CURVATURE INFLUENCE ON THE COOPERATIVITY AND THE PHASE TRANSITION ENTHALPY OF LECITHIN VESICLES

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1. Introduction

Small unilamellar vesicles exhibit a broader thermotropic phase transition than large liposomes. This is usually interpreted as a decrease in cooperativity. We have investigated single-shell dimyristoyl-lecithin vesicles of different diameters by calorimetry and 90° light-scattering techniques and found that the steepness of the lipid phase transition is predominantly determined by the transition enthalpy rather than by the cooperativity. This result is the more trustworthy as conditions were chosen to make vesicle aggregation effects negligible.

By means of a simple theoretical relation we obtain a parameter which represents cooperative interactions between lipid molecules. Throughout the considered range of vesicle radii this parameter remains very close to a value typical for first order transitions.

2. Experimental

The lipid L-β,γ-dimyristoyl-α-lecithin (DML) was used as purchased from Fluka (no detectable impurities in thin-layer chromatography). Vesicles of various radii were prepared by the method in [1]. The buffer was changed to 0.01 M Tris/HCl (pH 7.5), 0.1 M NaCl and 1 mM NaN_3. For the removal of ethanol the vesicle suspensions were dialysed against pure buffer solution for $\geqslant$8 h. Vesicle radii from 18–37 nm were determined by quasielastic light scattering.

Thermotropic phase transition curves were obtained by means of 90° light scattering intensity measurements. A Farrand MK1-spectrofluorometer with stabilized detection was used at 365 nm. Temperature was scanned at 12 $K.h^{-1}$.

Transition enthalpies were obtained by differential scanning calorimetry (DSC) as in [2,3]. The concentrations needed for calorimetry are 2–3 mM. To achieve this concentration, the suspensions of vesicles with the smallest radius were concentrated by ultrafiltration using polycarbonate membranes with a pore diam. 30 nm (Uni-Pore, Bio-Rad Labs). All concentrations were determined from phosphorous analysis.

Transition curves were evaluated by the usual assumption of linear plateaus, according to experimental evidence (for $T \lesssim 19$°C and $T \gtrsim 29$°C).

3. Results and discussion

Several attempts have been made to characterize small unilamellar phospholipid vesicles by DSC [4–7]. The results obtained so far, however, are quite controversial. Vesicle suspensions prepared by sonication always have contaminations with large multilamellar aggregates, which leads to the appearance of two peaks in the DSC curves [5,6]. From these scans it is impossible to determine the transition enthalpy for the small vesicles. On the other hand, the use of a type of calorimeter which requires high lipid concentrations for accurate measurements leads to rapid aggregation and fusion of vesicles, thus prohibiting accurate results [4,7]. Because of the low lipid concentration necessary for our DSC experiments, aggregation and fusion of vesicles can be neglected which

allows the determination of transition enthalpies of lipid vesicles with different diameters prepared by the injection method.

The enthalpy of transition obtained from calorimetry (ΔH_{cal}) shows a marked dependence on the vesicle size. In the case of our smallest vesicles (18 nm radius) it is reduced to ~1/3 of the value known for multilamellar liposomes [7,8] (fig.1a). A value of 3.8 kJ.mol^{-1} was estimated [6] for sonicated vesicles of ~11 nm radius. This value is slightly smaller, but not in contradiction with what one could extrapolate from our results. The midpoint of transition T_m shows a small, but systematic decrease with vesicle radius (24.3–23.3°C). Thus the transition entropy $\Delta S = \Delta H/T_m$ exhibits almost the same size dependence as ΔH_{cal}.

Transition curves obtained by light scattering and by integration of the excess heat capacity curves both showed an increasing steepness with increasing vesicle diameters. From the slope at the midpoint of transition the van't Hoff enthalpy ΔH_{vH} can be calculated. In analogy to a two-state model it is formally defined by:

$$\Delta H_{vH} = 4\,RT_m^2 \left(\frac{\partial \Theta}{\partial T}\right)_{T_m} \quad (1)$$

Figure 1b shows ΔH_{vH} as a function of vesicle radius. The discrepancy between the two sets of ΔH_{vH} may be due to experimental differences. The radius dependence, however, is very similar for both data sets.

Dividing ΔH_{vH} by ΔH_{cal} yields the so-called 'cooperative unit' (fig.2). This quantity appears to be relatively insensitive to the vesicle size in the investigated size range. It is, however, smaller than the value reported for large liposomes (200–300, [8,9]).

We conclude that the bilayer curvature has much less influence on the cooperativity of the transition than it had been thought so far [7], whereas enthalpy effects play a major role. The decrease of transition enthalpy may be attributed to packing constraints in vesicles due to the strong curvature. The fusion of vesicles in the gel phase has been found to be exothermic [6]. This may support the assumption that

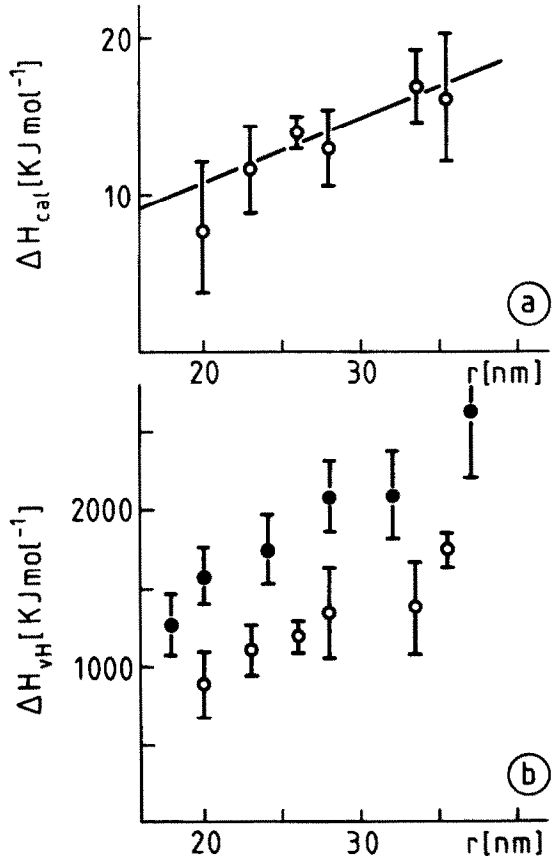

Fig.1. (a) ΔH_{cal} and (b) ΔH_{vH} as a function of vesicle radius, (•) refers to ΔH_{vH} obtained from light scattering and (○) to ΔH_{vH} obtained from the integrated excess heat capacity curves. The ΔH_{cal} values were averaged by giving a reduced weight to the 20 nm point (solid line).

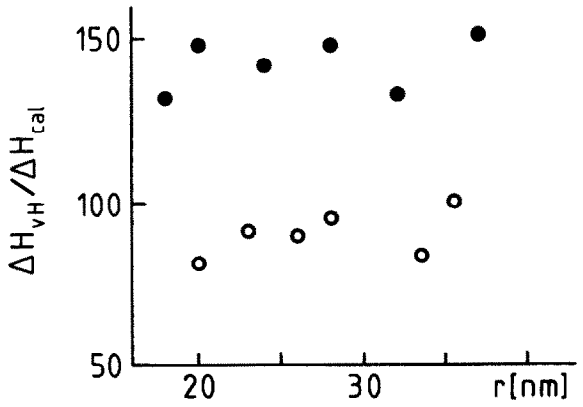

Fig.2. The cooperative unit $\Delta H_{vH}/\Delta H_{cal}$ as a function of vesicle radius. The experimental ΔH_{vH} values of fig.1b were divided by the averaged ΔH_{cal} values (solid line of fig.1a).

below T_m the lipid molecules are in a higher energetic state in small vesicles than in larger aggregates.

In addition, we should like to point out that even rather large variations of the cooperative unit does not necessarily have to correspond to analogous variations in the interaction energy between lipid molecules. In fact, it might be misleading to consider the simple relation:

$$\Delta H_{vH}/\Delta H_{cal} = 1/\sqrt{\sigma}$$

which has been suggested [10] in order to introduce a cooperativity parameter σ. This formula, taken from the linear Ising model [11] is certainly inadequate for two-dimensional systems. Treating the Ising model by the Bethe or quasichemical method (which yields the exact results for the linear case and is a good approximation for higher dimensions), we find for the two-dimensional hexagonal lattice [12]:

$$\frac{\Delta H_{vH}}{\Delta H_{cal}} = \frac{1}{3\sqrt{\sigma} - 2} \qquad (2)$$

From this expression it is easily seen that $\Delta H_{vH}/\Delta H_{cal}$ varies strongly as $\sqrt{\sigma}$ approaches 2/3 where $\Delta H_{vH}/\Delta H_{cal}$ equals infinity. (For $\sqrt{\sigma} < 2/3$ the theory predicts loops of the van der Waals type which correspond to discontinuous first-order transitions.)

All values of $\sqrt{\sigma}$ thus calculated from our cooperative units are <0.6692 for the light scattering data (<0.6708 for DSC). This means that the cooperative interaction energy $RT\ln\sigma$ deviates only by <1% (1.6%) from the range of values which corresponds to infinitely steep first-order transitions. Thus it seems plausible to consider the lipid bilayer as an ideal cooperative system which may exhibit a first-order transition (cf. [13]). In the case of small vesicles composed of only some 10^3 lipid molecules, however, a smoothing of discontinuities in the transition curve must be expected due to the finite size and possible lattice defects in the strongly curved bilayers.

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References

[1] Kremer, J. W. H., v.d. Esker, M. W. J., Pathmamaharan, C. and Wiersema, P. H. (1977) Biochemistry 16, 3932–3935.
[2] Blume, A. and Ackermann, Th. (1974) FEBS Lett. 43, 71–74.
[3] Grubert, M. and Ackermann, Th. (1974) Z. Phys. Chem. (Frankfurt) 93, 255–264.
[4] De Kruijff, B., Cullis, P. R. and Radda, G. K. (1975) Biochim. Biophys. Acta 406, 6–20.
[5] Suurkuusk, J., Lentz, B. R., Barenholz, Y., Biltonen, R. L. and Thompson, T. E. (1976) Biochemistry 15, 1393–1401.
[6] Kantor, H. L., Mabrey, S., Prestegard, J. H. and Sturtevant, J. M. (1977) Biochim. Biophys. Acta 466, 402–410.
[7] Van Dijck, P. W. M., de Kruijff, B., Aarts, P. A. M. M., Verkleij, A. J. and de Gier, J. (1978) Biochim. Biophys. Acta 506, 183–191.
[8] Mabrey, S. and Sturtevant, J. M. (1976) Proc. Natl. Acad. Sci. USA 73, 3862–3866.
[9] Hinz, H. J. and Sturtevant, J. M. (1972) J. Biol. Chem. 247, 6071–6075.
[10] Marsh, D., Watts, A. and Knowles, P. F. (1976) Biochemistry 15, 3570–3578.
[11] Zimm, B. H. and Bragg, J. K. (1959) J. Chem. Phys. 31, 526–535.
[12] Stankowski, S. and Gruenewald, B. (1979) in preparation.
[13] Mitaku, S., Ikegami, A. and Sakanishi, A. (1978) Biophys. Chem. 8, 295–304.