A Study on the Formation of Liquid Ordered Phase in Lysophospholipid/Cholesterol/1,3-Butanediol/Water and Lysophospholipid/Ceramide/1,3-Butanediol/Water Systems

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Abstract: Formation of a liquid ordered phase in lysophospholipid/cholesterol/1,3-butanediol/water and lysophospholipid/ceramide/1,3-butanediol/water systems was investigated. Differential scanning calorimetry confirmed that a liquid ordered phase was formed in the lysophospholipid/cholesterol/1,3-butanediol/water system similar to that in phospholipid systems. The structure of liquid ordered phase was analyzed by using X-ray scattering measurements. It was revealed that the liquid ordered phase has a lamellar structure in which the hydrophobic chains are less ordered than in \( \alpha \)-type crystals. On the other hand, in the lysophospholipid/ceramide/1,3-butanediol/water system, a liquid ordered phase was not formed.

Key words: lysophospholipid, cholesterol, ceramide, liquid ordered phase, lamellar structure, hydrated solid

1 INTRODUCTION

Ladbrooke et al. reported that the main transition peak in a differential scanning calorimetry (DSC) spectrum for the phase transition from a hydrated solid (gel) phase to a liquid crystalline phase disappeared when a part of the phospholipid was replaced with cholesterol (Chol) in a phospholipid/water system. Later, Demel and Kruyff suggested that the state of a lipid mixture was intermediate between a hydrated solid (gel) and a liquid crystal, and it was called the intermediate gel state. In recent studies of phospholipid/Chol mixtures, the term “liquid ordered phase” has been used instead of intermediate gel state. It is thought that the addition of Chol has the effect of softening the phospholipid bilayer below the transition temperature, and hardening it above the phase transition temperature. Liquid ordered phases have been studied by using various techniques such as X-ray diffraction, DSC, fluorescence, NMR, and other methods. Because of the unique chemical property of liquid ordered phases that they do not undergo phase transitions, they can be applied to manufacturing products, such as cosmetics, that require chemical stability under various environmental conditions. In fact, a liquid ordered phase that is formed by phospholipids, Chols, and water is applied to produce liposomes. Liposomes have attracted attention because of their effectiveness, stability, and safety, and have been applied to industrial products. However, little research has been conducted to examine mixture systems of Chol and surfactants other than phospholipids.

Lysophospholipids (LPLs) are obtained by the hydrolysis of phospholipids with phospholipases, and have structures in which alkyl chains esterified to the C2 of glycerol are converted to the hydroxyl group. Therefore they have excellent surfactant properties, and have been widely used in the food industry but not in the cosmetics industry. It has been reported that LPLs have lower surface and interfacial tensions compared to phospholipids. Krafft points and cloud points are observed in the phase diagrams of LPL/water systems. A micellar phase appears at low LPL concentrations (below 10%) and a lamellar liquid crystalline phase appears at high concentrations. Below the Krafft point, a \( \beta \)-type hydrated solid is formed but it changes to an \( \alpha \)-type solid upon mixing with long-chain alcohols. It has been shown that the effects of pH and of polyols and electrolytes, which are often added to cosmetics, on the phase behaviors of LPL/water systems are small. It has
also been reported that when an LPL was used as an emulsifier, the emulsion stability against electrolyte addition or pH change was improved.\textsuperscript{20,24}

Chol and also ceramide (Cer) are main components of intercellular lipids with contents of 26.9% and 40.0%, respectively. Intercellular lipids have the functions of holding moisture in the stratum corneum and effectively protecting against the intrusion of various water-soluble substances, and both of Chol and Cer are important for maintaining the moisture level in skin. Thus their solubilization at a molecular level in cosmetic formulations is important to functionalize cosmetic products. LPLs are formed through the hydrolysis of phospholipids and have small critical packing parameters. Therefore, LPLs could have solubilize larger amounts of Chol and Cer than phospholipids with large critical packing parameters. In this paper, we report the formation of a liquid ordered phase and the solubilization of Chol and Cer in aqueous LPL systems.

2 EXPERIMENTAL

2.1 Materials

The LPL was purchased from Nippon Fine Chemicals Co. Ltd., Osaka, Japan. This LPL was obtained by hydrolyzing phospholipids containing phosphatidylcholine (73.1%) and phosphatidylethanolamine (12.8%) as major components, with the minor components being phosphatidylinositol, phosphatidylserine and phosphatidic acid. The degree of hydrolyzation was 90%. Chol was obtained from Nippon Suisan Kaisha, Ltd., Tokyo, Japan. Cer was obtained from Takasago International Corporation, Tokyo, Japan. The Cer contained over 90% N-[15(2R)-2-hydroxy-1-(hydroxymethyl)heptadecyl]-octadecanamide. 1,3-Butanediol (1,3-BD) was obtained by Daicel Corporation, Tokyo, Japan. Water used was purified by an RO system (Elix 3, Millipore).

2.2 Sample preparation

Screw cap test tubes containing the required amounts of reagents were homogenized at 110°C and then were cooled at 25°C over 1 d. The samples were used for X-ray scattering and DSC measurements.

2.3 X-ray scattering measurement

X-ray scattering measurements were performed using a Kratky-type small angle scattering camera (SAXSsess, Anton Paar, Austria). Cu-Kα (λ = 0.1542 nm) radiation was used. An image plate was used as a detector and the scattering intensities of the X-rays were detected by a Cyclone image plate reader (Perkin Elmer Co. Waltham, MA, USA).

2.4 Differential scanning calorimetry measurements

DSC measurements were performed on a differential scanning calorimeter (DSC6200, SII Nano Technology Inc. Ciba, Japan). Samples of 10-20 mg were weighed in aluminum capsules and an empty aluminum capsule was used as the reference. The samples were cooled to −80°C before the measurements to avoid a super cooled state. The samples were heated from −10°C to 110°C at the rate of 10°C/min.

3 RESULTS AND DISCUSSION

3.1 Formation of liquid ordered phase in an LPL/Chol/1,3-BD/water and LPL/Cer/1,3-BD/water systems

To examine the formation of the liquid ordered phase, we performed DSC measurements for various mole fractions of Chol in mixtures of LPL and Chol(X\textsubscript{C}) in LPL/Chol/1,3-BD/water systems. The concentration of the aqueous solvent (1,3-BD + water) was fixed at 50 wt% in the system and the 1,3-BD concentration in the aqueous solvent was fixed at 10 wt%. In cosmetics applications, 1,3-BD is widely used as a moisturizer, texture conditioner and preservative. Figure 1 shows the results of the DSC measurements. At X\textsubscript{C} = 0, a large endothermic peak of the main phase transition can be observed approximately 50°C. The main phase transition temperature for the hydrated solid (gel) - liquid crystal transition gradually decreases with increasing X\textsubscript{C} and disappears above X\textsubscript{C} = 0.48. This result reveals the presence of a liquid ordered phase in the LPL-Chol mixed system, as is also found in phospholipid-Chol systems. Figure 2 shows a schematic diagram of the liquid ordered phase in the LPL/Chol/1,3-BD/water system.

Figure 3 shows similar DSC results for a system with Cer instead of Chol at different Cer mole fractions of in mixtures of LPL and Cer(X\textsubscript{C}) at X\textsubscript{C} = 0 and 0.16, a large endothermic peak for the main phase transition between hydrated solid (gel) and liquid crystals can be observed at approximately 50°C. This main transition peak shifts to a higher temperature of around 70°C at X\textsubscript{C} = 0.30-0.53 and then, splits into two peaks at X\textsubscript{C} = 0.63. A small peak appears at around 100°C and extensively grows when X\textsubscript{C} exceeds 0.72. The peak at around 100°C could be due to separated Cer. Although Cer molecules can be solubilized up to X\textsubscript{C} = 0.53, a liquid ordered phase is not formed in the Cer-containing system. Both Chol and Cer are the main components of intercellular lipids and act as moisturizing agents for skin. It is interesting, however, that the effects of those molecules on the liquid ordered phase formation are different. Further studies are necessary in the future to better understand this difference.

Figure 4 shows the main transition enthalpy, ΔH, in Fig. 1 as a function of X\textsubscript{C}. ΔH becomes zero at X\textsubscript{C} = 0.42, indicating a liquid ordered phase is obtained above this concentration. Figure 5 shows plots of the main phase transition
temperatures in the LPL/Chol/1,3-BD/water systems as a function of $X_1$. The transition temperature decreases from 51°C for the Chol-free system to 45°C at $X_1 = 0.37$ because of the softening effect of Chol on the bilayer for the LPL gel phase.

3.2 Structural analysis of the liquid ordered phase in the LPL/Chol/1,3-BD/water system

We performed small- and wide-angle X-ray scattering

Fig. 1  DSC curves as a function of $X_1$ obtained for LPL/Chol/1,3-BD/water system . $X_1$ indicates the mole fraction of Chol in the LPL + Chol mixture. 1,3-BD aqueous solution concentration is fixed at 50 wt% for the system and 1,3-BD concentration is fixed at 10 wt% in a 1,3-BD aqueous solution.

Fig. 2  Schematic diagram of liquid ordered phase.

Fig. 3  DSC curves as a function of $X_2$ obtained for LPL/Cer/1,3-BD/water system . $X_2$ indicates the mole fraction of Cer in the LPL + Cer mixture. 1,3-BD aqueous solution concentration is fixed at 50 wt% for the system and 1,3-BD concentration is fixed at 10 wt% in a 1,3-BD aqueous solution.

Fig. 4  Plot of main phase transition enthalpy as a function of $X_1$. 

measurements of the LPL/Chol/1,3-BD/water system. Figure 6-1 and 6-2 show the small-angle and wide-angle X-ray scattering spectra at 15°C and 80°C, respectively. The concentration of 1,3-BD + water was fixed at 50 wt% in the system and the 1,3-BD concentration was fixed at 10 wt% in the 1,3-BD + water.

At 15°C, a sharp peak at around $q = 15 \text{ nm}^{-1} (d = 0.42 \text{ nm})$ appears for $X_1 = 0.0-0.26$, indicating the formation of $\alpha$-type crystals in which the surfactant molecules are arranged in hexagonal arrays in the bilayer. Note that $\beta$-type crystals are formed in binary water/LPL systems but they change to an $\alpha$-type probably because of the presence of a hydrotrope (1,3-BD). At $X_1 = 0.37$ and 0.48, a peak still exists in the WAXS region, but it shifts to a slightly higher $q$ and becomes weak. A broad peak gradually evolves at around $q = 12 \text{ nm}^{-1}$ with increasing $X_1$ and only this peak remains at $X_1 = 0.58$, indicating that only the liquid ordered phase exists at this concentration. At $X_1 = 0.67$, a number of small peaks are observed, suggesting that Chol is separated from the liquid ordered phase and remains as Chol crystals. In other words, the Chol solubilization is possible only below $X_1 = 0.58$.

In the SAXS region, a set of peaks (a) for a lamellar structure is clearly observed in the Chol-free system and this set of peaks (a) remains up to $X_1 = 0.26$. Above $X_1 = 0.13$, another lamellar pattern (b) with larger interlayer spacing appears and it continues to grow in peak intensity up to $X_1 = 0.67$. Combining the information about WAXS region, it is determined that the lamellar structure with the smaller interlayer spacing is due to $\alpha$-type crystals and that with the larger interlayer spacing represents the liquid ordered phase. This difference becomes clearer by plotting the long spacing, $d$, as a function of $X_1$ as is shown in Fig. 7. The hydrophilic group of LPL is bulky, and Carnie has estimated that the area of the hydrophilic group is 71.7 Å². On the other hand, the area of the hydrophilic group of Chol is 19.0 Å². Therefore, the larger interlayer spacing for the liquid ordered phase is caused by the mixing of Chol molecules in the LPL bilayers since the water composition is constant for all mixture compositions of LPL and Chol. In other words, the $\alpha$-type crystals consist of pure LPL but the liquid ordered phase is formed by the LPL and Chol mixture.
A broad peak is observed in each of the WAXS spectra at 80 °C instead of the sharp peaks that appear at 15 °C and at low X₁. The broad WAXS peaks are located around q = 13 nm⁻¹ for X₁ below 0.26 but they shift to lower qₚ above X₁ = 0.37. As is found in the DSC results and the X-ray scattering results at 15 °C, a liquid ordered phase, which does not show a phase transition with temperature change, is formed above X₁ = 0.42. Therefore, the broad peaks for high and low X₁ originate from a liquid ordered phase and a liquid crystalline phase, respectively.

The SAXS spectrum for X₁ = 0 shows a set of peaks (c) for a hexagonal structure for which the interlayer spacing ratio is 1: √3. The hexagonal peaks remain up to X₁ = 0.26 but another set of peaks (d) also appear for a lamellar structure. For X₁ = 0.13 and 0.26, two different structures, a hexagonal and a lamellar structure, coexist at 80 °C. Above X₁ = 0.37, only lamellar peaks (d) appear.

Figure 8 depicts the phase behaviors of the LPL/Chol/1,3-BD/water systems as functions of composition and temperature. It is apparent that a liquid ordered (LO) phase is present across wide ranges of composition and temperature. However the LO phase only exists in a restricted range of between 0.42-0.63.

4 CONCLUSION

There are many publications that describe phospholipid-Chol mixed systems but few regarding LPL-Chol systems. It is known that the effects of Chol on the phospholipid bilayer are opposite above/below the phase transition temperature. Because of this interesting effect, a liquid ordered phase is formed by mixing Chol in the phospholipid bilayer. In this study, the sol-gel transition peak disappeared, which confirmed the formation of a liquid ordered phase, upon mixing Chol with LPL crystals. Cer and Chol are the main components of intercellular lipids. However, Cer does not have the same effect as Chol has although Cer can also be solubilized in the LPL bilayer. These findings are important for manipulating lipid aggregate structures and also important for the progress of cosmetic formulation technologies.

References


