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Lipid-Protein Interaction in Mixed Monolayers from Phospholipids and Proteins

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The thermotropic behavior of different proteins with phospholipids in monolayers was investigated by measuring the surface pressure and the surface viscosity. When phospholipids, such as dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC), were mixed with the hydrophobic proteins, such as hemoglobin and β-casein, below their phase transition temperatures (T_c), a considerable expansion of monolayers was observed. On the other hand, when phospholipids were mixed with the hydrophilic proteins, such as bovine serum albumin (BSA) and lysozyme below T_c, no expansion was observed. When DMPC was mixed with different proteins at T_c or above, all mixtures formed slightly deviated monolayers from ideal mixing, independent of a variety of proteins. This type of behavior suggests that the hydrophobic proteins perturb the structure of boundary lipids near the protein molecule below T_c, while the hydrophobic proteins do not perturb the phospholipid structure below T_c because of their low hydrophobicities. At T_c or above the membrane properties are independent of a variety of proteins, because all lipid hydrocarbon chains cause a disordering, even without proteins. In addition, when DPPC was mixed with a hydrophobic protein, such as hemoglobin, the fluidity of monolayer increased significantly.

The importance of studying mixtures of lipids and proteins in membranes is obvious when one considers the currently accepted fluid mosaic model of membranes.[1] Good knowledge concerning lipid-protein interaction is a key to understanding the structure and function of biological membranes. Intimately related to the above subject is the question how different proteins affect the physical properties of biological membranes, such as membrane fluidity.

From this viewpoint, various theoretical models have been proposed to study the lipid-protein interaction and its effect on the properties of biological membranes.[2-6] A large body of experimental data on the lipid-protein interaction in membranes has also been accumulated during the last two decades by using different experimental techniques; e.g., differential scanning calorimetry (DSC), electron spin resonance (ESR), Raman spectroscopy, deuterium magnetic resonance (^{2}H NMR), fluorescence anisotropy (FA). Chapman et al.[7] have investigated the effect of proteins on the phase behavior of membranes by using DSC and noted that cytochrome C decreases the phase-transition temperature (T_c) of a lipid membrane. On the contrary, Verkleij et al.[8] have reported that the basic myelin protein increases the T_c of a membrane. Papahadjopoulos et al.[9] have reported that a variety of proteins has very different effects on the lipid phase transition from gel to liquid crystalline. In addition, the effect of proteins on lipid order in membranes has been intensively investigated by means of the above-mentioned techniques. That is, the results based on ESR, FA, and Raman spectroscopy indicate that proteins increase the lipid order.[10] On the other hand, ^{2}H NMR indicates that proteins decrease the lipid order.[11] In this way, no consensus has been attained as to whether proteins in biological membranes change the T_c of lipids or not and cause an ordering or disordering of the lipids.

According to the criteria of Papahadjopoulos et al.[9] the physical properties of biological membranes, are characteristic of a hydrophobic interaction between protein and lipid molecules. In addition, Jähnig[12] has demonstrated that the hydrophobic effect of proteins provides the main driving force for insertion into the membrane. Then, many investigators have attempted to define the hydrophobicity of proteins. A fluorescence probe method has been proposed for the evaluation of protein hydrophobicity.[13] However, the above-mentioned hydrophobicity is susceptible to change by the unfolding of proteins, since it reflects the surface hydrophobicity of native proteins. On the other hand, Bigelow[14] has proposed the average hydrophobicity, $H\phi_{ave}$, based on Tanford's Gibbs energy of the transfer of amino acid side chains from an organic environment to an aqueous environment. This index is not influenced by any conformational changes of proteins. In a similar manner, Kyte and Doolittle[15] have proposed the hydropathy index, based upon the probability of being exposed to water from the hydrophobic parts of proteins as well as the Gibbs energy of transfer from water to an organic solvent. The former method for protein hydrophobicity is considerably simpler than the latter. Proteins with high hydrophobicity exhibit higher values of $H\phi_{ave}$ (over 4.6 kJ·res.$^{-1}$).

Previously, the author reported on the thermotropic behavior of different proteins in monolayers.[16] It was observed that the temperature-dependences on the π-A isotherms of hydrophobic proteins, such as hemoglobin ($H\phi_{ave}$=4.65 kJ·res.$^{-1}$) and β-casein ($H\phi_{ave}$= 5.57 kJ·res.$^{-1}$), were fairly different from those of hydrophilic proteins, such as lysozyme ($H\phi_{ave}$=4.06 kJ·res.$^{-1}$) and bovine serum albumin (BSA, $H\phi_{ave}$= 4.35 kJ·res.$^{-1}$), and that hydrophobicity of proteins was an important factor related to the thermal stability of proteins in membranes. Thus, the above-

mentioned confusion may be attributed to differences in the hydrophobicity of proteins.

In order to study the lipid-protein interaction in membranes, the spread monolayer technique is useful, since the orientation of lipid and protein molecules at the air-water interface can be precisely understood by using this technique and the area occupied by each molecule can be determined over a wide limit by careful compression of the monolayers. Then, the interaction of proteins with lipids at the air-water interface has been studied by many workers,[17-21] usually by the technique of injecting protein under preformed lipid monolayers and by measuring the increment of surface pressure during the interaction. However, the above injection technique has serious limitations in that the surface concentration of proteins is not known. Recently, the interaction of lipids with proteins at the air-water interface by spreading the components from a common solvent has been investigated.[22-24]

In this paper, we examined the hydrophobic effect of proteins on the thermotropic behavior of phospholipids, as measured by the miscibility in the mixed monolayers which are obtained by spreading the mixtures dissolved in a common solvent. These results were then discussed concerning the phase behavior of phospholipids. Although the proteins used were not membrane bound but, rather, water soluble, they were chosen because of their well-known amino acid sequences and their three-dimensional structures. The present work also investigated the rheological properties of mixed monolayers from phospholipids and proteins by using a surface viscometer.

Experimental

Reagents. All chemicals were of the highest purity available, used without further purification. Water was distilled once, passed through a mixed ion bed exchanger and redistilled.

Materials. The following high-purity proteins were used in this study from the suppliers: BSA (fatty acid free), bovine hemoglobin (2×crystallized), and bovine β-casein were from Sigma Chemical Co., and lysozyme (6×crystallized) was product of Seikagaku Kogyo Co. The phospholipids dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) (Sigma Chemical Co.) used were specified to be 99% pure and were used without further purification.

Surface Pressure. The film balance used for measuring the surface pressure has been described in detail elsewhere.[16] The trough was coated with Teflon and the entire balance was surrounded by a water jacket, providing temperature control within 1 °C. A 50% 1-propanol containing 0.5 mol·dm^{-3} sodium acetate was employed as a spreading solvent for proteins and phospholipids. After the respective proteins and phospholipids were dissolved in the solvent at a concentration of 0.05%, these solutions were stored in a refrigerator and used after about 24 h. For mixed monolayers, the mixtures of lipids and proteins were obtained by an appropriate volumetric mixing of solutions containing each component before application. In the experiments reported below, no buffer was used, in order to prepare a very simple subphase. The water was poured in the trough to a height of 1—2 mm above the rim. The surface of the subphase was cleaned by moving a Teflon barrier from one side of the trough to the other. The cleaning of the surface was repeated until the surface pressure of the subphase did not exceed 0.2 mN·m^{-1}. For the surface pressure-area (π-A) isotherm, these solutions (0.1 cm^3) were then spread onto an area of 897 cm^2. Monolayer spreading was made by a direct application of numerous small drops (≈0.0006 cm^3) of the solution to the surface of the subphase by using a microsyringe (Hamilton Co.), introduced by Ställberg and Teorell.[25] A time interval of 30 min was allowed for the equilibration of the monolayers before it was compressed. The π-A isotherms were obtained by using a compression velocity of 0.0567 m^2·mg^{-1}·min^{-1}.

Surface Viscosity. A Kyowa's surface viscometer (type SVR, Kyowa Surface Science Co.) was used. This apparatus was a rotational torsion surface viscometer of the type described by Karam.[26] The viscometer consisted of a knife-edged bob which hung freely from a torsion wire. A removable dish was placed on a turntable beneath the bob. The radii of the bob and dish were 2.5 and 7.5 cm, respectively. After the knife edge of the bob was placed on the surface of substrate, the monolayer spreading, based upon the recipe for measuring the surface pressure, was made. From the period and damping of the oscillation, the surface viscosity η_s expressed in mN·s·m^{-1} was calculated as follows:

$$\eta_s=\frac{2.303\,I}{2}\left(\frac{\lambda}{T}-\frac{\lambda_w}{T_w}\right)\left(\frac{1}{r_1^2}-\frac{1}{r_2^2}\right), \quad (1)$$

where I is the moment of interia, r_1 and r_2 the respective radii of the bob and dish, λ the (decadic) logarithmic decrement of the oscillation, and T their periodic time when the surface is covered by the monolayers; λ_w and T_w are the respective values for the clean surface.

Each part of all experiments was repeated at least three times and then averaged.

Results

Characteristics of Mixed Monolayers from DPPC and Proteins at an Air-Water Interface. Treatment of data involved adapting the theory of mixing in bulk to a two-dimensional situation. For ideal mixing, monolayers obey the following equation:[27]

$$A_{1,2}=n_1A_1+n_2A_2, \quad (2)$$

where $A_{1,2}$ is the average molecular area in the two-component film, n_1 and n_2 are the respective mole fractions, and A_1 and A_2 are the molecular areas in the two single-component films at the same surface pressure. In case the interaction between two components is detected, Eq. 2 is obeyed.

However, most biological membranes are composed of approximately 50% lipids and 50% proteins by weight,[28] and the molecular weights of proteins are extremely different from those of lipids. Then, Eq. 2

is modified as follows:

$$A_{l,p} = w_l A_l + w_p A_p, \quad (3)$$

where $A_{l,p}$ is the occupied area, expressed in $m^2 \cdot mg^{-1}$, in the mixed monolayer of lipid and protein, w_l and w_p are the respective weight fractions of lipid and protein, and A_l and A_p are the areas ($m^2 \cdot mg^{-1}$) in the lipid and protein monolayers at the same surface pressure.

Figure 1 shows typical π-A isotherms obtained at 25 °C for monolayers of hemoglobin, DPPC, and a mixture (1/1 in weight ratio) of the two spread on water, respectively. The calculated curve shown in Fig. 1 represents the behavior found when the additivity rule in Eq. 3 is obeyed. The result indicates that when DPPC is mixed with hemoglobin below its T_c (T_c=41 °C)[29] an expansion from the calculated curve occurs. Similar measurements were made with DPPC-β-casein, DPPC-BSA, and DPPC-lysozyme mixtures in various weight ratios. Although the deviation from ideal in the area of a mixed monolayer is observed by direct inspection of Fig. 1, a more convenient way of presenting the results of a number of runs on monolayers of the mixtures of different compositions is shown in Figs. 2 and 3. Here, the occupied area of the mixed monolayer at constant surface pressures is plotted as a function of the weight fraction of DPPC in the monolayers. The broken line on these plots represents the behavior found when the additivity rule is obeyed. When DPPC was mixed with such hydrophobic proteins as hemoglobin and β-casein[16] below T_c, an expansion or positive deviation from the additivity line occured. On the other hand, when DPPC was mixed with such hydrophilic proteins as BSA and lysozyme[16] below T_c, little deviation from the additivity line occured.

Phospholipids form a condensed monolayer or a gel

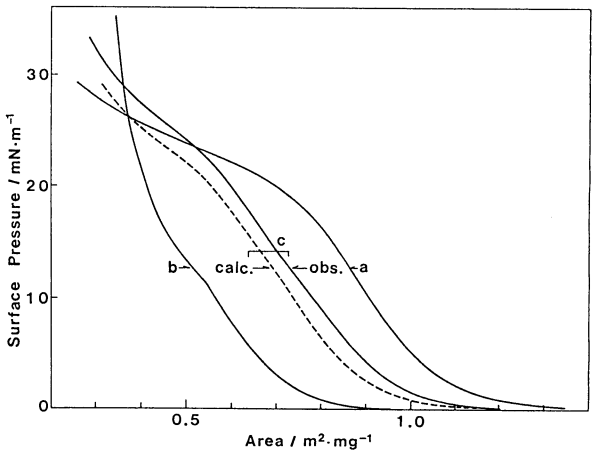

Fig. 1. π-A isotherms for monolayers of hemoglobin, DPPC, and a mixture of the two spread on water at 25 °C. a) hemoglobin, b) DPPC, c) DPPC-hemoglobin (1/1 in weight ratio); ——, experimental; ----, calculated from Eq. 3.

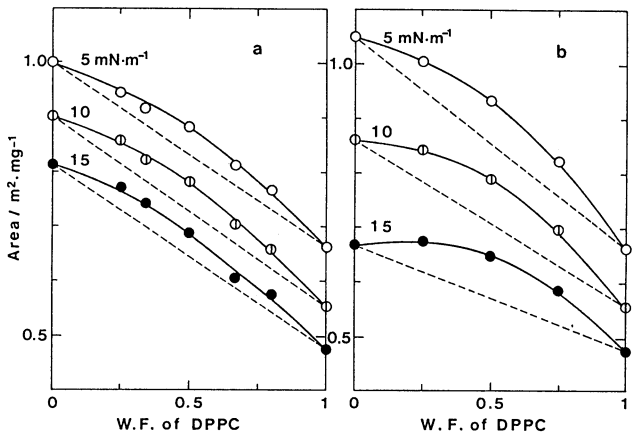

Fig. 2. Plots of mean area of mixed monolayers as a function of weight fractions of DPPC at 25 °C. a) DPPC-hemoglobin, b) DPPC-β-casein.

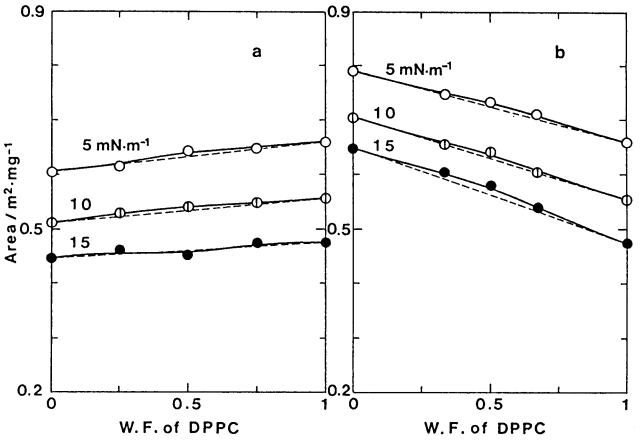

Fig. 3. Plots of mean area of mixed monolayers as a function of weight fractions of DPPC at 25 °C. a) DPPC-lysozyme, b) DPPC-BSA.

phase below T_c and form an expanded monolayer or liquid crystalline phase above T_c.[27] Accordingly, the temperature-dependence upon the lipid-protein interaction in membranes must be investigated. However, the T_c of DPPC is fairly higher than room temperature, and it is difficult to measure the surface pressure near T_c or above, since the surface evaporation of water from subphase occurs appreciably. Then, additional experiments were made by using DMPC (T_c=24 °C[29]).

Temperature-Dependence of Mixed Monolayers from DMPC and Proteins at an Air-Water Interface. Figures 4 and 5 show plots of the area of mixed monolayers composed of DMPC-hemoglobin and DMPC-β-casein at constant surface pressures as a function of the weight fractions of DMPC in their monolayers at different temperatures, respectively. When DMPC was mixed with hemoglobin below T_c (15 °C), a positive deviation from the additivity rule occured as well as DPPC. On the contrary, when DMPC was mixed with hemoglobin at T_c (24 °C) or

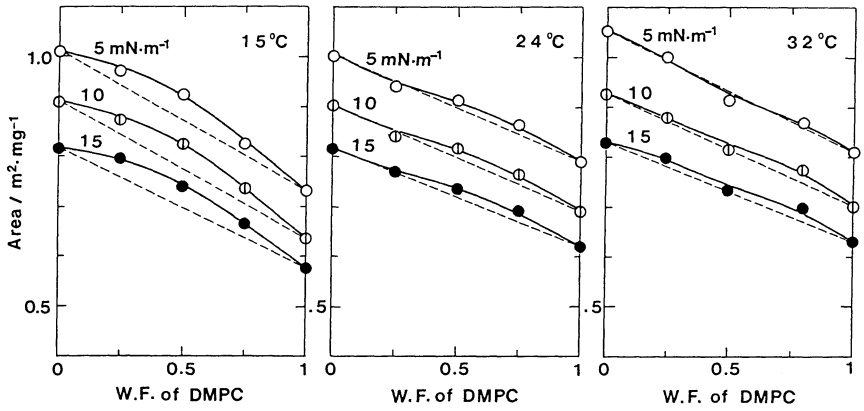

Fig. 4. Plots of mean area of DMPC-hemoglobin monolayer as a function of weight fractions of DMPC at different temperatures.

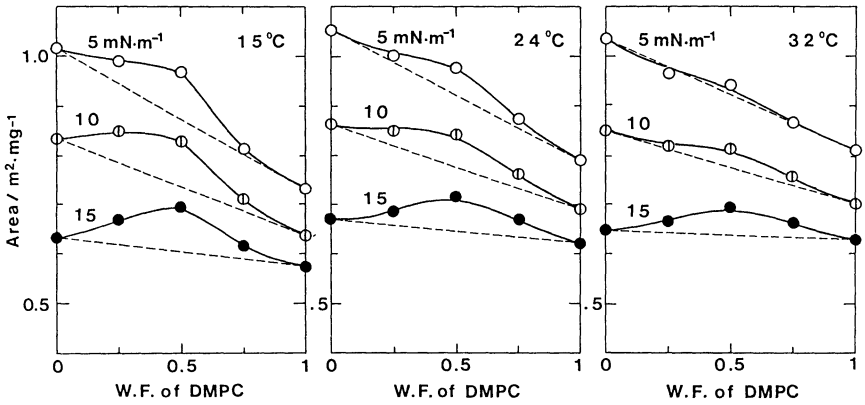

Fig. 5. Plots of mean area of DMPC-β-casein monolayer as a function of weight fractions of DMPC at different temperatures.

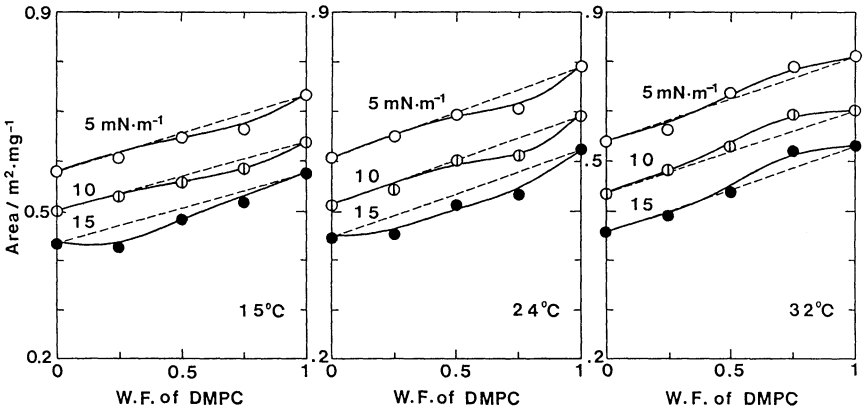

Fig. 6. Plots of mean area of DMPC-lysozyme monolayer as a function of weight fractions of DMPC at different temperatures.

above (32 °C), where DMPC was in a liquid crystalline state, little deviation from the additivity rule occured. When DMPC was mixed with β-casein below T_c, a large expansion from the additivity rule was observed. On the other hand, for the mixed monolayer of DMPC and β-casein, the magnitude of expansion decreased significantly at T_c or above. Figure 6 shows plots of area of the DMPC-lysozyme monolayer at constant surface pressures as a function of the weight fractions of DMPC at different temperatures. Strictly speaking, the DMPC-lysozyme mixture formed a slightly condensed monolayer from the ideal mixing at T_c or below, while the mixture formed a slightly deviated monolayer from the ideal mixing above T_c.

Surface Viscosity of Mixed Monolayers from DPPC and Proteins at an Air-Water Interface. The viscos-

ity, $\eta(c_A,c_B)$, of a solution containing two components, A and B, at concentrations c_A and c_B, is expressed by

$$\eta(c_A,c_B)=\eta_0\{1+([\eta_A]w_A+[\eta_B]w_B)c+s_mc^2+\cdots\}, \quad (4)$$

where $c=c_A+c_B$, $w_A=c_A/c$, $w_B=c_B/c$. η_0 is the solvent viscosity, $[\eta_A]$ and $[\eta_B]$ are the respective intrinsic viscosities of both components in a pure solvent, and s_m is the interaction parameter.[30] If there is no interaction between the two components, the third and following terms in the brace of the right-hand side of Eq. 4 may be ignored. Then, Eq. 4 can be approximated by

$$[\eta_{A,B}]=[\eta_A]w_A+[\eta_B]w_B, \quad (5)$$

where $[\eta_{A,B}]$ is the intrinsic viscosity of the solution containing two components. Accordingly, for ideal mixing without interaction between two components, the surface viscosity of monolayers may obey the additivity rule, as described in Eq. 3, although there are some problems in adapting the theory of mixing in bulk to the two-dimensional situation.

Figure 7 shows the surface viscosity-area (η_s-A) isotherms obtained at 25 °C for monolayers of hemoglobin, DPPC, and the mixtures of the two spread on water. The calculated curve for a mixture (1/1 in weight ratio) shown in Fig. 7 represents the behavior found when the additivity rule is obeyed. For the hemoglobin monolayer, η_s steeply appeared at a higher area than that where π was detectable. On the other hand, for the DPPC monolayer, η_s suddenly appeared at a slightly lower area than which corresponded to the limiting area (0.46 $m^2 \cdot mg^{-1}$). When DPPC was mixed with hemoglobin, a negative deviation from the additivity rule was observed, in contrast to the behavior of π-A isotherm. For example, when DPPC was mixed with hemoglobin at 1/1 in weight ratio, a considerable condensation from the calculated curve was observed. The above-mentioned result indicates that, when DPPC is mixed with hemoglobin below its T_c, η_s of the monolayer decreases by a fair amount. In addition, by using the mixture of DMPC and hemoglobin a measurement of η_s was attempted above T_c. Unfortunately, the measurement was unsuccessful because η_s of pure DMPC in a monolayer was very low.

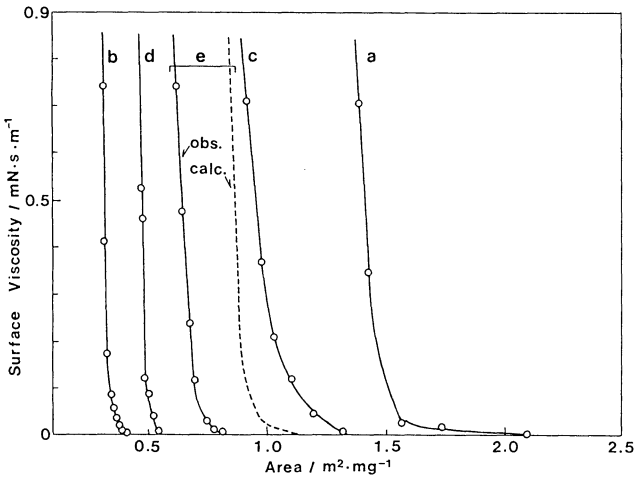

Fig. 7. η_s-A isotherms for monolayers of hemoglobin, DPPC, and mixtures of the two at 25 °C. a) hemoglobin, b) DPPC, c) DPPC-hemoglobin (1/3 in weight ratio), d) DPPC-hemoglobin (3/1), e) DPPC-hemoglobin (1/1); ——, experimental; ----, calculated from Eqs. 3 and 5.

Discussion

Because of the amphipathic nature of protein, the change to an extended conformation in which the protein molecule lowers the Gibbs energy of the system by maximizing contact of nonpolar side chains with the nonpolar phase is understandable. One must, therefore, consider whether the proteins denature irreversibly at the air-water interface or not. When the protein solutions are shaken, the insoluble protein due to the surface denaturation is occasionally seen to separate out. A comparison between the ease of coagulation (irreversible denaturation) by shaking a number of proteins has been carried out by Henson et al.[31] There is a significant difference in the ease of coagulation between the different proteins. Ovalbumin is especially susceptible, and β-lactoglobulin and γ-globulin also coagulate readily at the air-water interface. However, the β-casein, hemoglobin, BSA, and lysozyme, used in this study, are hardly influenced. According to MacRitchie,[32] every protein has a critical surface pressure, π_c, at the air-water interface, and will precipitate once this is exceeded. The coagulation may be observed, therefore, when the spread protein monolayers are compressed above π_c. However, our compression experiment was π_c. In addition, the view that the secondary structure of such proteins as the α-helix is stable at the air-water interface has been reported.[33] Accordingly, it is considered that surface denaturation did not occur during this study.

Biological membranes are sensitive to variation in the environment; for example, temperature essentially modifies such membrane characteristics as the permeability and excitability. Then, most lipids of biological membranes are normally in the liquid crystalline state at physiological temperatures and the lipids have the structure of a bilayer with low viscosity, deformability, and the ability to self-seal.[34] From this viewpoint, the effect of cholesterol on the membrane fluidity has been studied by a number of methods. The picture that emerges from these studies, summarized by Oldfield and Chapman,[35] is that cholesterol somehow fluidizes a lipid that is below the T_c, but rigidifies a lipid above T_c. This effect is called "dual effect."[36] Protein may also exhibit such a behavior as well as cholesterol in biological membranes. It has been found that boundary lipids near a

protein molecule have considerable differences in fluidity and conformation from those of the bulk lipids in the protein-free region and that the boundary lipids of many proteins are more fluid or have more gauche bonds in their hydrocarbon chains than the bulk lipids in the gel phase of the membranes, while the boundary lipids are more rigid or have more trans bonds than the bulk lipids in the liquid crystalline phase.[37-40]

When such phospholipids as DPPC and DMPC were mixed with such hydrophobic proteins as hemoglobin and β-casein below their T_c, a considerable expansion was observed (Figs. 2, 4, and 5), showing the lipid-protein interaction in membranes. On the other hand, when phospholipids were mixed with such hydrophilic proteins as BSA and lysozyme below T_c, no expansion was generally detected (Figs. 3 and 6), showing no interaction between lipids and proteins. Especially, the DMPC-lysozyme mixture formed a slightly condensed monolayer from the ideal mixing below T_c.

Papahajopoulos et al.[9] have extensively studied the effects of different proteins on the thermotropic phase transitions of phospholipid membranes, as detected by DSC and have indicated that ribonuclease leads to an increase in the enthalpy of the transition (ΔH) without any change in T_c, while hemoglobin and gramicidin induce a drastic decrease in ΔH accompanied by either a decrease or no change in T_c. Then, it is considered that a very hydrophilic protein, such as ribonuclease,[16] causes an ordering of the lipid hydrocarbon chains below the T_c of lipids, leading to an increase in ΔH, while such hydrophobic proteins as hemoglobin and gramicidin[14] cause a disordering of the chains below T_c, leading to a decrease in ΔH. In addition, O'Leary[41] has reported that proteins always weaken the intensity of the first-order lipid phase transition (pre-transition), and that they may cause it to broaden, and may induce other continuous transitions above or below it in temperature, depending on the system.

Accordingly, we consider that the hydrophobic proteins lead to an increase in the configurational freedom of lipid hydrocarbon chains, such as trans-gauche conversion,[6,42] due to a hydrophobic effect of proteins with lipid chains, resulting in an expansion of the monolayers composed of lipids and proteins, while that the hydrophilic proteins do not lead any configurational changes because of their low hydrophobicities, exhibiting ideal mixing. Very hydrophilic proteins, such as lysozyme, lead to an increase in the configurational restriction of lipid hydrocarbon chains, due to the electrostatic interaction of the polar head groups with the proteins, inducing the condensation of monolayers. On the other hand, when the phospholipid DMPC was mixed with different proteins near its T_c or above, all mixtures of DMPC-proteins formed little deviated monolayers from the ideal mixing, independent of a variety of proteins (Figs. 4—6). This suggests that the expansion behavior due to the hydrophobic effect does not occur, because all lipids are in the liquid crystalline state near T_c, or above.

The thermotropic behavior of phospholipid molecules in reconstituted glycophorin-containing vesicles has been investigated by means of DSC.[43] Each glycophorin molecule is able to perturb the properties of 80—100 phospholipid molecules, independent of the protein-to-lipid ratio in the membranes, in such a way that these lipid molecules no longer participate in the cooperative gel-to-liquid crystalline phase transition. A value of 15 lipid molecules per protein molecule has also been reported for lipophilin,[44] ten for melittin,[45] and six for gramicidin.[37]

The structural change of proteins owing to lipid-protein interaction in monolayers may be negligibly smaller than that of lipids. Then, the perturbed-to-unperturbed lipid ratios in the mixed monolayers are derived from Eq. 3,

$$A_{l,p} = w_l\{A'_l x + (1-x)A_l\} + w_p A_p, \quad (6)$$

where x is the mole fractions of the perturbed lipids in whole lipids, A'_l and A_l are the respective areas ($m^2 \cdot mg^{-1}$) in the perturbed and unperturbed lipid monolayers, A_p and $A_{l,p}$ are the respective areas in the protein and lipid-protein monolayers at the same surface pressure, and w_l and w_p are the respective weight fractions of lipid and protein. When the phospholipid DPPC is dispersed in excess water, the area per molecule in bilayers is about 4.8 nm^2 below T_c and about 7.0 nm^2 above T_c.[46] A fully-expanded DPPC monolayer above T_c occupies 7.0 $nm^2 \cdot molecule^{-1}$ when π is approximately 20 $mN \cdot m^{-1}$.[27] Since the value of the molecular weight of DPPC is 734, the areas expressed in 4.8 and 7.0 $nm^2 \cdot molecule^{-1}$ correspond to areas of 0.39 and 0.57 $m^2 \cdot mg^{-1}$, respectively. In Fig. 1, when π is 20 $mN \cdot m^{-1}$, hemoglobin and mixture (1/1 in weight ratio) occupy 0.70 and 0.60 $m^2 \cdot mg^{-1}$, respectively. This indicates that about 60% of all lipid molecules are perturbed in the mixed monolayer below T_c (25 °C). Then, it is understandable by the application of the molecular weight (64500) of hemoglobin that each hemoglobin molecule perturbs the properties of 50—60 phospholipid molecules.

Since the molecular weight of hemoglobin is about twice that of glycophorin,[47] and extremely larger than those of lipophilin, melittin, and gramicidin, the value of perturbed lipid molecules per hemoglobin molecule is slightly smaller than those of the integral proteins. This may be attributed not only to a difference in the hydrophobicity between the proteins, but also to a difference in the membrane properties between the monolayer and bilayer. When one considers a two-dimensional lattice of lipid molecules surrounding the protein, each hemoglobin molecule complexes 40—50 phospholipid molecules, independ-

ent of the protein-to-lipid ratio in the monolayer. However, this will be greatly diminished by the phase separation into the protein-rich and protein-free domain below T_c.[21] Then, the perturbation of the phospholipid structure near the hemoglobin molecule, while most pronounced within the annulus of the first neighbor molecules, will extend at least two layers beyond the annulus, coinciding with the result of Marčelja.[2]

Speth and Wunderlich[48] have shown by freeze-etch electron microscopy that, after chilling the cells of protozoan *Tetrahymena pyriformis,* the particles, which are randomly distributed on the outer faces of the fractured alveolar membranes, are largely aggregated, showing a clustering of membrane proteins. This indicates that, when the cells are rapidly cooled, the fluidity of biological membranes is regulated by the phase separation of membrane proteins.[49] The η_s-A isotherms for phospholipid-hemoglobin mixtures showed a considerable condensation below T_c, in contrast to the behavior of π-A isotherms (Fig. 7). Such behavior suggests that, when the phospholipids are mixed with the hydrophobic proteins such as hemoglobin below T_c, the fluidity of the monolayer increases significantly, depending on both the perturbation of lipid hydrocarbon chains and the phase separation of proteins in the monolayers. It is also related to the above-mentioned behavior that hemoglobin drastically increases the permeability of phospholipid vesicles.[50,51]

Finally, we consider the protein distribution is the mixed monolayers from phospholipids and proteins. According to Heckl et al.,[21] the high packing densities in the condensed lipid phases below T_c do not allow for a distribution due to protein penetration, while the low packing densities in the expanded lipid phases above T_c permit a homogeneous distribution of proteins in the monolayers. Accordingly, based on the lattice model of a phospholipid bilayer containing integral protein constructed by Lookman et al.,[52] the model shown in Fig. 8 is being proposed for monolayers composed of phospholipids and different proteins as a function of temperature. The black, open hexagons represent such hydrophobic proteins as hemoglobin and β-casein and such hydrophilic proteins as BSA and lysozyme, respectively. The dots represent perturbed lipids, and the blank areas represent unperturbed lipids. Below T_c the proteins are highly clustered, while at T_c or above they are more uniformly distributed. The hydrophobic proteins perturb the hydrocarbon chains of boundary lipids near the protein molecule below T_c. This perturbation, caused by the protein, extends at least to the second neighbor, leading to an expansion of the monolayers. On the other hand, the hydrophilic proteins do not perturb the phospholipid structure below T_c because of their low hydrophobicities, showing ideal mixing in monolayers. At T_c or above, the membrane properties are independent of a variety of proteins, since all lipid hydrocarbon chains cause a disordering, which results in ideal mixing.

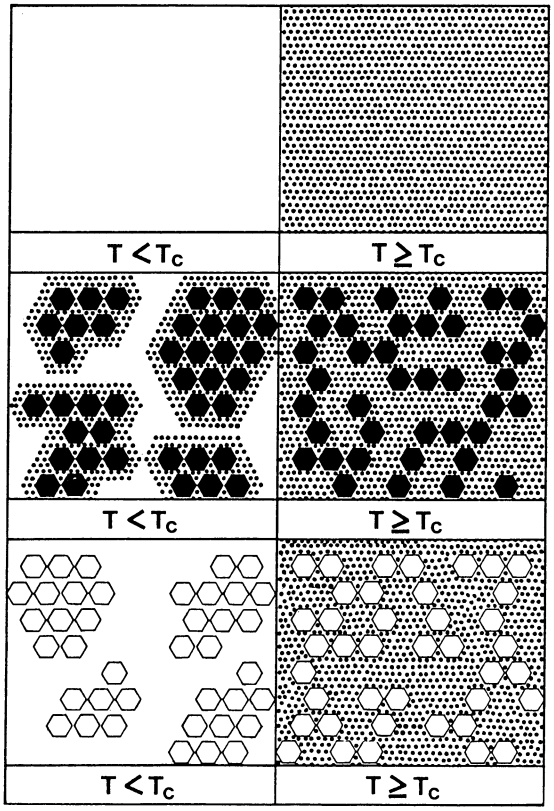

Fig. 8. A model of monolayers composed of phospholipids and different proteins as a function of temperatures, based on the lattice model of proteins and lipids in computer simulation by Lookman et al.[52] The black and open hexagons are the respective hydrophobic and hydrophilic proteins, the dots are perturbed lipids, and the blank areas are unperturbed lipids.

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