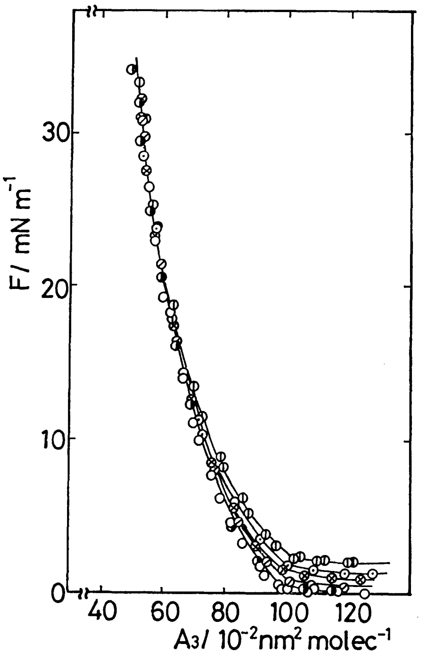

Fig. 2. F-A_3 curves of the DMPC monolayers on the leucine solutions.
C_2/mol dm^{-3}: ○; 0, ◑; 0.01, ⊘; 0.02, ⊗; 0.05, ⊙; 0.07, ⦶; 0.10.

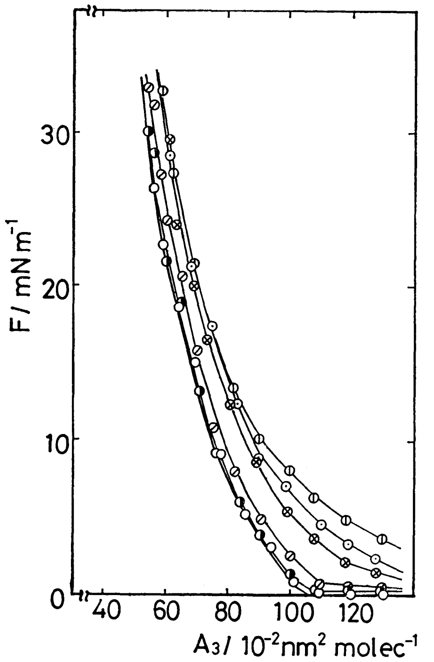

Fig. 3. F-A_3 curves of the DMPC monolayers on the norleucine solutions.
C_2/mol dm^{-3}: ○; 0, ◑; 0.01, ⊘; 0.02, ⊗; 0.05, ⊙; 0.07, ⦶; 0.10.

The Amount Which Had Been Adsorbed onto the Solution Surface or Which Had Penetrated into a DMPC Monolayer. The change of the surface pressure against the concentration of solution was obtained from the surface pressure-area curves (Figs. 2 and 3); this change is shown in Fig. 4 for leucine or in Fig. 5 for norleucine. The closed circles represent the values when no mono-

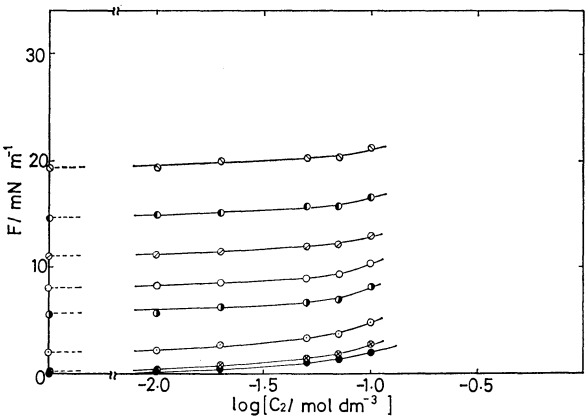

Fig. 4. The relation of the surface pressure, F, and the concentration of leucine solution, C_2, at various areas per molecule of DMPC, A_3.
$A_3/10^{-2}$ nm^2 molecule^{-1}: ●; no insoluble monolayer, ⊗; 100, ⊙; 90, ◑; 80, ○; 75, ⊘; 70, ◐; 65, ⦸; 60.

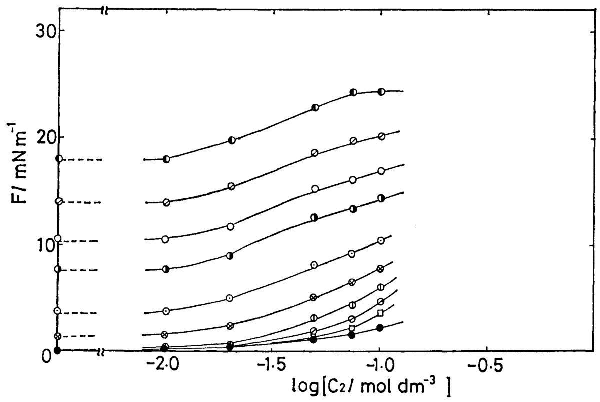

Fig. 5. The relation of the surface pressure, F, and the concentration of norleucine solution, C_2, at various areas per molecule of DMPC, A_3.
$A_3/10^{-2}$ nm^2 molecule^{-1}: ●; no insoluble monolayer, □; 130, ⊖; 120, ⦶; 110, ⊗; 100, ⊙; 90, ◑; 80, ○; 75, ⊘; 70, ◐; 65.

layer exists at the interface, as obtained from Fig. 1. It is seen from these two figures (Figs. 4 and 5), that the change of the surface pressure is very small in the case of leucine, and that in the case of norleucine the surface pressure increases with the increase in the concentration, C_2.

The amount which had been adsorbed onto the liquid-air interface or which had penetrated into a DMPC monolayer, Γ_2, was calculated by the following equation:

$$\Gamma_2 = (1/RT)(1-\overline{A}_3/A_3)(\partial F/\partial \ln C_2)_{A_3}, \qquad (2)$$

where R, T are the gas constant and the absolute temperature, respectively, $\overline{A}_3$ is the partial molecular area of DMPC which was approximated by the molecular area of DMPC on pure water, $A_3°$, by Pethica.[5] This equation, proposed by Pethica, has been used in order to calculated the amount of solute molecules penetrated into lipid monolayers.[6,7] Values of Γ_2 thus calculated are shown in Figs. 6 (a) and (b). It is seen that the amounts adsorbed onto the liquid-air interface of leucine and norleucine are nearly equal and are larger than that of lysine, whereas the amount of leucine penetrated into the DMPC monolayer is remarkably small and that of norleucine is not so small but is still smaller than that of lysine.

On the other hand, both the amount of leucine and of norleucine penetrated into a monolayer vary with the area per molecule of lecithin at constant C_2, as is shown in Figs. 7 (a) and (b). It is seen that the amount which penetrated is larger at the higher concentrations, and it is smaller at the smaller areas per molecule of lecithin, the latter suggesting that the molecules which have penetrated are excluded by the compression of the monolayer. It is also found that the amount of leucine which penetrated is always smaller than that of lysine, but the amount of norleucine which penetrated becomes larger than that of lysine when the area per molecule of lecithin is large.

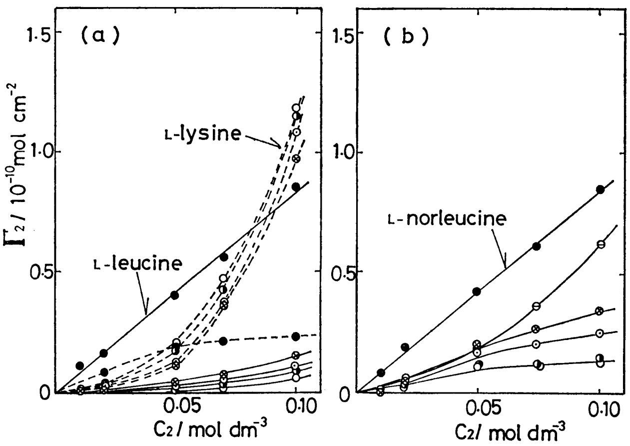

Fig. 6. The amount of amino acid penetrated into DMPC monolayer, Γ_2, *vs.* C_2.
(a): Leucine, (b): norleucine. The broken lines show the amounts of lysine in Ref. 4. $A_3/10^{-2}$ nm² molecule⁻¹: ●; no insoluble monolayer, ⊖; 120, ⊗; 100, ⊙; 90, ◑; 80, ○; 75.

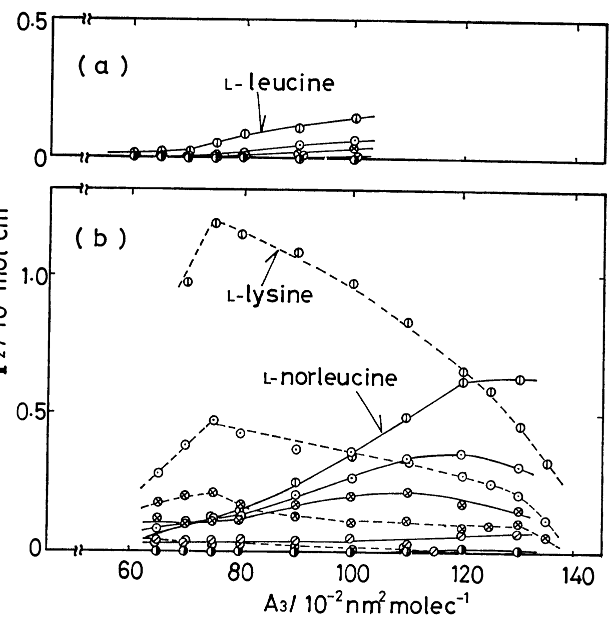

Fig. 7. The relation of the amount of amino acid penetrated, Γ_2, and the area per molecule of DMPC, A_3.
(a): Leucine, (b): norleucine. The broken lines show the amounts of lysine in Ref. 4. C_2/mol dm⁻³: ◑; 0.01, ⊘; 0.02, ⊗; 0.05, ⊙; 0.07, ⦶; 0.10.

Discussion

The Mixed Monolayers of DMPC and Leucine or Norleucine. It may be considered that a mixed monolayer of DMPC and leucine or norleucine is formed as the result of the penetration of these amino acids into a DMPC monolayer. The mole fraction of leucine or norleucine in this mixed monolayer, X_2, and the average area per molecule of the monolayer, A_{23}, are given by:

$$X_2 = \frac{\Gamma_2}{\Gamma_2 + \Gamma_3} \tag{3}$$

and

$$A_{23} = \frac{1}{(\Gamma_2 + \Gamma_3)N_A} \tag{4}$$

respectively, where Γ_3 is the moles of DMPC per unit area $(=1/(A_3 \times N_A))$, and N_A is the Avogadro number. The relation between X_2 and A_{23} is shown in Fig. 8 (a) in the case of leucine and in Fig. 8 (b) in the case of norleucine. The values of A_{23} vary with the surface pressure and with the mole fraction. The value of A_{23} increases with the increase in X_2 at each surface pressure in the case of leucine, and also in the case of norleucine, although the values of A_{23} stop increasing and start decreasing at a large X_2, as is shown for norleucine at the surface pressure of 5 mN m⁻¹. The curve with a maximum point is of the same shape as lysine,[4] although the slope of this curve is not so sharp as in the case of lysine.

The partial molecular area of DMPC on the solution, $\overline{A}_3$, obtained from Fig. 8, was not equal to that on pure water, $A_3°$, in the cases of both leucine and norleucine. The amount which penetrated, Γ_2, was,

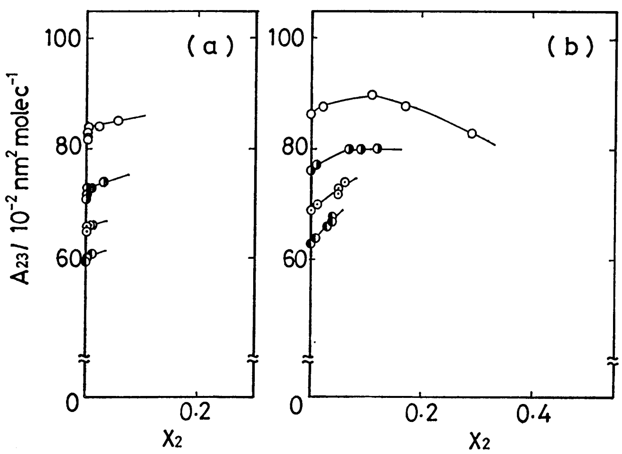

Fig. 8. The relation of the average area per molecule of an amino acid–DMPC monolayer, A_{23}, and the mole fraction of amino acid, X_2.
Obtained from Fig. 7. (a): Leucine, (b): norleucine. F/mN m⁻¹: ○; 5, ◑; 10, ⊙; 15, ◐; 20.

therefore, recalculated by substituting in Eq. 2 the value of $\overline{A}_3$ instead of $A_3°$, as described in Ref. 4. The amount thus obtained is shown in Figs. 9 (a) and (b). The features of the curve of Γ_2 as a function of A_3 did not vary, although the value of Γ_2 was a little smaller than that in Figs. 7 (a) and (b).

The Interaction between Leucine or Norleucine and DMPC. The mole fraction of leucine or norleucine, X_2, and the average area per molecule of the monolayer, A_{23}, were calculated again, by using recalculated values of Γ_2. The recalculated relation between X_2 and A_{23} is shown in Figs. 10 (a) and (b) in the cases of leucine and norleucine, respectively. From this figure, the relation between X_2 and A_{23} can be described as follows:

(1) for leucine,

$$A_{23} = A_3° + 1.7 + 38.4X_2 \tag{5}$$

at each surface pressure, F, when the values of X_2 are smaller than 0.10, and

(2) for norleucine,

$$A_{23} = A_3° + 13.0 - 27.0X_2 \tag{6}$$

in the region where F is smaller than 10 mN m^{-1} and the values of X_2 are from 0.04 to 0.30.

From these equations, the partial molecular area of amino acid, $\overline{A}_2$, and that of DMPC, $\overline{A}_3$, are written as follows:

$$\begin{cases} \overline{A}_2 = A_3° + 40.1 \\ \overline{A}_3 = A_3° + 1.7 \end{cases} \quad \text{(for leucine)} \tag{5a}$$

$$\begin{cases} \overline{A}_2 = A_3° - 14.0 \\ \overline{A}_3 = A_3° + 13.0 \end{cases} \quad \text{(for norleucine).} \tag{6a}$$

These are shown in Fig. 11. The values of $\overline{A}_2$ and $\overline{A}_3$ in the case of lysine as well as the molecular area of DMPC on pure water $A_3°$ (▲) are also shown in this figure. It is concluded from Fig. 11 that the interaction between leucine or norleucine and DMPC can be compared with that of lysine and DMPC as follows:

(1) The partial molecular area of amino acid, $\overline{A}_2$, is the largest in the case of leucine, and the smallest in the case of lysine. This would mean that the area occupied by lysine is smaller than that of norleucine, because the interaction of ε-amino group with DMPC contracts the mixed monolayer, while leucine occupies a larger area because of the branching of the carbon chain.

(2) The increase in the partial molecular area of DMPC, $\overline{A}_3$, due to the interaction with amino acid, is not large in comparison with the change in $\overline{A}_2$. This means that the change in the state of DMPC itself is not large, irrespective of the kind of amino acid.

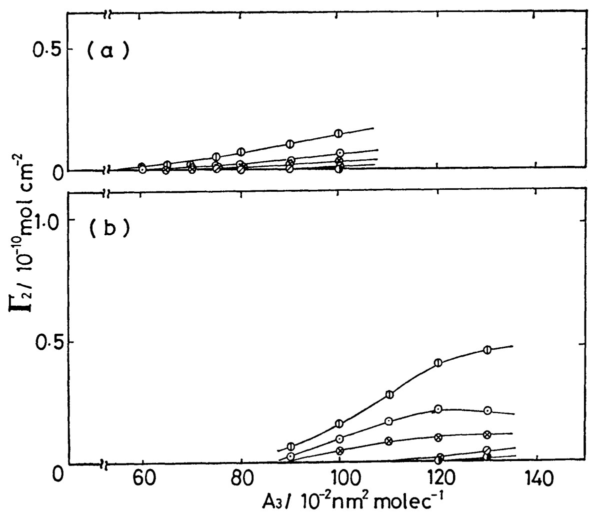

Fig. 9. The relation of the amount of amino acid penetrated, Γ_2, obtained by using the corrected value of $\overline{A}_3$, and the area per molecule of DMPC, A_3. (a): Leucine, (b): norleucine. C_2/mol dm^{-3}: ◑; 0.01, ⌀; 0.02, ⊗; 0.05, ⊙; 0.07, ⦶; 0.10.

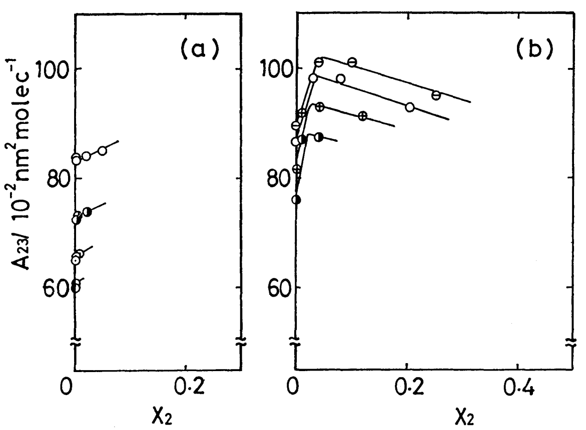

Fig.10. The relation of the average area per molecule of an amino acid–DMPC monolayer, A_{23}, and the mole fraction of amino acid, X_2.
Obtained from Fig. 9. (a): Leucine, (b): norleucine. F/mN m^{-1}: ⊖; 4, ○; 5, ⊕; 7, ◑; 10, ⊙; 15, ◐; 20.

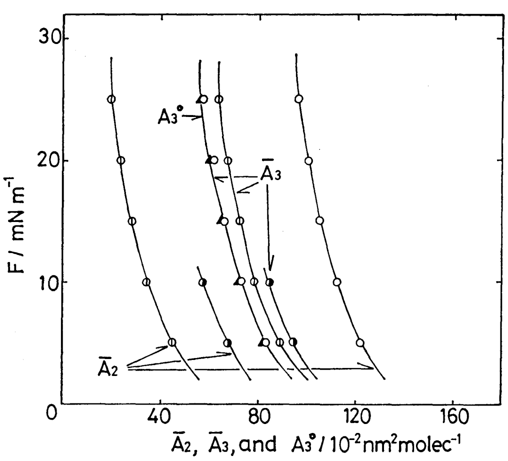

Fig.11. The relation of the surface pressure, F, and the partial molecular areas of amino acid, $\overline{A}_2$; DMPC on solution, $\overline{A}_3$; and DMPC on water, $A_3°$.
○: Leucine, ◑: norleucine, ⦶: lysine. Symbols(▲) designate the values of $A_3°$.

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