Mixed Langmuir monolayer properties of sphingoglycolipids (cerebrosides) and lipids

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Surface pressure (π) - , surface potential (ΔV) - , and dipole moment ($\mu_{\perp}$) - area (A) isotherms were obtained for Langmuir monolayers made from sphingolipids(cerebrosides) (LMC-2), cholesteryl sodium sulfate (Ch-S) and cholesterol and their combinations.

1. INTRODUCTION

The interactions of animal cells with surfaces regulate such fundamental biological processes as growth, differentiation and motility. Although the nature of the interactions is not understood at the molecular level it is thought that the complex glycolipid and glycoprotein molecules which lie at the outer surface of the cells are involved.

Taking into account the above phenomena, as the first attempt, the surface pressure (π) and the surface potential(ΔV)- area (A) isotherms were obtained for mixed monolayers of different glucocerebrosides with lipids on substrate solution of 0.5M NaCl as a function of compositions in the mixture by employing the Langmuir method and the ionizing electrode method.

2. EXPERIMENTAL PART

Sphingoglycolipid was isolated from starfish *Luidia maculata*:LMC and purified as reported previously [1]. 1-O-(β-D-glucopyranosyl)-(1S,3S,4R)-2-[(2R)-2-hydroxy-carbonylamino]-alkane-1,3,4-triol (LMC-2) is chemical species whose chain lengths are 22, 23, and 24 on carbonyl group and 17, 18, and 19 chain lengths on alkane, respectively. Cholesteryl sodium sulfate (Ch-S) is the by-product from LMC. Its purity was checked by TLC and showed single spot. Cholesterol (Ch) was purchased from Nu-Chek-Prep, Inc., and also checked by TLC. The pure compounds or their mixtures were spread from a *n*-hexane/ethanol mixture (7/3) at the air /aqueous solution interface. While Ch-S was spread from a chloroform / methanol mixture (2/1) which are both Merck (Uvasol).

Other experimental conditions were the same as described in the previous papers |2|.

3.RESULT AND DISCUSSION

3.1.Surface pressure (π) -, surface potential (ΔV) -, and dipole moment ($\mu_\perp$) - area (A) isotherms

The π-A, ΔV-A, and $\mu_\perp$-A isotherms of monolayers made from pure LMC-2 and Ch-S spread on a 0.5 M NaCl substrate (at 298.2 K) are shown on Figure 1a-b. LMC-2 was stable up to 47.2mN m^{-1} with a liquid-expanded (LE) phase (Fig. 1a). The extrapolated area in the condensed state was 0.65 nm^2 and the collapse area 0.44 nm^2. Cholesteryl sodium sulfate (Ch-S) isotherm was condensed, indicating that the monolayer was in a liquid condensed (LC) phase. It collapsed at 52.2 mN m^{-1} (0.30 nm^2) and the extrapolated area was 0.36 nm^2. These values indicate that the steroid skeletons are at close contact at high pressure.

The surface potentials (ΔV) of LMC-2 and Cholesteryl sodium sulfate (Ch-S) show positive change (Fig. 1b). The Cholesteryl sodium sulfate (Ch-S) monolayer showed an increase of ΔV under compression. ΔV became gradually jump and reached the value of about 280 mV (starting from 40 mV, an absolute difference of 240 mV) at the collapse area (0.30 nm^2). The LMC-2 monolayer displayed a much smaller variation of ΔV (from 0 mV to 100 mV) at the collapse area (0.44 nm^2). The small variation of ΔV(LMC-2) reflects the orientation change during compression. The LMC-2 head area is much larger than the
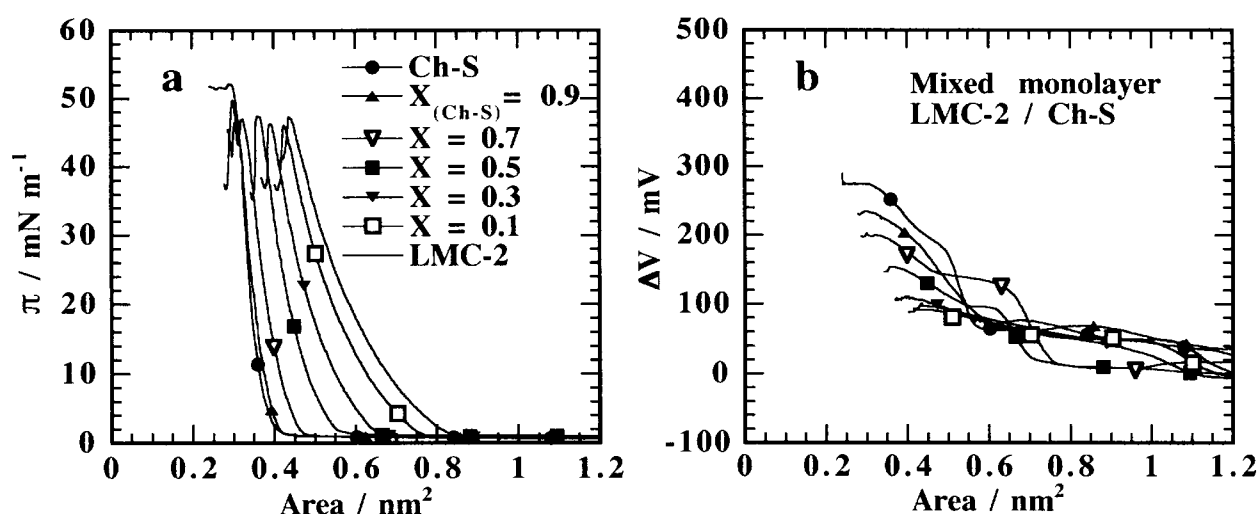
Fig. 1. Surface pressure (π) - area (A) isotherms (a), and surface potential (ΔV) - area (A) isotherms (b) on 0.5 M NaCl at 298.2 K.

cholesteryl sodium sulfate (Ch-S) head area, which results in a loose packing of LMC-2 chains. The larger increase of ΔV observed in the case of Ch-S can be explained by the higher organization of the monolayer, as compared to that of LMC-2, due to increased mutual polarization.

The variations of the vertical component of the surface dipole moment, $\mu_\perp$, of LMC-2 and Ch-S monolayers under compression are obtained. $\mu_\perp$ strongly depends on the polar head group's nature. Upon compression, $\mu_\perp$ (Ch-S) increased from about 100 mD to 215 mD, while $\mu_\perp$(LMC-2) only increased from 40 mD to 110 mD.

3.2. Ideality of the mixture

The two-component mixed monolayer system composed of LMC-2 and Ch-S was studied in order to assess the impact of the molecular structure of the amphiphiles on their miscibility in the monolayer, and on the state of the monolayer. For the above purpose, the π - A , ΔV-A and $\mu_\perp$- A isotherms of the LMC-2 and Ch-S mixed monolayers were measured for various Ch-S molar fractions (X_{Ch-S}) (298.2K, 0.5M NaCl substrate). Results are shown in Figure 1. All the curves of the mixed system exist between those of the respective pure components, and they successively change with increasing mole fraction.

An understanding of the interactions between LMC-2 and Ch-S is provided by examining whether the variation of the mean molecular area as a function of X_{Ch-S} does satisfy the additivity rule or not. For all surface pressures (5, 15, 25, and 35 mN m^{-1}), it clearly shows a negative deviation between the theoretical and experimental curves, indicating some interactions between LMC-2 and Ch-S. These interactions may likely result from attractive interactions between LMC-2 and Ch-S polar heads.

We have also examined the influence of X_{Ch-S} on the ΔV-A and $\mu_\perp$ - A curves. Both ΔV and $\mu_\perp$ reflected the π-A behavior: the higher the collapse pressure, the larger the ΔV and $\mu_\perp$ values. An analysis of the surface potential (ΔV) and of the surface dipole moment ($\mu_\perp$) of the monolayer was also made in terms of the additivity rule. For LMC-2/Ch-S mixed system, assuming the additivity rule, variations of ΔV and $\mu_\perp$ at various surface pressures (15, 25 and 35 mN m^{-1}) showed a significant negative deviation.

3.3.Two-Dimensional Phase Diagram

Two-dimensional phase diagrams of LMC-2 and Ch-S monolayers were constructed by plotting the values of collapse pressures as a function of Ch-S mole fractions. The coexistence phase boundary between the expanded phase of the LMC-2 and Ch-S mixture and the bulk phase can be theoretically simulated by the Joos equation [3]. It is noteworthy that the LMC-2 /Ch-S system produced a positive interaction parameter ($\xi = 1$). Such a positive parameter implies that the interaction energy between LMC-2 and Ch-S (which was calculated to be 413 J mol^{-1}) is lower than the mean energy between similar molecules. That means that the two components are completely miscible in the expanded state but are not miscible in the condensed state. However their mutual interaction is weaker than the mean of interactions between pure component molecules themselves [4]. The above procedures were performed for LMC-2/ Ch system.

To ascertain the above phenomena, the miscibility of LMC-2 / Ch-S and LMC-2 / Ch systems have to be further studied by other techniques such as Brewster angle microscopy, and fluorescence microscopy which will be reported in a separate paper.

In conclusion, the new finding of this study is that cerebrosides (LMC-2) and Cholesteryl sodium sulfate (Ch-S) can be spread as a stable monolayer at 298.2 K on a 0.5M NaCl subphase. Judging from the collapse pressure, our phase diagrams might be classified into two types. *The first* might be an eutectic type, which the two combinations of cerebroside (LMC-2) with cholesteryl sodium sulfate (Ch-S) is assigned; they are miscible in the expanded state but immiscible in the condensed state. *The second* is a completely immiscible type in the expanded state and in the condensed state; the combination of cerebroside (LMC-2) with cholesterol (Ch) .

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