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TEMPERATURE DEPENDENCE OF THE SIZE OF PHOSPHOLIPID VESICLES

BRUCE A. CORNELL [a], GUY C. FLETCHER [b], JACK MIDDLEHURST [a] and FRANCES SEPAROVIC [a]

[a] *Food Research Laboratory, CSIRO Division of Food Research, P.O. Box 52, North Ryde and* [b] *School of Mathematics and Physics, Macquarie University, North Ryde, N.S.W., 2113 (Australia*

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Summary

We report measurements of the size of dimyristoyl phosphatidylcholine vesicles over a temperature range that includes the main transition temperature and show that any change in average diameter is less than ±3%.

Many conflicting accounts have been published of the variation in the size of phospholipid vesicles over a range of temperatures including the $L_{\beta'}$ to L_{α} phase transition temperature found in extended multilamellar dispersions. One of the earliest of these studies was by Mishima [1] who reported that vesicles of dipalmitoyl phosphatidylcholine vary from 169.4 nm diameter below the transition temperature to 194 nm diameter above the transition temperature. Smaller diameters have been obtained by Ostrowsky and Hesse-Bezot [2] who found that over the same range of temperatures dipalmitoyl phosphatidylcholine vesicles varied from approx. 50 nm to 90 nm diameter. The latter authors suggest that the size of vesicles of synthetic lipids is naturally larger than that of vesicles prepared from a natural heterogeneous lipid such as hen's egg yolk phosphatidylcholine. In a similar study by Watts et al. [3] the diameter of vesicles prepared from dimyristoyl phosphatidylcholine has been observed to increase from 19 to 26 nm on heating from 10 to 30°C. This result contradicts the contemporary report of Aune et al. [4] in which it is concluded that the diameter of similarly sized vesicles is independent of temperature in the range 4 to 34°C.

The disagreement which exists within the published literature on this subject has been discussed recently by Ceuterick et al. [5]. These authors have inde-

pendently found the size of dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine vesicles to be constant over a range of temperatures spanning their $L_{\beta'}$ to L_{α} phase transition. However, owing to the heterogeneity of their solutions these authors did not quote a vesicle diameter.

In the present article we report a series of estimates of the size of dimyristoyl phosphatidylcholine vesicles from 10 to 30°C using the photon-counting autocorrelation light-scattering technique. Our results support the more recent estimates of vesicle size for we observed synthetic phospholipid vesicles with a diameter of approximately 20 nm which is in agreement with the 21 nm diameter obtained by Huang [6] for vesicles of the natural phospholipid egg yolk phosphatidylcholine. Furthermore, as in the studies of Ceuterick et al. [5] and Aune et al. [4] we failed to observe any changes in the dimensions of vesicles of dimyristoyl phosphatidylcholine over the temperature interval 10 to 30°C.

The dimyristoyl phosphatidylcholine vesicles were prepared by ultrasonic irradiation and separated from traces of larger unilamellar and multilamellar aggregates by ultracentrifugation following a minor modification of the procedure described by Barenholz et al. [7].

Dimyristoyl phosphatidylcholine was purchased from Calbiochem, La Jolla, U.S.A. and was used without further purification. The dimyristoyl phosphatidylcholine was judged to be a single compound by thin-layer chromatography (TLC) both prior to and following ultrasonic irradiation. The TLC checks were run using SiO_2 plates and a solvent of $CHCl_3/CH_3OH/H_2O$ in the ratio 65 : 25 : 4. Iodine vapour was used to develop the TLC plates.

Ultrasonic irradiation used in the formation of the lipid vesicles was supplied by a Branson type B12 sonifier fitted with a 12 mm diameter macroprobe. Continuous irradiation was applied to the lipid solutions under a stream of oxygen-free nitrogen for a period of approximately 10 min. Self heating in the solutions held the temperature at approx. 30°C throughout the sonication procedure. The samples were subsequently transferred to a series of 10-ml cellulose nitrate centrifuge tubes and spun at 47 000 rev./min for 70 min in a Beckman TY50 rotor. The supernatants were then drawn off and spun for a further 70 min in 2-ml tubes.

Measurements of the vesicle diameter were made by locating the upper fraction of the supernatant in the focussed beam of a He-Ne Spectra Physics type 125, 50 mW laser, and observing the 90° scattered light using an EMI type 9863(B) photomultiplier mounted in an R.F. shielded RFI-B263 F stainless steel housing. Throughout the entire preparation, centrifugation, and transfer to the light-scattering cell the sample temperature was maintained at 30°C. All subsequent measurements were made as the sample temperature was cycled down to 12°C and back to 30°C. Measurements of the photocount correlation function were made with a Langley Ford Inst. Correlator (with a sample time of 5 μs) connected to an HP 9835A computer. The data were interpreted according to the method of cumulants proposed by Koppel [8] and Brown et al. [9]. From the basic principles of the light-scattering technique, for a polydisperse solution containing a distribution of scatterers $G(\Gamma)$ each having a decay constant Γ, the mean decay constant $\bar{\Gamma}$ is given by

$$\bar{\Gamma} = \int_0^\infty \Gamma G(\Gamma)\, d\Gamma$$

where $G(\Gamma)$ is related to the optical correlation function, $g^{(1)}(\tau)$, according to

$$|g^{(1)}(\tau)| = \int_0^\infty G(\Gamma) \exp(-\Gamma\tau)\, d\Gamma$$

and is normalised so that

$$\int_0^\infty G(\Gamma)\, d\Gamma = 1$$

Expanding $\exp(-\Gamma\tau)$ about the mean decay rate $\bar{\Gamma}$ results in

$$|g^{(1)}(\tau)| = \exp(-\bar{\Gamma}\tau)\left\{1 + \frac{\mu_2\tau^2}{2!} - \frac{\mu_3\tau^3}{3!} + \frac{\mu_4\tau^4}{4!} \cdots\right\}$$

where the moments of the distribution function $G(\Gamma)$,

$$\mu_2 = \int_0^\infty (\bar{\Gamma} - \Gamma)^2\, G(\Gamma)\, d\Gamma$$

$$\mu_3 = \int_0^\infty (\bar{\Gamma} - \Gamma)^3\, G(\Gamma)\, d\Gamma\,, \qquad \text{etc.}$$

are a measure of the departure of the system from a single exponential. By convention we define the polydispersity as

$$\frac{\mu_2}{\bar{\Gamma}^2}$$

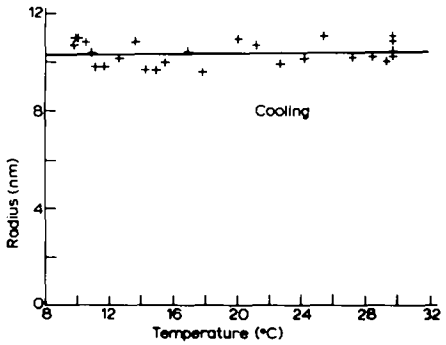
In the case of the vesicles reported here, a polydispersity of 0.18 was obtained. A measure of the mean hydrodynamic radius $\bar{R}_H$ of the vesicles was obtained from the Stokes-Einstein relationship

$$\bar{R}_H = kT/6\pi\eta_T\bar{D}$$

where k is Boltzmann's constant, T is the absolute temperature and η_T is the viscosity of water at temperature T derived using the formula of Korson et al. [10].

A measure of the mean translational diffusion coefficient $\bar{D}$ obtained from $\bar{\Gamma}$ is usually taken at several different sample concentrations and extrapolated to zero concentration before calculating the mean hydrodynamic radius of the solute molecules. In the solutions of vesicles studied here the concentrations after centrifugation were already so low as to make further dilution impracticable. It was, therefore, assumed that the measured values correspond in good approximation to the 'infinite dilution' condition. The effect of this approximation will be to underestimate very slightly $\bar{D}$ and thus to overestimate the absolute value of the Stokes radius. The solute concentration in the studies reported here was typically 0.3 mg/ml.

Figs. 1 and 2 show the effect of temperature on the measured radius during the initial cooling cycle for a suspension of dimyristoyl phosphatidylcholine



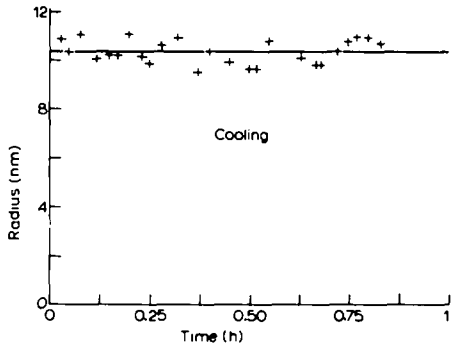


Fig. 1. Estimate of vesicle radius in nm as a function of temperature in °C during the initial cooling cycle of a suspension of dimyristoyl phosphatidylcholine vesicles. The linear regression fit to the data is discussed in the text. In this case the radius has been calculated from the second-order cumulants fit.

Fig. 2. The same data as for Fig. 1, however here presented as a function of the time from the start of cooling.

vesicles. In Fig. 1 the data are presented as a function of the temperature at which the estimate of radius was made and in Fig. 2 as a function of the time of the estimate following the completion of centrifugation. These data have been fitted by a linear regression procedure to expressions for the radius of

$$r = 10.29 + 0.005\ T$$

and

$$r = 10.38 + 0.011\ t$$

as a function of temperature T (°C) and time t (h), respectively. These fitting procedures indicate an estimated vesicle radius of 10.3 ± 0.3 nm (one S.D.) which is essentially independent of both temperature and time over the ranges studied here.

Measurements were also made of the effect of temperature on the subsequent heating cycle for the same samples. Again the data were analysed as a function of temperature and time, yielding expressions for the radius of

$$r = 10.24 + 0.02\ T$$

and

$$r = 10.5 + 0.5\ t,$$

respectively. Averaging the extrapolated size estimates we obtain an initial vesicle radius of 10.35 ± 0.3 nm. The slightly greater temperature- and time-dependence of the radius seen during the heating cycle indicates the onset of some form of aggregation or growth in the vesicle diameter.

These results are in reasonable agreement with those of Aune et al. [4] who

found a temperature-independent radius of 12.5 nm.

Similar sizes have been reported by Watts et al. [3] for temperatures just below the multilamellar phase transition. However, these authors report a 30% change in radius over the temperature range 10 to 30°C and give no indication of the polydispersity of their samples. The dilatometric data of Nagle and Wilkinson [11], made on dispersions of dimyristoyl phosphatidylcholine show a change in the specific volume of approx. 5% on passing through the transition temperature. As we have shown previously [12], the constraints imposed by the spherical geometry of a small lipid vesicle containing a fixed number of molecules prevent major changes in the area per lipid molecule and the bilayer thickness on passing through the bilayer phase transition temperature. If the phospholipid molecular partial specific volume in a small vesicle changes with temperature in a similar manner to a phospholipid within a planar bilayer the spherical geometry constrains the change in vesicle diameter on passing through the phase transition temperature to approximately 1.5%. This magnitude of change would give the null result demonstrated in our experiments.

The absence of a size change in the vesicle preparations studied here is not due to the presence of impurities in the phospholipid bilayer. As seen in Figs. 3a and 3b the calorimetric behaviour of the reclaimed and resuspended dispersions of lipid from the vesicle preparations reported here is the same as for the unsonicated dimyristoyl phosphatidylcholine and is in agreement with the calorimetric behaviour of dimyristoyl phosphatidylcholine reported elsewhere [13]. We conclude, therefore, that the geometrical constraints imposed on the lipid bilayer within a small spherical vesicle prevent the changes in the area per molecule and the bilayer thickness which are known to occur in essentially planar multilamellar systems. The large changes in vesicle radius observed by Watts et al. [3] are inconsistent with our results and may result from an aggregation of their vesicles.

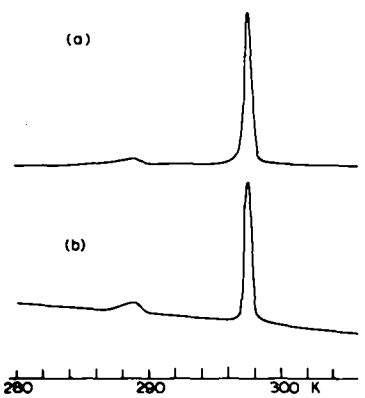


Fig. 3. (a) Calorimetric scan of a dispersion of dimyristoyl phosphatidylcholine resuspended in H_2O following its reclamation by lyophylization from the vesicle solution used to obtain the measures shown in Figs. 1 and 2. (b) Calorimetric scan of a dispersion of dimyristoyl phosphatidylcholine in H_2O prior to being used for the preparation of lipid vesicles. The calorimetry was carried out using a Perkin Elmer DSC-2 calorimeter operating at 2.5°C/min. The lipid dispersions (50% w/w) was sealed in an air-tight aluminium sample pan.

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