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RAMAN SCATTERING STUDY OF EFFECT OF CALCIUM AND MAGNESIUM ON PHOSPHATIDYLSERINE VESICLES

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SUMMARY: Raman scattering has been used to study the effect of divalent cations of phosphatidylserine vesicles. The data show that magnesium does not significantly alter the acyl chain conformation and lateral packing of the bilayers other than shifting the transition temperature, while calcium results in highly rigid acyl chains with a gradual temperature dependence in the trans-gauche isomerization. The results suggest that Raman scattering is a potentially useful probe of calcium-induced membrane fusion and phase separation.

INTRODUCTION

The effect of divalent cations on the structure and thermal properties of acidic phospholipid bilayers has been a subject of intense current interest (1-4). Of particular significance is the ability of Ca^{2+}, but not Mg^{2+}, to induce fusion of acidic phospholipid vesicles (2,5) and phase separation in mixed vesicles (3,6-9). Despite reservations about the possible lack of vesicle integrity prior to fusion (10), research in these model systems is likely to increase our understanding of the specific role played by Ca^{2+} in regulating many cellular and subcellular activities (11).

Earlier studies of the interaction between divalent cations and acidic phospholipid vesicles employed such macroscopic techniques as differential scanning calorimetry and freeze-fracture electron microscopy (2,5). It has become apparent that a detailed understanding of the phenomena depends on additional microscopic information about the structure of the bilayers. Recent studies in this direction include X-ray and NMR measurements of the bilayer spacing and the degree of hydration in phosphatidylserine in the presence of divalent cations (12-14). The results suggest that Ca^{2+} induces the formation of a complex composing of almost anhydrous bilayers, while Mg^{2+} causes a milder perturbation of the bilayer structure. These studies provide only an indirect picture of the fluidity and packing of the bilayers.

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There has been, however, no measurement on a molecular level to study directly the conformation and lateral packing of the acyl chains in acidic phospholipids in the presence of divalent cations.

We report here the first attempt to use Raman scattering to study the microscopic properties of phosphatidylserine vesicles in the presence of Ca^{2+} and Mg^{2+}. Raman scattering has been shown to be a powerful and quantitative probe of biomembrane structure (15-18). The C-C stretching modes around 1100 cm^{-1} and the C-H stretching modes around 2900 cm^{-1} are found to be sensitive indicators of the bilayer phase transitions. Our objectives are to study the effect of the divalent cations on the conformation and packing of the phosphatidylserine molecules and to explore the use of Raman scattering as a measure of the extent of Ca^{2+}-induced vesicle fusion.

METHODS

Bovine brain phosphatidylserine was obtained from Avanti Biochemicals. Vesicles were prepared in a buffer solution containing 0.1 M NaCl, 2 mM N-tris-(hydroxymethyl)methyl-2-amino-ethanesulfonic acid, 2 mM L-histidine, and 0.1 mM EDTA, adjusted to pH 7.4. Typically, 25 mg of phosphatidylserine was dispersed in 0.3 ml of buffer and shaken mechanically for 10 min. Raman spectra in the absence of divalent cations were taken with the dispersion in a sealed 1 mm capillary tube. Sonicated vesicles were used in the studies with divalent cations. The dispersion was sonicated in a bath-type sonicator under nitrogen at 25°C for at least 2 h until a clear solution was obtained. About 50 µl of the sonicated vesicles in buffer was transferred to a capillary tube and mixed with an equal volume of buffer containing 0.1 M $CaCl_2$ or $MgCl_2$. The final concentrations of both phosphatidylserine and either Ca^{2+} or Mg^{2+} are typically 50 mM. The capillary tube was sealed under nitrogen and centrifuged to pack the precipitate.

Raman spectra were taken with a Spex 14018 double monochromator at 3 cm^{-1} resolution. The 514.5 nm radiation from a Coherent CR-4 argon ion laser was used as the light source. The typical intensity at the sample was 300 mW. The 90° scattering geometry was used. The temperature of the sample in the capillary tube was controlled above and below ambient to a stability of 0.1°C by means of a Cambion thermoelectric module with a Kepco bipolar power supply as the current source. The detector was a cooled Hamamatsu R955 photomultiplier. Photon counting was used to monitor the detector output. Band intensities were taken as peak heights measured from a consistently chosen baseline. All the temperature scans were repeated at least once to test for repeatability. For the precipitated samples in the presence of Ca^{2+} or Mg^{2+}, spectra with steady baselines were difficult to obtain above 50°C, possibly because of increased motion of the precipitate relative to the incident laser beam.

RESULTS AND DISCUSSION

The hydrocarbon skeletal C-C stretching modes between 1050 and 1150 cm^{-1} have been used as convenient indicators of the trans-gauche conformation of the acyl chains (15,17,19). The intensities of the 1063

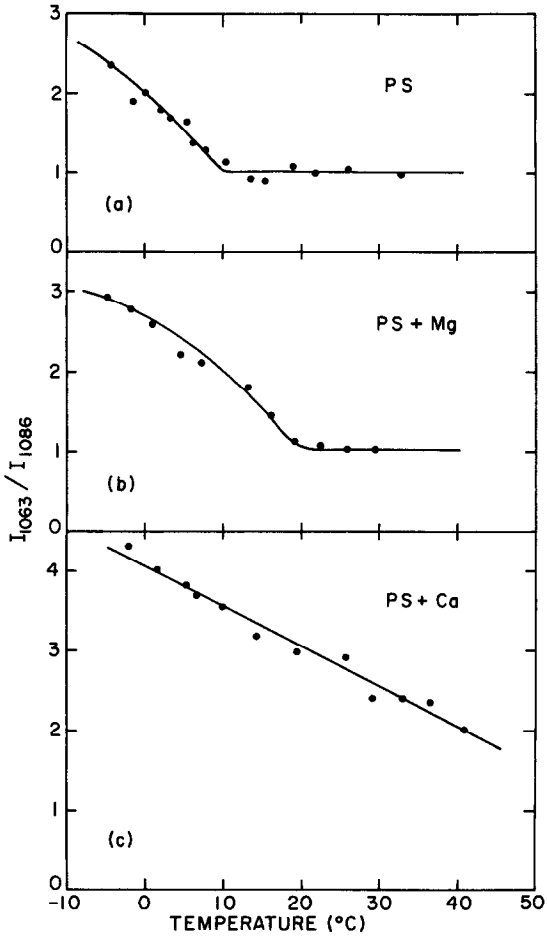

Fig. 1. Temperature dependence of the ratio of the Raman peak intensity at 1063 cm^{-1} to that at 1086 cm^{-1} in (a) 100 mM phosphatidylserine dispersion, (b) 50 mM sonicated phosphatidylserine vesicles with 50 mM Mg^{2+}, and (c) 50 mM sonicated phosphatidylserine vesicles with 50 mM Ca^{2+}.

cm^{-1} and the 1086 cm^{-1} bands are known to be a measure of the number of trans and gauche bonds, respectively. Fig. 1 shows the temperature dependence of the I_{1063}/I_{1086} intensity ratio in phosphatidylserine dispersion and in sonicated vesicles in the presence of Mg^{2+} and Ca^{2+}. The data for phosphatidylserine dispersion in Fig. 1(a) show a phase transition around 10°C, in good agreement with differential scanning calorimetry results (3,13). The transition is considerably broader than that in synthetic phosphatidylcholines (15). This is understandable in view of the heterogeneous composition of bovine brain phosphatidylserine. For sonicated vesicles in the presence of Mg^{2+}, the intensity ratio curve in Fig. 1(b) shows a transition at 20°C, again in good agreement with

differential scanning calorimetry data (3,13). It is significant to note that the melting curves in Fig. 1(a) and 1(b) are identical quantitatively except for a relative shift in the temperature scale. It is known that Mg^{2+} results in the formation of complicated vesicle aggregates and a reduced water content between bilayers (14). Our results show clearly that, apart from an upward shift in the transition temperature by 10°C, Mg^{2+} does not result in any change in the molecular conformation of the phosphatidylserine bilayers despite the macroscopic changes brought about by aggregation.

The temperature dependence of the I_{1063}/I_{1086} intensity ratio in the presence of Ca^{2+}, as shown in Fig. 1(c), is of particular interest. Within the temperature range studied, our data are consistent with the absence of a phase transition of the fused cochleate cylinders (3,14). More significantly, the Raman intensity ratio shows a degree of rigidity of the acyl chains in the presence of Ca^{2+} which is considerably higher than that in Mg^{2+} below 20°C. This is not obvious from X-ray data (12,14). Furthermore, while there appears to be no temperature dependence in the X-ray diffraction spacings of the phosphatidylserine-Ca^{2+} system between 5°C and 60°C (14), the Raman results clearly show a gradual temperature dependence of the intensity ratio, implying a steady increase in acyl chain trans-gauche isomerization as the temperature of the crystalline phosphatidylserine-Ca^{2+} system is raised. The X-ray spacings are apparently not sensitive to such changes.

The C-H stretching mode at 2880 cm^{-1} has been found to be sensitive to both the lateral packing of the acyl chains and to the number of gauche bonds, while that at 2930 cm^{-1} is a measure of the trans bonds (15,16,19,20). Fig. 2 shows the temperature dependence of the I_{2880}/I_{2930} intensity ratio in phosphatidylserine with and without Ca^{2+} or Mg^{2+}. Again, the addition of Mg^{2+} shifts the phase transition temperature upward by about 10°C, while there is no phase transition in the data in the presence of Ca^{2+}. The interesting information provided by Raman scattering is that the C-H intensity ratio with Ca^{2+} in Fig. 2(c) has a range of values similar to those in Fig. 2(a) (without divalent cations) and Fig. 2(b) (with Mg^{2+}). This is in contrast to the much higher C-C intensity ratio with Ca^{2+} in Fig. 1(c) as compared to Fig. 1(a) and 1(b). We believe this is due to the sensitivity of the C-H stretching modes to the lateral packing, which is adversely affected by the heterogeneity of the acyl chain length in bovine brain phosphatidylserine.

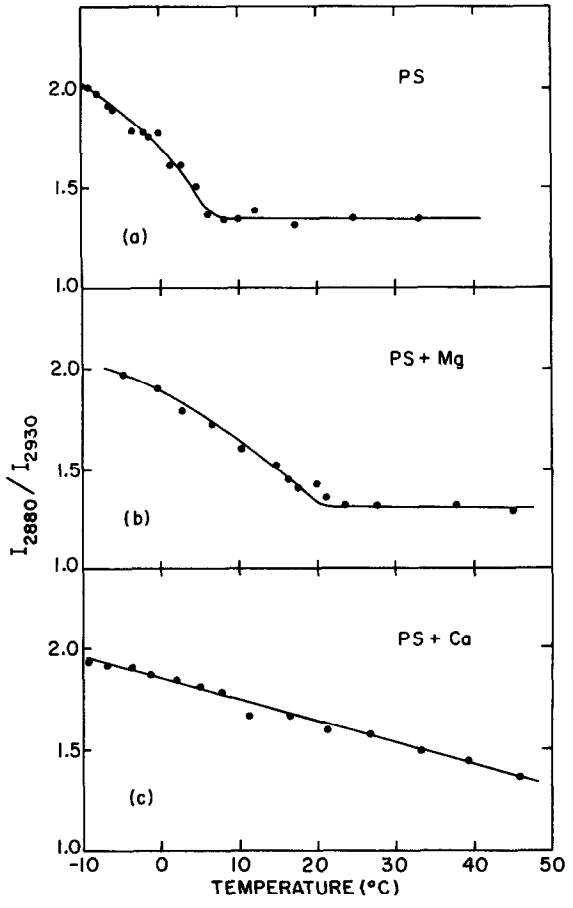

Fig. 2. Temperature dependence of the ratio of the Raman peak intensity at 2880 cm^{-1} to that at 2930 cm^{-1} in (a) 100 mM phosphatidylserine dispersion, (b) 50 mM sonicated phosphatidylserine vesicles with 50 mM Mg^{2+}, and (c) 50 mM sonicated phosphatidylserine vesicles with 50 mM Ca^{2+}.

Finally, our results suggest the possible use of Raman scattering in research on the fusion of model membranes. There is a significant difference between the Raman spectra obtained in the presence of Ca^{2+}, which induces vesicle fusion, and those of Mg^{2+}, which induces only vesicle aggregation. At 25°C, for example, Fig. 1 shows that the I_{1063}/I_{1086} intensity ratio remains unchanged with the addition of Mg^{2+}, but increases by a factor of 3 with the addition of Ca^{2+}. This implies Raman scattering can serve as an accurate and convenient alternative to other techniques, such as dynamic light scattering (21,22), to study the extent of Ca^{2+}-induced fusion in acidic phospholipid vesicles. It may be particularly useful in studies involving fusion kinetics, or Ca^{2+}-induced phase separation of a binary system in which one of the components is deuterated.

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