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THE EFFECT OF SURFACE CURVATURE ON THE HEAD-GROUP STRUCTURE AND PHASE TRANSITION PROPERTIES OF PHOSPHOLIPID BILAYER VESICLES

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Summary

Proton nuclear magnetic resonance spectra at 360 MHz of small sonicated distearoyl phosphatidylcholine vesicles show easily distinguishable resonances due to choline N-methyl head-group protons located in the inner and outer bilayer halves. A study of the chemical shift of these resonances as a function of temperature reveals that the splitting between them increases below the phase transition. This occurs as a result of an upfield shift of the inner layer resonance at the phase transition. Consideration of the possible causes of this effect results in the conclusion that, at the phase transition, there is a change in the organization of the inner layer head-groups which does not occur for the outer layer head-groups.

Biological membranes often contain regions which have a small radius of curvature. It has been suggested [1,2] that this curvature could modify membrane properties, thereby providing a means of regional differentiation of membrane function. The effect of curvature on the phase behavior of phospholipid bilayer membranes has been demonstrated by a variety of techniques. Differential scanning calorimetry [3—6] and fluorescence studies [6,7] have shown that the phase transition temperature, enthalpy and entropy are all lower in small sonicated vesicles than in planar bilayers. NMR [1,8—12] and Raman studies [13—15] have shown that this is partly due to packing differences of the hydrocarbon chains as a result of the structural asymmetry

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imposed by a small radius of curvature. Because of this asymmetry, it is expected that curvature will affect the organization of the head-group region as well.

In this communication we present the results of nuclear magnetic resonance experiments which suggest a substantial difference in the organization of inner and outer head-groups of small vesicles below the thermal phase transition. This difference is reflected in the changes in chemical shift of inner and outer choline head-group resonances as the phase transition is traversed. In particular, the chemical shifts indicate that the inner layer lipids undergo at the phase transition a structural change not observed for the outer layer.

Small single-walled bilayer vesicles were prepared by sonicating a dispersion of distearoyl phosphatidycholine (30 mg/ml in ^2H_2O) at a temperature above the phase transition for 10 min. Following sonication the solution was centrifuged to remove any remaining large bilayer structures and titanium particles released from the sonicator tip. Previous studies [16] have shown that this treatment results in a fairly homogeneous population of small vesicles with an outer diameter of approx. 250 Å.

Fourier transform ^{1}H-NMR spectra at 360 MHz of the resulting vesicle suspension were obtained at 2° C intervals of temperature from 64 to 36° C. A total of 100 transients were collected for each temperature using 16K data points (0.30 Hz digital resolution) with 2-s delay between pulses. The probe temperature was determined using ethyleneglycol and can be considered reliable to within 0.5° C. A coaxial capillary containing 1% $(CH_3)_4Si$ in $CHCl_3$ was used as an external reference.

The spectra of the vesicles show the characteristic splitting of the choline N-methyl resonance which has previously been assigned to head-groups on the inner and outer surfaces of the bilayer vesicle [1,17]. At the magnetic field of this experiment, 84.56 kG, the inner and outer peaks can be resolved throughout the temperature range of the experiment. At the lower temperatures the splitting becomes much more apparent. Resonances due to choline N-methylene and PO-methylene protons are also perceptibly split at the lower temperatures in the same manner as the choline N-methyl resonance, although the splittings of these resonances are either unresolved or absent at the higher temperatures. The relative intensities of the inner and outer peaks reflect the number of molecules in the inner and outer bilayer halves [1] and can be used to estimate the average outer radius of the vesicles to be 120 Å. The outer-to-inner choline intensity ratio is about 2.7 at both limits of the temperature range studied, indicating that the size distribution of the vesicle population has not changed during the course of the experiment. In addition, the total area of the choline peak remains constant with temperature, as was previously observed for dipalmitoyl phosphatidylcholine vesicles [1].

Fig. 1 shows the portion of the spectrum due to choline N-methyl protons at various temperatures. It can be seen that the chemical shift difference between the inner and outer choline N-methyl resonances increases as the temperature is lowered. In Fig. 2 the difference in chemical shift of the two resonances is plotted vs. temperature. The data show an abrupt change in magnetic inequivalence over a relatively narrow range of temperatures near 50° C, decreasing from about 25 Hz below 50° C to about 9 Hz at higher tempera-

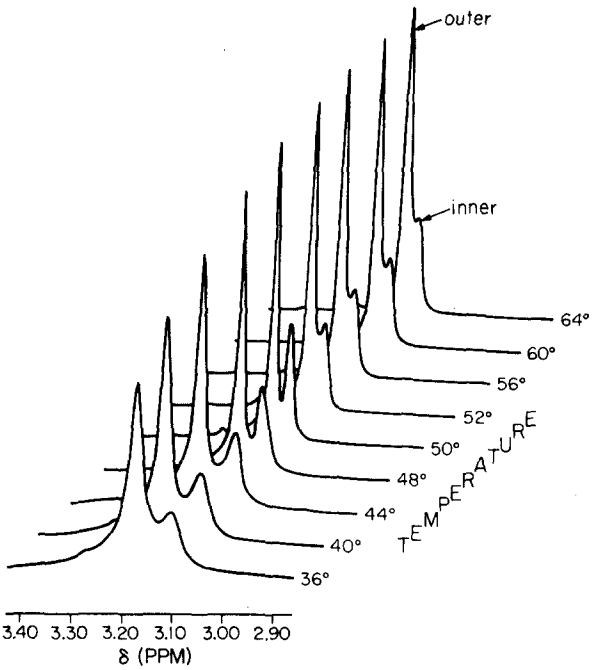

Fig. 1 Choline N-methyl portion of the 360 MHz ^{1}H-NMR spectrum of small sonicated vesicles at various indicated temperatures (° C). Resonances due to molecules on the inner and outer halves of the bilayer are indicated by the arrows.

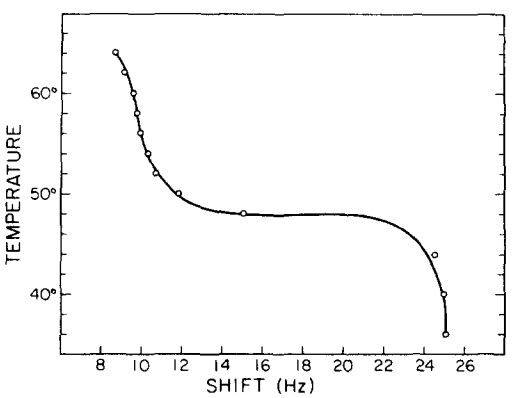

Fig. 2 Chemical shift difference in Hz between inner and outer choline N-methyl proton resonances at various temperatures ranging through the thermal phase transition of the bilayer vesicles. (360 Hz = 1 part per million).

tures. The resonance linewidths also begin to increase at about 50° C, in agreement with previous observations that lipid resonances broaden below the thermal phase transition. These observed changes correlate well with the phase transition temperature of 51.3° C and width of 7.3° C reported for distearoyl phosphatidylcholine vesicles by fluorescent probe studies [7].

In Fig. 3, the resonance frequencies of the individual inner and outer choline N-methyl protons are plotted vs. temperature. These data reveal a change in the shift of inner layer head-groups at the phase transition which

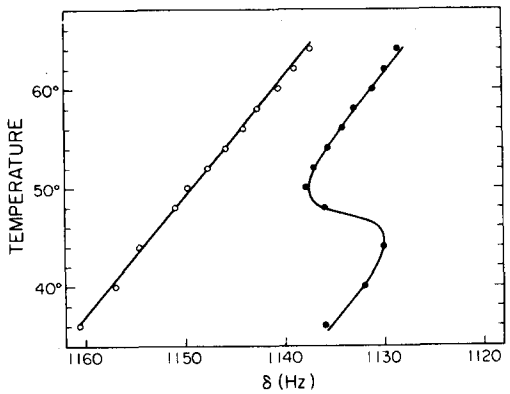

Fig. 3 Resonance position (in Hz relative to $(CH_3)_4Si$) of outer (o—o) and inner (•—•) choline N-methyl protons plotted vs. temperature.

does not occur for the outer layer head-groups. Inasmuch as these chemical shifts are referenced to external $(CH_3)_4Si$, the data also reflect the change in solution bulk susceptibility with temperature. This effect is nearly linear over the temperature range of our experiment [18] and causes a small slope in the data. If the hydrocarbon methylene resonance is used as an internal reference, the outer layer shifts become constant with temperature, as do the inner layer resonances except for the inner layer transition at 50° C. Whilst it has been known previously that the chemical shifts of inner and outer layer choline N-methyl resonances are different, the data of Fig. 2 and 3 show that there is an increase in the splitting below the phase transition and, furthermore, that this increase is the result of an upfield shift of the inner layer resonance.

As Kostelnik and Castellano [17] have pointed out, chemical shift differences of the magnitudes shown in Fig. 2 and 3 cannot be accounted for solely by the shielding at the inner surface due to the bilayer (estimated to be less than $1 \cdot 10^{-5}$ ppm). Any deviation of the vesicle shape from spherical symmetry could result in a more substantial shift between inner and outer layers since the induced field at the outer layer does not average to zero over all orientations of the non-spherical surface. This possibility can be considered unlikely because the surface free energy of the vesicle favors a uniform spherical surface [19]. In this context it is pertinent that the ^{31}P spectrum of vesicles also consists of two resonances due to molecules on the inner and outer layers [20] and that the splitting between them is substantially larger than that observed for the choline N-methyl proton resonance. Thus, bulk magnetic effects may be excluded as a cause of the magnetic inequivalence between layers. This leaves local magnetic effects due to head-group organization as the important determinant of the difference in shift between inner and outer layers.

A difference in head-group organization, reflecting differences in head-group conformation or packing, may be a result of variations in the intermolecular electrostatic interactions of the zwitterionic head-groups. Yeagle [21] has established that phosphatidylcholine head-group packing consists of the positively charged quaternary amine of each lipid associating with the negatively charged phosphates of adjacent neighbors. This interaction is electrostatically favorable and orients the head-groups parallel to the membrane

surface [22]. Although the exact distance between intermolecular charged pairs is not known, ^{31}P nuclear Overhauser experiments suggest that it is less than 3 Å. It is known that under conditions which neutralize the electrostatic binding, this interaction can be disrupted [23—26]. This point is relevant because lipid packing is very different for the inner and outer halves of small bilayer vesicles. In particular, the area occupied by each lipid head-group in the outer layer of the vesicle is some 12—21% larger than for the inner layer, depending on the lipid. In vesicles of dipalmitoyl phosphatidylcholine, the area per head-group at the inner surface [27] (68 Å^2) is near to the value for planar bilayers [29], while the larger area at the outer surface (76 Å^2) indicates that the outer layer is somewhat expanded by curvature. This 12—21% difference in head-group area translates to a 6—10% linear expansion between outer layer head-groups, irrespective of the packing lattice assumed by the lipid molecules. Assuming a reasonable distance of 8—10 Å between head-groups, this 6—10% difference in the average lipid-lipid distance would imply that head-groups are stretched almost 1 Å farther apart in the outer layer. Such an expansion would unquestionably influence the electrostatic interactions of the head-groups in the outer layer, causing them to much weaker. If the interaction is sufficiently weakened, it might be expected that the head-groups of the outer layer could adopt some new, more energetically favored conformation in which the head-groups do not electrostatically interact to the same extent. In fact, minimum energy calculations of head-group conformation predict an extended conformation of the head-group when electrostatic interactions are excluded [30]. The conformation of the inner layer head-groups remains unperturbed by curvature since the inner head-group areas remain roughly the same as in a planar bilayer.

The results of Fig. 3 suggest that there is no structural change in the outer layer head-groups over the temperature range of the thermal phase transition of the bilayer. This conclusion may have some bearing on the apparent discrepancy between transition enthalphies of vesicles and flat multilamellar bilayer (multilayers). The transition enthalpy of small vesicles is about one-third of that for multilayers [4,5], and increases with vesicle size [5]. Coincidentally, the fraction of molecules in the inner layer of small vesicles is about one-third and this fraction increases with vesicle size in a manner consistent with the dependence of enthalpy on vesicle size. It might be that the variation in enthalpies is a reflection of the different head-group organization of the inner and outer layers and it is conceivable that the different surface curvatures of the two layers can affect their thermal melting differently.

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