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PRECISE DETERMINATION OF THE HYDRODYNAMIC RADIUS OF PHOSPHOLIPID VESICLES NEAR THE PHASE TRANSITION

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Using dynamic light scattering we have been able to determine precisely the hydrodynamic radius of L-α-dimyristoylphosphatidylcholine (DMPC) vesicles as a function of temperature. We have detected a sharp, thermally reversible change in the vesicle radius at a phase transition temperature 24°C, corresponding to an approximate 11% increase in surface are. In the range 10–20°C, the change in radius is less than 1%.

Cell membranes exhibit the physically interesting and biologically significant phenomenon of an order-disorder transition of the constituent hydrocarbon chains [1]. Investigations of concentrated multibilayer phospholipid dispersions, a membrane model, suggest the phase transition is accompanied by a change in surface area per lipid molecule [2]. In this model, however, interactions between adjacent bilayers complicate the analysis [3]. Therefore it is preferable to study the structural changes in unilamellar systems, such as phospholipid vesicles [4].

The phase transition in lipid bilayers has been studied using differential scanning calorimetry and X-ray diffraction [5,2,1]. In the case of DMPC bilayers these techniques have revealed a main transition at 24°C, which, according to the X-ray results, is accompanied by a 20% change in the surface area of the bilayer. In addition, a secondary transition at a lower temperature, marked by a 6% decrease in the surface area with increasing temperature, was observed. Both techniques are limited by their sensitivity only to concentrated multilamellar systems. In contrast, dynamic light scattering is very sensitive, noninvasive and can measure the size of small particles with high precision. It is therefore well suited for the desired investigation of single bilayer vesicles. Previous measurements performed on DMPC or dipalmitoylphosphatidylcholine (DPPC) vesicles prepared by sonication yielded controversial results. Most authors observed no dimension change at the phase transition [6–8]. Others reported changes in size which were complicated by fusion of the vesicles [9] or by sample polydispersity [10]. These conflicting studies associated with sonicated vesicles prompted us to use another method of preparation to study the phase transition.

Vesicles used in our experiments were prepared by the ether injection technique [11,12]. L-α-Dimyristoylphosphatidylcholine (DMPC) (Avanti Polar Lipids) was dissolved in an diethyl ether/methanol mixture (9 : 1, v/v) and injected at 70°C into a buffer consisting of 350 mM LiCl, 10 mM Tris/Tris-HCl (pH 7), 1 mM EDTA and 0.02% NaN_3. EDTA was added to prevent aggregation of vesicles induced by Ca^{2+}. The resulting suspension was dialyzed, filtered trough a Millex SR filter (0.5

μm), concentrated and fractionated using Sephacryl S1000 gel filtration. The concentration of phospholipid in each fraction was determined by phosphorus analysis [13]. Finally, the samples were diluted to a concentration of approx. 0.02 mg/ml and filtered into the scattering cell. The samples were maintained at 4°C from fractionation to final filtering.

Dynamic light scattering experiments [14] have been performed on an apparatus described elsewhere [15] in the homodyne configuration at a 90° scattering angle. The temperature was controlled to 0.05°C.

The measured correlation function was fitted using

$$G(t) = A \exp(-2\Gamma t - \Delta t^2) + B$$

From the decay parameter, Γ, the hydrodynamic radius is calculated using the Stokes-Einstein formula [14]. The parameter Δ is a measure of the polydispersity of the sample [14,16]. A comparison has shown our samples to be nearly as monodisperse as a dilute suspension of standard polystyrene spheres of radius 550 ± 15 Å.

The data obtained with vesicles having a radius in the range 330–430 Å are shown in Fig. 1. We observe a 5–6.5% increase in the hydrodynamic radius when the temperature is increased from 22 to 26°C, the transition being centered at T_c = 24.2°C. Such a change in the radius corresponds to a 10–13% increase in surface area.

The position of the transition and its extent do not depend on the size of the vesicles, as seen in Fig. 1b. This result is in contrast to the findings on DPPC vesicles obtained by a different method [17].

The phase transition is reversible. However, when the transition is scanned repeatedly or when the sample is kept for prolonged period at temperatures above 30°C, the radius below T_c decreases slightly (1–3%) and the extent of the radius change varies between 5 and 6.5%. The data shown in Fig. 1 correspond to the initial scan made with increasing temperature. The measurements did not depend on whether the temperature was raised or lowered during the experiments, nor on the rate of heating or cooling. Each measurement was carried out 15 minutes after a sample was set to a new temperature.

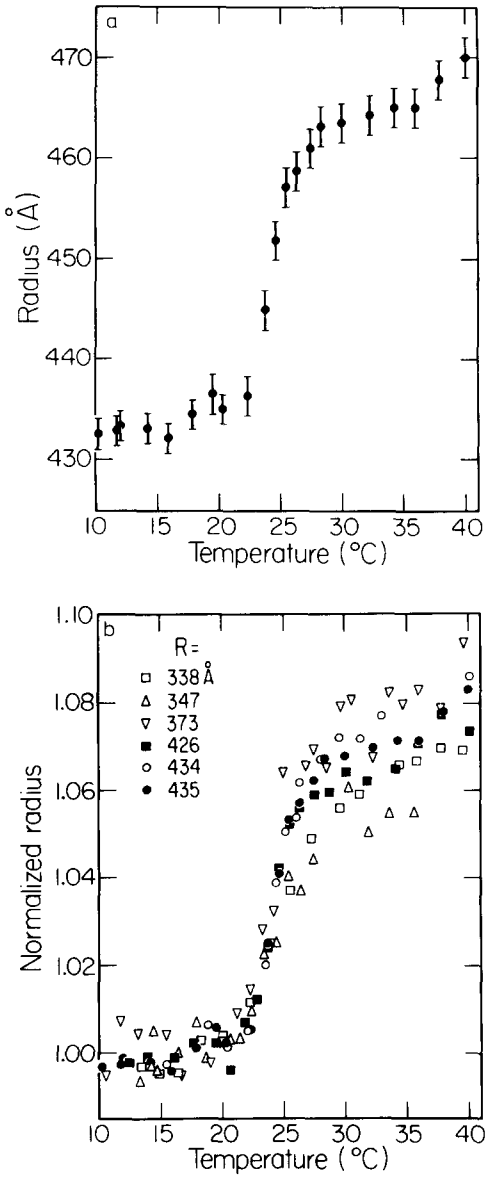

Fig. 1. (a) The temperature dependence of the hydrodynamic radius of DMPC vesicles. (b) The temperature dependence of the hydrodynamic radius from different fractions. The data are normalized by R, the average radius below the transition temperature.

As the reversibility indicates, the samples were sufficiently dilute to prevent fusion or aggregation of the vesicles. That samples stored at 4°C (concentration approx. 0.2 mg/ml) showed no significant size changes during a period of 4 weeks is

further evidence of the complete absence of fusion and aggregation in our system.

The quality of the data is sufficient to extract even more detailed information. In the crystalline state below T_c, the size of the vesicles is almost independent of temperature. In the range 27–40 °C the thermal area expansivity, defined as [18]

$$C = (\partial A/\partial T)/A$$

can be estimated to be $2.5 \pm 0.7 \cdot 10^{-3}$ deg.$^{-1}$.

Our data differ somewhat from the results of X-ray measurements on multilamellar phospholipid suspensions [2]. In contrast to the X-ray investigations, we did not observe any pre-transition in the range 10–20 °C, which would be accompanied by a decrease in the surface area with increasing temperature [2]. Also, the area change at the primary transition was only 10–13%, not 20%.

On the other hand, we find excellent qualitative and quantitative agreement between our results and the available data on monolamellar DMPC systems. Evans and Kwok [18] have measured the temperature dependence of the size of very large (diameter $> 2 \cdot 10^{-3}$ cm) vesicles using microscope observations. Although the size of their vesicles is several orders of magnitude larger than ours, and the experimental methods are quite different, their results are almost identical with our findings. They observed no pretransition, a main transition at 24.2 °C, a surface change at the transition of 12–13%, thermal area expansivity of $(4–6) \cdot 10^{-3}$ deg^{-1}. and much smaller expansivity below the T_c.

Evans and Kwok [18] suggest the large single bilayer DMPC vesicles undergo a transition directly from the $L_{\beta'}$ to L_α phase, without entering the 'rippled' $P_{\beta'}$ phase [2].

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