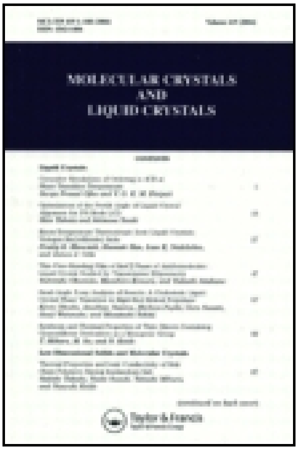

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Effects of Chaotropic and Kosmotropic Solutes on the Structure of Lipid Cubic Phase: Monoolein-Water Systems

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Effects of Chaotropic and Kosmotropic Solutes on the Structure of Lipid Cubic Phase: Monoolein-Water Systems

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Using X-ray diffraction technique, we investigated the modulation of the bicontinuous cubic structure of monoacylglycerol (monoolein)-water systems by the presence of solutes. X-ray diffraction results demonstrated that chaotropic solutes (NaSCN, NaI, and urea) expand the lattice constants of fully hydrated monoolein in Pn3m cubic phase and on the other hand, that kosmotropic solutes (Na_2SO_4, NaCl, and sucrose) reduce the lattice constants. These results can be interpreted as suggesting that kosmotropes reduce the interfacial area and that chaotropes expand the interfacial area, by assuming independence of the molecular length of monoolein on the presence of solutes.

Keywords: lipid cubic phase; monoacylglycerol; hofmeister effect; x-ray diffraction; lyotropic liquid crystal

INTRODUCTION

Lipid aqueous dispersions are one of the most intensive studied biological lyotorpic liquid crystal systems. One of the reasons is that a lipid bilayer is the fundamental structure of biological membranes. From also the viewpoint of physics on phase transitions, lipid systems have been paid much attention, because the systems exhibit various polymorphic phase behavior (lamellar, hexagonal, cubic phases, etc.) [1,2]. The physical studies on the lipid phase behavior have revealed that many factors affect the polymorphism of lipids, for example, temperature, pH, ionic strength, etc. To study in detail the effects of ions and small molecules on the lipid phase behavior is of interest from the both biological and physical viewpoints, since many kinds of ions and low molecular weight substances exist in cells.

For proteins, which are another main components of biological membranes, the systematic effects of different neutral solutes on the solubility (salting out of proteins) are well known as the Hofmeister effect [3]. In the framework of the Hofmeister effect, substances are divided into two groups, i.e., kosmotropic substances (kosmotropes) and chaotropic substances (chaotropes). Kosmotropes (Na_2SO_4, NaCl, sucrose, etc.) have a great ability of salting out of proteins and stabilize the native state of proteins. On the other hand, chaotropes (NaSCN, NaI, urea, etc.) destabilize the native state of proteins.

The effects of kosmotropes and chaotropes on the phase stability of lipid-water systems have been investigated for the lamellar gel, lamellar liquid-crystalline and the inverted hexagonal phases of various phospholipids and glycolipids [4, 5 and references in [5]]. These studies have showed that kosmatropes stabilize the lamellar gel and inverted hexagonal phases and chaotrpes stabilize the lamellar liquid crystalline phase. These results have been interpreted as follows [4,5]: Kosmotropes tend to be excluded from interfacial regions between water and proteins or lipids and to reduce the amount of interfacial water molecules, since they stabilize the structure of bulk water [3]. Hence, it is expected that they tend to reduce the interfacial area of water and lipids. Consequently, kosmotropes stabilize the phases having smaller area per a lipid molecule (lamellar gel and inverted hexagonal phases) and destabilize the phase having larger area (lamellar liquid-crystalline phase). For chaotropes, the opposite situation happens. Several structural evidences supporting above interpretation have been reported for phosphatidylcholines and phosphatidylethanolamines [4,6,7].

To our knowledge, no systematic studies on the effects of solutes have been conducted for lipid systems forming cubic phases, although lipid cubic phases have recently become an interested subject in connection with the success of the crystallization of membrane proteins using the lipid cubic phase [8] and the attempt to use the lipid cubic phase for drug delivery [9]. The structures and properties of lipid cubic phases have been described in detail in several review articles [10-12]. The effect of several kinds of sodium salts on the lattice constant of cubic phases induced by subjecting to a thermal cycling treatment in fully hydrated phosphatidylethanolamines has been reported [13] and the result is inconsistent with above interpretation. For the results, however, there is a question whether the cubic phases formed after the thermal cycling treatment are thermodynamically stable phases or not.

This study was carried out in order to determine the effects of kosmotropic and chaotropic solutes on the structure of lipid cubic phase. Monoacylglycerol (monoolein) was chosen because its phase behavior has been revealed in detail [14-19] and it has been used in the studies of proteins crystallization [8] and drug delivery [9]. X-ray diffraction was used to monitor the structural changes of the cubic phase of monoolein, which is formed under excess water condition at room temperature [14-19], by various solutes. Three kosmotropes (Na_2SO_4, NaCl, and sucrose) and three chaotropes (NaSCN, NaI, and urea) were examined.

MATERIALS AND METHODS

The monoacylglycerol, 1-monooleoyl-*rac*-glycerol (monoolein) was obtained from Sigma Chemical Co. (St. Lois, USA). It had a purity >99% and was used without further purification. Reagent grade chemicals of NaSCN, NaI, Na_2SO_4, NaCl and urea were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sucrose was obtained from Sigma Chemical Co. Water used in this study was purified using Mill-Q System (Millipore Corp., Bedford, USA).

The hydrated monoolein samples were prepared using the method reported by Rummel et al. [20]: The lipid/solution mixture was put into an Eppendorf tube (2 ml) and centrifuged in a fixed angle rotor in a temperature-controlled centrifuge (MX-150, Tomy Ltd., Tokyo, Japna) with 10,000 rpm for about 5 min at 30 °C. Thereafter, the tube was rotated by 180° and recentifuged again with the same condition described above. This procedure was repeated 5 to 10 times to get homogeneous samples. The sample was sealed in a fine wall glass capillary with 1 mm or 1.5 mm diameter. The lipid concentration was about 30 wt% and the samples were visually inspected before use to ensure that they satisfied the excess water condition.

X-ray diffraction data collection was carried out using a Ni-filtered CuK_α radiation source from a rotating anode X-ray generator (RU200BEH, Rigaku, Tokyo, Japan) running at 40 kV and 30 mA and a two-dimensional area detector (Imaging plate, Fuji Photo Film Co. Ltd., Tokyo, Japan). X-ray beam was focused with a double-mirror optical system. The capillary containing sample was fixed to a brass hollow holder. Temperature of the sample was controlled within ± 0.1 °C by circulating water from a temperature-controlled water bath (B. Braun, Melsungen, Germany) to the sample mount.

The data on imaging plates were digitized with a BAS2000 System (Fuji Photo Film Co. Ltd., Tokyo). The diffraction spacings were calibrated using the diffraction peaks from anhydrous cholesterol [21]. The typical exposure time and sample-to-detector distance were 4 hours and 420 mm, respectively. For several samples, high-resolution measurements using synchrotron X-ray radiation source were also performed at beamline 15A of the Photon Factory in High Energy Accelerator Research Organization (KEK) at Tsukuba, Japan [22]. The patterns of X-ray scattering were recorded using a one-dimensional position sensitive proportional counter with 300 mm effective length (Rigaku). The exposure time was less than 60 sec.

RESULTS

All X-ray diffraction measurements were performed at 20 °C. At this temperature, under excess water condition, monoolein forms a bicontinuous cubic phase with the space group of Pn3m in pure water [15-19]. The Pn3m cubic phase gives rise to the diffraction series with the reciprocal spacing ratio of $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}$ ...., according to the extinction rule for symmetry [10]. The space groups of samples dispersed in various solutions were determined based on the ratios of observed spacings. It was found that monoolein forms the Pn3m cubic phase at 20 °C in all solutions examined in this study within the solute concentration of less than 0.75 M. For the samples dispersed in NaSCN solution of more than 1M concentration, it was impossible to determine the structure owing to the broadness of the diffraction patterns.

The lattice constants of the cubic phase of monoolein varied, depending on the kinds of the solutions. Typical X-ray diffraction patterns are shown in Figure1. In pure water, the lattice constant of monoolein was 10.0 nm, agreeing with the previous reported value [16-19]. While, the lattice constant for the sample dispersed in 0.5 M NaSCN solution was 13.7 nm and the lattice constant was 8.99 nm for 0.5 M Na_2SO_4 solution. The dependence of the lattice constants on the kinds and concentration of the solutes is illustrated in Figure 2. It is evident from this figure that NaSCN, NaI, and urea increase the lattice constants of Pn3m cubic phase of monoolein with increasing the concentration and on the other hand, that Na_2SO_4, NaCl and sucrose decrease the lattice constants with increasing the concentration.

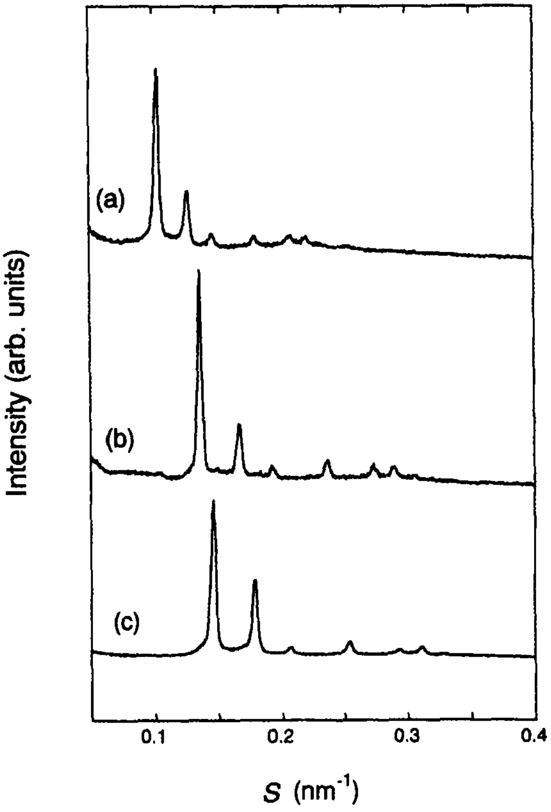

FIGURE 1 X-ray diffraction patterns of monoolein dispersed in (a) 0.5 M NaSCN, (b) pure water and (c) 0.5 M Na_2SO_4 recorded at 20 °C. The horizontal axis represents the reciprocal space ($S = 2\sin\theta/\lambda$, 2θ = scattering angle, λ = x-ray wavelength). These patterns were recorded at the beamline 15A of the Photon Factory.

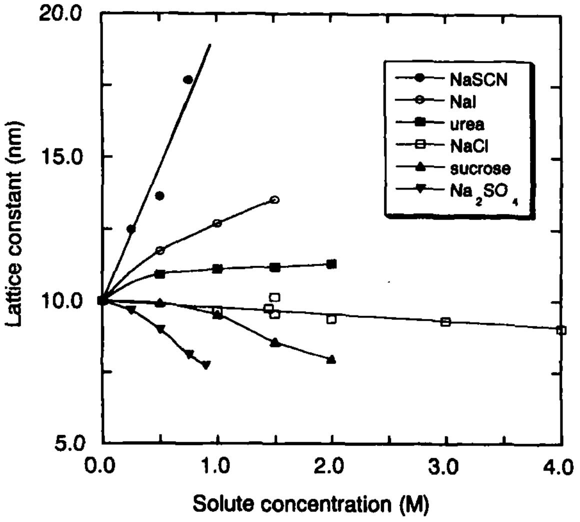

FIGURE 2 The dependence of the lattice constants of the Pn3m cubic phase of momoolein at 20 °C on the concentrations of various salutes. NaSCN (●), NaI (○), urea (■), NaCl (□), sucrose (▲) and Na_2SO_4 (▼).

DISCUSSION

According to the strength of stabilizing effect on proteins, a representative Hofmeister series for anions is given in the order: $SO_4^{2-} > F^- > Cl^- > Br^- > I^- > SCN^-$ [23]. In this series, the left side is kosmotropic and the right side is chaotropic. The changes of the lattice constants of cubic phase observed in this study are in line with the Hofmeister series for sodium salt. Sucrose and urea are recognized as kosmotrope and chaotrope, respectively, from the effect on the denaturation of proteins [3]. Hence, using the terms of Hofmeister seris, the present results are expressed as follows: Chaotropes expand the lattice constants of the cubic phase of monoolein, while kosmotropes reduce the lattice constants.

In the previous studies [4,5], the stabilization or destabilization of the lamellar and inverted hexagonal phases of phospholipids and glycolipids by kosmotropes and chaotrpes has been discussed based on the tendencies which kosmotropes reduce the interfacial area and chaotropes expand the interfacial area as described in the introduction section. In order to determine whether the present X-ray diffraction results of the cubic phase agree with above interpretation, we consider the relation between the lattice constants and the interfacial area in the Pn3m cubic phase of monoolein.

From the analysis of the dependence of the lattice constants on the hydration level for the Ia3d cubic phase of monoolein-water system, Chung and Caffrey [24] have shown that the length of the lipid molecule hardly varies with the change of the lattice constant. Engblom and Hyde [25] have proposed a model for the dependence of the lattice constants on the hydration level for cubic phase systems, assuming a constant lipid length, and they have reported that the experimental data can be fitted reasonably using the model for the Pn3m cubic phase of monoolein-water systems. Taking into these results consideration, it would be reasonable to assume that the length of monoolein molecule in the Pn3m cubic phase is constant for the case of the presence of solutes.

Based on this assumption and the geometrical relation of the cubic structure [26], it can be concluded that the increase and decrease of the lattice constants of the Pn3m cubic phase correspond to the increase and decrease of the area at the water/lipid interface, respectively. Hence, the present X-ray diffraction data suggest that kosmotropic solutes reduce the interfacial area of the Pn3m cubic phase of monoolein and chaotropic solutes expand the interfacial area. In other words, the present study implies that the effects of kosmotropes and chaotropes are the same not only for the lamellar and inverted hexagonal phases but also the cubic phase of lipid aggregations.

Acknowledgments

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