

Cerebroside Langmuir monolayers originated from the echinoderms: II. Binary systems of cerebroside and steroids

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Abstract

Two-component Langmuir monolayers formed on a subphase of 0.5 M sodium chloride solution were investigated for two different cerebroside (LMC-1 and LMC-2) with steroids of cholesterol (Ch) and cholesteryl sodium sulfate (Ch-S); i.e. LMC-1/Ch, LMC-1/Ch-S, LMC-2/Ch, and LMC-2/Ch-S were examined in terms of surface pressure (π), the surface potential (ΔV) and the dipole moment (μ_{\perp}) as a function of surface area (A) by employing the Langmuir method, the ionizing electrode method, and the fluorescence microscopy. Surface potentials (ΔV) of steroids were analyzed using the three-layer model proposed by Demchak and Fort [R.J. Demchak, T. Fort Jr., *J. Colloid Interface Sci.* 46 (1974) 191–202]. The miscibility of cerebroside and steroids in the insoluble monolayers was examined by plotting the variation of the molecular area and the surface potential as a function of the steroid molar fraction (X_{steroid}) based upon the additivity rule. From the $A-X_{\text{steroid}}$ and $\Delta V_m-X_{\text{steroid}}$ plots, partial molecular surface area (PMA) and apparent partial molecular surface potential (APSP) were determined at the different surface pressures. The PMA and APSP with the mole fraction were discussed for the miscible system. Judging from the two-dimensional phase diagrams, they can be classified into two types. The first is a completely immiscible type; the combination of cerebroside with cholesterol. The second is a negative azeotropic type, where cerebroside and cholesteryl sodium sulfate are completely miscible both in the expanded state and in the condensed state. In addition, a regular surface mixture (the Joos equation for the analysis of the collapse pressure of two-component monolayers) allowed calculation of the interaction parameter (ξ) and the interaction energy ($-\Delta\varepsilon$) between the cerebroside and Ch-S. The miscibility of cerebroside and steroid components in the monolayer state was also supported by fluorescence microscopy.

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1. Introduction

Sphingolipids, e.g. ceramides, sphingomyelin, cerebroside and gangliosides, are important constituents of cellular membranes. The principal component of sphingolipids is the

long-chain base, sphingosine. Although the functional implications of these structural variations are largely unknown, some of them are obviously involved in defining antigenic specificities of cells. We have reported that surface behavior of some pure cerebroside and of two-component monolayers made from cerebroside (LMC-1 and LMC-2) and phospholipids (DPPC and DPPE) at the air–water interface in the first paper in series [1]. In order to know the detailed interactions of sphingolipids and their roles in the cell membrane, it is necessary to collect more information on their dependence on the

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molecular structure difference, i.e. the number, location and orientation of hydroxyl groups attached to the acyl chains with or without double bond. Due to the physicochemical importance of Ch molecules in cell membrane, the interaction between Ch and other lipids has been studied by various physicochemical methods [2–5]. Then, steroids (in particular, Ch) were found to be widely distributed in all cells of the organism. It is a principal component of cellular membranes and plasma lipoproteins. In addition, Ch plays an important role in modifying the physicochemical properties of cellular membranes [6–8].

As long as we know, reports on cerebroside are still much fewer in number than reports on gangliosides [9]. In one of the fewer papers, the adsorption of Ca^{2+} to the monolayer of pure cerebroside or pure cerebroside sulfates was reported [10]. The report showed that cerebroside was a liquid-condensed (LC) film and cerebroside sulfate was a typical liquid-expanded (LE) film, indicating that membrane properties of cerebroside were sensitive to their molecular structure such as the length of hydrophobic chains and the size of head groups. However, the report about cerebroside–steroid interaction using the monolayer study does not seem to exist.

In this study, we have focused on characterizing the Langmuir behavior of pure steroids of cholesterol (Ch) and cholesteryl sodium sulfate (Ch-S) and two-component systems of cerebroside and steroids at the air/water interface. Surface pressure (π), surface potential (ΔV), and dipole moment (μ_{\perp})–area (A) isotherms were obtained for the pure compounds and their two-component systems. The surface potentials were analyzed using the three-layer model proposed by Demchak and Fort [11]. The phase behavior of two-component monolayers was examined in terms of additivity of molecular surface area and of surface potential. Furthermore, it was analyzed employing the partial molecular surface area (PMA) and apparent partial molecular surface potential (APSP). The molecular interaction between monolayer components was investigated using the Joos equation. Finally, the monolayer behaviors were examined by fluorescence microscopy.

2. Experimental

2.1. Materials

The cerebroside (LMC-1 and LMC-2) possess β -*O*-glucosyl head group linked to the terminal hydroxyl group of ceramide. The procedures of extraction and purification were described in detail in the preceding paper in series [1]. Cholesterol (Ch) was purchased from Nu-Chek-Prep Inc. (USA). Its purity was >99%. Ch was checked by TLC just before its use and used without further purification. Cholesteryl sodium sulfate (Ch-S) was obtained from lipid extracts of the echinoderm [12]. The purity was checked by TLC and it showed one spot at normal phase chromatography. The chemical structure of two cerebroside used in this work was shown in the preceding paper [1].

Ch was dissolved in *n*-hexane/ethanol mixture (7/3, v/v; the former from Cica-Merck, Uvasol, and the latter from Nacalai Tesque). On the other hand, Ch-S was dissolved in chloroform/methanol mixture (2/1, v/v; both two from Cica-Merck, fluorometry), because Ch-S was insoluble in *n*-hexane/ethanol mixture. The preparing for the subphase (0.5 M NaCl solution) was described in the first paper in series [1].

2.2. π - A and ΔV - A measurements

The π - A measurement was performed by the automated Langmuir film balance whose resolution is 0.01 mN m^{-1} . The trough ($500 \text{ mm} \times 150 \text{ mm}$) was made from aluminum coated with Teflon. After spreading, 15 min of time was allowed for the solvent to evaporate. The monolayer was compressed at a constant rate of $1.00 \times 10^{-1} \text{ nm}^2 \text{ mol}^{-1} \text{ min}^{-1}$.

The ΔV - A measurement was simultaneously carried out during the π - A measurement. The potential was measured with an electrometer (Keithley, 614) and ^{241}Am air-ionizing electrode at 1–2 mm above the interface, while a reference electrode was dipped in subphase. Other experimental conditions were the same as described in the previous paper [1,13].

2.3. Fluorescence microscopy

Fluorescence microscopy observation was made by using the same film balance equipped with a fluorescence microscope (BM-1000, U.S.I. system, Japan). The fluorescent probe (1 mol%) was a xanthylum 3,6-bis(diethylamino)-9-(2-octadecyloxycarbonyl) phenyl chloride (R18, Molecular Probes). All the experiments were carried out on 0.5 M NaCl at 298.2 K. Image analysis was performed using NIH (National Institutes Health) image. Other experimental conditions were the same as described in the preceding paper [1].

3. Results and discussion

3.1. π - A and ΔV - A isotherms of steroid monolayers

The π - A , ΔV - A and μ_{\perp} - A isotherms of LMC-1 and LMC-2 monolayer were discussed in the preceding paper [1]. Fig. 1 shows those of steroids (Ch and Ch-S) spread on 0.5 M NaCl solution at 298.2 K. Ch and Ch-S exhibited a liquid-condensed (LC) monolayer, their collapse pressures were 42 and 50 mN m^{-1} , and the extrapolated areas were 0.39 and 0.38 nm^2 , respectively.

The surface potentials (ΔV) of Ch and Ch-S showed positive values, and the Ch monolayer showed the larger variation of ΔV under compression than Ch-S'. The ΔV value of Ch and Ch-S reached 385 and 256 mV at the closest packed state, respectively. It seemed that this difference in ΔV ($\sim 130 \text{ mV}$) resulted from the difference in polar head group between Ch and Ch-S.

The vertical component of surface dipole moment, μ_{\perp} was calculated from the Helmholtz equation using the measured

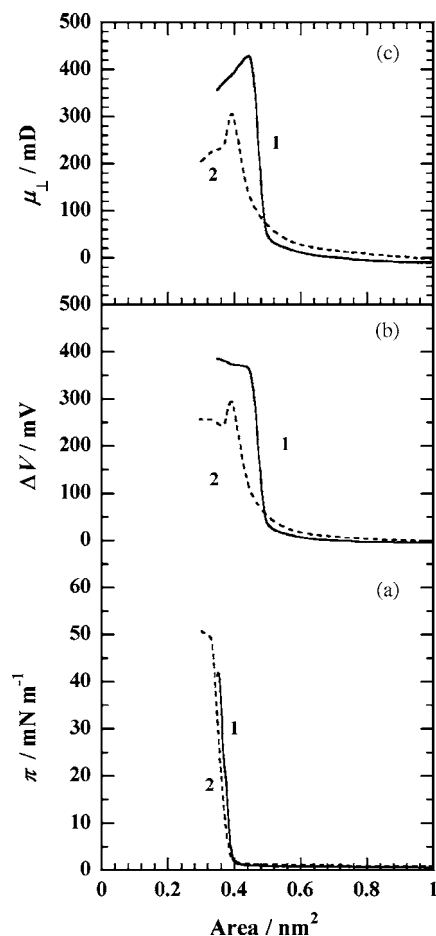


Fig. 1. Surface pressure (π)–area (A) isotherms (a), surface potential (ΔV)– A isotherms (b), and surface dipole moment (μ_{\perp})– A isotherms (c) of steroids on 0.5 M NaCl solution at 298.2 K ((1) Ch and (2) Ch-S).

ΔV values:

$$\Delta V = \mu_{\perp} / \epsilon_0 \epsilon A \quad (1)$$

where ϵ_0 is the permittivity of a vacuum and ϵ is the mean permittivity of the monolayer (which is assumed to be 1). A is the area occupied by the molecule. The ΔV values involve the resultant of the dipole moments accompanied by the polar head group, the C–H bond group, and the subphase.

3.2. The surface dipole moments (μ_{\perp}) of steroids

As is the first paper in series [1], the surface potential of monolayers was analyzed using the three-layer model proposed by Demchak and Fort [11].

We have compared the experimental values of μ_{\perp} in the most condensed state of the monolayer with those calculated $\mu_{\perp, \text{calc}}$ by the three-layer model-based equation:

$$\mu_{\perp, \text{calc}} = \mu_1 / \epsilon_1 + \mu_2 / \epsilon_2 + \mu_3 / \epsilon_3 \quad (2)$$

where μ_1 / ϵ_1 , μ_2 / ϵ_2 , and μ_3 / ϵ_3 are the contributions of the subphase, polar head group, and hydrophobic chain group, respectively.

We want to determine the contribution of the steroid segment (Ch and Ch-S) and sulfate group (Ch-S). The contributions of hydrophobic chains and hydrophilic head groups of some cerebroside were determined in the preceding article [1]. There are some parameter values for the three-layer model; $\mu_1 / \epsilon_1 = 0.040$ D, $\epsilon_2 = 7.6$, $\epsilon_3 = 5.3$ [11], $\mu_1 / \epsilon_1 = 0.025$ D, $\epsilon_2 = 7.6$, $\epsilon_3 = 4.2$ [14], and $\mu_1 / \epsilon_1 = -0.065$ D, $\epsilon_2 = 6.4$, $\epsilon_3 = 2.8$ [15]. We have used a set of values introduced by Taylor and Oliveira because our experimental conditions have resembled to their ones. Then, we made sure that the parameter values provided good agreements with our experimental conditions by using the data of stearic acid in the preceding paper [1].

Firstly, we evaluated the contribution of the hydrophobic steroid segment of Ch from the following equation (see Table 1):

$$\mu_{\perp}(\text{Ch}) = \mu_1 / \epsilon_1 + \mu_2^{\text{OH}} / \epsilon_2 + \mu_3^{\text{steroid}} / \epsilon_3 = 0.36 \text{ D} \quad (3)$$

Surface dipole moment values have been proposed for μ_2 for the different conformations of the OH group: $\mu_2(\text{OH-gauche}) = 1.00$ D, $\mu_2(\text{OH-trans}) = -0.63$ D, and $\mu_2(\text{OH-free}) = 0.18$ D. We have employed the above $\mu_2(\text{OH-gauche}) = 1.00$ D, as many studies support the gauche conformation for condensed alkanol monolayers. Using the experimentally determined ΔV values (see Table 1) and assuming the set of the values ($\mu_1 / \epsilon_1 = -0.065$ D, $\epsilon_2 = 6.4$, and $\epsilon_3 = 2.8$), we were able to obtain $\mu_3^{\text{steroid}} / \epsilon_3 = 0.264$ D, so that $\mu_3^{\text{steroid}} = 0.74$ D.

Secondly, we similarly evaluated the contribution of the sulfate group of Ch-S:

$$\mu_{\perp}(\text{Ch-S}) = \mu_1 / \epsilon_1 + \mu_2^{\text{sulfate}} / \epsilon_2 + \mu_3^{\text{steroid}} / \epsilon_3 = 0.23 \text{ D} \quad (4)$$

Then, we obtained the following value, $\mu_2^{\text{sulfate}} = 0.22$ D ($\mu_2^{\text{sulfate}} / \epsilon_2 = 0.034$). The value of μ_2^{sulfate} group is about one-fifth of $\mu_2(\text{OH-gauche})$.

3.3. Compression isotherms of cerebroside/steroids two-component monolayers

Next, turning to the discussion toward two-component systems, four combinations of two-component monolayer systems composed of the two cerebroside (LMC-1 and

Table 1
Surface potential data used for dipole moment evaluation

Sample	A_0 (nm ²)	ΔV (mV)
LMC-1	0.42	249
LMC-2	0.39	104
Ch	0.36	385
Ch-S	0.33	256

A_0 is the molecular surface area obtained by extrapolating the high-pressure portion of the π – A isotherms to zero pressure. ΔV is the surface potential at maximum compression. In all cases, the subphase was 0.5 M NaCl at 298.2 K.

LMC-2) and two steroids (Ch and Ch-S) have been studied in order to clarify the effect of molecular structure, the molecular interaction between two components, and the miscibility on the monolayer state. For the above purpose, the π - A , ΔV - A , and μ_{\perp} - A isotherms were measured at various compositions at 298.2 K on a 0.5 M NaCl subphase for two-component systems of LMC-1/Ch, LMC-2/Ch, LMC-1/Ch-S, and LMC-2/Ch-S. The isotherms of all two-component systems are shown in Fig. 2. The isotherms of five two-component at discrete mole fractions are also inserted in the corresponding figures. All the curves of the two-component systems exist between those of the respective pure components, and they successively change with the increasing mole fraction of steroids.

3.3.1. Cerebrosides (LMC-1 and LMC-2)/Ch systems

Fig. 2A and B shows π - A , ΔV - A , and μ_{\perp} - A isotherms of binary LMC-1/Ch and LMC-2/Ch systems, respectively. Ch had no transition point on their π - A isotherms, and also the π - A isotherms and fluorescence images (later section) indicated LC film behavior. For cerebrosides, both LMC-1

and LMC-2 were found to be typical LE film in the preceding paper [1].

The interaction between LMC-1 or LMC-2 and steroid molecules was investigated by examining whether the variation of the mean molecular areas as a function of X_{Ch} or $X_{\text{Ch-S}}$ satisfies the additivity rule [16]. Comparison between the experimental mean molecular areas and the mean molecular areas based on ideal mixing is shown in Fig. 3A and B at four surface pressures (5, 15, 25, and 35 mN m^{-1}). The A - X_{Ch} (Fig. 3A) shows a big negative deviations at all surface pressures, and also A - X_{Ch} does negative deviations at all surface pressures as shown in Fig. 3B. It can be said from the figures that these negative deviations result from good fit in size between the small polar head group (Ch) and big polar head group (cerebrosides) and between the big steroid segment (Ch) and almost straight chain (cerebrosides). For ΔV - X_{Ch} in Fig. 4A and B, both LMC-1/Ch and LMC-2/Ch systems indicate positive deviations at all surface pressures. This result suggests that these phenomena arise from the diminished interaction in the ΔV due to the above-mentioned packing between cerebroside and cholesterol molecules.

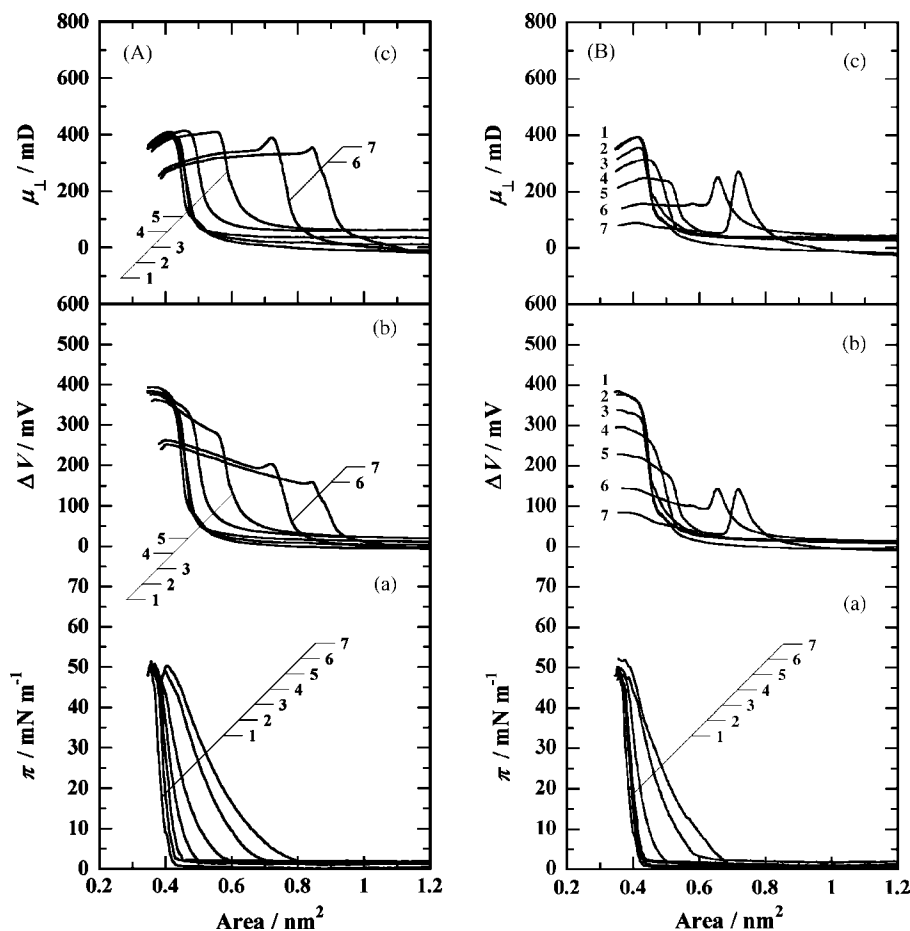


Fig. 2. Surface pressure (π)-area (A) isotherms, surface potential (ΔV)- A isotherms, and surface dipole moment (μ_{\perp})- A isotherms of the two-component systems on 0.5 M NaCl at 298.2 K: (A) LMC-1/Ch, (B) LMC-2/Ch, (C) LMC-1/Ch-S, and (D) LMC-2/Ch-S systems.

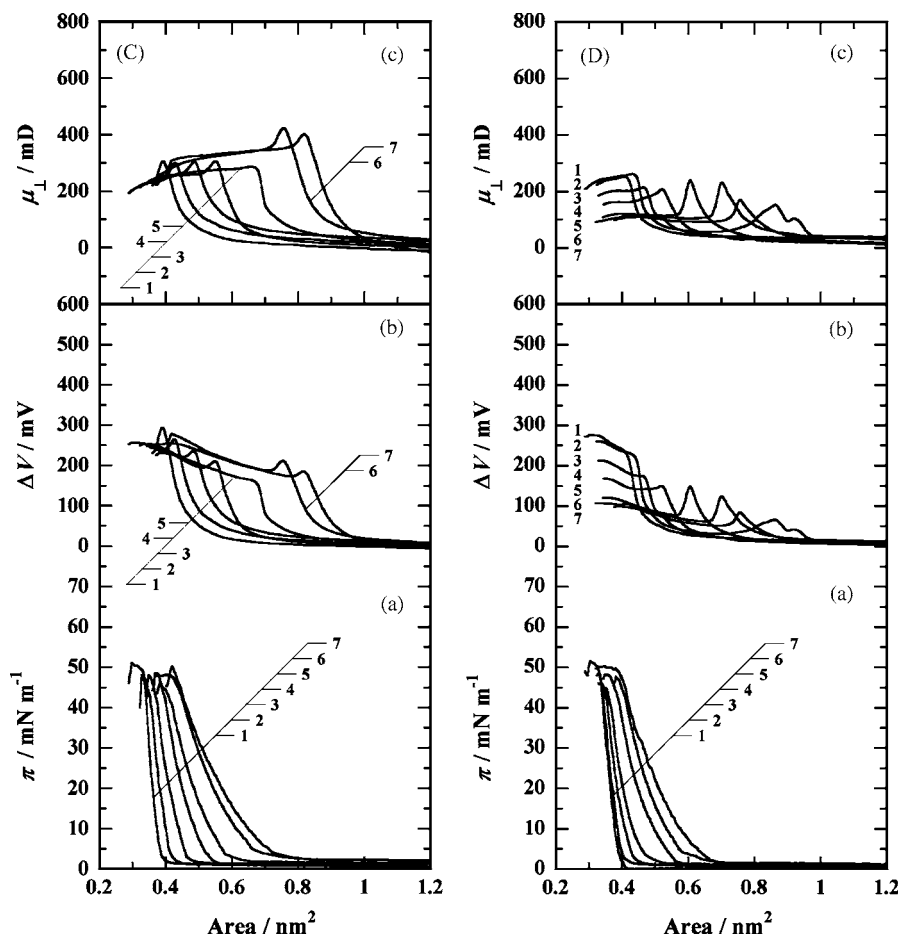


Fig. 2. (Continued).

3.3.2. Cerebrosides (LMC-1 and LMC-2)/Ch-S systems

The π - A isotherms of two-component monolayers for the cerebrosides (LMC-1 and LMC-2) and Ch-S systems are shown in Fig. 2C and D. Ch-S had no transition pressures and shows LC type on its π - A isotherm, and also fluorescence images (later section) indicated LC film. The interaction between LMC-1 or LMC-2 and Ch-S molecules was analyzed in the same procedures as Section 3.3.1 (additivity rule of area and ΔV). For $\pi = 5$ mN m⁻¹ of LMC-1/Ch-S system (Fig. 3C), experimental values show a negative deviation from the theoretical line, indicating enhanced attractive interaction between LMC-1 and Ch-S. The A - $X_{\text{Ch-S}}$ shows good agreements with ideal lines at $\pi = 25$ and 35 mN m⁻¹. For LMC-2/Ch-S system, the A - $X_{\text{Ch-S}}$ indicates negative deviations at 5–25 mN m⁻¹ and the good agreement at 35 mN m⁻¹. These results indicate that cerebrosides and Ch-S are almost ideally mixed in the monolayer state.

Analysis of the surface potential (ΔV) of the two-component monolayers in terms of the additivity rule is presented in Fig. 4C and D. For LMC-1/Ch-S system (Fig. 4C), comparison of the experimental data versus calculated variations clearly indicated negative deviations from

the ideal line at all surface pressures. LMC-2/Ch-S system was also the same tendency as LMC-1/Ch-S system. Comparing the ΔV - X_{steroid} plots of cerebrosides/Ch with that of cerebrosides/Ch-S in Fig. 4, the adverse deviation is observed, indicating that the difference in head group of steroids considerably affects the electrical properties of cerebrosides.

To understand the contribution of each component in binary systems in more detail, we also employed partial molecular surface areas (PMAs) and apparent partial molecular surface potentials (APSPs) as the same of the first article [1]. When PMA and APSP are evaluated, the molecular occupation and orientation behavior at the air/solution interface can be more clearly seen, respectively [17,18].

3.3.2.1. Mean surface areas (A_m), partial molecular surface areas (PMAs), mean surface potentials (ΔV_m) and apparent partial molecular surface potential (APSP).

Although we will discuss the miscibility of these systems in more detail in the next section, cerebrosides/Ch-S systems had an evidence of miscibility from the change of the collapse pressures with $X_{\text{Ch-S}}$ and fluorescence images (FM: as discussed latter). So, the analyses of PMA and APSP were made

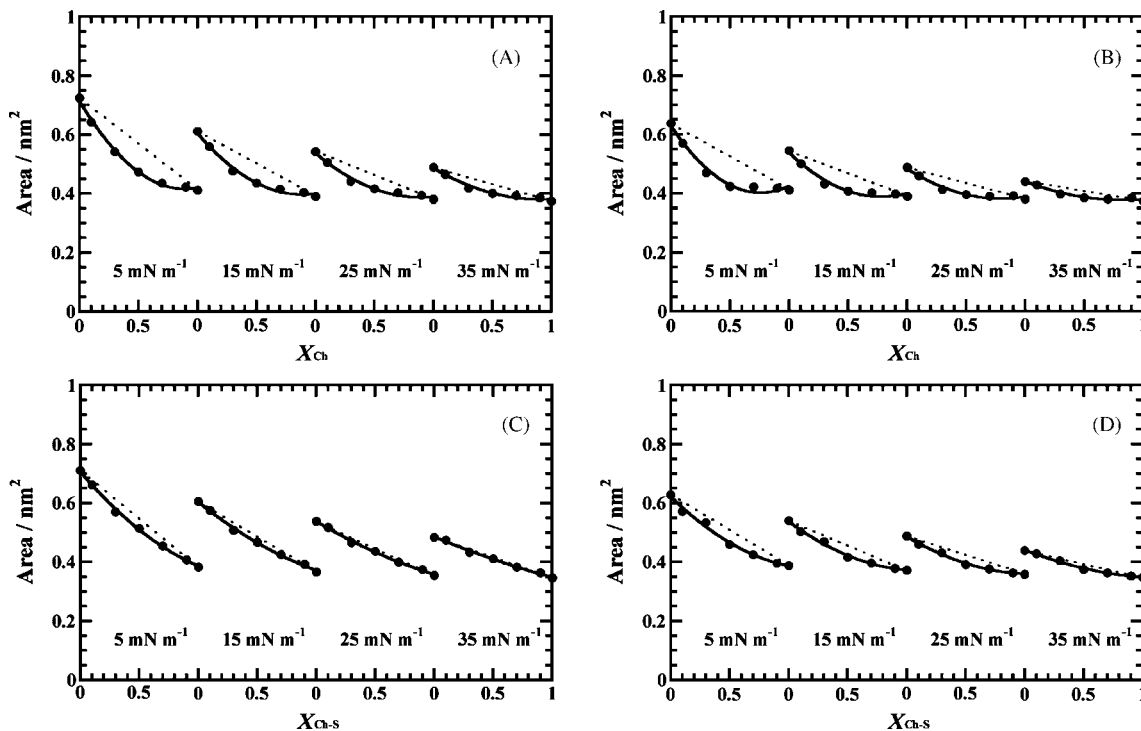


Fig. 3. Mean molecular area (A) of two-component monolayer as a function of X_{steroid} at four surface pressures. Dashed lines, theoretical variation assuming the additivity rule; closed circles, experimental value: (A) LMC-1/Ch, (B) LMC-2/Ch, (C) LMC-1/Ch-S, and (D) LMC-2/Ch-S systems.

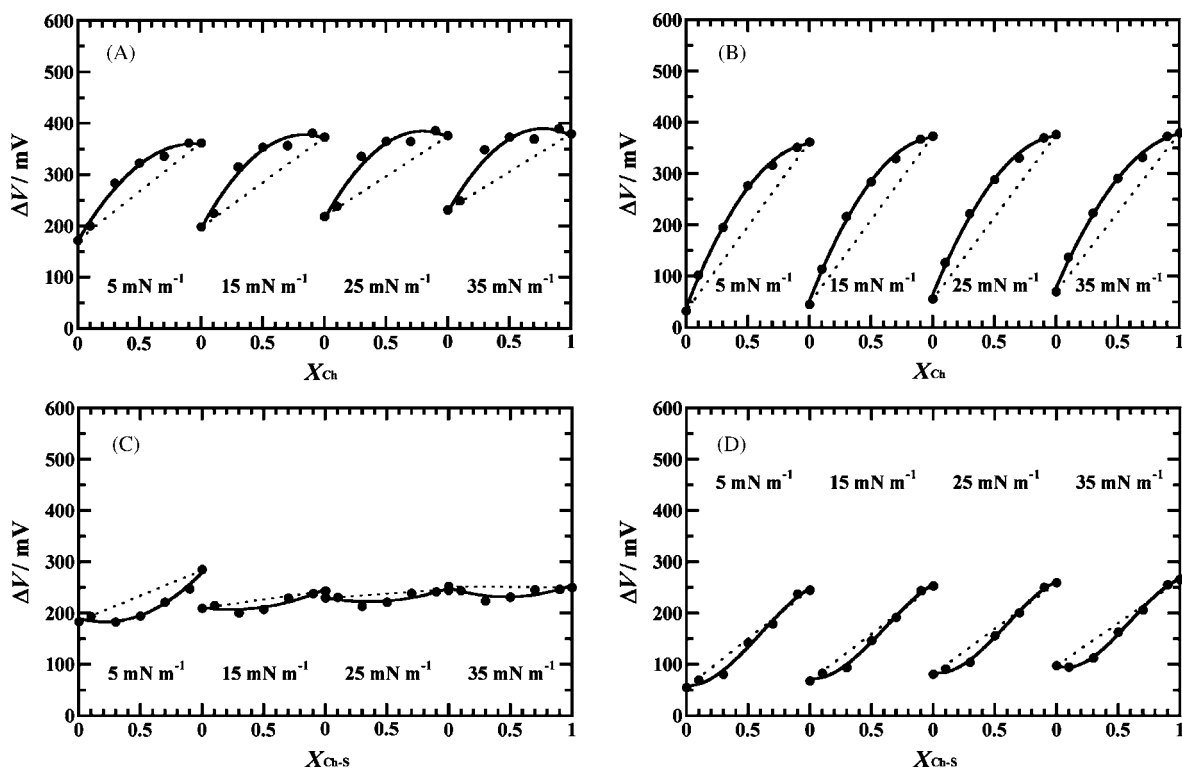


Fig. 4. Mean surface potential (ΔV) of two-component monolayer as a function of X_{steroid} at four surface pressures. Dashed lines, theoretical variation assuming the additivity rule; closed circles, experimental value: (A) LMC-1/Ch, (B) LMC-2/Ch, (C) LMC-1/Ch-S, and (D) LMC-2/Ch-S systems.

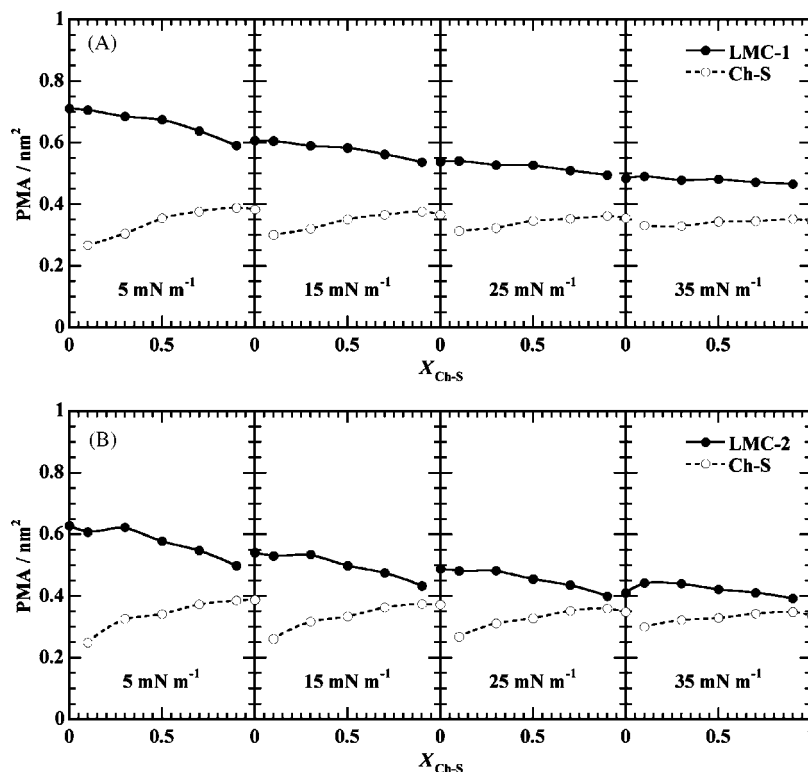


Fig. 5. Variation of partial molecular area (PMA) for two-component cerebrosides and Ch-S as a function of $X_{\text{Ch-S}}$ at surface pressures of 5, 15, 25, and 35 mN m^{-1} : (A) LMC-1/Ch-S: closed circle: LMC-1, open circle: Ch-S; (B) LMC-2/Ch-S: closed circle: LMC-2, open circle: Ch-S.

for the cerebrosides/Ch-S systems as the first paper in series [1]. The PMA- $X_{\text{Ch-S}}$ curves for cerebrosides/Ch-S systems are shown in Fig. 5. It is noted that if the two-component systems are ideal mixing, the PMA and APSP should be parallel to the axis of $X_{\text{Ch-S}}$ (the additivity rule). The PMA for both cerebrosides/Ch-S systems indicates the similar contracting behavior over the whole mole fraction at 5–25 mN m^{-1} . At 35 mN m^{-1} , it is found that Ch-S molecules occupy almost same surface area in binary LMC-1 or LMC-2/Ch-S systems.

In contrast to PMA, the $\Delta V_{\text{m}}-X_{\text{DPPC}}$ curves for cerebrosides/Ch-S systems suggest the similar interaction of Ch-S between LMC-1 and LMC-2 as shown in Fig. 6. The APSP for each cerebroside/Ch-S system indicates the similar behavior at different surface pressures as shown in Fig. 7. For LMC-1/Ch-S system, it is found that APSP of LMC-1 molecules decreased with increasing the mole fraction of $X_{\text{Ch-S}}$, while the that of Ch-S increases with increasing $X_{\text{Ch-S}}$ from 0.1 to 0.7, and reaches the individual one at the mole fractions above 0.7 ($X_{\text{Ch-S}} > 0.7$). Especially, at $X_{\text{Ch-S}} = 0.3$ and 0.5, the orientation of LMC-1 tends to tilt by the addition of Ch-S. On the contrary, for LMC-2/Ch-S system, the APSP of LMC-2 decreases from its own value over the whole mole fraction and its original value increases from about 35 times to about $45 \times 10^{-14} \text{ mV mol}^{-1}$ with increasing surface pressure. On the other hand, the Ch-S values remain almost constant over the $X_{\text{Ch-S}}$ range from 0.5 to 1. However, the Ch-S value decreases from the original one with decreasing mole fraction of Ch-S over $X_{\text{Ch-S}}$ range from 0.1 to 0.5. The

magnitude of APSP variation is about $40 \times 10^{-14} \text{ mV mol}^{-1}$ even at $\pi = 35 \text{ mN m}^{-1}$. In consequence, the surface orientation of Ch-S molecules is affected more strongly by LMC-1 chains than by LMC-2 ones due to the matching between unsaturated hydrophobic tails and steroid group and to the electrical interaction between hydroxyl group of LMC-1 and sulfate group of Ch-S.

3.4. Two-dimensional phase diagram

From the π - A isotherm for the binary systems of LMC-1/Ch, LMC-2/Ch, LMC-1/Ch-S, and LMC-2/Ch-S, their two-dimensional phase diagrams were constructed by use of the collapse pressure (π^c) changes at various molar fractions of steroids. Representative phase diagrams at 298.2 K are shown in Fig. 8.

3.4.1. Cerebrosides/Ch

By the same procedure as the preceding paper [1], the first type of phase diagram is constructed in Fig. 8A for LMC-1/Ch and 8 B for LMC-2/Ch. We recognized that Ch is completely immiscible with cerebrosides. For example, in the phase diagrams for LMC-1/Ch at lower π values, LE film of cerebrosides (LMC-1) is formed independent upon Ch; the film is separated into LMC-1 domains and steroids (Ch) domains, like island and sea. Their region is expressed as $M.(\text{LMC-1}) + M.(\text{Ch})$. If further compression of the film is made up to the collapse pressure of the given Ch, then the Ch starts to

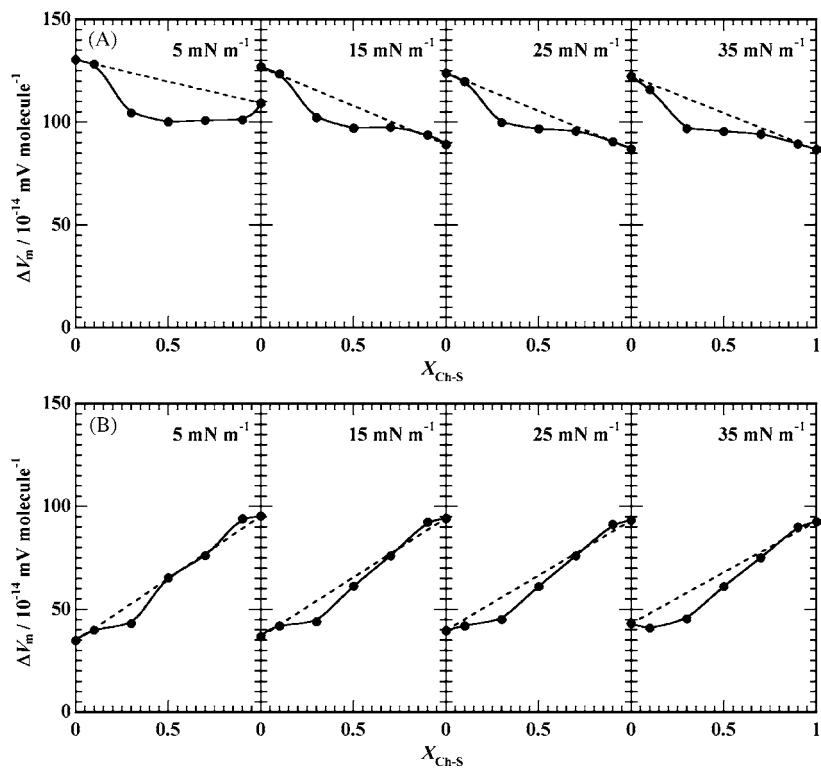


Fig. 6. Variation of the molecular surface potential (ΔV_m) with Ch-S mole fraction for the cerebroside/Ch-S mixtures at surface pressures of 5, 15, 25, and 35 mN m⁻¹: (A) LMC-1/Ch-S, and (B) LMC-2/Ch-S.

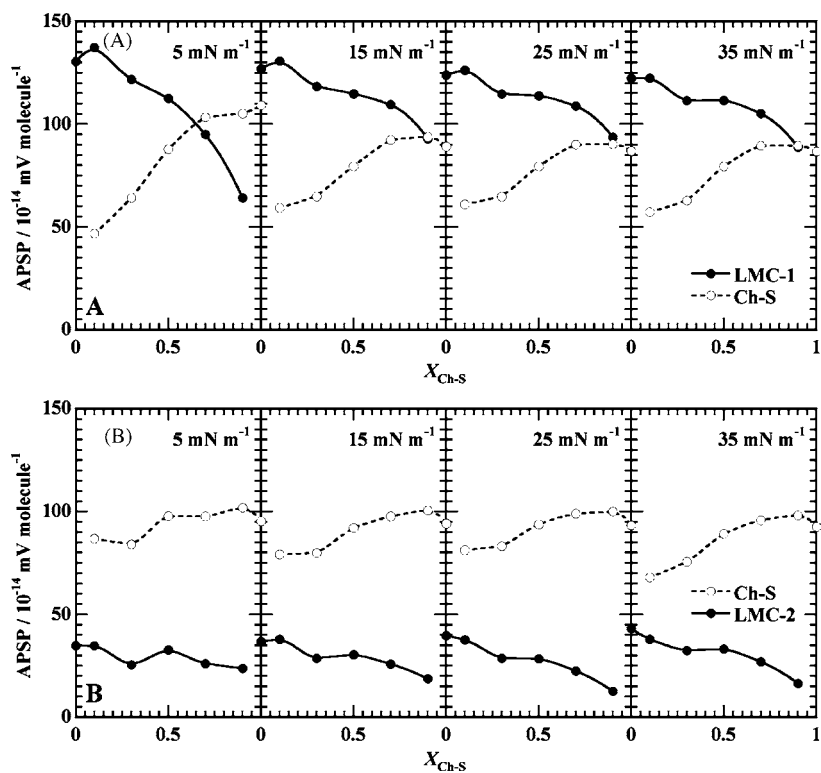


Fig. 7. Variation of apparent partial molecular surface potential (APSP) for two-component cerebroside and Ch-S as a function of $X_{\text{Ch-S}}$ at surface pressures of 5, 15, 25, and 35 mN m⁻¹: (A) LMC-1/Ch-S: closed circle: LMC-1, open circle: Ch-S; (B) LMC-2/Ch-S: closed circle: LMC-2, open circle: Ch-S.

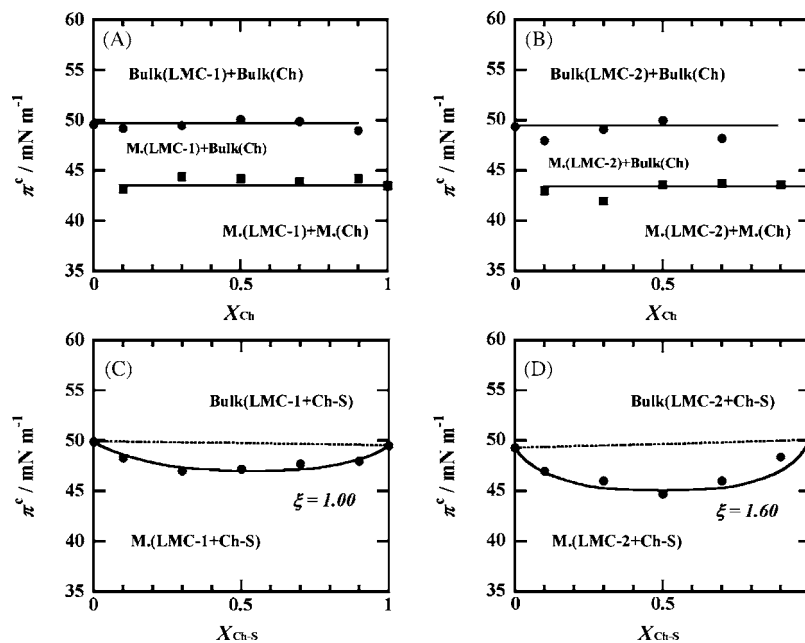


Fig. 8. Variation of the collapse pressure (π^c) as a function of X_{steroids} on 0.5 M NaCl at 298.2 K. The dashed line was calculated according to Eq. (5) for $\xi = 0$: (A) LMC-1/Ch, (B) LMC-2/Ch, (C) LMC-1/Ch-S, and (D) LMC-2/Ch-S systems.

form a solid film (Bulk) of its own (denoted Bulk(Ch) in the figure). Until the Ch completes its solid formation, the surface pressure remains constant. At much higher pressures of π , bulks of LMC-1 and Ch coexist independently, as shown by Bulk(LMC-1) + Bulk(Ch). In the middle surface pressure region, the monolayer (LE) of LMC-1 coexists with bulk state of the Ch.

The above implies that cerebrosides and Ch are not mixed in the monolayer state. This means that the lateral interaction between cerebrosides and Ch is extremely small. Then, two components are completely separated, and they form patched monolayers. Therefore, this phase diagram is divided into three parts by double parallel lines.

3.4.2. Cerebrosides/Ch-S

Next, the second type of phase diagram is constructed, and then, the coexistence phase boundary between the ordered monolayer phase and the bulk phase can be theoretically simulated by the Joos equation [1,17], which is the same as the preceding paper in series [1].

$$1 = x_1^s \gamma^1 \exp\{(\pi_m^c - \pi_1^c)\omega_1/kT\} \exp\{\xi(x_2^s)^2\} + x_2^s \gamma^2 \exp\{(\pi_m^c - \pi_2^c)\omega_2/kT\} \exp\{\xi(x_1^s)^2\} \quad (5)$$

where x_1^s and x_2^s denote the mole fraction in the two-component monolayer of components 1 and 2, respectively, and π_1^c and π_2^c are the corresponding collapse pressures of components 1 and 2, π_m^c is the collapse pressure of the two-component monolayer at given composition of x_1^s and x_2^s , ω_1 and ω_2 are the corresponding limiting molecular surface area at the collapse points, γ^1 and γ^2 are the surface activity coefficients at the collapse point, ξ is the interaction param-

eter, and kT is the product of the Boltzmann constant and the Kelvin temperature.

In Fig. 8, M. indicates a two-component monolayer formed by cerebrosides and Ch-S species, while Bulk denotes a solid phase of cerebrosides and Ch-S (“bulk phase” may be called “solid phase”). The collapse pressure π^c determined at each molar fraction is indicated by filled circles, where the dotted line shows the case where the interaction parameter (ξ) is zero.

As shown in Fig. 8C and D, the two-dimensional phase diagrams of the binary LMC-1/Ch-S and LMC-2/Ch-S systems show completely different phase behavior compared with cerebrosides/DPPC systems, as mentioned in the preceding paper [1]. That is, the interaction parameter (ξ) are 1.00 for LMC-1/Ch-S and 1.60 for LMC-2/Ch-S. The positive interaction parameter implies that the interaction energy between different molecules is smaller than the mean energy of interactions. Its interaction energy was calculated to be -413 and -661 J mol $^{-1}$ for LMC-1/Ch-S and LMC-2/Ch-S, respectively. That is, they are completely miscible, because interaction energy $-\Delta\varepsilon < 2RT (=4958.7$ J mol $^{-1})$. Two components are miscible each other in both the expanded state and the condensed state. This system is likely to be the negative azeotropic type, and the phase diagram is completely constructed.

3.5. Fluorescence microscopy of cerebrosides/steroids two-component monolayers

In order more to interpret the phase behavior on the π -A isotherms, we investigated the monolayers by fluorescence microscopy. The contrast is due to difference in dye solubility between disordered (or LE) and ordered phases (or

LC). Representative fluorescence micrographs (FMs) of pure systems (Ch and Ch-S) and binary systems (LMC-2/Ch and LMC-2/Ch-S) spread on 0.5 M NaCl at 298.2 K are shown in Figs. 9 and 10 at various surface pressures.

3.5.1. Cerebroside/Ch-S

Fluorescence micrographs of pure Ch-S and LMC-2/Ch-S two-component monolayers spread on 0.5 M NaCl are shown in Fig. 9 as a function of molar fraction of Ch-S at discrete surface pressures. The numerical value shows surface pressure in the figures. Essentially, two phases of bright and dark contrast were observed for pure Ch-S at low surface pressure (Fig. 9e), which are corresponding to the LE (white image) and gaseous (dark image) regions. The Ch-S monolayer on the 0.5 M NaCl substrate forms LC phase at the high sur-

face pressure (Fig. 9f). In binary LMC-2/Ch-S monolayer, the morphologies of white image do not change up to 50 mN m^{-1} in the FM images. The addition of small amount of cerebroside to Ch-S induced the phase separation at the zero surface pressure. Morphology of the separated phase was changed to homogeneous images by film compression up to high surface pressure. It suggests that cerebroside becomes miscible with Ch-S at high pressure.

3.5.2. Cerebroside/Ch

Fluorescence images of LMC-2/Ch monolayers on 0.5 M NaCl subsolution are shown in Fig. 10. FM images of the pure Ch showed some large domains at zero surface pressure (Fig. 10e), where the dark black regions at low surface pressure are the gaseous phase because fluorescence is completely quenched. The medium gray regions are the LC phase

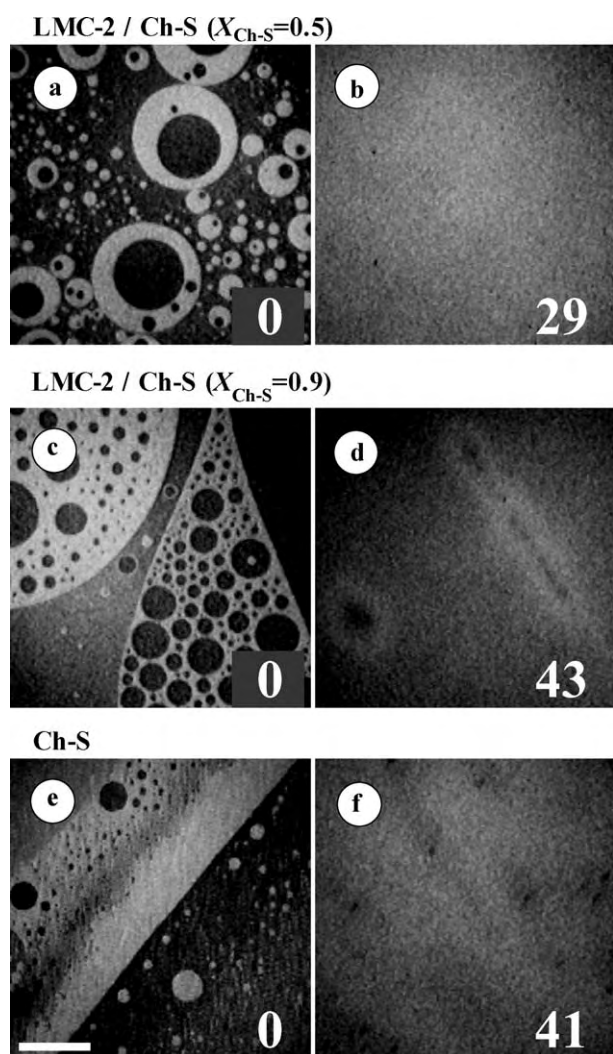


Fig. 9. Fluorescence micrographs of LMC-2/Ch-S two-component monolayer ($X_{\text{Ch-S}}=0.5$) (a: 0 mN m^{-1} , b: 29 mN m^{-1}), LMC-2/Ch-S two-component monolayer ($X_{\text{Ch-S}}=0.9$) (c: 0 mN m^{-1} , d: 43 mN m^{-1}) and Ch-S monolayer (e: 0 mN m^{-1} , f: 41 mN m^{-1}) observed at a compression rate of $1.0 \times 10^{-1} \text{ nm}^2 \text{ mol}^{-1} \text{ min}^{-1}$ at 298.2 K on 0.5 M NaCl. The monolayer contained 1 mol% of fluorescent probe. The number in these images indicates the surface pressure (mN m^{-1}). Scale bar represents $50 \mu\text{m}$.

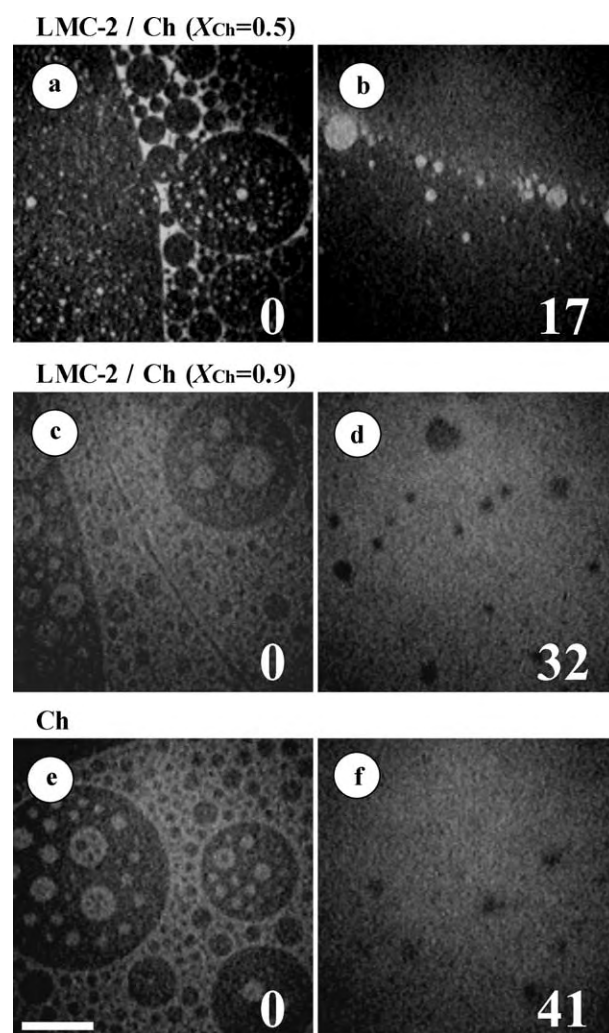


Fig. 10. Fluorescence micrographs of LMC-2/Ch two-component monolayer ($X_{\text{Ch}}=0.5$) (a: 0 mN m^{-1} , b: 17 mN m^{-1}), LMC-2/Ch two-component monolayer ($X_{\text{Ch}}=0.9$) (c: 0 mN m^{-1} , d: 32 mN m^{-1}) and Ch monolayer (e: 0 mN m^{-1} , f: 41 mN m^{-1}) observed at a compression rate of $1.0 \times 10^{-1} \text{ nm}^2 \text{ mol}^{-1} \text{ min}^{-1}$ at 298.2 K on 0.5 M NaCl. The monolayer contained 1 mol% of fluorescent probe. The number in these images indicates the surface pressure (mN m^{-1}). Scale bar represents $50 \mu\text{m}$.

in Fig. 10f. In the cases of cerebroside/Ch of $X_{\text{Ch}} = 0.5$ and 0.9, even at low surface pressure ($0\text{--}2 \text{ mN m}^{-1}$) and at high surface pressure (17 or 32 mN m^{-1}) the two phases coexist in Fig. 10a–d; that is, two components are immiscible each other, indicating that the two-dimensional phase diagram is reasonable.

According to the cerebroside/Ch-S two-component systems, cerebroside showed miscibility with Ch-S over all surface pressures. Therefore, it turned out that the miscibility of cerebroside with Ch or Ch-S results from the difference of the polar head group.

4. Conclusion

The cerebroside derived from echinoderm can be spread with steroids as stable monolayers on 0.5 M NaCl solution at 298.2 K. The $\pi\text{--}A$ and $\Delta V\text{--}A$ isotherms of cerebroside/Ch-S mixtures show that the two components are miscible in the monolayer state over the whole range of Ch-S mole fractions and of surface pressures investigated. From the $A\text{--}X_{\text{Ch-S}}$ and $\Delta V_{\text{m}}\text{--}X_{\text{Ch-S}}$ plots, partial molecular surface area (PMA) and apparent partial molecular surface potential (APSP) were determined at the discrete surface pressure. Each PMA behavior changes with the mole fraction and becomes the individual one at high surface pressure for the miscible system. On the other hand, the APSP shows almost the same behavior over the whole mole fraction regardless of the surface pressure for cerebroside/Ch-S systems. The binary systems of cerebroside/Ch suggest that the two components are completely immiscible in the monolayer state. The two-dimensional phase diagram and the Joos equation allowed calculation of the interaction parameter (ξ) and interaction energy ($-\Delta\varepsilon$) between cerebroside and Ch-S for miscible binary systems. The three types of phase diagrams were obtained and were classified as follows: a positive azeotropic (cerebroside/DPPC), a negative azeotropic (cerebroside/Ch-S) and a phase separate types (cerebroside/Ch and cerebroside/DPPE in the preceding paper). The sulfate polar head group and hydrophobic steroid segments strongly influence the surface potential. The Demchak and Fort treatment was applied to analyze the surface potential of steroids, from which the dipole moments of the polar head group and hydrophobic tail were determined to be 0.22 and 0.74 D, respectively. In fluorescence microscopy observation, the cerebroside/Ch-S systems on 0.5 M NaCl showed homogeneous FM images, while that of the cerebroside/Ch system showed immiscible pattern. These phenomena indicate that the miscibility of two-component system is influenced by hydrophilicity of polar head group (hydroxyl group and sulfate group).

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