Biochimica et Biophysica Acta 861 (1986) 237–242
Elsevier

BBA 73292

Thiocyanate and bromide ions influence the bilayer structural parameters of phosphatidylcholine bilayers

B.A. Cunningham and L.J. Lis

Department of Physics and The Liquid Crystal Institute, Kent State University, Kent, OH 44242 (U.S.A.)

(Received 5 February 1986)
(Revised manuscript received 13 May 1986)

Key words: Phosphatidylcholine bilayer; Monovalent ion; Membrane structure; X-ray diffraction; Differential scanning calorimetry

The influence of monovalent cations and anions on the structural parameters of dipalmitoylphosphatidylcholine (DPPC) bilayers was examined at 25°C using X-ray diffraction. It was shown that monovalent salts, in general, have little effect on lipid packing within the bilayer. However, fully hydrated DPPC bilayers in 1 M KSCN pack in an interdigitated acyl chain phase. This is the first observation of an ion-induced interdigitated bilayer phase in a zwitterionic lipid. In addition, gel state DPPC bilayers in 1 M KBr imbibe approx. 10 Å more solvent than bilayers in water. The influence of these same salts on the phase transitions of DPPC bilayers was also examined using high-resolution differential scanning calorimetry. These results are discussed in terms of ion-induced changes in solvent and solvent/bilayer structure.

Introduction

The presence of ions influences the structures and properties of model and biological membranes. It is the general consensus that divalent and trivalent cations bind to phosphatidylcholine bilayers [1–5] to the extent that large electrostatic forces are produced which cause bilayers to swell to large separation distances [6–8]. These ions are thought to bind to the phosphate moiety of the phosphatidylcholine head group, thereby allowing the choline moiety to infer a net positive charge to the bilayer surface. The influence of monovalent cations and anions on phosphatidylcholine structures [9–15] and interactions [16,17] has not been as well documented. There is recent evidence to support the recurring view that monovalent salts bind to some extent to phosphatidylcholine bilayers resulting in changes in the bilayer packing of the lipid molecules.

Electrophoretic mobility measurements [18] and chromatography [19] have provided direct evidence for the binding of monovalent ions to phosphatidylcholine bilayers when one molar salt solutions are present. Various calorimetric [9–11] and spectroscopic [12–15] studies have indicated that the presence of monovalent ions influence lipid bilayer packing. We have examined the influence of monovalent cations and anions in 1 M solutions on phosphatidylcholine bilayer structural parameters. Similar studies on the influence of divalent metal ions on dipalmitoylphosphatidylcholine bilayer separations [6] were instrumental in phenomenologically predicting the extent of Me^{2+} binding to DPPC bilayers. The choice of 1 M solutions is related to our previous observations [16,17] that these solutions change the repulsive force between DPPC bilayers. Preliminary results indicate that KBr and KSCN are the only salts in this study that cause a dramatic change in the

Correspondence address: Dr. L.J. Lis, Department of Physics and The Liquid Crystal Institute, Kent State University, Kent, OH 44242, U.S.A.

0005-2736/86/$03.50 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

bilayer repeat spacing. A previous study [20], in which an analogue of phosphatidylcholine containing mixed acyl chains was used, may not be descriptive of all ionic influences on zwitterionic phospholipid bilayers. In particular, the presence of 1 M KSCN is found to induce an interdigitated acyl chain phase in DPPC bilayers. The presence of most monovalent salts was also found to increase the pre- and main DPPC bilayer phase-transition temperature. However, the pretransition was not observed for DPPC bilayers in the presence of 1 M KSCN. The transition to an interdigitated bilayer phase caused the main phase transition to occur at a lower temperature with a higher transition enthalpy.

Materials and Methods

L-α-Dipalmitoylphosphatidylcholine was obtained from Avanti Polar Lipids (Birmingham, AL). Salts were reagent grade and obtained from Fisher Chemical Co. All lipids were used without further purification.

Chloroform was removed from the lipid using a rotovaporator, followed by drying under vacuum for at least 2 h to ensure complete removal of the chloroform. 1 M salt solutions were prepared using distilled water.

X-ray samples were prepared by mixing known amounts of DPPC in a 1 M salt solution and allowing equilibration to occur over 48 h. The lipid-salt solution samples were then transferred to X-ray sample holders and placed in Guinier-type cameras to obtain the X-ray powder pattern. The Cu $K\alpha_1$ line ($\lambda = 1.540$ Å) from a Dunlee X-ray tube connected to a Picker Instruments 6238 diffraction generator was isolated using nickel foils. A Philips X-ray film reader was used to measure the diameters of the circular diffraction patterns. Powdered Teflon was mixed in the samples to provide an internal camera standard. The lattice repeat spacing, d, is calculated directly from our film readings. With less than full hydration, the d-spacing can be converted into the bilayer thickness, d_L, and the water layer thickness, d_w from the volume fraction of the lipid in the sample (ϕ) where: $d_L = \phi d$ and $d_w = d - d_L$. The volume fraction of the lipid is determined by the expression:

$$\phi = \left[1 + \frac{(1-c)\bar{\nu}_w}{c\bar{\nu}_L}\right]^{-1}$$

where c is the weight fraction of lipid in the sample, and $\bar{\nu}_w$ and $\bar{\nu}_L$ are the partial specific volumes of water and phospholipid, respectively [21].

Calorimetry samples were prepared by initially pipetting known weights of DPPC dissolved in chloroform in a culture tube and evaporating the chloroform under a stream of nitrogen gas. During this process, the culture tube was rotated by hand to produce a thin layer of lipid on the bottom of the tube. The lipid was then dried under vacuum for 1 h to remove the chloroform completely. An appropriate amount of salt solution was added to the lipid to make a dispersion of 5 mg lipid per 1 ml salt solution. A dispersion was formed by sonicating the lipid salt solutions in a bath sonicator (Bransonic 220) for approx. 1 min. The samples were incubated in an oven at 60°C for 1 h with additional sonication for a few minutes to completely disperse the lipid.

Sample and salt solution reference were delivered to the respective cells by syringe and the cells were tightly sealed. The calorimeter cells were then equilibrated for a minimum of 30 min before measurements were taken. High sensitivity differential scanning calorimetry was performed on a MicroCal MC-2. All calorimetry scans on the MC-2 were performed at a scan rate of 12 Cdeg/h. After the first scan, the sample and reference were cooled in the calorimeter cells and rescanned to check reproducibility. All samples were run under a nitrogen pressure of 15 lb/inch^2 to minimize bubble formation in the sample and reference.

The lipid dispersion and the reference solution were extracted from the calorimeter cells using methanol.

The lipid concentration of the dispersion was determined by ashing the sample in a warm oven (approx. 45°C) for several hours.

Thermograms obtained with the MicroCal MC-2 were analyzed with the MicroCal DA2 software package using an IBM PC interfaced to the calorimeter. Enthalpy was calibrated using an internal electronic calibration. The onset temperature of the thermogram was used as the transition temperature.

Results and Discussion

DPPC bilayers swell continuously in water and salt solutions until the limiting bilayer repeat distance (d-spacing) is obtained. In this regime, the d-spacing increases as the solvent content increases entailing a combination of a decreasing bilayer thickness and an increasing water separation between bilayers. The limiting d-spacing is produced for neutral phospholipid bilayers at specific solvent contents which defines the limiting bilayer thickness (d_L) and water separation (d_w). DPPC bilayers in 1 M chloride salts of various monovalent cations and the divalent cation Ba^{2+} swell in the same manner as DPPC bilayers in water (i.e., limiting d-spacing of 62–63 Å at approx. 70 volume% DPPC). These results (Table I) are in contrast to our observation that the phase transition temperatures are increased in the presence of these salt solutions (Table II). The presence of 1 M LiCl causes the DPPC pretransition to increase by approx. 5 Cdeg. This result is similar to the previous observation of the effect of 30 to 50 mM $CaCl_2$ on this same transition [25]. We can model the extent of Li^+ binding to DPPC bilayers. If we assume that ion binding to DPPC influences the thermogram for the gel to liquid-crystal phase transition, then we can infer that Li^+ (in the presence of 1 M LiCl) and Ca^{2+} (in the presence of 30 to 50 mM $CaCl_2$) bind in similar quantities to the DPPC headgroups, since their influence on the thermograms is similar. It was determined previously [7,8] that Ca^{2+} binding to DPPC bilayers in the presence of 30 mM $CaCl_2$ was at a ratio of one ion per 20 lipid headgroups at the maximum hydration examined. We can infer that at least one Li^+ binds to 20 DPPC headgroups under the conditions we employed.

TABLE I

BILAYER STRUCTURAL PARAMETERS FOR DIPALMITOYLPHOSPHATIDYLCHOLINE IN VARIOUS SALT SOLUTIONS. ALL MEASUREMENTS WERE AT ROOM TEMPERATURE (25°C) WITH THE BILAYERS IN THE GEL STATE

Salt solution	Limiting parameters			
	ϕ	d (Å)	d_L (Å)	d_w (Å)
H_2O	70.3	62.0 [a]	43.6 [a]	18.4 [a]
1 M KCl	70.0	63.1 [a]	44.2 [a]	18.9 [a]
1 M NaCl	71.3	62.8	44.8	18.0
1 M CsCl	69.7	64.4 [a]	44.9 [a]	19.6 [a]
1 M LiCl	70.7	63.1 [a]	44.6	18.5 [a]
1 M NH_4Cl	72.6	62.8	45.6	17.2
1 M $BaCl_2$	71.5	62.0	44.3	17.7
1 M potassium acetate	69.9	62.8	43.9	18.9
0.5 M K_2SO_4	68.9	62.8	43.3	19.5
1 M KBr	60.0	71.9	43.1	27.8
1 M KSCN	52.9	52.3	27.7	24.6

[a] Values taken from Ref. 24.

TABLE II

THERMODYNAMIC PARAMETERS FOR DPPC BILAYERS IN IONIC SOLUTIONS WHEN THE SCAN RATE IS 12 Cdeg/h

Salt solution	Pretransition		Main transition	
	T_p (K)	ΔH_p (kcal/mol)	T_m (K)	ΔH_m (kcal/mol)
H_2O	306.5	0.96	313.1	7.80
1 M KCl	307.0	0.70	314.0	6.05
1 M NaCl	308.1	0.85	314.7	6.30
1 M CsCl	307.2	0.91	314.1	6.00
1 M LiCl	311.7	0.94	314.9	6.43
1 M NH_4Cl	307.8	0.51	314.0	7.53
1 M $BaCl_2$	308.6	0.63	314.0	5.07
1 M potassium acetate	309.7	1.10	314.4	7.14
0.5 M K_2SO_4	307.3	0.99	314.1	6.61
1 M KBr	305.1	0.72	313.7	6.49
1 M KSCN	–	–	311.5	8.34

The lack of extensive solvent uptake for DPPC bilayers on 1 M LiCl is probably due to the screening of the electrostatic repulsion between the (now) charged lipid bilayers by the high molar concentration of salt present.

The interaction of monovalent anions and the divalent sulphate anion with DPPC bilayers appears more complex. Although the majority of potassium salts studied had no effect on the net bilayer repeat spacing (Table I), DPPC bilayers in 1 M KBr or KSCN showed dramatic changes in the bilayer repeat spacing (Table I and Fig. 1). The change in *d*-spacing for DPPC bilayers in 1 M KBr is due solely to a change in the interbilayer separation. This additional swelling is unique among the salts studied, and must be caused by an increase in the repulsion between DPPC bilayers in the presence of 1 M KBr. One can simplistically assume that this increased repulsion is due solely to an increase in the electrostatic repulsion between bilayers caused by K^+ and/or Br^- binding

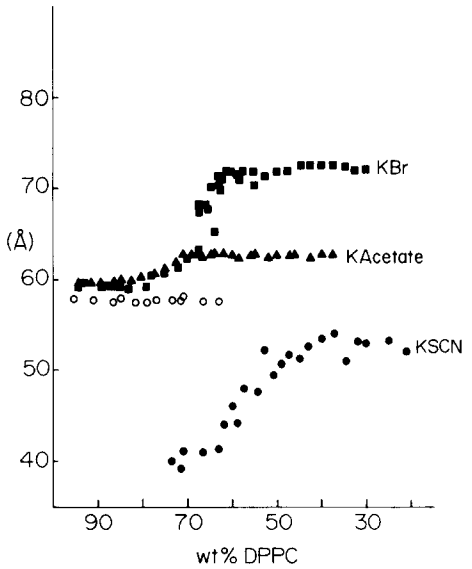

Fig. 1. The swelling curves (*d*-spacing as a function of lipid volume fraction) for DPPC bilayers in 1 M potassium acetate (▲), KBr (■), and KSCN (●, ○). DPPC bilayers in 1 M potassium acetate and KBr are in the gel state. The swelling curve for DPPC bilayers in 1 M KSCN is biphasic. The gel lamellar phase exists at low solvent contents (○) and the interdigitated acyl chain lamellar phase exists at high solvent contents (●). There is a region between these single phase regions, where X-ray diffraction lines from both phases are observed.

to the lipid head groups. This would require that more than 1 charge per five lipid head groups be present in order to obtain an increase in the bilayer separation of 10 Å [7,8]. Historically, the influence of monovalent ions on DPPC bilayers has also been correlated with the ion's influence on the hydrogen bonding structure of water. These changes in water structure are related to changes in the polarizability of the water and therefore influence the hydration repulsive force between bilayers [26]. It has been shown that the influence of ions on the repulsive forces between DPPC bilayers can be partially explained by the interaction of salts on water structure [17]. A major difference between monovalent cations in general and some anions is in their influence on the water hydrogen bonding structure [9,11,12]. Monovalent cations are believed to disorder the structure of water, whereas monovalent anions are known to order the water structure [27]. This change in the entropy of the water when anions are present would, however, result in an increased repulsion between bilayers [16] based on the polarization model for the hydration force. It must be concluded, then, that the increase in repulsion between DPPC bilayers in the presence of KBr is caused by extensive ion binding to the lipid headgroups and a change in the polarization of the water layer between the bilayers. This additional repulsive force arising from the water structure is not sufficient by itself to produce the swelling properties that are observed but is a necessary condition allowing electrostatic repulsion to effectively propagate over the distances observed. We have, at this time, no explanations as to why DPPC bilayers swell differently in 1 M KBr than in the other salt solutions studied.

The DPPC bilayer pretransition temperature in the presence of potassium salts increases in the order:

$$Br^- < Cl^- < SO_4^{2-} < \text{acetate}^-$$

and the main transition temperature increases in the order:

$$SCN^- < Br^- < Cl^- < SO_4^{2-} < \text{acetate}^-$$

It can be inferred that the presence of the above anions causes an increase in the degree of K^+

binding to the DPPC bilayers in the same order as the increases in phase-transition temperatures (Table II). This is also additional evidence that the increase in the DPPC bilayer repeat spacing in the presence of 1 M KBr is due in part to a change in the polarization of the water between the bilayers rather than solely to K^+ binding to the DPPC bilayer. The change in the DPPC bilayer thermogram in 1 M KSCN (Fig. 2) is due to the presence of an interdigitated bilayer phase as shown in the X-ray data for this system (Fig. 1).

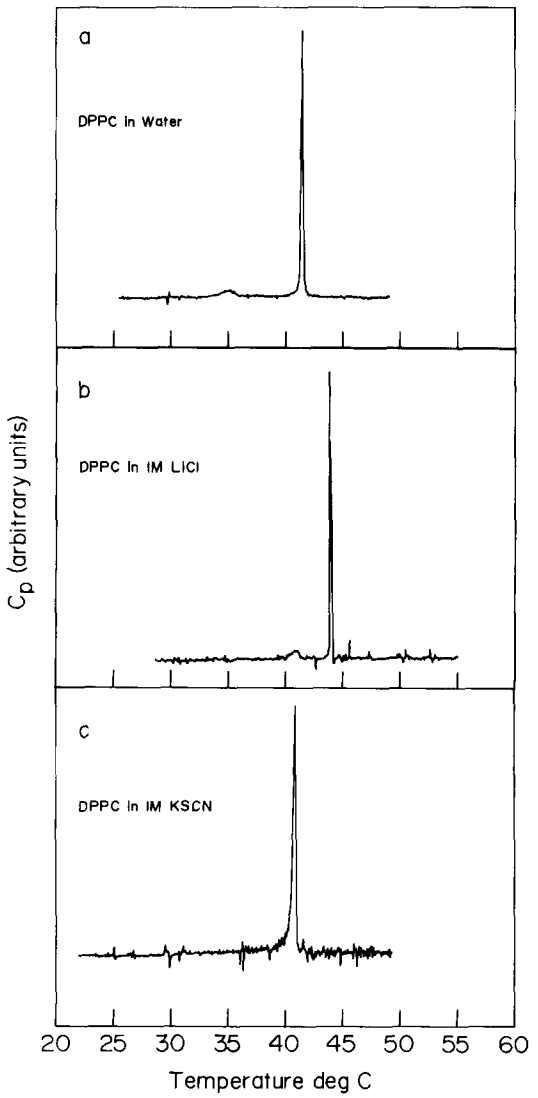

Fig. 2. Calorimetric curves of excess specific heat versus temperature for the pre- and main transitions of fully hydrated DPPC bilayers in (a) H_2O, (b) 1 M LiCl and (c) 1 M KSCN.

The change in the *d*-spacing for DPPC bilayers in 1 M KSCN is due to the formation of a bilayer phase with interdigitated acyl chains and an increase in the solution between bilayers. The X-ray patterns have the characteristic [28–34] small *d*-spacing (52.3 Å at full hydration), small limiting bilayer thickness (27.7 Å) and 4.09 Å wide-angle X-ray scattering from the acyl chains. This phase is dependent on the amount of solvent present (Fig. 1) and the concentration of KCNS. Fully hydrated DPPC bilayers in 10 or 100 mM KSCN produce X-ray patterns characteristic of the usual gel phase. The formation of interdigitated phases in phosphatidylcholines typically requires a change in the solvent structure adjacent to the bilayer [32,34]. The presence of solvents such as benzyl alcohol [32], phenyl alcohol [32], chlorpromagene [32] and ethanol [34] is required to induce the DPPC interdigitated bilayer phase. One can assume that the change in the solvent structure or water polarization caused by the presence of 1 M KSCN is responsible for the appearance of the interdigitated phase. Further evidence that solvent structure is significantly modified is the additional repulsion between the bilayers resulting in an increase in interbilayer separation to 24 Å.

In summary, the interaction of some monovalent cations and anions with DPPC bilayers involves ion binding to the lipid headgroups. Modification of the interactions between bilayers and the molecular packing within a bilayer may also result from the influence ions exert on the water structure in the double layer region.

Acknowledgements

This research was supported in part by a research grant from the National Institutes of Health (GM 33570).

References

1 Gradalsen, H., Eriksson, L.E.G., Westman, J. and Ehrenberg, A. (1977) Biochim. Biophys. Acta 469, 151–162
2 Hauser, H., Hinkley, C.C., Krebs, J., Levine, B.A., Phillips, M.C. and Williams, R.J.P. (1977) Biochim. Biophys. Acta 468, 364–377
3 Hutton, W.C., Yeagle, P.L. and Martin, R.B. (1977) Chem. Phys. Lipids. 9, 255—265

4 McLaughlin, A.C., Grathwohl, C. and McLaughlin, S. (1978) Biochim. Biophys. Acta 513, 338–357
5 Lau, A.L.Y., McLaughlin, A.C., MacDonald, R.C. and McLaughlin, S.G.A. (1980) Adv. Chem. Ser. 188, 49–56
6 Ohshima, H., Inoko, Y. and Mitsui, T. (1981) J. Colloid Interface Sci. 86, 57–72
7 Lis, L.J., Parsegian, V.A. and Rand, R.P. (1981) Biochemistry 20, 1761–1770
8 Lis, L.J., Lis, W.T., Parsegian, V.A. and Rand, R.P. (1981) Biochemistry 20, 1771–1777
9 Simon, S.A, Lis, L.J., Kauffman, J.W. and MacDonald, R.C. (1975) Biochim. Biophys. Acta 375, 317–326
10 Chapman, D., Urbina, J. and Keough, K.M. (1974) J. Biol. Chem. 249, 2512–2521
11 Chapman, D., Peel, W.E., Kingston, B. and Lilley, T.H. (1977) Biochim. Biophys. Acta 464, 260–275
12 Jendrasiak, G.L. (1972) Chem. Phys. Lipids 9, 133–146
13 Lis, L.J., Kauffman, J.W. and Shriver, D.F. (1975) Biochim. Biophys. Acta 406, 453–464
14 Loshchilova, E. and Karvaly, B. (1978) Biochim. Biophys. Acta 514, 274–285
15 Karvaly, B. and Loshchilova, E. (1977) Biochim. Biophys. Acta 470, 492–496
16 Afzal, S., Tesler, W.J., Blessing, S.K., Collins, J.M. and Lis, L.J. (1984) J. Colloid Interface Sci. 97, 303–307
17 Kwok, W.M., Shimotake, J.E. and Lis, L.J. (1985) Mol. Cryst. Liq. Cryst. 132, 181–188
18 Tatulian, S.A. (1983) Biochim. Biophys. Acta 736, 189–195
19 Cserhati, T. and Szogi, M. (1983) Chem. Phys. Lipids 34, 93–99
20 Gottlieb, M.H. and Eanes, E.D. (1972) Biophys. J. 12, 1533–1548
21 Levine, Y.K. (1973) Prog. Surf. Sci. 3, 279–352
22 Lis, L.J., McAlister, M., Fuller, N., Rand, R.P. and Parsegian, V.A. (1982) Biophys. J. 37, 657–666
23 Tardieu, A., Luzzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733
24 Cunningham, B.A., Shimotake, J.E., Tamura-Lis, W., Mastrin, T., Kwok, W.-M., Kauffman, J.W. and Lis, L.J. (1986) Chem. Phys. Lipids 39, 135–143
25 Ganesan, M.D., Schwinke, D.L. and Weiner, N. (1982) Biochim. Biophys. Acta 686, 245–248
26 Guren, D.W.R. and Marcelja, S. (1983) J. Chem. Soc. Faraday Trans. 2, 79, 225–242
27 Leyendekkers, J.V. (1983) J. Chem. Soc. Faraday Trans. 1, 79, 1123–1134
28 Ranck, J.L., Keira, T. and Luzzati, V. (1977) Biochim. Biophys. Acta 488, 432–441
29 Ranck, J.L. and Tocanne, J.F. (1982) FEBS Lett. 143, 171–174
30 Ranck, J.L. and Tocanne, J.F. (1982) FEBS Lett. 143, 175–178
31 McDaniel, R.V., McIntosh, T.J. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 97–108
32 McIntosh, T.J., McDaniel, R.V. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 109–114
33 Serrallach, E.N., Dijkman, R., DeHaas, G.A. and Shipley, G.G. (1983) J. Mol. Biol. 170, 155–174
34 Simon, S.A. and McIntosh, T.J. (1984) Biochim. Biophys. Acta 733, 169–172