

Salt Effects on Lamellar Structure of Nonionic Surfactants

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Received: 16 November 2015 / Accepted: 26 September 2016 / Published online: 12 October 2016
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Abstract Effects of monovalent and monatomic salts on lamellar repeat distances d of nonionic surfactants (monomyristolein and $C_{12}E_2$) are investigated using small-angle X-ray diffraction. The lamellar repeat distances (sum of thicknesses of a bilayer and a sandwiched water layer) increase with increasing salt concentration with a strong anion dependence ($Br^- > Cl^-$). The increase of the thickness of the water layer is found to dominate the increase in d . Since the anion dependence is inconsistent with the ion dependence of the strength of the primary hydration, we reported previously (Hishida et al. J Chem Phys 142:171101, 2015), the hydration force classically considered is not the origin of the increase in d . This means the increase in d cannot be explained by the existing model of the forces between neutrally charged bilayers. The temperature dependence of d also supports the necessity for a new mechanism of the effect of ions. The new mechanism seems to be related to the water structure beyond the primary hydration water, i.e., the secondary hydration water, which depends on the ion species.

Keywords Nonionic surfactant · Lamellar repeat distance · Monovalent ion · Role of water structure

1 Introduction

The self-assembly of soft materials such as surfactants, lipids and polymers in water is widely used in cosmetics, detergents, foods, etc. It is also important for the proper functioning of biomolecules in living cells. In many cases salts have drastic influences. Charged soft materials are dispersed in water without salt due to the electrostatic repulsion, while

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they are aggregated due to charge-screening by salts [1]. The effects of salts on the self-assembly, however, still have many mysteries. In 1888, Hofmeister [2] reported that the aggregation of soft materials by addition of salts exhibits strong ion dependence, which is called the Hofmeister effect. Since the Hofmeister effect is also recognized for uncharged soft materials [3, 4], in reality the aggregation is not induced by the charge-screening. Although the mechanism of the Hofmeister effect still remains unclear, a vast number of studies have suggested that interactions between soft materials are altered somehow by the perturbation of water structure (hydrogen-bond network) by ions, which depends on the ion species [5, 6].

At the surface of solvated soft materials in water, there exist hydrating water molecules that have different physical properties from bulk water. Apart from the Hofmeister effect, the effects of the hydration water on the interactions between soft materials have been widely investigated [7–10]. The assumed force, related to the existence of the hydration water, is usually called a hydration force (or solvation force): hydration water of two surfaces repel each other because of opposite orientation of water molecules at each surface [7, 11]. Previously, strongly bound hydration water has been considered as the sole origin of the repulsion [7, 11]. Since the strongly bound hydration water molecules form almost a single layer of water molecules, the decay length of the hydration force has been estimated about 2 Å [7]. Recently, however, another, relatively longer-ranged, hydration force ($\sim 10\text{--}20$ Å) has been suggested [8–10, 12].

The short-range force is often called the primary hydration force and the long-ranged one the secondary hydration force. It has been claimed that the secondary hydration force becomes clearer when salts are added to the aqueous phase [5, 8, 13, 14]. The strength and decay length of the primary/secondary hydration forces seem to be involved in the mechanism of the Hofmeister effect. However, the relation between the ion-induced changes in the hydration states and forces between the solutes has not been fully clarified.

The effect of ions on the hydration water of a nonionic surfactant, monomyristolein (MM, Fig. 1a), which forms a lamellar structure when dispersed in water, has recently been investigated by us [15]. Measurements of ζ potentials indicated that anions are distributed closer to the surface than cations. The small ion dependence of the ζ potentials indicated that Br^- and Cl^- bind similarly. Furthermore, water orientation at the surface was investigated using heterodyne-detected vibrational sum frequency generation spectroscopy (HD-VSFG). While water molecules are randomly oriented at the surface without salt, they are oriented in the presence of salts. The degree of the orientation was stronger with Cl^- than Br^- . It is plausible that the hydration water observed by this technique was within the primary hydration layer, since this technique only observes obvious orientation of water molecules at the surface. Thus, the HD-VSFG result implies that the primary hydration force should be stronger with Cl^- ions.

In the present study, the Hofmeister effect for MM is investigated to clarify the relation between the hydration water structure and the Hofmeister effect. The Hofmeister effect for

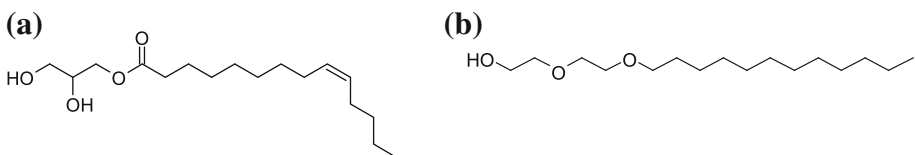


Fig. 1 Nonionic surfactants used: **a** monomyristolein (MM), **b** diethylene glycol monododecyl ether (C₁₂E₂)

the nonionic surfactant is investigated on the basis of the lamellar repeat distance d , which is determined by the balance of forces between bilayers in the presence of excess water. Compared to zwitterionic lipids, with which the present authors studied the Hofmeister effect [16], the nonionic surfactant should be better for the study because the interaction between a head group and ions seems to be quite small. It thus becomes easier to extract solely the effect of the change in the water structure. In addition to MM, we auxiliary use diethylene glycol monododecyl ether ($C_{12}E_2$, Fig. 1b).

2 Materials and Methods

Monomyristolein (MM) and diethylene glycol monododecyl ether ($C_{12}E_2$) were purchased from NU-CHEK PREP, INC. and Wako Pure Chemical Industries, Ltd., respectively. Six salts with monovalent and monatomic ions, LiCl (>98 %), LiBr (>98 %), NaCl (>99.5 %), NaBr (>99.5 %), KCl (>99 %) and KBr (>99 %) were purchased from NACALAI TESQUE, INC. These were used without further purifications. For preparing the salt solutions, ultra pure water (MilliQ, >18.2 M Ω ·cm) was used.

Lamellae of the surfactant with excess water are the best to investigate the interactions between the bilayers, since the lamellar repeat distance is determined by the balance of the interactions between bilayers [17].

Since lamellae of MM and excess water are separated when the MM concentration is less than 70 wt% at 20 °C [18], MM is mixed with salt solutions so that the MM concentration is 30 wt%. To make ions distributed homogeneously in the sample, the mixed solutions were treated as follows: (1) the sample was frozen at -25 °C and fused; (2) the fused solution was homogenized using ultrasound (UH-50, SMT Corporation); (3) the treatments (1) and (2) were repeated over four times; (4) the samples were stored at <5 °C. The MM solution has a phase transition to the cubic phase at about 20–30 °C [18], and the relaxation time from the cubic phase to the lamellar phase was found to be very long (over one day) when it was cooled from the cubic phase. Therefore, we took great care so that the temperature of samples did not exceed 20 °C.

The concentration of $C_{12}E_2$ was 15 wt%, for which excess water exists at 0 °C [19]. The sample preparation procedures were the same as for MM. We also took great care that the temperature did not to exceed 10 °C before the measurement (we poured the samples into our sample cells in a low temperature room at 4 °C).

To examine the effects of ions on the bilayer thickness of MM, concentrated MM solutions (75 wt%) were also prepared. At this concentration no excess water exists [18] and the lamellar repeat distance is determined by the lipid concentration. The bilayer thickness d_b is calculated by the Luzzati's method [20] as:

$$d_b = \frac{v_{MM}}{v_{MM} + v_{sol}} d, \quad (1)$$

where the volume of MM, v_{MM} , and a solution, v_{sol} , were calculated from the mass and densities of MM (we assumed it is similar to that of monoolein [21]) and solution [22].

For the concentrated samples, freeze/fuse treatments were performed five times. Then, they were stored in a freezer for about one month before the measurement to make the ion distribution homogeneous. The salt concentrations were 300 mmol·dm $^{-3}$.

The lamellar repeat distances of the nonionic surfactants were measured using small-angle X-ray diffraction (SAXD). For the samples with excess water, SAXD were performed at the BL6A, Photon Factory, KEK, Japan. The wavelength of X-ray was 1.5 Å and

the sample-to-detector distances were 1.2–2.5 m, which was calibrated with a standard sample (silver behenate). The detectors were PILATUS 300K and PILATUS 1M (DECTRIS Ltd.). SAXD for determining the lamellar repeat distances of 75 wt% of MM in 300 mmol·dm⁻³ salt solutions were performed using a laboratory instrument NANO-viewer with MicroMax007HF (Rigaku Corporation) and a PILATUS 100 K detector (DECTRIS Ltd.). The wavelength of the X-ray was 1.54 Å and the sample-to-detector distance was 0.7 m, which were also calibrated with silver behenate. The temperatures of the samples were controlled using a hot stage (FP82HT, METTLER TOLEDO) in both experiments.

3 Results and Discussion

Figure 2 shows the SAXD results for the lamellae of MM in salt solutions at 20 °C. The peaks in the SAXD profiles shown in Fig. 2a correspond to regular stacking of MM bilayers. The peak shifts to lower q with increasing the salt concentration. From the peak position q_0 , the lamellar repeat distance d is obtained as $d = 2\pi/q_0 = d_b + d_w$ as shown in Fig. 2b, where d_b and d_w are the thickness of the bilayer and sandwiched water layer, respectively. For all salts, d increases as the salt concentration increases. The increase in d shows anion dependence, i.e., salts with Br⁻ increase d to a greater extent than salts with Cl⁻. In contrast, cation dependence is weak. Strong anion dependence of the lamellar repeat distance was also observed in the case of phospholipid bilayers [16]. The anion dependent increase in d is also observed in the case of C₁₂E₂ at 0 °C (Fig. 3).

The measured d is the sum of the bilayer thickness d_b and the thickness of the water layer d_w . To make clear which of them dominates the increase in d , d_b in 300 mmol·dm⁻³ salt solutions were estimated using Luzzati's method (Eq. 1) [20] for 75 wt% MM solutions, since only the first Bragg peak was observed in the present SAXD experiments. The resultant d_b values are summarized in Table 1. The d_b values in 300 mmol·dm⁻³ salt solutions differ little from that without salt, indicating that the increase in d is mainly caused by the increase in d_w . This is consistent with the case of the phospholipid bilayers

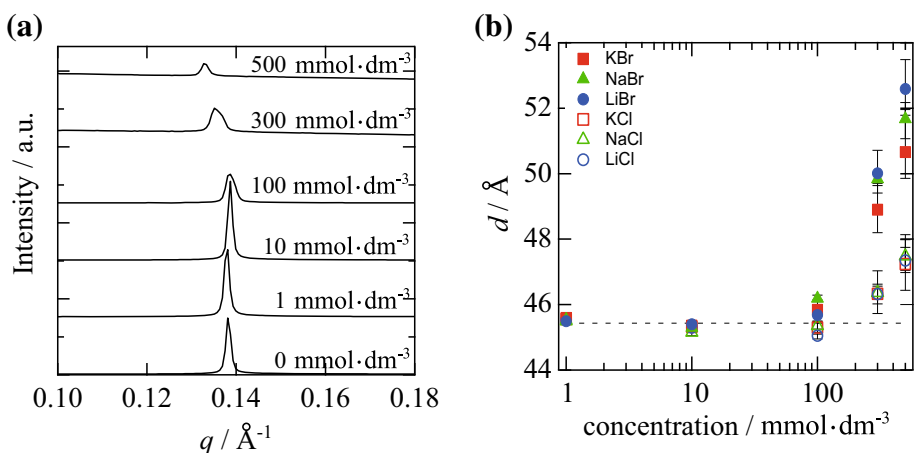


Fig. 2 **a** SAXD profiles of MM in the lamellar phase with NaCl. Peaks around $q = 0.14 \text{\AA}^{-1}$ are the first Bragg peak due to the lamellar structure. **b** Lamellar repeat distances d of MM in every salt solutions against salt concentrations where MM denotes mmol·dm⁻³. The dashed line is d without salt

Fig. 3 Lamellar repeat distance d of $C_{12}E_2$ in salt solutions. The dashed line is d without salt

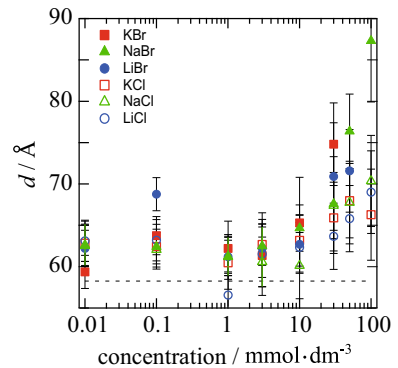


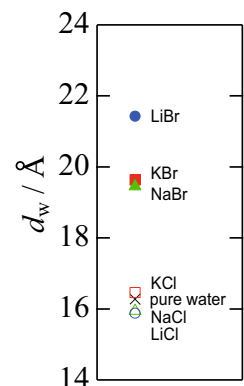
Table 1 Bilayer thicknesses d_b of MM in pure water and 300 $\text{mmol}\cdot\text{dm}^{-3}$ salt solutions obtained by Luzzati's method

$d_b/\text{Å}$ without salt	$d_b/\text{Å}$ with salt	Li	Na	K
39.5	Cl	40.6	39.5	39.5
	Br	37.3	40.1	38.8

[16]. The values of d_w at 300 $\text{mmol}\cdot\text{dm}^{-3}$ salt concentrations are obtained by subtracting d_b of 75 wt% MM samples from d of 30 wt% MM samples as shown in Fig. 4. The anion dependence is now clearer than in d . It is interesting to note that d_w scarcely changes in the Cl^- cases.

When excess water exists, d_w has been considered to be determined by the balance of attractive and repulsive forces between bilayers [17, 23]. While the attractive force is the van der Waals force, three repulsive forces have been considered: so-called electrostatic repulsion, steric repulsion (so-called “Helfrich repulsion”) due to the undulation motion of bilayers [24], and the hydration force. In the case of forces between bilayers, only the primary hydration force has been taken into account with a decay length scale of 2 Å, and the secondary hydration has not been formulated so far.

Fig. 4 Thickness of the water layer d_w ($= d - d_b$) of MM in 300 $\text{mmol}\cdot\text{dm}^{-3}$ salt solutions



When salts are added to the solution, the van der Waals force should change since the dielectric constant of the solution is altered. However, the effect is expected to dominate at much higher salt concentrations ($\sim 1 \text{ mol}\cdot\text{dm}^{-3}$) than the concentrations in this study. Furthermore, the change was found to exhibit little ion dependence [25]. For neutrally charged bilayers, the electrostatic repulsion is negligible without ions, since the repulsion originates from the entropic contribution arising from the heterogeneous distribution of ions. On the other hand, heterogeneous distribution has been reported when the same monovalent ions as used in the present study are added to the solution of MM [15]. Thus, the electrostatic repulsion should contribute to the present case. In the case of ionic surfactants, it has been reported that the counter ion binds to the head group differently, depending on the ion species, leading to different repulsive forces, i.e., Br^- ion that binds strongly on the surface reduces the repulsive force [26]. It is thus possible that a similar mechanism works in the present case, that is, Br^- may bind more strongly leading to strong electrostatic repulsion. However, the heterogeneous distributions exhibited only minor ion-dependence both for cations and anions and the surface electrostatic potentials (ζ potential) were similar for all samples [15]. These previous results indicate that anion binding on the surface (due to the specific chemisorption or van der Waals adsorption) are similar for Cl^- and Br^- , leading to the conclusion that the electrostatic repulsions are also similar. Further, at high salt concentrations, the electrostatic repulsion is screened by the ion atmosphere, and d_w should decrease with increasing salt concentration in contrast to the present results. Finally, monovalent ions seem to have only a small effect on the “Helfrich repulsion” [27].

In the model of the forces between bilayers [17], therefore, the hydration force is the only candidate that is strongly modified by ions. Our previous study of the lamellar phase of MM [15] showed that no notable ion-dependence was observed in the ion distributions, but was recognized in the hydration water structure. Namely, the structure strongly depends on the size of ions [15]. Water orientation is stronger with Cl^- than with Br^- , while it exhibits weaker cation dependence. This result can be interpreted in two ways; the amount of the hydration water is larger with Cl^- or the degree of water orientation is stronger with Cl^- . It seems natural to assume that the stronger the hydration (the larger the amount and/or the stronger the orientation), the stronger is the short-range repulsion caused by steric repulsion between the primary hydration water layers, i.e., the primary hydration force.

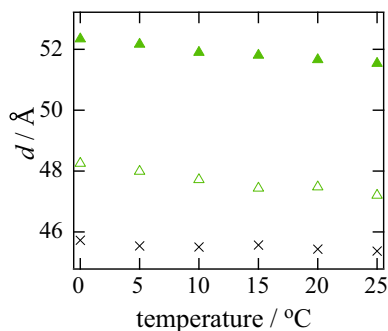
This trend is opposite to the present experimental finding, in which d_w is larger with Br^- . Our results clearly demonstrate that the strength of the primary hydration does not directly relate to the increase in d_w .

In the existing model of the forces between bilayers, the secondary hydration force has been rarely discussed [17]. Thus, the effect of ions observed in this study cannot be explained by the existing model.

The temperature dependence of d (Fig. 5) is also not explained by the existing model. With ions, d decreases as temperature increases, while it changes little without ions. Within the four interactions in the existing model, “Helfrich repulsion” depends most significantly on temperature [17]. “Helfrich repulsion” becomes stronger at higher temperatures [24], resulting in the increase in d_w with increasing temperature, contrary to the results.

The secondary hydration force [8–10] seems to be key to understanding the differences between the experimental results and the existing model. The secondary hydration force has recently been claimed to exist but has not been formulated yet [8]. If we assume that the increase in d_w with increasing salt concentration and the ion dependence of the increase arise from the change in the secondary hydration, our result indicates that the secondary

Fig. 5 Temperature dependences of the lamellar repeat distances d of 30 wt% MM in pure water (*cross*), 500 mmol·dm⁻³ NaCl (*open triangle*), and 500 mmol·dm⁻³ NaBr (*closed triangle*)



hydration force is stronger with Br⁻ and at lower temperature. Ions with different sizes have different charge densities, resulting in different effects on the water structure [28]. It is interesting that the tendency is in contrast to the primary hydration, which is weaker with Br⁻.

4 Conclusion

Effects of monovalent and monatomic ions on the lamellar repeat distances d of two nonionic surfactants were investigated. d increases with the increase in salt concentration, depending strongly on anion species. The increase in d is the result of the increase in the thickness of water layer d_w in the lamellae. With increasing temperature, d decreases in the presence of ions. The ion-dependence of the change in d (Br⁻ > Cl⁻) and the temperature dependence of d cannot be explained by the existing model of forces between bilayers, which has not taken the secondary hydration force into account. Our previous report [15] on the primary hydration of MM with salts has shown that the strength of the primary hydration force has opposite tendency to the experimental results of d . It is thus suggested that the inconsistency between our experimental results and the existing model is likely related to the change in the secondary hydration force. The intrinsic relation between the water structure in the secondary hydration layer and the forces between bilayers remains unclear. The present results imply that the secondary hydration force is stronger with Br⁻ and at lower temperatures. Formulation of the secondary hydration force is strongly desired. Measurements of the secondary hydration forces in various salt solutions by a surface force apparatus should give crucial clues for proper theoretical formulation.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from JSPS (Grant Nos. 24740289, 15K13546) for M.H. The SAXD experiments were performed under the approval of the Photon Factory Program Advisory Committee (Proposal Nos. 2013G525 and 2013G530).

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