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Effects of electrostatic interaction on the phase stability and structures of cubic phases of monoolein/oleic acid mixture membranes

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Abstract

To elucidate effects of electrostatic interactions due to the surface charges on structures and phase stability of cubic phases of lipids, the mixture membranes of monoolein (MO) and oleic acid (OA) (OA/MO membrane) have been investigated by small-angle X-ray scattering method. As increasing OA concentration in the OA/MO membrane, a phase transition from Q^{224} to Q^{229} phase occurred, and above 1.0 mol% OA, OA/MO membranes were in the Q^{229} phase. However, when NaCl concentration in the bulk phase is large, the Q^{224} phase is more stable than the Q^{229} phase in the OA/MO membranes containing high concentrations of OA. These results indicate that the electrostatic repulsion between the headgroups of these membranes makes the Q^{229} phase more stable than the Q^{224} phase. We have also found that lowering pH induces a phase transition from Q^{229} to Q^{224}, and at lower pH a phase transition from Q^{224} to H_{II}. These results are discussed in terms of the spontaneous curvature of the monolayer membrane and a lipid packing parameter of the membrane. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cubic phase; Monoolein; Surface electrostatics; Phase transition; Low pH; X-ray diffraction

1. Introduction

Recently, nonbilayer membranes such as several kinds of cubic phases and inverted hexagonal (H_{II}) phase have attracted much attention in both biological and physicochemical aspects. These nonbilayer structures have been postulated to play several important biological roles in membrane fusions, a control of functions of membrane proteins, ultrastructural organizations inside cells, and crystallization of membrane proteins [1–4]. Phase stability of these nonbilayer membranes, and phase transitions between different nonbilayer structures and also between these nonbilayer phases and liquid-crystalline (L_α) phase have also been interesting subjects for understanding mechanisms of the lipid polymorphism [5–10].

As three-dimensional nonbilayer membranes, cubic phases are well known [1,5,6]. So far, many kinds of cubic phases such as Q^{230}, Q^{224}, Q^{229}, Q^{225}, Q^{223}, Q^{227}, Q^{212} have been found in lipids extracted of cells and also pure lipids in water by X-ray diffraction and freeze-fracture electron microscopy. One family of cubic phases has an infinite periodic minimal surface (IPMS) consisting of bicontinuous regions of water and hydrocarbon. That is, the surface of this family has a negative Gaussian curvature and zero mean

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Abbreviations: MO, 1-monoolein; OA, oleic acid; SAXS, small-angle X-ray scattering

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curvature at all points. The other family of cubic phases consists of discrete micelles packed in cubic symmetry. Several physicochemical studies on these cubic phases such as determination of temperature–water concentration phase diagrams and lateral diffusion of lipids have been done. However, several important points in these cubic phases such as a determinant of structure and phase stability are not well understood.

The monoacylglyceride 1-monoolein (C18:1), MO, in excess water is well known to form a cubic phase Q^{224} (space group Pn3m) at wide regions of temperature (10–92°C). At 92°C, a phase transition from Q^{224} to H_{II} phase occurs [11]. This cubic phase has an IPMS consisting of bicontinuous regions of water and hydrocarbon, which corresponds to Schwartz F surface (sometimes denoted the D-surface) [5,6,12]. Two interwoven tetrahedral networks of rodlike water-channels are arranged on a double-diamond lattice. Several kinds of lipids such as DDPE (didodecyl phosphatidylethanolamine) [13], monoelaidin, and also lipid extracts from *Sulfolobus solfataricius* [14] form this cubic Q^{224} phase. Effects of temperature and water concentration on these Q^{224} phases have been investigated. However, phase stability, structure determinant, and a mechanism of phase transitions involving this Q^{224} phase are not well understood.

In this report, we have investigated effect of electrostatic interaction due to the surface charges on structures and phase stability of the cubic phase Q^{224} composed of MO by small-angle X-ray scattering method (SAXS). To change the surface charge density in the MO membrane, we add small amounts of oleic acid (OA) into the MO membrane. We have investigated effects of the electrostatic interaction in these OA/MO membranes on their structures and phase stability by changing systematically the OA concentration, salt (NaCl) concentration, and pH in bulk phase. We have found that as increasing the OA concentration, a phase transition from Q^{224} to Q^{229} phase occurred, and above 1.0 mol% OA, OA/MO membranes were in the Q^{229} phase. However, when NaCl concentration in the bulk phase is large, the Q^{224} phase is more stable than the Q^{229} phase in the OA/MO membranes containing high concentrations of OA. These results indicate that the Q^{229} phase is more stable than the Q^{224} phase due to the electrostatic repulsion between the headgroups of these lipids. We have also found that lowering pH induces a phase transition from Q^{229} to Q^{224}, and a phase transition from Q^{224} to H_{II}. We have discussed these new results in terms of the spontaneous curvature of the monolayer membrane and a lipid packing parameter of the membrane.

This research was presented at the 36th Annual Meeting of the Biophysical Society of Japan [15].

2. Materials and methods

2.1. Materials and sample preparation

1-Monoolein (MO) was purchased from Sigma Chemical. Oleic acid (OA) (purity $>99.0\%$) was purchased from Wako Chemical.

Lipid dispersions were prepared by adding appropriate amounts of various pH buffer to dry lipids, i.e., mixtures of MO and OA (in large excess water), and the suspensions were vortexed for about 30 s at room temperature several times. For pH $6.0 \leq \text{pH} \leq \text{pH}\ 7.0$, 10 mM PIPES buffer, and for pH $2.5 < \text{pH} < \text{pH}\ 6.0$, 50 mM citrate buffer were used. These buffers contained various concentrations of NaCl (from 0 to 2.5 M). For measurements of X-ray diffraction, pellets after centrifugation ($13\,000\times g$, 1 h at 20°C; Tomy, MR-150) of 1-ml suspensions of 10 mM lipids were used. In most cases, the pH values of the suspensions were a little different from those of the used buffers. Therefore, to determine exact pH values of the suspensions, pH of their supernatants after the centrifugation were measured.

2.2. X-ray diffraction

X-ray diffraction experiments were performed by using Nickel filtered Cu K_α-radiation ($\lambda = 0.154$ nm) from rotating anode type X-ray generator (Rigaku, Rotaflex, RU-300, 50 kV × 300 mA). Small-angle X-ray scattering (SAXS) data were recorded using a linear (one-dimensional) position-sensitive proportional counter (PSPC) (Rigaku, PSPC-5) [16] with camera length of 350 mm and associated electronics (multichannel analyzer, etc., Rigaku). In all cases, samples were sealed in a thin-walled glass capillary tube (outer diameter 1.0 mm) and mounted in a thermostatable holder whose stability was ±0.2°C [17].

3. Results

3.1. *Effect of concentrations of oleic acid (OA) on structures of OA/MO membranes at neutral pH*

We have investigated effects of concentration of oleic acid (OA) in OA/MO membranes on their structures in 10 mM PIPES buffer (pH 7.0) at 20°C by the SAXS method. In the SAXS pattern of pure MO membrane (Fig. 1a), several peaks had spacings in the ratio of $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{9}\cdots$, which were indexed as (110), (111), (200), (211), and (221) reflections on a three-dimensional cubic phase of space group Pn3m (Q^{224}) (Fig. 2). The reciprocal spacing, S, of the cubic phase is connected with the lattice constant, a, by $S(h,k,l) = (1/a)\cdot(h^2+k^2+l^2)^{1/2}$, where h, k, and l are Miller indices [6]. The lattice constant, a, of this MO membrane determined by the gradient of the plot in Fig. 2 was 10.4 nm. Addition of small amounts of OA into the MO membrane changed this cubic structure. As shown in Fig. 1b, in the SAXS pattern of 6.0% OA/94% MO (molar ratio) membrane, several peaks had spacings in the ratio

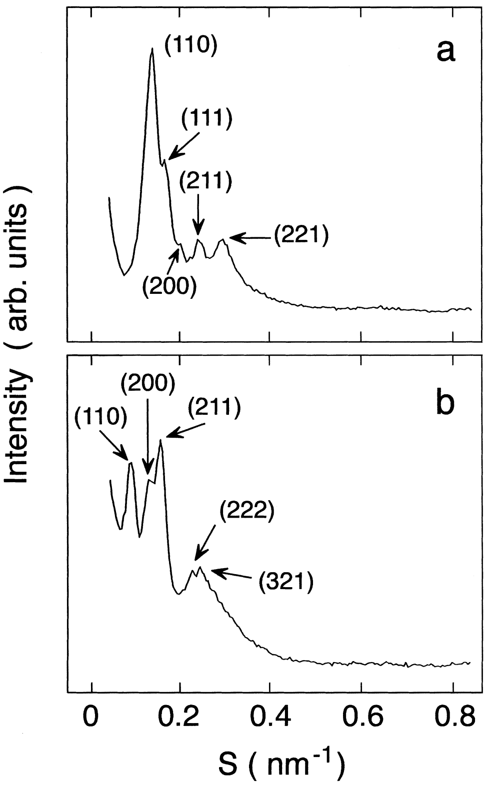

Fig. 1. X-ray diffraction profile of the 100% MO membrane (a) and 6.0% OA/94% MO membrane (b) in 10 mM PIPES buffer (pH 7.0) at 20°C.

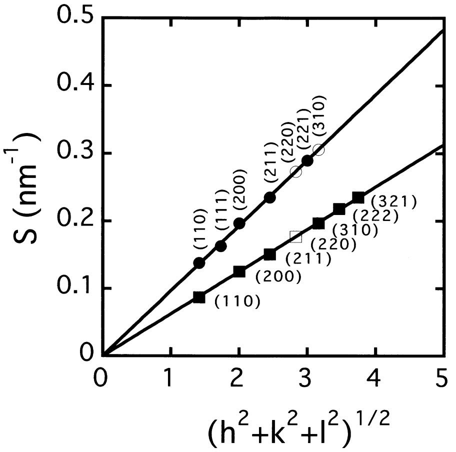

Fig. 2. Indexing of the X-ray diffraction (SAXS) data of the 100% MO membrane (●) and 6.0% OA/94% MO membrane (■) in 10 mM PIPES buffer (pH 7.0) at 20°C. The open symbols (○,□) show reflections which, although allowed by the space group, were not clearly observed due to weak intensities.

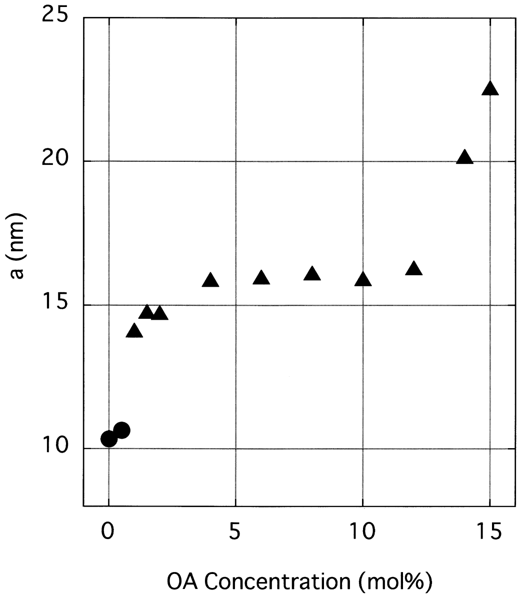

Fig. 3. The lattice constant a of cubic phases of OA/MO membranes containing various concentrations of OA (mol%) in 10 mM PIPES buffer (pH 7.0) at 20°C determined by SAXS. ●, Q^{224} phase; ▲, Q^{229} phase.

of $\sqrt{2}:\sqrt{4}:\sqrt{6}:\sqrt{10}:\sqrt{12}:\sqrt{14}\cdots$. They were indexed as (110), (200), (211), (310), (222), and (321) reflections on a cubic phase of space group Im3m (Q^{229}). The lattice constant of this Q^{229} phase was 16.0 nm. Fig. 3 shows that dependence of the lattice constant and kind of cubic phase on the contents of OA in OA/MO membranes. Less than 1.0 mol% OA, OA/MO membranes were in Q^{224} phase. At 1.0 mol% OA, a phase transition from Q^{224} to Q^{229} occurred, and above 1.0 mol% OA, OA/MO membranes were in Q^{229} phase. The lattice constant of the membranes gradually increased with an increase in OA concentrations.

3.2. *Effect of salt concentration on the lattice constants and kinds of cubic phase in OA/MO membranes at neutral pH*

To elucidate effects of the electrostatic interaction on the phase stability and the lattice constant of OA/MO membranes, we have investigated dependence of NaCl concentration in the bulk phase on structures of these membranes. As shown in Fig. 4, 10% OA/90% MO (molar ratio) membrane in 10 mM PIPES buffer (pH 7.0) was in Q^{229} phase, and on the other hand, the same membrane in 10 mM PIPES buffer (pH 7.0) containing 0.1 M NaCl was in Q^{224} phase. Fig. 5 shows in detail a dependence of the lattice constant and kinds of phase of this membrane on NaCl concentration. At less than 0.1 M NaCl, they were in Q^{229} phase. At 0.1 M NaCl, a phase transition from Q^{229} to Q^{224} occurred, and above 0.1 M NaCl, they were in Q^{224} phases. The lattice constant gradually decreased with an increase in NaCl concentration in both the phases.

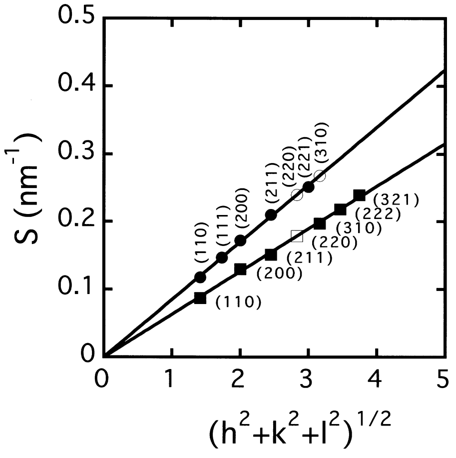

Fig. 4. Indexing of the X-ray diffraction (SAXS) data of the 10% OA/90% MO membranes in 10 mM PIPES buffer (pH 7.0) (■) and in 10 mM PIPES buffer (pH 7.0) containing 0.1 M NaCl (●) at 20°C. The open symbols (○,□) show reflections which, although allowed by the space group, were not clearly observed due to weak intensities.

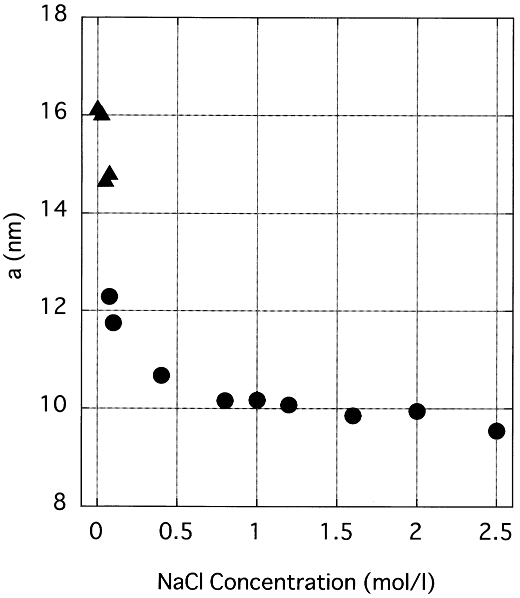

Fig. 5. The lattice constant *a* of cubic phases of the 10% OA/90% MO membranes in 10 mM PIPES buffer (pH 7.0) containing various concentrations of NaCl (mol/l) at 20°C determined by SAXS. ●, Q^{224} phase; ▲, Q^{229} phase.

3.3. *pH dependence of the lattice constants and kinds of cubic phase in OA/MO membranes*

To elucidate effects of the electrostatic interaction on the phase stability and the lattice constant of OA/MO membranes, we have also investigated pH dependence on structure of these membranes. As mentioned before, 10% OA/90% MO (molar ratio) membrane at pH 7.0 in 0M NaCl was in Q^{229} phase. At pH 5.6 in 0 M NaCl, it was in Q^{224} phase, judging from the SAXS pattern. On the other hand, at pH 4.0, several peaks in SAXS pattern had spacing in the ratio of $1:\sqrt{3}:2:\sqrt{7}\cdots$, corresponding to the (10), (21), (20), and (31) reflections on a two-dimensional inverted hexagonal (H_{II}) phase. The reciprocal spacing, *S*, of the cubic phase is connected with the lattice constant, *a*, by $S\ (h,k)=(2/\sqrt{3}a)\bullet(h^2+k^2-hk)^{1/2}$, where *h* and *k* are Miller indices. The lattice constant, *a*, of this H_{II} phase was 6.5 nm. Fig. 6 shows in detail a pH dependence of the lattice constant and kinds of phase of this membrane in various buffers containing 0 M NaCl. As pH in the bulk phase decreased, two kinds of phase transition occurred: at pH 5.8 a phase transition from Q^{229} to Q^{224} occurred, and at pH 5.3 a phase transition from Q^{224} to H_{II} phase occurred (Fig. 6). At low pHs from pH 3.0 to pH 5.0, they were in H_{II} phase. On the other hand, in the presence of 0.5 M NaCl, the same membrane (10% OA/90% MO) was in Q^{224} phase, and as pH decreased, a phase transition from Q^{224} to H_{II} phase occurred at pH 5.6 (data not shown).

A pH dependence of structures and phase behaviors of 6% OA/94% MO (molar ratio) membrane in 0 M NaCl was also investigated (Fig. 6). At pH 7.0, it was in Q^{229} phase, and as pH decreased, a phase transition from Q^{229} to Q^{224} occurred at pH 5.8. However, no phase transition from Q^{224} to H_{II} phase occurred until pH 3.0.

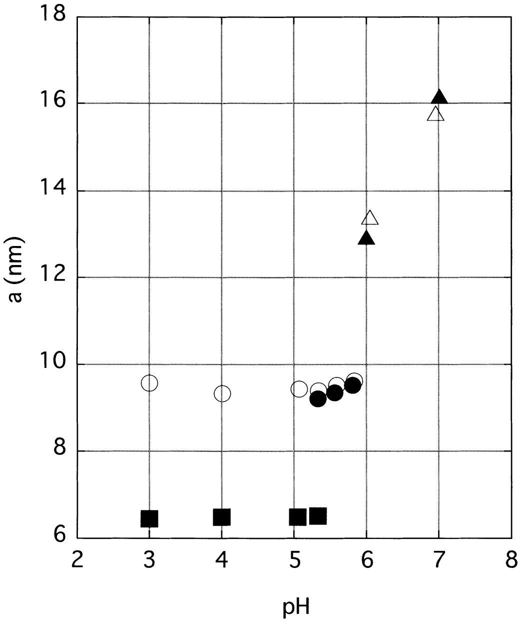

Fig. 6. The lattice constant *a* of nonbilayer phases of the 10% OA/90% MO membranes (closed symbols) and 6.0% OA/94% MO membranes (open symbols) in various pH buffers at 20°C determined by SAXS. ▲,△: Q^{229} phase; ●,○: Q^{224} phase; ■: H_{II} phase.

4. Discussion

4.1. The effect of electrostatic interaction on phase stability and structure of OA/MO membranes

The results of X-ray diffraction experiments clearly show that, in OA/MO mixture dispersion in excess water at neutral pH, the Q^{229} phase is more stable than the Q^{224} phase when OA concentration is high, i.e., the surface charge density is large, and salt concentration in the bulk phase is low (Figs. 3 and 5). Under these conditions, the electrostatic repulsive interaction due to the surface charges of these membranes is large [18]. Therefore, we can say that the larger electrostatic repulsive interaction in the membrane interface increases a stability of the Q^{229} phase rather than the Q^{224} phase. This effect of the electrostatic interaction on phase stability of the OA/MO membrane can be considered as follows.

The spontaneous (or intrinsic) curvature of a single monolayer membrane, H_0, is a useful parameter characterizing non-bilayer membranes, and expressed as $H_0 = 1/R_0$, where R_0 is the spontaneous radius of curvature[8,9,19–21]. Inverted curved structures such as H_{II} phase and cubic phases where the spontaneous curvature of the monolayer is toward the water region, have a large negative H_0 values, and on the other hand, normal structures such as micelles where the spontaneous curvature of the monolayer is toward the alkyl chain region, have a large positive H_0 values. As the repulsive interaction between the headgroups due to electrostatic interaction or steric interaction increases, the absolute value of H_0 decreases to increase the average area of the lipid headgroup. On the other hand, the decrease in the repulsive interaction increases the absolute value of H_0. Marsh has recently shown that H_0 can be expressed in terms of the packing parameter, and that in the inverted curved structures the absolute value of H_0 increases with an increase in the packing parameter [9].

In OA/MO membranes in excess water, when the OA concentration in the membrane is high, i.e., the surface charge density of the membrane is large, and the salt concentration in the bulk phase is low, the electrostatic repulsive interaction between the headgroups is large. Under these conditions, the average area of the lipid headgroup, *a*, is large (i.e., the packing parameter is small) and the absolute value of H_0 is small. Therefore, our results in this report show that the absolute value of H_0 of the Q^{229} phase is smaller than that of the Q^{224} phase in the OA/MO membranes.

The bicontinuous infinite periodic minimal surface (IPMS) such as the Q^{224} and the Q^{229} phase has a negative Gaussian curvature and zero mean curvature at all points. In this kind of membrane, a lipid molecular packing parameter or a surfactant parameter, v/al, where v is the average volume of the alkyl chains of the lipid, l is its average chain length, and a is the average area of the lipid headgroup, is related with the surface-averaged Gaussian curvature, $\langle K\rangle$, as [22],

$$\frac{v}{al}=\frac{1+\langle K\rangle l^2/3}{1+\langle K\rangle l^2} \tag{1}$$

Eq. 1 shows that in the surface where the surface-average Gaussian curvature is negative, i.e., $\langle K\rangle<0$, the lipid molecular packing parameter, v/al, must be larger than 1, and that the absolute value of $\langle K\rangle$, $|\langle K\rangle|$, increases monotonously with an increase in v/al. As discussed above, the average area of the lipid headgroup, a, of the OA/MO membrane in the Q^{229} phase is larger than that of the Q^{224} phase due to the electrostatic interaction, and thereby, v/al in the Q^{229} phase is smaller. Therefore, the membrane in the Q^{229} phase has a smaller negative value of the surface-average Gaussian curvature, $\langle K\rangle$, than that of the Q^{224} phase, i.e., the membrane in the Q^{229} phase, has a flatter surface than that of the Q^{224} phase on average. This is consistent with the above conclusion that the absolute value of H_0 of the Q^{229} phase is smaller than that of the Q^{224} phase.

4.2. *The pH effect on phase stability and structure of the OA/MO membranes*

The results of Fig. 6 clearly show that in the 10% OA/90% MO membranes, at neutral pH, the Q^{229} phase is the most stable, and that with decreasing pH, at pH 5.8 the Q^{229} to Q^{224} phase transition occurs, and at low pH less than pH 5.0, the H_{II} phase is the most stable. What is the mechanism of these pH-induced phase transitions? At neutral pH, the OA has a negative charge, and thus, in the 10% OA/90% MO membranes, the large electrostatic repulsion between the headgroups stabilizes the Q^{229} phase as discussed in the previous section. The carboxyl group of the OA has an intrinsic p$K\sim5$. In the negatively charged membranes, pK values of phosphate groups and carboxyl groups at the membrane interfaces are larger than their intrinsic pK values [23]. As pH of the solution decreases, the protonation of the carboxyl group occurs, decreasing the surface charge density of the OA/MO membranes. The decrease in the surface charge density will decrease the electrostatic repulsive interaction in the membrane interface, which decreases the area a and increases the absolute value of H_0. At a critical pH, i.e., a critical value of H_0, the Q^{229} to Q^{224} phase transition occurs.

The protonation of the carboxyl group of the OA at low pH decreases the surface charge density of the membrane, and also decreases the polarity of the membrane interface, i.e., the interaction free energy of the headgroup segment of the OA with water increases. Therefore, the number of water molecules interacting with the headgroup decreases. Moreover, the headgroup segment of the OA itself is smaller than that of MO (i.e., glycerol). Hence, the protonated OA has a smaller repulsive interaction between the headgroups compared with the MO, and thereby, a smaller average area of the headgroup, a. Hence, the absolute value of H_0 and also the lipid packing parameter increases with a decrease in pH. At a critical pH (in this case, pH 5.0), i.e., at a critical value of H_0, the Q^{224} to H_{II} phase transition occurs (Fig. 6). In the OA/MO membrane which has a lower content of the protonated OA (6.0% OA in Fig. 6), the absolute value of H_0 cannot surpass the critical value, and thereby, the Q^{224} to H_{II} phase transition does not occur.

Summarizing the results of the 10% OA/90% MO membrane, as the absolute value of H_0 increases with a decrease in pH, two kinds of phase transitions occur, i.e., $Q^{229}\Rightarrow Q^{224}\Rightarrow H_{II}$. In the dispersion of DDPE (didodecyl phosphatidylethanolamine) in excess water, the phase sequence: $L_\alpha\Rightarrow Q^{229}\Rightarrow Q^{224}\Rightarrow H_{II}$ was observed as temperature increased [13]. The molecular motion of the alkyl chain increases with an increase in temperature, and thereby, the spontaneous curvature H_0 may increase. The mechanism of this phase transition of DDPE membrane and that of the OA/MO membranes in this report may have some common aspects. A more quantitative analysis in terms of a free energy of the membranes including a curvature elastic energy, an interaction energy between the headgroups, and a packing energy of the alkyl chains is necessary as a next step.

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