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Temperature- and ionic strength-induced conformational changes in the lipid head group region of liposomes as suggested by zeta potential data

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Abstract

Neutral liposomes composed of DMPC (dimyristoylphosphatidylcholine), DPPC (dipalmitoylphosphatidylcholine) or DSPC (distearoylphosphatidylcholine) are found to exhibit non-zero zeta potentials in an electric field even when they are dispersed in solution at pH 7.4. A model for the orientation of lipid head groups is proposed to explain the observed non-zero zeta potentials. The dependence of the zeta potential on temperature and ionic strength is analyzed via this model to obtain the information on the direction of the lipid head group in the liposome surface region. The direction of the lipid head group is found to be sensitive to the temperature and the ionic strength of the medium. At low ionic strengths, the phosphatidyl groups are located at the outer portion of the head group region. At constant temperature, as the ionic strength increases, the choline group approaches the outer region of the bilayer surface while the phosphatidyl group hides behind the surface. At the phase transition temperature of the lipid, the phosphatidyl group lies in the outer-most region of the surface and the choline group is in the inner-most region.

Keywords: Conformational change; Lipid head group; Liposome; Zeta potential; Phase transition

1. Introduction

The arrangement of lipid molecules in the lipid bilayers has often been studied by ESR [1–3], NMR [4,5], FTIR [6] and X-ray diffraction [7,8]. The purposes of the present paper are to demonstrate that zeta potential measurements of lipid vesicles provide us with direct information on the structure of lipid head group and to predict how the lipid head groups on the liposome surface move depending on temperature and ionic strength. The zeta potential of liposomes [9–14], microcapsules [15,16] and biological cells [17] has extensively been measured to determine the charge density of their surfaces and the binding affinity of various ions [4–10]. In particular, Tatulian [9] has obtained a sequence for anion affinities to DMPC liposome surface as follows, trini-

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trophenol > ClO_4^- > I^- > SCN^- > Br^- > NO_3^- > $Cl^- \approx SO_4^{2-}$. In the present work, we report the results of zeta potential measurements of liposomes dispersed in a phosphate buffer solution containing no Cl^- to eliminate the possible anion adsorption onto the liposome surface. Pearson and Pascher [18] and Hauser et al. [19] have studied the temperature dependence of the orientation of polar head groups of DMPC. Cevc pointed out the significance of the degree of hydration of the lipid polar surface in the lipid phase transition temperature [20]. We investigate the temperature dependence of the zeta potential of DMPC, DPPC and DSPC liposomes to obtain information on changes in the orientation of the lipid head groups in the lipid bilayer by analyzing the data with the help of a simple model.

2. Materials and methods

2.1 Materials

DMPC (dimyristoylphosphatidylcholine), DPPC (dipalmitoylphosphatidylcholine) and DSPC (distearoylphosphatidylcholine) were purchased from Nihon Yushi Co. Ltd. These lipids have the phase transition temperatures at 23 °C, 42 °C, and 55 °C, respectively. All other chemicals were of reagent grade.

2.2 Preparation of liposomes containing water

DMPC, DPPC or DSPC was dissolved in chloroform to give a 5% (w/v) solution. Five milliliters of distilled water was dispersed in 5 ml of the phospholipid solution at a temperature 10 °C lower than the phase transition temperature of the lipid to yield a water/oil (w/o) emulsion. Then, the emulsion was poured into 50 ml of a phosphate buffer solution of pH 7.4 with an ionic strength of 0.154 (0.0475 *M* Na_2HPO_4–0.0116 *M* KH_2PO_4) and the complex emulsion formed was stirred at 313 rpm for 10 min. Another 50 ml of the phosphate buffer solution was then added to the complex emulsion, which was kept being stirred for 3 hours to remove the organic solvent. The liposome suspension was centrifuged at 3500 rpm for 10 min. The supernatant was collected and was centrifuged at 15000 rpm for 10 min. The precipitates were washed three times with phosphate buffer and then redispersed in the same buffer. This liposome suspension was used to prepare samples for zeta potential measurements.

2.3 Measurements of zeta potential

We measured the zeta potentials of the above-prepared liposomes in the phosphate buffer solutions with various ionic strengths by using an electrophoresis apparatus PEN KEM System 3000 at various temperatures. Each measured value of the zeta potential corresponds to a statistical average over at least 1000 liposomes. The measurement was repeated three times. For measurement of the zeta potential, the prepared liposome suspension was centrifuged and the collected liposomes were redispersed in the phosphate buffer solutions with various ionic strengths. The ionic strength was adjusted by dilution with distilled water.

3. Results and discussion

Figures 1, 2 and 3 show the observed dependence on the ionic strength of the zeta potential of liposomes composed of DMPC, DPPC and DSPC, respectively. The standard errors for the

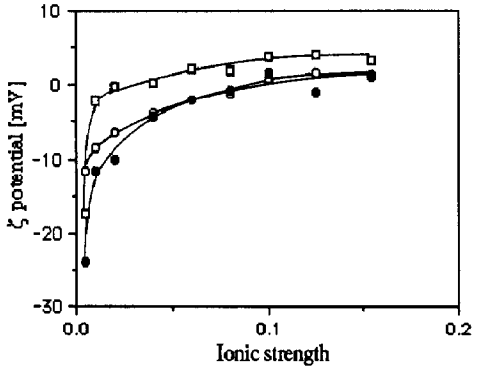

Fig. 1. Ionic strength dependence of the zeta potential of DMPC liposomes at several temperatures: (○) 13 °C, (●) 23 °C, and (□) 33 °C.

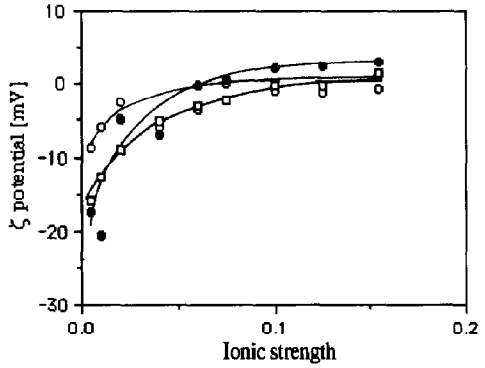

Fig. 2. Ionic strength dependence of the zeta potential of DPPC liposomes at several temperatures: (○) 33 °C, (●) 38 °C, and (□) 53 °C.

measured values of the zeta potential are less than the size of the data symbols in the figures and therefore are not shown. In solutions of low ionic strength, the zeta potential is negative and decreases in magnitude with increasing ionic strength. With further increase of ionic strength, the zeta potential reverses its sign in some cases. Figures 1, 2 and 3 also show the temperature dependence of the zeta potential of the liposomes. Figure 1 shows that the zeta potential of DMPC liposomes is more negative at 23 °C than those at 13 °C and 33 °C. The zeta potentials at 18 °C and 28 °C were almost the same as those at 13 °C and 33 °C, respectively (data are not shown). This tendency was seen more clearly in solutions with ionic strengths of 0.005, 0.01 and 0.02, while the zeta potential slightly changed depending on the temperature in solutions with ionic strengths of 0.1, 0.125 and 0.154.

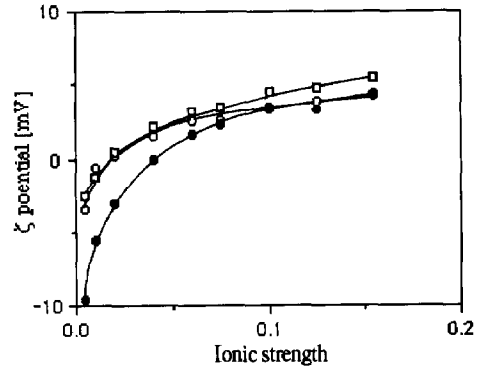

Fig. 3. Ionic strength dependence of the zeta potential of DSPC liposomes at several temperatures: (○) 45 °C, (●) 50 °C, and (□) 55 °C.

Figure 2 shows that the zeta potential of DPPC liposomes is most negative at 38 °C in solutions of low ionic strengths (0.005 and 0.01). In solutions with higher ionic strengths, the zeta potential is more negative at 33 °C and 53 °C than at 38 °C. The zeta potential becomes less negative as the ionic strength increases. At 38 °C, in particular, it shows a stepwise change at the ionic strength of 0.05. The zeta potentials at 43 °C and 48 °C were almost the same as that at 38 °C (data are not shown).

Figure 3 shows that the zeta potential of DSPC is most negative at 50 °C. At temperatures above 55 °C, the zeta potential remains almost the same as that at 50 °C (data are not shown).

Liposomes used in these experiments are composed of neutral lipids, each having one phosphatidyl group and one choline group in their molecules. In spite of this fact, the liposomes exhibit non-zero mobilities in an external electric field. This means that these liposomes have non-zero surface electric potentials, even though the liposomes are composed of neutral lipids. One possible explanation for this observation is that some anions can bind to neutral liposomes, making their surface negatively charged. Tatulian obtained a sequence for anion affinities to DMPC liposome surface in the order, trinitrophenol > ClO_4^- > I^- > SCN^- > Br^- > NO_3^- > Cl^- ≈ SO_4^{2-} [9]. In the present experiments, we chose the phosphate buffer solution containing no Cl^- as the dispersing medium of liposomes to exclude the possibility of anion adsorption onto the liposome surfaces. Another reason why we did not take into account the adsorption of anions is that the adsorption of anions onto liposomes can only explain the negative zeta potential but cannot account for the sign reversal of the zeta potential caused by changes in ionic strength. To explain the ionic strength dependence of the zeta potential of liposomes, we made a model shown in Fig. 4. In this model, changes in the zeta potential caused by increases of ionic strength and temperature, which were observed in the phosphate buffer solutions containing no Cl^- ions, are con-

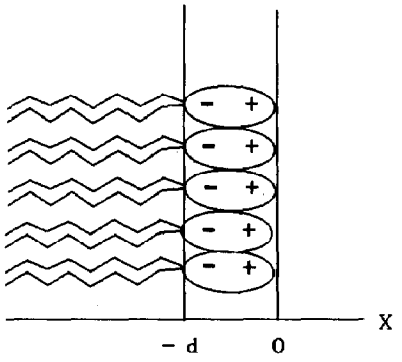

Fig. 4. Schematic representation of the head group region.

sidered to be due to a structural change of the head-group region of the liposomes. We explain the reversal of the zeta potential depending on ionic strength to be caused by changes in the direction of the dipole connecting the negative charge of phosphatidyl group and the positive charge of choline group in the head group of a lipid molecule [20]. Several studies [21–24] have been made on the dependence of the surface potential and surface pressure of various lipid monolayers upon temperature, giving information about the direction of the lipid dipole moments with respect to the monolayer surface. In the surface area of liposomes, lipid molecules are considered to be arranged in such a way that the hydrophilic groups are located on the surface of liposomes. We describe our model, which is schematically shown in Fig. 4, below in more detail.

Consider a membrane immersed in an electrolyte solution of bulk concentration n. The membrane has a surface layer of thickness d. In this surface layer we assume that two charge sheets are located parallel to the surface on the membrane core/solution interface at a separation of d, with charge densities σ_1 and σ_2, respectively, and that one lies on the outer surface with σ_1 and the other on the inner surface with σ_2. Here, $\sigma_1 = -\sigma_2$, since the number of negatively charged groups and that of positively charged groups are equal. We also assume that electrolyte ions can penetrate into the region between the two sheets. Since the radius of the liposomes used in the present study ($\approx 1\ \mu$m) is much larger than $1/\kappa$ (≈ 8–43 Å), κ being the Debye–Hückel parameter, the liposome surface can be regarded as planar. We take the x-axis perpendicular to the membrane with its origin $x = 0$ at the outer sheet so that the region $x > 0$ corresponds to the electrolyte solution and $x < 0$ to the membrane core and the region between the two sheets (Fig. 4). We further assume that the electric potential $\psi(x)$ at position x, which is measured relative to the bulk solution phase (where $\psi(\infty) = 0$), satisfies the following linearized Poisson–Boltzmann equation:

$$\frac{d^2\psi}{dx^2} = \kappa^2\psi \tag{1}$$

with

$$\kappa = \sqrt{\frac{e^2 \sum_{i=0}^{N} v_i^2 n_i}{\epsilon_r \epsilon_0 kT}} \tag{2}$$

the Debye–Hückel parameter, v_i and n_i are, respectively, the valence and bulk concentration of the ith ionic species ($1 \le i \le N$), e is the elementary electric charge, ϵ_r and ϵ_0 are, respectively, the relative permittivity of the electrolyte solution and the permittivity of vacuum, k is the Boltzmann constant, and T is the absolute temperature. The boundary conditions are as follows.

$$\left.\frac{d\psi}{dx}\right|_{x=-d+0} = -\frac{\sigma_1}{\epsilon_r\epsilon_0} \tag{3}$$

$$\left.\frac{d\psi}{dx}\right|_{x=-0} - \left.\frac{d\psi}{dx}\right|_{x=+0} = +\frac{\sigma_2}{\epsilon_r\epsilon_0} \tag{4}$$

and

$$\lim_{x\to+\infty} \psi(x) = 0 \tag{5}$$

The solution to eq. (1) is thus given by

$$\psi(x) = \psi(0)\, e^{-\kappa x}, \qquad x > 0 \tag{6}$$

$$\psi(x) = \{-\psi(-d)\ \sinh \kappa x + \psi(0) \times \sinh \kappa(x+d)\}\{\sinh \kappa d\}^{-1}, \quad -d < x < 0 \tag{7}$$

where

$$\psi(0) = \frac{1}{\epsilon_r \epsilon_0 \kappa}\left[\sigma_1 \, e^{-\kappa d} + \frac{\sigma_2}{2}(1 + e^{-2\kappa d})\right] \qquad (8)$$

is the potential at $x = 0$, which we define as the liposome surface potential. We introduce the surface area S occupied by one charged group so that when $\sigma_1 = e/S$ and $\sigma_2 = -e/S$, i.e. when the positive sheet faces the bulk solution,

$$\psi(0) = \frac{e}{2\epsilon_r \epsilon_0 \kappa S}(1 - e^{-\kappa d})^2 \qquad (9)$$

and for the inverse situation when $\sigma_1 = -e/S$ and $\sigma_2 = e/S$, (i.e. when the negative sheet lies outside)

$$\psi(0) = -\frac{e}{2\epsilon_r \epsilon_0 \kappa S}(1 - e^{-\kappa d})^2 \qquad (10)$$

As will be seen later, κd is found to be much less than unity so that $1 - e^{-\kappa d}$ can be approximated by κd. Under this condition, eqs. (9) and (10) are simplified into

$$\frac{d}{\sqrt{S}} = \mathrm{sgn}(\zeta)\sqrt{\frac{2\epsilon_r \epsilon_0 |\zeta|}{e\kappa}} \qquad (11)$$

where ζ is the zeta potential which can be equated to $\Psi(0)$ and $\mathrm{sgn}(\zeta)$ is $+1$ if $\zeta > 0$ and -1 if $\zeta < 0$. Note that the separation d is the distance from the position of the choline group to that of the phosphatidyl group in the direction normal to the surface of liposomes as shown in Fig. 5. Let the actual distance between the phosphatidyl group and the choline group be given by l. Then, the value of d lies between $-l$ and $+l$ depending on the direction of the head group to the surface. As shown in Fig. 6, when the head group is perpendicular to the surface in such a way that the choline group is outside and the phosphatidyl group is inside, the value of d is positive and maximum in magnitude $(+l)$. When the head group is parallel to the surface, d becomes 0. When the head group is perpendicular to the surface in.such a way that the phosphatidyl group is outside with the choline group inside in the head group, d is negative and minimum $(-l)$. In eq. (11), S is the surface area occupied by one

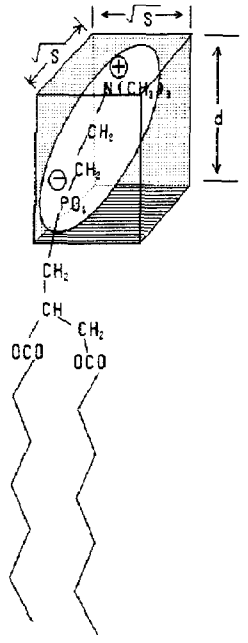

Fig. 5. Lipid head group region.

head group. The head group occupies a block with the volume of $d \times \sqrt{S} \times \sqrt{S}$. The quantity on the left-hand side of eq. (11), $d/\sqrt{S}$, serves as a measure which indicates the direction of the head group and the packing state of lipid molecules in liposomes.

We plotted $d/\sqrt{S}$ against the ionic strength of the medium for DMPC, DPPC and DSPC liposomes in Figs. 7, 8, and 9, respectively. At constant temperature as ionic strength decreases, $d/\sqrt{S}$ increases in magnitude becoming more negative. In Fig. 7, at 33° C in a solution with an

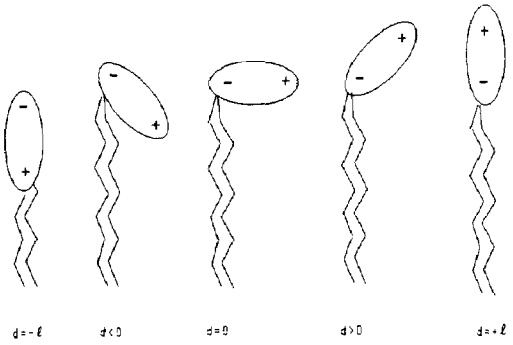

Fig. 6. Model for the conformation of lipid head groups.

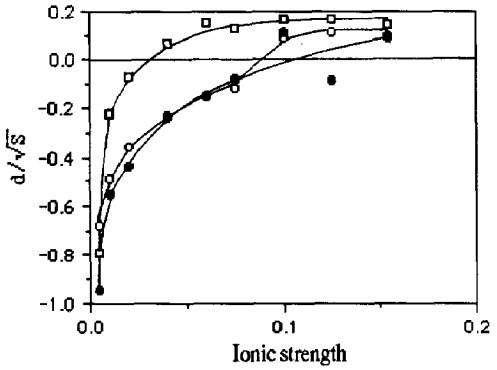

Fig. 7. Ionic strength dependence of $d/\sqrt{S}$ of DMPC liposomes at several temperatures: (○) 13 °C, (●) 23 °C, and (□) 33 °C.

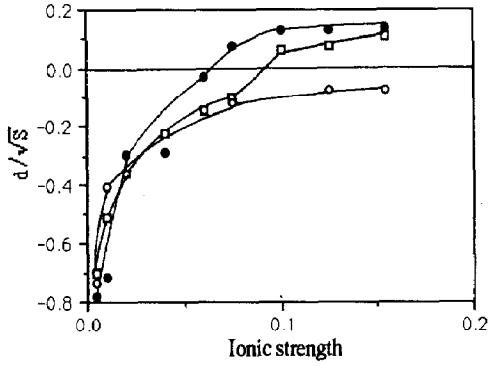

Fig. 8. Ionic strength dependence of $d/\sqrt{S}$ of DPPC liposomes at several temperatures: (○) 23 °C, (●) 38 °C, and (□) 48 °C.

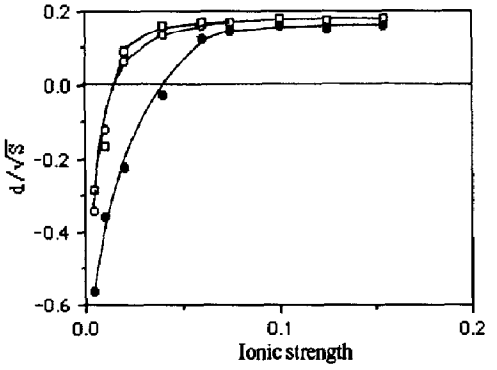

Fig. 9. Ionic strength dependence of $d/\sqrt{S}$ of DSPC liposomes at several temperatures: (○) 45 °C, (●) 50 °C, and (□) 55 °C.

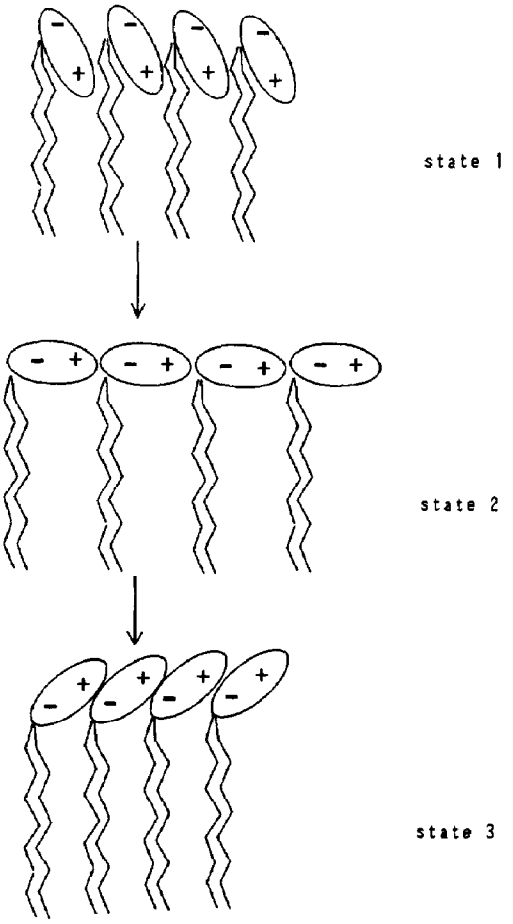

Fig. 10. Change of the direction in the head group of lipid depending on ionic strength.

ionic strength of 0.03, we have $d/\sqrt{S} = 0$. In solutions with higher ionic strengths than 0.03, $d/\sqrt{S}$ positive. Also in solutions with lower ionic strengths it is negative. At 13 °C and 23 °C, in a solution with an ionic strength of about 0.09, $d/\sqrt{S} = 0$. This means that the packing state of lipid molecules alters from state 1 to state 3 through state 2 with increasing ionic strength, as indicated in Fig. 10. At constant temperature, $\sqrt{S}$ is considered to be constant independent of the ionic strength, because the movement of the acyl chains in lipid molecules is controlled by temperature. Therefore, the change in magnitude of $d/\sqrt{S}$ with its sign unchanged is explained by the angle change of the head group to the surface or

by the length (l) change of the head group. As ionic strength increases, the head groups of the lipids can be located closely to each other, because the electric repulsion between the charged groups with the same sign is decreased by increases of the counterion concentration around the charged groups. Therefore, the head group can become parallel to the surface, which explain the change from state (1) to state (2) in Fig. 10. During this state change, a decrease of l might occur at the same time. Figure 8 shows that although $d/\sqrt{S}$ becomes less negative as the ionic strength increases at 23 °C, it does not become positive in solutions of high ionic strengths. At 38 °C and 48 °C, as ionic strength increases, $d/\sqrt{S}$ increases in solution with the an ionic strength of about 0.07 $d/\sqrt{S} = 0$, and $d/\sqrt{S}$ is positive in solutions with higher ionic strengths. These phenomena indicate that the angle between the head groups of lipids and the membrane surface alters at 38 °C and 48 °C while they do not move so much at 23°C. Figure 9 demonstrates that the head groups of DSPC change their direction depending on ionic strength. From Figs. 7, 8, and 9, it is clear that $d/\sqrt{S}$ is dependent on ionic strength and temperature.

We plotted $d/\sqrt{S}$ as a function of temperature for DMPC, DPPC and DSPC liposomes in Figs. 11, 12, and 13, respectively. As shown in Fig. 11, when ionic strength is less than 0.02, and temperature is between 13 °C and 33 °C, $d/\sqrt{S}$ negative and is most negative at 23 °C. On the other hand, when ionic strength is more than 0.1, $d/\sqrt{S}$ always positive and is less positive at 23 °C. When ionic strength is between 0.04 and 0.075 at 23 °C, $d/\sqrt{S}$ has the smallest value. At higher temperatures than 23 °C it increases as temperature increases, while it becomes 0 at 25 °C, 30 °C and 32 °C when ionic strength is 0.04, 0.06, and 0.075, respectively. Figures 12 and 13 show that DPPC and DSPC liposomes exhibit the same dependence of $d/\sqrt{S}$ on ionic strength and temperature as DMPC liposomes (Fig. 11). In Fig. 12, when ionic strength is less than 0.01, and temperature lies between 23 °C and 53 °C, $d/\sqrt{S}$ is always negative and is most negative at 38 °C. When ionic strength is greater than 0.1, $d/\sqrt{S}$ is

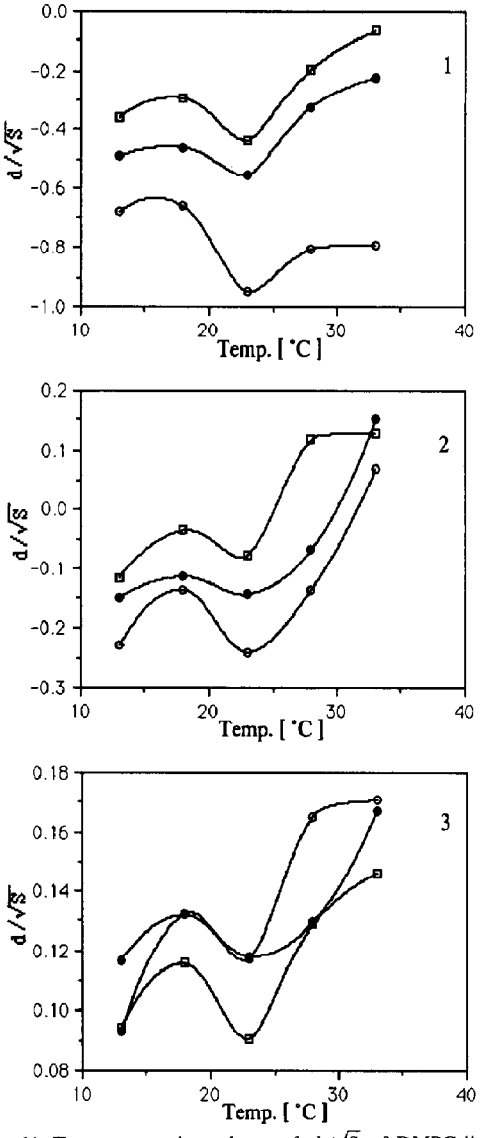

Fig. 11. Temperature dependence of $d/\sqrt{S}$ of DMPC liposomes at various ionic strengths: (○) 0.005, (●) 0.01, (□) 0.02 (in Fig. 11-1); (○) 0.04, (●) 0.06, (□) 0.075 (in Fig. 11-2); and (○) 0.1, (●) 0.125, (□) 0.154 (in Fig. 11-3).

most negative at 38 °C. Also in Fig. 13, when ionic strength is less than 0.01, and temperature is between 45 °C and 65 °C, $d/\sqrt{S}$ is always negative and is most negative at 50 °C. When ionic strength is 0.02 or 0.04, $d/\sqrt{S}$ is negative only at 50 °C. When ionic strength is greater than 0.06, $d/\sqrt{S}$ is positive between 45 °C and 65 °C, and reaches its minimum at 50 °C. As a result, it

becomes clear that the head group of these three types of lipids can take one of the directions shown in Fig. 14 depending on the magnitude of temperature and ionic strength. Figures 14-1, 14-2, and 14-3 show changes of the direction of the head group depending on temperature in solutions with low, intermediate, and high ionic strengths, respectively. It is interesting that around the phase transition temperature, the

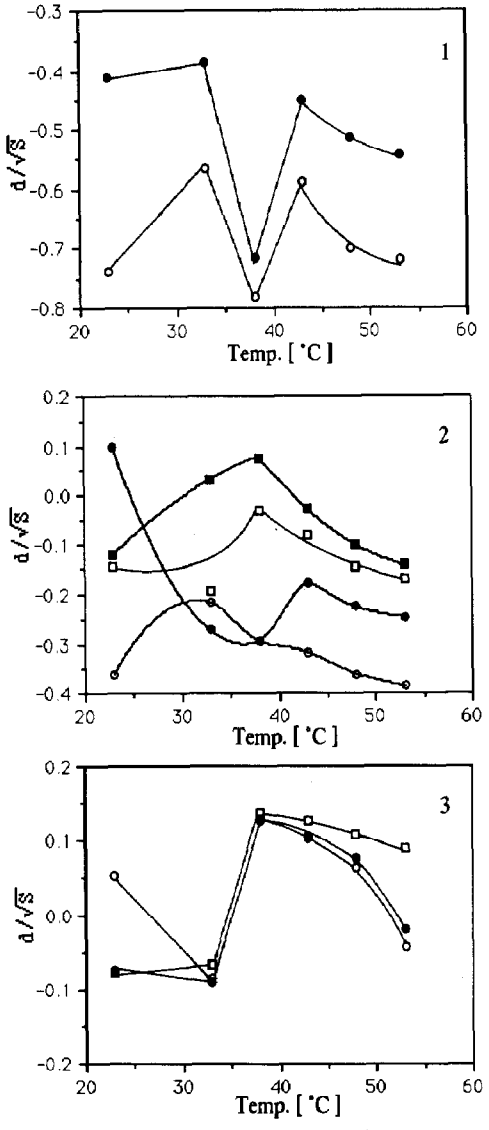

Fig. 12. Temperature dependence of $d/\sqrt{S}$ of DPPC liposomes at various ionic strengths: (○) 0.005, (●) 0.01 (in Fig. 12-1); (○) 0.02, (●) 0.04, (□) 0.06, (■) 0.075 (in Fig. 12-2); and (○) 0.1, (●) 0.125, (□) 0.154 (in Fig. 12-3).

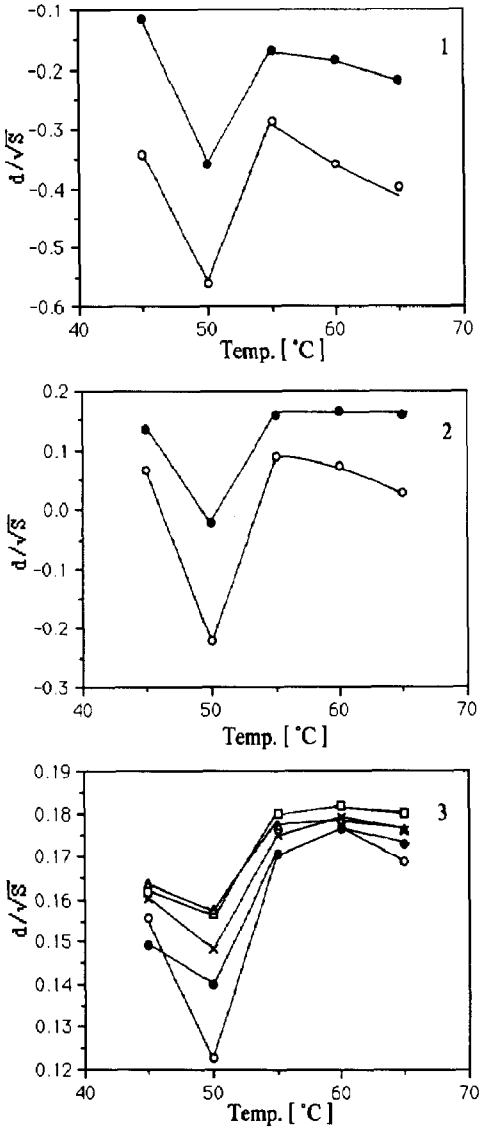

Fig. 13. Temperature dependence of $d/\sqrt{S}$ of DSPC liposomes at various ionic strengths: (○) 0.005, (●) 0.01 (in Fig. 13-1); (○) 0.02, (●) 0.04 (in Fig. 13-2); and (○) 0.06, (●) 0.075, (□) 0.1, (×) 0.125, (△) 0.154 (in Fig. 13-3).

phosphatidyl group lies closest to the liposome surface and the choline group is most inside in solution at constant ionic strength. It has been reported that ion binding to the lipid head groups affects the phase transition temperature of liposomes [1,11–13]. Also, this ion binding affinity and water structure in the double layer region significantly change at the pre- or main-phase transition temperature. Conformation of the head

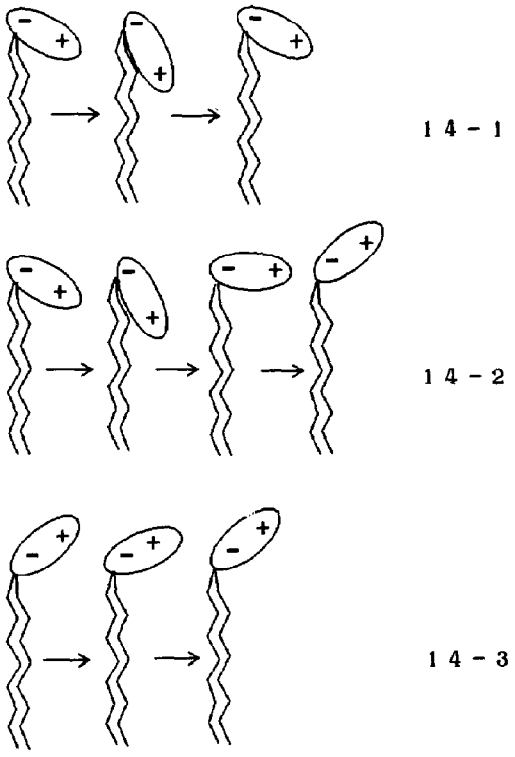

Fig. 14. Change of the direction in the head group of lipid depending on temperature.

group of DMPC was well defined and it was reported that the orientation of the polar head group changes with respect to the bilayer planes depending on temperature [18,19]. The conformational change of lipid head groups depending on temperature shown in this report corresponds with the changes of the ion binding affinity to lipid head group and of the water structure at the phase transition temperature. $d/\sqrt{S}$ can alter its magnitude by changes of the length of the head group, however the sign of $d/\sqrt{S}$ cannot change. During the change of packing state both the length and the direction of the head group are expected to change. The parameter d is the projection of the distance between the choline group and the phosphatidyl group of a lipid molecule onto the direction normal to the membrane surface. If the value of S is set equal to 70 Å^2, then one has $d = 1.7$–8.4 Å. These values of d agree with those predicted from the molecular structure of the head group of DMPC, DPPC and DSPC. However, the reason why the direction of the head group changes depending on temperature and ionic strength remains the subject of further study.

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