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# RAMAN STUDY OF EFFECT OF PHOSPHOLIPID CHAIN UNSATURATION ON BILAYER PHASE TRANSITIONS

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SUMMARY: Raman scattering has been used to study the gel to liquid-crystalline transition in several unsaturated mixed-chain, saturated mixed-chain, and unsaturated symmetric phospholipids. The results show that the large transition temperature widths in membranes containing unsaturated mixed-chain phospholipids in which the two acyl chains do not differ significantly in length are due mainly to the presence of the double bond and not to the asymmetry in the chains. 

It is well known that in phospholipids found in biological membranes, the two acyl chains attached to carbons 1 and 2 of the glycerol backbone are usually different (1). There is also a strong preference for natural phospholipids to possess a saturated fatty acyl chain at position 1 and an unsaturated one at position 2 of the glycerol moiety. Earlier model membrane studies have been mostly performed using synthetic phospholipids containing symmetric saturated chains. More recently, there have been increasing interest in investigating the gel to liquid-crystalline phase transitions in bilayers formed from saturated mixed-chain phospholipids (2-4). These studies have revealed how the packing of the bilayer and therefore its transition entropy can be affected by the extent of interdigitation of the acyl chains across the center of the bilayer, and by the disruptive effect of the terminal methyl groups of the chains.

The presence of double bonds in biological membranes is potentially important for at least two reasons. It enables the membrane to remain

Abbreviations: POPS, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine; OSPC, 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine; PSPC, 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine; SPPC, 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine; DNPC, L-$\alpha$-dinervonoyl-phosphatidylcholine.

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in the liquid-crystalline or fluid state at a lower temperature (5), thus providing the proper physical environment for many membrane functions to take place. There is also evidence for the existence of a specific interaction between enzymatic recogition sites and cis double bonds that can affect the activity of certain membrane-bound enzymes (6). There have been to date only a few studies of the effect of the presence of an unsaturated acyl chain on the physical properties of a phospholipid bilayer (7-9). We report here the results of a study using Raman spectroscopy of synthetic membranes containing unsaturated chains. Raman scattering has been shown to be a powerful and quantitative probe of biomembrane structure (10-12). 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 membrane fluidity. Our objective is to examine the effect of chain unsaturation in the phospholipid molecules on the cooperativity of the membrane phase transition.

## METHODS

Synthetic phospholipids were obtained from Avanti Polar Lipids. Symmetric lipids were 99% pure, while mixed-acid lipids contained up to 19% of randomization of the position of the two acyl chains (W. A. Shaw, private communication). 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 phospholipid was dispersed in 0.3 ml of buffer and shaken mechanically for 10 min. The dispersion was then sealed in 1 mm capillary tube.

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 100 mW. The right-angle 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. Multi-scan signal averaging was performed using an IMB PC computer. Band intensities were taken as peak heights measured from a consistently chosen baseline.

## RESULTS AND DISCUSSION

The C-H stretching mode at 2883 $cm^{-1}$ 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 $cm^{-1}$ can be used as a measure of the

number of trans bonds (13,14). In this report, the $I_{2883}/I_{2930}$ intensity ratio is primarily used as a convenient indicator of the degree of order of the bilayer. We have also data on the C-C stretching modes near 1100 $cm^{-1}$ which corroborate our conclusions here. To minimize the effects of the interdigitation of the acyl chains across the center of the bilayer (4), we have limited our studies to phospholipids in which the two acyl chains do not differ sigificantly in length.

Fig. 1 contains the results obtained in two unsaturated mixed-acid phospholipids, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS) and 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (OSPC). The transition temperature $T_c$ and the width of the transition in OSPC found with Raman scattering are consistent with those reported using differential scanning calorimetry (8). The important result here is the striking similarity, apart from the difference in $T_c$, in the overall behavior in POPS and OSPC. The phase transitions in both phospholipids are characterized by an $I_{2883}/I_{2930}$ intensity ratio of 1.4 above $T_c$ and 2.0 immediately below $T_c$. The relatively low value of the intensity ratio below $T_c$ is probably due to the disruptive nature of the double bond in one of the acyl chains on the ability of the bilayer to achieve

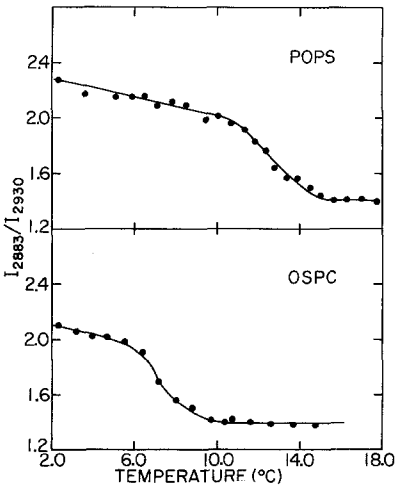


Fig. 1. Temperature dependence of the C-H Raman peak intensity ratio in dispersions of the unsaturated mixed-chain phospholipids POPS and OSPC.

a highly ordered and closely packed arrangement. More significantly, both transitions take place in a large temperature width of about 4°C. In contrast, Raman studies on symmetric saturated phospholipids with acyl chains containing 16 or 18 carbons typically yield a transition width of less than 1°C (13,15). As demonstrated in earlier studies (8,9), the large transition widths in POPS and OSPC appear to be an intrinsic property of these membranes and are not solely attributable to the possible presence of a small amount of the reversed isomer. In addition, our results show that the large transition width occurs independently of the location of the unsaturated acyl chain relative to the glycerol backbone. The nature of the headgroup, not surprisingly, does not seem to play a role either.

We have examined other phospholipids in order to find out whether it is the asymmetry in the two acyl chains or the presence of the double bond that is responsible for the large transition widths in the two unsaturated mixed-acid phospholipids. Fig. 2 shows the results in 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (PSPC) and 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (SPPC). Our observation of a higher $T_c$ in PSPC than in SPPC is consistent with published results using differential scanning calorimetry (2,3) and Raman scattering (16). The difference in $T_c$ between PSPC and SPPC here is somewhat less than that reported earlier, possibly due to the presence of a small fraction of the reverse isomer in these samples. However, the transition temperature widths in the two saturated mixed-acid phospholipids are both only about 1°C. These results suggest that asymmetry in the two acyl chains alone, without the presence of any double bond, does not necessarily lead to a phase transition with low cooperativity. The large values of the Raman intensity ratio below $T_c$ in PSPC and SPPC compared to those in POPS and OSPC also indicate a higher order and better packing when the double bond is not present. Finally, Fig. 3 shows the results obtained in L-α-dinervonoyl-phosphatidylcholine (DNPC),

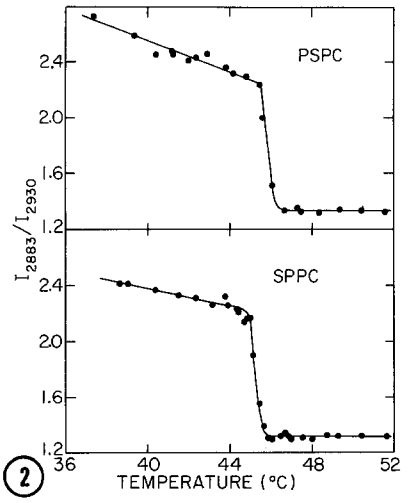


Fig. 2. Temperature dependence of the C-H Raman peak intensity ratio in dispersions of the saturated mixed-chain phospholipids PSPC and SPPC.

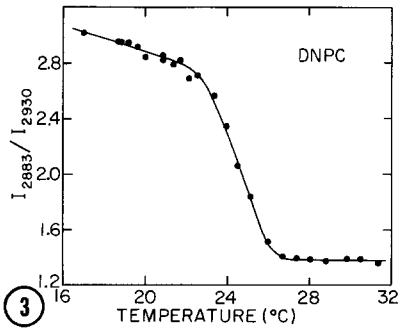


Fig. 3. Temperature dependence of the C-H Raman peak intensity ratio in dispersions of the unsaturated symmetric phospholipid DNPC.

which contains two symmetric 24-carbon acyl chains each with one double bond. Two striking observations can be made about the data in DNPC. First, the large transition width of 4°C is almost identical to those in POPS and OSPC, showing that the effect on the transition width is similar whether there is a double bond in one or both acyl chains. Secondly, the Raman intensity ratio just below $T_c$ is as high as 2.8, indicating the higher degree of order in this long chain system despite the presence of a double bond in both chains.

In summary, this project represents the first study using Raman scattering designed to elucidate the effect of chain unsaturation in the biophysical properties of phospholipid bilayers. The subject of asymmetric or unsaturated phospholipids has emerged recently as one of intense current interest (9,16). Unlike previous studies using predominantly differential scanning calorimetry, Raman scattering provides additional valuable information about chain ordering and molecular packing. Our results are the first to suggest that the large transition widths, and hence low transition cooperativity, in membranes containing

unsaturated mixed-chain phospholipids are mainly due to the presence of the double bond and not to the asymmetry of the chains. This property should be relevant in explaining the prevalent unsaturation of natural phospholipids, and must also be considered one of the essential features in any theoretical model of the bilayer phase transition.

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