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THE INTERNAL AQUEOUS VOLUME OF SMALL UNILAMELLAR VESICLES CHANGES AT THE PHASE TRANSITION TEMPERATURE OF THE PHOSPHOLIPID

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Changes in the fluorescence of partially self-quenched 5(6)-carboxyfluorescein trapped within the internal aqueous compartment of small unilamellar dipalmitoylphosphatidylcholine vesicles indicate that the trapped volume of these vesicles decreases when the phospholipid undergoes the liquid crystalline to gel state transition. This volume change is completely reversible and is not caused by vesicle-vesicle fusion. Furthermore, this decrease in volume of the internal aqueous compartment may be attributed to a change in vesicle shape upon undergoing the phase transition.

Recently, the effect of the phospholipid phase transition upon the internal aqueous volume of large single bilayer dipalmitoylphosphatidylcholine vesicles (LUV) has been determined by examining the changes in the fluorescence self-quenching of a fluorophore trapped within this internal compartment [1]. The origin of the volume change rests in the decrease in molecular cross-section and increase in molecular length which accompany the liquid crystalline to gel strate transition of the phospholipid shell. These molecular packing changes result in a reduction of the outer surface area of the vesicle with a concomitant decrease in the radius of this surface. In addition, the increase in molecular length causes a thickening of the bilayer shell with a concomitant decrease in the radius of the internal surface of the phospholipid shell. The net result is a decrease in both the external radius of the shell and the volume of the internal aqueous compartment [1]. In a detailed discussion of this volume change presented elsewhere, it was shown that if the reduction of the polar headgroup cross-sectional area is independent of the vesicle size, the fractional change in the internal volume should also be independent of vesicle size. This is true provided that the vesicle remains spherical and no lipid flip-flop occurs during the lipid phase transition [1].

It was reported recently that the Stokes' radius of small unilamellar phospholipid vesicles (SUV) prepared by sonication does not change when vesicles pass through the gel to liquid crystalline transition [2]. Since many of the physical properties of these minimum radius vesicles differ from the corresponding properties of larger vesicles [3], we decided to measure the volume of the interior aqueous compartment in gel and liquid crystalline phase SUV.

Using an improved technique similar to that employed previously [1], we have determined that the internal aqueous volume of small unilamellar vesicles is in fact reduced when the bilayer lipid goes from liquid crystalline to gel state. Fig. 1 shows the temperature dependence of the fluores-

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Abbreviations: LUV, large unilamellar vesicle(s); SUV, small unilamellar vesicle(s); DPPC, dipalmitoylphosphatidylcholine.

cence intensity for 5(6)-carboxyfluorescein trapped within the internal aqueous compartment of small unilamellar vesicles. A large decrease in the fluorescence occurs when these vesicles are cooled

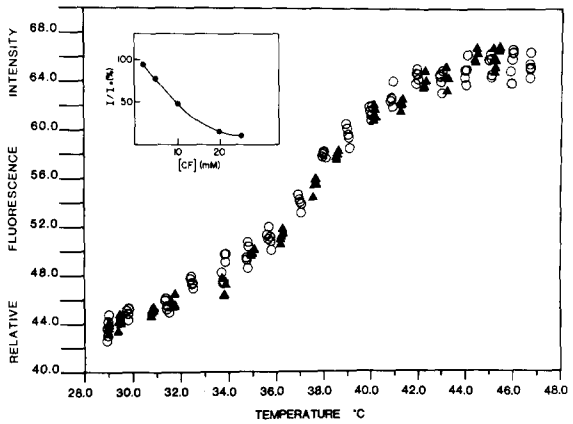

Fig. 1. Temperature dependence of the fluorescence intensity of small sonicated unilamellar dipalmitoylphosphatidylcholine vesicles containing trapped 5(6)-carboxyfluorescein. Small DPPC unilamellar vesicles were prepared by co-sonicating 20 mM 5(6)-carboxyfluorescein with 20 mM DPPC in buffer 1 (100 mM KCl/20 mM NaH_2PO_4/5 mM NaN_3 pH 8.2). The sonicated mixture was fractionated by ultracentrifugation following the method of Barenholz et al. [10]. The upper one-third of the resulting supernatant was carefully removed for further study. These fractionated vesicles were chromatographed on Sephadex G-25 equilibrated with buffer 1 with 30 mM sucrose in order to separate the vesicles from any fluorophore which was untrapped during sonication. Osmotic pressure measurements indicated that buffer 1/sucrose is approximately isotonic with 20 mM 5(6)-carboxyfluorescein in buffer 1. The phosphor content of the vesicle solution used in fluorescence measurements was determined by the Bartlett method [11] to be 385 μM. The temperature dependence of the steady-state fluorescence intensity of these small vesicles was determined by using an SLM 4800 Spectrofluorimeter. The sample was excited at 492 nm and the emission as a function of temperature was recorded at 520 nm. The sample temperature was regulated by a NESLAB ETP-3 Temperature Programmer and a NESLAB DCR-1 Digital Controller/Readout to control the temperature of a circulating water bath. The actual sample temperature was monitored by inserting a probe from the DCR-1 into the same cuvette holder which held the sample. The sample temperature was varied linearly at 4 deg. per h, and the excitation slit remained closed except when actually acquiring data. Each datum point in the figure represents a cumulative average of 100 data acquisitions. The open circles (○) denote the initial cooling scan; the dark triangles (▲) represent a subsequent heating scan performed on the same sample. The insert shows the relation between relative fluorescence intensity and the concentration of carboxyfluorescein [1]. The non-quenched fluorescence at 45.6°C was 97.4.

below the phase transition temperature. This change is reversed by returning the vesicles to the liquid crystalline state. When compared with the fluorescence changes seen in LUV [1], these smaller vesicles exhibit the change over a wider temperature range and the midpoint temperature of the change is lower. The breadth and midpoint of this fluorescence change correlate well with the breadth and midpoint of the liquid crystalline to gel phase transition previously established for SUV using differential scanning calorimetry and diphenylhexatriene fluorescence anisotropy (Table I).

Small unilamellar vesicles spontaneously fuse below the phase transition temperature to give large unilamellar vesicles [4,5]. Although fusion is not reversible, significant amounts of large vesicles contaminating the sample could contribute to the fluorescence change shown in Fig. 1. Precautions were taken during the preparative steps to minimize fusion by insuring that the vesicles remained above the phase transition temperature prior to measurement of fluorescence. Quasi-elastic light scattering measurements of these vesicles revealed that the hydrodynamic radius was 160 Å or less before measuring the fluorescence. Although this radius is slightly larger than expected for small vesicles, quasi-elastic light scattering studies on binary mixtures of large fused DPPC vesicles and small sonicated POPC vesicles have shown that this larger hydrodynamic radius may result when the vesicle sample contains one percent of these large vesicles [6]. In addition, molecular exclusion chromatography using Sepharose CL-2B, which can separate large from small vesicles [5], was performed after the fluorescence measurements. Within the limits of resolution of this method (better than $\pm$ 3% of the total lipid), no large vesicles were detected before or after the fluorescence measurements. A simple calculation indicates that the presence of 3% large vesicles cannot be responsible for more than 15% of the fluorescence change appearing in Fig. 1.

Temperature-dependent changes in buffer pH and fluorescence quantum yield may also contribute to the reversible fluorescence changes in Fig. 1. Solution studies using 5(6)-carboxyfluorescein dissolved in the buffer used to obtain the data in Fig. 1 showed the fractional change in fluorescence intensity to be approximately 0.5% per Celsius

TABLE I

MIDPOINT TEMPERATURE AND TEMPERATURE RANGE OF THE LIQUID CRYSTALLINE TO GEL STATE TRANSITION IN SMALL AND LARGE UNILAMELLAR DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES

Values are °C.

Method	Large vecicles		Small vesicles	
	Temperature range	Midpoint	Temperature range	Midpoint
Differential scanning calorimetry (3)	39–42	41.2	33–39	36.9
DPH fluorescence anisotropy (3)	38–42	41.1	34–41	37
6-Carboxyfluorescein fluorescence intensity (1)	39–41.5	40.5	34–41	37.5

degree. This change corresponds well with the slope of the curve in Fig. 1 before and after the phase transition. Therefore, the fluorescence change observed in the phase transition region is almost certainly caused by the change in self-quenching of the fluorophore resulting from the change in the internal aqueous volume.

If calculations are made as previously described [1], the data in Fig. 1 correspond to a 34% decrease in internal volume when SUV go from liquid crystalline to the gel state. This contrasts with a 25% decrease observed for LUV [1].

In SUV above the phase transition temperature, the cross sectional headgroup area per molecule is smaller for those molecules residing on the inner monolayer than those on the outer monolayer. Furthermore, the polar headgroups of the inner monolayer may already be tightly packed together above the phase transition temperature [7,8]. This further restricts the ability of the inner monolayer in small unilamellar vesicles to undergo a phase transition-related decrease in surface area. Yet it is precisely the disposition of this inner monolayer which governs the magnitude of the trapped volume change. Since the reduction in the inner monolayer surface area for small unilamellar vesicles must be less than the surface area reduction for large vesicles, the small vesicles should experience a correspondingly smaller reduction of the internal aqueous volume. This prediction is, however, contrary to fact. Thus the reduction in surface area alone cannot account for the entire trapped volume change observed in these small vesicles.

It is possible that in small vesicles, as the outer monolayer surface area decreases and the lipid acyl chains approach a rigid all-*trans* configuration in going into the gel state, the mandatory increase in the bilayer thickness which accompanies these changes can be accommodated only if some regions of the inner monolayer become planar. As a result, thicker bilayer regions would then exist in the form of planar facets at the expense of some lipid which remains as a non-bilayer edge to hold the facets together. The result would be a polygonal shell. A morphological change of this type would decrease the internal aqueous volume, since even at constant inner monolayer surface area, any deviation from a spherical shape would decrease the volume of the internal compartment.

A polygonal shape for gel-phase SUV was suggested several years ago by Blaurock and Gamble based on large-angle X-ray diffraction data [9]. These authors suggested that their data were most consistent with a model in which each vesicle has between 30 and 40 planar facets with the balance of the lipid sealing the edges of the facets to give a topologically closed surface. Since it is well known that the breadth of the phase transition is inversely proportional to the number of molecules comprising the individual cooperative units, this model is also consistent with the increased breadth of the phase transition in small vesicles, if each facet acts as a cooperative unit undergoing the transition.

Furthermore, the molar enthalpy of melting for small vesicles is a fraction of that for the large vesicles [3]. This would be the case if the acyl chains of the lipid bordering the facets were not in the all-*trans* configuration in the gel state.

Observations on small vesicles using dynamic light scattering suggest that no detectable change occurs in the Stokes' radius of small vesicles as they are cooled below the phase transition temperature [2]. This observation is not inconsistent with our volume data. Since for a fixed particle mass, deviation from a spherical shape will in general lead to an increase in the hydrodynamic frictional coefficient, the apparent constancy of the Stokes' radius in liquid crystalline and gel states may be the result of the change in shape to an irregular polygonal shell which compensates for the volume decrease.

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