Interaction between Hydrophilic Ionic Liquid and Phospholipid/Cholesterol Mixed Film

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Abstract: Surface pressure (π)-area (A) isotherms were studied to analyze the interactions between a hydrophilic ionic liquid (IL) (ethyl(2-hydroxyethyl)dimethylammonium methanesulfonate) and a pure dipalmitoylphosphatidylcholine (DPPC) film or a DPPC-cholesterol mixed film. When the hydrophilic IL was added to an underlayer solution, the isotherm shifted toward higher areas. Intriguingly, when the hydrophilic IL was added, the packing of the film materials became loose and the elastic modulus decreased, resulting in increased flexibility. This phenomenon was most evident under a cholesterol mole fraction of 0.2. This composition resembles that of cell membranes, which typically comprise phospholipids and cholesterol, suggesting that this hydrophilic IL may be able to interact significantly with biological membranes.

Key words: hydrophilic ionic liquid, phospholipid, cholesterol, surface pressure-area (π-A) isotherm, fluorescence anisotropy

1 Introduction

Salts featuring bulky organic cations and anions occasionally have low melting points and remain in a molten state even at low temperatures. Specifically, those with a melting point of 100°C or lower are called ionic liquids (ILs), and those that remain in a liquid state at room temperature in particular are called room-temperature ionic liquids (RT-ILs). ILs typically exhibit extremely low vapor pressure, flame retardancy, high thermal stability, high ionic conductivity, and high ionic atmospheres. ILs can dissolve various organic and inorganic compounds, and therefore provide promising replacements for conventional organic solvents and water. For example, ILs could potentially be used as solvents for inorganic nano-ordered material preparations, separation operations, organic synthetic reactions, and catalytic reactions.

Research concerning the use of ILs in colloid and interface chemistry began in the early 2000s, and is divided into the following four streams: (i) utilization of ILs as novel surfactants, (ii) addition of ILs to water-soluble substances, (iii) replacement of water with ILs in microemulsion systems, and (iv) study of self-assembly of surfactants in ILs.

Because of their negligible vapor pressures, ILs cannot evaporate even in vacuum. Furthermore, owing to their high ionic conductivities, ILs are used as conductivity-imparting agents for sample pretreatment in electron microscopy. Owing to their ability to infiltrate even samples with complicated and uneven surfaces, they can prevent sample shrinkage or deformation due to water evaporation in a vacuum state, thereby enabling highly accurate observation when using electron microscopes.

In the case of biological samples, replacing their water content with an IL means that certain steps (e.g., dehydration, replacement, and drying) can be omitted, reducing the overall work time before microscope observation. Conventionally, when observing an insulated sample with an electron microscope, it is necessary to coat the surface of the sample with a thin layer of a metal, such as gold or platinum, in order to prevent the reduction of image quality due to charging. In addition, it is typically necessary to strictly regulate the observation conditions, such as by lowering the acceleration voltage of the radiated electron beam and keeping the sample chamber in a low vacuum. Furthermore, in order to maintain the morphology of a water-containing sample, fixation/dehydration/substitution...
pretreatment is required, followed by critical point drying and freeze-drying. Therefore, understanding the interactions between ILs and biological membranes is crucial from both physical and engineering perspectives.

Regarding the interactions between ILs and biological phospholipid bilayers, there have been several reports on the utilization of imidazolium-type hydrophobic ILs, however, studies on hydrophilic ILs and their impacts on human health and the global environment are limited. In order to use ILs as pharmaceutically active ingredients or excipients, it is necessary to understand their interaction mechanisms with biological membranes.

In this study, the effect of adding a hydrophilic IL to a pure phospholipid film or a mixed film comprising a phospholipid and cholesterol was investigated to examine the interactions between hydrophilic ILs and biological membranes. During this investigation, the surface pressure of the monolayer at the air-water interface and the fluorescence anisotropy of the bilayer constituting the liposome were measured.

2 Experimental Procedures
2.1 Materials

1-α-Dipalmitoylphosphatidylcholine (DPPC) and cholesterol (both of >99% purity, Fujifilm Wako Pure Chemical) were used as the film materials. The chemical structures of these materials are displayed in Fig. 1. Chloroform (99.8% purity, Fujifilm Wako Pure Chemical) was used as the developing solvent.

The hydrophilic IL used in our experiments was ethyl (2-hydroxyethyl) dimethylammonium methanesulfonate (>99% purity, Miyoshi Oil & Fat), which was diluted with water to 0.01 and 0.1 wt%. Ammonium methanesulfonate (96% purity, Miyoshi Oil & Fat) and sodium methanesulfonate (96% purity, Fujifilm Wako Pure Chemical) were used for comparative purposes, and their chemical structures (shown in Fig. 2) were identical to that of the anion in the hydrophilic IL.

The hydrophilic IL and comparative substances: (a) ethyl (2-hydroxyethyl) dimethylammonium methanesulfonate (IL), (b) ammonium methanesulfonate, and (c) sodium methanesulfonate.

2.2 Methods
2.2.1 Surface pressure measurements

The surface pressure was measured using a trough (KSV NIMA Small) equipped with a Wilhelmy plate. A monolayer was prepared by applying 17 μL of a chloroform solution (0.5 mmol/dm³) with a cholesterol mole fraction of 0, 0.2, 0.4, 0.6, or 1.0 onto the underlayer solution. The underlayer liquid (approximately 39 cm³) was pure water, aqueous hydrophilic IL solution (0.01 or 0.1 wt%), aqueous ammonium methanesulfonate solution (0.1 wt%), or aqueous...
sodium methanesulfonate solution (0.1 wt%). Under these experimental conditions, the IL and comparative materials in the aqueous solutions were in excess with respect to the film materials. The monolayer was allowed to stand at 25°C for 10 min, and the surface pressure was measured at a barrier compression rate of 5 cm/min.

2.2.2 Fluorescence anisotropy measurements

For these measurements, 10 μL of a solution of DPH (10 mmol/dm³), which is a hydrophobic fluorescent probe, was added to 3 cm³ of a liposome dispersion (10 mmol/dm³) using tetrahydrofuran as a solvent; the cholesterol mole fraction was 0 or 0.2. Then, the fluorescence was measured at an excitation wavelength of 360 nm and a fluorescence wavelength of 500 nm using a Hitachi F-2700 fluorescence spectrometer after incubation at 25°C for 2 h under shade.

The liposome dispersions with cholesterol mole fractions of 0 (pure DPPC) and 0.2 were prepared via the following procedure. DPPC and cholesterol were weighed in a 50 cm³ screw tube and then dissolved in 1 cm³ of chloroform. While the screw tube containing this solution was being rotated, nitrogen was blown to volatilize the chloroform. The system was further dried under reduced pressure for approximately 25°C, until a thin film was obtained. Subsequently, an aqueous medium was added to the screw tube: pure water, an aqueous hydrophilic IL solution (0.1 wt%), an aqueous ammonium methanesulfonate solution (0.1 wt%), or an aqueous sodium methanesulfonate solution (0.1 wt%). After it was heated at 80°C for 10 min, the mixture was mixed at 3000 rpm for 3 min using a vortex mixer until a liposome dispersion equivalent to 10 mmol/dm³ was obtained.

Fluorescence anisotropy (γ) is defined by the following equation:

\[
γ = \frac{I^\parallel - I^\perp}{I^\parallel + 2I^\perp}
\]  

where, \(I^\parallel\) and \(I^\perp\) are the respective fluorescence intensities of the components parallel and perpendicular to the polarization plane of the incident light.

3 Results and Discussion

3.1 Surface pressure-area (π-A) isotherms with hydrophilic IL in underlayer solutions

The surface pressure-area (π-A) isotherms were measured with and without a hydrophilic IL (0.01 and 0.1 wt%) dissolved in aqueous underlayer solutions. We note that the surface tension of the aqueous IL solution (0.1 wt%) was measured to be 72.1 mN/m, equal to that of pure water. This indicates that the hydrophilic IL sample employed in this study is essentially dissolved in aqueous media and does not form a monolayer at the air-aqueous solution interface. The π-A isotherms are shown in Fig. 3. The mole fraction of cholesterol, with respect to the total amount of film materials (DPPC and cholesterol), was (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, or (e) 1.0. In the pure DPPC system (Fig. 3a), the transition from the liquid expansion phase to the liquid condensation phase occurred at approximately 10 mN/m, and a solid phase was subsequently formed. Notably, when the hydrophilic IL was introduced, the isotherm of DPPC shifted toward higher areas, indicating that the hydrophilic IL had interacted with DPPC. Furthermore, at higher hydrophilic IL concentrations, the shift toward higher areas was even more evident, confirming the concentration dependence of this phenomenon.

Hereafter, we focus on the influence of the cholesterol mole fraction on the monolayer system. In the absence of the IL, the liquid expansion phase transitioned to the liquid condensation phase under a low cholesterol mole fraction (Fig. 3b), as was observed in the case without cholesterol addition. The addition of the IL at 0.1 wt% also caused a phase transition at approximately 10 mN/m, while no transition was detected at an IL concentration of 0.01 wt% under experimental conditions.

As the cholesterol mole fraction increased, the liquid phase region became narrower and transition to the solid phase began to occur at lower areas (Figs. 3c and 3d). In the absence of DPPC (Fig. 3e), only the solid phase was observed. When the hydrophilic IL was added to these systems, the π-A isotherm of the film materials shifted toward higher areas. From these observations, it is suggested that the hydrophilic IL can interact with both substances (DPPC and cholesterol) in the pure and mixed monolayers.

To verify the interaction between the hydrophilic IL and the film substances, the area per molecule was plotted against the cholesterol mole fraction at low and high surface pressures (5 and 20 mN/m) (Fig. 4). At both surface pressures and without IL addition, the molecular occupied area decreased as the cholesterol mole fraction increased. In other words, the addition of cholesterol resulted in a closely packed monolayer. When the hydrophilic IL was added, the molecular occupied area increased under all tested cholesterol mole fractions. In particular, when the cholesterol mole fraction was 0.2, the molecular occupied area increased remarkably. Thus, it was concluded that the interaction between the hydrophilic IL and DPPC was maximized at this mole fraction.

This mole fraction closely resembles the composition of the phospholipids and cholesterol comprising cell membranes, indicating increased interaction efficiency between the hydrophilic IL used in our experiments and biological membranes.

The Gibbs elastic modulus (Cs⁻¹) of the monolayer was calculated from the π-A isotherm according to the following formula:

\[
Cs^{-1} = -A \left( \frac{\partial \pi}{\partial A} \right)_T
\]

where, A is the molecular occupied area at surface pres-
sure $\pi$, and $T$ is the temperature. Overall, the addition of cholesterol increased the $C_{s^{-1}}$ value (Figs. 5 and S1), indicating an increase in the elasticity of the film. This result was consistent with the findings in a previous report. It has also been reported that a hydrogen bond is formed between the carbonyl group of DPPC and the hydroxy group of cholesterol, resulting in enhanced interaction. More importantly, at all cholesterol mole fractions, the addition of the hydrophilic IL decreased the $C_{s^{-1}}$ value, indicating that it reduces the elasticity of the monolayer, causing it to become "soft."

It is interesting to note that at a given hydrophilic IL concentration, the $C_{s^{-1}}$ value calculated at a cholesterol mole fraction of 0.2 was lower than that without cholesterol. This is particularly obvious at a high surface pressure (20 mN/m, Fig. 5b). Moreover, the addition of the hydro-

![Fig. 3](image-url)  
Fig. 3 $\pi$-$A$ isotherms of pure DPPC and DPPC-cholesterol mixed system with hydrophilic IL in underlayer solutions. Mole fraction of cholesterol: (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, or (e) 1.0.
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We hypothesize the following to interpret the observed result. The hydrophilic IL contains a hydroxy and sulfonyl group in the cation and anion, respectively, and these functional groups may interact with the two film substances (DPPC and cholesterol) through hydrogen bonding. The bulkiness of the IL cation causes looser packing of the film substances, thereby decreasing elasticity and increasing flexibility.

3.2 \( \pi - A \) isotherms with comparative materials in underlayer solutions

The \( \pi - A \) isotherms measured with the comparative materials (ammonium methanesulfonate and sodium methanesulfonate) dissolved in aqueous underlayer solutions (0.1 wt\%) are shown in Fig. 6. As mentioned in section 2.1, the structures of these comparative materials are identical to that of the anion in the hydrophilic IL. Fig. S2 shows the molecular occupied areas at surface pressures of 5 and 20 mN/m, and Fig. S3 presents the corresponding Cs\(^{-1}\) values at these surface pressures. Similar to the case where the hydrophilic IL was added, the isotherm shifted toward higher areas when either comparative substance was introduced, implying that they both interacted with the film materials. It should be noted that when the same mass percent concentration was applied (0.1 wt\%), the measured shifts for the comparative substances were smaller than that for the IL. The molar concentrations of ammonium methanesulfonate and sodium methanesulfonate were 8.8 and 8.5 mmol/dm\(^3\), respectively, notably higher than...
that of the hydrophilic IL (4.7 mmol/dm$^3$). Regardless, compared to the other substances, the addition of the hydrophilic IL induced a larger shift in the isotherm toward higher areas, suggesting a strong effect on the properties of the monolayer.

In the systems with cholesterol mole fractions of 0.4 and 0.6, the addition of a comparative substance increased the $C_{s^{-1}}$ value (Fig. S3). It has been previously reported that the addition of calcium chloride tightens the packing of film materials containing cholesterol$^{26}$, which is consistent with our findings. In contrast, the $C_{s^{-1}}$ value decreased for the system with hydrophilic IL added (Fig. 5), indicating that the hydrophilic IL loosened the packing of the film. As discussed in the previous section, the cation and anion of the hydrophilic IL contain a hydroxy and sulfonyle group, respectively, and can interact simultaneously with DPPC

Fig. 6  $\pi$-$A$ isotherms when comparative substances were added to the 0.1 wt% underlayer aqueous solution. Mole fraction of cholesterol: (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, or (e) 1.0.

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Fig. 7  Fluorescence anisotropy results when hydrophilic IL or comparative substance was added to the 0.1 wt% underlayer aqueous solution: pure DPPC (white) and DPPC-cholesterol mixed systems (mole fraction of cholesterol = 0.2) (black).

and cholesterol. Additionally, the IL cations are bulkier than those of the comparative substances, which contributed significantly to this result.

3.3 Fluorescence anisotropy of bilayer

The fluorescence anisotropy was measured for liposomes prepared with both pure DPPC and a mixture of DPPC and cholesterol (mole fraction of 0.2). A higher fluorescence anisotropy value corresponds to lower fluidity of the bilayer constituting the liposome. As shown in Fig. 7, the additions of sodium methanesulfonate and ammonium methanesulfonate (both 0.1 wt% aqueous solutions) made limited impacts on the fluorescence anisotropy when error is taken into consideration. Importantly, the addition of the hydrophilic IL (0.1 wt% aqueous solution) resulted in decreased fluorescence anisotropy. This indicates increased fluidity of the bilayer caused by the loose packing of the monolayer (Fig. 3). This result further supports the decreased Cs value, as observed in Fig. 5.

4 Conclusions

π-A isotherms were studied to analyze the interactions between a hydrophilic IL (ethyl(2-hydroxyethyl)dimethylammonium methanesulfonate) and a pure DPPC film or DPPC-cholesterol mixed film. The addition of the hydrophilic IL to an aqueous underlayer solution caused the isotherm to shift toward higher areas, a phenomenon that was maximized under a cholesterol mole fraction of 0.2. Furthermore, when the hydrophilic IL was added, the packing of the film materials became loose, decreasing the elastic modulus (or increasing the flexibility) in the process. This was supported by fluorescence anisotropy measurements of the DPPC and DPPC-cholesterol liposome systems. The bulky, hydroxy-group-containing cations and sulfonylether-group-containing anions of the hydrophilic IL enabled interaction with both DPPC and cholesterol, leading to looser molecular packing. The composition of cholesterol with a mole fraction of 0.2 closely resembles that of cell membranes comprising phospholipids and cholesterol, suggesting high interaction efficiency between the hydrophilic IL studied here and biological membranes. The findings reported in this paper will potentially deepen the understanding of the structural and packing properties of hydrophilic ILs at cell membrane interfaces and aid in the rational design of functional materials.

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Supporting Information
This material is available free of charge via the Internet at doi: 10.5650/jos.ess21261

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