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# EFFECT OF LIPID PHASE TRANSITION ON THE BINDING OF ANIONS TO DIMYRISTOYLPHOSPHATIDYLCHOLINE LIPOSOMES

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**Temperature dependence of the electrophoretic mobility of multilamellar liposomes prepared from dimyristoylphosphatidylcholine was measured in the presence of salts with different anions in aqueous solutions. It was established that specific binding of anions to liposome surface induced a pronounced zeta potential (electrostatic potential at the hydrodynamic plane of shear). A combination of Langmuir, Gouy-Chapman, and Boltzmann equations was used to describe the dependence of the zeta potential on the concentration of anions. The values of binding constants ($K$) and maximum numbers of binding sites per unit area ($\sigma_{max}$) were determined by this method. The sequence for anion affinities to liposome surface was found to be as follows: trinitrophenol > $ClO_4^-$ > $I^-$ > $SCN^-$ > $Br^-$ > $NO_3^-$ > $Cl^- \cong SO_4^{2-}$. A sharp increase in the negative zeta potential was detected at the temperature of phase transition of the lipid from the gel to liquid-crystalline state. It was found that the parameter $K$ did not change at lipid phase transition and the shifts in zeta potential might be due to alterations of $\sigma_{max}$. The binding sites were considered as defects in the package of lipid molecules in membranes.**

## Introduction

The importance of thermotropic phase transitions of lipids in the regulation of transport and enzymatic processes in cell membranes has been demonstrated by a number of recent studies. Discontinuities in the Arrhenius plots of succinic-dichloroindophenol reductase activity, the rates of *o*-nitrophenyl galactoside hydrolysis and sugar transport in *Escherichia coli* membranes have been shown to be caused by the gel to liquid-crystalline phase transitions of membrane lipids [1–3]. It has been detected that ($Mg^{2+}$ + $Ca^{2+}$)-ATPase, ($Na^+$ + $K^+$)-ATPase, and adenylate cyclase in plasma membranes of various cells are only active when surrounding lipids are in fluid, liquid-crystalline state [4–6]. Lee has demonstrated that the addition of local anaesthetics to nerve membranes triggers a transition of boundary lipids from the gel to liquid crystalline state, allowing the $Na^+$-channels to close [7]. These findings show that the activity of membrane proteins could be affected by structural changes in lipid matrix of the membranes.

On the other hand, the influence of negatively charged lipids of cell membranes (e.g., phosphatidylserine) on the conductivity of ion channels via modulation of local electrostatic potential and, consequently, ionic concentrations at the channel orifice seems quite reasonable. In fact, dependence of the conductivity of ion channels in both biological and artificial lipid membranes on the membrane surface charge density has been demonstrated [8,9]. The observed dependences have been explained successfully with the aid of the diffuse

double layer theory of Gouy-Chapman. None the less, in some cases it has been pointed out that the application of Gouy-Chapman equation alone fails to describe the experimentally found dependences of membrane surface potential on the electrolyte concentration in bathing aqueous solutions. Specific interactions of ions with membranes not taken into account by the Gouy-Chapman theory were found to be responsible for the discrepancies. In such instances the use of a combination of Gouy-Chapman and Boltzmann equations with one of specific adsorption isotherms (more often Henry's or Langmuir's) has proved more successful. Dependence of the surface potential on the ionic species present in aqueous phase has been recently shown for phospholipid planar bilayers [10,11] and vesicles [11–14], synaptic vesicles and synaptosomal membranes [15], mollusc neuron [16] and some other membranes. The data have been described in terms of specific binding of ions to membranes.

At present little information is available about the relation of membrane surface potential to the temperature-dependent state of lipids. Sharp reduction of the Volta potential of monolayers formed from both dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) at the air/water interface, as well as an increase in the zeta potential of dipalmitoylphosphatidylserine vesicles concurrent with the phase transitions of these lipids from the gel to liquid crystalline state have been detected [17–19]. However, complete understanding of the mechanisms of these phenomena is not yet achieved and further research in this direction is needed.

The present work was undertaken to investigate the influence of the lipid phase transition on the surface potential of DMPC liposomes induced by the adsorption of anions. It was supposed to help in understanding the nature of anion binding to lipid membranes.

A preliminary communication of the work has been published elsewhere [20].

## Materials and Methods

Liposomes for microelectrophoresis experiments were prepared from DMPC synthesized in this laboratory by Dr. Albina E. Sokolova. A single spot characteristic for phosphatidylcholine was obtained for the lipid by thin-layer chromatography. The gas-liquid chromatographic analysis showed that the fatty acid content of the lipid was 99.3% myristic and 0.7% palmitic. Thus, neither any free fatty acid nor other lipids than phosphatidylcholine were detected and the 0.7% admixture was completely attributed to DPPC. The lipid was dissolved in spectroscopic grade ethanol and dried in a rotary evaporator at about 50°C. Then several glass beads and a salt solution of appropriate concentration buffered to pH 7.2 or 7.4 with Tris-HCl were added and the system thermostated at 40°C for 10 min. Dispersions of multilamellar liposomes of the radius from 1 to 10 $\mu$m were prepared by shaking the flask for a few min just after thermostating. The salts used were mostly of 'chemically pure' or 'extra pure' grade obtained from commercial suppliers (Reachim, U.S.S.R.). The salts of lower degree of purity were twice recrystallized from spectroscopic grade ethanol. Twice distilled water was used in all the experiments. Measurements of electrophoretic mobility were carried out on an automatic apparatus 'Parmoquant-2' (Carl Zeiss, Yena, D.D.R.). Measurements were made at successively altered temperatures maintained with an accuracy of $\pm 0.1$ deg.C after equilibrating the sample at a given temperature for 10 min. Special care was taken to focus at the stationary layer in the measuring chamber and mean values of electrophoretic mobility for 100 or more liposomes were measured at each temperature. The usual S.D. values were 5 to 20%. As the radii of liposomes were always large enough compared with the Debye-Hückel parameter, the zeta potentials were calculated directly from the Helmholtz-Smoluchowski equation:

$$\zeta = \eta u / \varepsilon_0 \varepsilon \quad (1)$$

where $\zeta$ is the zeta potential, $u$ is the electrophoretic mobility, $\eta$ and $\varepsilon$ are the viscosity and dielectric constant of the aqueous solution, respectively, $\varepsilon_0$ is the permittivity of free space. The parameters $\eta$ and $\varepsilon$ were taken as equal to their bulk values.

## Results

The temperature-dependence curves of the electrophoretic mobility of DMPC liposomes in solutions of different potassium salts are presented in Fig. 1. The zwitterionic lipid DMPC should not bear any net charge at pH 7.4. However, the data of Fig. 1 point to the fact that liposomes possess substantial surface potential (at 20°C the value of $u = 1\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$ corresponds to the $\zeta = 14$ mV). The magnitude of the potential shows a pronounced dependence on the anion species present in the medium. These findings indicate that the zeta potential evidently arises from the specific binding of anions to the liposome surface.

Fig. 1 demonstrates sharp changes in electrophoretic mobility values at the temperature of $23.6 \pm 0.3$°C, which was found previously to be the temperature of the gel to liquid-crystalline phase transition ($T_c$) of DMPC water dispersions [21] and specially checked by DSC in this laboratory for DMPC preparations used in the experiments *. As the data show in the case of liposomes in $K_2SO_4$, KCl, and $KNO_3$ solutions the sign at electrophoretic mobility reverses concurrently with the lipid phase transition.

Curves of the temperature-dependence of electrophoretic mobility of liposomes in the presence of different concentrations of trinitrophenol ($TNPh^-$) are shown in Fig. 2. These curves also demonstrate that abrupt changes in electrophoretic mobility values occur at $T_c$ similar to those found for inorganic anions.

The results presented in Figs. 1 and 2 were austerely reproducible irrespective of the direction of temperature change and no hysteresis was detected.

It is of interest to note that in all curves of Figs. 1 and 2 (except of those indicating alteration of the sign at electrophoretic mobility) the ratio of zeta potentials $\zeta_a/\zeta_b$ determined just 'after' and 'before' the phase transition is constant and equals $1.6 \pm 0.2$.

Nearly linear changes of electrophoretic mobil-

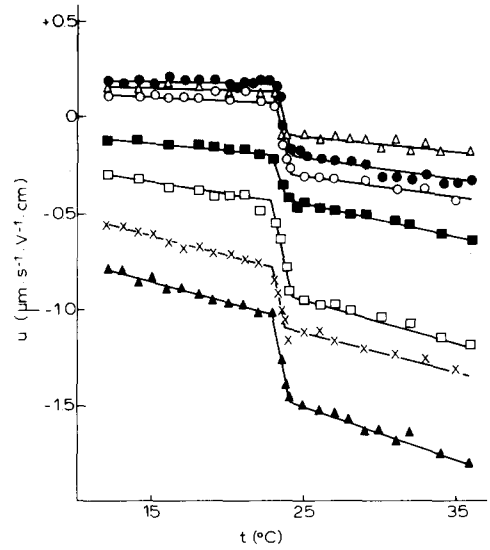


Fig. 1. Temperature dependence of the electrophoretic mobility of DMPC liposomes in 0.01 M solutions of $K_2SO_4$ (△), KCl (●), $KNO_3$ (○), KBr (■), KSCN (□), KI (×), and $KClO_4$ (▲) buffered to pH 7.4 with 5 mM Tris-HCl.

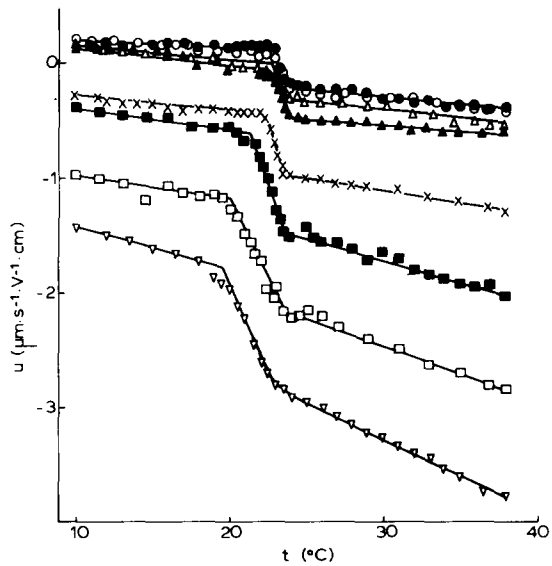


Fig. 2. Temperature dependence of the electrophoretic mobility of DMPC liposomes in aqueous solutions containing $TNPh^-$ (its potassium salt) in concentrations 0 (●), $10^{-7}$ (○), $10^{-6}$ (△), $10^{-5}$ (▲), $3 \cdot 10^{-5}$ (×), $10^{-4}$ (■), $3 \cdot 10^{-4}$ (□), and $10^{-3}$ M (▽). The aqueous solutions were buffered to pH 7.4 with 5 mM Tris-HCl and the ionic strength was held constant at 0.01 M with KCl.

* The width of DSC endothermic peaks at their half-height ($w_{1/2h}$) was of 0.6 deg. C which demonstrated additionally the high-enough purity of the lipid used.

ity with temperature in the regions below and above $T_c$ (Figs. 1 and 2) might be caused by the temperature-dependence of the viscosity of aqueous solutions. As a matter of fact, the viscosity of water changes by a factor of about 1.25 per 10 deg.C in the temperature range from 10 to 40°C, coinciding with changes in electrophoretic mobility at $T \neq T_c$.

For quantitative description of anion binding to liposomes the Langmuir adsorption isotherm was used:

$$\sigma_{ads} = K(\sigma_{max} - \sigma_{ads})[A^-]_0 \quad (2)$$

where $\sigma_{ads}$ is the density of surface charge induced by anion adsorption, $K$ is the binding constant, $\sigma_{max}$ is the maximum number of binding sites per unit area, $[A^-]_0$ is the concentration of the anion at the membrane-solution interface. The interfacial concentration of the monovalent anion, $[A^-]_0$, is related to its value in the bulk aqueous phase, $[A^-]$, through the Boltzmann equation:

$$[A^-]_0 = [A^-] \exp(F\psi/RT) \quad (3)$$

where $\psi$ is the potential of diffuse double layer, $F$, $R$, and $T$ have their usual significance. The total charge density of the membrane surface, $\sigma$, was considered as consisting of two components:

$$\sigma = \sigma_{ads} + \sigma_0 \quad (4)$$

where $\sigma_0$ is the surface charge density at $[A^-] = 0$. On the other hand, $\sigma$ must obey the Gouy-Chapman equation which can be written for the case of 1 : 1 electrolytes present in the medium as:

$$\sigma = \sqrt{8\varepsilon_0 \varepsilon RTC}\ \mathrm{sh}(F\psi/2RT) \quad (5)$$

where $C$ is the total concentration of 1 : 1 electrolytes or the ionic strength of the aqueous solution. The combination of Eqns. 2–5 gives:

$$\frac{\sigma_{max}}{1+\exp(-F\psi/RT)/K[A^-]} + \sigma_0 = \sqrt{8\varepsilon_0 \varepsilon RTC}\ \mathrm{sh}(F\psi/2RT) \quad (6)$$

The Eqn. 6 was used to describe experimental dependences of the surface potential of liposomes on the concentration of adsorbing anions.

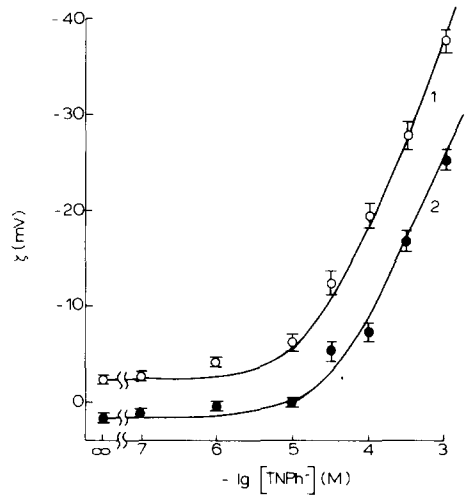


Fig. 3. Dependence of the zeta potential of DMPC liposomes on the concentration of $TNPh^-$ at the temperatures above (curve 1, $t = 26$°C) and below $T_c$ (curve 2, $t = 18$°C). The aqueous solutions were buffered to pH 7.4 with 5 mM Tris-HCl and the ionic strength was held constant at 0.01 M with KCl. The curves through the points were drawn according to Eqn. 6 using the mean values of the parameters $K^{-1}$ and $\sigma_{max}$ presented in Table I.

In Fig. 3 curves of the dependence of zeta potential on trinitrophenol ($TNPh^-$) concentration at a constant ionic strength are plotted for the temperatures above and below $T_c$ ($t = 26$ and 18°C, respectively). These temperatures were chosen inasmuch as the increase of $[TNPh^-]$ led to some broadening of the temperature range of phase transition and a slight reduction of $T_c$ (see Fig. 2). Monotonous curves predicted by Eqn. 6 for this case ($C$ = const) were built using the mean values of $K^{-1}$ and $\sigma_{max}$ presented in Table I. Eisenberg et al. [13] showed that the width of hydrodynamic plane of shear of liposomes in aqueous solutions of alkaline metal chlorides was of 2 Å. Using this value we calculated the surface potentials, $\psi$, from corresponding zeta potentials, When $C = 1.5 \cdot 10^{-2}$ M (Fig. 3) the difference between $\zeta$ and $\psi$ was shown to be less than 10%. Therefore, for the case the values of zeta potentials instead of $\psi$ were used in Eqn. 6. The experimentally found values of $\sigma_0 = -6.3 \cdot 10^{-2}$ and $+5.03 \cdot 10^{-2}$ $\mu C/cm^2$ were used at 26 and 18°C, respectively.

When the ionic strength of solutions is not constant and varies as $[A^-]$ does ($[A^-] = C$) the

TABLE I

THE PARAMETERS $K^{-1}$ (mM) AND $\sigma_{max}$ ($\mu C/cm^2$) USED IN Eqn. 6 TO DESCRIBE THE BINDING OF SEVERAL ANIONS TO DMPC LIPOSOMES ABOVE AND BELOW $T_c$

| Anion | $T = 25°C$ | | $T = 22°C$ | |
|---|---|---|---|---|
| | $K^{-1}$ | $\sigma_{max}$ | $K^{-1}$ | $\sigma_{max}$ |
| $TNPh^-$ [a] | 0.20 ± 0.07 | 2.3 ± 0.7 | 0.22 ± 0.08 | 1.4 ± 0.3 |
| $ClO_4^-$ | 4.5 ± 0.3 | 1.0 ± 0.1 | 4.3 ± 0.3 | 0.6 ± 0.04 |
| $I^-$ | 25 ± 3.5 | 2.0 ± 0.2 | 27 ± 4.0 | 1.2 ± 0.1 |
| $SCN^-$ | 100 ± 7.0 | 5.5 ± 0.4 | 110 ± 10 | 3.2 ± 0.2 |
| $Br^-$ | 280 ± 25 | 2.6 ± 0.3 | 280 ± 25 | 1.7 ± 0.2 |
| $NO_3^-$ | 480 ± 40 | 1.8 ± 0.2 | 500 ± 60 | 1.1 ± 0.1 |

[a] The parameters presented correspond to 26 (left) and 18°C (right).

Eqn. 6 predicts $\psi$ against $[A^-]$ curves with a maximum in the value of $\psi$. Such curves are presented in Fig. 4 to describe the adsorption of several anions to liposome membranes at the temperatures above and below $T_c$ ($t = 25$ and 22°C, respectively). The value of $\sigma_0$ was suggested in this case to be zero ($\sigma_0 \ll \sigma_{max}$). Since the concentrations of 1 : 1 electrolytes reached 1.5 M, which might lead to a considerable difference between $\zeta$ and $\psi$, the values of $\psi$ calculated from corresponding values of $\zeta$ were used in Eqn. 6.

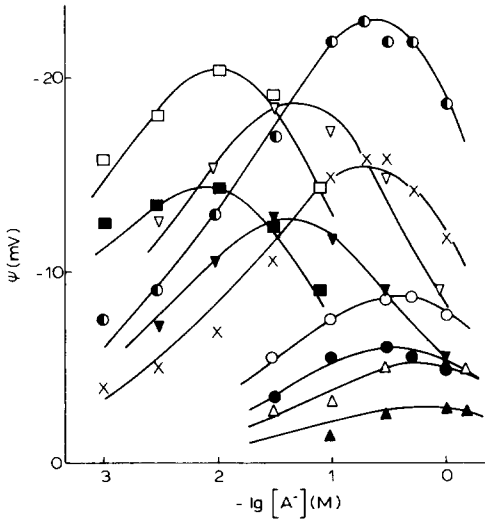


Fig. 4. Dependence of the surface potential of DMPC liposomes on the concentration of $KClO_4$ (□, ■), KI (▽, ▼), KSCN (◐, ×), KBr (○, ●), and $KNO_3$ (△, ▲). The curves marked by symbols □, ◇, ◐, ○, and △ correspond to the temperature above $T_c$ ($t = 25°C$), the others to the temperature below $T_c$ ($t = 22°C$). The aqueous solutions were buffered to pH 7.2 with 1 mM Tris-HCl. The curves through the points were drawn according to Eqn. 6 using the mean values of the parameters $K^{-1}$ and $\sigma_{max}$ presented in Table I.

It is worthwhile noting that the increase of the negative surface potential up to a maximum with increasing electrolyte concentration shown in Fig. 4 confirms the suggestion that the observed potentials were created just by the adsorption of anions to liposomes. The decrease of $\psi$ after reaching its maximum value, when $\sigma_{ads} = 0.5\sigma_{max}$, obviously results from the screening by counterions the surface charge induced by anion binding.

The values of $K^{-1}$ for different anions summarized in Table I together with the data of Fig. 1 give the following sequence for the affinities of anions to liposome membranes:

$$TNPh^- > ClO_4^- > I^- > SCN^- > Br^- > NO_3^- > Cl^- \cong SO_4^{2-}$$

## Discussion

Several studies of electrokinetic properties of liposomes prepared from zwitterionic lipids were carried out over the past two decades. Hanai et al. [22] measured zeta potentials of multilamellar liposomes made of egg yolk phosphatidylcholine (PC) in NaCl solutions at 20°C. The mean values of zeta potentials were found to be near zero but the S.D. values were large. In particular, zeta potential of liposomes in 0.01 M NaCl solution varied from +3.4 to −2.8 mV. In fact, the liposomes contained PC molecules with different fatty acid chains and at 20°C some portion of the lipid could be in

the gel state and the other portion in liquid-crystalline state. As follows from Fig. 1, the sign of zeta potential of PC liposomes in 0.01 M chloride solution depends on the state of the lipid. Hence, these authors could in principle observe both negatively and positively charged particles when the lipid composition of different preparations of liposomes was not quite the same.

McLaughlin et al. [11] determined zeta potentials of phosphatidylethanolamine vesicles in 0.25 M solutions of NaCl, NaSCN, and $NaClO_4$ and found mean values of $-1$, $-11$, and $-14$ mV, respectively. Hauser et al. [23] established the following sequence for the effectiveness of anions in reducing the positive zeta potential of egg PC vesicles with $La^{3+}$ bound: $ClO_4^- > I^- > SCN^- > NO_3^- > Br^- > Cl^-$. Using microelectrophoresis and $^1$H-NMR techniques Barsukov et al. [14] revealed specific binding of $Cl^-$, $NO_3^-$, and $SCN^-$ to the surface of egg PC vesicles. The binding constants of the anions determined by the latter authors were 1.67, 4.03, and 23.5 $M^{-1}$, respectively.

The values of binding constants of $SCN^-$ and $NO_3^-$ reported by Barsukov et al. [14] exceed those found in this work about two times. Hauser et al. [23] showed that the affinity of $NO_3^-$ for PC membranes was greater than that of $Br^-$, which is inconsistent with our results. In other respects data presented in this study are in good agreement with those obtained by others.

The difference between our data and those of Barsukov et al. [14] and Hauser et al. [23] might be explained taking into account that both the groups mentioned used egg PC whereas we used pure DMPC. In fact, as Schneider and Wolff [24] showed, the values of distribution coefficients of various monovalent anions between aqueous and lipid-containing organic phases, as well as the sequence for the coefficients, depended on the lipid content of the organic phase. The data of Jendrasiak [25] on the influence of $Cl^-$, $Br^-$, $I^-$, and $SCN^-$ on $^1$H-NMR signal from PC $N^+(CH_3)_3$-group demonstrated that for a given anion the peak-separation was different for $^2H_2O$ dispersions of egg PC, egg lyso-PC, and dioleoyl-PC.

The change of the sign of electrophoretic mobility at $T_c$ detected for liposomes in $K_2SO_4$, KCl, and $KNO_3$ solutions (Fig. 1) and small positive zeta potentials observed below $T_c$ could be possibly due to a weak adsorption of $K^+$ to DMPC membranes. This suggestion is consistent with the results reported on the binding of monovalent cations to egg PC vesicles [23] and DPPC planar bilayer membranes [10]. To verify the idea for DMPC we measured zeta potentials of liposomes from this lipid in 0.01 M $NH_4ClO_4$ solution in the temperature range from 10 to 40°C (data not shown) and obtained an increment of about $+3$ mV in respect to liposomes in 0.01 M $KClO_4$ solutions.

Taking into account the data presented in Figs. 1 and 4 and Table I it seems reasonable to assume that the binding constant of $K^+$ is comparable with those of $SO_4^{2-}$ and $Cl^-$ and smaller than that of $NO_3^-$, i.e. $0 < K_{K^+} < 2$ $M^{-1}$. Since in deriving Eqn. 6 the binding of the counterion (cation) was ignored, or the binding constant of the cation was assumed to be zero, only curves for the anions, the binding constants of which appeared greater than that of $K^+$, are presented in Fig. 4. The lack of $K^{-1}$ and $\sigma_{max}$ parameters for $SO_4^{2-}$ and $Cl^-$ in Table I is connected with the fact that the binding of $K^+$ could no longer be neglected in describing theoretically the adsorption of these anions. Binding of cations to lipid membranes is the subject of our current research.

Evidence presented in Table I indicates that the binding constants, $K$, obviously depend on the anion species present in the medium, whereas the values of $\sigma_{max}$ for different anions are approximately the same. On the other hand, it can be clearly seen that it is the $\sigma_{max}$ which changes at the lipid-phase transition while $K$ remains almost constant. This statement, however, can hardly be so much substantiated for the case of $TNPh^-$ as for other anions studied because of the vagueness and overlapping of both $K^{-1}$ and $\sigma_{max}$ values estimated for $TNPh^-$ above and below $T_c$ (see Table I). This uncertainty was due to the absence of saturation of zeta potential with increasing $TNPh^-$ concentration and, consequently, the lack of any experimental value for $\sigma_{max}$. Nevertheless, the above corroboration is well-enough supported for all the other anions summarized in Table I and still feasible for $TNPh^-$. It might be assumed, therefore, that the $(1.6 \pm 0.2)$-fold shifts in zeta potential at the lipid-phase transition were caused by similar changes in $\sigma_{max}$.

These conclusions suggest that the lipid phase transition results in an alteration of the number of binding sites but does not affect their qualitative characteristic, namely, their affinity to anions.

The binding sites of DMPC liposomes to anions can be presumably regarded as disordered boundary regions between domains of regularly arranged lipid molecules, or, in other words, as defects in molecular organisation of the lipid membrane. According to this assumption the increase in $\sigma_{max}$ and zeta potential would be considered as a result of the increase in the number of defects at the lipid transition from the gel to liquid crystalline phase.

The existence of ordered lipid domains in the membranes of dimyristoyl-, dipalmitoyl-, and distearoyl-PC vesicles has been demonstrated by Lawaczeck et al. [26]. Electron-microscopic data reported by these authors have supported the idea of cracks between closely packed clusters in lipid bilayers. An increase in $Na^+$ permeation through the membranes of both DPPC and dipalmitoylphosphatidylglycerol vesicles at the phase-transition temperatures of the lipids has been attributed to the formation of imperfect boundaries between the solid and liquid-crystalline phases of membranes [27]. Sackmann and Träuble [28] found an increase in the surface density of binding sites of DPPC vesicles to Bromothymol blue and 1-anilino-8-naphthalene sulfonate by a factor of about 3 at the lipid transition from the gel to liquid-crystalline state. Marsh et al. [29] described their data on the enhancement of Tempocholine spin label binding to DMPC vesicles at thermotropic phase transition of the lipid in terms of increase in the number of binding sites. They regarded the binding sites as boundaries between differently packed domains.

These findings provide additional evidence in favour of the proposition we use to explain the shifts in zeta potential of liposomes at the lipid phase transition.

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## References

1 Esfahani, M., Crowfoot, P.D. and Walik, S.J. (1972) J. Biol. Chem. 247, 7251–7256
2 Overath, P. and Träuble, H. (1973) Biochemistry 12, 2625–2634
3 Linden, C.D., Wright, K.L., McConnell, H.M. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2271–2275
4 Grishman, C.M. and Barnett, R.E. (1973) Biochemistry 12, 2635–2637
5 Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 622–626
6 Poon, R., Richards, J.M. and Clark, W.R. (1981) Biochim. Boiphys. Acta 649, 58–66
7 Lee, A.G. (1976) Nature (London) 262, 545–548
8 Gilbert, D.L. and Ehrenstein, G. (1969) Biophys. J. 9, 447–463
9 Apell, H.J., Bamberg, E. and Läuger, P. (1979) Biochim. Biophys. Acta 552, 369–379
10 Abidor, I.G., Vanýsek, P., Tatulian, S.A. and Chernomordik, L.V. (1981) Elektrokhimiya 17, 1844–1851 (Russ.)
11 McLaughlin, S., Bruder, A., Chen, S. and Moser, C. (1975) Biochim. Biophys. Acta 349, 304–313
12 McLaughlin, A., Grathwohl, C. and McLaughlin, S. (1978) Biochim. Biophys. Acta 513, 338–357
13 Eisenberg, M., Gresalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5224
14 Barsukov, L.I., Volkova, V.I., Shaprio, Yu.E., Viktorov, A.V., Bystrov, V.F. and Bergelson, L.D. (1977) Bioorg. Khimiya 3, 1355–1361 (Russ.)
15 Siegal, D.P. and Ware, B.R. (1980) Biophys. J. 30, 159–171
16 Kostyuk, P.G., Mironov, S.L., Doroshenko, P.A. and Ponomarev, V.N. (1982) J. Membrane Biol. 70, 171–179
17 Simon, S.A., Lis, L.J., Kauffman, J.W. and MacDonald, R.C. (1975) Biochim. Biophys. Acta 375, 317–326
18 McIntosh, T.J., Simon, S.A. and MacDonald, R.C. (1980) Biochim. Biophys. Acta 597, 445–463
19 MacDonald, R.C., Simon, S.A. and Baer, E. (1976) Biochemistry 15, 885–891
20 Tatulian, S.A. (1983) Dokl. Akad. Nauk S.S.S.R. 268, 230–233 (Russ.)
21 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–356
22 Hanai, T., Haydon, D.A. and Taylor, J. (1965) J. Theor. Biol. 9, 278–296
23 Hauser, H., Hinckley, C.C., Krebs, J., Levine, B.A., Phillips, M.C. and Williams, R.J.P. (1977) Biochim. Biophys. Acta 468, 364–377
24 Schneider, P.B. and Wolff, J. (1965) Biochim. Biophys. Acta 94, 114–123
25 Jendrasiak, G.L. (1972) Chem. Phys. Lipids 9, 133–146
26 Lawaczeck, R., Kainosho, M., Girardet, J.-L. and Chen, S. (1975) Nature (London) 256, 584–586
27 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348
28 Sackmann, E. and Träuble, H. (1972) J. Am. Chem. Soc. 94, 4482–4491
29 Marsh, D., Watts, A. and Knowles, P.F. (1976) Biochemistry 15, 3570–3578