Interbilayer Cluster-Cluster Interaction in Multilamellar Vesicles: Thermodynamic Approach

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Abstract. A simple thermodynamic model of the main transition in pure lipid bilayers is presented. The basic relations for cooperativity, aggregation degree and fractional number of molecules are derived. The experimental heat capacity measurements of uni- and multilamellar phospholipid vesicles are analysed within the context of this model, and the transition characteristics are obtained. It is demonstrated that they differ from each other, and these differences are discussed according to the system configuration. The derived cluster configuration entropy supports the idea of strong interbilayer cluster-cluster cooperation.

Key words: Lipid bilayer — Phase transition — Interbilayer cooperativity — Cluster model

Introduction

A great biological importance of cell membrane fluidity and other cell membrane properties are the reasons of intensive experimental and theoretical study of phospholipid bilayer state in the gel-fluid transition region. The molecular interactions responsible for the rise of the main transition in pure lipid bilayers are well known. Trans-gauche excitations in the lipid chains at this transition lead to an increase of internal energy which is compensated for by the entropy of their conformational states (Nagle 1980). The process of transition itself is more intricated. The influence of the system configuration on transition properties has not been completely explained, either (e.g. interbilayer cooperation in multilamellar vesicles).

The appearance of fluctuations around the transition point in the form of molecular clusters of the different phases is a common feature of the group of cluster models. A fraction of the cluster boundary lipids passes through a maximum at the transition temperature, which is dependent on the vesicle size (Marsh et al. 1977) and contents of impurities such as anaesthetics (Tsong et al. 1977). Freire and Biltonen (1978), using their deconvolution theory, have demonstrated that average

cluster size is strongly affected by the radius of curvature of the bilayer and by addition of small molecular weight compounds to the system. The mismatch in molecular packing between gel and fluid domains in the transition region has been interpreted as the origin of the enhanced ion permeability (Cruzeiro-Hansson and Mouritsen 1988) and passive diffusion of some molecules (Kanehisa and Tsong 1978). The multistate lattice model of the bilayer, based on computer simulations, has been used to examine the interaction with foreign molecules (Mouritsen 1984, 1991; Jorgensen et al. 1991a,b; Corvera et al. 1992). Morrow et al. (1992) have used the phenomenological Landau model to examine dependence of lipid bilayer properties on chain length.

In this paper we present a thermodynamical model of the main transition in pure lipid bilayers; the model belongs to the class of cluster models. We have defined a new phenomenological function to describe qualitatively the melting process during the transition, taking into account cooperativity and transition curve shape. The relations derived allow us to acquire global information about the transition process from heat capacity measurements. It is dèmonstrated that the system configuration may account for the pronounced decrease of the cluster configuration entropy in the case of multilamellar vesicles.

The Model

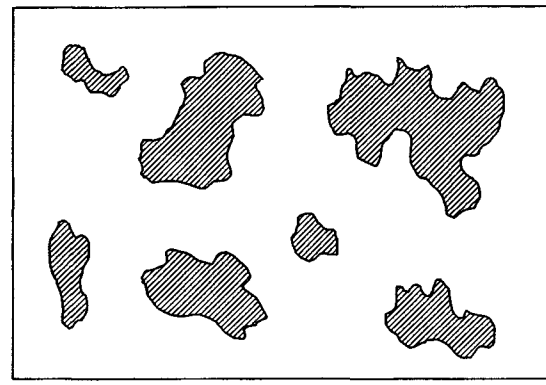
The existence of the minority phase domains surrounded by the majority phase is a common basis of all cluster models. Fig. 1 shows a schematic illustration of cluster distribution in the bilayer plane. Three states are available to lipid molecules only: solid-like gel state, fluid-like liquid crystal state, and intermediate state in the cluster boundaries. Packing constraints cause the interfacial region to be more disordered and energetically unfavourable which brings the molecules in the same state group together and thus to minimalize the length of the boundary region. On the other hand, cumulation of molecules reduces the cluster configuration entropy, and the equilibrium state depends on the ultimate balance between the above forces.

A change in the Gibbs free energy of the model bilayer relatively to the gel phase is given by

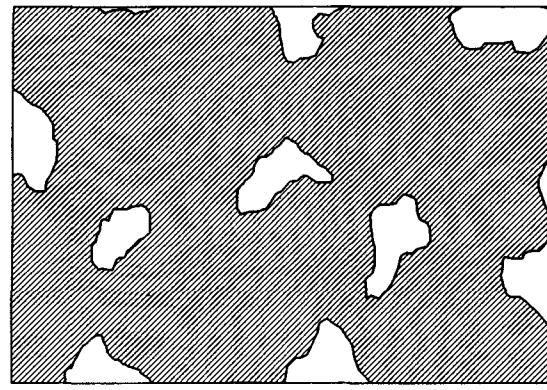
$$\Delta G = \Delta G_{21} + \Delta G_I, \tag{1}$$

where ΔG_{21} and ΔG_I are free enthalpy differences between fluid-gel and interface-gel states, respectively. At any temperature, the configurational state of the bilayer can be described in terms of the fractional numbers of molecules in the liquid and intermediate states, n, n_I. It should be noted that $n + n_I + n_g = 1$, where n_g is the gel fraction. Eq. 1 will get the form

$$\Delta g = n\ \Delta g_{21} + n_I \Delta g_I - T\ S_C, \tag{2}$$



a)



b)

Figure 1. A schematic illustration of cluster distribution in the bilayer-plane (gel phase, white; fluid phase, shaded area; intermediate boundary region, black contour) a) $T < T_m$ b) $T > T_m$.

where Δg, Δg_{21} and Δg_I are the molar free enthalpies, and S_c is the cluster configuration entropy represented as

$$S_C = \mathrm{R}\ \ln\ W(n, n_I). \tag{3}$$

Here, R is the universal gas constant and W is the statistical weight (per molecule) of the configurational state of the bilayer, and it arises from many different ways of distributing a given number of liquid molecules in clusters within the bilayer plane (at a given amount of boundary lipids). The possibilities of an exact theoretical

treatment of this model are limited by the fact that the latter quantity cannot be calculated exactly. We circumvented these difficulties through the introduction a phenomenological function δ as follows

$$W(n, n_I) = \delta(n, n_I)\ W_0(n), \tag{4}$$

where W_0 is the statistical weight (per molecule) in the case of non-interacting molecules (i.e. distribution of liquid molecules is not affected by an interfacial energy), since W_0 can be expressed analytically using combinatorial laws and Stirling's formula

$$\ln W_0 = \frac{1}{n_0} \ln \binom{n_0}{n'} = n \ln \left(\frac{1}{n} - 1 \right) - \ln (1 - n) \tag{5}$$

where $n = n'/n_0$ and n', n_0 are fluid fraction and total numbers of molecules, respectively. Eq. 2 will be rewritten using Eqs. 3, 4 and 5

$$\Delta g(n, n_I) = n\Delta g_{21} + n_I \Delta g_I - \mathrm{R}T \left(\ln \delta(n, n_I) + n \ln \left(\frac{1}{n} - 1 \right) - \ln(1 - n) \right) \tag{6}$$

Equilibrium conditions are given by partial derivatives of Eq. 6 via n and n_I.

$$\frac{\partial \Delta g}{\partial n_I} = 0, \qquad \frac{\partial \Delta g}{\partial n} = 0 \tag{7}$$

The solutions to Eq. 7, corresponding to the minimum of Δg are readily obtained as

$$\begin{aligned} \frac{\partial \ln \delta}{\partial n} &= \frac{\Delta g_{21}}{\mathrm{R}T} - \ln \left(\frac{1}{n} - 1 \right) \\ \frac{\partial \ln \delta}{\partial n_I} &= \frac{\Delta g_I}{\mathrm{R}T}. \end{aligned} \tag{8,9}$$

Using these equations and noting that

$$\frac{\mathrm{d} \ln \delta}{\mathrm{d}T} = \frac{\partial \ln \delta}{\partial n} \frac{\mathrm{d}n}{\mathrm{d}T} + \frac{\partial \ln \delta}{\partial n_I} \frac{\mathrm{d}n_I}{\mathrm{d}T} \tag{10}$$

we obtain

$$\delta(T) = \exp \left\{ \int \frac{\mathrm{d}n}{\mathrm{d}T} \left[\frac{\Delta g_{21}}{\mathrm{R}T} - \ln \left(\frac{1}{n} - 1 \right) \right] \mathrm{d}T + \int \frac{\mathrm{d}n_I}{\mathrm{d}T} \left[\frac{\Delta g_I}{\mathrm{R}T} \right] \mathrm{d}T \right\} \tag{11}$$

which allows calculation of δ as a function of temperature from the known temperature dependencies of fractions n, n_I. We consider this quantity a cumulation

coefficient reflecting its definition as the ratio between real and ideal statistical weights, which characterise the degree of molecular aggregation in the clusters.

Eq. 8 may be used to give the expression for the fluid phase fraction temperature dependence, also called degree of transition or melting profile

$$n(T) = \frac{1}{1 + \exp\left(\alpha - \dfrac{\partial \ln \delta}{\partial n}\right)} \tag{12}$$

where

$$\alpha = \frac{\Delta g_{21}}{RT}. \tag{13}$$

We find it convenient to introduce the quantity ν defined as follows

$$\nu = 1 - \frac{1}{\alpha}\frac{\partial \ln \delta}{\partial n} = \frac{1}{\alpha}\ln\left(\frac{1}{n} - 1\right), \tag{14}$$

and to rewrite Eq. 12 as

$$n(T) = \frac{1}{1 + \exp(\alpha\nu)} \tag{15}$$

The sense of the foregoing transformation will be highlighted through the evaluation of ν at the centre of transition. The transition point temperature, T_m is given by

$$\Delta g_{21}(T_m) = \Delta h_{21} - T_m \Delta s_{21} = 0, \tag{16}$$

where Δh_{21} and Δs_{21} are molar enthalpy and entropy changes, respectively. From Eq. 14 using l'Hospital's rule, we obtain

$$\nu(T_m) = \lim_{T \to T_m}\left\{\frac{1}{\alpha}\ln\left(\frac{1}{n} - 1\right)\right\} = \frac{4RT_m^2}{\Delta h_{21}}\left(\frac{dn}{dT}\right)_{T=T_m} \tag{17}$$

The preceding form of Eq. 17 is consistent with similar expressions given in the literature (Marsh et al. 1977; Kanehisa and Tsong 1978), and is often referred to as the size of the cooperative unit. In such a way Eq. 14 is a generalisation of this quantity at any temperature in transition region. This is in agreement with Sugar (1989) who derived the same $\nu(n)$ dependence in a different way, on the basis of the idea of the smallest independent subsystem. The interpretation of the latter quantity is ambiguous and model-dependent. This should not be regarded as the cluster size but could be taken as the effective number of molecules that simultaneously participate in the phase transition (Cevc and Marsh 1987; Sugar 1989). We rewrite Eq. 14 by substituting Eq. 13 in order to understand the physical sense of the above quantity

$$\nu = 1 + \frac{1}{\Delta g_{21}}\frac{\partial \Delta g_c}{\partial n} \tag{18}$$

where

$$\Delta g_c = -\mathrm{R}T \ln \delta \tag{19}$$

is the loss of the molar Gibbs free energy due to the cumulation of molecules into clusters, and

$$\begin{aligned} \mu_c &= \frac{\partial \Delta g_c}{\partial n} \\ \mu_{21} &= \frac{\partial \Delta G_{21}}{\partial n} = \Delta g_{21} \end{aligned} \tag{20}$$

may be considered as the chemical potential of the cumulation of molecules into clusters and the fluid-gel difference of chemical potentials, respectively. Note that μ_c is the result of an interplay between entropy and energy of the cluster configuration, and thereby depends on the interfacial properties. In this way, a cooperative unit is associated with chemical potential by equation

$$\nu = 1 + \frac{\mu_c}{\mu_{21}} \tag{21}$$

It is possible to derive an analytical expression for δ in the case of highly cooperative systems such as high purity phospholipid bilayers (i.e. $\nu \gg 1$, $n_I \ll n$). Transformation of Eq. 11 to n-scale (by neglecting of 'n_I' term) and using Eq. 14 gives

$$\delta(n) = \exp\left\{\int \left[\alpha - \ln\left(\frac{1}{n} - 1\right)\right] \mathrm{d}n\right\} = \exp\left\{\int \alpha(1-\nu)\mathrm{d}n\right\} \tag{22}$$

By integrating Eq. 22 we obtain (for $\nu \gg 1$; high cooperativity approximation)

$$\delta(n) \approx n^n \, (1-n)^{1-n} \tag{23}$$

The relation for the cluster configuration entropy can be derived in the following way: Inserting Eq. 4 into Eq. 3 gives

$$S_c = \mathrm{R}(\ln \delta + \ln W_0),$$

from Eqs. 5, 14 follows

$$\begin{aligned} \frac{\mathrm{d} \ln W_0}{\mathrm{d}n} &= \ln\left(\frac{1}{n} - 1\right) = \alpha\nu \\ \ln W_0 &= \int \alpha\nu \, \mathrm{d}n \end{aligned}$$

and using the foregoing relations and Eq.22 we obtain

$$S_c = \mathrm{R} \int \alpha \mathrm{d}n. \tag{24}$$

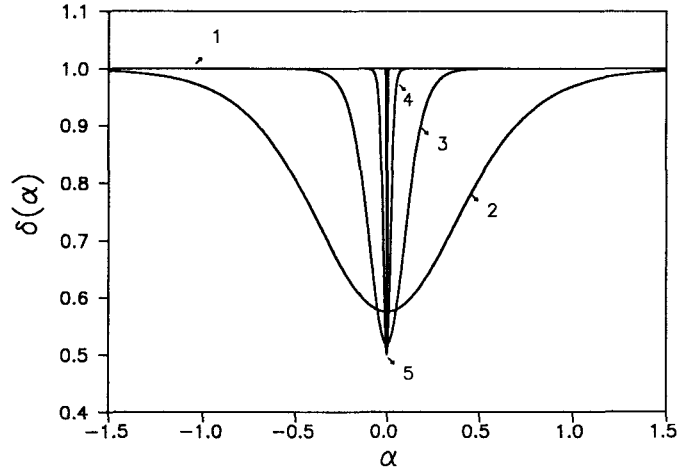


Figure 2. Demonstration of the influence of cooperativity on the shape of cumulation coefficient $\delta(\alpha)$ for various values of cooperative unit ($\nu = 1 \rightarrow 1$, $5 \rightarrow 2$, $20 \rightarrow 3$, $100 \rightarrow 4$, $1000 \rightarrow 5$).

According to Eqs. 13 and 16,

$$\alpha = \frac{\Delta h_{21}}{\mathrm{R}} \left(\frac{1}{T} - \frac{1}{T_m} \right). \tag{25}$$

Representative dependencies of the cumulation coefficient δ were calculated from Eqs. 15 and 22 for several values of the cooperative unit ν in order to demonstrate cooperativity effect on the transition process (Fig. 2). As the size of the cooperative unit increases, the peak height of the cumulation coefficient rapidly increases, while the peak width is reduced, which corresponds to a smaller number of larger clusters. According to Eq. 22 the magnitude of δ never exceeds 1 and is never smaller than 0.5 throughout the transition region. The upper value of δ (Eq. 22 for $\nu = 1$) indicates that the system undergoes a continuous transition without any aggregation of the molecules into the domains of the same state molecules, which means that subsystems are totally independent. In this case, Eq. 15 provides a good description of the melting process of some globular proteins and is identical with known vant'Hoff equation. Eqs. 3, 4, 5 and 15 may be used to obtain the molar statistical weight at the transition centre

$$W_{(\alpha=0)} = (2\delta)^{\mathrm{N_A}}, \tag{26}$$

which yields the lower border of $\delta = 0.5$ if $\nu \rightarrow \infty$ (only one state is available for molecules to access, gel or fluid, which corresponds to $W = 1$; $\mathrm{N_A}$ is Avogadro

number) The degree of transition, n as well as volume and entropy change discontinuously in this case ($n = 0$ if $T < T_m$, $n = 1$ if $T > T_m$, Eqs 15, 25) This means that transition with $\delta = 1/2$ is of the first order type The phospholipid bilayers are "nearly" first order systems due to the finite value of cooperative interaction between molecules and $\delta \approx 1/2$ Decreasing of cooperativity (e g by adding impurities to the bilayer) changes the nature of transition, and δ lies somewhere between the above mentioned limits Therefore, the temperature dependence of δ may be used to indicate the nature of the transition

Results and Discussion

Two experimental heat capacity temperature dependencies were used to calculate transition characteristics using the derived relations Biltonen (1990) has reported a high sensitivity heat capacity measurement of DPPC (dipalmitoylphosphatidylcholine) multilamellar vesicles (MLV) He has used high purity samples, performed measurements at very low scan rates (27 μKs^{-1}) in order to exclude the influence of all possible effects on the transition broadening He found that the finite width of the specific heat curve, which was a few tenths of a degree (a half width 0 076K), could not be a result of an experimental artefact, and that this transition was of nearly first order The same calorimetric curve (without baseline) is given in the paper by van Osdol et al (1991) together with heat capacity measurements of large unilamellar vesicles (ULV) (Fig 3)

The cooperative unit can be calculated directly from Eq 14 if the melting

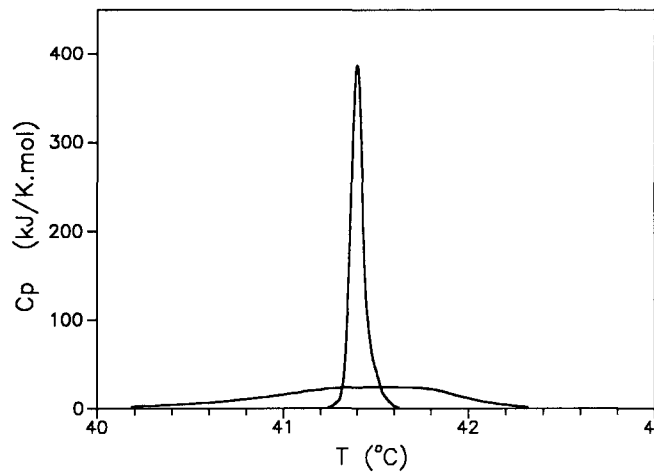


Figure 3. Experimentally obtained heat capacities of DPPC multilamellar vesicles (Biltonen 1990, van Osdol et al 1991, sharp peak) and DPPC unilamellar vesicles (van Osdol et al 1991, wide peak)

profile $n(T)$ is known. It is possible to obtain the latter quantity by numerical integration of $c_p(T)$ curve measured. The formal term for $\nu(n)$ in Eq. 14 diverges if n-values are very close to 0 or 1 in consequence of the model approximation. Regions of divergence are shifted towards the centre and deform the rounds of obtained curves, if borders of integration are not precisely at the beginning and the end of the process. It appears to be the case for negative values of MLV entropy

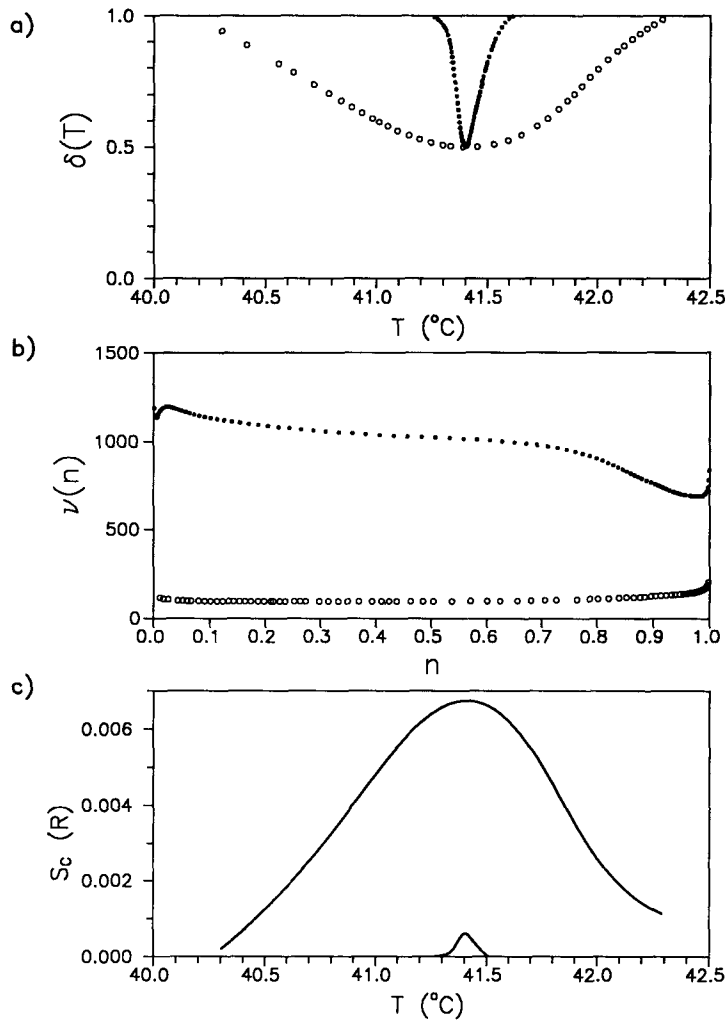


Figure 4. The melting process characteristics obtained from experimental heat capacities of DPPC multilamellar vesicles (Biltonen 1990; van Osdol et al. 1991, •, smaller peak in c); and unilamellar DPPC vesicles (van Osdol et al. 1991, o, higher peak in c). *a*) Temperature dependence of cumulation coefficient; *b*) Dependence of cooperative unit on the fraction melted; *c*) Temperature dependence of cluster configuration entropy.

near to the right transition edge (not shown).

We have used the foregoing experimental c_p data to determine by numerical integration the fraction melted and to calculate transition characteristics using Eqs. 14, 23, 24 and 25. Fig. 4*b* shows the cooperative unit in n-scale which is more suitable for shape comparison. There are significant differences between ULV and MLV, as shown in Figs. 4*a*, 4*b* and 4*c*. Much lower values of ULV cooperative unit indicate strong interbilayer cooperation in multilamellar vesicles which amplifies the effect of the own cooperative process in the bilayer. The type of this interaction can be deduced from extracted cluster configuration entropies (Fig. 4*c*). The very close ULV and MLV transition temperatures indicate that internal bilayer properties did not change significantly. In such a way a marked reduction in MLV cluster configuration entropy as compared to ULV must be connected with a reduction of the degree of freedom of cluster displacement. This means that cluster distributions are not independent in neighbouring bilayers. This idea is supported by the estimation of cluster configuration entropies (see Appendix). Estimated MLV entropy (a14) in the case of strong interbilayer cluster cooperation is in good agreement with experimentally obtained values (a15), contrary to the case of independent clusters. In view of the fact that the entropy is proportional to $1/\sigma$, it is more sensitive to the accuracy of σ for small values of σ. Probably, it causes a less accurate entropy estimation of ULV.

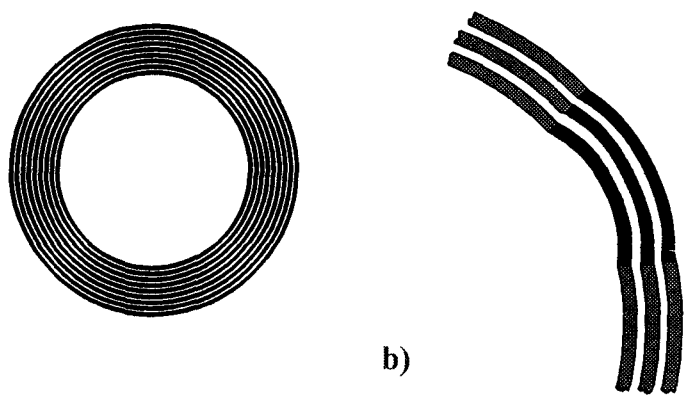


Figure 5. Schematically illustrated cross section of *a*) a model multilamellar vesicle; *b*) a detail of cooperating clusters (gel phase, grid; fluid phase, black).

The possible mechanism of cluster-cluster interaction arises from area expansion at gel to fluid phase transition ($\sim$ 25%). A fluid domain is created in solid matrix and this is accompanied by a high lateral pressure at the cluster boundary. In the case of ULV the latter is compensated for by cluster undulations. However,

in MLV (Fig. 5*a*), the distance shortening produced by undulation induces a considerable steric repulsion between the cluster and the neighbouring bilayer surfaces (for a review of interbilayer forces, see Cevc and Marsh 1987). According to simple geometric calculations, this shortening is comparable with interbilayer distance. From point of view of internal MLV energy, the group of interbilayer clusters is preferable state (Fig. 5*b*). The cluster formed in one layer drives clusters to form in all layers above and below this place. This strong cluster-cluster cooperation may account for a great extension of the cooperative unit and a reduction of cluster entropy which is proportional to the number of layers. A very slow relaxation process has been observed in multilamellar vesicles near the transition point (Yao et al. 1994). We suppose that it may be connected with interbilayer cooperation mentioned above.

Conclusion

A thermodynamical model of the melting process in pure lipid bilayers is presented in this paper. On the basis of derived relations we have obtained cooperative unit, cumulation coefficient and clusters configuration entropy dependencies from the heat capacity measurements of multi- and unilamellar DPPC vesicles. It was demonstrated that cluster-cluster interaction between neighbouring bilayers may account for a large cooperativity extension of multilamellar vesicles. In such a way, the transition cooperativity of multilamellar vesicles is not dependent on internal bilayer properties only but it also has a considerable external component. The fact mentioned above should not be omitted in interpreting the results obtained from experiments using multilamellar vesicles as a model system.

Appendix

Estimation of ULV, MLV entropies

ULV and MLV cluster configuration entropies will be estimated in both cases for dependent and independent clusters, respectively. These entropies are given by the respective statistical weights of cluster displacement on a spherical vesicle. In the case of a single bilayer the number of possible ways to distribute k clusters on a spherical surface is approximately

a1) $$w = \binom{N}{k},$$

where N is the number of molecules in a monolayer.
The number of vesicles with size $N_V = 2N$ is $M = N_A/N_V$ (per mol) and the

molar entropy of ULV vesicles is

$$S_{\mathrm{ULV}} = (k_B \ln w) \cdot M = \frac{\mathrm{R}}{N_V} \ln w.$$

Using Eq. a1 we obtain

a2) $$S_{\mathrm{ULV}} = \frac{\mathrm{R}}{N_V} \ln \binom{N}{k}.$$

MLV vesicles are composed of single bilayers separated by water. The number of MLV vesicles with size N_V is $M = N_A/N_V$ (per mol), where

a3) $$N_V = 2 \sum_{i=1}^{m} N_i$$

and N_i is the number of molecules in i-th monolayer ($i = 1, 2, \cdots, m$).

I. Independent clusters

The statistical weight is a product of statistical weights in different layers due to no correlation existing between them, and their molar entropy is given by

a4) $$S^I_{\mathrm{MLV}} = \left(k_B \ln \prod_{i=1}^{m} w_i \right) \cdot M$$

where is $w_i = \binom{N_i}{k_i}$ the statistical weight in i-th layer.

II. Dependent clusters

The cluster displacement is the same in all layers due to a tight correlation between clusters in neighbouring layers, and the statistical weight can be estimated by calculating statistical weight for average number $\langle N \rangle$ of molecules in a layer,

a5) $$\langle N \rangle = \frac{1}{m} \sum_{i=1}^{m} N_i.$$

The molar entropy of dependent clusters is then given by

a6) $$S^D_{\mathrm{MLV}} = (k_B \ln \langle w \rangle) \cdot M, \qquad \text{where } \langle w \rangle = \binom{\langle N \rangle}{k}.$$

Substituting preceding weights gives

a7) $$S^I_{\mathrm{MLV}} = \frac{\mathrm{R}}{N_V} \sum_{i=1}^{m} \ln \binom{N_i}{k_i}.$$

a8) $$S^D_{\mathrm{MLV}} = \frac{\mathrm{R}}{N_V} \ln \binom{\langle N \rangle}{k}.$$

Using combinatorial laws, Stirling's formulas and the fact that $N \gg k \gg 1$, we can derive

a9) $$\ln \binom{N}{k} = k \ln \frac{N}{k}.$$

The cluster number k at transition temperature (fraction melted $n = 1/2$) is expressed through the average cluster size, σ

a10) $$k = \frac{N}{\sigma}.$$

Eqs. a2, a7, a8 will be rewritten according to Eqs. a3, a5, a9, a10 as

a11) $$S_{\mathrm{ULV}} = \mathrm{R} \frac{\ln \sigma}{\sigma} \frac{N}{N_V} = \frac{\ln \sigma}{2\sigma} \mathrm{R}$$

a12) $$S^I_{\mathrm{MLV}} = \mathrm{R} \frac{\ln \sigma}{\sigma} \frac{\sum_{i=1}^{m} N_i}{N_V} = \frac{\ln \sigma}{2\sigma} \mathrm{R}$$

a13) $$S^D_{\mathrm{MLV}} = \mathrm{R} \frac{\ln \sigma}{\sigma} \frac{\frac{1}{m} \sum_{i=1}^{m} N_i}{N_V} = \frac{1}{m} \frac{\ln \sigma}{2\sigma} \mathrm{R}$$

The average cluster sizes can be calculated from heat capacity curves of ULV and MLV using deconvolution method (Freire and Biltonen 1978). This gives $\sigma_{\mathrm{ULV}} \approx 180$ and $\sigma_{\mathrm{MLV}} \approx 1150$ at transition temperature. Now we can evaluate entropy estimations according to Eqs. a11, a12, a13 and compare them with entropies obtained. The number of bilayers in MLV $m \geq 5$ (Biltonen, 1990), and

a14) $$S^D_{\mathrm{MLV}} \leq 0.0006\,\mathrm{R} \qquad S^I_{\mathrm{MLV}} \approx 0.0031\,\mathrm{R} \qquad S_{\mathrm{ULV}} \approx 0.0144\,\mathrm{R}$$

The entropies obtained from experimental curves (Fig. 3) at transition temperature are

a15) $$S^{\mathrm{ex}}_{\mathrm{MLV}} = 0.0006\,\mathrm{R} \qquad S^{\mathrm{ex}}_{\mathrm{ULV}} = 0.0067\,\mathrm{R}$$

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