Effect of Electrostatic Interactions on Phase Stability of Cubic Phases of Biomembranes

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Abstract. We investigated effect of electrostatic interactions due to surface charges on structures and stability of cubic phases of monoolein (MO) membrane using the small-angle X-ray scattering method. Firstly, we changed the surface charge density of the membrane by using dioleoylphosphatidic acid (DOPA). As increasing DOPA concentration in the membrane at 30 wt % lipid concentration, a Q^{224} to Q^{229} phase transition occurred at 0.6 mol % DOPA, and at and above 25 mol %, DOPA/MO membranes were in the L_α phase. NaCl in the bulk phase reduced the effect of DOPA. These results indicate that as the electrostatic interactions increase, the most stable phase changes as follows: $Q^{224} \Rightarrow Q^{229} \Rightarrow L_\alpha$. The increase in DOPA concentration reduced the absolute value of spontaneous curvature of the membrane, $|H_0|$. Secondly, we changed the surface charge of the membrane by adding a *de novo* designed peptide, which has net positive charges and a binding site on the electrically neutral membrane interface. The peptide-1 (WLFLKKKK) induced a Q^{224} to Q^{229} phase transition in the MO membrane at low peptide concentration. As NaCl concentration increases, the MO/peptide-1 membrane changed from Q^{229} to Q^{224} phase. The increase in peptide-1 concentration reduced $|H_0|$. Based on these results, the stability of the cubic phases and the mechanism of phase transition between cubic phase and L_α phase are discussed.

Key words: a cubic to L_α phase transition, cubic phases, curvature elastic energy, infinite periodic minimal surface, membrane interface, peptide-membrane interaction, spontaneous curvature, X-ray diffraction

1. Introduction

Cubic phases of lipid membranes have attracted much attention in both biological and physicochemical aspects [1, 2]. One family of cubic phases such as Q^{224} , Q^{229} , and Q^{230} has an infinite periodic minimal surface (IPMS) consisting of bicontinuous regions of water and hydrocarbon [2]. In these cubic phase membranes, the minimal surface is located at the bilayer midplane, which is the interface of two monolayer membranes, and the minimal surface has a negative Gaussian curvature and zero mean curvature at all points. Cubic phases of biomembranes have been postulated to play several important biological roles in biomembranes such as

membrane fusions, a control of functions of membrane proteins, and ultrastructural organizations inside cells [3–6]. 3-D regular structures of biomembranes similar to cubic phases have been often observed in various cells by the transmission electron microscopy (TEM) [6]. Especially, a prolamellar body (PLB) in plant cells is one of the most famous examples. When plants are grown in the dark, a paracrystalline-like structure called as PLB is formed in etioplasts in their leaf cells [7]. After absorbing light, the PLB transforms into the lamellae of thylakoids whose membranes are in the bilayer liquid-crystalline (L_α) phase, and then, the etioplasts become chloroplasts. The PLB structures have been considered as Q^{224} or Q^{229} phase based on the analysis of TEM [6]. This transformation is considered as a kind of a cubic to L_α phase transition, but its mechanism is not well understood.

Several peptides such as so-called fusion peptides of membrane proteins in virus induce cubic phases in phospholipid membranes [e.g., 5]. In cells, proteins and peptides may change the stability of cubic phases of biomembranes. However, the mechanism of effects of peptides and proteins on the stability of cubic phases is not well understood [8]. Recently, it was elegantly shown that the cubic phases are also very useful for crystallization of membrane proteins [9, 10]. To increase its applicability, the elucidation of its mechanism is essential. Therefore, it is very important to elucidate the mechanism of stability of cubic phases and also to determine the factor to control structures of cubic phases. Especially, elucidation of the mechanism of phase transitions between cubic phases and L_α phase is essential for understanding of dynamical structural change of biomembranes and also the development of the new crystallization technique.

Several physicochemical studies on these cubic phases such as determination of temperature-water concentration phase diagrams and lateral diffusion of lipids have been reported. Especially, effect of water contents and temperature on the phase stability of cubic phases of monoolein (MO) (C18:1), a typical monoacylglycerol, has been extensively investigated [e.g., 11]. In excess water, the MO membrane is in the Q^{224} phase (space group $Pn3m$) at wide temperature range. This cubic phase has an IPMS consisting of bicontinuous regions of water and hydrocarbon, which corresponds to Schwartz D surface [2]. Two interwoven tetrahedral networks of rodlike water-channels are arranged on a double-diamond lattice. However, effect of other factors on the phase stability and the structure determinant of the cubic phase are still not well understood.

In this report, we describe the effect of electrostatic interactions due to surface charges of the membrane on structures and phase stability of the cubic phase of biomembranes determined by the small-angle X-ray scattering method (SAXS). We used the MO membrane as a model of cubic phases of biomembrane, because it is one of the best cubic phases whose physical property has been thoroughly investigated. In the first experiment, to change the surface charge density in the MO membrane, we added various amounts of a negatively-charged phospholipid, dioleoylphosphatidic acid (DOPA) into the MO membrane from 0 to 40 mol % DOPA [12]. In the second experiment, we changed the surface charge of the mem-

Figure 1. The structure parameter of DOPA/MO membranes containing various concentrations of DOPA (mol %) in 10 mM PIPES buffer (pH 7.0) at 30 wt % lipid condition at 20 °C determined by SAXS. (○), (□), and (◇) denote the Q²²⁴ phase, Q²²⁹ phase, and L_α phase, respectively.

brane by adding *de novo* designed peptides, which has positive charges and also a binding region on the lipid membrane interface composed of electrically neutral lipids such as phosphatidylcholine (PC) and MO. Based on these new results, we discuss the mechanism of the phase transition between cubic phase and the L_α phase, and also that of the phase stability of the cubic phase of the MO membrane.

2. Materials and Methods

X-ray diffraction experiments were performed by using Nickel filtered Cu K_α X-ray ($\lambda = 0.154$ nm) from rotating anode type X-ray generator (Rigaku, Rotaflex, RU-300) at the operating condition (40 kV $\times$ 200 mA). Small-angle X-ray scattering (SAXS) data were recorded using a linear (one-dimensional) position sensitive proportional counter (PSPC) with camera length of 350 mm [12].

Peptides with an amide-blocked C terminus were synthesized by the FastMoc method and purified by reversed-phase HPLC. The purified peptides were analyzed by the electrospray ionization mass spectrometry [13].

3. Results and Discussion

3.1. EFFECT OF ELECTROSTATIC INTERACTIONS ON PHASE STABILITY OF CUBIC PHASES OF MEMBRANES OF MONOOLEIN/DIOLEOYLPHOSPHATIDIC ACID MIXTURES

Effect of DOPA concentration and NaCl concentration on structures of DOPA/MO membranes

We investigated effect of DOPA concentration (mol %) in DOPA/MO membranes on their structures in 10 mM PIPES buffer (pH 7.0) at high lipid concentrations (30 wt % lipids) at 20 °C by SAXS (Figure 1). SAXS pattern shows that MO membrane is in a primitive cubic phase of space group Pn3m (Q^{224}). On the other hand, DOPA/MO membranes containing 1.0–15% DOPA were in a body-centered cubic phase of space group Im3m (Q^{229}). At 0.6% DOPA, a phase transition from Q^{224} to Q^{229} phase occurred, and both the phases coexisted, where the ratio of the lattice constant (Q^{229}/Q^{224}) was 1.27. It is close to the theoretical value (1.28), which is determined by the analysis of the coexisting cubic phases based on the Bonnet transformation [14]. The lattice constant (i.e., the structure parameter) of the DOPA/MO membrane in the Q^{229} phase gradually increased from 14.6 nm to 20.5 nm with an increase in DOPA concentration from 1.0 to 15%. At 16 ~ 24% DOPA, it was difficult to specify the phase. At and above 25% DOPA, a new set of SAXS peaks appeared with a large spacing (11.3 ± 0.5 nm) in the ratio of 1:2:3, which is consistent with an L_α phase. The spacing (i.e., the structure parameter) of the L_α phase was almost constant with an increase in DOPA concentration from 25 to 40%. The condition of these membranes in the L_α phase was not in excess water, because the intermembrane distance of the DOPA/MO-multilamellar vesicle was very large due to the electrostatic repulsion between the membranes and also contents of water was not enough (70 wt % water). Thereby, the spacing did not change as the DOPA concentration increased.

The phase and structure of the DOPA/MO membranes containing less than 12% DOPA in excess water were almost the same as those at 30 wt % lipids, but above 12% DOPA, peaks in SAXS pattern became broad, and thereby it is difficult to analyze it. To investigate the structure of DOPA/MO membranes containing high concentration of DOPA in excess water condition, we tried to make a giant unilamellar vesicle (GUV) of these membranes. The observation by Hoffman modulation contrast method shows that many 30%-DOPA/70%-MO GUVs were formed in 10 mM PIPES buffer (pH 7.0). In most cases, these GUVs were spherical vesicles with 20 ~ 50 μ m diameter. This result indicates that the DOPA/MO membranes containing high concentration of DOPA in excess water were in the L_α phase. It supports the above conclusion based on the SAXS experiments.

To elucidate effects of the electrostatic interactions on the phase stability and the lattice constant of DOPA/MO membranes, we investigated effect of NaCl concentration in the bulk phase on structures of these membranes in excess water

condition. At less than 60 mM NaCl, 10% DOPA/90% MO membranes in 10 mM PIPES buffer (pH 7.0) were in the Q^{229} phase. At 60 mM NaCl, a phase transition from Q^{229} to Q^{224} phase occurred, and at 60 mM and 70 mM NaCl, both the phases coexisted. The lattice constant of the Q^{224} phase gradually decreased with an increase in NaCl concentration. At 1.2 M NaCl, a phase transition from Q^{224} to H_{II} phase occurred, and above 1.5 M NaCl the membrane was in the H_{II} phase.

Next, we investigated effect of NaCl concentration on structures of 30%-DOPA/70%-MO membrane in excess water condition. As the previous data show, in 0 M NaCl, it was in the L_α phase. In the presence of low NaCl concentration below 0.37 M, after the centrifugation of the samples, pellets were not observed, and the SAXS measurement of the suspension gave a broad peak, whose analysis was difficult. However, judging from the analysis of the membrane in 0 M NaCl in the previous section, we consider these membranes were in the L_α phase. At and above 0.50 M NaCl (less than 0.75 M NaCl), the SAXS pattern showed that the membranes were in the Q^{224} phase and that the lattice constant of the Q^{224} phase gradually decreased with an increase in NaCl concentration. At the intermediate concentration ($0.37 \text{ M} \leq \text{NaCl} < 0.50 \text{ M}$), there were several peaks in SAXS pattern. They contained peaks due to the L_α phase and also due to the cubic phase which could not be indexed with reliability. At 0.65 M NaCl, a phase transition from Q^{224} to H_{II} phase occurred, and at and above 0.75 M NaCl, the 30%-DOPA/70%-MO membranes were in the H_{II} phase.

The results of X-ray diffraction experiments clearly show that, in DOPA/MO membranes in excess water at neutral pH, the Q^{229} phase is more stable than the Q^{224} phase when the surface charge density due to the DOPA is relatively high and salt concentration in the bulk phase is low. Under this condition, the electrostatic interactions due to the surface charges of these membranes are large. Therefore, we can say that these electrostatic interactions in the membrane interface increase the stability of the Q^{229} phase with respect to that of the Q^{224} phase. This is the same conclusion as that of our previous paper on the MO/oleic acid membranes in excess water [15]. As the electrostatic interactions due to the surface charge become larger, the L_α phase becomes more stable than the cubic phases (i.e., Q^{224} and Q^{229} phases). Therefore, we can conclude that as the electrostatic interactions increases, the most stable phase of DOPA/MO membranes changes as follows: $Q^{224} \Rightarrow Q^{229} \Rightarrow L_\alpha$.

Spontaneous curvature of DOPA/MO membranes

The spontaneous curvature of a single monolayer membrane, H_0 , is a useful parameter characterizing nonbilayer membranes, and expressed as $H_0 = 1/R_0$, where R_0 is the radius of spontaneous curvature [16, 17]. H_0 is defined as a curvature to minimize the curvature elastic energy of the monolayer membrane, and determined by physical properties of the monolayer by itself without the interaction of other monolayer membrane [16, 17]. Thereby, it is a kind of an ideal curvature of the monolayer membrane. In most lipid membranes, monolayer membranes cannot

Figure 2. The basis vector length, d , of H_{II} phases of DOPA/MO membrane containing 16 wt % tetradecane vs. DOPA (mol %) concentration in 10 mM PIPES buffer (pH 7.0) in excess water condition at 20 °C determined by SAXS. (▲) in 0 M NaCl and (△) in 1.0 M NaCl.

have their spontaneous curvature, because the interaction between two monolayer membranes plays an important role in the determination of the curvature. It is important to note that H_0 depends on both the molecular structure of the constituent lipids and various external conditions, such as temperature and kinds of solution [17, 18].

To consider the mechanism of the phase transitions above mentioned, we investigated effects of DOPA concentration and NaCl concentration in the bulk phase on H_0 of MO membrane. To allow the lipid monolayer membranes to express H_0 , the addition of alkanes such as tetradecane to the membranes is required, because they fill the interstitial region of the H_{II} phase and relax the alkyl chain packing stress [18]. At first, we investigated effect of concentration of tetradecane in the membrane on structure of the MO membrane in excess water condition at 20 °C in 10 mM PIPES buffer (pH 7.0). Above 8% (w/w) tetradecane, the MO membranes were in the H_{II} phase. Thereby, to get information on the dependence of H_0 of the MO membrane on DOPA concentration, we investigated the structure of DOPA/MO membrane containing 16 wt % tetradecane in excess water condition at 20 °C (Figure 2). The basis vector length, d , of the DOPA/MO/tetradecane membrane in 10 mM PIPES buffer (pH 7.0) gradually increased from 6.5 nm to 9.4 nm with an increase in DOPA concentration from 0 to 40 mol %. On the other hand, d of the DOPA/MO/tetradecane membrane in 10 mM PIPES buffer (pH 7.0)

containing 1.0 M NaCl gradually decreased from 6.7 nm to 6.2 nm with an increase in DOPA concentration from 0 to 30 mol %. We also investigated the structure of 30%-DOPA/70%-MO membrane containing 16 wt % tetradecane in 10 mM PIPES buffer (pH 7.0) containing various NaCl concentrations. In 0 M NaCl, the 30%DOPA/70%-MO membrane without tetradecane was in the L_α phase (Figure 1), but the 30%DOPA/70%-MO membrane containing 16 wt % tetradecane was in the H_{II} phase with a basis vector length of 8.7 nm. This value gradually decreased from 8.7 nm to 6.4 nm with an increase in NaCl concentration from 0 M to 1.0 M.

The basis vector length of the H_{II} phase, d , is expressed as a sum of the radius to the neutral plane (or pivotal plane), R_{pp} , and the distance between the bilayer midplane and the neutral plane, ξ , i.e., $d = 2(R_{pp} + \xi)$ [14]. In excess water, the DOPA/MO membrane containing 16 wt % tetradecane in the H_{II} phase has H_0 , and thereby, $R_{pp} \approx R_0$. The increase in d of the DOPA/MO/tetradecane membrane induced by the increase in content of DOPA is attributed to the increase in R_{pp} , since the change in ξ is considered to be small. Thus, the result of Figure 2 shows that R_0 of the DOPA/MO membranes increases with an increase in DOPA concentration. It indicates that the increase in electrostatic interactions reduces the absolute value of spontaneous curvature, $|H_0|$, of the membrane to increase the average area of the lipid headgroup of the membrane. Therefore, the experimental results clearly show that the change of $|H_0|$ of the monolayer membrane is deeply correlated with the stability of cubic phases; as $|H_0|$ decreases, the most stable phase of the lipid membrane changes as follows; $Q^{224} \Rightarrow Q^{229} \Rightarrow L_\alpha$.

As described above, when the surface charge density of the membrane due to DOPA is high and the salt concentration in the bulk phase is low, $|H_0|$ is low. This is mainly due to two kinds of electrostatic interactions. One is the repulsive electrostatic interaction between the headgroups, which may reduce $|H_0|$ to increase the distance between the headgroups. The other is the attractive electrostatic interaction between negative charge of the headgroup of DOPA and a dipole moment of water molecule. It attracts more water molecules near the headgroup, and thereby the effective volume of the headgroup increases, which may reduce $|H_0|$ [19, 20]. However, at present, we don't have enough experimental evidence to determine which electrostatic interaction is dominant one to reduce $|H_0|$.

Phase stability of cubic phases

The difference of the chemical potential of the phospholipid membrane in the non-bilayer phase such as the cubic (Q^{224} , Q^{229}) phase and the H_{II} phase (μ^{nonbil}) and in the bilayer liquid-crystalline (L_α) phase (μ^{bil}), $\Delta\mu$, is expressed as follows:

$$\begin{aligned}\Delta\mu &= \mu^{nonbil} - \mu^{bil} \\ &= (\mu_{curv}^{nonbil} - \mu_{curv}^{bil}) + (\mu_{ch}^{nonbil} - \mu_{ch}^{bil}) \\ &= \Delta\mu_{curv} + \Delta\mu_{ch}\end{aligned}\tag{1}$$

where $\Delta\mu_{curv}$ is a term due to the curvature elastic energy, and $\Delta\mu_{ch}$ is a term due to interstitial chain packing of the nonbilayer phase. The curvature elastic energy of the membrane, μ_{curv} , in Equation 1 can be expressed as [21]

$$\mu_{curv} = 2\kappa\langle H - H_0 \rangle^2 + \kappa_G\langle K \rangle \quad (2)$$

where κ is the elastic bending modulus, H the mean curvature, K the Gaussian curvature, κ_G the Gaussian curvature modulus, and $\langle \rangle$ means the average value over the area of the unit cell. The bicontinuous infinite periodic minimal surface (IPMS) such as the Q^{224} and the Q^{229} phase has a negative Gaussian curvature, i.e., $K < 0$. It has zero mean curvature at all points in the bilayer midplane (i.e., the minimal surface), but the neutral plane of the membrane has a constant nonzero mean curvature since it has a fixed distance from the minimal surface [21]. Since $H = K = 0$ in the L_α phase,

$$\Delta\mu_{curv} = 2\kappa\{\langle H - H_0 \rangle^2 - H_0^2\} + \kappa_G\langle K \rangle. \quad (3)$$

Since $H_0 < H < 0$ for these nonbilayer membranes, Equation 3 shows that $\Delta\mu_{curv}$ is always negative ($\Delta\mu_{curv} < 0$), and thereby, is an important factor stabilizing the nonbilayer phases. On the other hand, in the H_{II} phase, alkyl chains of lipids have to extend to different lengths to fill the interstitial hydrocarbon region, which increases the free energy of the membrane. This situation is almost the same in the cubic phases. Therefore, this packing energy of the alkyl chains unstabilizes the nonbilayer phases, and thus $\Delta\mu_{ch}$ is always positive ($\Delta\mu_{ch} > 0$). In most cases, phase transitions between the cubic phases and the L_α phases are determined by the interplay of these two factors, $\Delta\mu_{curv}$ and $\Delta\mu_{ch}$. Equation 3 shows that $|\Delta\mu_{curv}|$ decreases with a decrease in $|H_0|$.

For the MO membrane, $|H_0|$ is large and thereby $\Delta\mu_{curv}$ has a large negative value. Therefore, $\Delta\mu < 0$, indicating that the cubic phase is stable. As the electrostatic interactions increase owing to the charged DOPA, $|H_0|$ of the membrane decreases, inducing the decrease in $|\Delta\mu_{curv}|$. At the critical value of the electrostatic interactions, $\Delta\mu = 0$, and thereby the cubic to L_α phase transition occurs. Above the critical value of the electrostatic interactions, $\Delta\mu > 0$, and thereby the L_α phase is stable. Hence, the decrease in $|H_0|$ with an increase in the electrostatic interactions induces the cubic to L_α phase transition in the DOPA/MO membrane.

3.2. EFFECT OF *de novo* DESIGNED PEPTIDES ON STABILITY OF CUBIC PHASES OF MONOOLEIN MEMBRANES

Recent biophysical studies indicate that the lipid membrane interface is composed of hydrophilic segments (so-called head group), hydrophobic alkyl chains, and water molecules because of large thermal motions of the membranes such as undulation and protrusion [22, 23]. Peptides can be partitioned into the membrane interface or bound on the membrane interface. The thickness of the membrane interface of dioleoyl-PC (DOPC) membrane was ~ 1.5 nm, showing that an α -helix

can be partitioned parallel to the bilayer plane into the membrane interface. Free energies of transfer of short peptides (Ac-WL-X-LL with X being any of the twenty amino acids) from the membrane interface of palmitoyl-oleoylphosphatidylcholine (POPC) to water were obtained experimentally, and from this data an interfacial hydrophobicity scale of amino acids was determined [24]. According to their data, aromatic residues such as Trp (W) and Phe (F) have high interfacial hydrophobicity, meaning that partition of these residues into the lipid membrane interface is large.

As the *de novo* designed peptide, we designed and synthesized a peptide with the sequence of WLFLKKK (peptide-1). The N-terminal region (WLFL) is the binding site of the peptide on the membrane interface, since an experimental result shows that WLFL peptide locates at the membrane interface and also F has a high interfacial hydrophobicity [24]. The purpose of the design of this peptide is that it is bound on the membrane interface and gives positive charges on the membrane interface. We investigated effect of the peptide-1 on the stability of cubic phases of the MO membrane.

Effect of peptide-1 on MO membrane

We investigated effect of peptide-1 on the structure of MO membrane in 10 mM PIPES buffer (pH 7.0) in excess water condition at 20 °C by SAXS. Figure 3(a) shows that dependence of the lattice constant and kind of cubic phase on the concentration of peptide-1. Less than $R = 0.011$, MO membranes were in the Q^{224} phase. At and above $R = 0.011$, MO membranes were in the Q^{229} phase. Hence, at $R = 0.011$, a phase transition from Q^{224} to Q^{229} phase occurred. The lattice constant of the Q^{224} phase immediately before the phase transition ($R = 0.010$) and that of the Q^{229} phase immediately after the phase transition ($R = 0.011$) were 11.0 nm and 14.2 nm, respectively. The ratio of the lattice constant (Q^{229}/Q^{224}) was 1.29, which is close to the theoretical value (1.28). The lattice constant of the membranes gradually increased with an increase in peptide-1 concentration. As a control experiment, effect of peptide-2 (LLKKK) on structure of MO membranes was investigated (Figure 3(a)). The structure of MO membrane did not change in the presence of the peptide-2 up to $R = 0.1$.

To elucidate effects of the electrostatic interactions on the phase stability of the MO/peptide-1 membrane, we investigated effect of NaCl concentration in the bulk phase on the structure of the MO membrane/peptide-1 mixture ($R = 0.020$) in excess water condition. Figure 3(b) shows a detailed dependence of the lattice constant and kinds of phase of this membrane on NaCl concentration. Less than 20 mM NaCl, they were in the Q^{229} phase. At 20 mM NaCl, a phase transition from Q^{229} to Q^{224} occurred, and the lattice constant of both the phases gradually decreased with an increase in NaCl concentration.

The results of X-ray diffraction experiments clearly show that the *de novo* designed peptide-1 induced a phase transition from Q^{224} to Q^{229} phase in the MO membranes in excess water. The binding of the peptide-1 on the membrane interface of the MO membrane occurs at the N-terminus region (WLFL) of the

Figure 3. (a) The structure parameter (i.e., lattice constant) of cubic phases of MO membranes containing various concentrations of peptide-1 (●, ■) and peptide-2 (○) in 10 mM PIPES buffer (pH 7.0) in excess water condition at 20 °C determined by SAXS. R is the molar ratio of peptides to MO. (●, ○) denote the Q^{224} phase and (■) denotes the Q^{229} phase. (b) The structure parameter of cubic phases of the MO-membrane/peptide-1 mixture ($R = 0.020$) in 10 mM PIPES buffer (pH 7.0) containing various concentrations of NaCl (M) at 20 °C determined by SAXS. (●) denotes the Q^{224} phase and (■) denotes the Q^{229} phase.

peptide-1 due to its high interfacial hydrophobicity [24] (Figure 4). The binding of the peptide-1 increases the surface charge density of the MO membrane, since the peptide-1 has three positive charges at the C-terminus site and one positive charge at the N-terminus. As the peptide-1 concentration increases and thereby the surface charge density of the membrane increases, the electrostatic repulsive interaction between the positive charges increases. On the other hand, in the membrane of the MO membrane/peptide-1 mixture, as the salt concentration in the bulk phase increases, the electrostatic repulsive interaction decreases due to the screening of the charges of the peptides. Therefore, these results clearly show that the Q^{229}

Figure 4. Scheme of the binding of peptide-1 on the membrane interface due to the high interfacial hydrophobicity.

phase is more stable than the Q^{224} phase in the MO membrane in excess water when the electrostatic repulsive interactions between the positive charges of the peptides bound on the membrane interface is large.

Preliminary data show that at high concentrations of peptide-1 ($0.075 \leq R \leq 0.15$) the MO membranes in 10 mM PIPES buffer (pH 7.0) at 30 wt % lipid are in the $L\alpha$ phase, indicating that the peptide-1 also induced a cubic to $L\alpha$ phase transition in the MO membrane.

Effect of peptide-1 on spontaneous curvature of MO membrane

To consider the mechanism of the peptide-1-induced phase transition, we investigated effects of the peptide-1 on the spontaneous curvature of the MO monolayer membrane. For this purpose, we investigated the structure of MO/peptide-1 mixture membrane containing 16 wt % tetradecane in excess water condition at 20 °C (Figure 5). The basis vector length, d , of the MO/peptide-1/tetradecane membrane in 10 mM PIPES buffer (pH 7.0) gradually increased from 6.7 nm to 8.2 nm with an increase in peptide-1 concentration from $R = 0$ to 0.040.

The increase in d of the MO/peptide-1/tetradecane membrane with an increase in peptide-1 concentration is attributed to the increase in R_{pp} , since the change in ξ is considered to be small. Thus, the result of Figure 5 shows that R_o of the MO/peptide-1 membrane increases with an increase in peptide concentration. It indicates that the increase in electrostatic repulsive interaction reduces $|H_o|$ of the membrane to increase the distance between the positive charges of different peptides bound on the membrane interface. Based on this analysis, we can indicate that the decrease in $|H_o|$ is deeply correlated with the phase transition from Q^{224} to Q^{229} phase in the MO membrane in excess water.

Figure 5. The basis vector length, d , of H_{II} phases of MO membranes containing 16 wt % tetradecane vs. peptide-1 concentration (R) in 10 mM PIPES buffer (pH 7.0) in excess water condition at 20 °C determined by SAXS.

4. Conclusion

The effects of water contents and temperature on the phase stability of cubic phases have been vigorously investigated. However, effect of electrostatic interactions due to the surface charge on the structure and stability of cubic phase membranes has never been investigated systematically. This report and our previous report [15] indicated that the structure and the phase stability of cubic phases and the cubic to L_{α} phase transition can be controlled by the electrostatic interactions due to the surface charge of membranes. Two methods to change the surface charge of the membrane were demonstrated; one is the change of lipid composition such as the concentration of negatively-charged lipids and the other is the binding of peptides with net charges to the membrane interface. We can conclude that as the electrostatic interactions increases, the most stable phase of MO membrane changes as follows: $Q^{224} \Rightarrow Q^{229} \Rightarrow L_{\alpha}$. Moreover, the experimental results clearly show that the change of $|H_0|$ of the monolayer membrane is deeply correlated with the stability of cubic phases; as $|H_0|$ decreases, the most stable phase of the lipid membrane changes as follows; $Q^{224} \Rightarrow Q^{229} \Rightarrow L_{\alpha}$. A more quantitative analysis of the effects of the electrostatic interactions on the cubic phase stability is necessary as a next step, and in progress in our laboratory.

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