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The effect of peptides and ions interacting with an electrically neutral membrane interface on the structure and stability of lipid membranes in the liquid-crystalline phase and in the liquid-ordered phase

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Abstract

We investigated the effects of a *de novo* designed peptide, WLFLLKKK (peptide-1) and La^{3+}, which can bind with the electrically neutral lipid membrane interface, on the stability of the phosphatidylcholine (PC) membrane in the L_α phase and that of the liquid-ordered (lo) phase membranes. The results of spacing of the multilamellar vesicle and shape changes of the giant unilamellar vesicle (GUV) indicate that the peptide-1 can be partitioned into the membrane interface in the L_α phase but not into that in the lo phase. La^{3+} induced shape changes of GUVs of the lo phase membrane, which are the same as those of GUVs in the L_α phase. This indicates that the binding of La^{3+} induced an increase in the lateral compression pressure of the membrane, which decreased the surface area of the membrane in the lo phase. The difference of the membrane interface between the L_α phase and the lo phase is discussed.

1. Introduction

Interactions of proteins with lipid membranes play important roles in the static and dynamic structures of biomembranes and their functions. It is reported that many water-soluble proteins

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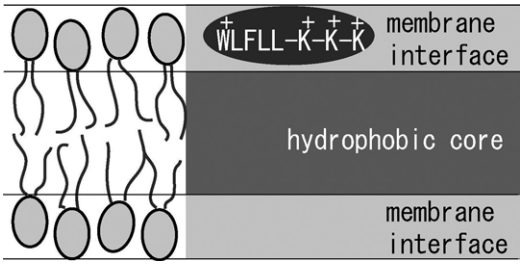

Figure 1. A schematic view of the membrane interface of a PC membrane in the L_α phase, and the partition of peptide-1 into the membrane interface.

can be bound with lipid membrane regions in biomembranes reversibly and that their binding depends on their concentration in the aqueous phase, conformation, and local net charge. Especially, the electrostatic interaction between a cluster of basic amino acid residues of proteins and negatively charged lipid membranes and also the hydrophobic interaction between a fatty acid covalently linked protein and lipid membranes are well understood [1, 2]. For example, myristoylated alanine-rich C kinase substrate (MARCKS) and src ($pp60^{src}$) can be bound with lipid membranes using both electrostatic attractive interaction and hydrophobic interaction due to the covalently linked fatty acid. However, the interaction between substances and the membrane interface of electrically neutral lipids such as phosphatidylcholine (PC) and its effect on the structure of lipid membranes are not well understood.

Recent biophysical studies indicate that the lipid membrane interface is composed of hydrophilic segments (so-called head groups), hydrophobic acyl chains, and water molecules incorporated as a result of large thermal motions of membranes such as undulation and protrusion [3–5]. The thickness of the membrane interface of a dioleoyl-PC (DOPC) membrane is $\sim$1.5 nm, indicating that an α-helix can be partitioned in the membrane interface parallel to the bilayer plane [6]. Factors other than electrostatic attraction between peptides and membranes are involved in partitioning of peptides and proteins in the membrane interface of electrically neutral lipid membranes. Free energies of transfer of short peptides from the membrane interface of palmitoyloleoyl-PC (POPC) to water have been obtained experimentally; from the resulting data, an interfacial hydrophobicity scale of amino acid residues (i.e., free energies of transfer of amino acid residues from the membrane interface to water, ΔG_{tr}) has been constructed [7]. These data show that aromatic amino acid residues such as Trp (W) and Phe (F) have high interfacial hydrophobicity (ΔG_{tr} of Trp and Phe are 1.85 and 1.13 kcal mol^{-1}, respectively), indicating strong partitioning of these residues in the lipid membrane interface. Using this interfacial hydrophobicity data, we have designed and synthesized a *de novo* designed peptide, WLFLLKKK (peptide-1), which has positive charges and also can be partitioned into the lipid membrane interface composed of electrically neutral lipids (figure 1), and investigated the effect of peptide-1 on the stability of electrically neutral lipid membranes in the liquid-crystalline (L_α) phase [8]. The free energy of transfer of peptide-1 from the membrane interface to water is 1.69 kcal mol^{-1} (using the following ΔG_{tr} values: Leu (L), 0.56 kcal mol^{-1}; Lys (K), -0.99 kcal mol^{-1}) (we neglected effects of peptide termini; i.e., NH^{3+} and $CONH_2$), indicating that peptide-1 can be partitioned in the PC membrane interface. In contrast, the free energy of transfer of peptide-2 (LLKKK, used for control experiments) from the membrane interface to water was estimated to be -1.85 kcal mol^{-1}, indicating that peptide-2 is not partitioned in the PC membrane interface. Peptide-1 was designed to bring positive charges to the membrane interface of electrically

neutral lipid membranes, as a result of its partition. Peptide-1 increased the intermembrane distance of PC multilamellar vesicles (PC-MLVs) and induced various shape changes in giant unilamellar vesicles (GUVs) of PC membranes, whereas peptide-2 did not have any effects on PC membranes, indicating that peptide-1 was appropriately designed [8].

We also investigated the effects of peptide-1 on the stability of the cubic phase of monoolein (MO) membranes. One family of cubic phases, which includes Q^{224} phase (Schwartz's D surface), Q^{229} phase (P surface) and Q^{230} phase (G surface), has an infinite periodic minimal surface (IPMS) consisting of bicontinuous regions of water and hydrocarbon [9]. Transmission electron microscopy has revealed regular 3D structures of biomembranes similar to cubic phases in various cells [10]. It has been postulated that cubic phases play important roles in biomembrane dynamics such as membrane fusion, control of membrane protein functions, and intracellular various structures of membranes. Recent elegant experiments have shown that cubic phases are very useful for the crystallization of membrane proteins [11]. Elucidation of the mechanisms of transitions between the cubic phase and L_α phase, and between different cubic phases, is essential for the understanding of biomembrane dynamics and the development of new crystallization techniques. However, there has been only limited research on the phase transitions and stability of cubic phases. The effects of water content and temperature on the stability of cubic phases have been investigated. Recently, we have systematically investigated the effects of electrostatic interactions due to surface charges on the structure and stability of cubic-phase membranes, and have found that, in lipid membranes, electrostatic interactions due to surface charges induce transitions between the cubic phase and L_α phase, and between different IPMS cubic phases [12, 13]. As electrostatic interactions increase (i.e., surface charge density increases or salt concentration in bulk phase decreases), the most stable phase of the MO membrane changes: $Q^{224} \Rightarrow Q^{229} \Rightarrow L_\alpha$. We also found that the electrostatic interactions greatly reduced the absolute value of the spontaneous curvature of the single monolayer MO membranes, which is defined as its radius of curvature of the monolayer membrane to minimize its curvature elastic energy without the interaction of other monolayer membrane (note: the spontaneous curvature of the monolayer is a different concept from that of a bilayer) [14]. The spontaneous curvature of the monolayer is determined by the packing parameter of lipids (V/Al), which depends on the molecular structure of the lipids and also on external conditions such as temperature and solvents [14, 15]. We can reasonably consider that the curvature elastic energy of the membrane due to the spontaneous curvature of the monolayer membrane plays an important role in the phase transition between cubic phases and the L_α phase, although its quantitative analysis is necessary as a next step [13]. We also investigated the effects of peptide-1 on the structures and stability of the cubic phase of MO membranes, because we expected that peptide-1 would be partitioned in the membrane interface of the MO membrane and bring positive charges to the membrane interface, which would change the stability of the cubic phase due to electrostatic interactions. We found that, as the peptide-1 concentration increased, a phase transition from Q^{224} to Q^{229} occurred. At higher peptide-1 concentration, MO/peptide-1 membranes were in the L_α phase [15]. Salts in solution inhibited these phase transitions, indicating that the electrostatic interaction due to the adsorbed peptide-1 induces these phase transitions. Increased peptide-1 concentration reduced the absolute value of spontaneous curvature of the MO monolayer membrane. These results show that peptide-1 can be partitioned into the MO membrane interface. The common segments of the membrane interface of PC and MO membranes are hydrocarbon chains and glycerol, and therefore we can consider that these segments play an important role in the recognition of peptide and amino acid residues in the membrane interface.

As for the effects of the interaction between substances and the electrically neutral membrane interface on the structure and stability of the lipid membranes, we also investigated

the effects of La^{3+} and Gd^{3+} on membranes composed of phospholipids such as PC and phosphatidylethanolamine (PE), which have no net charge at neutral pH. The binding of La^{3+} and Gd^{3+} on PC membranes has been indicated by several experimental techniques such as NMR, titration calorimetry, and measurement of the ζ-potential. We found that the chain-melting transition temperatures of PC and PE membranes increased with an increase in La^{3+} concentration, and even at low concentrations (100 μM–1 mM) their increments were evident, indicating that the lateral compression pressure of the membrane increases with an increase in La^{3+} concentration [16, 17]. The addition of 10–100 μM La^{3+} (or Gd^{3+}) through a micropipette near a DOPC-GUV induced several kinds of shape change. In contrast, the addition of 1 mM NaCl did not induce any shape changes of the GUVs, indicating that osmotic pressure due to the difference of $LaCl_3$ concentration between the inside and the outside of the GUV is not an important factor of these shape changes. We can reasonably explain that La^{3+}-induced shape changes of the GUVs occurred by the decrease in the area of the outer monolayer membrane of the GUV, which is induced by the lateral compression pressure of the membrane due to the adsorbed La^{3+} in the PC membrane interface [16]. We also found that La^{3+} stabilizes the H_{II} phase rather than the L_α phase in the PE membrane, which can be explained by the increase in the lateral compression pressure of the membrane at the local sites [17]. More recently, we found that low concentrations of La^{3+} induced membrane fusion of two GUVs composed of DOPC and dipalmitoleoyl-PE (DPOPE) [18]. In this membrane fusion, the lateral compression pressure of the membrane plays an important role.

In this report, we investigated the effect of peptide-1 and La^{3+} on the liquid-ordered (lo) phase membranes which also have an electrically neutral membrane interface, and compared them with those of the L_α phase membrane. The lo phase of lipid membranes is formed in binary mixture membranes of cholesterol and saturated PCs such as dipalmitoyl-PC (DPPC), and also of cholesterol and sphingomyelin (SM) [19]. DPPC/chol membranes containing $\geqslant$25 mol% cholesterol and SM/chol membranes containing $\geqslant$30 mol% cholesterol are in the lo phase. In the lo phase, hydrocarbon chains of PC and SM have high orientational order, but the lateral diffusion coefficient of lipids in the membrane is relatively high [19]. The rafts in cell membranes are considered the lo phase [20]. We have found interesting physical properties of GUVs of the lo phase-membranes [21, 22]. However, the characteristics of the membrane interface of the lo phase-membranes are not well known. It is important to elucidate them for understanding the interaction of substances with the lo phase-membranes as well as the rafts, and also for the practical purpose of liposomes of the lo phase-membranes.

2. Experimental methods

2.1. Materials and peptide synthesis

DOPC, POPC, DPPC, and SM from the brain were purchased from Avanti Polar Lipids Inc. The peptides were synthesized by the FastMoc method using a peptide synthesizer 433A (PE Applied Biosystems, Foster city, CA). The sequence of peptide-1 is WLFLLKKK, and that of peptide-2 is LLKKK. These peptides have an amide-blocked C-terminus. The methods of purification and identification of peptides were described in our previous paper [8, 15].

2.2. Formation and observation of GUVs and method of addition of peptides or La^{3+}

GUVs were prepared by the natural swelling of dry lipid films [16, 22], and were observed by a phase-contrast microscope using the standard method described in our previous paper [16, 22].

Various kinds of concentrations of peptides and La^{3+} in 0.1 M glucose aqueous solution were added into the neighbourhood of a GUV through a 10 μm diameter glass micropipette [8, 16].

2.3. Preparation of MLV and SAXS measurement

Preparation of MLVs was described in our previous paper [8]. X-ray diffraction experiments were performed using nickel filtered Cu Kα x-rays from a rotating anode type x-ray generator (Rigaku, Rotaflex, RU-300). Details were given in our previous papers [13–15].

3. Results

3.1. Effect of peptides on the structure of lipid membranes in the L_α phase and those in the lo phase

The intermembrane distance of MLV is a good measure of the intermembrane interaction and, therefore, of the interaction of a substance with the membrane [23]. We investigated the effect of peptide-1 on the spacing of the POPC-MLV, which membrane is in the L_α phase. As shown in figure 2, the spacing (d_l) of POPC-MLV gradually increased from 6.3 to 8.8 nm with an increase in peptide-1 concentration (R: molar ratio of peptides to lipid). On the other hand, peptide-2 (LLKKK), which does not have the partitioning site, did not change the spacing. The spacing is determined by the summation of the intermembrane distance (d_f) and the membrane thickness (d_m); i.e., $d_l = d_\mathrm{f} + d_\mathrm{m}$. The values of d_f and d_m can be determined by the electron density profile of the membranes. In the presence of peptide-1 only two diffraction peaks were obtained, and, therefore, it was difficult to obtain the electron density profile of the POPC-MLV. In spite of this result, we can get qualitative information on the intermembrane distance (d_f) from figure 2. The membrane thickness of the L_α phase membrane cannot change greatly [23]. Therefore, the large increase in the spacing of POPC-MLV is due to the increase in the intermembrane distance. Moreover, the spacing of the POPC-MLV in the presence of peptide-1 ($R = 0.030$) decreased greatly with an increase in NaCl concentration in solution [8]. This result indicates that the increase in the intermembrane distance of POPC-MLV by peptide-1 is due to electrostatic repulsive interaction. Based on these results, we can reasonably consider that peptide-1 is partitioned into the membrane interface and gives positive charges to the membrane surface, resulting in the increase in electrostatic repulsive interaction between membranes in the MLV. In figure 2, the spacing shows saturation at high concentration of peptide. The peptide-1 adsorbed in the membrane interface produces the electric field, decreasing the partition of other peptide-1 molecules due to the electrostatic repulsive interaction. Therefore, the apparent partition coefficient of peptide-1 into the membrane interface decreased with an increase in peptide-1 concentration, which is a similar phenomenon to the binding of La^{3+} to a PC membrane [16]. In contrast, peptide-1 did not change the spacing of 70 mol% DPPC/30 mol% chol-MLV and 70 mol% SM/30 mol% chol-MLV, which membranes are in the liquid-ordered (lo) phase (△ and ○ in figure 2). These data indicate that peptide-1 could not be partitioned into the membrane interface of the lo phase-membranes.

Next, the effect of peptide-1 on the shape of GUVs was investigated using phase-contrast microscopy, because the observation of shape change of a GUV has been recently considered as a highly sensitive method of detecting the interaction between substances and lipid membranes [8, 16]. At first, we investigated the effect of peptide-1 on the shapes of DOPC-GUVs in the L_α phase. Figure 3 shows two kinds of shape changes of a DOPC-GUV induced by the addition of 5 μM of peptide-1 through a 10 μm diameter micropipette near

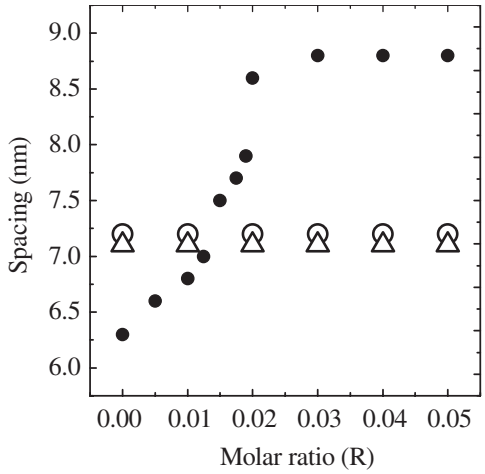

Figure 2. (A) Spacing of POPC-MLV (●), 70%DPPC/30%chol-MLV (△), and 70%SM/30%chol-MLV (○) in the presence of various concentrations of peptide-1 in 10 mM PIPES buffer (pH 7.0) in excess water condition at 20 °C. R is the molar ratio of peptide-1 to lipids.

the GUV. At first (in the absence of peptide-1), the GUV was a prolate (figure 3(A)-(1)). After the addition of peptide-1, the shape changed into a dumbbell (figure 3(A)-(3)) and then into two spheres connected by a neck ($n = 5$) (figure 3(A)-(5)). In the case of 0.5 μM peptide-1, this type of shape change was not observed. When we added 5 μM of peptide-1 near a cylindrical shape of GUV (figure 3(B)-(1)), the shape changed into a GUV made of a series of many spherical vesicles connected by a narrow tube (so called 'pearls on a string') ($n = 8$) (figure 3(B)-(4)). In the case of 0.5 μM of peptide-1, this type of shape change was not observed. As a control experiment, peptide-2 was added near the GUV, but no shape changes of the GUV such as figures 3(A) and (B) were observed. When 5 μM of peptide-1 was added near a spherical DOPC-GUV, a narrow tube suddenly budded into the outside of the GUV and this tube changed into the pearls on a string [8]. These shape changes of GUVs were almost reversible.

What kind of effects of the peptide on the PC membranes can induce such shape changes of the GUV? The shapes of GUVs of lipid membranes are determined by the minimum of the elastic energy of the closed membrane of the GUV (W_{el}). It is considered that the area-difference-elasticity model ⟨ADEmodel⟩ (i.e., the generalized bilayer-couple model) can reasonably explain the shape changes of the GUV [24, 25]. In the ADE model, the area of each monolayer is not fixed to the equilibrium area, but the monolayer membrane can stretch elastically to increase the nonlocal elastic energy of the membranes. Hence the elastic energy of the GUV (W_{el}) can be expressed as a sum of the membrane bending energy (W_{b}) and the energy of the relative monolayer stretching (W_{r}) as follows:

$$\begin{aligned} W_{\mathrm{el}} &= W_{\mathrm{b}} + W_{\mathrm{r}} \\ &= \frac{\kappa_{\mathrm{c}}}{2}\int (C_1 + C_2 - C_0)^2\,\mathrm{d}A + \frac{\kappa_{\mathrm{r}}}{2Ah^2}(\Delta A - \Delta A_0)^2 \end{aligned} \tag{1}$$

where κ_{c} is the local bending modulus of the membrane, C_1 and C_2 are two principal curvatures of the monolayer membrane, C_0 is the spontaneous curvature of the bilayer membrane, the integration is over the whole area of the neutral surface, κ_{r} is the nonlocal bending modulus of the membrane and $\kappa_{\mathrm{r}} = K_{\mathrm{a}}h^2/2$ (K_{a} is the area expansivity modulus), ΔA is the area difference between the two monolayers in the bilayer membrane, and ΔA_0 ($=A_0^{\mathrm{ex}} - A_0^{\mathrm{in}}$) is

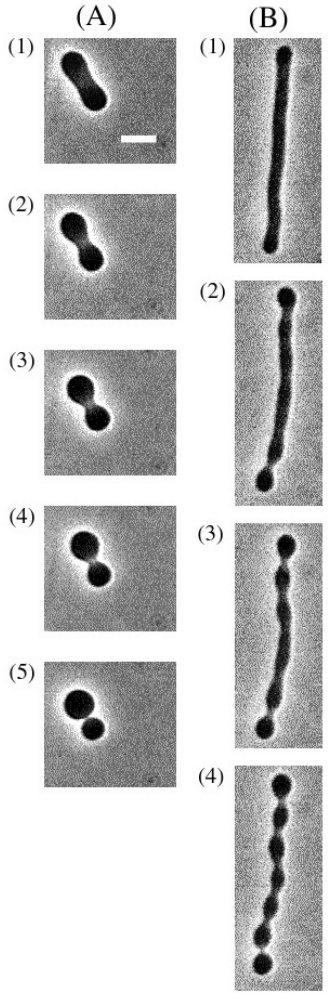

Figure 3. Shape change of DOPC-GUV induced by the addition of 5 μM of peptide-1 at 20 °C. (A) A prolate was changed into a dumbbell and then into two spheres connected by a neck. (B) The cylinder to pearls on a string transformation. The time after starting injection of the peptide-1 solution is (1) 0 s, (2) 11 s, (3) 14 s, (4) 17 s, and (5) 19 s for (A), and (1) 0 s, (2) 23 s, (3) 27 S, and (4) 42 s for (B). The bar in the picture corresponds to 10 μm.

the area difference between the two monolayers in the bilayer membrane under nonstretched conditions. In the ADE model, the shape of the GUV is determined by the minimization of the membrane elastic energy (W_{el}) for a given area A, a given volume V, and also a given area difference between the two monolayers under the relaxed condition ΔA_0. The analysis based on the ADE model shows that, under the condition of constant volume of the GUV, the shape changes as follows: with an increase in ΔA_0, (1) a prolate $\rightarrow$ a pear (i.e., asymmetric prolate) $\rightarrow$ two spheres connected by a narrow neck, (2) a dumbbell (i.e., symmetric prolate) $\rightarrow$ two spheres connected by a narrow neck, and (3) a cylinder $\rightarrow$ a pearls on a string. In the experiments of the shape change of the GUVs, peptide-1 was added into the neighbourhood of GUVs from outside the GUVs, and was partitioned into the membrane interface of the external monolayer membrane of the GUV. The partition of peptide-1 into the membrane interface increases the

area of the membrane. Therefore, only the area of the external monolayer membrane of the DOPC-GUV under the relaxed condition, A_0^{ex}, increases due to the partition of peptide into the membrane interface, while the area of the internal monolayer, A_0^{in}, does not change. Hence, the interaction of peptide-1 with the GUV induces an increase in ΔA_0 without a change of volume of the GUV. Therefore, the peptide-1-induced shape changes of DOPC-GUV shown in figure 3 can be reasonably explained by the ADE model. However, at present we do not have a reasonable mechanism for the shape change of the spherical GUV described above.

In contrast, peptide-1 did not change any shapes of 60%DPPC/40%chol-GUV, which membrane is in the lo phase. These data also indicate that peptide-1 cannot be partitioned into the membrane interface of the lo phase-membranes. We can consider that in the lo phase-membrane the probability of the existence of hydrocarbon chains in the membrane interface is very low, because the hydrocarbon chains are highly ordered, and therefore amino acid residues with high interfacial hydrophobicity cannot be partitioned into the membrane interface of the lo phase-membrane. This characteristics of the membrane interface of the lo phase-membrane is very different from that of the L_α phase-membrane.

3.2. Effect of La^{3+} on the shapes of DPPC/chol-GUVs in the lo phase

Figures 4(A) and (B) show the shape changes of a 60%DPPC/40%chol-GUV induced by the addition of 10 μM of La^{3+} through a 10 μm diameter micropipette near the GUV. At first (in the absence of La^{3+}), the GUV was a discocyte. After the addition of La^{3+}, the shape changed into the stomatocyte (figures 4(A)-(4), (B)-(3)). On further addition of La^{3+}, the stomatocyte invagination became unstable and a small vesicle budded into the inside of the GUV ($n = 20$) (figures 4(A)-(5), (B)-(4)). When 1 μM of La^{3+} was added near the GUV for 5 min, this type of shape change was not observed. In order to determine the reversibility of the shape change induced by La^{3+}, the addition of these ions through the micropipette was stopped after the shape change of the GUV completed. Figures 4(A)(6)–(9) and (B)(5)–(8) show the time course of the shape change of the GUV once the addition of La^{3+} was stopped; the inside budded shape became the stomatocyte, and then changed into the discocyte. In this experiment, 10 μM of La^{3+} was added to the vicinity of the GUV through the micropipette, and therefore, while the addition was stopped, La^{3+} diffused away from the vicinity of the GUV, inducing the decrease in La^{3+} concentration near the GUV. Therefore, these results indicate that the shape change induced by La^{3+} was reversible ($n = 20$), indicating that no vesicle fission occurred. Figure 4(C) shows another type of shape change of 60%DPPC/40%chol-GUV induced by the addition of 10 μM of La^{3+}. When we added 10 μM of La^{3+} near GUVs made of three or a series of many spherical vesicles connected by a narrow tube (so-called 'pearls on a string') (figure 4(C)-(1)), the shape changed into a cylinder (or tube) ($n = 10$) (figure 4(C)-(3)). When 1 μM of La^{3+} was added near the GUV for 5 min instead of 10 μM of La^{3+} in this experiment, this type of shape change was not observed. This shape change from the pearls on a string to cylinder transformation induced by La^{3+} was also reversible.

These La^{3+}-induced shape changes of GUVs of the lo phase-membrane are almost the same as those of the L_α phase [17]. The mechanism of the latter case can be considered as follows. The chain-melting phase transition temperature of DPPC-MLV increased with an increase in La^{3+} concentration, indicating that the lateral compression pressure of the membrane increases with an increase in La^{3+} concentration. Hence, the interaction of La^{3+} on the external monolayer membrane of the GUV induces a decrease in its area (A_0^{ex}), whereas the area of the internal monolayer membrane (A_0^{in}) keeps constant. Therefore, the shape changes of the GUV induced by these lanthanides can be reasonably explained by the decrease in the area difference between two monolayers, ΔA_0 ($=A_0^{ex} - A_0^{in}$), on the basis of the ADE

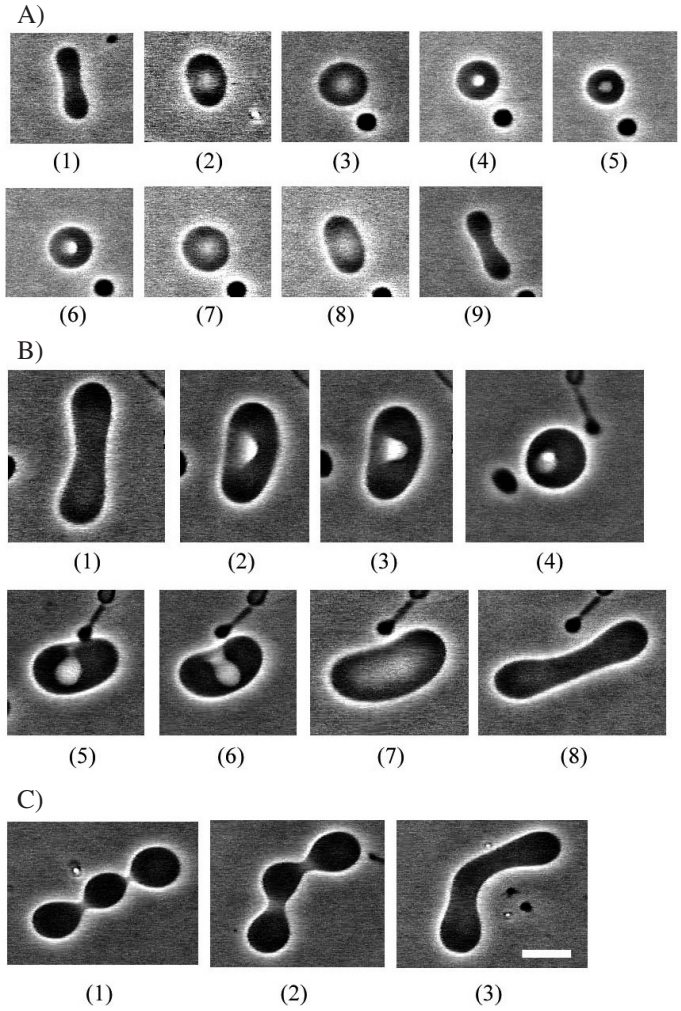

Figure 4. Shape change of 60%DPPC/40%chol-GUV induced by the addition of 10 μM of La^{3+} at 20 °C. ((A), (B)) The discocyte via stomatocyte to inside budded shape transition occurred. The time after starting injection of the La^{3+} solution through the micropipette is (1) 0 s, (2) 65 s, (3) 70 s, (4) 120 s, and (5) 121 s for series (A), and (1) 0 s, (2) 5 s, (3) 6 s, and (4) 10 s for series (B). The reversibility of these shape changes was also investigated. After the addition of La^{3+} was stopped, the shape change was reversed. The time after stopping injection of La^{3+} solution through the micropipette is (6) 10 s, (7) 13 s, (8) 45 s, and (9) 50 s for series (A), and (5) 14 s, (6) 45 s, (7) 60 s, and (8) 80 s for series (B). (C) The pearls on a string to cylinder (or tube) transformation occurred. The time after starting injection of La^{3+} solution through the micropipette is (1) 0 s, (2) 60 s, and (3) 100 s. The bar in the picture corresponds to 10 μm.

model [24, 25]. We consider the mechanism of the La^{3+}-induced lateral compression pressure as follows: a PC headgroup has one positive charge (N^+) and one negative charge at the phosphate group, and therefore it has a large electric dipole moment of 20 D. In the absence of La^{3+}, the lateral component of the dipole moment of PC headgroups orients randomly in the membrane due to thermal motion (figure 5(A)). When La^{3+} binds with the phosphate group of the PC headgroup, it causes the lateral polarization of dipole moments of its neighbouring PC

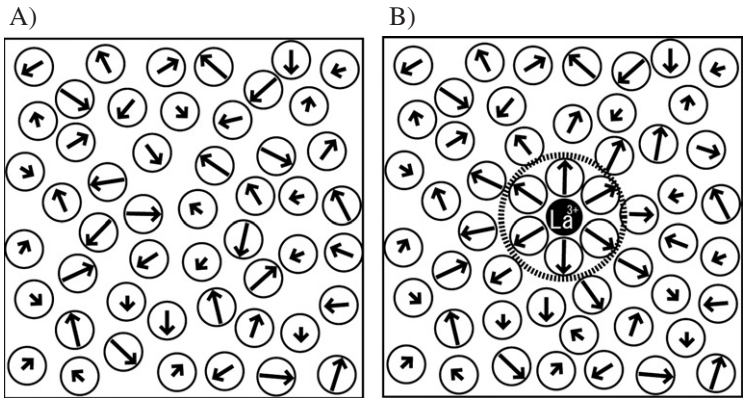

Figure 5. View of lipid membrane interface from the above. The PC molecule is represented by a circle, and an arrow in the circle is the lateral component of the electric dipole moment of the PC headgroup. (A) In the absence of La^{3+}, the lateral component of the dipole moment orients randomly. (B) When La^{3+} binds with the phosphate group of the PC, it induces the lateral polarization of dipole moments of its neighbouring PC molecules.

molecules, because the electrostatic interaction of surface charge (La^{3+}) with neighbouring dipoles of PC headgroups tends to orient the dipoles in a certain direction (figure 5(B)). This lateral polarization can increase the lateral compression pressure of the membrane [26]. In the case of the lo phase-membrane, we could not investigate the effect of La^{3+} on the chain-melting phase transition temperature, because there is no phase transition in the lo phase-membrane. Therefore, we do not have direct evidence of the La^{3+}-induced lateral compression pressure. However, the results of La^{3+}-induced shape changes of the GUV of the lo phase-membrane clearly show that the lateral compression pressure increased by the interaction of La^{3+} with the membrane. We can reasonably consider that, in the case of the lo phase-membrane, La^{3+} binds with the phosphate group of the PC headgroup and it induces the lateral polarization of dipole moments of its neighbouring PC molecules, which increases the lateral compression pressure of the membrane. The results of the La^{3+}-induced shape changes of the GUV of the lo phase-membrane indicate that the lo phase-membrane can change its area depending on the interaction of substances with the membrane.

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References

[1] Kim J, Blackshear P J, Johnson D and McLaughlin S 1994 *Biophys. J.* **67** 227
[2] Buser C A, Sigal C T, Resh M D and McLaughlin S 1994 *Biochemistry* **33** 13093
[3] Israelachvili J 1992 *Intermolecular & Surface Forces* 2nd edn (New York: Academic)
[4] Kinoshita K, Furuike S and Yamazaki M 1998 *Biophys. Chem.* **74** 237
[5] Nagle J F and Tristram-Nagle S 2000 *Biochim. Biophys. Acta* **1469** 159
[6] Wiener M C and White S H 1992 *Biophys. J.* **61** 434
[7] Wimley W C and White S H 1996 *Nat. Struct. Biol.* **3** 842

[8] Yamashita Y, Masum S M, Tanaka T and Yamazaki M 2002 *Langmuir* **18** 9638
[9] Seddon J M and Templer R H 1995 *Structure and Dynamics of Membranes* ed R Lipowsky and E Sackmann (Amsterdam: Elsevier Science B. V.) p 97
[10] Hyde S, Andersson S, Larsson K, Blum Z, Landh T and Ninham B W 1997 *The Language of Shape* (Amsterdam: Elsevier Science B.V)
[11] Pebay-Peyroula E, Rummel G, Rosenbusch J P and Landau E M 1997 *Science* **277** 1676
[12] Aota-Nakano Y, Li S J and Yamazaki M 1999 *Biochim. Biophys. Acta* **1461** 96
[13] Li S J, Yamashita Y and Yamazaki M 2001 *Biophys. J.* **81** 983
[14] Kinoshita K, Li S J and Yamazaki M 2001 *Eur. Biophys. J.* **30** 207
[15] Masum S M, Li S J, Tamba Y, Yamashita Y, Tanaka T and Yamazaki M 2003 *Langmuir* **19** 4745
[16] Tanaka T, Tamba Y, Masum S M, Yamashita Y and Yamazaki M 2002 *Biochim. Biophys. Acta* **1564** 173
[17] Tanaka T, Li S J, Kinoshita K and Yamazaki M 2001 *Biochim. Biophys. Acta* **1515** 189
[18] Tanaka T and Yamazaki M 2004 *Langmuir* **20** 5160
[19] Bloom M, Evans E and Mouritsen O G 1991 *Q. Rev. Biophys.* **24** 293
[20] Ahmed S N, Brown D A and London E 1997 *Biochemistry* **36** 10944
[21] Tanaka T, Sano R, Yamashita Y and Yamazaki M 2004 *Langmuir* **20** 9526
[22] Tamba Y, Tanaka T, Yahagi T, Yamashita Y and Yamazaki M 2004 *Biochim. Biophys. Acta* **1667** 1
[23] Kinoshita K, Furuike S and Yamazaki M 1998 *Biophys. Chem.* **74** 237
[24] Heinrich V, Svetina S and Zeks B 1993 *Phys. Rev.* E **48** 3112
[25] Miao L, Seifert U, Wortis M and Döbereiner H-G 1994 *Phys. Rev.* E **49** 5389
[26] Levadny V and Yamazaki M 2005 submitted