Biochimica et Biophysica Acta, 1025 (1990) 117–121
Elsevier

BBAMEM 74874

Effect of high electrolyte concentration on the phase transition behaviour of DPPC vesicles: a spin label study

R. Bartucci, N. Gulfo and L. Sportelli

Dipartimento di Fisica, Laboratorio di Biofisica Molecolare, Università della Calabria, Arcavacata di Rende (Italy)

(Received 12 February 1990)

Key words: DPPC; Phase transition; Electrolyte; Spin label; ESR

We have investigated by Electron Spin Resonance spectroscopy, the effects of high electrolyte concentration on the phase transitions of unilamellar vesicles of dipalmitoylphosphatidylcholine at the pH values of 5.0 and 9.0. Using the 5-nitroxide stearic acid as spin probe we have found that, at both pH values, the lipid main phase transition is not quite affected by variations of the electrolyte concentration up to the value of 3 M. Instead, the pretransition at pH 5.0 disappears in the presence of 1 M electrolyte, and at pH 9.0, the pretransition temperature shifts upward from 25.5 to 31.0 °C when the electrolyte concentration reaches the value of 3 M. The observed results on the pre- and main phase transition widths, transition temperatures and their cooperativity indicate that the presence of salt in the bulk solution leads to structural changes of the lipid bilayer which essentially concern either the polar zone or the hydrogen belt region of the DPPC vesicles. The extent of observed perturbation depends on salt concentration.

Introduction

Studies on the phase transitions of lipid bilayers have shown that these play an important role in determining the function of biological membranes. It has been also reported that the physical state of phospholipid bilayers, i.e., their structure, packing density and fluidity, can be affected both by physical and chemical agents. Among the latter, the ionic character of the suspension medium.

In this connection, the effects of various ions on proteins and charged natural membranes as well as their related model systems have been extensively studied both experimentally and theoretically [1–5]. In fact, it has been found that ions are able to influence the physiological and biochemical processes occurring in membranes either by interacting directly with the membrane components or by inducing structural changes of the hydration water layers bound to the membrane surface.

More controversial is the nature and the extent of the interaction occurring between highly concentrated electrolyte aqueous solutions and membrane model systems formed with neutral lipids, such as phosphatidylcholine and phosphatidylethanolamine [2,6–15]. These works on the interaction ions-uncharged systems suggest that the ions influence essentially the interfacial region of the lipid bilayer to an extent which is less than that observed on charged phospholipids. Moreover, all investigations have been confined to systems dispersed in aqueous media containing up to 1 M salt of mono-, bi- and trivalent cations [6–10].

Neutral phospholipids have been found in high percentages in natural membranes of several microorganisms living in extreme conditions of pH, ionic strength and temperature. Halobacteria can be an example [16]. It is, therefore, of great interest to investigate the effects of the high electrolyte concentration on the structural properties of neutral systems.

In this respect, we have investigated with ESR spectroscopy and 5-NSA, the effects of high sodium chloride concentrations on the endothermic phase transitions of a suitable membrane model system, i.e., ULV of DPPC. We performed the measurements at pH 5.0 and 9.0 in the temperature range 20–50 °C and varied the electrolyte concentration from 0 to 3 M.

Due to the different vertical location of the label in the interfacial region of DPPC-ULV at all salt con-

Abbreviations: ESR, electron spin resonance; 5-NSA, 5-nitroxide stearic acid spin-labelled molecule; ULV, unilamellar vesicles; DPPC, dipalmitoylphosphatidylcholine; HB, hydrogen belt; PZ, polar zone.

Correspondence: L. Sportelli, Dipartimento di Fisica, Laboratorio di Biofisica Molecolare, Università della Calabria, I-87036 Arcavacata di Rende, Italy.

centrations considered according to the pH, the nitroxide probes the HB region at pH 5.0, and the PZ at pH 9.0. The label investigates the same regions also in the presence of the highest electrolyte concentration. We find that the main transition is not affected by variations of the electrolyte concentration; this always remains centered at $T_m = 37°C$. Moreover, at pH 5.0 the pretransition disappears for high electrolyte concentration and at pH 9.0 the pretransition temperature is shifted from 25.5 to 32°C as the electrolyte concentration increases from 0 to 3 M. On the other hand, when the temperature goes through the phase transition, the outer hyperfine splitting decreases much more at pH 5.0 than at pH 9.0.

Our results emphasize a perturbation of the hydrogen bonds network and an increase in the packing of the polar zone of the lipid bilayer which depend on the electrolyte concentration in the dispersion medium.

Experimental

Materials

Synthetic L-α-dipalmitoylphosphatidylcholine was used as obtained from Sigma. The 2-(*n*-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl spin label positional isomers ($n = 3$ and 5; 5- and 7-nitroxide stearic acid, 5- and 7-NSA, respectively) were Aldrich products stored at 4°C in ethanol solution. Reagent grade sodium chloride and salts for citrate and borate buffer preparation at pH 5.0 and 9.0 were from C. Erba. Distilled water was used throughout.

Sample preparation

For DPPC-ULV preparation, lipids in chloroform were first dried in a rotavapor under reduced pressure at 45°C, and then the residual traces of solvent were removed with a stream of dry nitrogen. After that, the dried lipids were dispersed in spin-labeled solution containing 0, 1, 2 or 3 M NaCl prepared in 10 mM citrate and borate buffer (pH 5.0 and 9.0, respectively). The final lipid concentration was $4 \cdot 10^{-2}$ M and the label-to-lipid molar ratio of 1:150. The phospholipid-water system so prepared was then sonicated (30 s sonication followed by 30 s pause) in a MSE instrument (100 W, 20 kHz, 9 μm amplitude) at a temperature above the main phase transition of DPPC until a translucent suspension of single walled vesicles was obtained.

ESR measurements

ESR measurements were performed in the temperature range of 20–50°C with a BRUKER Mod ER 200D-SRC 9 GHz spectrometer equipped with an ER 4111VT variable temperature control system (accuracy ±0.3° C). Samples were inserted in sealed off capillary tubes accommodated within a standard 4 mm quartz ESR tube containing silicon oil to avoid temperature gradient and were positionated in a TE_{102} ESR cavity. Measurements on freshly prepared samples were performed at thermal equilibrium and the spectrometer setting was: 10 mW microwave power, 1.2 G_{pp} field modulation amplitude, 0.1 s time constant and 500 s scan time.

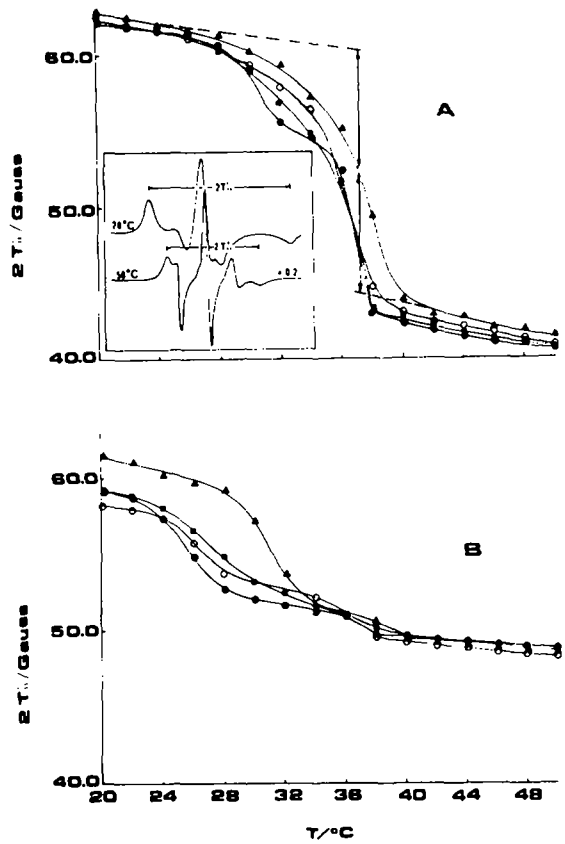

Fig. 1. (A) $2T'_{II}$ values vs. temperature of the spin label 5-NSA in DPPC-ULV dispersed in solutions with different contents of salt at the pH value of 5.0: ●———●, no NaCl; ■———■, 1 M NaCl; ○———○, 2 M NaCl; ▲———▲, 3 M NaCl. Inset: ESR spectra of 5-NSA in DPPC-ULV at pH 5.0 with 0 M NaCl at 20 and 50°C, respectively. (B) As in (A) but at pH 9.0. The transition temperatures are defined as the temperatures where the measured curves (———) bisect the vertical distance between the extrapolated straight lines (- - -).

Results

The ESR spectra of the spin label 5-NSA incorporated into DPPC-ULV dispersed in buffer solution in absence of electrolyte at pH 5.0 and recorded at 20 and 50°C are reported in the inset of Fig. 1A. As can be seen, at both temperatures they are one-component powder-like ESR spectra of spin labels undergoing temperature-dependent anisotropic motion. Further, when the dispersion medium of the DPPC – ULV contains 1, 2 or 3 M NaCl at the same pH, the lineshape of the ESR signal remains almost unchanged.

TABLE I

Temperatures of the pre-, T_p and main, T_m, endothermic phase transitions for unilamellar vesicles of dipalmitoylphosphatidylcholine dispersed in media with different NaCl contents at pH 5.0 and 9.0, respectively

n.d., not determined.

NaCl (M)	pH 5.0		pH 9.0	
	T_p (°C)	T_m (°C)	T_p (°C)	T_m (°C)
0	30.5	37.0	25.5	37.0
1	n.d.	37.0	26.5	37.0
2	n.d.	37.0	26.5	37.0
3	n.d.	37.0	31.0	37.0

Quantitatively, the thermotropic behaviour of the unilamellar vesicles of DPPC dispersed in media at different electrolyte concentration can be compared by means of the spin label outer resonance hyperfine splitting, $2T'_{\parallel}$. This is a spectral parameter that gives a relative measure of the local fluidity of the membrane: large $2T'_{\parallel}$ values correspond to a rigid spin label environment, while small values correspond to a more fluid one.

The plots of $2T'_{\parallel}$ vs. temperature for 5-NSA in DPPC-ULV dispersed in 0, 1, 2 and 3 M NaCl buffer solution at pH 5.0 are reported in Fig. 1A. It is interesting to note that the label in the lipid bilayer undergoes the same large variation of the degree of its anisotropic motion at all electrolyte concentrations considered. In fact, $2T'_{\parallel}$ decreases from about 62 to 40 G as the temperature is raised from 20 to 50 °C, respectively. Moreover, with 0 M NaCl the label reveals the main phase transition, i.e., the $P'_{\beta} \rightarrow L_{\alpha}$ transition of DPPC, as a drop around $T_m = 37$ °C and the pre-transition, i.e., the $L'_{\beta} \rightarrow P'_{\beta}$ one, as another step drop at about $T_p = 30.5$ °C (Table I).

The presence of 1, 2 or 3 M NaCl in the dispersion medium suppresses the pretransition, while it has no effect on the temperature as well as on the amplitude of the major transition (Table I).

In the case in which the dispersion media of DPPC-ULV were adjusted at pH 9.0, the ESR spectra at all the temperature and salt concentration ranges investigated, result again to be one component powder-like. Moreover, at this pH, the variation of the magnetic parameter is less pronounced than that observed at pH 5.0. In fact, as can be seen from Fig. 1B, $2T'_{\parallel}$ reduces from 58 G at 20 °C to 47 G at 50 °C. The most obvious features of the plots of $2T'_{\parallel}$ vs. temperature given in Fig. 1B are that in all the cases only a trace of the DPPC main phase transition remains at $T_m = 37$ °C, while the pre-transition temperature shifts from 25.5 °C in absence of electrolyte to 31 °C as the salt concentration reaches the value of 3 M NaCl (Table I).

Discussion

The aim of our study is to investigate the effect of high electrolyte concentration (up to 3 M NaCl) on the interfacial region of a single lipid model system, i.e., ULV-DPPC, using 5-NSA in the temperature range 20–50 °C. In order to locate precisely the position of the reporter group of the label in the bilayer, we have performed the measurements at pH 5.0 and 9.0.

In all the experimental conditions of the suspension medium, there is no variation of the electric state of the polar head of DPPC; it always remains zwitterionic. At pH 5.0, the carboxylic group of 5-NSA is fully protonated and, therefore, anchored in the region of negatively charged phosphate groups of DPPC (see Fig. 5 in Ref. 17). The nitroxide moiety probes the HB region of the bilayer, i.e., the region between the glycerol backbone and the beginning of the fatty acid acyl chains of DPPC. At pH 9.0, instead, the interaction between the fully dissociated -COO^- group and the positive charge on the choline quaternary ammonium group forces the probes $\equiv 5$ Å outside into the interfacial region of lipid bilayer, namely, the PZ. At both pH values, the position of the probe within the bilayer is not changed when the electrolyte is present in a different concentration in the suspension medium. In fact, although the pK_a of the dissociation equilibrium of the carboxylic group can undergo variations with ionic strength, the pH values used are too far removed from it. The conventional ESR spectra of 5-NSA in ULV prepared with media with different NaCl contents have powder-like character and, importantly, at both pH values in the investigated temperature range are single component (inset of Fig. 1A). If the protonation state of the carboxylic group would depend on the ionic strength or temperature, composite ESR spectra should be recorded with the features of the simulaneous anchoring of the ionized and non-ionized form of 5-NSA in the two different loci of the membrane.

From our experimental findings we can say that the position of the probe within the bilayer is determined only by the pH and there is no effect of high electrolyte concentration. The variation of the spectral parameter seen in Fig. 1A and B should be due only to the different dynamic properties of the micro-environments tested by the nitroxide moiety of 5-NSA anchored into two different loci of the interfacial region of vesicles. The pH-dependent location of the probe within the bilayer is further supported by measurements (data not shown) performed using 7-NSA in which the nitroxide group is slightly down to the acyl chain. In fact, when this label is incorporated in DPPC-ULV dispersed at pH 9.0, both the spectral features and the transition characteristics are somewhat similar to those obtained with 5-NSA at pH 5.0. This means, that at pH 9.0, the 7-NSA probes the HB region again.

The different vertical location of the probe in the bilayer gives differences in the variation of $2T'_{\parallel}$ values between 20 and 50 °C. In fact, as can be seen from Fig. 1A, the variation of the hyperfine splitting of 5-NSA probing (pH 5.) the HB region of the DPPC-ULV dispersed in solution without NaCl, $\Delta(2T'_{\parallel}) = 2T'_{\parallel}$ (20 °C) − $2T'_{\parallel}$ (50 °C), is large. The decrease in spectral anisotropy with the temperature increase reflects the increase in the angular amplitude of the motion of the DPPC molecules, and, in turn, the increase in formation of rotational isomerisms along the acyl chains of lipids. On the other hand, the $\Delta 2T'_{\parallel}$ value found when the label investigates the PZ region of the DPPC vesicles is smaller than that observed with 5-NSA in the HB region. It is noteworthy, that in the differences in $\Delta 2T'_{\parallel}$ the $2T'_{\parallel}$ contribute much more at 50 °C than at 20 °C when the spectra in both situations are in the slow motion regime on the ESR time scale. It is also evident from a comparison between Fig. 1A and B that at 20 °C, $2T'_{\parallel}$(HB) > $2T'_{\parallel}$(PZ), while at 50 °C the situation is reversed, i.e., $2T'_{\parallel}$(PZ) > $2T'_{\parallel}$(HB).

The observations suggest, from a molecular dymanics point of view that in the gel phase in spite of the existing defects in the structure the region of the choline portion of the DPPC vesicles is more fluid than the glycerol backbone environment. In the liquid crystalline state, the structural arrangement of the polar heads results relatively independent of the temperature. In fact, the label records only a small reduction of its initial anisotropic motion (Fig. 1B). The two abrupt changes of $2T'_{\parallel}$ vs. temperature seen at both pH values and with no NaCl (Fig. 1A and B) correspond to the main and the pretransitions of DPPC. These phase transitions have been widely investigated both in different lipid mesophases and with diverse techniques (see Ref. 18 and references therein).

Back to our results, the main transition, associated with the hydrocarbon chain melting, is more evident at pH 5.0 when the nitroxide radical is located at the border of the hydrophobic core of DPPC than at pH 9.0 when the probe investigates an environment about 5 Å outside of the border of the HB region [17]. At the latter pH value it appears to a very small extent. Further, the $P'_{\beta} \rightarrow L_{\alpha}$ main transition is centered at $T_m = 37$ °C, as previously reported for sonicated DPPC vesicles [18–20]. The $L'_{\beta} \rightarrow P'_{\beta}$ transition, known as pretransition, occurs at $T_p = 30.5$ °C and 25.5 °C at pH 5.0 and 9.0, respectively (Table I). The difference of about 5 °C in the T_p values found at the two pH values in absence of NaCl in the vesicles dispersion media, supports the existence, for $T < T_p$, of a larger disorder in the polar region with respect to that in the hydrogen belt one. The presence of 1, 2 or 3 M 1 : 1 electrolyte in the vesicles dispersion media at pH 5.0, leaves almost unchanged the $2T'_{\parallel}$ value. At pH 9.0, instead, it increases to some extent at the highest NaCl concentration (Fig. 1A and B). This dependence of $2T'_{\parallel}$ on NaCl concentration at pH 9.0, observed particularly in the gel phase, does not result from a dependence on the position/degree of anchoring of the label, as no similar results are seen at 50 °C. It is evident from data in Table I, that the main transition always remains centered at $T_m = 37$ °C at both pH values. The T_p value, instead (pH 9.0), shifts upwards from 25.5 °C with 0 M NaCl to 31 °C with 3 M, while, at pH 5.0 it already disappears when the NaCl concentration reaches 1 M, probably under the main transition (Fig. 1A and B).

From a phenomenological point of view, the data clearly suggest that the ionic strength influences, although in a different way, both of the microenvironments at the DPPC interface investigated by 5-NSA. The well known Stern-Gouy-Chapman [5] theory of lipid double layers cannot be invoked in this case, to explain the experimental data; this is because of the high salt concentrations used in this work and the neutral electric state of DPPC polar heads forming the membrane model. The theory, in fact, can predict the decay of the electric potential from the membrane surface and in presence of low ionic strength values.

Although our own ^{31}P-NMR measurements on the same samples (unpublished data) exclude specific binding of the ions to the polar head, as suggested by other authors for mono-, bi- and trivalent ions [6,8,11,13–15], i.e., neither variation in the anisotropy of the chemical shift, $\Delta\sigma = \sigma_{\parallel} - \sigma_{\perp}$, nor changes in the shape of the ^{31}P resonance signal in presence of NaCl have been observed at the highest concentration, an explanation can be given by considering a diffuse interaction of Na^+ and Cl^- ions with the $-PO^-$ and $-N^+(CH_3)_3$ charges.

Experimental findings claim a step-by-step action of ions on the interfacial region of DPPC vesicles. At low ion concentration the structure of water around the approx. 4Å thick HB region should be concerned. In fact, due to their small hydration radius, the Na^+ ions reach the HB region and rearrange the hydrogen bond network around the glycerol backbone. The rearrangement induces packing of the head group causing the disappearance of the pretransition observed at pH 5.0 with 1 M NaCl (Fig. 1A) and the increase of 1 °C in the T_p value found at pH 9.0 with the label located about 5 Å outside the border of the HB region [17]. On increasing ionic strength, the targets of the Na^+ and Cl^- ions become the positive $-N^+(CH_3)_3$ and negative $-PO^-$ charges in the 10 Å thick PZ region, forming the dipole present on the polar head of each zwitterionic DPPC molecule. The net charges forming the electric dipole present at the DPPC polar head are screened and, consequently, the electrostatic interactions between them are reduced. Attractive dispersion forces between less charged polar heads together with changed hydration forces at the surface of the bilayer could be at the origin of the stabilization of the packing of the PZ region. This

is supported by the increase of the $2T'_{\parallel}$ value observed at pH 9.0 and 20 °C when the gel structure predominates as well as, from the increase of the pretransition temperature value from $T_p = 25.5$ to 31 °C in the presence of 3 M NaCl in the dispersion medium (Fig. 1B and Table I).

The hydrophobic core of the lipid bilayer seems not to be affected as much by the high salt concentration, as the spin label, at both pH values, does not reveal any significant variation of the hydrophobic chain melting temperature, T_m.

At present, a lot of questions remain unanswered: (i) does the membrane surface become weakly charged with salt concentration increase?; (ii) how does the dynamic of the choline portion of DPPC change?; and (iii) how is the hydration shell of the DPPC vesicles changed by the salt?

Infrared, deuterium-NMR and dielectric constant measurements are in progress with the aim of answering some of these questions.

Acknowledgments

The authors thank the Consiglio Nazionale delle Ricerche and the Ministero della Pubblica Istruzione for financial support.

References

1 Helm, C.A., Laxhuber, L., Losche, M. and Mohwald, H. (1986) Colloid Polymer Sci. 264, 46–55.
2 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152–161.
3 Janigh, F. (1976) Biophys. Chem. 4, 309–318.
4 Trauble, H. and Eibl, H. (1974) Proc. Natl. Acad. Sci. USA 71, 214–219.
5 Trauble, H., Teubner, M., Woolley, P. and Eibl, H. (1976) Biophys. Chem. 4, 319–342.
6 Akutsu, H. and Seelig, J. (1981) Biochemistry 20, 7366–7373.
7 Chapman, D., Peel, W.E., Kingston, B. and Lylley, T.H. (1977) Biochim. Biophys. Acta 464, 260–275.
8 Cunningham, B.A., Shimotake, J.E., Tamura-Lis, W., Mastram, T., Kwok, W.M., Kauffman, J.W. and Lis, L.J. (1986) Chem. Phys. Lipids 39, 135–143.
9 Loshchilova, E. and Karvaly, B. (1978) Biochim. Biophys. Acta 514, 274–285.
10 Simon, S.A., Lis, L.J., Kauffman, J.W. and MacDonald, R.C. (1975) Biochim. Biophys. Acta 375, 317–326.
11 Cserhati, T. and Szogyi, M. (1983) Chem. Phys. Lipids 34, 93–99.
12 Lis, L.J., Kauffman, J.W. and Shriver, D.F. (1975) Biochim. Biophys. Acta 406, 453–464.
13 Lis, L.J., Parsegian, V.A. and Rand, R.P. (1981) Biochemistry 20, 1761–1770.
14 Lis, L.J., Lis, W.T., Parsegian, V.A. and Rand, R.P. (1981) Biochemistry 20, 1771–1777.
15 Tatulian, S.A. (1983) Biochim. Biophys. Acta 736, 189–195.
16 Stoeckenius, W., Lozier, R. and Bogomolni, R. (1979) Biochim. Biophys. Acta 505, 215–278.
17 Sanson, A., Ptak, M., Rigaud, J.L. and Gary-Bobo, C.M. (1976) Chem. Phys. Lipids 17, 435–444.
18 Silvius, J.R. (1982) in Lipid-protein Interaction, (Jost, P.C. and Griffith, O.H., eds.), Vol. 2, pp. 239–281
19 Marsh, D., Watts, A. and Knowles, P.F. (1977) Biochim. Biophys. Acta 465, 500–514.
20 Suurkuusk, J., Lentz, B.R., Barenholz, Y., Biltonen, R.L. and Thompson, T.E. (1976) Biochemistry 15, 1393–1401.