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EFFECT OF IONS ON PHOSPHOLIPID LAYER STRUCTURE AS INDICATED BY RAMAN SPECTROSCOPY

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SUMMARY

Various anions and cations are found to induce changes in the layered structure of phosphatidylcholine-water systems as indicated by Raman Spectroscopy. From the ratio of Raman intensities, I_{1064}/I_{1089}, it is inferred that dipositive ions decrease the proportion of gauche character in the hydrocarbon chains, with the relative influence being: $Ba^{2+} < Mg^{2+} < Ca^{2+} \approx Cd^{2+}$. Unipositive ions ($Li^+$, K^+ and Na^+) produce no observed changes in the Raman spectrum of the lecithin dispersion. The proportion of gauche character of the hydrocarbon chains is found to be nearly independent of the anion for: Br^-, Cl^-, acetate$^-$, I^-, ClO_4^-, CNS^- and SO_4^{2-}. Dispersions prepared with a solution of $KI+I_2$ produced Raman spectra in which the 1089 cm^{-1} peak, which is characteristic of random lipid chains, was greatly intensified, presumably because of the presence of I_3^- which is known to penetrate the lipid lamellae. The observed trends are discussed.

INTRODUCTION

Despite the important role of membranes in living systems, there is still little detailed knowledge on the molecular level of the structural interactions responsible for their function. Biological membranes are affected by the ionic character of the surrounding media, via alteration of the lipid lamellar system. This alteration has been attributed to direct ion-membrane interaction and also to an indirect effect arising from an ion-induced structural change of the water surrounding the membrane [1, 2]. Physical techniques such as surface pressure [3–5], differential scanning calorimetry [6], X-rays [7], ESR [8], NMR [9, 10], and fluorescence spectroscopy [11] have been used to study the extent and nature of the interactions between lipids and aqueous salt solutions.

Raman spectroscopy is the most recent physical method to be used in the study of lipid systems and biological membranes [12–17]. This technique provides detailed vibrational information of modes associated with the C-C skeleton of fatty acids and lipids which has been shown to reflect the degree of order of the hydrocarbon chains.

Raman spectroscopy unlike infra-red spectroscopy, which would in principle provide similar information, provides detailed vibrational information without interference due to the presence of water. In this paper, we present a Raman spectral study of the influence of ions on lamellar dispersions of phosphatidylcholine by examining the peak intensity ratio of the all trans C-C vibration to the O-P-O stretch plus random (gauche) C-C vibration, I_{1064}/I_{1089}.

MATERIALS AND METHODS

1,2 L-α-Dimyristoyl phosphatidylcholine, 1,2 L-α-dipalmitoyl phosphatidylcholine, 1,2 L-α-distearoyl phosphatidylcholine and phosphatidylethanolamine (palmitoyl) were used as obtained from Calbiochem. Phosphatidylserine was the gift of Dr Erich Baer. Unless otherwise stated, aqueous solutions consisted of reagent grade material washed in purified chloroform to remove nonpolar impurities at one molar concentrations in doubly distilled and deionized water. A 4 : 1 weight ratio of solvent to lipid was used throughout. Dispersions were prepared by heating the mixture to 60 °C for 1 h, air cooling, and sampling in 1 mm diameter capillary tubes. The Raman spectra were obtained with an Argon ion laser (Spectra Physics 164) operating at 488 or 514.5 nm. with a typical power of 0.45 W. An interference filter was employed in the laser beam to reject argon atomic lines. Scattered light was collected at 90° to the incident light, and was imaged into an 0.85 nm. Spex 1401 double monochrometer. A cooled RCA C31034 GaAs photomultiplier and photon counting electronics were employed for detection. All spectra taken for quantitative purpose were obtained with a band pass approx. 2.0 cm^{-1}, and intensities were judged by peak heights. At least two independent runs were made for each system and a maximum difference of ± 0.1 was obtained. Care was taken to eliminate effects due to background noise in calculating the intensity ratios.

RESULTS AND DISCUSSION

The Raman spectra of fatty acids [12], lipids[13, 14], and membranes [17] have been reported and the influence of temperature and cholesterol on the Raman spectra of lecithin has been studied [15, 16]. Mendelsohn [15] and Lippert and Peticolas [16] have used the intensity ratio of the approx. 1128 and approx. 1089 cm^{-1} peaks which they respectively assign to a skeletal mode for the carbon chain in an all trans conformation and in a gauche conformation. Lippert and Peticolas [16] reported that the intensity ratio I_{1128}/I_{1089} decreased with the addition of cholesterol to phosphatidylcholine multilayers, while Mendelsohn [15] found that the addition of cholesterol to egg lecithin increased the ratio I_{1128}/I_{1089}. The difference is attributed by Mendelsohn [15] to dipalmitoyl phosphatidylcholine being in a crystalline phase at room temperature while egg lecithin is in a fluidized state. Lippert and Peticolas [16] gave tentative assignment to the peaks in the 1000–1200 cm^{-1} region but later reassigned the approx. 1089 cm^{-1} peak of dipalmitoyl phosphatidylcholine to include the head group O-P-O stretch mode, as well as the random hydrocarbon motion [12]. This reassignment, does not negate the conclusions in their earlier paper.

The criterion used in this study is the I_{1064}/I_{1089} ratio which appears to reflect the proportion of trans hydrocarbon chains because the approx. 1064 cm^{-1}

band is a skeletal mode characteristic of the all trans hydrocarbon chains, while the approx. 1089 cm^{-1} feature appears to arise from the superposition of the O-P-O symmetric stretch mode with a skeletal mode for random chains [12]. The O-P-O symmetric stretch is assumed to make a constant intensity contribution at all temperatures and therefore does not seriously affect conclusions drawn about the hydrocarbon chain conformation from the I_{1064}/I_{1089} ratio. Any relative change in the approx. 1089 cm^{-1} mode intensity is attributed to a change in the amount of randomness in the chains. Considerable care was taken in analysis involving the approx. 1089 cm^{-1} peak since this peak in particular is influenced by background interference, such as arises from grating ghosts and free phosphate symmetric stretch which occurs in an intense band around 1080 cm^{-1}. In particular, the 514.5 nm. Argon ion laser line was used to eliminate grating ghosts present when the 488 nm, line is used in the approx. 1089 cm^{-1} peak. Also, inorganic phosphate was not found to be a problem in our samples as the relative intensity in the 1080 cm^{-1} region did not vary from one sample source to another. The intensity ratio I_{1128}/I_{1089} which was used by others [15, 16], produced general trends similar to the I_{1064}/I_{1089} ratio.

Pure lipids

The dimyristoyl, dipalmitoyl and distearoyl derivatives of 1,2 L-α-phosphatidylcholine were studied in powder form (Fig. 1) and in water dispersions ((Fig. 2) in the region 1000–1200 cm^{-1}. These lipids were examined in order to find a lipid for which there is a clear separation of the diagnostic hydrocarbon peaks at approx. 1064 cm^{-1} and approx. 1089 cm^{-1}. The derivatives were all found to satisfy this condition.

A subtle feature observed in lipid spectra is the small frequency shifts observed in the 1089 cm^{-1} region. The influence of high temperature or cholesteric interaction shifts the approx. 1089 cm^{-1} feature of the lipid dispersion to a somewhat lower frequency, and produces a broader Raman band [16]. This shift is thought to result from the increase in the amount of gauche C-C conformers producing a band which dominates the previously dominant O-P-O stretch band. A similar trend is observed when comparing spectra of dispersions of lipids varying hydrocarbon chain length. It can be seen in Fig. 2 that this band for the short chain lipid dimyristoyl phosphatidylcholine falls at a lower frequency than it does for the longer chain dipalmitoyl phosphatidylcholine and distearoyl phosphatidylcholine dispersions. Both the shift of this feature to a lower frequency and its high relative intensity reflect the fact that the dimyristoyl phosphatidylcholine dispersion is near its transition temperature, 23 °C, while the dipalmitoyl phosphatidylcholine and distearoyl phosphatidylcholine dispersions are well below their transition temperatures of 41° and 58 °C respectively [18]. Dipalmitoyl phosphatidylcholine was chosen for this study because the extensive literature on this system allows the comparison of the Raman results with those from other physical techniques.

It is shown in Fig. 3 that pH does not affect the lipid hydrocarbon peaks in the region 1000–1200 cm^{-1} in the pH range 5 to 8 for dipalmitoyl phosphatidylcholine. This result is in agreement with the report by Chapman [18] of a study by Trauble in which the effect of pH on the transition temperature (T_m) of dipalmitoyl phosphatidylcholine is examined using fluorescence spectroscopy. Trauble found that there was little change in T_m for dipalmitoyl phosphatidylcholine in the pH range 4 to 12.

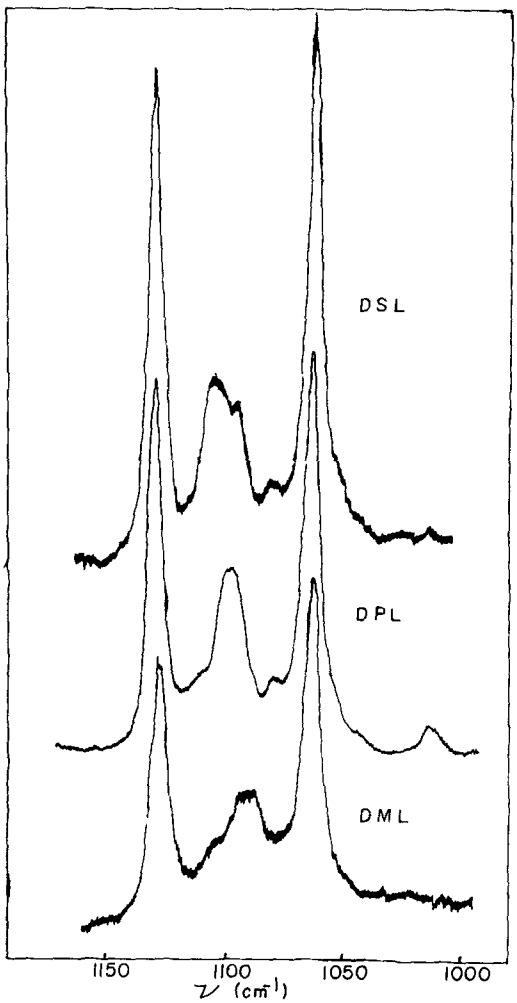

Fig. 1. Spectra of dimyristoyl (DML), dipalmitoyl (DPL) and distearoyl (DSL) phosphatidylcholine powders in the Raman spectral range 1000–1150 cm^{-1}.

Various diacylphosphoglycerides were studied in the powder form. The lipids varied in the head group structure, the fatty acid component being palmitic acid. Phosphatidylcholine and phosphatidylethanolamine showed peaks at 725 and 763 cm^{-1} respectively, while phosphatidylserine produced a triplet of overlapping peaks at 785, 795 and 805 cm^{-1}. The 725 and 763 cm^{-1} peaks of phosphatidylcholine and phosphatidylethanolamine were assigned to the deformation vibration of the choline and ethanolamine groups by Mendelsohn [13]. By analogy, the phosphatidylserine triplet should be due to the deformation vibrations of the serine head group.

Lipid-^2H_2O interaction

The Raman spectral positions corresponding to the hydrocarbon chains of phosphatidylcholine in ^2H_2O and H_2O are the same and it is difficult to discern any

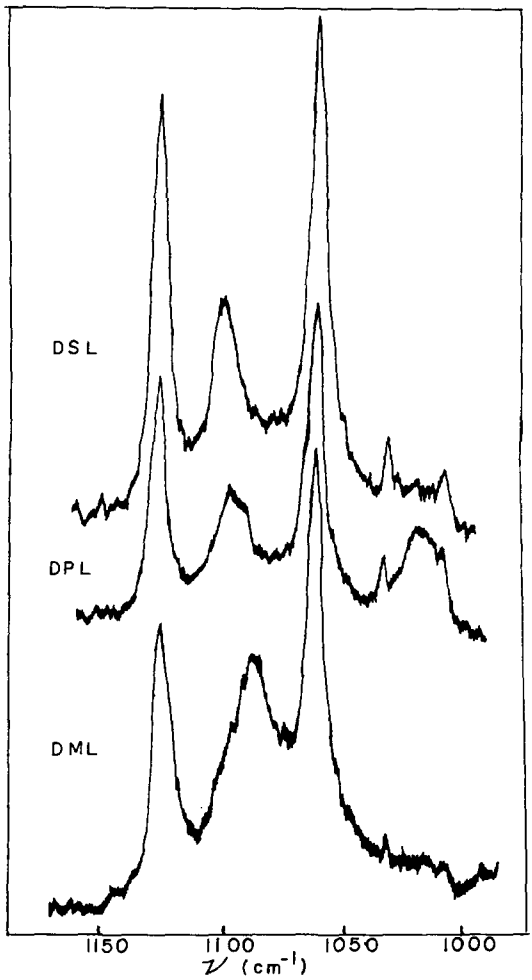

Fig. 2. Spectra of dimyristoyl (DML), dipalmitoyl (DPL) and distearoyl (DSL) phosphatidylcholine-water unsonicated dispersions in the Raman spectral range 1000–1150 cm^{-1}.

change in the intensity ratio I_{1064}/I_{1089} (Fig. 4). Previously Walter and Hayes [19] found a change in hydrocarbon motion using NMR, and have ascribed it to a decrease in the hydrogen bonding between the lipid and the solvent in a ^2H_2O solution as compared with an H_2O solution.

Lipid-cation interaction

The interaction of cations with lipids is thought to be electrostatic in nature with dipositive ions especially interacting coulombically with the negative charge on the lipid head group structure. This interaction is facilitated by the outward direction of the hydrogen atoms of the water in the cation hydration shell [2]. These interactions are important in biological systems. For example, Tasaki and Hallett [20] have found that dipositive ions are necessary in the external media to produce nerve

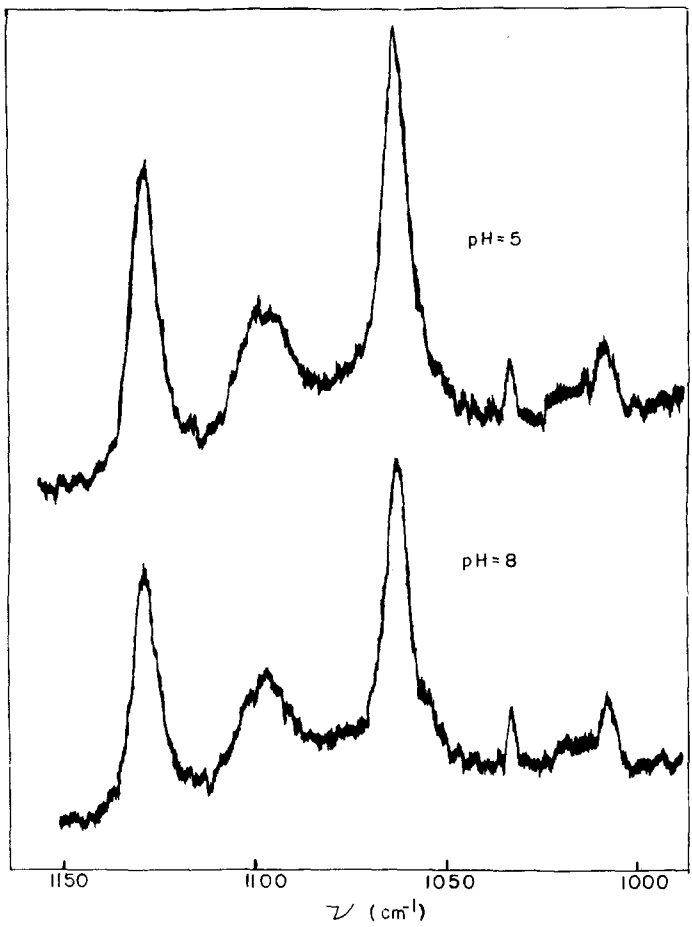

Fig. 3. Spectra of dipalmitoyl phosphatidylcholine in unsonicated dispersions made with 10^{-1}M KCl at different pH values in the Raman spectral range 1000–1150 cm^{-1}.

excitation, and Reynolds [1] has found that the structural integrity of the human red blood cell is directly related to the presence of inorganic cations. Various authors have studied lipid-cation interactions using many physical techniques in an attempt to characterize this interaction. In an X-ray study of electrolyte effects on a plasmalogen. Gottlieb and Eanes observed thinning of the bilayer which they attributed to the repulsion between bilayers caused by the ions disrupting the electrical neutrality of the zwitterionic lipid molecules [7]. Shah and Schulman [4], and Papahadjopoulos [5] have shown in monolayer studies that cations expand the layer area of lipids at large ion concentrations and have found this effect to increase with cation charge. It has been hypothesized by Shah and Schulman [4] that cations bind to the phosphate group of phosphatidylcholine in competition with the quarternary ammonium group.

In this Raman study, we have found the influence of the dipositive ions of chloride salts on the I_{1064}/I_{1089} of phosphatidylcholine to be: $Cd^{2+}(3.6) \gtrsim Ca^{2+}(3.5) > Mg^{2+}(2.8) \gtrsim Ba^{2+}(2.5) \gtrsim H_2O(2.4)$. Fig. 5 shows some typical spectra of

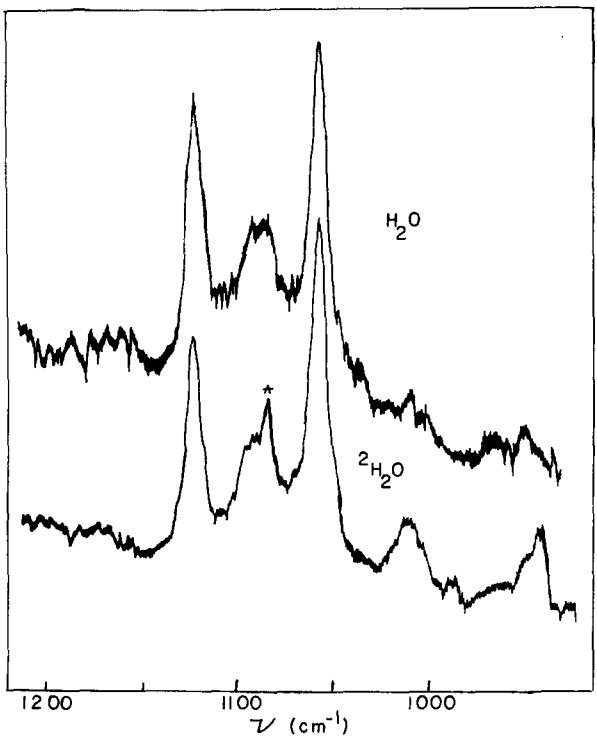

Fig. 4. Spectra of dipalmitoyl phosphatidylcholine in unsonicated dispersions made with ^2H_2O or H_2O in the Raman spectral range 1000–1200 cm^{-1}. *denotes grating ghost.

dipositive ion-lipid-water dispersions. The ionic radii of Cd^{2+} and Ca^{2+} are approximately the same; therefore, one might conclude from the observed results that in the dipositive ion-lipid interaction simple electrostatic forces are important. However, only cadmium halide salts increase the ratio I_{1064}/I_{1089} (Fig. 6). The order of the intensity ratio I_{1064}/I_{1089} for cadmium salts is: $CdCl_2(3.6) > CdI_2(2.7) >$ Cd-Acetate$_2(2.6) \gtrsim CdSO_4(2.3)$. The effect of $Cd(NO_3)_2$ on the lipid hydrocarbon chains Raman bands could not be separated from the large band at 1050 cm^{-1} due to the NO_3^-. Further, the influence of CdI_2 on the lipid hydrocarbon chain bands cannot be determined via examination of peak heights since CdI_2 affects both the shape and height of these bands. Fig. 7 shows a comparision of the strong influence of 1 M CdI_2 on the lipid hydrocarbon chain bands as opposed to that of 0.1 M CdI_2 which has an I_{1064}/I_{1089} intensity ratio of the same value as a lipid-water dispersion. Similarly, differential scanning calorimetry studies in this laboratory of phosphatidylcholine-water dispersions have shown that cadmium halides affect the thermalproperties of phosphatidylcholine more than the other cadmium salts studied [6]. This difference may be accounted for by the high propensity for cadmium to form complexes with halides. From published equilibrium data [21], it is found that in a one molar solution of $CdCl_2$ approximately 65 % of the cadmium is present as $CdCl^+$ with most of the remainder present as $CdCl_2$, while in a one molar solution of CdI_2 approx. 70 % of the cadmium is present as CdI^+ with the remainder divided between

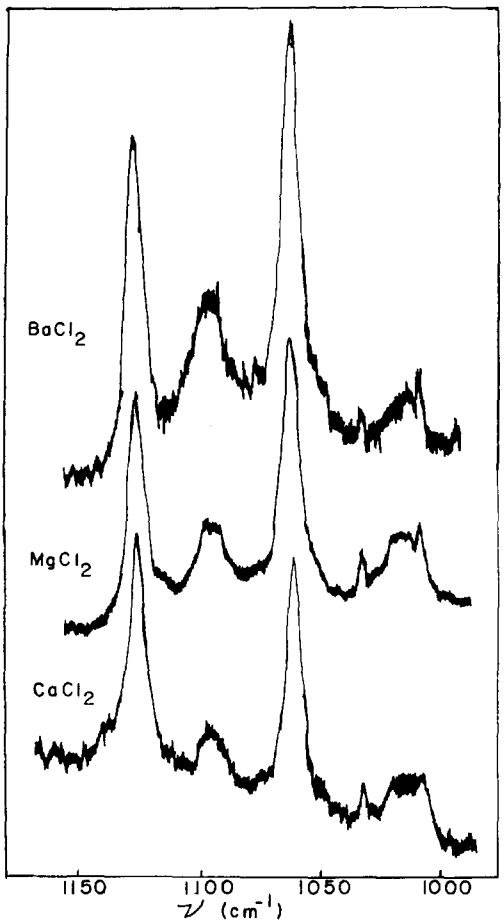

Fig. 5. Spectra of dipalmitoyl phosphatidylcholine in unsonicated dispersions made with 1 M concentrations of chloride salts of various dipositive ions in the Raman spectral range 1000–1150 cm^{-1}.

CdI_2 and CdI_3^- (or CdI_4^-). Under these same conditions calcium is not appreciably complexed by halide. We conclude that the influence of the various cations or their complexes is highly specific. The approx. 720 cm^{-1} band assigned to the lipid C-N stretch (Spiker, R. C., personal communication) has also been studied for $CaCl_2$ and the various cadmium salts. It was found that $CaCl_2$, $CdCl_2$ and CdI_2 increase the half-width of this band as well as changing the frequency of the band. These changes tend to confirm the idea that the ions are interacting with the head groups, perhaps in the manner discussed by Sundaralingam and Jenson [22].

In agreement with differential scanning calorimetry [6] and surface pressure studies [3], monopositive ions (Li^+, Na^+ and K^+) were found to have negligible effects on the lecithin Raman spectrum.

Lipid-anion interaction

Anions are thought to affect membrane structure by their influence on hydrogen bonding between the membrane and the surrounding water structure. Accord-

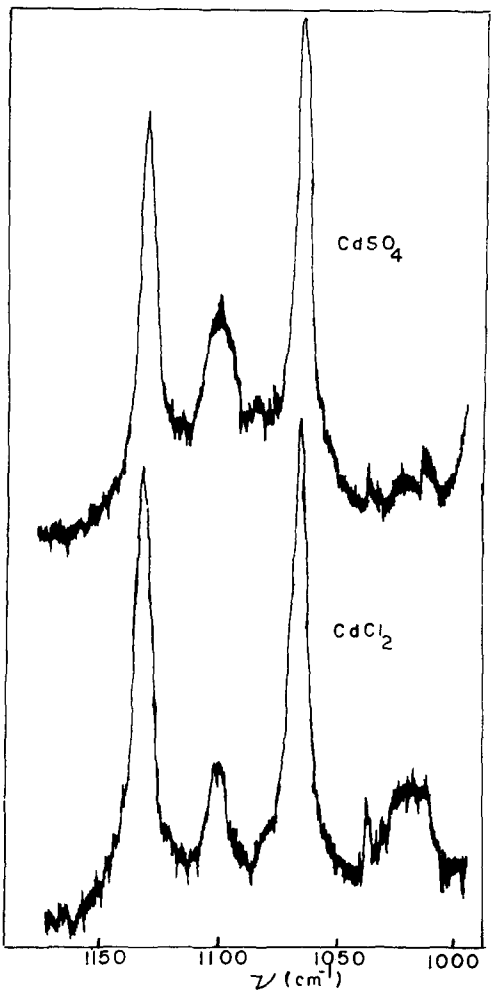

Fig. 6. Spectra of dipalmitoyl phosphatidylcholine in unsonicated dispersions made with 1M $CdCl_2$ or 1 M $CdSO_4$ in the Raman spectral range 1000–1150 cm^{-1}.

ing to this model anions decrease the polarity of the surrounding water because the hydrogen atoms in the anion hydration shell are directed inward, resulting in a water-anion aggregate less polar than the OH-O of water itself [2]. The decrease in hydrogen bonding between the membrane and water leads to an increase water solubility of the non-electrolyte components of the membrane.

The simpler problem of the interaction of anions with lipid bilayers has received relatively little attention [7, 9, 10]. It is possible that the water structure arguments outlined above for membranes also apply to the pure lipid systems. Alternatively, Parseghian's statistical thermodynamic model describes the influence of ions on the bilayer structure in terms of long range coulombic interactions [23]. Another possibility is highly specific ion pairing between head groups and anions in the fashion already discussed for cations. Specific interactions of this sort would be dependent on both anion and cation.

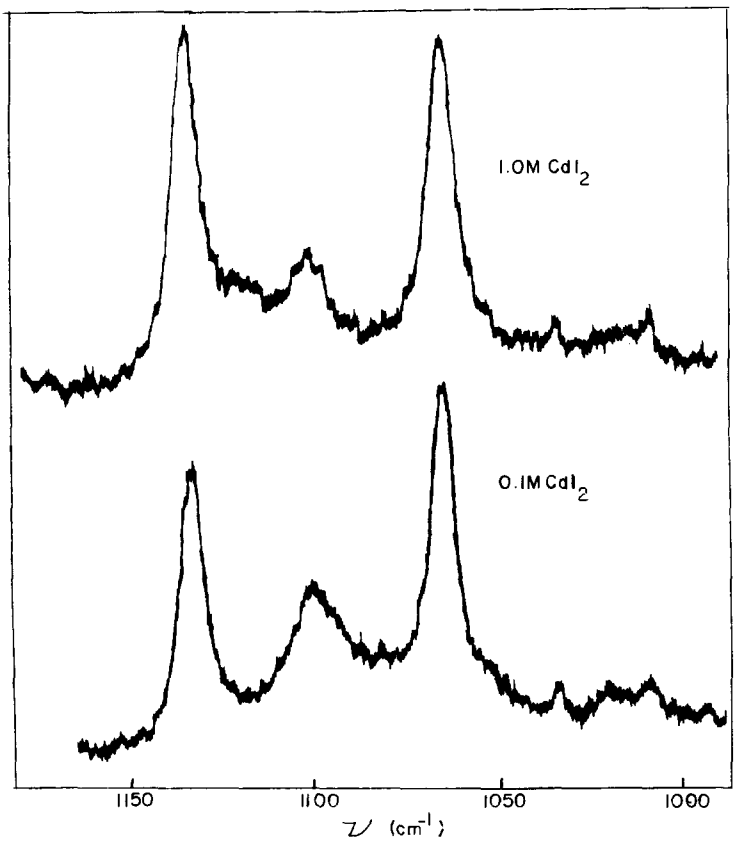

Fig. 7. Spectra of dipalmitoyl phosphatidylcholine in unsonicated dispersions made with 1 M or 0.1 M CdI_2 in the Raman spectral range 1000–1150 cm^{-1}.

The relative proportions of trans to random chain (as judged by I_{1064}/I_{1089}) is only slightly affected by the nature of the anions. The observed order for potassium salts (0.5 M K_2SO_4 was used) and lithium perchlorate being: $Br^-(2.5) \approx Cl^-(2.4) =$ acetate$^-(2.4) = I^-(2.4) = H_2O(2.4) \approx ClO_4^-(2.2) \approx CNS^-(2.1) = SO_4^{2-}(2.1)$. We conclude that below the transition temperature these anions have negligible influence on the hydrocarbon chain packing. This result contrasts with the influence of cholesterol which increases the lipid fluidity below the transition temperature [15, 16]. Our results give no evidence for strong and specific interaction between the anions and dipalmitoyl phosphatidylcholine.

Lipid-tri-iodide interaction

A solution of 1 M KI and 10^{-3} M I_2 was used to form lipid dispersions. Although 1 M KI appears to have no effect on the dipalmitoyl phosphatidylcholine-Raman spectrum, the addition of 10^{-3} M I_2 into the dipalmitoyl phosphatidylcholine KI dispersion produces both a broadening and a downward shift in the frequency of the approx. 1089 cm^{-1} dipalmitoyl phosphatidylcholine Raman band (Fig. 8). The

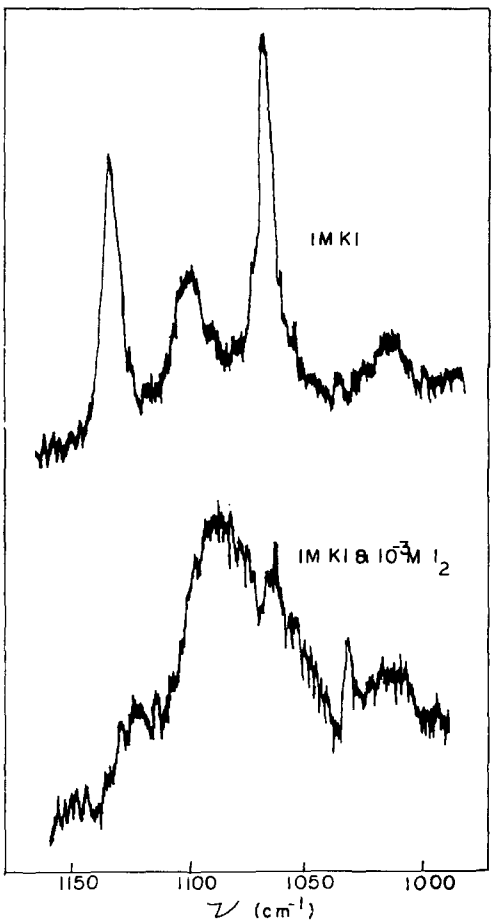

Fig. 8. Spectra of unsonicated dipalmitoyl phosphatidylcholine-KI dispersions with and without I_2 added in the Raman spectral range 1000–1150 cm^{-1}.

$KI+I_2$ solution causes the dipalmitoyl phosphatidylcholine Raman spectrum to have a high background noise which makes the system unsuitable for quantitative analysis. However, the broadening and the frequency shift in the approx. 1089 cm^{-1} band is apparent and is similar to that observed for the interaction of cholesterol with dipalmitoyl phosphatidylcholine [12]. The tri-iodide ion, I_3^-, is generated by the combination of KI and I_2, and so it is this ion which greatly influences the dipalmitoyl phosphatidylcholine structure. Penetration of lecithin lamellae by I_3^- was previously inferred from electrical conductivity measurements [24, 25].

CONCLUSIONS

In this study we have shown by Raman spectroscopy that calcium ion and cadmium halide complexes affect the packing of phosphatidylcholine.These interactions appear to arise from highly specific interactions with the lipid head group.

The monopositive cations and anions which were studied have relatively little influence on the lamellar structure at room temperature. The tri-iodide ion, I_3^-, greatly affects the lipid hydracarbon chain Raman bands in the region 1000–1200 cm^{-1}.

NOTE ADDED IN PROOF (Received August 28th, 1975)

The recent assignment of phosphatidylcholine Raman peaks by Spiker and Levin [26] reaffirm our use of the I_{1064}/I_{1089} intensity ratio as a measure of the randomness of the hydrocarbon chain since they have also assigned the approx. 1089 cm^{-1} dipalnitoyl phosphatidylcholine Raman peak to the gauche C-C and O-P-O diester stretches.

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