

STATISTICAL THERMODYNAMIC ANALYSIS OF THE EXCESS HEAT CAPACITY
FUNCTION OF MACROMOLECULAR SYSTEMS

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Abstract- The deconvolution theory of thermal transitions has proven to be a powerful method with which to analyze the heat capacity function of macromolecular systems. In this article, the basic results of the theory will be presented and their application to multistate transitions and general cooperative transitions of biopolymers and phospholipid membranes will be discussed.

INTRODUCTION

The development of highly precise differential scanning calorimeters has made possible the accurate definition of the heat capacity function associated with thermally-induced transitions of proteins, polypeptides, nucleic acids, lipid bilayers and other macromolecular systems. The importance of having experimental access to the exact shape of this function is that it contains all the information necessary to develop a complete thermodynamic description of a thermally-induced transition. In fact, it has been demonstrated that the excess heat capacity function can be appropriately transformed to yield the partition function of such a system and that this partition function can be used to deduce the microscopic mechanism of the transition (Refs. 1-6).

In this article the analytical methods directed to obtaining a detailed statistical thermodynamic description of complex macromolecular systems will be presented. These methods constitute the basis of the deconvolution theory of thermal-transitions in macromolecules and, thus far, have been applied to the study of protein unfolding reactions, helix-coil transitions in polynucleotides, thermal-transitions of transfer ribonucleic acids (tRNA) and biomembrane phase transitions.

MACROMOLECULAR CONFORMATIONAL TRANSITIONS

Most theories directed toward describing the molecular basis of function and modulation of biochemical systems include structural variations of the relevant macromolecules. Thus important questions relate to what are the characteristics of the equilibrium and dynamic fluctuations within an ensemble of such states.

In general, the accessible structural states of a macromolecular system can be represented by the following reaction scheme:



where the indexing of states is such that the enthalpy of state i (H_i) is greater than the $i-1$ state. (i.e. $H_i > H_{i-1}$). Thus as temperature is increased the population distribution monotonically progresses toward state n . A normalized partition function for this system can be written in terms of the Gibbs energy differences as:

$$Q = \sum_{i=0}^n e^{-\Delta G_i/RT} \quad (2)$$

where

$$\Delta G_i = \bar{G}_i - \bar{G}_0 \quad (3)$$

Since the excess enthalpy function

$$\langle \Delta H \rangle = \langle H \rangle - \bar{H}_0 = RT^2 \frac{d(\ln Q)}{dT} \quad (4)$$

the partition function for such a system can be evaluated as

$$Q = \exp \int_{T_0}^T \frac{\langle \Delta H \rangle}{RT^2} dT \quad (5)$$

where T is a temperature at which $Q \approx 1$ (i.e. all molecules exist in state A_0). For most protein unfolding reactions, the helix-coil transitions of polypeptides and nucleic acids, tRNA unfolding and the gel-liquid crystalline transitions of model bilayer membranes, the latter condition exists in the temperature range of 0 to 100°C. Thus the partition function related to changes in the conformational state of such systems can be obtained directly from differential scanning calorimetric data.

The simplest question to ask concerns the existence of intermediate states during a conformational change of a macromolecule. The probability of the initial state, F_0 , is related to Q as

$$F_0 = Q^{-1} \quad (6)$$

Similarly, giving the final state a statistical weight equal to one, another partition function can be defined such that

$$Z = \exp \int_{T_f}^T \frac{(\Delta H_t - \langle \Delta H \rangle)}{RT^2} dT \quad (7)$$

where $\Delta H_t = H_t - H_0$ and T_f is a temperature at which $F_f = 1$. Therefore the probability of the existence of the final state is $F_f = Z^{-1}$. This immediately leads to the result that the Gibbs energy change for the transition $A_0 \rightarrow A_n$

$$\Delta G_t^0 = -RT \ln (Q/A) \quad (8)$$

Further, the probability of the existence of intermediate states is,

$$\sum_{i=1}^{n-1} F_i = 1 - Q^{-1} - Z^{-1} \quad (9)$$

For most globular proteins it has been found that $\sum_{i=1}^{n-1} F_i < 0.05$ at all degrees of transition. This result is consistent with such reactions being approximately two state in nature. The results of this type of analysis for Ribonuclease A are shown in Fig. 1.

MULTISTATE TRANSITIONS

Many biological macromolecules, such as transfer ribonucleic acids, do not undergo two-state transitions. In this case, the unfolding reaction is characterized by the presence of stable intermediate states and a general description of the transition requires the identification and enumeration of the various enthalpically distinct states, and their transition tempera-

tures. The heat capacity function of these multistate transitions is generally very complex. However, these curves can be deconvoluted by noting the following.

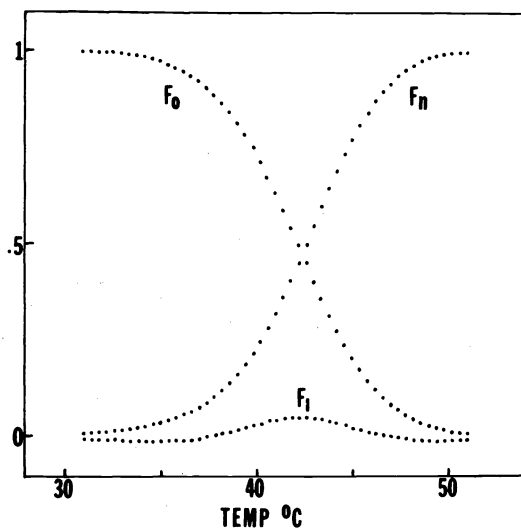


Fig. 1. The experimentally calculated relative population of states associated with the thermal unfolding of Ribonuclease A. F_0 and F_n are the fraction of molecules populating the initial and final states, respectively. F_1 is the summed population of all intermediates. (From Freire, E. and Biltonen, R., *Biopolymers* 17, 463 (1978) with permission).

The quantity

$$\frac{\langle \Delta H \rangle}{(1 - F_0)} = \frac{\sum_{i=1}^n \Delta H_i e^{-\Delta G_i/RT}}{\sum_{i=1}^n e^{-\Delta G_i/RT}} + \Delta h_1 \quad (10a)$$

$$= \langle \Delta H_1 \rangle + \Delta h_1 \quad (10b)$$

The function $\langle \Delta H_1 \rangle$ defines a new, average excess enthalpy, which is averaged over all energy states except the first. In this sense, $\langle \Delta H_1 \rangle$ can be used to define a second partition function, Q_1 , which is obtained by summing over the statistical weights of all energy states except the first. Q_1 and $\langle \Delta H_1 \rangle$ are related by the integral equation,

$$Q_1 = \exp \left(\int_{T_0}^T \frac{\langle \Delta H_1 \rangle}{RT^2} dT \right) \quad (11)$$

Q_1 can then be used to define a subfraction ($F_1' = Q_1^{-1}$) of molecules in the first intermediate state. It must be noted that F_1' is not an average over all the accessible energy states of the system but only over those states included in Q_1 . The above mathematical procedure is equivalent to elim-

inating the contributions of the initial state to the thermodynamic parameters describing the transition. From a mathematical standpoint, we have reduced the number of energy states by one, and all remaining functions are analogous to those that one would find if the first intermediate state (state 1) was the lowest enthalpy state and the initial state (state 0) did not exist.

This procedure can be successively repeated to yield a set of recursion relations from which all the $\Delta h_i \equiv \Delta H_i - \Delta H_{i-1}$, F'_i , and Q_i can be estimated.

$$\langle \Delta H_{i+1} \rangle = \frac{\langle \Delta H_i \rangle}{1 - F'_i} - \Delta h_{i+1} \quad (12)$$

$$F'_i = Q_i^{-1} = \exp \left(- \int_{T_0}^T \frac{\langle \Delta H_i \rangle}{RT^2} dT \right) \quad (13)$$

from which all the thermodynamic parameters describing each transition of the unfolding reaction can be evaluated. Each $T_{m,i}$ is the temperature at which $F_{i-1} = F_i$, so that $\Delta s_i = \Delta h_i / T_{m,i}$. Each of the equilibrium constants K_i , can be calculated by

$$K_i = \exp \left(- \frac{\Delta h_i}{RT} + \frac{\Delta s_i}{R} \right) \quad (14)$$

or by use of the relation:

$$K_i = \frac{Q_{i-1}^{-1}}{Q_i} \quad (15)$$

Absolute fractions of molecules in each particular state i , can be expressed in a recursive form as

$$F_i = F_{i-1} K_i \quad (16a)$$

$$= F_{i-1} \frac{(Q_{i-1})^{-1}}{Q_i} \quad (16b)$$

from which the relative population of each state as a continuous function of the temperature is obtained. In this form, a complete characterization of a multistate transition can be obtained, as it has been demonstrated for the cause of tRNA (Ref. 4).

CONFORMATIONAL TRANSITIONS COUPLED TO ASSOCIATION REACTIONS

Many biological macromolecules consist of two or more molecular subunits and their conformational transitions are often coupled to association-dissociation reactions. One example of this type of process is the double stranded - single stranded transition of nucleic acids and synthetic polynucleotides. DNA and synthetic polynucleotides of complementary sequence form well defined double-helical structures in aqueous solution. Disruption of these helical structures occurs upon increasing the temperature, giving rise to anomalous heat capacity curves such as that shown in Figure 2.

The strand separation reaction occurs abruptly near the maximum in the heat capacity function. The gradual increase in C_p prior to strand separation is due to the partial unraveling at the ends of the double strand and the formation of non-base-paired loops within the helical duplex.

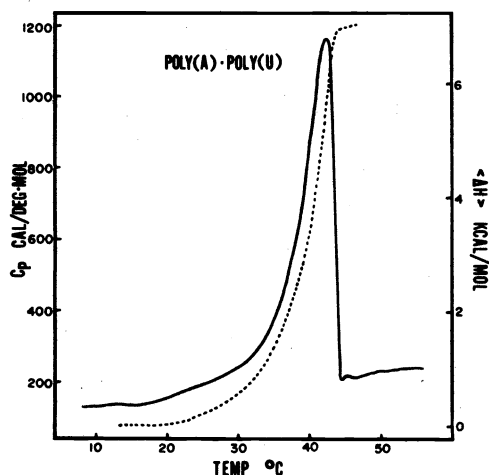
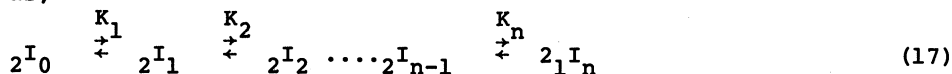


Fig. 2. C_p vs. temperature (Solid line) for the helix-coil transition of poly (A) · Poly (U) at neutral pH and $[Na^+] = 0.016M$. The dashed line ($\langle \Delta H \rangle$) is the average excess enthalpy calculated by direct integration of C_p after subtraction of $C_{p,0}$. (From Suurkuusk, J., Alvarex, J., Freire, E., and Biltonen, R., *Biopolymers*, 16, 2641, (1977) with permission.)

In general, a double-stranded to single-stranded transition can be represented as,



where all the species up to ${}_2I_{n-1}$ inclusive are double-stranded forms characterized by different degrees of base pairing and ${}_1I_n$ representing all the possible forms of the single-stranded conformation. Here ${}_xI_y$ refers to a species of x strands with y broken base pairs. The partition function, Q , of this system is represented by

$$Q = 1 + \sum_{i=1}^n \omega_i \exp(-\Delta G_i/RT) \quad (18)$$

the concentration dependence for double-strand formation being included in the last term of the summation, i.e.,

$$\frac{\Delta G_n}{RT} = \frac{(\Delta H_n - T\Delta S_n)}{2RT} + \frac{1}{2} \ln({}_2I_0) + \ln 2 \quad (19)$$

where $({}_2I_0)$ is the concentration of molecules in the initial state expressed in moles of double-stranded polymers. ΔG_i is the Gibb's energy difference between states i and 0. The fraction of molecules in the i^{th} state, defined as

$$F_i = \frac{{}_2I_i}{({}_2I_0) + ({}_2I_1) + \cdots + ({}_1I_n)/2} \quad (20a)$$

$$F_n = \frac{1I_n}{2\{(2I_0) + (2I_1) + \dots + (1I_n)/2\}} \quad (20b)$$

is given by

$$F_i = \exp(-\Delta G_i/RT)/Q \quad (21)$$

and together with the partition function can be calculated with the deconvolution equations.

The overall fraction, $(\theta_h)_t$, of base pairs can be written as

$$(\theta_h)_t = (\Delta H - \langle \Delta H \rangle) / \Delta H \quad (22)$$

and the fraction θ_h of base pairs among the double-stranded molecules is equal to

$$\theta_h = (\theta_h)_t / (1 - F_n) \quad (23)$$

DOUBLE-STRANDED PARTITION FUNCTION

The average excess enthalpy of the system $\langle \Delta H \rangle$ can be written as

$$\langle \Delta H \rangle = \sum_{i=0}^{n-1} F_i \Delta H_i + F_n \Delta H_n \quad (24)$$

from which the average excess enthalpy function associated with the double-stranded molecules ($\langle \Delta H \rangle^*$) can be calculated

$$\langle \Delta H \rangle^* = \frac{\sum_{i=0}^{n-1} \Delta H_i \exp(-\Delta G_i/RT)}{Q^*} \quad (25a)$$

$$= \frac{\langle \Delta H \rangle - F_n \Delta H}{1 - F_n} \quad (25b)$$

Q^* is the partition function only for double-strand forms and can be numerically obtained from the relation

$$Q^* = \exp\left(\int_{T_0}^T \frac{\langle \Delta H \rangle^*}{RT^2} dT\right) \quad (26)$$

Having Q^* , the thermodynamics of the double-stranded molecules can be described in an analogous form to that derived for the case of multistate transitions.

STRAND SEPARATION

The strand-separation step in the general reaction mechanism [Eq. 17] can be analytically isolated from the whole transition by considering the equilibrium



with the equilibrium constant K defined as

$$K = (I_n)^2 / \sum_{i=0}^{n-1} (I_i) \quad (28a)$$

$$K = 2C_t F_n^2 / (1 - F_n) \quad (28b)$$

where C_t is the total concentration of polymeric molecule expressed in moles of single-stranded forms. The apparent enthalpy change for the strand separation reaction, defined in terms of the classical van't Hoff equation, is equal to

$$\Delta H_{vh} = -R \frac{d \ln K}{d(1/T)} = \Delta H_n - \langle \Delta H \rangle^* \quad (29)$$

where ΔH_n is the total heat for the transition and $\langle \Delta H \rangle^*$ is the average excess enthalpy of the double-stranded form at the point where strand separation occurs. If the usual assumption that the only contribution to the total enthalpy change is the disruption of base pairs is made, it follows that

$$\Delta H_{vh} = \Delta h (N - \langle n \rangle^*) \quad (30)$$

where Δh is the enthalpy change associated with breaking one base pair, N is the total number of base pairs per polymer chain, and $\langle n \rangle^*$ is the average number of broken base pairs in the double-stranded chain immediately before the strand separation. In other words, $(N - \langle n \rangle^*)$ is a measure of the size of the cooperative unit required for double-strand formation.

This analysis has been applied to the double stranded-single stranded transition of Poly (A) · Poly (U) under different ionic conditions (Ref.3). In Figure 3 the fraction of broken base pairs and the fraction of single stranded molecules estimated from the heat capacity function shown in Figure 2 have been plotted as a function of the temperature.

GENERAL COOPERATIVE TRANSITIONS

In many systems of biological interest (e.g., polypeptides, polyribonucleotides, DNA, and phospholipid bilayers), the individual macromolecular states differ only slightly in energy so that the discrete steps tend to merge into a near continuum of states. In such cases, it is not possible to deconvolute the transition curve into distinct transitions but, having the partition function, it is possible to describe the overall thermodynamic changes and calculate molecular distribution functions. In these types of systems, the individual macromolecular states are not uniquely defined, and therefore a better description is obtained by considering the energy states accessible to a single residue (e.g. helix, coil, etc.). Macromolecular states are then defined in terms of statistical averages and distribution functions. Cooperative transitions are generally described by specifying the relative population of residues populating a particular energy state and the statistical distribution of such residues within the macromolecular moiety. The distribution of residues is not random but is constrained by the extent and magnitude of the cooperative interactions existing within the system. The greater the cooperativity, the greater the tendency of residues in the same state to group together, and, therefore, the smaller the probability of finding a pair of neighboring residues in different states. A highly cooperative transition is then characterized by the presence of large clusters or domains of residues in the same state. Conversely, in a poorly cooperative system, the size of these clusters tends to a minimum. A two-state transition as defined previously is, within this context, a limiting case of a highly cooperative transition, i.e., one in

which the probability of finding unlike neighbors is always zero.

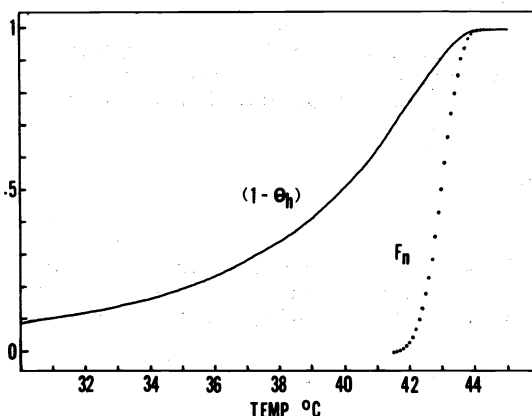


Fig. 3. Experimental fraction of broken base pairs ($1-\theta_h$) (solid line) and fraction of single-stranded molecules (f_n) vs temperature, for the helix-coil transition of poly(A) . poly(U) at $[Na^+] = 0.16M$. (From Freire, E. and Biltonen, R., *Biopolymers* **17**, 497 (1978) with permission).

Helix-coil transitions in polypeptide and polynucleotide systems, as well as the gel-liquid transition in phospholipid bilayers, are examples of these types of cooperative transitions. In such transitions, a precise evaluation of the fractional degree of melting, the relative population of residues in the various accessible energy states, and the statistical distribution of residues within the molecular array is of primary interest. The knowledge of this statistical distribution will provide information regarding the size of the clusters and the equilibrium fluctuations about the statistical averages.

RESIDUE PARTITION FUNCTIONS

For a system in which the macromolecule is composed of N identical units or residues (e.g., an amino acid residue comprising a polypeptide chain), a "residue partition function" can be defined as

$$q(n) = (Q)^{\frac{1}{N}} = \exp \int_{T_0}^T \frac{\langle \Delta h \rangle}{RT^2} dT \quad (31)$$

where $\langle \Delta h \rangle \equiv \langle \Delta H \rangle / N$ is the excess enthalpy per mole of residues. As defined, the molecular partition function, Q , will always be recovered by raising $q(N)$ to the N^{th} power. It is also true that as N becomes large, $q(N)$ approaches a limit, q , in which it is independent of N . This situation defines the thermodynamic limit in the canonical ensemble. In an analogous way a second residue partition function $z = (Z)^{1/n}$ can be defined, where Z is given by Eq. 7. It can also be shown that

$$\frac{z}{q} = e^{-\Delta g/RT} \quad (32)$$

where Δg is the Gibbs energy difference per mole of residues between the initial and final states. This relation allows calculation of Δg as a continuous function of temperature without assuming a particular model for the transition. The volume change for the transition can also be calculated if Δg is known at different pressures, as we have recently demonstrated for the gel-liquid crystalline transition of dipalmitoyl phosphatidylcholine liposomes (Ref. 6).

CLUSTER DISTRIBUTIONS

In the thermodynamic limit canonical and grand canonical ensemble averages are identical for all the properties of a system that are bounded functions of N . In general, the grand canonical partition function, $\Xi(\alpha)$, can be represented as,

$$\Xi(\alpha) = \sum e^{-\alpha N} z^N \quad (33)$$

and diverges as $e^\alpha + e^{\alpha_0} = z$. This divergence condition defines the thermodynamic limit in the grand canonical ensemble. For the cluster model, $\Xi(\alpha)$ can also be written formally in terms of cluster grand partition functions Ω_i

$$\Xi(\alpha) = \sum_{\{n_i\}} \prod_i (\Omega_i)^{n_i} \quad (34)$$

where the product runs over all different types of clusters i , and the summation runs over all sets of number of clusters $\{n_i\}$ consistent with the specification of the system. The cluster grand partition functions Ω_i are defined by the equation

$$\Omega_i = \sum_{j=1} e^{-G_{i,j}/RT} e^{-\alpha j} \quad (35)$$

where $G_{i,j}$ is the Gibbs energy of a cluster of type i containing j residues. This formalism is an extension to any dimensions of the method originally proposed by Lifson and Zimm for the helix-coil transition of DNA (Refs. 7,8). For a transition in which each residue exists in either state A or state B we can write

$$\Omega_A = \sum_{j=1} \left(\frac{z}{q}\right)^j e^{-j\alpha} \quad (36)$$

and

$$\Omega_B = \sum_{j=1} e^{-j\alpha} \quad (37)$$

where, by convention, the state stable above the transition temperature, B, has been chosen as reference state. The average size of clusters of type i is defined as

$$\langle l_i \rangle = - \left(\frac{\partial \ln \Omega_i}{\partial \alpha} \right)_{\alpha = \alpha_0} \quad (38)$$

from which it follows that

$$\langle l_A \rangle = \frac{q}{q-1} ; \quad \langle l_B \rangle = \frac{z}{z-1} \quad (39)$$

The above equations allow direct calculation of the average length of helical and coil sequences in the case of helix-coil transitions in polypeptides and polynucleotides; and of the average size of gel and liquid crystalline clusters in phospholipid membranes. Typical results of such calculations are shown in figures 4 and 5.

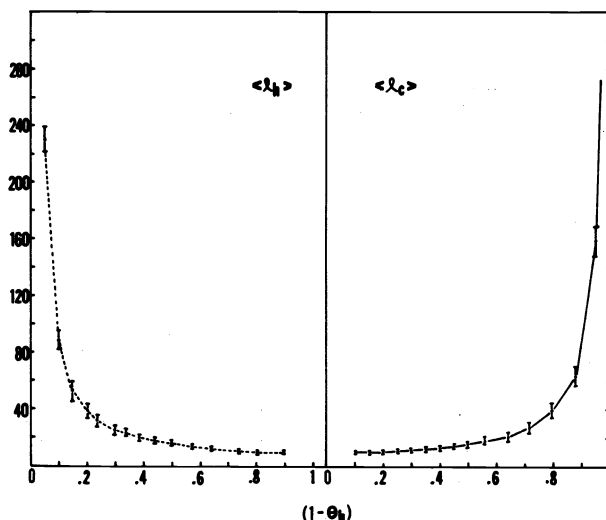


Fig. 4. Experimental average helical- and coil-sequence lengths ($\langle l_h \rangle$ and $\langle l_c \rangle$) associated with the helix-coil transition of poly (A) . poly (U) at $[Na^+] = 0.016M$. (From Freire, E. and Biltonen, R., *Biopolymers* 17, 497 (1978) with permission).

EQUILIBRIUM FLUCTUATIONS

In addition to calculation of cluster averages, the exact energy distribution functions can be calculated from excess heat capacity function by considering the following. The relative probability, $F_A(l)$, of finding an A cluster of size l among all A clusters can be written in terms of the residue partition function q as,

$$F_A(l) = \frac{q-1}{q^l} \quad (40)$$

The k^{th} moment of the cluster distribution is

$$\langle l_A^k \rangle = (q-1) \sum_{l=1}^{\infty} l^k q^{-l} \quad (41)$$

From which

$$\langle l_A^0 \rangle = 1 \quad (42)$$

$$\langle l_A^1 \rangle = \frac{q}{q-1} = \langle l_A \rangle \quad (43)$$

$$\langle l_A^2 \rangle = \frac{q(q+1)}{(q-1)^2} \quad (44)$$

The dispersion of the distribution, Δ^2 , is

$$\Delta^2 = \langle l_A^2 \rangle - \langle l_A \rangle^2 = \langle l_A \rangle (\langle l_A \rangle - 1) \cong \langle l_A \rangle^2 \quad (45)$$

and indicates a broad distribution with an average fluctuation, Δ , approximately equal to the average cluster size.

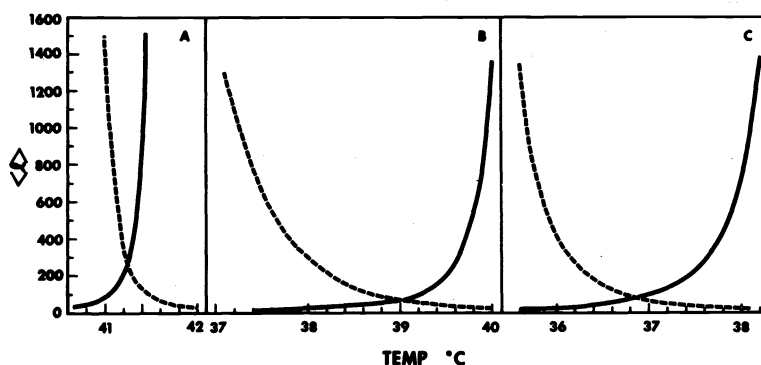


Fig. 5. Average size of gel (-----) and liquid crystal (——) clusters as a function of temperature for (A) multilamellar dipalmitoyl phosphatidylcholine liposomes, (B) multilamellar dipalmitoyl phosphatidylcholine liposomes in equilibrium with 0.047 atm halothane, and (C) sonicated dipalmitoyl phosphatidylcholine vesicles. (From Freire, E. and Biltonen, R., *Biochim. Biophys. Acta* 514, 54 (1978) with permission).

At $\Delta g = 0$, the residue partition function, q , is bounded in the interval $1 \leq q \leq 2$. It is equal to one for a classical first order phase transition and equal to two for a non-cooperative transition. According to Eq. 45, the fluctuations associated with a first order phase transition are of infinite size, in agreement with the predictions of classical theories of critical phenomena. In real systems these fluctuations are, however, finite due to such factors as the finite size of the system, the presence of impurities and most importantly, to the existence of molecular interactions not contemplated in model hamiltonians which reduce the effective magnitude of the cooperative interaction energies. Since q is directly calculated from experimental data, it implicitly contains all these possible contributions

and, as such, allows calculation of the effective range of the fluctuations in any physical system.

In phosphatidylcholine bilayers, for example, the magnitude of the cooperative interactions varies inversely with the length of the hydrocarbon chain. This effect is illustrated in Figure 6, where the average cluster size has been plotted as a function of the length of the lipid acyl chains. Since the experimental lipid systems are very similar in size and the samples are equivalent with regard to their chemical purity, this phenomena most likely reflects an intrinsic characteristic of such systems.

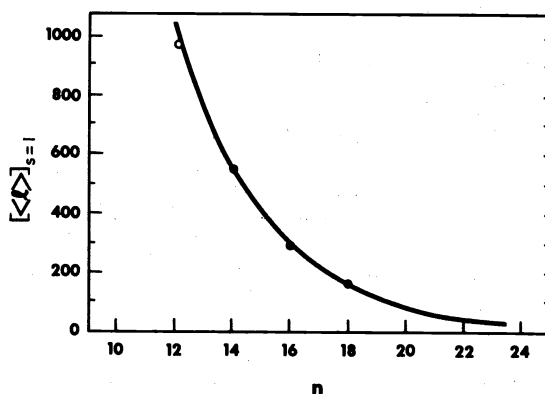


Fig. 6. Average cluster size at $\Delta g = 0$ as a function of acyl chain length for phosphatidylcholine liposomes. The value for $n = 12$ (open circle) was calculated from the heat capacity data obtained by Mabrey and Sturtevant (Ref.9).

VOLUME CHANGES

It was recognized in the first paper dealing with the deconvolution analysis (Ref. 1), that the volume changes associated with a conformational transition can also be used to investigate the microscopic evolution of a system, adding a new dimension to this type of analysis. Consider, for example, the partition function Q defined by Eq. 2. At constant temperature, the pressure derivative ($-\partial \ln Q / \partial P$) defines the excess volume function, $\langle \Delta V \rangle$, and therefore,

$$Q(P) = \exp - \int_{P_0}^P \langle \Delta V \rangle dP \quad (46)$$

where P_0 is a pressure at which all molecules exist in the initial state A. This isothermal partition function, $Q(P)$, can be deconvoluted following the same procedures developed for Q , to yield information regarding the number of intermediate states, their partial specific volumes and their corresponding transition pressures. Thus, having a family of P-V isotherms obtained at different temperatures and knowing $Q(T)$ at constant pressures as a function of temperature from scanning calorimetric data, the system partition function in the P-V plane can be defined. From this a complete thermodynamic mapping of the system can be performed.

The excess volume function, $\langle \Delta V \rangle$, can also be measured as a function of temperature, at constant pressures. In such cases, the simultaneous knowledge of $\langle \Delta V \rangle$ and $\langle \Delta h \rangle$ provides important information to characterize phase transitions in two-component systems, such as in phospholipid mixtures of various composition. For such mixtures one can write the following set of simultaneous equations,

$$\langle \Delta h \rangle = X_A \Delta h_A F_A + X_B \Delta h_B F_B \quad (47)$$

$$\langle \Delta V \rangle = X_A \Delta V_A F_A + X_B \Delta V_B F_B \quad (48)$$

where X_A and X_B are the mole fractions of components A and B. These equations can be solved at each temperature to yield F_A and F_B , the fraction of A and B molecules in the melted state. Further analysis of F_A and F_B allows calculation of the thermodynamic and cooperative behavior of each component within the mixture.

CONCLUDING REMARKS

The potential of this analytical procedure to obtain molecular information about complex systems appears to be almost unlimited. It is to be noted, however, that the validity of this analysis requires that the systems studied be homogeneous, but not necessarily pure; that each system in the ensemble exist in distinct thermodynamic states or that each molecular unit of the system exists in only two defined states; and that the data are obtained under equilibrium conditions. The ultimate usefulness of this approach will be realized when the energy fluctuations obtained can be quantitatively correlated with structural fluctuation which in turn can be related to variation in other physical or functional properties of the system of interest.

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