

A RAMAN STUDY OF THE INTERACTION OF Mg^{2+} , Ca^{2+} , AND Ba^{2+} IONS WITH AN ACIDIC MODEL MEMBRANE

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The interaction of dipalmitoylphosphatidylglycerol (DPPG) liposomes with divalent ions of magnesium, calcium and barium has been investigated with laser-Raman spectroscopy over the temperature range of 0–60°C. The effect of Ca^{2+} ions was also investigated as a function of concentration. At a Ca^{2+} /DPPG molar ratio of 0.1, the number of *trans* carbon to carbon bonds in the hydrocarbon domain of the phospholipid and the lateral order of the hydrocarbon chains was increased both below and above the gel to liquid crystal transition. At higher Ca^{2+} concentrations the number of *trans* bonds and the lateral order is further increased over the entire temperature range studied, while the transition disappears. Magnesium and barium ions have a much smaller ordering effect on the side-chain packing of DPPG liposomes. At a molar ratio of 0.3, the gel to liquid crystal transition is still discernible for DPPG liposomes in the presence of Ba^{2+} ions, but not in the presence of Mg^{2+} ions.

Keywords: Raman; membrane; phospholipid interaction.

Introduction

Divalent alkaline earth cations, in particular the Ca^{2+} ion, exhibit interactions with liposomes composed of acidic phospholipids, such as DPPG and phosphatidylserine (PS), but have no significant effect on liposomes of neutral phospholipids such as dipalmitoylphosphatidylcholine (DPPC) [1–9]. The presence of Ca^{2+} is thought to be essential for the fusion of biological membranes [4,6], which has long been recognized as an important event in a variety of cellular activities [10]. In model membranes composed of several phospholipids, divalent alkaline earth ions can lead to segregation and phase separation [3,7–9]. The nature of the induced changes in bilayers containing negatively charged phospholipids is strongly dependent on the character of the polar head groups involved [11], as well as on the nature of the divalent ion [3]. Fusion of membranes by Ca^{2+} and Mg^{2+} ions is not thought to be a result of simple charge neutralization, but is probably

related to the ability to induce isothermal phase separations in phospholipid membranes [4]. Several spectroscopic techniques, including nuclear magnetic resonance (NMR) [12,13], electron spin resonance (ESR) [14–16], and laser-Raman [17,18] spectroscopy, have been used in recent years to investigate these phenomena.

Raman spectroscopy yields direct information about aspects of molecular structure of the hydrocarbon side-chains of phospholipids under various conditions [19,20]. In particular, it provides information about *trans-gauche* isomerism of the side-chains, and about their mobility and lateral packing [19,21]. These characteristics have been employed to investigate phospholipid–polypeptide interactions [22] as well as phospholipid–protein interactions [23,24]. Recently, Raman spectroscopic techniques have shown that the *trans-gauche* isomerism as well as lateral packing and mobility of the hydrocarbon side-chains of PS liposomes are profoundly influenced by the presence of Ca^{2+} ions and in a milder way by Mg^{2+} ions [17]. It has been demonstrated that it is possible to investigate calcium-induced fusion and molecular segregation of PS/dimyristoylphosphatidylcholine- d_{54} (DMPC) membranes by means of Raman spectroscopy [18].

The present investigation is concerned with the interaction of DPPG liposomes with Ca^{2+} , Mg^{2+} and Ba^{2+} ions as studied by laser-Raman spectroscopy. Unlike PS, which has mixed hydrocarbon side-chains and, therefore, a very broad gel to liquid crystal transition range, DPPG exhibits a sharp phase transition between the gel state and the liquid crystalline state.

Materials and Methods

High purity (better than 99%) L- α -dipalmitoylphosphatidyl-DL-glycerol ammonium salt was purchased from the Sigma Chemical Company*. Calcium chloride, magnesium chloride and barium chloride were 'Baker Analyzed' reagents. To provide a sample of pure phospholipid liposomes, 0.015 g ($2.3 \cdot 10^{-5}$ mol) of DPPG in 0.060 ml of 'Trizma' buffer (pH 7.5) (Sigma Chemical Co.) with 0.1 M KCl, was shaken for 15 min on a vibrator type mixer. Samples containing calcium, magnesium and barium ions were prepared by adding the proper amount of salt to the buffer solution to provide concentrations in the range of 0.05–0.2 M. The solution (0.060 ml) was then added to 0.015 g of phospholipid and the mixture shaken as previously on a vibrator type mixer.

Laser-Raman spectra were obtained with a Spex Ramalog system equipped with a Model 1401 monochromator, an RCA C-31034 photomultiplier, photon counting, a Spectra-Physics Model 165-8 argon ion laser, and a Spex 180° viewing platform. A brass-jacketed cell designed for 180° excitation, connected with a

thermostated water bath, was employed. The temperature was measured with a thermocouple located in the jacket, close to the cell. Such a 180° excitation cell has the advantage over conventional capillaries that only a shallow cavity has to be filled with the highly viscous suspension. The laser power was 300 mW at the sample. The spectral slit width was 5 cm⁻¹. The green 514.5 nm laser line was used for excitation. Local heating of the sample by the laser beam was estimated to be 2°C over the studied temperature range, as previously determined [22].

Raman spectra were obtained in the frequency-shift range of 2800–3050 cm⁻¹ (C–H stretching region) and 1000–1170 cm⁻¹ (C–C stretching region) over the temperature range of 0–60°C. Data obtained at temperatures higher than 60°C tended to be irreproducible and are therefore not given. Peak intensities were measured from a linear baseline defined by points at 2800 cm⁻¹ and 3020 cm⁻¹ for the C–H stretching region, and at 1020 cm⁻¹ and 1160 cm⁻¹ for the C–C stretching region.

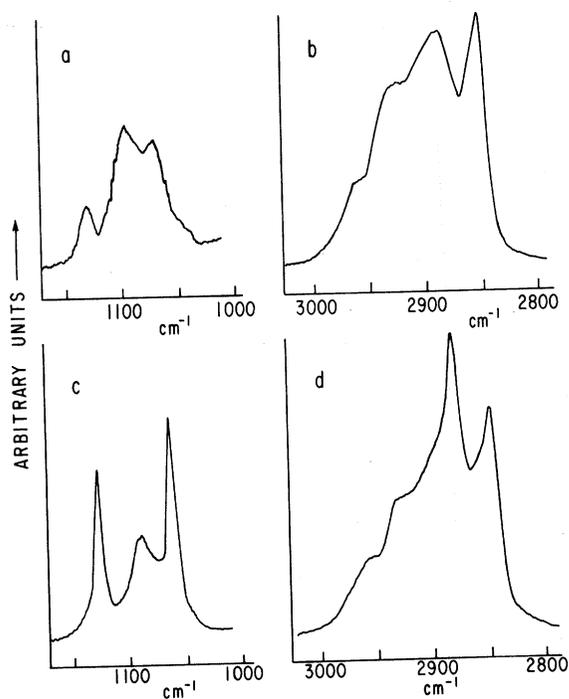


Fig. 1. Representative sample of laser-Raman data. (a) C–C stretching region and (b) C–H stretching region of DPPG liposomes at 45°C and pH 7.5. (c) C–C stretching region and (d) C–H stretching region of DPPG liposomes at 45°C in the presence of 0.1 M Ca²⁺ ions at pH 7.5.

Results and Discussion

Effect of Ca^{2+} ions

Figure 1 presents typical Raman spectra of DPPG liposomes, and of DPPG liposomes in the presence of Ca^{2+} ions, in the C–C stretching region ($1000\text{--}1170\text{ cm}^{-1}$), and in the C–H stretching region ($2800\text{--}3050\text{ cm}^{-1}$). The sample of DPPG had a very low fluorescence background; the spectra could therefore be recorded with a favorable signal-to-noise ratio.

Figure 2 presents the peak intensity ratio, I_{1130}/I_{1090} , of pure DPPG liposomes and of DPPG liposomes suspended in CaCl_2 solutions ranging from 0.037 M to 0.19 M , which corresponds to molar ratios, $n(\text{CaCl}_2)/n(\text{DPPG})$, from 0.1 to 0.55 . The Raman band centering at 1130 cm^{-1} has been assigned to in-phase C–C stretching modes of *trans* segments of the hydrocarbon side-chains of phospholipids [19,20]. To obtain a measure for *trans-gauche* isomerism in the hydrocarbon side-chains of phospholipids, the intensity of the 1130 cm^{-1} band of phospholipids like DPPC

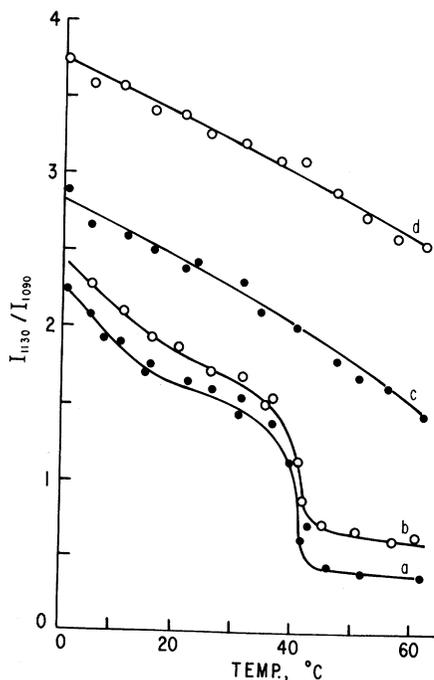


Fig. 2. The peak intensity ratio, I_{1130}/I_{1090} , of C–C stretching bands of pure DPPG liposomes and DPPG liposomes in the presence of Ca^{2+} ions at pH 7.5. (a) pure DPPG liposomes (15 mg suspended in 60 mg buffer); (b) DPPG liposomes suspended in 0.037 M CaCl_2 (molar ratio, $n(\text{CaCl}_2)/n(\text{DPPG}) = 0.1$); (c) DPPG liposomes suspended in 0.1 M CaCl_2 (molar ratio 0.3); (d) DPPG suspended in 0.19 M CaCl_2 (molar ratio 0.55).

or of the corresponding dimyristoyl compound, DMPC, is frequently ratioed to the intensity of the 715 cm^{-1} C–N stretching band of the choline group, which is independent of side-chain conformation and temperature [25,26]. Unfortunately, the Raman spectrum of DPPG does not exhibit a conformation- and temperature-independent band. We have, therefore, taken the peak intensity ratio of the 1130 cm^{-1} band to the 1090 cm^{-1} band as an indicator of *trans-gauche* isomerism of the hydrocarbon side-chains, as previously suggested by several authors [17, 19,20]. The major portion of the Raman intensity of the 1090 cm^{-1} band is contributed by stretching vibrations of *gauche* C–C bonds, although small contributions from *trans* C–C bonds and some other modes are also present [19,27].

As seen in Fig. 2b, a 0.1 molar ratio of Ca^{2+} to DPPG is sufficient to increase the *trans* content of the hydrocarbon side-chains of DPPG over the entire temperature range of $0\text{--}60^\circ\text{C}$, i.e., to increase the orderliness of the hydrocarbon domains of the phospholipid. We notice this effect on the hydrocarbon side-chain structure despite the likelihood that the Ca^{2+} ions interact primarily with the polar head groups. (A 'stiffening' effect is also observed in interactions of some polar polypeptides and extrinsic proteins with phospholipid liposomes [22,28].) The gel to liquid crystal transition is still clearly exhibited at this solute concentration and is observed at roughly the same temperature as in pure DPPG liposomes. The transition temperature is in good agreement with calorimetric data [28].

At a molar ratio, $n(\text{Ca}^{2+})/n(\text{DPPG})$, of 0.3, as shown in Fig. 2c, the *trans* content of the hydrocarbon side-chains is considerably increased and no gel to liquid crystal phase transition is observed over the investigated $0\text{--}60^\circ\text{C}$ temperature range. It is interesting to note that the I_{1130}/I_{1090} ratio for the sample with a molar ratio of 0.3 at 60°C is roughly the same as the corresponding ratio of pure DPPG liposomes just above the gel to liquid crystal transition. The approximately linear nature of the curve in Fig. 2c is quite similar to corresponding data obtained by Raman spectroscopic studies of PS [17]. At a molar ratio of 0.55, the temperature profile of the I_{1130}/I_{1090} ratio is further displaced to considerably higher values, as seen in Fig. 2d. (This molar ratio is slightly higher than the stoichiometric amount required to neutralize all negative charges of the phospholipid.)

Figure 3 presents the peak intensity ratio I_{2885}/I_{2850} of pure DPPG liposomes and of DPPG liposomes suspended in CaCl_2 solutions of different solute concentrations, in analogy to Fig. 2. The Raman bands exhibited by hydrocarbon chains at 2885 cm^{-1} and 2850 cm^{-1} can be assigned as being primarily caused by anti-symmetric and symmetric CH_2 stretching vibrations, although Fermi resonance with CH_2 bending overtones is also observed [29,30]. The ratio I_{2885}/I_{2850} is very sensitive to the physical state of the hydrocarbon chains and is affected by chain packing [25] and, as recently determined, by chain mobility [21]. The mobility is associated with the freedom of an extended chain to rotate and twist about its main axis [21]. In general, chain mobility affects the 2885 cm^{-1} band, chain packing (intermolecular coupling), the 2850 cm^{-1} band. The two effects can not

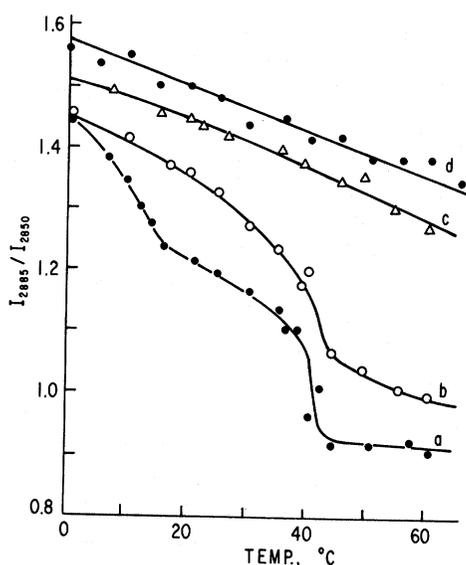


Fig. 3. The peak intensity ratio of C-H stretching bands, I_{2885}/I_{2850} , of pure DPPG liposomes and in the presence of Ca^{2+} ions. (a) pure DPPG liposomes; (b) DPPG liposomes suspended in 0.037 M CaCl_2 (molar ratio 0.1); (c) DPPG liposomes suspended in 0.1 M CaCl_2 (molar ratio 0.3); (d) DPPG liposomes suspended in 0.19 M CaCl_2 (molar ratio 0.55).

be separated by Raman studies alone and the ratio I_{2885}/I_{2850} reflects both [21]. In this communication, we refer to the combined effect as 'lateral order'.

As seen in Fig. 3, the lateral order follows, in a general sense, the *trans-gauche* isomerism as reflected in Fig. 2. A 0.1 molar ratio of $n(\text{Ca}^{2+})/n(\text{DPPG})$ increases the value of I_{2885}/I_{2850} over the entire temperature range of 0–60°C, but at this concentration the gel to liquid crystal transition is still clearly discernible, at a temperature close to the corresponding temperature of pure DPPG liposomes. At a molar ratio of 0.3, the lateral order is substantially increased and no transition can be detected in the experimentally covered temperature range. At a molar ratio of 0.55, the I_{2885}/I_{2850} ratio is further displaced to higher values. The plot 2d is linear as observed for the corresponding parameter of PS [17]. The lateral order, as reflected in Figs. 3c and 3d, at 60°C is higher than the corresponding value for pure DPPG liposomes just above the gel to liquid crystal transition, showing the drastic ordering effect of divalent calcium ions. The change from Fig. 3c to 3d, however, is much smaller than for corresponding lines of the I_{1130}/I_{1090} ratio (see Fig. 2). This suggests that at a molar ratio of 0.3 the lateral order has proceeded farther than the *trans-gauche* isomerism toward the state reached at a molecular ratio of 0.55.

Effect of Ba^{2+} and Mg^{2+} ions

Figure 4 shows the ratio I_{1130}/I_{1090} , which reflects *trans-gauche* isomerism,

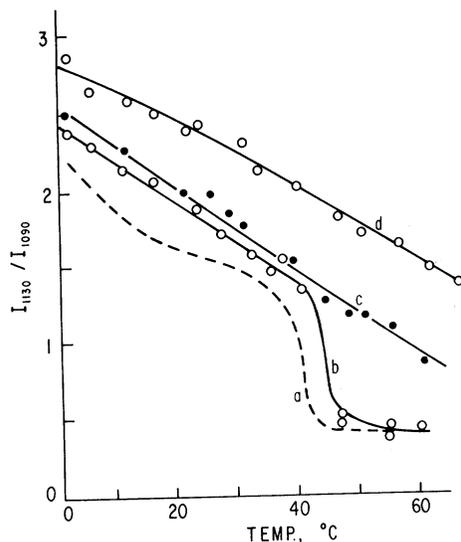


Fig. 4. The peak intensity ratio of C-C stretching bands, I_{1130}/I_{1090} , of DPPG liposomes and DPPG liposomes in the presence of different divalent ions. (a) pure DPPG liposomes; (b) DPPG liposomes suspended in 0.1 M BaCl_2 solution (molar ratio 0.3); (c) DPPG liposomes suspended in 0.1 M MgCl_2 solution (molar ratio 0.3); (d) DPPG liposomes suspended in 0.1 M CaCl_2 solution (molar ratio 0.3).

for pure DPPG liposomes as compared to the corresponding peak height ratios of liposomes prepared in the presence of 0.1 M Mg^{2+} , Ca^{2+} and Ba^{2+} ions. It is immediately evident that the effect of Ca^{2+} ions is much more pronounced than the effect of either Mg^{2+} or Ba^{2+} ions. The temperature profile of the I_{1130}/I_{1090} ratio of the sample containing Mg^{2+} ions (Fig. 4c) is similar to the corresponding profile of the Ca^{2+} -containing sample, but the intensity ratio has a much lower value over the entire studied temperature range, indicating weaker interactions between the Mg^{2+} ions and DPPG liposomes than in the case of Ca^{2+} ions. Studies on PS [17] have shown that Mg^{2+} ions shift the transition temperature to higher values. To find if this is also true for the present system, attempts were made to prolong curve c in Fig. 4 beyond the 60°C limit. In some runs a weak transition could be observed around 70°C, but this was not clearly reproducible. (At temperatures of about 60°C the DPPG suspension tends to become inhomogeneous and flaky.) Ba^{2+} ions appear to have the weakest effect on the orderliness of the DPPG liposomes, as seen in Fig. 4b. A slight increase of *trans* content is apparent above the gel to liquid crystal transition, and the transition itself, which is now clearly distinguishable, is shifted by $\sim 5^\circ\text{C}$ to higher temperatures. Above the transition there is little difference between pure DPPG liposomes (Fig. 4a) and liposomes in the presence of Ba^{2+} ions (Fig. 4b).

The peak height ratio of the C-H stretching band, I_{2885}/I_{2850} (Fig. 5), resembles qualitatively the I_{1130}/I_{1090} ratio as shown in Fig. 4. Curve 5d, representing inter-

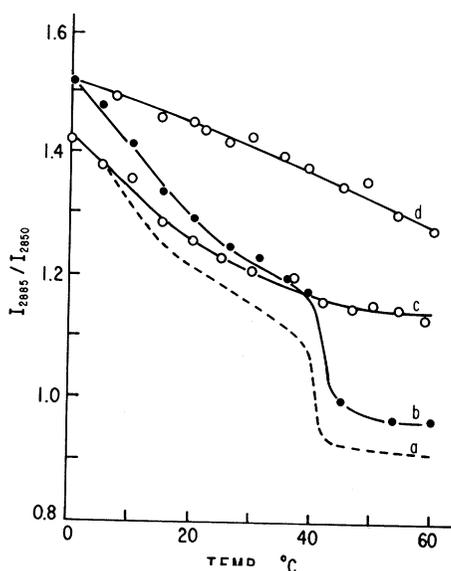


Fig. 5. The peak intensity ratio of C-H stretching bands, I_{2885}/I_{2850} , of DPPG liposomes and DPPG liposomes in the presence of different divalent ions. (a) pure DPPG liposomes (pH 7.5) for comparison; (b) DPPG liposomes suspended in 0.1 M BaCl_2 solution (molar ratio 0.3); (c) DPPG liposomes suspended in 0.1 M MgCl_2 solution (molar ratio 0.3); (d) DPPG liposomes suspended in 0.1 M CaCl_2 solution (molar ratio 0.3).

actions with Ca^{2+} ions exhibits by far the highest ratios of I_{2885}/I_{2850} . The heights of curves 5b (Ba^{2+} ions) and 5c (Mg^{2+} ions) are reversed above the transition temperature, but both are considerably lower than curve 5d. The gel to liquid crystal transition is clearly distinguishable in the sample with Ba^{2+} ions (Fig. 5b) but is not observed with Mg^{2+} ions in the studied temperature range, in agreement with the data in Fig. 4.

Conclusion

The Raman data have shown that Ca^{2+} ions at a molar ratio as low as 0.1 do increase the number of *trans* C-C bonds and the lateral order of DPPG liposomes both below and above the gel to liquid crystal transition temperature. The transition temperature remains essentially unchanged at this Ca^{2+} ion concentration. Higher concentrations of Ca^{2+} ions wipe out the transition in the 0–60°C range, and result in a markedly higher number of *trans* bonds, as well as a higher lateral order, over the entire studied temperature range. A comparison between the effects of Mg^{2+} , Ca^{2+} and Ba^{2+} ions at a molar ratio of 0.3 shows that the Ca^{2+} ions exhibit by far the strongest effect on the hydrocarbon side-chain packing of DPPG liposomes as judged by both the I_{1130}/I_{1090} and the I_{2885}/I_{2850} ratios. Ba^{2+} ions

appear to leave the transition intact while slightly increasing its temperature; Mg^{2+} ions wipe it out within the studied temperature range.

Raman data do not provide a mechanism for these observations. They do, nevertheless, show that group IIA cations have a pronounced effect on the conformation of the phospholipid both above and below the transition temperature; that the effects induced by Ca^{2+} ions are much more pronounced than the effects produced by Mg^{2+} and Ba^{2+} ions; and that the influence of these ions goes beyond simple interaction with the charged head groups of the phospholipid. Reference is made in this connection to the charged-induced tilt of the long, aliphatic chains, which has been established also for DPPG [31]. Charge compensation, which is one effect of divalent cations among others, leads to a vanishing of the tilt and the results obtained by Raman spectroscopy may be correlated to this mechanism.

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