Elucidating the Effects of Cholesterol on the Molecular Packing of Double-chained Cationic Lipid Langmuir Monolayers by Infrared Reflection-absorption Spectroscopy

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Abstract: Cholesterol has been suggested to play a role in stable vesicle formation by adjusting the molecular packing of the vesicular bilayer. To explore the mechanisms involved in adjusting the bilayer structure by cholesterol, the molecular packing behavior in a mimic outer layer of cationic dialkyldimethylammonium bromide (DXDAB)/cholesterol vesicular bilayer was investigated by the Langmuir monolayer approach with infrared reflection-absorption spectroscopy (IRRAS). The results indicated that the addition of cholesterol in the DXDAB Langmuir monolayers not only restrained the desorption of the DXDAB with short hydrocarbon chains, such as ditetradecyldimethylammonium bromide or dihexadecyldimethylammonium bromide, into the aqueous phase but also induced a condensing effect on the DXDAB monolayers. At a liquid-expanded (LE) state, the ordering effect of cholesterol accompanying the condensing effect occurred in the mixed DXDAB/cholesterol monolayers due to the tendency of maximizing hydrocarbon chain contact between cholesterol and the neighboring hydrocarbon chains. However, for the mixed monolayers containing the DXDAB with long hydrocarbon chains, such as dioctadecyldimethylammonium bromide (DODAB), the disordering effect of cholesterol took place at a liquid-condensed (LC) state. This was related to the molecular structure of cholesterol and hydrocarbon chain length of DODAB. The rigid sterol ring of cholesterol hindered the portion of neighboring hydrocarbon chains from motion. However, the flexible alkyl side-chain of cholesterol along with the corresponding portion of neighboring hydrocarbon chains formed a fluidic region, counteracting the enhanced conformational order induced by the sterol ring of cholesterol. Furthermore, the long hydrocarbon chains of DODAB possessed a more pronounced motion freedom, resulting in a more disordered packing of the monolayers.

Key words: air/water interface, cholesterol, dialkyldimethylammonium bromide, infrared reflection-absorption spectroscopy, Langmuir monolayer

1 Introduction

In recent years, the potential of using cationic lipids as non-viral vectors in gene therapy has been widely studied involving cationic lipid-DNA complexes, known as lipoplexes. The structure of cationic lipids has been recognized as a major factor to influence the transfection efficiency. To improve the transfection efficiency, newly developed cationic compound and/or neutral helper lipids, such as cholesterol and dioleoyl phosphoethanolamine (DOPE), have been adopted in the preparation of lipoplexes.

Dialkyldimethylammonium bromide (DXDAB), one kind of synthesized cationic lipids, has been used instead of natural lipids as model biomembranes for investigating the biological processes. DXDAB has also been applied in gene therapy and genetic engineering. For DXDAB with various alkyl chain lengths, dioctadecyldimethylammonium bromide (DODAB) was the most frequently studied one and was used to prepare cationic vesicles in order to examine the formation of lipoplexes. It has been demonstrated that lipoplexes composed of DODAB were successful in delivering nucleic acid in vitro and in vivo, and the effectiveness was increased in the presence of neutral helper lipids.

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lipids, such as cholesterol and DOPE\textsuperscript{12,14–16}.

Cholesterol, as a neutral helper lipid, is believed to improve the transfection efficiency by reducing the binding of serum proteins and protecting the DNA from DNase degradation\textsuperscript{16–18}. One of the possible reasons is that cholesterol altered the molecular packing of vesicular bilayers and assisted the formation of stable liposomes\textsuperscript{19,20}. Several studies have pointed out that cholesterol can rigidify membranes and induce ordered bilayer structures in the liquid-crystalline phase, while fluidize membranes and/or induce disordered bilayer structures in the gel phase\textsuperscript{21–23}. This characteristic of cholesterol seems to play an important role in the transfection efficiency.

Adding cholesterol in cationic vesicles or lipoplexes composed of DODAB has also been proven effective in altering the membrane fluidity\textsuperscript{10,24–26} and improving the transfection efficiency\textsuperscript{15,16}. To explore the effect of cholesterol on bilayer structures of cationic vesicles composed of DXDAB, a Langmuir monolayer is a suitable model to mimic half of a vesicular bilayer\textsuperscript{27}. The structure of a DODAB Langmuir monolayer containing cholesterol has been investigated\textsuperscript{28}, and the interactions between cholesterol and DXDABs have also been studied by the monolayer approach\textsuperscript{29}. However, effects of cholesterol on the molecular packing of DXDAB Langmuir monolayers, especially on the hydrocarbon chain conformation of DXDAB molecules, have not been fully understood. To elucidate the mechanisms involved in adjusting DXDAB bilayer structures by cholesterol, molecular packing characteristics of DXDAB monolayers containing cholesterol at the air/water interface were analyzed by infrared reflection-absorption spectroscopy (IRRAS).

2 Materials and methods

2.1 Materials

Diallyldimethylammonium bromide (DXDAB) (\( \sim 98\% \)) with different alkyl chain lengths including ditetradecyldimethylammonium bromide (DTDAB), dihexadecyldimethylammonium bromide (DHDAB), and dioctadecyldimethylammonium bromide (DODAB) were supplied by TCI, Japan. Cholesterol (\( \sim 99\% \)) and \( n \)-hexane (\( \sim 99.3\% \)) were purchased from Sigma, USA. Ethanol (\( \sim 99\% \)) was obtained from J. T. Baker, Malaysia. Chloroform of HPLC analytical grade was supplied by Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA. All the chemicals were used as received without further purification. The water used in all experiments was purified by a Milli-Q plus purification system (Millipore, USA) with a resistivity of 18.2 M\( \Omega \)-cm.

2.2 Surface pressure-area isotherms

Surface pressure-area (\( \pi-A \)) isotherms of monolayers at the air/water interface were obtained with a Langmuir trough (type 601BAM, Ninfa, England) equipped with a Wilhelmy plate accessory for surface pressure measurement. The Wilhelmy plate was made of a filter paper (10 mm \&times; 24 mm \&times; 0.15 mm) and was provided by the Langmuir trough manufacturer. For all experiments, the Teflon trough (trough size 700 \&times; 70 mm\(^2\)) was filled with purified water as the subphase, and the temperature was maintained at 20 \( \pm \) 1\(^\circ\)C by an external circulator.

To form a monolayer at the air/water interface, a 60–80 \( \mu \)l solution (1 mM), which was prepared by dissolving the monolayer-forming materials in an ethanol/\( n \)-hexane (1:9, v/v) mixture, was spread at the interface by using a 100-\( \mu \)l microsyringe (Hamilton Co., USA). After waiting 20 min for solvent evaporation, the monolayer at the interface was continuously compressed by Teflon barriers at a rate of 12 cm\(^2\)/min (2.3-3A\(^2\)/molecule-min) to obtain the \( \pi-A \) isotherm.

2.3 Infrared reflection-absorption spectroscopy

An IRRAS analysis was performed by using a PerkinElmer FTIR spectrometer (model Spectrum GX) with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector. IRRAS measurements were taken by using unpolarized light with an incident angle of 40\(^\circ\) from the surface normal. The sample chamber of the FTIR spectrometer was continuously purged with dry air from a purge gas generator (model 2002N-8B1-BX, Parker Hannifin, corp., USA). All spectra were collected at 8 cm\(^{-1}\) resolution with a scan number of 128 and were reported by subtracting the water spectrum from the measured spectra.

When the IRRAS analysis of a monolayer at a continuously compressed air/water interface was conducted, the removable Langmuir trough was filled with 16 mL of purified water, and a proper amount of 1 mM chloroform solution containing the monolayer-forming materials was spread at the interface. It is noted that the mixed solvent of ethanol/\( n \)-hexane (1:9, v/v) evaporated very quickly under the purge of dry air in the IRRAS analysis, which was not suitable for the spreading of the monolayer-forming molecules at the air/water interface. Therefore, chloroform was adopted as a spreading solvent for the IRRAS measurement. After spreading monolayer-forming materials at the interface and waiting 30 min for solvent evaporation, the monolayer at the interface was continuously compressed by a barrier at a rate of 0.22 cm\(^2\)/min (0.6-1.2 A\(^2\)/molecule-min) and the IR spectra were collected. The slower compression rate adopted for the IRRAS analysis than for the surface pressure-area measurement is to avoid a pronounced change of molecular packing during the collection of an IR spectrum. It should be noted that an iso-
therm for the monolayers from a gas state to collapse state was unable to obtain in one compression stage due to the limitation of a minimum relative area of 52.5% for the compression stage. Thus the isotherm was constructed by the data collected from experiments with the spreading solvent of different volumes (3, 4.2, and 6 μL). Repeated experiments were performed to ensure the reproducibility of the isotherms.

3 Results and discussion

3.1 π-A isotherm

Figure 1 shows π-A isotherms of mixed DXDAB/cholesterol monolayers at the air/water interface. In the isotherms of pure DTDAB and DHDAB monolayers, the surface pressure slowly increased upon the interface compression without the presence of a plateau characteristic of liquid-expanded (LE) to liquid-condensed (LC) phase transition. This indicates that DTDAB and DHDAB formed expanded Langmuir monolayers. However, the isotherm of a DODAB Langmuir monolayer at the interface shows distinct liquid and solid regions. For a cholesterol Langmuir monolayer, no plateaus was detected in the π-A isotherm and an abrupt rise of surface pressure with decreased interface area was observed at ~38 Å²/molecule, demonstrating the typical characteristic of a condensed monolayer. The π-A isotherms for pure DXDAB and cholesterol Langmuir monolayers are in agreement with those obtained in the literature.

When DXDAB was mixed with cholesterol at the interface, π-A isotherms of the mixed Langmuir monolayers mostly located between those of the two pure component monolayers and gradually shifted toward the smaller molecule-occupied area regions with the increased mole fraction of cholesterol, X_{Chol}. Moreover, the LE/LC phase transition plateau observed in the isotherm of a DODAB monolayer became insignificant when cholesterol was added. The isotherms of mixed DXDAB/cholesterol Langmuir monolayers shown in Fig. 1 are somewhat different from those reported by Hac-Wydro et al. The difference probably resulted from a slower monolayer compression rate applied in this study.

To investigate the miscibility of DXDAB and cholesterol in the mixed monolayers, the excess area, A_{ex} for a mixed monolayer was analyzed by using the following equation:

\[ A_{ex} = A_{12} - (x_1A_1 + x_2A_2) \]

where A_{12} is the mean area per molecule in the mixed monolayer at a particular surface pressure, A_1 and A_2 are the areas per molecule for pure DXDAB and cholesterol monolayers, respectively, and x_1 and x_2 are the mole fractions of the two components in the mixed monolayer. Figure 2 plots the excess areas of mixed DXDAB/cholesterol Langmuir monolayers at surface pressures of 5, 10, 20, and 30 mN/m. The non-zero excess areas for the mixed monolayer systems indicated that DXDAB and cholesterol were miscible and formed non-ideal monolayers at the interface.

For most of the mixed DXDAB/cholesterol monolayers, negative values of A_{ex} were found at various surface pres-
ures. However, at lower surface pressures, slightly positive deviations were observed in the mixed monolayers of DTDAB or DHDAB with 10 mol% cholesterol. For a mixed monolayer, a positive value of $A_{\text{ex}}$ suggested that the interaction between the molecules of the two components was weaker than that between the molecules of pure component or the molecules of the two components were in a more disordered state than an ideal one. In contrast, negative $A_{\text{ex}}$ is ascribed to an enhanced intermolecular attraction or the favorable geometric accommodation of the molecules in the mixed monolayer\(^{42}\). Thus the slightly positive excess areas for the mixed monolayers of DTDAB or DHDAB with 10 mol% cholesterol implied that the interaction between DTDAB and cholesterol or between DH DAB and cholesterol was repulsive at lower surface pressures, while attractive at higher surface pressures. It is confusing that the two components possessed interactions with opposite characteristics at different surface pressures. One may consider that, in comparison with DODAB with longer hydrocarbon chains, DTDAB and DH DAB with shorter hydrocarbon chains are slowly dissolved into the water subphase upon the interface compression at a slow compression rate. According to the quantitative analysis of IRRAS discussed in the next section, molecules in the pure DTDAB and DHDAB monolayers were proven to dissolve into the water subphase under the interface compression and the presence of cholesterol could restrain the molecules from desorption. The molecule loss under the interface compression led to the underestimation of $A_{\text{ex}}$ for the pure monolayer of DTDAB or DHDAB and thus resulted in positive deviations of $A_{\text{ex}}$ for the mixed monolayers.

With increasing $X_{\text{Chol}}$ from 10 to 43 mol%, more negative values of $A_{\text{ex}}$ for the mixed DXDAB/cholesterol monolayers were detected and are shown in Fig. 2, which were indicative of enhanced intermolecular attraction induced by cholesterol. Thus the addition of cholesterol in the Langmuir monolayers not only restrained the desorption of DTDAB or DHDAB molecules from the interface but also induced a condensing effect on the DXDAB monolayers. The condensing effect of cholesterol usually accompanies with the ordering effect\(^{34}\). To clarify the effects in terms of orientational and conformational orders of the mixed monolayers, an IRRAS analysis was performed.

### 3.2 IRRAS analysis

The molecular packing of mixed DXDAB/cholesterol monolayers at the air/water interface was analyzed by IRRAS. From the original IRRAS data, the reflectance-absorbance (RA) intensity data were obtained with an uncertainty of $2 \times 10^{-4}$ for a major IR peak of alkyl chain, the antisymmetric methylene stretching vibration ($\nu_s\text{CH}_2$) at $\sim$ 2920 cm$^{-1}\text{.}^{35, 36}$ Negative absorption bands of a monolayer at the air/water interface were observed in the spectra, and the bases of the negative absorbance have been explored and explained in the literature\(^{36, 37}\). The IRRAS theory shows that the RA intensity mainly depends on the surface density and molecular orientation of a monolayer during the interface compression\(^{35, 36, 38-42}\). A higher surface density or a lower tilt angle of the molecules would lead to an increase in the RA value. Furthermore, the wavenumber...
of να-CH₂ band is related with the conformational order (trans/gauche ratio) of molecular alkyl chains. A higher wavenumber of να-CH₂ band (＞2924 cm⁻¹) is characteristic of dominated gauche conformers or highly conformational disorder of the alkyl chains, while the wavenumber decreases with increasing the fraction of trans conformers.⁵⁵ The να-CH₂ RA intensity-area and wavenumber-area isotherms of mixed DXDAB/cholesterol monolayers at the air/water interface are plotted in Figs. 3-5. The να-CH₂ wavenumbers of the DTDAB and DHDAB monolayers were near or larger than 2924 cm⁻¹ upon the interface compression, demonstrating the expanded monolayer characteristic with disordered molecular arrangement. For the pure DODAB monolayer, the να-CH₂ wavenumber shifted from 2926 to 2921 cm⁻¹ when the molecule-occupied area was reduced from 84 to 51 Å². This implies that the molecular packing in the monolayer changed from a disordered state to an ordered state in this region, which corresponds to the transition from LE phase to LC phase (Fig. 1).

A quantitative RA analysis was adopted to evaluate the molecule loss of the monolayers at the air/water interface. It has been reported that the RA intensity is in a linear relationship with the molecular surface density under the similar molecular orientation or conformation. The relationship is reasonably described by:

\[
RA = aN + b
\]

Here, \( N \) is the number of CH₂ group per molecule, \( \Gamma \) is the molecular surface density at the interface, and \( a \) and \( b \) are constants.

The diagram of RA intensity-surface density obtained for a hexadecyltrimethylammonium-dodecylsulfate (HTMA-
DS) monolayer in a previous study\textsuperscript{[31]} was chosen as the calibration curve. The linear relationship of RA value versus molecular surface density was demonstrated when the $\nu_a$-CH\textsubscript{2} wavenumbers were near or larger than 2924 cm\textsuperscript{-1}. The parameters $a$ and $b$ were thus determined to be 0.0476 and -0.4639, respectively, from the calibration curve. It is noted that equation (2) is obtained based on the assumption of dominated gauche conformation of molecules with a similar orientational order. The assumption can be applied in pure DXDAB monolayers at a LE state, at which the measured $\nu_a$-CH\textsubscript{2} wavenumbers of the monolayers were near or larger than 2924 cm\textsuperscript{-1} and thus the estimated RA value could be used to compare with measured RA value. Figure 6c demonstrates that the RA value obtained for a DODAB Langmuir monolayer at a molecule-occupied area of 80 Å\textsuperscript{2} was slightly larger than the corresponding estimated RA value. A deviation of $1 \times 10^{-4}$ between the measured and estimated RA values of the DODAB monolayer could be ascribed to the experimental error. For the pure DTDAB and DHDAB monolayers, that is, $X_{\text{Chol}}$=0, RA values obtained at a molecule-occupied area of 66 Å\textsuperscript{2} (Figs. 6a and 6b) were significantly lower than the corresponding calibrated RA values, implying the molecule loss from the interface. The results indicated that the positively charged DTDAB and DHDAB molecules with comparatively short hydrocarbon chains desorbed from the interface and dissolved into the water subphase with a certain extent during the interface compression.

In Fig. 6, positive deviations between the measured and estimated RA values were observed for the mixed DXDAB/cholesterol Langmuir monolayers. The larger RA values in comparison with the corresponding estimated RA values at the same molecular-occupied areas were attributed to the lower tilt angle of the molecules at the interface. It is worthy to note that the mixed monolayers possessed dominated gauche conformation at the selected molecule-occupied areas. In addition, small aggregated 2-D domains for the mixed monolayers of DXDAB with 43 mol\% cholesterol at large molecule-occupied areas have been observed with Brewster angle microscopy\textsuperscript{[30].} Thus it is suggested that the addition of cholesterol in the DXDAB monolayers could enhance the intermolecular attraction and promote the formation of aggregated 2-D domains at large molecule-occupied areas, leading to the decrease in the tilt angle of the monolayer.
molecules. However, the electrostatic repulsion between charged head groups of DXDAB would restrain DXDAB molecules from approaching, resulting in a disordered packing of molecules in spite of the formation of aggregated 2-D domains. As a result, the addition of cholesterol in the DXDAB monolayers increased the orientational order of the molecules but did not significantly alter the molecular conformation at large molecule-occupied areas.

When DXDAB was mixed with cholesterol at the interface, the $\nu_{\text{a-CH2}}$ wavenumber-area isotherms were obviously changed in comparison with those of pure DXDAB Langmuir monolayers (Figs. 3-5). At small molecule-occupied areas (<60 Å$^2$), $\nu_{\text{a-CH2}}$ wavenumbers of the mixed DTDAB/cholesterol and DHDAB/cholesterol monolayers were lower than those of pure DTDAB and DHDAB monolayers, suggesting that cholesterol induced a more ordered packing of DTDAB and DHDAB molecules at the interface. However, in comparison with a pure DODAB monolayer, the larger $\nu_{\text{a-CH2}}$ wavenumbers observed for the mixed DODAB/cholesterol monolayers at small molecule-occupied areas were indicative of a more disordered packing at the interface with the presence of cholesterol. This implies that cholesterol-induced ordering or disordering effects on the molecular packing of DXDAB monolayers are related to the hydrocarbon chain length of DXDAB.

To further explore the ordering/disordering effects of cholesterol on the DXDAB monolayers, $\nu_{\text{a-CH2}}$ wavenumbers of the mixed DXDAB/cholesterol monolayers are plotted as a function of surface pressure in Fig. 7. A decrease in the $\nu_{\text{a-CH2}}$ wavenumber for the mixed monolayers was observed with increased $X_{\text{Chol}}$ at various surface pressures, except for that obtained for the mixed DODAB/cholesterol monolayers at surface pressures larger than 20 mN/m. It is noteworthy that, according to the $\pi$-A isotherms (Fig. 1), DXDAB monolayers were at a LE phase at various surface pressures except for the DODAB monolayer at $\pi > 20$ mN/m. Therefore, the results shown in Fig. 7 suggest that the incorporation of cholesterol in the monolayers at a LE phase apparently reduced gauche defects in the hydrocarbon chains of the molecules. The rigid nucleus of cholesterol has been suggested to play an important role in the condensing effect of cholesterol on a fluidic monolayer$^{[48]}$. The rigid nucleus of cholesterol would maximize the hydrocarbon chain contact with neighboring molecules$^{[49]}$. Thus cholesterol drove the condensation of hydrocarbon chains and induced a more conformational order of the hydrocarbon chains, resulting in the cholesterol-induced condensing effect accompanying with the ordering effect on the monolayers at a LE state.

It is noted that the plasma membranes contain 30-40% cholesterol and cholesterol can rigidify the plasma membranes$^{[50,51]}$. Figure 7 shows that the pronounced ordering effect of cholesterol occurred in the mixed monolayers with 30-43 mol% cholesterol at a LE state. This implies

\[ X_{\text{Chol}} \]

\[ \nu_{\text{a-CH2}} \]

\[ \text{RA value} \]

\[ \text{composition} \]

\[ \text{molecule-occupied area of 66 Å}^2/\text{molecule} \]

\[ \text{molecule-occupied area of 80 Å}^2/\text{molecule} \]

\[ \text{calibrated RA value} \]

\[ \text{as a function of composition for the mixed monolayers.} \]
that the presence of 30-40% cholesterol in the plasma membranes can prevent the membranes from being too fluidic and provide the membranes enough rigidity.

When surface pressures were higher than 20 mN/m, ν\textsubscript{\text{a}}-CH\textsubscript{2} wavenumbers of the DODAB monolayer were not significantly changed with the addition of 10 or 30 mol% cholesterol (Fig. 7). This indicates that adding these amounts of cholesterol in the DODAB monolayer did not change the molecular packing at high surface pressures. This phenomenon was also observed by Ohe et al.\textsuperscript{52} in the mixed monolayers of dipalmitoylphosphatidylcholine (DPPC) and cholesterol. However, with increasing X\textsubscript{Chol} from 0.3 to 0.5, ν\textsubscript{\text{a}}-CH\textsubscript{2} wavenumbers of the monolayers were significantly increased at high surface pressures (Fig. 7). This is indicative of a disordering effect caused by cholesterol on the monolayer. It should be mentioned that the analysis of A\textsubscript{ex} in the previous section showed the condensing effect of cholesterol on the DODAB monolayer at all surface pressures. It is an interesting finding that adding a high content of cholesterol (X\textsubscript{Chol} ≥ 0.43) into the DODAB monolayer at a LC state induced the condensing and dis ordering effects simultaneously.

Combining the results obtained for mixed monolayers of DPPC\textsuperscript{52} and DODAB with cholesterol, one may consider that disordering effect of cholesterol under tight packing might be attributed to molecular structure characteristic of cholesterol. It has been demonstrated that cholesterol could maximally interact with 17-carbon acyl chains of phosphatidylcholine (PC) and a length of adjacent planar sterol rings was equivalent to ~9 methylene units\textsuperscript{53-55}. The planar sterol ring of cholesterol might immobilize the portion of the hydrocarbon chains of neighboring molecules due to rigidity of the sterol ring. However, the alkyl sidechain of cholesterol is somewhat flexible and cannot affect neighboring hydrocarbon chains as well as sterol ring\textsuperscript{56}. The condensation of hydrocarbon chains could only take place at the portions that directly contact with the rigid nucleus of cholesterol, thus increasing the conformational order. On the contrary, the alkyl side-chain of cholesterol together with the portion of neighboring hydrocarbon chains could form a flexible and disordered region, countering the conformational order of hydrocarbon chains induced by the rigid sterol ring. Therefore, the molecular packing of a tightly packed monolayer, such as a DPPC monolayer at high surface pressures, is likely to show insignificant change with the incorporation of cholesterol.

Moreover, owing to the long hydrocarbon chains of DODAB, the terminal part of the DODAB hydrocarbon chain in mixed DODAB/cholesterol monolayers would protrude and wag under the tight packing\textsuperscript{56}. At high X\textsubscript{Chol}, the vacancies of mismatch in the hydrophobic region were increased and the motion freedom of the hydrocarbon chains was thus enhanced\textsuperscript{57}. As a result, the cholesterol-induced disordering effect on the DODAB monolayer at a LC state could overwhelm the condensing as well as ordering effects induced by cholesterol at high X\textsubscript{Chol}.

![Fig. 7](image-url)
4 Conclusion

The effects of cholesterol on the molecular packing of Langmuir monolayers of double-chained cationic surfactants, DTDAB, DHDAB, and DODAB, were investigated. The results indicated that the addition of cholesterol into the Langmuir monolayers could restrain the desorption of DTDAB or DHDAB molecules with comparatively short hydrocarbon chains from the interface due to the enhanced intermolecular attraction. Furthermore, cholesterol could induce both condensing and ordering effects on the molecular packing of DODAB monolayers at a LE state, while induce a disordering effect along with the condensing effect on the comparatively long-chained DODAB monolayer at a LC state. The condensing effect of cholesterol was ascribed to the cholesterol-enhanced intermolecular attraction in the monolayers. The sterol ring of cholesterol tended to maximize the contact with neighboring hydrocarbon chains and hinder the portion of neighboring hydrocarbon chains from motion, leading to an increase in conformational order of the molecules. However, at a LC state, the flexible alkyl side-chain of cholesterol along with the corresponding portion of neighboring hydrocarbon chains formed a fluidic region, counteracting the enhanced conformational order of the molecules in the monolayers induced by the sterol ring of cholesterol. In addition, the protrusion of the comparatively long hydrocarbon chains of DODAB contributed a more pronounced motion freedom at high X\textsubscript{Chol} resulting in a more disordered packing of the monolayer. In this study, the disordering effect of cholesterol on the molecular packing of a DODAB monolayer, which was seldom reported in other monolayer studies, was observed and explained. These effects induced by cholesterol would result in different variations of molecular packing in fluid and rigid bilayers, respectively, which can explain how cholesterol adjusts bilayer structures of cationic vesicles composed of DXDAB.

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