Biochimica et Biophysica Acta, 375 (1975) 317–326

BBA 76855

A CALORIMETRIC AND MONOLAYER INVESTIGATION OF THE INFLUENCE OF IONS ON THE THERMODYNAMIC PROPERTIES OF PHOSPHATIDYLCHOLINE

S. A. SIMON[a,*], L. J. LIS[a], J. W. KAUFFMAN[b] and R. C. MACDONALD[c]

[a]*Department of Materials Science*, [b]*Departments of Materials Science and Biological Sciences, and* [c]*Department of Biological Sciences, Northwestern University, Evanston, Ill. 60201 (U.S.A.)*

(Received July 29th, 1974)

SUMMARY

The effects of various ions and ^2H_2O on the thermal properties of phosphatidylcholine dispersions were studied using differential scanning calorimetry and the change in the surface potential of monolayers with temperature. The phosphatidylcholine in ^2H_2O dispersion exhibits a slightly higher transition temperature and lower enthalpy of melting than a phosphatidylcholine in H_2O dispersion. Monovalent (H^+, Na^+, and Li^+) and some divalent cations of chloride salts (Ba^{2+}, Mg^{2+}, and Sr^{2+}) have no effect on the thermal properties of phosphatidylcholine, while halide salts of the di-positive ions Cd^{2+} and Ca^{2+} have an effect on both the enthalpy of melting and transition temperature. No effect attributable to the metal ion was observed in non-halide salts of cadmium. The chloride salt of La^{3+} has no effect on lipid thermal properties whereas that of Fe^{3+} affects the transition temperature. The enthalpy of melting of phosphatidylcholine in one molar solutions of potassium salts increases in the order: $CNS^- >$ acetate $> I^-$. Such large, polarizable anions clearly interact with phosphatidylcholine and must therefore also confer a negative charge on the lipid. The potassium salt of SO_4^{2-} has no effect. Possible origins of the observed trends are discussed.

INTRODUCTION

Recent investigations of the mesomorphic phase transitions of lipid dispersions [1, 2] have led to a better understanding of the physical properties of lipids in biological membranes. It is now recognized that both natural and synthetic phospholipids may exist in any of several different phases. The particular phase a given phospholipid prefers, at a given pressure and temperature, will depend on the amount of water present, ionic strength and pH of the aqueous phase, length and degree of unsaturation

* Present address: Department of Physiology, Duke University School of Medicine, Durham, N.C. 27710, U.S.A.

of the fatty acids, and presence of other molecules such as proteins and organic solvents [1, 3–6].

According to elementary considerations of solution theory, phase relationships in a two-component system may be expected to be altered by the addition of a third component. This paper will deal with the influence of certain common electrolytes on the phase behavior of aqueous dispersions of dipalmitoyl- and dimyristoylphosphatidylcholines. As most biological membranes are bathed by salt solutions, an objective of this work was to determine the influence of salts on biological membranes.

The phase relationships of an aqueous dispersion of phospholipid can be affected by the addition of a solute to the aqueous medium in one or more of five ways. First, the addition of a solute will alter the structure of water itself and this may be translated, via changes in the strength of hydrophobic interactions, into an influence on membrane structure [7]. Second, the addition of electrolytes may dehydrate the membrane. Third, solutes may bind to the membrane surface and impart a surface change to the formerly neutral membrane. Fourth, some solutes may partition into the hydrophobic region and destroy the long range order of the hydrocarbon region. Fifth, electrolytes may modify lipid polar group interactions by ionic strength effects. In any event, one may anticipate that the presence of high electrolyte concentrations in the aqueous phase will have a demonstrable effect on the structure of lipid bilayer membranes. Qualitatively at least, a similar influence on biological membranes might be expected. In addition, lipid dispersions, unlike most biological membranes, are multilamellar structures and are, therefore, potentially subject to interbilayer effects. For example, high concentrations of electrolytes in a lipid dispersion may cause adjacent bilayers to move closer together by removing water between them to the extent that their proximity might be sufficient for their mutual interaction [8]. In order to determine whether the effects on transition parameters, as observed by calorimeters, might be attributed to interbilayer interactions, studies of monolayers (in which no such interactions may occur) were performed in parallel with the calorimetric studies. If precautions are observed to prevent spreading solvents from remaining in the monolayer, phosphatidylcholine monolayers exhibit phase transitions, indicated by a rather sharp decrease in surface potential, at the same temperature as corresponding bilayers.

MATERIALS AND METHODS

L-α-Dipalmitoylphosphatidylcholine was purchased from Schwartz-Mann (Rockville, Md.) and was used without further purification. Thin-layer chromatography revealed a single component. L-α-Dimyristoylphosphatidylcholine was purchased from Nutritional Biochemicals (Cleveland, Ohio) and purified by standard column-chromatographic techniques so that it also exhibited a single spot by thin-layer chromatography. The water was twice distilled, the second time over glass. All salts were roasted at 600 °C for 24 h or washed in purified chloroform to remove nonpolar impurities. The solvents used in spreading monolayers were passed through an alumina column. Unless otherwise specified, dicyclohexyl was the spreading solvent. The ^2H_2O was 99.7 mole %.

Calorimetric measurements were performed with a Perkin-Elmer DSC 1 modified to give the characteristics of a DSC 1B. The temperature scale was calibrated

from melting point standards (James Hinton, Columbia, S.C.) whereas the enthalpy per unit area was calibrated using both indium (99.9999 % pure, Fisher Scientific Co.) and stearic acid (James Hinton). Calibrations were performed prior to each experiment. The temperature scale was accurate to ± 0.3 °C. The variation of the enthalpy measurements was ± 4 %. The transition temperature(s), T_m, used here corresponds to the temperature at the apex of the transition peak. This choice gave much better reproducibility than the temperature of the onset of melting. The enthalpy of transition(s), ΔH_m, was obtained by measuring the area under the transition peak, and the entropy of the transition(s) was calculated from $\Delta S = \Delta H_m / T_m$. As the systems which we dealt with were all multicomponent, ΔH and ΔS represent partial molar quantities.

The lipid was added to a volatile sample holder with enough electrolyte solution so that a solution: lipid weight ratio of 2 : 1 (unless otherwise specified) was obtained. The lid was then hermetically sealed onto the holder and the sample was allowed to equilibrate for two hours at 60 °C and usually another hour at room temperature. The heating and cooling rate was 5 °C/min unless otherwise noted. All samples were cycled at least three times or until reproducible results were obtained on consecutive cycles.

Surface potential measurements were carried out in an 8-ml teflon trough. The surface potential was measured between a polonium air electrode and a grounded calomel electrode in the aqueous phase. The polonium electrode was connected to the input of a Keithley 610C electrometer, the output of which was connected to the Y terminal of an X–Y recorder. The trough had a false bottom so that the temperature could be varied by circulating water from a controlled temperature bath through the bottom compartment. The temperature control of the bath was rotated by synchronous motors geared to give the desired scanning rate (2 °C/min) on heating. The monolayer transition temperature was obtained by taking the arithmetic mean of the temperature between onset and end of melting for the ΔV vs T curves. The temperature was measured with a linear thermistor (Yellow Springs Instrument Co., Yellow Springs, Ohio) inserted 2 mm into the aqueous phase. The output of the thermistor network was connected to the X terminal of the recorder. The temperature was calibrated before each run and was accurate to ± 0.3 °C. The entire unit was enclosed in an aluminum box which provided electrostatic and air current shielding. A syringe needle attached to an aspirator was placed near the air electrode to remove water vapor which otherwise would have condensed on the electrode. The presence of the aspirator did not affect any monolayer properties.

The concentration of lipid in spreading solvent was 10 mg/ml, 5 μl of which solution was injected with a microliter syringe along the side of the trough. This is a convenient way to produce monolayers at collapse pressure since there remains a reservoir of lipid in the lenses of spreading solvent that is contiguous with the monolayer. We have also observed lipid transitions at the appropriate temperature in monolayers spread from petroleum ether–ethanol (9 : 1, v/v) to an area of 50 Å^2 per molecule. Since in these cases there is no excess lipid and the solvent is lost from the monolayer very quickly, it is evident that the transitions under the usual conditions are not artifacts attributable to either the presence of lenses or an excess of lipid. Dimyristoylphosphatidylcholine, rather than dipalmitoylphosphatidylcholine, was chosen as the monolayer for routine measurements because its transition temperature

occurs near room temperature, and the temperature gradient between the aqueous phase and the air as well as the condensation of water on the electrode would therefore be minimal.

RESULTS AND DISCUSSION

Phase transition parameters in the presence of H_2O and ^2H_2O alone

The thermodynamic parameters, ΔH_m, ΔS_m, T_m and $W_{\frac{1}{2}H}$ (the transition width at half peak height of the heating cycle), for the phase transition of the dipalmitoylphosphatidylcholine-heating cycle in excess H_2O and ^2H_2O are given in Table I. The results obtained for H_2O agree reasonably well with previously reported values. Table I also illustrates that the transition temperature is slightly increased in ^2H_2O as opposed to H_2O and the peak is also somewhat broadened. It has been demonstrated that ^2H_2O and H_2O interact differently with the polar region of the phospholipid so as possibly to affect the packing of the hydrocarbon tails [9]. The differences between these modes of interaction are presumably due to structural differences between ^2H_2O and H_2O at interfaces [10], dipalmitoylphosphatidylcholine has also been found to have a higher T_m in ^2H_2O than in H_2O by nuclear magnetic resonance (Davis, D. G., personal communication).

TABLE I

THERMODYNAMIC DATA FOR UNSONICATED DISPERSIONS OF DIPALMITOYLPHOSPHATIDYLCHOLINE IN EXCESS H_2O AND ^2H_2O

Solvent	T_m (°C)	ΔH_m (cal/g)	ΔS_m (cal/degree per mole)	$W_{\frac{1}{2}H}$ (°C)
H_2O	42.1	10.0	23.4	0.6
^2H_2O	43.5	9.07	21.4	0.8

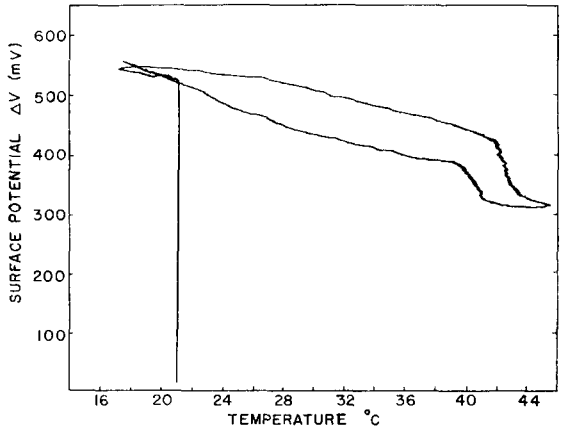

Fig. 1. Surface potential vs. temperature curve of a monolayer of dipalmitoylphosphatidylcholine spread from a 10 mg/ml mixture of *n*-hexane–ethanol (9 : 1, v/v).

The calorimetric experiments in Table I complement the monolayer experiment in Fig. 1, in which the surface potential of a monolayer of dipalmitoylphosphatidylcholine spread on water from a solution of hexane–ethanol (9 : 1, v/v) is plotted as a function of temperature. It can be seen that there is only a slight decline in surface potential up to about 41 °C. The slope of the curve prior to the transition is due to the change in the surface potential of water with increased temperature. The midpoint of the abrupt change in surface potential occurs at 42.6 °C which is in good agreement with the transition temperature obtained by calorimetry. The change in surface potential accompanying the transition is 90 mV, which is 16 % of the total surface potential. This percent change corresponds with that reported in area per molecule (from 48 to 58 $Å^2$) for the transition of lipid bilayers, as determined by X-ray diffraction analysis [11]. The surface potential change of the monolayer was therefore sufficient to indicate a membrane expansion but insufficient to indicate a radical reorientation of dipoles. The fact that both the magnitude of the surface potential change and the transition temperature of lipid monolayers agree with data obtained from lipid bilayer dispersions suggests that a bilayer behaves like two monolayers. This evidence and that obtained through X-ray [11] analysis do not imply appreciable (if any) chain interdigitation.

It should be noted that a monolayer of dipalmitoylphosphatidylcholine does not exhibit the characteristic pre-transition peak (Fig. 1) that has been observed for dipalmitoylphosphatidylcholine dispersions previously by differential scanning calorimetry [2] and light scattering [3]. This peak was first thought to result from a cooperative rotation of the polar head groups [2]. Another interpretation is that the lattice is more expanded above than below the pretransition [4]. If such a change occurred by any of these mechanisms, it certainly should be detected by means of surface potential measurements. A third suggestion which is consistent with the present finding, attributes this peak to the aggregation and disaggregation of small vesicles [13].

Phase transition parameters in the presence of aqueous solutions of 1 : 1 electrolytes

Uni-positive chloride salts. Table II shows the calorimetric data for dipalmitoylphosphatidylcholine in the presence of monovalent salts. The transition parameters in 10^{-4} M HCl (pH 4.0), 1 M LiCl, and 1 M NaCl are virtually identical to those of pure water, in spite of the "structure-making" properties of these cations [14, 15]. In solutions of these electrolytes, therefore, changes in osmotic pressure, hydrocarbon thickness, dielectric constant, surface tension, surface viscosity and differences in ion binding between the gel and liquid crystal phases must be considered secondary to interaction energies within either the gel or the liquid crystal phase. Evidence will be presented below that other salts which have a greater influence on such parameters are also without influence on membrane transition parameters. No obvious aggregation of lipid dispersions occurred in solutions of these or any other monovalent salts at 23 °C.

Concentrated KCl solutions raise the transition temperature slightly and lower the transition enthalpy slightly. It may also be noted that between 1 M and 3.5 M KCl there is little difference in the transition parameters. These effects, while not large, contrast with the lack of effect of LiCl and NaCl. It is difficult to rationalize a difference between salts as similar as NaCl and KCl. There is evidence, however, that the structure of lipid bilayers is slightly different in 1 M KCl than in 1 M NaCl. By X-ray diffraction,

TABLE II

THERMODYNAMIC DATA FOR DIPALMITOYLPHOSPHATIDYLCHOLINE–MONOVALENT SALT SYSTEMS WITH RELEVANT PHYSICAL PARAMETERS

Solvent	T_m (°C)	ΔH_m (cal/g)	ΔS_m (cal/degree per mole)	$W_{1/2H}$ (°C)	Activity coefficient*
10^{-4} M HCl	40.2	10.0	2.4	0.8	0.98
1 M LiCl	40.8	10.1	24.0	1.0	0.77
1 M NaCl	41.0	9.9	23.7	1.0	0.67
1 M KI	40.0	11.2	26.8	0.6	0.64
3 M KI	43.7	12.7	30.2	0.8	0.65
1 M KCNS	40.8	14.7	34.3	0.6	0.59
5 M KCNS	45.2	14.4	33.9	0.9	0.52
1 M KCl	43.6	9.1	21.6	0.8	0.60
3.5 M KCl	42.0	8.5	20.2	1.0	0.56
1 M potassium acetate	41.1	11.4	27.2	0.5	–

* Data from ref. 23.

Gottlieb and Eanes [8] found the thickness of an ether analog of phosphatidylcholine (choline plasmalogen) containing one saturated and one unsaturated fatty acid to be different in the two electrolytes. These workers also found the water content of such lipid dispersions to be similar in 1 M LiCl and 1 M NaCl solutions, but slightly higher (approximately 20 %) in 1 M KCl solutions.

Uni-negative potassium salts. In contrast to the absence of transition anomalies of the dipalmitoylphosphatidylcholine dispersion in the presence of small hydrated cations at 1 M concentrations, the thermodynamic parameters of the lipid phase transition are altered in the presence of solutions containing large, polarizable anions such as I^- and CNS^-. Table II shows that both 1 M CNS^- and 1 M I^- slightly lower the transition temperature and significantly increase the enthalpy of the transition. At concentrations greater than 1 M, CNS^- and I^- raise the transition temperature as well. Although the effect of thiocyanate is greater, the effects of both salts on the enthalpy is well beyond experimental error. Indeed, in 1 M KCNS the transition enthalpy of the lipid is increased by 47 %. This corresponds to about 3.5 kcal/mole, roughly the enthalpy of breaking a hydrogen bond.

The cooling curves of lipid dispersions in 3 M KI and 5 M KCNS revealed two peaks, the smaller of which corresponded to the normal transition temperature. It is possible, therefore, that there is some specific binding between I^- or CNS^- and the lipid and that there is some phase separation of the uncomplexed portion upon cooling. It is also possible that two peaks would be observed in the heating if the temperature were raised very slowly.

I^- and CNS^- have been termed "chaotropic" [7, 16], a word that describes their effect on hydrophobic interactions, and also on biological structures such as membranes that owe their stability to apolar bonds. Since in those instances where the T_m is unaffected, the effect of these ions is to raise ΔS in proportion to ΔH. Such an entropy increase would be consistent with the known effects of chaotropic ions on biological structures [7]. Lipids in biological membranes are in the liquid-crystalline

phase [3], so it is only necessary to postulate that such ions exert a lesser influence on lipids in the gel phase than on those in the liquid crystalline phase to account for the calorimetric results, i.e. that there are more binding sites for the chaotropic agents above the transition than below it. This idea is also consistent with the idea that the excess enthalpy may be due in part to a difference in surface charge density of the bilayer above and below the transition [17].

In cases (like dipalmitoylphosphatidylcholine dispersed in 1 M KCNS) where the transition temperature is slightly lower but where the enthalpy of transition is considerably greater than that of the dispersion in pure water, two non-exclusive interpretations can be given: (1) The gel phase in 1 M KCNS has a lower enthalpy than the corresponding gel phase in pure water and the lipid crystal phases in both systems have the same enthalpy. (2) The gel phases in both systems have the same enthalpies but the enthalpy of the liquid crystal phase in KCNS is greater than the corresponding phase in pure water. Thermodynamic parameters such as transition enthalpies and temperatures only reflect differences and by themselves do not distinguish between the possible interpretations. The answer to this and related questions must await either a determination of the heat capacities of the lipids in both phases (hence, the entropy) in the presence and absence of salts or a direct measurement of ion binding by other techniques.

At 1 M, the acetate ion, a "structure maker" in water, also acts like CNS^- and I^-. The interaction of these ions with the dipalmitoylphosphatidylcholine dispersion appears to be determined by how these ions interact with bulk water, insofar as anions (being generally more lipophilic than cations) will tend to reside at non-polar interfaces [18]. The order in which monovalent anions increase ΔH_m of dispersions in 1 M solutions is also the order in which the hydrophobicity of the anions increases, i.e. $CNS^- >$ acetate $\approx I^-$.

Thermodynamic data on the phase transitions of dipalmitoylphosphatidylcholine dispersions and dimyristoylphosphatidylcholine monolayers in aqueous solutions containing at least one divalent ion

Table III contains thermodynamic data on the phase transition of dipalmitoylphosphatidylcholine bilayers in aqueous solutions of at least one divalent ion, some transition temperatures of dimyristoylphosphatidylcholine monolayers spread on the corresponding salt solution, and the mean activity coefficients of solutions of the salts. It may be seen that Mg^{2+}, Sr^{2+}, and Ba^{2+} have negligible influence on the phase changes of monolayers and bilayers. Comparison of the data in Tables I and II with the data in Table III for the chlorides of Mg^{2+}, Sr^{2+}, and Ba^{2+} also reveals that for these ions, very large differences in mean activity coefficients do not necessarily affect the thermodynamic parameters of the lipid phase transition. In addition, it is evident that other effects produced by the addition of these ions to an aqueous dispersion (e.g. changes in bulk aqueous dielectric constant, ionic strength, surface viscosity and potential, osmotic pressure, bilayer thickness, and bilayer–bilayer interaction) likewise have minimal importance for the main lipid phase transition.

In striking contrast to these electrolytes, some cadmium salts appear to interact specifically with the lipid (Table III). The effects of $CdCl_2$ are clearly due to interactions at the lipid–aqueous interface and not due to interbilayer interactions, since both monolayers and bilayers are affected in the same manner by this salt. In 1 M

TABLE III

THERMODYNAMIC DATA FOR DIPALMITOYLPHOSPHATIDYLCHOLINE–DIVALENT ION SYSTEMS AND TRANSITION TEMPERATURES OF DIMYRISTOYLPHOSPHATIDYLCHOLINE (DML) MONOLAYERS SPREAD FROM A 10 mg/ml MIXTURE OF DICYCLOHEXYL (DCH) OVER VARIOUS DIVALENT SALT SUBPHASES

Solvent	T_m (°C)	ΔH_m (cal/g)	ΔS_m (cal/degree per mole)	$W_{1/2H}$ (°C)	Activity coefficient*	Change in T_m of DML–DCH monolayer (°C)
1 M $MgCl_2$	43.1	9.55	22.6	0.8	0.57	+1.3
1 M $SrCl_2$	41.2	9.27	22.1	1.0	0.46	−2.3
1 M $BaCl_2$	41.2	10.1	24.0	1.2	0.39	−0.5
10^{-4} M $CaCl_2$	39.0	10.1	24.4	0.6	—	—
10^{-3} M $CaCl_2$	39.2	10.2	24.4	0.8	0.88	—
10^{-2} M $CaCl_2$	39.8	10.4	24.9	0.8	0.73	—
10^{-1} M $CaCl_2$	39.3	10.1	24.2	0.8	0.51	−1.1
1 M $CaCl_2$	44.8	10.2	24.1	1.2	0.50	−5.5
2 M $CaCl_2$	53.0	10.8	25.0	5.0	0.79	+6.7
3 M $CaCl_2$	64.9	15.4	34.2	2.0	1.48	+11.7
1 M cadmium acetate	40.4	13.7	32.8	0.5	—	—
1 M $CdSO_4$	42.4	10.3	24.6	1.0	0.04	—
1 M $Cd(NO_3)_2$	42.6	6.38	15.2	4.1	0.43	—
	44.0	3.14	7.4			
0.1 M CdI_2	54.5	5.42	5.62	7.5	0.10	—
1 M CdI_2	38.5	0.87	2.1	3.2	0.25	—
	43	5.23	12.4			
10^{-3} M $CdCl_2$	41.3	9.41	22.5	0.9	0.81	—
10^{-2} M $CdCl_2$	42.0	8.51	20.3	1.0	0.52	—
10^{-1} M $CdCl_2$	41.9	9.93	23.6	0.9	0.22	—
1 M $CdCl_2$	57.0	13.0	27.6	8.0	0.66	+24.0
2 M $CdCl_2$	48.7	4.78	11.2	3.5	0.0441	—
0.5 M K_2SO_4	39.4	10.0	24.0	0.9	0.264	—

* Data from ref. 23.

$CdCl_2$ the transition temperature of dipalmitoylphosphatidylcholine bilayers increases by 18 °C, the enthalpy decreases by 30 %, and the peak becomes very broad, $W_{\frac{1}{2}H}$ being 8 °C. None of these changes occur in 0.1 M $CdCl_2$. Likewise, the transition temperature of dimyristoylphosphatidylcholine monolayers is 24 °C higher when spread over a 1 M $CdCl_2$ subphase than one of pure water. 1 M CdI_2 lowers the transition temperature and decreases the peak width to the same extent as 2 M $CdCl_2$. Similar temperature effects are also produced by both 1 M $CdCl_2$ and 0.1 M CdI_2. This difference in effective concentration was expected in view of the differences in behavior observed for these two salts on egg lecithin vesicles [19]. An additional effect appears in dispersions of 1 M CdI_2, namely the emergence of two separate phase transitions upon heating. Since the sharpness of the transition is a measure of molecular cooperativity [12], the increased breadth of the transition peaks in the presence of the cadmium halides suggests that the packing of the lipids is altered by high concentrations of these salts.

Cadmium halide solutions are able to form a series of cadmium halide complexes [20] which may be related to the observed effects of these solutions on the

thermodynamic properties of phosphatidylcholine. The lack of effect of $CdSO_4$, a salt that does not form complex ions [20], on transition parameters suggests that it is the cadmium halide complex, rather than cadmium ion by itself, that alters these parameters. We, as well as others [19], have observed that cadmium halides but not calcium halides cause precipitation of phosphatidylcholine vesicles. This is a clear indication that a species present in cadmium halide solutions has an affinity for the phospholipid. The effect of 2 M cadmium acetate, on the other hand, is attributable to the acetate anion, since 1 M potassium acetate, but not 1 M KCl, also increased ΔH_m (Table II). Similarly, the effect of 1 M $Cd(NO_3)_2$ is probably due to the 2 M NO_3^- present.

Calcium, unlike cadmium, does not form halide complex ions [20]. Table III shows that at concentrations of $CaCl_2$ from 10^{-4} to 1 M, ΔH_m and $W_{\frac{1}{2}H}$ do not appreciably change, and the transition temperature increases moderately from 39.0 to 44.8 °C. At 3 M Ca^{2+}, both the transition temperature and enthalpy exhibit dramatic increases of about 25 °C and 50 %, respectively. The peak has a well defined maximum and $W_{\frac{1}{2}H} = 2.0$ °C. At high concentrations, therefore, Ca^{2+} will bind to the surface of lecithin and does not dehydrate the membrane as previously suggested [21, 22], assuming that the dehydration that is produced by binding a large fraction of the water to ions is equivalent to reducing the percentage of water in the dispersion (elevation of the transition temperature and a diminution of the transition enthalpy) [1]. The increase in T_m of the monolayers in the presence of Ca^{2+} parallels that in the calorimetric experiments.

Similarly, minimal effects on the transition parameters were observed with K_2SO_4, the only salt used which contained a monovalent cation and a divalent anion.

Thermodynamic parameters for the phase transition of dipalmitoylphosphatidylcholine in solution with at least one trivalent ion

Transition parameters for dipalmitoylphosphatidylcholine in solution with trivalent cations are given in Table IV. The fact that the transition was unchanged by 1 M $LaCl_3$ has significance since at 25 °C one mole of $LaCl_3$ per l lowers the dielectric constant of water to 36, which is lower than most monovalent and divalent electrolytes studied [5]. This means either that this electrolyte does not influence the dielectric constant of water at the lipid surface or that electrostatic interactions between lipid molecules are of minimal significance for their phase transitions. If the transition is unaffected by lanthanum, it is highly unlikely that the alteration of transition para-

TABLE IV

THERMODYNAMIC DATA FOR DIPALMITOYLPHOSPHATIDYLCHOLINE–TRIVALENT ION SYSTEMS

Solvent	T_m (°C)	ΔH_m (cal/g)	ΔS_m (cal/degree per mole)	$W_{1/2H}$ (°C)
0.5 M $FeCl_3$	40.2	1.08	2.58	1.5
	44.5	6.66	15.8	4.1
1 M $FeCl_3$	48.8	10.1	23.6	2.1
1 M $LaCl_3$	42.0	10.5	25.0	1.2
3 M $LaCl_3$	47.2	7.57	17.6	3.5

meters by similar ions is related to a low dielectric constant. This is apparent with respect to 3 M $LaCl_3$, in which the peak retains its shape although it is broadened. Here, however, dehydration of the membrane is indicated by an increased T_m and $W_{\frac{1}{2}H}$ and decreased ΔH_m.

At 0.5 M $FeCl_3$ the transition displays two distinct peaks quite close to each other during the heating and cooling cycles. This type of behavior is usually obtained when two distinct phases are present and melting separately [2]. The first transition peak has a transition temperature akin to that of pure water, whereas the second peak is greatly broadened and exhibits a 2.5 °C increase in transition temperature. In 1 M $FeCl_3$ a single peak appears with a higher transition temperature and enthalpy than either of those observed in 0.5 M $FeCl_3$. Presumably this represents a single phase.

It would appear that the difference between these trivalent ions is related to the same factors that contribute to the different effect of Ca^{2+} and Cd^{2+} salts on the lipid phase transition parameters, i.e. Fe^{3+} forms complex ions with both Cl^- and OH^- whereas lanthanum exhibits little tendency to form complexes [20]. Like the cadmium halides, $FeCl_3$ solutions cause precipitation of lecithin dispersions [6].

ACKNOWLEDGEMENT

This work was supported in part by N.I.H. grant No. NS09448.

REFERENCES

1 Ladbrooke, B. D. and Chapman, D. (1969) Chem. Phys. Lipids, 3, 304–367
2 Abramson, M. B. (1970) in Surface Chemistry of Biological Systems (Blank, M., ed), pp. 37–53, Plenum Press, New York
3 Sackmann, E., Träuble, H., Galla, H. and Overath, P. (1973) Biochemistry 12, 5360–5369
4 Shimshick, E. J. and McConnell, H. M. (1973) Biochemistry 12, 2351–2360
5 Bockris, J. O. M. and Reddy, A. K. N. (1970) Modern Electrochemistry, Vol. I, p. 157, Plenum Press, New York
6 MacDonald, R. C. and Thompson, T. E. (1972) J. Membrane Biol. 7, 54–87
7 Hatefi, Y. and Hanstein, W. G. (1969) Proc. Natl. Acad. Sci. 62, 1129–1136
8 Gottlieb, M. H. and Eanes, E. D. (1972) Biophys. J. 12, 1533–1547
9 Walter, W. V. and Hayes, R. G. (1971) Biochim. Biophys. Acta 249, 528–538
10 Parsons, R., Reeves, R. M. and Taylor, P. N. (1974) Electron. Anal. Chem. Interfacial Electro. 50, 149–152
11 Chapman, D., Williams, R. M. and Ladbrooke, B. D. (1967) Chem. Phys. Lipids 1, 445–447
12 Hinz, H. J. and Sturtevant, J. M. (1972) J. Biol. Chem. 247, 6071–6075
13 Yi, P. N. and MacDonald, R. C. (1973) Chem. Phys. Lipids 11, 114–134
14 Engel, G. and Hertz, H. G. (1968) Ber. Bunsenges. Phys. K. Chem. 71, 808–834
15 Stokes, R. H. (1964) Aust. J. Chem. 20, 2087–2100
16 Mitchell, C. D. and Hanahan, D. J. (1966) Biochemistry 5, 51–57
17 Tokiwa, F. (1972) Adv. Colloid Interface Sci. 3, 389–424
18 Miller, K. W. and Hildebrand, J. H. (1968) J. Am. Chem. Soc. 35, 1281–1285
19 Jendrasiak, G. I. (1972) Chem. Phys. Lipids 9, 133–146
20 Sillen, L. G. and Martell, A. E. (1964) Stability Constants of Metal Ion Complexes, pp. 271–301, 340, The Chemical Society, London
21 Cerbon, J. (1967) Biochim. Biophys. Acta 144, 1–9
22 Papahadjopoulos, D. and Weiss, L. (1969) Biochim. Biophys. Acta 183, 417–426
23 Parsons, R. (1959) Handbook of Electrochemical Constants, Butterworths, London