Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Version of record first published: 20 Apr 2011.

To cite this article: W. M. Kwok , J. E. Shimotake & L. J. Lis (1986): Effect of Ion Species on Interactive Forces Between Phosphatidylcholine Bilayers, Molecular Crystals and Liquid Crystals, 132:1-2, 181-188

To link to this article: http://dx.doi.org/10.1080/00268948608079538

Mol. Cryst. Liq. Cryst., 1986, Vol. 132, pp. 181–188
0026-8941/86/1322–0181/$15.00/0

Printed in the United States of America

Effect of Ion Species on Interactive Forces Between Phosphatidylcholine Bilayers†

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(*Received October 1, 1984*)

We have investigated the effect of varying cation species in salt solutions on the hydration force acting between neutral lipid surfaces using multilamellar arrays of biological liquid crystals. The osmotic pressure technique of Rand and Parsegian [LeNeveu et al., *Nature* **259**, 601, (1976)] was used to obtain the net repulsive pressure between lipid bilayers and X-ray diffraction was used to determine the bilayer structure parameters. Net repulsive pressure versus lamellar separation relationships for dipalmitoylphosphatidylcholine (DPPC) bilayers in *1 M*. KCl, NaCl, LiCl, or CsCl solutions are compared. Preliminary results indicate a difference in the apparent force decay for DPPC bilayers in LiCl when compared to bilayers in NaCl, KCl, and CsCl.

INTRODUCTION

The investigation of interactive forces acting between phospholipid bilayers has generated much interest due to its importance in our understanding of cell-cell and vesicle-cell interactions, and colloidal stability. The forces are generally characterized as 1.) the electrostatic force, 2.) the van der Waals force, and 3.) the hydration force. The long range repulsive electrostatic force and attractive van der Waals force are relatively well understood both experimentally and theoretically. The short range repulsive hydration forces known to have an exponentially decaying form with an effective range of approximately 3 Å have been experimentally observed for phospholipids

†Presented at the Tenth International Liquid Crystal Conference, York, July 15–21, 1984.

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and mica surfaces.[1-3] The exact origin of this force, however, has yet to be determined. Several theories have been proposed[4-11] including surface-induced local polarization of water structure in aqueous media[4-6] and image-charge effects at the lipid bilayer's surface[7,8] to account for the presence of this short range repulsive force.

The works of Rand, Parsegian and co-workers[1,12-19] have expanded our knowledge of interactive forces between lipid bilayers. The initial observation that dextran can be used to produce an osmotic pressure[1,12] which can then dehydrate lipid bilayers (whose structural parameters can be determined from x-ray diffraction) greatly facilitated the studies of interactive forces both quantitatively and qualitatively. The initial reports[1,12] and subsequent characterization of the hydration repulsive force,[13,14] the determination of theoretical approaches to characterize electrostatic forces,[15-18] the determination of Hamaker constants[12,14] and the characterization of bilayer compressibilities[19] have shown the usefulness of this technique in describing fundamental phenomena of interest in colloid science and biophysics.

Afzal et al.[20] have previously investigated the effects of varying ion concentrations on the interactive forces acting between dipalmitoylphosphatidylcholine (DPPC) bilayers. The qualitative results obtained indicate that the hydration force and van der Waals forces decrease with increasing ion concentration. These results were hypothesized to be due to decreased structuring of the solvent structure between the lipid bilayers as the ionic concentration increases.

This report is a continuation of the above work. The influence of cation species on the hydration force between phosphatidylcholine bilayers was studied. Specifically, the influence of one molar chloride solutions of K^+, Na^+, Li^+, and Cs^+ on the hydration force between DPPC bilayers was determined. Preliminary osmotic pressure measurements indicate that the bilayer repulsive force at any bilayer separation is different for bilayers in LiCl when compared to bilayers in NaCl, KCl, and CsCl. We can hypothesize that Li^+ either has no effect on water structure, or produces an increased structuring of the water which balances the effect of increasing the ion concentration in solution.

MATERIALS AND METHODS

Dipalmitoylphosphatidylcholine was obtained from the Sigma Chemical Company (St. Louis, Mo, USA) and Avanti Polar Lipids (Bir-

mingham, Al. USA) and used without further purification. Dextran 2000 was obtained from Pharmacia Fine Chemical of Uppsala, Sweden. All salts were reagent grade. The salts were used in one molar solutions. Distilled water was used for all lipid and dextran samples.

The x-ray diffraction method was used to determine the lipid bilayer structural parameters.[12-14] The lipid repeat spacing, d, was obtained by Bragg diffraction, and the bilayer thickness, d_1, and bilayer separation, d_w, were inferred by the following relationships: $d_l = \phi d$ and $d_w = d - d_l$, where ϕ is the volume concentration of lipid in the sample. The volume fraction of lipid is approximated by the weight percentage of lipid in the solution.

Gravimetric results were obtained by adding a known amount of lipid to varied amounts of solution and allowing the sample to equilibrate for 36 to 46 hours at room temperature. The samples were then placed in aluminium x-ray holders and the bilayer repeat distance was obtained by Bragg diffraction. A collimated CuK_α beam was placed before the sample and focused onto a film cassette. Exposures were for 4–14 hours.

The osmotic pressure technique of Rand and Parsegian[1,12-14] was used to apply an external pressure to the system. Dextran was mixed with each salt solution and added to DPPC in weighing bottles. The dextran molecules, which have a high molecular weight, and the lipid bilayers compete for available water in the system. This competition is measured as the osmotic pressure of the dextran solution. The samples are then equilibrated at room temperature for 36 to 48 hours. Upon reaching equilibrium, the samples are transferred to aluminium sample holders. By measuring the refractive index of dextran, the concentration of dextran in each sample can be determined which, in turn, is used to infer the osmotic pressure.

As before, Bragg diffraction was used to determine the bilayer repeat distance. The values of d_l and d_w were inferred by comparing the values of d thus obtained with the d-spacings obtained earlier gravimetrically.[1,12-14]

The applied pressure versus lamellar separation relationships were obtained for the same lipid in different salt solutions by combining the net repulsive force exerted by dextran with the bilayer repeat distance. The general trend of the data fits an exponentially decaying function: $P = P_o \exp(-d_w/\lambda)$ where P_o is the pressure needed to remove water completely from the bilayer separations and $1/\lambda$ is the rate of change of repulsive force with d_w.

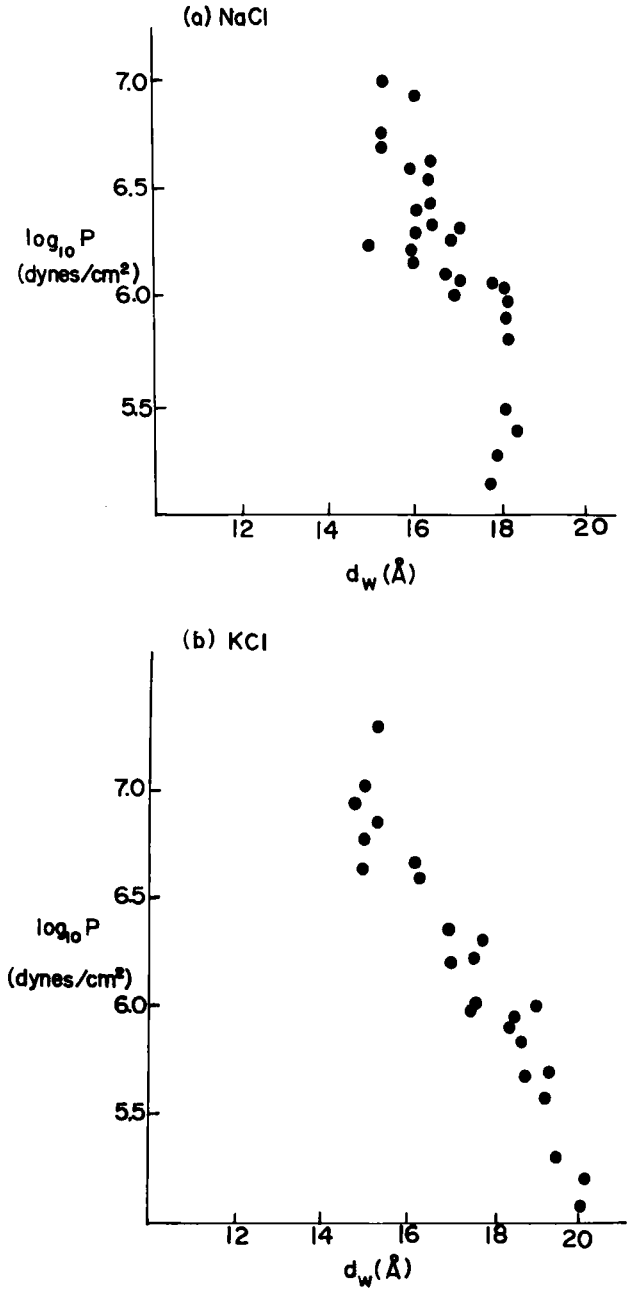

FIGURE 1. Applied osmotic pressure, $\log_{10}P$, as a function of bilayer separation, d_w, for DPPC bilayers in 1 M solutions of a) NaCl, b) KCl, c)LiCl, and d) CsCl.

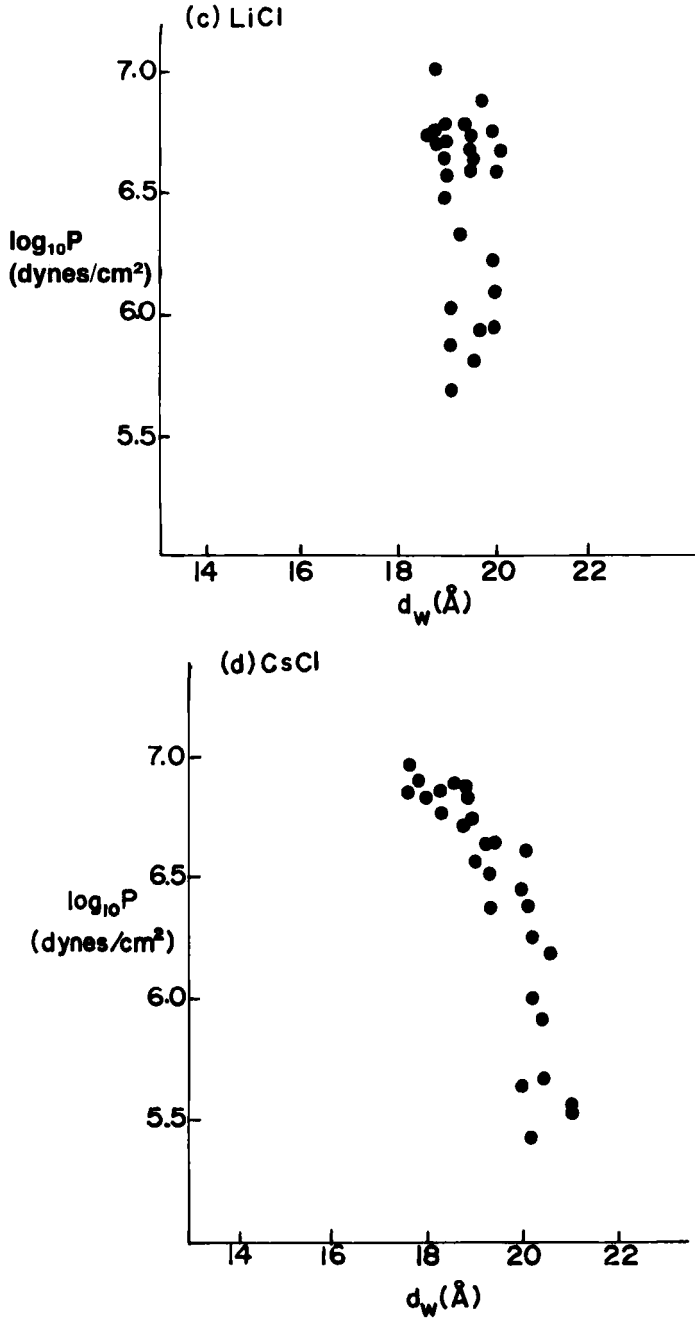

FIGURE 1 continued

RESULTS AND DISCUSSIONS

Known quantities of DPPC and 1 M solutions of LiCl and CsCl were mixed and allowed to equilibrate. The repeat spacings as a function of weight percentage lipid were obtained for each system by Bragg diffraction. The swelling behavior of the DPPC bilayers in LiCl and CsCl is similar to that of DPPC in water and 1 M solutions of KCl and NaCl.[14,20] Gottlieb and Eanes[21] have observed decreases in bilayer thickness in systems of synthetic lecithin (1-octadec-9-enyl-2-hexadecylglycerophoscholine) in 1 M solutions of LiCl, NaCl, KCl, and CsCl as compared to the bilayer thickness in water. The greatest decrease occurred in KCl and CsCl solutions. Such decreases are not apparent for DPPC in any of the one molar chloride solutions used in these studies. The difference is probably due to the types of lecithin used.

The repulsive pressure resulting from the osmotic pressure of the dextran molecules between the lipid bilayers was also measured. Figure 1 shows our preliminary data for the change in bilayer separation as a function of applied osmotic pressure for DPPC in various 1 M ionic solutions. The curves for DPPC bilayers in 1 M NaCl and KCl solution were originally obtained by Afzal et al.[20] We can hypothesize that the influence of the monovalent salts on DPPC swelling is primarily non-electrostatic since hydration forces dominate in the region of separation (<20Å), and we observe no break between hydration and electrostatic force curves as seen when other ions bind to DPPC bilayers and cause electrostatic swelling.[15-18]

Cations are thought to "break" the structure of water.[22] Table I shows the decay rates of the repulsive force with respect to the interbilayer separation d_w exhibited by the DPPC bilayers in all the ionic solutions studied. The values for NaCl and KCl solutions were obtained by Afzal et al.[20] These data indicate that the decay of force

TABLE I

Slopes and intercepts of $\log_{10}P$ versus d_w with the corresponding standard deviation

Salt	Slope ($\log_{10}P/d_w$)	Intercepts ($\log_{10}P$)
NaCl	-0.25 ± 0.21[a]	10 ± 3[a]
KCl	-0.30 ± 0.20[a]	11 ± 2[a]
LiCl	-0.20 ± 0.06	10.4 ± 1.1
CsCl	-0.23 ± 0.03	11.1 ± 0.5

[a]Values taken from reference 20.

with interbilayer separation for DPPC bilayers in NaCl, KCl, and CsCl is greater than for DPPC bilayers in pure water or 1 M LiCl. The influence of Li^+ thus appears to counter the influence of increasing ion concentration on the hydration force between bilayers resulting in a force versus separation relationship similar to that of bilayers in water[14] in the force region studied. These results are consistent with the interpretation of Li^+ binding to a greater extent than Na^+, K^+, or Cs^+ to DPPC bilayers,[23] and producing an additional (electrostatic) repulsion force. However, we cannot completely rule out the influence of monovalent cations on the water structure affecting the net repulsive force between bilayers. The next stage of this study will involve changing anionic species since $SO_4^=$ and possibly $CO_3^=$ are thought to be "structure makers" of water.[22] This later work could lead to a confirmation of the prediction that the hydration force is influenced by the structure of the water between the surfaces studied.

ACKNOWLEDGMENTS

We thank J. M. Collins for valuable discussions throughout the course of this work. This research was supported by NIH grant GM 33570.

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