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Adsorption of *N*-Dodecyl-β-alanine to the Spread Monolayer of Lecithin or Dilaurin

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The adsorption of *N*-dodecyl-β-alanine (NDA) was studied at the air–solution interface with the spread film of L-α,β-dimyristoyllecithin (DML) or α,α'-dilaurin (DIL). The adsorbed amounts were measured by radiometry using tritium-labelled NDA, combined with surface pressure measurements. Measurements of IR, DSC, and NMR were made in order to determine the bulk properties of amphoteric moieties in the mixture of DML and NDA. For the mixed monolayer of NDA and DML or DIL, the surface composition *vs.* area curves were obtained at various surface pressures. The molecular interaction in monolayer was discussed on the basis of the excess free energy of mixing, which was calculated at various surface pressures and compositions. As a result, the mixing of DIL and NDA in monolayer was ideal at surface pressures less than 5 mN m^{-1}, whereas the mixed monolayer of DML and NDA showed a remarkable condensation in surface molecular area. Such a large condensation at low surface pressure was concluded to be due to the formation of ion pairs between the choline and alanine moieties on the water surface, by referring to the findings obtained by studying the lipid properties in the bulk phase. It was also found that the DML–NDA monolayer near close packing exhibited various types of complicated behavior, depending on the surface compositions. The appreciable differences in the nature of the amphoteric moieties in DML and NDA molecules can explain these findings.

Studies of the monolayer behavior of phospholipids on the surface of surfactant solution are very useful for understanding some of the actions of surfactant on the biomembrane. Nevertheless, few studies on the interaction of membrane lipids with surfactant have been performed, due to some experimental and theoretical restrictions. Studies of mixing in the insoluble lipid films[1] or of binding properties of lipid and metallic ions[2] are more numerous. The thermodynamic approach to analyzing the nature of penetrated films must be based on precise information about the monolayer composition, especially for the penetrated films with the ionic interactions between the polar groups of lipids.[3] Recently, Hendrikx, and Ter-Minassian-Saraga,[4] who studied the adsorption of a cationic surfactant to a lecithin monolayer, found that the mixed monolayer formed a stable two-dimensional micelle on the water surface. The use of ionic surfactant as penetrant, however, would not be convenient for the thermodynamic analysis of the monolayers, because it is almost impossible to distinguish the hydrophobic interaction in the penetrated monolayer with the ionic interaction caused by the surfactant (and/or spread) film ions and their gegen ions.

In the present study, experiments are carried out on the spread monolayer of lecithin and dilaurin into which an ampholytic surfactant is penetrated. By determining the adsorbed amounts by radiometry, attempts are made to elucidate the behavior of the penetrated monolayers as a function of the surface composition or the surface pressure. Further, the work is discussed about the interaction between the polar groups of lecithin and surfactant in monolayer, combined with the observations on their mixtures in the bulk phase.

Experimental

Materials. After the usual purifications, L-α,β-dimyristoylphosphatidyl choline (DML), which had been purchased from Fluka Co., showed a single spot in the TLC analysis. Dilaurin (DIL), *i.e.* glycerol α,α'-dilaurate, was purified by the recrystallization with hexane. *N*-Dodecyl-β-alanine (NDA), *i.e.* 3-(dodecylammonio)propionate, was employed as an ampholytic surfactant. For the radiometry, the alkyl chain in NDA was labelled with tritium.[5] The solution was prepared by using water which was distilled thrice after being refluxed with acidic permanganate solution overnight. Chloroform and hexane were used for the spreading solvent.

Measurements of Surface Properties. Adsorbed amounts of NDA were determined by the radiotracer method. The radioactivity of tritium β-rays at the air-solution interface was detected by a plastic scintillation counter.[6] Equilibrium surface tension was measured by the Wilhelmy plate method. All measurements were made at 30±0.5 °C, above the gel-liquid transition temperature of DML on the water surface.[7] The pH of the solution was at 5.8±0.2 throughout the experiments: at this value NDA[8] and DML[9] in water possess an amphoteric structure.

Preliminary experiments indicated that the surface pressure *vs.* mean molecular area curves which were obtained by the compression method, coincided well with those obtained by the successive additions of spread solution onto the water surface. All the spread monolayers used in present work, then, were formed by the addition method on the solution surfaces at which NDA had already been adsorbed.

Measurements of Bulk Properties. In order to elucidate the polar interaction between DML and NDA in the bulk state, DSC, IR, and NMR measurements were carried out. In the DSC measurements (Rigaku Denki 8056), the lipids were mixed up well in an aluminium DSC pan with chloroform, allowed to stand overnight *in vacuo* to evaporate the solvent, and sealed in nitrogen gas. The measurements were made as a function of mole fraction of NDA. The specimen for the IR measurement (Hitachi EPI G-2) was prepared in the same manner as that used in the DSC analyses. The absorption spectra were taken as functions of the temperature and mole fraction by the Nujol method. The NMR spectra (JEOL FX-100) were also taken for the 0.1 mol dm^{-3} deuteriochloroform solutions of DML, NDA, and their equimolar mixture at 25 °C.

Results and Discussion

Figure 1 shows the relationship between the surface pressure, π, and spread amount, Γ_1, of DML or DIL monolayer formed on the solution surface of NDA at various concentrations. Figure 2 exhibits the plot of the surface excess, Γ_2, of NDA against the spread amounts of DML or DIL. The subscripts 1 and 2 mean the film-forming substance and surfactant, respectively. In Fig. 2-b, a little hump was observed on the desorption isotherms of NDA at the intermediate region of concentrations, upon spreading small amounts of DML. This means that DML strongly interacts with the adsorbed NDA on the water surface. On the other hand, the isotherms of NDA for the DIL films simply decreased with increasing spread amounts for all concentrations studied. Thermodynamical discussion for these adsorption isotherms will be made elsewhere.

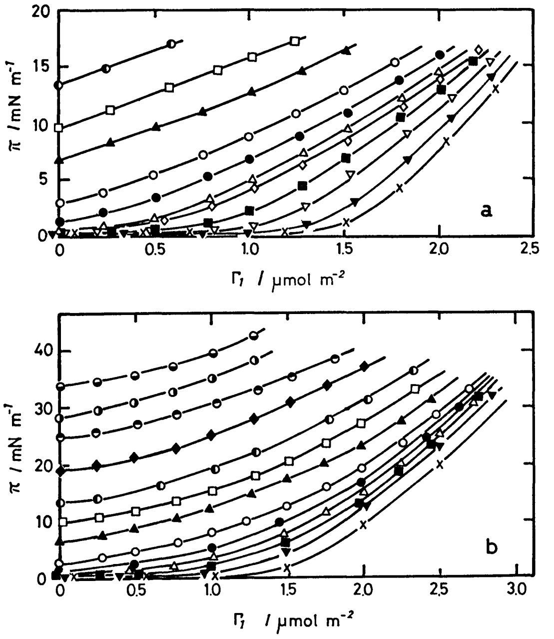

Fig. 1. π *vs.* Γ_1 curves for DIL–NDA (a) and DML–NDA (b) at the following concentrations of NDA ($10^5\ C_2$/mol dm^{-3}).
×: 0, ▼: 0.10, ▽: 0.20, ■: 0.30, ◇: 0.40, △: 0.50, ●: 0.70, ○: 1.0, ▲: 2.0, □: 3.0, ◐: 5.0, ◆: 10, ◓: 20 ◑: 30, ◒: 50.

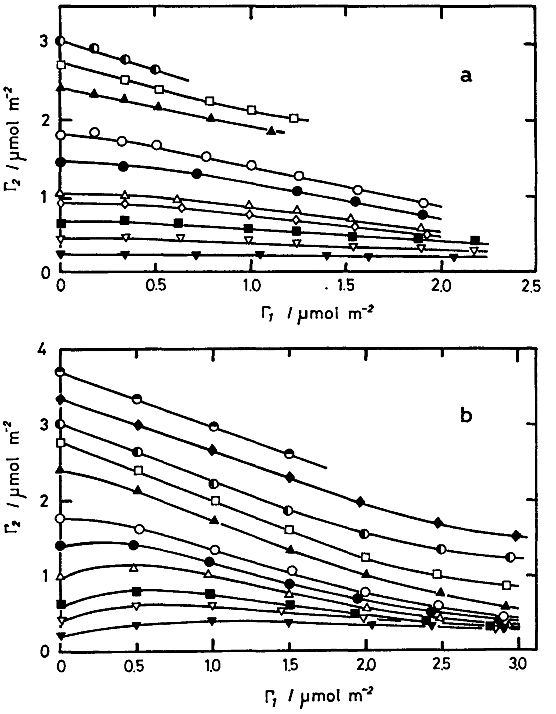

Fig. 2. Γ_1 *vs.* Γ_2 curves for DIL–NDA (a) and DML–NDA (b) at various concentrations of NDA. Signs for each curve are identical with those of Fig. 1 independently of (a) and (b) curves,

Miscibility of Monolayers. Before studying the properties of the penetrated monolayers, the miscibility in monolayer was examined for the DML–NDA and DIL–NDA systems. According to the two-dimensional phase rule,[10] two degrees of freedom are available for one surface phase at constant temperature and pressure in the present systems. Thus the criterion of miscibility is easily made from the relationship in surface pressure and surface fraction at constant total amounts of the monolayers, whose fraction may be chosen by variation in amounts of the spread films and bulk concentration. Here, the surface fraction of NDA, X_2, in the monolayer was obtained by dividing Γ_2 by Γ_t, which was a sum of Γ_1 and Γ_2. The results are shown in Fig. 3. Consequently, the monolayers of both systems were confirmed to be miscible in all surface fractions. In mixing of DML and NDA, there was no indication of the occurrence of a phase separation such as has been observed in mixing of DML and cholesterol on the water surface adsorbed from the dispersed solution.[11]

Area Condensation in Monolayer. The plot of mean molecular area, A, against X_2 in the mixed monolayers of DML–NDA or DIL–NDA is shown at various surface pressures in Fig. 4. The mixing of DIL and NDA in the monolayer was almost ideal at surface pressures lower than 5 mN m^{-1}, but allowed a slight condensation in surface molecular area when surface pressure increased, and approached to the equilibrium spreading pressure of DIL (16.6 mN

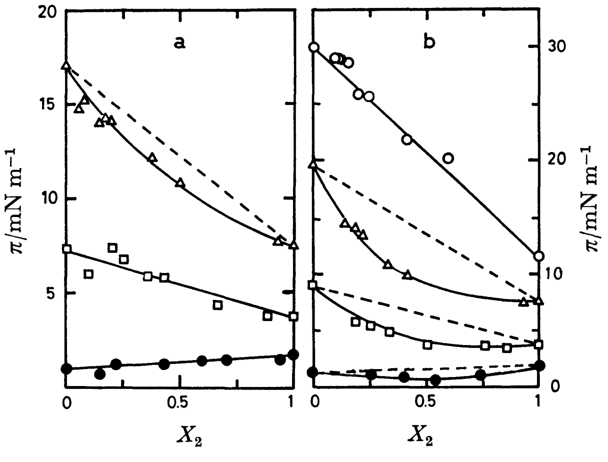

Fig. 3. π *vs.* X_2 curves for DIL–NDA (a) and DML–NDA (b) at the following constant surface excess (Γ_t/μmol m^{-2}).
●: 1.5, □: 2.0, △: 2.5, ○: 3.0,

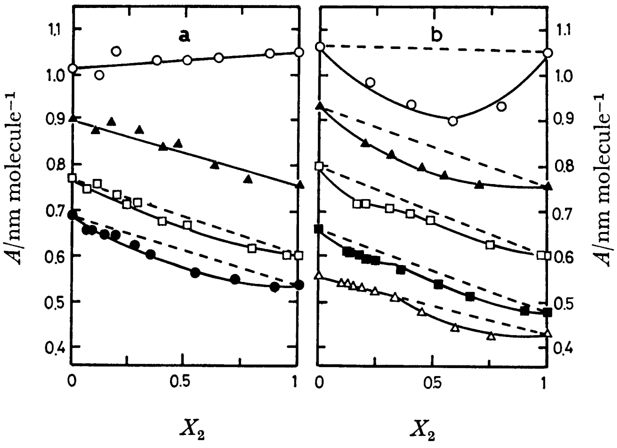

Fig. 4. A *vs.* X_2 curves for the DIL–NDA (a) and DML–NDA (b) monolayers at various surface pressures (π/mN m^{-1}).
○: 2, ▲: 5, □: 10, ●: 15, ■: 20, △: 30.

m^{-1}). Since the mixing of DIL and NDA does not include the process of interionic interaction, the area condensation observed can be considered to arise mainly from the van der Waals interaction in hydrocarbon chains. On the other hand, the mixing in DML and NDA monolayers is likely to depend in a complicated way on the surface pressure and compositions: for instance, there is the remarkable deviation from the additivity in molecular area at low surface pressure, and also a characteristic kink appears at about 0.3 fraction of NDA, as may be seen in Fig. 4-b. Such behavior in mixing cannot be explained without taking into account the interactions between polar groups as well as hydrocarbon chains in the monolayers. Because both the choline moiety in DML and alanine moiety in NDA possess an amphoteric structure under the conditions of the present work, the ionic interaction can be supposed to occur directly between these moieties. In order to confirm the occurrence of such a binding, some experiments were carried out on the mixture of DML and NDA in the bulk phase.

Confirmation of Ionic Binding of DML and NDA in the Bulk Phase. *DSC Analysis*: The endothermic peak at 63.5 °C was found independently of the mole fraction of the mixture. The plot of the heat of transition at 63.5 °C *vs.* the composition is shown in Fig. 5. Since no peaks are observed at 63.5 °C for each single component, the endothermic peak observed seems to be due to the formation of intermolecular compounds in the DML and NDA mixture. The heat of the corresponding transition was endothermically 20.1 kJ mol^{-1} without any medium.

IR Analysis: IR absorption for the ethyl group in the gauche structure of β-alanine appears at 860 cm^{-1}.[12] Thus, the IR spectra for the mixture of DML and NDA were measured at the various temperatures and compositions. The spectra from 780 to 940 cm^{-1} are shown in Fig. 6. For the equimolar mixture the gauche structure almost disappears near the temperature at which the endothermic peak has been found by the DSC measurement. On 68.4% NDA mixture, however, the corresponding absorption was still observed at 73 °C. This signifies that NDA molecules in more than equimolar quantities exist in the mixture as single molecules. The reason is that the absorption at 860 cm^{-1} for pure NDA remains unchanged while the temperature is increased up to the melting point of NDA, except for the negligible temperature dependence of absorption intensity for each group. It may be concluded that the mixture of DML and NDA converts into an intermolecular compound of 1:1 by heating.

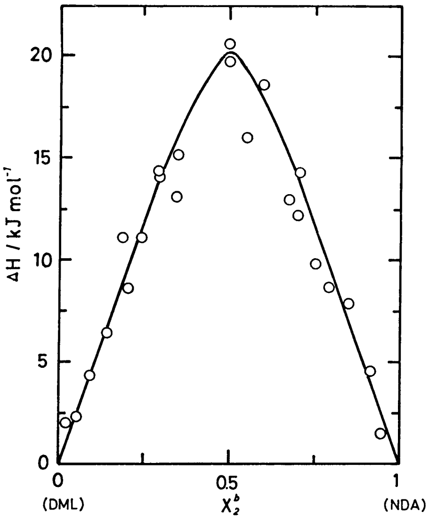

Fig. 5. Heat *vs.* NDA fraction, $X_2{}^b$, curve relation to endothermic peaks at 63.5 °C by DSC analysis for DML–NDA mixture.

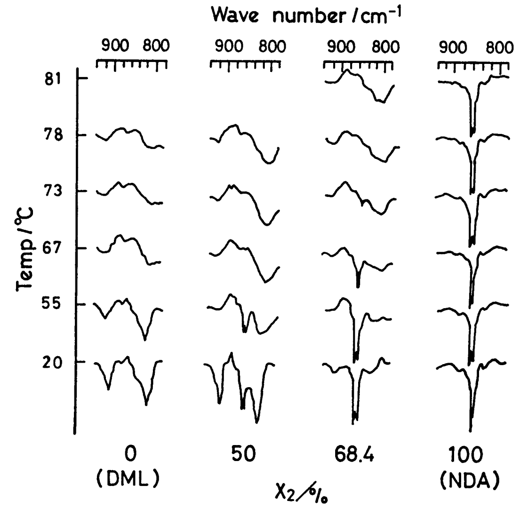

Fig. 6. IR spectra from 780 to 940 cm^{-1} for DML–NDA mixture at various temperatures and compositions.

NMR Spectrum: In order to elucidate the contribution of the polar groups to the formation of ionic binding, measurements were made on proton chemical shift for DML, NDA, and their equimolar mixture in the deuteriochloroform solutions. Table 1 shows the proton chemical shift before and after mixing of the DML and NDA solutions. By mixing, –N$^+$H$_2$– spectrum of NDA shifted towards the low field, while

Table 1. Proton chemical shift for polar groups of DML, NDA, and the equimolar mixture in $CDCl_3$ (ppm) (reference TMS, 25 °C, 100 MHz)

Groups	DML	NDA	Mixture	$\Delta\delta^{a)}$
$=N^+H_2$		−8.751	−6.106	+2.65
$-CH_2COO^-$		−2.577	−2.587	−0.01
$\equiv P^-OCH_2-$	−4.118		−4.341	−0.22
$-N^+(CH_3)_3$	−3.340		−3.333	+0.01

a) $\Delta\delta$: Differences of proton chemical shift.

$\equiv P^- -O-CH_2-$ spectrum of DML was oppositely shifted towards the high field. However, no difference in chemical shifts was observed for other groups in these compounds within the experimental error (±0.002 ppm). This indicates that the ionic interaction in solution takes place only between the $\equiv P^- -O-CH_2-$ group of DML and the $-N^+H_2-$ group of NDA.

From all these experiments, we may conclude that the molecules of DML and NDA, whose polar groups possess both amphoteric structures, form stoichiometrically an intermolecular salt when their mixture is dissolved in a polar solvent or heated up to about 63.5 °C. This may imply the favorable formation of ionic binding with 1:1 of DML and NDA in the penetrated monolayer, provided that the ionic properties of the lipids do not change essentially between the surface and the bulk phases.

Effects of Polar Groups on "Condensing." The condensation at high surface pressure in Fig. 4-b cannot be explained by only the formation of the DML(1): NDA(1) complex as found in the bulk phase. In order to elucidate the unique mixing of DML and NDA in monolayer, one must take into account the difference in the behavior of their polar groups, which depends on the surface pressure and the surface composition. Then the excess free energy of mixing Δg^v, defined by Goodrich,[13] was calculated at 2:1, 1:1, and 1:2 surface compositions at various surface pressures for the DML–NDA and DIL–NDA monolayers. The results are shown in Table 2. A clear difference was found in the mixing behavior of these monolayers. For DML and NDA monolayers, considerably large Δg^v values were appraised even at low surface pressure, compared to those for DIL and NDA monolayer. Since the behavior of hydrocarbon chains in the mixed monolayers is regarded as to be almost the same between DML–NDA and DIL–NDA, such large values may be considered to be due to the occurrence of ionic binding between DML and NDA in monolayer, as depicted in Fig. 7-a. This consideration coincides with the conclusion obtained in the bulk experiments mentioned above.

Table 2. Surface pressure dependence of Δg^v ($-J\ mol^{-1}$) at constant surface compositions

π	DML-NDA			DIL-NDA		
$mN\ m^{-1}$	0.33	0.50	0.66	0.33	0.50	0.66
2	134	182	169	0	0	0
5	138	159	154	0	0	0
6	159	87	86	11	12	11
10	218	148	151	96	120	97
15	244	235	235	307	372	307
20	216	325	337			
25	135	421	550			
30	0	543	795			

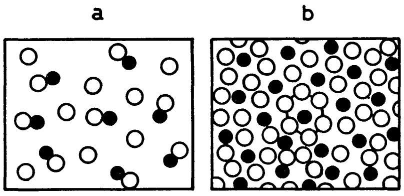

Fig. 7. Schematic representations for mixed monolayer of 2:1 of DML(○) and NDA(●) at low (a) and high (b) surface pressures.

The properties of mixed monolayer at high surface pressure are discussed. In the equimolar mixed monolayer at 6 mN m^{-1} the Δg^v values attain a minimum. This is explained by two different origins for the interactive energies caused in mixing: namely, one comes from the ionic binding between polar groups in monolayers and is seen in the low surface density, while the other arises from the condensation between hydrocarbon chains and increases with increasing monolayer density, as may be expected from the Δg^v values for the DIL–NDA system. The surface pressure dependence of Δg^v in the 0.33 fraction monolayer is also interpretable in view of the interaction between the ampholytic moieties of DML and NDA. With increasing film density, the mixed monolayer in all cases is considered to form an ionic network structure which would be more stable rather than remaining in the pair binding of DML(1):NDA(1). Structural formation in monolayer has been also found by Ter-Minassian-Saraga for the mixed monolayer of DML and hexadecyltrimethylammonium chloride.[4] Such a phenomenon turns out to make a unique kink on the *A vs.* X_2 curves in Fig. 4, because the structural monolayer is probably less compressible. A model of mixing at DML(2):NDA(1) composition at high surface pressure is depicted in Fig. 7-b. In DML and cholesterol system, however, the formation of molecular complex of DML(2):CHOL(1) at the surface[11] and bulk[14] phases has been confirmed. Approaching the close packing at about 30 mN m^{-1}, the monolayer which makes a two-dimensional structure becomes so relatively tight and incompressible that the whole system apparently behaves as an ideal one. At 0.66 fraction of NDA the mixed monolayer, however, still keeps the tendency to condense. This is probably because the ionic network structure is gradually broken down by the increases of NDA fraction in the monolayer: the monolayer finally consists of the mixture of DML(1):NDA(1) binding and NDA. Because the dissociation constants of both ampholites at the water surface are $pK_a=3.5$ and $pK_b=3.11$ for NDA[8] and $pK_a=3.32$ and $pK_b=3.55$ for DML[9] at 30 °C, the β-alanine moiety in NDA is considered scarcely to dissociate in water and then to make a hydrated ring structure, compared to the choline moiety in DML. Eventually, NDA molecules in mixed monolayer at high fraction of NDA tend to be present with the nature like nonionic molecules. Therefore, the maximum condensation occurs at about 0.66 fraction, corresponding to the equimolar mixture of DML–NDA ion pair and NDA. The complicated bahavior in mixing at high surface pressure results from the difference characters in the ampholytic moieties of DML and NDA.

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