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LASER RAMAN STUDIES OF MOLECULAR INTERACTIONS WITH PHOSPHATIDYLCHOLINE MULTILAYERS

II. EFFECTS OF MONO- AND DIVALENT IONS ON BILAYER STRUCTURE

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Summary

Laser Raman spectroscopy was applied to characterize structural behavior of dipalmitoyl phosphatidylcholine multibilayer systems in the presence of several cations (K^+, Na^+, Cs^+, Rb^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Ba^{2+}) and anions (Cl^-, Br^-, I^-, NO_3^-, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$). To evaluate the Raman-spectroscopical data quantitatively, characteristic intensity ratios, lateral and *trans* order parameters were used and compared. It was shown that the different *trans* order parameters are rather sensitive to ion-polar head group interactions and thus, they cannot give unequivocal information on the *trans-gauche* isomerization of hydrocarbon chains of phospholipids. The observed effects of ions on Raman spectra of phospholipid multilayers could not be explained simply on the basis of electrostatic interactions. The possible involvement of other factors (changes in polarizability, hydrogen bonds, etc.) is also discussed. It was demonstrated that the order parameters defined in different ways may result in different effectiveness sequences of ions. Of monopositive ions Na^+ was found to be the most effective to influence the bilayer structure. For dipositive ions, of which Ca^{2+} proved to be the most effective, concentration-dependent effectiveness sequences were obtained. A plausible interpretation and some consequences of the concentration-dependent two-step binding of divalent cations were also outlined. Bilayered phospholipid structures turned out to be more responsive to anions than to most cations investigated. Interdependent actions of cations and anions, as well as the possible relevance of the charge distribution on anions are postulated.

Introduction

There is understandable interest in the interactions of phospholipids with ions as regards excitability, ion permeation, ion binding and ion exchange properties at the surfaces of biological membranes. The lipid moiety of membranes is affected directly by ionic interactions, depending upon the chemical nature of the polar head groups, and indirectly by the charge-induced structural changes in the vicinal and absorbed water. Although the influences of ions on phospholipid-water systems have been extensively investigated, the results obtained by different authors with various techniques are rather contradictory. Several investigators have observed in mono-, bi- and multibilayer studies [1—9], thermal phase transition [10,11] and X-ray diffraction [12] experiments, NMR [13,14] measurements that divalent cations interact directly with neutral phospholipids, Ca^{2+} having been found especially effective. Other authors failed to observe any cation binding [15—19], or it appeared only at fairly high ionic concentrations [20].

Recent studies have shown the applicability of Raman spectroscopy to structural investigations of both model [21—26] and natural membranes [27—29]. The conformational sensitive regions of the lipid spectra furnish information on the fatty acid chains [21—23,25], packing density of lipid molecules [24,30] and the conformational changes of the polar groups of the membrane phospholipids [25,31] under different conditions. Recently an effort was made by Gaber and Peticolas [32] to evaluate the laser Raman spectra of lipids and biomembranes quantitatively, introducing appropriate (*trans* and lateral) order parameters characteristic of the lipid bilayer structure. Severe difficulties arise, however, in the application of these order parameters to non-thermally induced structural changes of lipid dispersions [33]. Because of the diverse experimental antecendents we have reexamined the effects of various mono- and divalent ions on the lipid conformation and conformational changes at constant temperature by using laser Raman technique.

Materials and Methods

High-purity synthetic β,γ-dipalmitoyl-DL-α-phosphatidylcholine, exhibiting a single spot in thin-layer chromatography, was purchased from Fluka A.G. (Switzerland) and was used without further purification. Aqueous solutions containing analytical grade purity salts (Reanal, Hungary) were made in tri-distilled water. Lipid dispersions were prepared in a 4 : 1 weight ratio of water-to-lipid, and were sealed in capillary tubes as described previously [34]. Raman spectra were recorded with a Cary 82 Raman spectrophotometer with a Spectra Physics 164 argon ion laser operating at 448 nm and 500 mW. The scattered light was collected at right angles to both the incident laser beam and the capillary axis. The scan speed was 0.4 cm^{-1}/s and the count rate ranged between 1000 and 15 000 counts/s full scale. All spectra were obtained with a band pass of 4 cm^{-1} and the band intensities were judged by peak heights. 2—3 independent sample preparations were made for each system studied and at least three spectra were taken of every sample. The numerical data given below are averages of at least six measurements. The standard error of the mean of all intensity ratios was ±0.02. All the measurements were carried out at 23 ± 1°C.

Results and Discussion

In the Raman spectra of phospholipids the conformational sensitive regions at about 1100 and 3000 cm^{-1} are particularly useful for detecting structural changes in the hydrocarbon chains. The two intense bands at 1066 and 1128 cm^{-1} are C-C stretching vibrations of the rigid *all-trans* configuration of the chain. The band at about 1100 cm^{-1} is a superposition of the contributions from C-C stretching modes of *all-trans* fatty acid chains (if the system is in a gel or crystalline state), fluid chains with *gauche* rotations and the symmetric PO_2^- stretching mode [26]. The relative intensities I_{1066}/I_{1100} and I_{1128}/I_{1100} are frequently used as relative measures of the number of *gauche* structures, i.e. the degree of *trans* order in the fatty acid region of different phospholipid systems [21,22,24,32]. The spectral region at about 3000 cm^{-1} is attributed to C-H stretching vibrations; the symmetric methylene vibration at 2850 cm^{-1} dominates in the liquid state, whereas the symmetric methylene mode at about 2880 cm^{-1} is peculiar to the highly ordered state of the apolar region [24,26, 31,32].

Quantitation of Raman-spectroscopic data

Two order parameters were introduced to characterize the membrane structure, the *trans* order parameter S_T and the lateral order parameters S_L [32]:

$$S_T = \left(\frac{I_{1128}}{I_{ref}}\right)_{sample} \times \left(\frac{I_{1128}}{I_{ref}}\right)^{-1}_{crystalline} \tag{1}$$

and

$$S_L = \frac{(I_{CH_2})_{sample} - 0.7}{1.5} \tag{2}$$

where either the 722 cm^{-1} or the 1100 cm^{-1} line were proposed as reference and, $(I_{CH_2}) = I_{2880}/I_{2850}$. (The 722 cm^{-1} line is due to the C-N symmetric stretch in the choline group.) The meaning of probability was attributed to both parameters: $0 \leqslant S_T,\ S_L \leqslant 1$. There is, however, only a restricted correspondence between the structural transitions and intensity ratios I_{1128}/I_{722} and/or I_{1128}/I_{1100}, they being determined not only by the *trans-gauche* relationship but even by the changes in the C-N stretching modes and PO_2^- vibrations, etc. as well. Thus, the concerted action of the different events may result in an unreal variation of S_T with respect to the *trans-gauche* isomerization and therefore, the comaprison of different structures, on the basis of S_T with either I_{722} or I_{1100} as reference may lead to misinterpretation of the *trans* order situation.

The structural arrangements of fatty acid chains in the solid and differently hydrated states are rather multifarious as revealed by X-ray diffraction technique [12,35-38]. For this reason the quantity

$$\alpha = \frac{\left(\frac{I_{1128}}{I_{ref}}\right)_{sample}}{\left(\frac{I_{1128}}{I_{ref}}\right)_{hydrated\ lipid}} - 1 \tag{3}$$

is somewhat more correct from the viewpoint of physics than the analogous S_T parameter, and may give more realistic measure of the *trans* structure-modifying effectiveness of the solute. α is only a phenomenological parameter for monitoring the conformational states of fatty acid residues and polar head groups in membranes, as compared to those of an identically hydrated lipid sample at a fixed reference temperature. In the following apparent ordering means that $\alpha > 0$, while apparent disordering is used when $\alpha < 0$. In the effectiveness sequences to be introduced below $H_2O < \underline{A}$ denotes if ion $\underline{A}$ causes apparent ordering, and $\underline{A} > H_2O$ applies apparent disordering. Otherwise the ions will be arranged according to $|\alpha|$. Clearly, no probability meaning is ascribed to α.

The intensity ratios I_{1128}/I_{722}, I_{1128}/I_{1100} and I_{2880}/I_{2850}, as well as the parameters α and S_L will be primarily used to evaluate the effects of various cations and anions on the structures of the aqueous dispersions of lecithin at constant temperature. For the sake of comparison the S_T values will also be given in the tables below. S_T^1 and α_1 were obtained with $I_{ref} = I_{722}$, while S_T^2 and α_2 correspond to $I_{ref} = I_{1100}$.

Monovalent cations

Due to the slight changes in the spectra, monovalent salt effects were examined only at high salt concentrations, such as 1 M. The experimental results show that in 1 M concentration all of them caused detectable alterations in the Raman spectra [33] and order parameters (Table I). On the basis of the phenomenological parameter α_1 the following effectiveness order can be defined:

$$Na^+ > H_2O < K^+ < Cs^+ < Rb^+ \qquad (I)$$

The same sequence is obtained with S_T^1 and I_{1128}/I_{722} (see Table I). Since S_T^1 was originally interpreted as a measure of the *all-trans* probability the obtained numerical values for it, for K^+, Rb^+ and Cs^+, are lacking in meaning, they being higher than the theoretical maximum, unity. This is probably due to the interactions between the polar head groups of lecithin molecules and cations as it

TABLE I

Characteristic intensity ratios and order parameters (S_T^1, S_T^2, α_1 and α_2 for the *all-trans* configuration of hydrocarbon chains; S_L for the lateral packing density) for dry dipalmitoyl phosphatidylcholine and aqueous dipalmitoyl phosphatidylcholine dispersions in the presence of 1 M chloride salts of different monovalent cations. Phospholipid : water = 1 : 4 (w/w).

Composition of lipid sample	$\frac{I_{1100}}{I_{722}}$	$\frac{I_{1128}}{I_{722}}$	$\frac{I_{1128}}{I_{1100}}$	$\frac{I_{2880}}{I_{2850}}$	S_T^1	S_T^2	α_1	α_2	S_L
Dry	1.18	1.76	1.49	1.37	1.00	1.00	—	—	0.45
H_2O	1.20	1.74	1.45	1.28	0.99	0.97	0.00	0.00	0.39
NaCl	1.10	1.49	1.35	1.28	0.85	0.91	−0.15	−0.07	0.39
KCl	1.38	1.92	1.39	1.28	1.09	0.93	+0.10	−0.04	0.39
RbCl	1.45	2.10	1.45	1.28	1.19	0.97	+0.21	0.00	0.39
CsCl	1.45	1.96	1.35	1.28	1.12	0.91	+0.12	−0.07	0.39

was suggested earlier [33]. The effectiveness sequence (I) also suggests that the interaction of Na^+ with hydrated lecithin may be different from those of K^+, Cs^+ and Rb^+, respectively.

Another effectiveness sequence for monovalent cations can be given on the bases of the intensity ratios I_{1128}/I_{1100} and the corresponding order parameters S_T^2 and α_2 as follows

$$Na^+ \approx Cs^+ > K^+ > Rb^+ \approx H_2O \qquad \text{(II)}$$

This is in agreement with that of differential scanning calorimetry [11] except for Na^+, but non of the criteria suggested in ref. 11 could explain ours. According to effectiveness order (II) Na^+ and Cs^+ are the most effective in producing structural changes in the phospholipid multibilayer system. These behaviors of Na^+ and Cs^+ are, at first glance, in line with their structure breaking effects on water [39]. Most plausibly, the monopositive ions interact with the hydrated polar head both electrostatically and by changing its hydration shell. This latter may alter both the hydrogen bond network at the interface [38,40] and the polarizability of some Raman-active groups (probably the phosphoryl group, etc.) in the polar head [33], and lead to an apparent enhancement in *gauche* formation as revealed by the decreasing values of I_{1066}/I_{1100}, I_{1128}/I_{1100}, S_T^2 and α_2, respectively. Since no appreciable change in S_L accompanied the decrease and/or constancy of the intensity parameters used above monovalent salt exposure, one inclines to believe that the effectiveness order (II) is determined by both cation-head group interactions and the perturbation of the acyl chains and, in fact, a smaller increase, if any, in the *gauche* formation occurs than would follow from the corresponding intensity ratios and order parameters. Interestingly, the order parameter S_T^2 does not exhibit any anomaly, indicating that the 1100 cm^{-1} line as reference is probably less sensitive to ion-head group interactions and can be, at least for monovalent cations, more suitable than the 722 cm^{-1} line.

The intensity ratios of the two reference lines, I_{1100}/I_{722} vary ion to ion and depend also upon concentration (see Tables I—III). They can be considerably higher and lower than that for hydrated phosphatidylcholine, 1.18, and exhibit the following sequence:

$$Na^+ < H_2O \approx \text{dry dipalmitoyl phosphatidylcholine} < K^+ < Cs^+ \approx Rb^+ \qquad \text{(III)}$$

This reminds very much of sequence I, indicating that sequences I and III are determined basically by the same interactions. Considering the origins of the Raman lines at 722 and 1100 cm^{-1} sequence II seems to reflect the effectiveness of the different monopositive ions in their interactions with the polar head of lecithin. If so the changes in all the quantities defined by means of I_{722} may be dominated by the cation-head group interactions.

Divalent cations

The influence of the divalent cations Ca^{2+}, Cd^{2+}, Mg^{2+} and Ba^{2+} on the structures of dipalmitoyl phosphatidylcholine-water lamellar phases were investigated in the concentration range 0.5 mM—1 mM of their chloride salts (ref. 33 and Fig. 1). Detectable changes in the Raman spectra occurred even at lower salt concentrations, depending upon the dipositive ion.

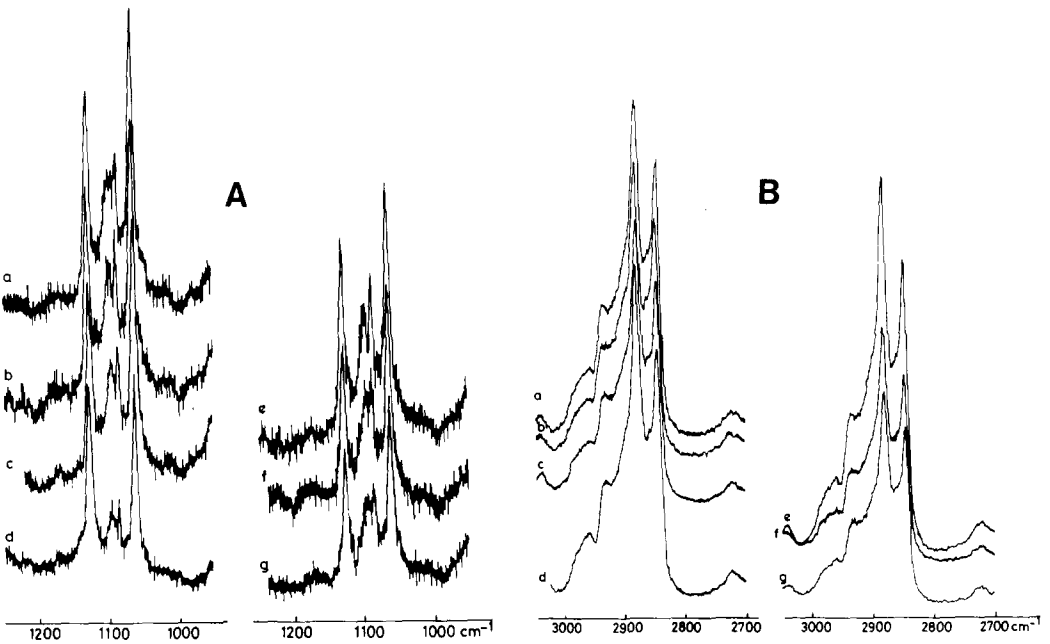

Fig. 1. Laser Raman spectra of dipalmitoyl phosphatidylcholine in lamellar aqueous dispersions containing $CaCl_2$ in different concentrations, in the Raman spectral ranges 950—1250 cm^{-1} (A) and 2700—3050 cm^{-1} (B). a, 1 M; b, 100 mM; c, 50 mM; d, 10 mM, e, 5 mM; f, 1 mM; g, 0.5 mM. Phosphatidylcholine : water = 1 : 4 (w/w).

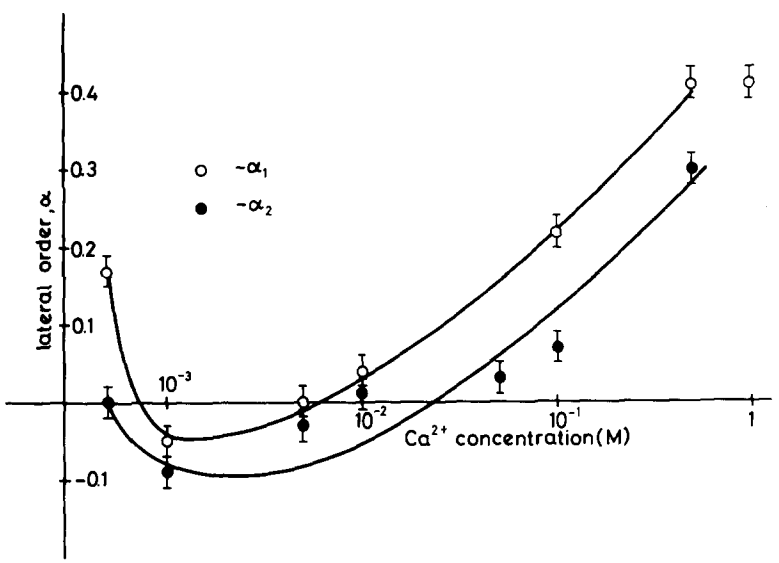

Fig. 2. Dependences of the phenomenological order parameters α_1 and α_2 for dipalmitoyl phosphatidylcholine in lamellar aqueous dispersions upon the $CaCl_2$ concentration. Phosphatidylcholine : water = 1 : 4 (w/w).

At 1—5 mM and/or 1—10 mM Ca^{2+}, depending upon the reference line chosen (Fig. 2 and Table II), a well-pronounced apparent disordering was observed in the hydrocarbon chains, which is probably related to the partial desctruction of the lamellar phase revealed by X-ray diffraction measurements [12]. At higher concentrations the intensity ratios were close to those of ion-free phospholipid-water systems, indicating the 'reappearance of the lamellar phase' [12]. It should be emphasized here that the *trans* order parameters calculated from Eqn. 1 with either $I_{ref} = I_{722}$ or $I_{ref} = I_{1100}$ exceed the theoretical maximum value, unity, in the concentration range above 10 mM. On the basis of the corresponding α values, strong and concentration-dependent interactions with the head groups can be deduced, the intensity ratios and order parameters used being affected by the changes in head-group polarizability and the PO_2^- vibrations as well [33], but very probably not in the same way as in the case of monovalent cations. An essential difference between mono- and divalent cations in their overall interactions with polar heads of phosphatidyl-

TABLE II

Characteristic intensity ratios and order parameters (S_T^1, S_T^2, α_1 and α_2 for the *all-trans* configuration of hydrocarbon chains; S_L for the lateral packing density) for dry dipalmitoyl phosphatidylcholine and aqueous dipalmitoyl phosphatidylcholine dispersions in the presence of chloride salts of different concentrations. Phospholipid : water = 1 : 4 (w/w).

Composition of lipid sample	$\frac{I_{1100}}{I_{722}}$	$\frac{I_{1128}}{I_{722}}$	$\frac{I_{1128}}{I_{1100}}$	$\frac{I_{2880}}{I_{2850}}$	S_T^1	S_T^2	α_1	α_2	S_L
Dry	1.18	1.76	1.49	1.37	1.00	1.00	—	—	0.45
H_2O	1.20	1.74	1.45	1.28	0.99	0.97	0.00	0.00	0.39
Ca^{2+}									
0.5 mM	1.40	2.01	1.45	1.28	1.15	0.97	+0.17	0.00	0.39
1 mM	1.25	1.65	1.32	1.25	0.94	0.89	—0.05	—0.09	0.37
5 mM	1.24	1.74	1.41	1.22	0.99	0.95	0.00	—0.03	0.35
10 mM	1.23	1.81	1.47	1.25	1.02	0.99	+0.04	+0.01	0.37
50 mM	1.20	1.79	1.49	1.26	1.02	1.00	+0.03	+0.03	0.37
100 mM	1.36	2.12	1.56	1.28	1.20	1.05	+0.22	+0.07	0.39
500 mM	1.30	2.46	1.89	1.30	1.40	1.27	+0.41	+0.30	0.40
1 M	0.98	2.46	2.50	1.32	1.39	1.68	+0.41	+0.72	0.42
2 M	1.07	2.35	2.20	1.46	1.34	1.48	+0.35	+0.52	0.51
Mg^{2+}									
1 mM	1.38	2.00	1.45	1.28	1.14	0.97	+0.15	0.00	0.39
5 mM	1.12	1.58	1.41	1.25	0.90	0.95	—0.08	—0.03	0.37
10 mM	1.17	1.67	1.43	1.25	0.95	0.96	—0.04	—0.01	0.37
50 mM	1.11	1.66	1.49	1.25	0.94	1.00	—0.05	+0.03	0.37
100 mM	1.39	2.16	1.55	1.25	1.22	1.14	+0.24	+0.07	0.37
1 M	1.42	2.30	1.61	1.30	1.30	1.19	+0.31	+0.11	0.40
Ba^{2+}									
1 mM	1.34	2.06	1.54	1.28	1.17	1.03	+0.18	+0.06	0.39
10 mM	1.24	1.88	1.52	1.28	1.07	1.02	+0.08	+0.05	0.39
100 mM	1.47	2.20	1.49	1.28	1.24	1.00	+0.26	+0.03	0.39
1 M	1.38	2.03	1.47	1.26	1.15	0.92	+0.17	+0.01	0.37
Cd^{2+}									
10 mM	1.46	2.09	1.43	1.25	1.18	0.96	+0.20	—0.01	0.37
100 mM	1.11	1.61	1.45	1.26	0.92	0.97	—0.07	0.00	0.37
1 M	0.98	1.79	1.82	1.41	1.01	1.22	+0.02	+0.25	0.47

choline molecules is exhibited in the opposite behavior of the 722 cm^{-1} line.

The lower values of the *trans* order parameters α_1 and α_2 in the concentration range of 1--10 mM strongly suggest the existence and the competition of two opposing forces, both Ca^{2+}-dependent; an attractive force favoring close-packing (self-association) of the membrane constituents, and a repulsive force that is essential to keep the lipid molecules apart and maintains membrane stability. The van der Waals forces, the hydrogen bond network due to the hydration of the polar heads, as well as the blanketing cloud of counter ions provide the main contributions to the resultant attractive force. Primarily, the electrostatic charges on the head groups, which may come either from zwitterionic charges, or from adsorption of foreign ions from the surrounding solution furnish the repulsive interactions. (A further aspect of the hydration of the polar head is similar to the role of identical electric charges; it tends to cause the lipid molecules to keep apart.) Obviously, the balance between net attractive and repulsive forces, as well as the hydration shell will determine the existing structure of lipid-water dispersion.

The measurements of Seimiya and Ohki [6] and those of Belmonte et al. [41] indicate that: (a) when the interlipid spacing is relatively large the divalent cation interacts only with one lipid molecule; (b) when the interlipid distance is relatively small the dipositive ion binds two lipid molecules. Keeping these in mind our observations presented in Fig. 2 can be qualitatively explained in the following way. In the absence of electrolyte the structure of the lipid dispersion is essentially determined by van der Waals interactions and the hydrogen bond network (as attractive forces), and by the electrostatic and hydration effects tending to separate the lipid molecules. As a consequence, a rather loose bilayer structure with relatively large intermolecular spacing will be formed. If a small amount of Ca^{2+} (in chloride form) is introduced, divalent cations will be adsorbed on single lipid molecules, partially dehydrating the polar head and converting the head group's charge into positive. Although the dehydration of the polar moiety and the presence of the Cl^- (as counter ions) favor close packing of the lipid molecules, the electrostatic repulsion due to the large positive charge on the head groups surmounts the attractive forces and a larger equilibrium distance between lipid molecules with adsorbed Ca^{2+} will be established, resulting in the disordering of hydrocarbon chains ($\alpha < 0$, Fig. 2). If the electrolyte concentration is increased, the chloride counter-ions present in sufficient amounts can effectively mask the charge of the adsorbed ion and will not allow the Ca^{2+}-induced disordering to proceed. A further rise in the electrolyte concentration leads to the interpenetration of the blanketing counter-ion clouds of neighbouring lipid molecules with adsorbed Ca^{2+} and hence, to the approach of the bilayer constituents. This process must be manifested in a moderate chain ordering as it was in fact observed (Fig. 2 and Table II). At a critical interlipid distance (commensurable with the order of magnitude of the ionic radius) a fairly sudden drop can be expected in the binding of Ca^{2+} [6], which must be reflected in increases in the order parameters α and S_L. It is believed that this transition occurs already at the concentration of 100 mM and the bridging interaction promotes ordering of hydrocarbon chains (Fig. 2 and Table II). The effects of Cd^{2+} and Mg^{2+} and, their concentration dependences are similar to those of Ca^{2+}, but somewhat weaker

(Table II). Ba^{2+} practically does not affect the lateral structure of the phospholipid multibilayers, but the tendency of the concentration dependence of α_1 is also qualitatively similar to those for Ca^{2+}, Cd^{2+} and Mg^{2+}, with the difference that the minimum apparent order is at higher salt concentrations. This again provides arguments in favor of the possible two-step binding of divalent cations to bilayered neutral phospholipids.

A great variety of effectiveness sequences can be constructed on the basis of intensity ratios and order parameters given in Table II. These sequences depend, however, not only upon the reference line as was the case for monovalent cations, but even upon the concentration of salt. The sequence of divalent cation effectiveness for lecithin found above 5 mM (Table II) is in good agreement with the results obtained with various other techniques [10,11, 25], though some authors reported the reverse order of Mg^{2+} and Ca^{2+} [42] or the same magnitude of the effect for these two divalent cations [14]. These latter observations can perhaps be attributed to the concentration-effect mentioned. The differences between the influences of various divalent cations on membrane may be due to differences in coordination numbers, geometrical requirements for binding ligands, etc. as suggested by Williams [43].

Since practically no frequency shift was found for the 2880 cm^{-1} line, S_L defined as in Eqn. 2 could be used to evaluate the influences of dipositive ions on the lateral packing of the lipid molecules. There are mostly insignificant differences in the lateral orders of hydrocarbon chains for difference divalent cations, in comparison with aqueous dispersions, even at high ionic concentrations. This suggests that either the parameter S_L is rather insensitive to the packing density or these cations interact with the head groups and directly affect mainly the polar region of the bilayers. In this latter case the ordering effect on the fatty acid residues is indirect and their structure may be quite close to that in the ion-free phospholipid-water dispersions. If so, this means that Ca^{2+} and other dipositive ions control the permeability properties primarily not via the structure of the membrane interior, but rather at the interface.

Mono- and divalent anions

We have investigated the structural influences of 1 M sodium salts of the anions, Cl^-, Br^-, I^-, NO_3^-, SO_3^{2-}, SO_4^{2-}, and the very effective $S_2O_3^{2-}$ and $S_2O_8^{2-}$ in 10 mM concentrations (Fig. 3 and Table III). In the following it will be assumed, as a first approximation, that anions and cations affect the lecithin molecules independently of each other, i.e. the changes observed are to be ascribed to anions, the sodium concentration being the same in all the cases, except for $S_2O_3^{2-}$ and $S_2O_8^{2-}$.

Several aspects of the iodide effect have already been discussed in detail in the first part of this series [34]. The results obtained (Table III) show that the intensity ratios for various mono- and divalent anions mirror different anion effects. That the anions NO_3^-, SO_3^{2-}, $S_2O_3^{2-}$ and $S_2O_8^{2-}$ may in fact give rise to higher *trans* order follows from the slightly enhanced packing densities monitored by S_L (Table III) and from the appearances of the corresponding spectra (Fig. 3). It is very likely that all the anions act as counter-ions for the trimethylammonium groups and they change the conformational states of head

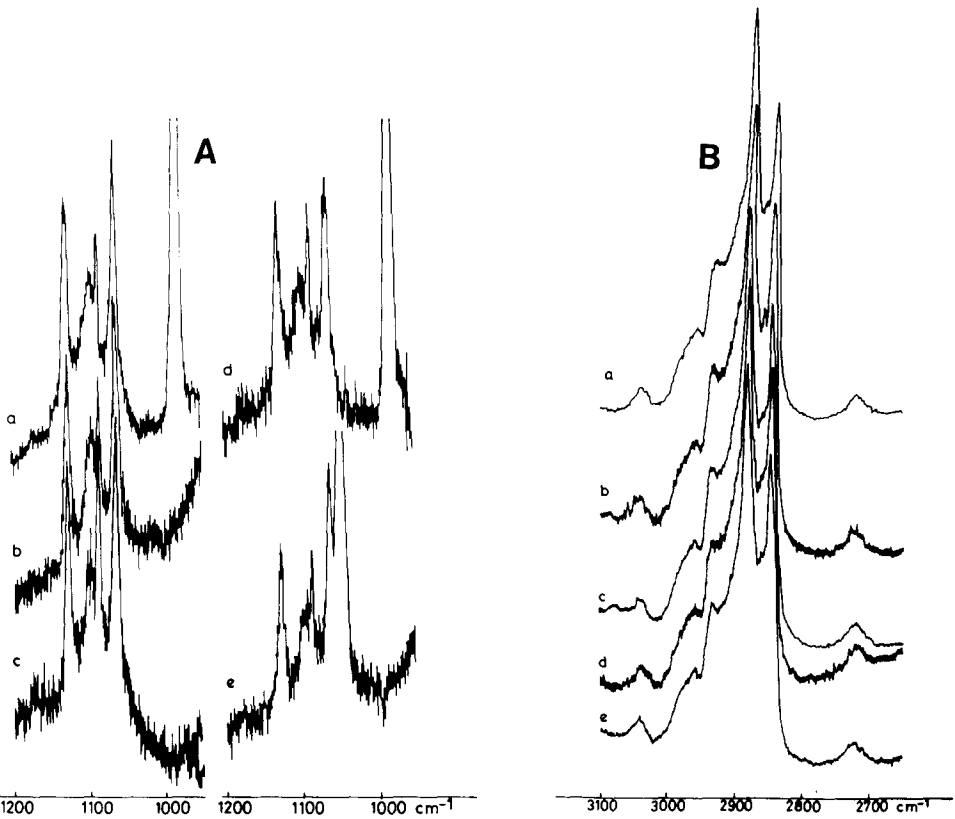

Fig. 3. Representative laser Raman spectra of dipalmitoyl phosphatidylcholine in lamellar dispersions containing 1 M sodium salts of different anions, in the Raman spectral ranges 950—1200 cm^{-1} (A) and 2650—3100 cm^{-1} (B). a, Na_2SO_4; b, NaBr; c, NaCl; d, Na_2SO_3; e, $NaNO_3$. Phosphatidylcholine : water = 1 : 4 (w/w).

TABLE III

Characteristic intensity ratios and order parameters (S_T^1, S_T^2, α_1 and α_2 for the *all-trans* configuration of hydrocarbon chains; S_L for the lateral packing density) for dry dipalmitoyl phosphatidylcholine and aqueous dipalmitoyl phosphatidylcholine dispersions in the presence of sodium salts of various mono- and divalent anions. Phosphatidylcholine : water = 1 : 4 (w/w).

Composition of lipid sample	$\frac{I_{1100}}{I_{722}}$	$\frac{I_{1130}}{I_{722}}$	$\frac{I_{1130}}{I_{1100}}$	$\frac{I_{2880}}{I_{2850}}$	S_T^1	S_T^2	α_1	α_2	S_L
Dry	1.18	1.76	1.49	1.37	1.00	1.00	—	—	0.45
H_2O	1.20	1.74	1.45	1.28	0.99	0.97	0.00	0.00	0.39
1 M NaCl	1.10	1.49	1.35	1.28	0.85	0.91	—0.15	—0.07	0.39
1 M NaBr	1.27	1.93	1.52	1.28	1.10	1.02	+0.11	+0.05	0.39
1 M NaI *	5.3	4.1	0.77	1.02	2.33	0.52	+1.36	—0.47	0.21
1 M $NaNO_3$	1.06	1.58	1.49	1.30	0.90	1.00	—0.09	+0.03	0.40
1 M Na_2SO_3	1.46	2.06	1.41	1.30	1.17	0.95	+0.18	—0.03	0.40
1 M Na_2SO_4	1.44	2.09	1.45	1.28	1.19	0.97	+0.20	0.00	0.39
10^{-2} M $Na_2S_2O_3$	1.48	2.38	1.61	1.32	1.35	1.08	+0.37	+0.11	0.41
10^{-2} M $Na_2S_2O_8$	1.46	2.08	1.49	1.25	1.18	0.95	+0.02	—0.20	0.37

* For particular experimental difficulties and problems see ref. 34.

groups, leading to an altered freedom in charge displacement of polarization origin. Besides this, at least NO_3^- and the divalent species have a further particular effect on the polar head motion and polarizability, α being positive. Just for structural reasons, it is plausible that, in the presence of divalent anions, the phosphatidylcholine molecules can rearrange themselves so that a divalent anion can be shared by trimethylammonium groups on neighbouring phospholipid molecules and thus, a more closely packed bilayer structure emerges.

The spectral data suggest that cations, especially the monovalent ones, and anions may have opposite effects on the structures of phospholipid-water systems; anions appear more effective. The structure of the zwitterionic head groups of neutral phosphatidylcholine and their orientation indicate that the negative groups, being closer to the hydrocarbon chains and partially neutralized by the quaternary nitrogen of the same molecule [4], are rather well masked and thus, less accessible for cations than the trimethylammonium groups for anions. No correlation seems to exist between (either effective or crystal) ionic radii and strengths of interaction of anions with lecithin multibilayers, indicating that their effects are rather specific and not entirely of an electrostatic nature. It appears also that the charge distribution on the anion is an important factor in the structural effects. In contrast to the observations of Lis et al. [25] on the structure influencing effects of potassium salts of various anions, we found anion-dependent structural changes for the anions investigated. The origin of this discrepancy is not yet well understood. A possible explanation offers itself in the difference in the cations used: potassium by Lis et al. [25] and sodium by us. As mentioned, sodium was the most effective in altering the structure of our system, and thus, it can be imagined that the anion effects are manifested differently for sodium and potassium. This would mean that the structures and structural transitions of lipid dispersions are determined and governed by cations and anions mutually rather than independently [25]. Whether the effects of anions and cations are interlinked at the level of a certain kind of competition or somehow else, the possible significance of the interactions postulated in the transport and regulatory processes, etc. all remain open, and are interesting tasks of further investigations.

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