First- or Second-Order Transition in the Melting of Repeat Sequence DNA

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ABSTRACT Both theoretical analysis and observation of the continuity of the melted fraction of base pairs indicate that the melting transition in DNA is second order. Analysis of the salt dependence of the transition by polyelectrolyte limiting laws, however, has first-order dynamics imbedded in the analysis. This paper proposes that the observation taken to be a latent heat of melting in the limiting law analysis could instead be a specific heat anomaly associated with a second-order transition. The limiting laws can be reconstructed based on a second-order transition with a specific heat anomaly. The T_M^2 dependence of this excess heat is also consistent with its being a specific heat anomaly of a system displaying classical critical behavior. Classical critical behavior indicates that theoretical mean field approaches such as MSPA should be particularly appropriate to helix melting studies.

INTRODUCTION

An early study of the order of the denaturation or melting of the double helix was conducted by Poland and Scheraga (1966). They concluded theoretically that a transition was possible and that it would be a second-order transition. A number of other theoretical papers concluded that critical point melting occurs in DNA (Fisher, 1966; Azbel, 1974) and such melting is necessarily second order. More recent analysis has been done at a microscopic level getting away from the Ising models of earlier work using a self-consistent phonon approximation (Chen and Prohofsky, 1993a, b, c), which also found the transition to be second order. Wells et al. (1970) have measured hyperchromicity curves through the transition for many DNA polymers and found continuous curves. Wartell and Benight (1985) have written an extensive review that summarized much theoretical work and compared the results with experimental observation. In that work they determined the fraction of disrupted base pairs as a function of temperature through the transition from experimental observation and found it to be continuous across the melting transition.

The shift in fraction of base pairs dissociated is therefore both calculated to be, and observed to be, a continuous function of temperature at the transition point. Modern theories of transitions define an order parameter for the transition, and the most definitive factor in assigning the order of a transition is whether the change in order parameter is continuous or not (Landau and Lifshitz, 1958; Callen, 1985). Because the fraction of open base pairs is simply related to the order parameter and is observed to change continuously, the transition is likely a second-order transition. It is possible but unlikely that the observed continuity of the hyperchromicity curves are artifacts due to heterogeneity of the samples.

A good order parameter for DNA denaturation would be $(1 - P^{op})$, or some simply related function, where P^{op} is the probability that a given base pair is open. This factor follows the convention that it is zero in the more homogeneous phase, the melted phase, and is finite in the more structured phase, the helical phase. If the melted fraction P^{op} is continuous as observed experimentally the order parameter $(1 - P^{op})$ is also continuous and a second-order transition is indicated.

In at least one area of DNA research, the assumption that the denaturing transition is first order is made, and that assumption is embedded in the analysis. That area is the study of the salt dependence of the melting temperature by use of Manning limiting laws based on Manning condensation theory (Manning, 1972; Record et al., 1976). In that work a thermodynamic relation was found for the shift of melting temperature as a function of salt concentration by equating the free energy of the intact helix phase to that of the coil phase at the assumed first-order transition temperature. The observed excess heat was then assumed to be a latent heat of the first-order transition. The free energy for each phase is then broken down into two contributions, one due to differences in the polyelectrolyte free energy of the two phases as determined by Manning theory, and the second due to structural differences in free energy of the helix itself. The assumption of an enthalpy of melting or latent heat between two distinct phases at the melting temperature imposes an assumption of first-order dynamics. The limiting law developed predicted variation of melting temperature with salt, as well as ph dependence, and other control variables in good agreement with observation.

First-order transitions have a latent heat associated with them, second-order transitions have a specific heat anomaly associated with them. When the specific heat anomaly is narrow, i.e., extends over a small region of temperature at the transition temperature, it can look very much like a latent heat and it is often difficult to tell them apart. The observed width of the heat anomaly is about 1 K. For this reason the observation of the continuity of the order parameter is accepted as the most definitive factor in determining the order of the transition. It is the contention of this paper that the

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transition is more likely second order as indicated by the observed continuity of the melted fraction of base pairs through the transition. We propose that the observation assumed to be an enthalpy of transition is a standard integrated specific heat anomaly associated with the second-order transition. We show that a thermodynamic limiting law identical to that found by Manning can be constructed based on second-order dynamics. Therefore, the agreement of experiment with the Manning limiting law analysis cannot be used to argue that the transition is first order.

This paper also discusses some differences between first- and second-order transitions and the significance of these differences for DNA denaturation. We find that the experimentally observed T_M^2 dependence of the excess specific heat implies that the transition displays classical critical behavior and discuss why this may be.

FIRST- AND SECOND-ORDER TRANSITIONS

Modern approaches to the dynamics of phase change take into account the role of fluctuations. When two phases of a system are close to a transition point, fluctuations can introduce elements of the nonequilibrium phase into the dominant or equilibrium phase. The dynamics of the transition is analyzed in terms of the growth of these fluctuations. In particular a correlation length for these fluctuations is introduced. The preferred theoretical definition of a second-order transition is one in which the correlation length of the other phase fluctuation goes to infinity at the critical temperature and fills the volume (Toulous and Pfuety, 1977; Landau and Lifshitz, 1958; Callen, 1985). A second-order transition is then one in which the fluctuations dominate and the transition dynamics is driven by the fluctuational breakdown of one phase into a second phase. The preferred theoretical definition of a first-order transition is one in which the transition occurs before the correlation length goes to infinity and can be considered to be a prematurely aborted second-order transition (Toulous and Pfuety, 1977).

The most well known example of the critical behavior associated with this fluctuational breakdown is found in the critical point behavior of a liquid-gas transition. A coexistence curve exists for the transition only up to a critical temperature. The transition is first order along the coexistence curve but becomes second order at the critical point. At this point the transition has zero latent heat and is continuous. The fluctuational breakdown of the liquid state has gone to completion, the correlation length for gas-like fluctuations has spread continuously throughout the volume. There is no jump to a second phase as the fluctuations have already accomplished the change in phase. The large increase in fluctuations is directly observed in the phenomenon of critical opalescence that follows the growth of fluctuations on approach to the critical point.

An alternate but related approach to the study of transitions is based on Landau theory (Landau and Lifshitz, 1958; Callen, 1985). There, one defines a generalized thermodynamic configuration space in which one usually studies the free energy of the entire system for a given set of control variables such as temperature, pressure, salt concentration, etc., displayed as a function of the order parameter associated with the transition studied. In this analysis both first-order and second-order transition behavior can be analyzed. First-order transitions require two distinct local minima to be present in the free energy for the set of control variables at the transition. The two local minima relate to the two distinct phases, both of which are stable against small fluctuations. The stable or equilibrium configuration for one set of control variables on one side of the transition point is that local minimum, which is the global minimum. The transition occurs when the other minimum becomes the new global minimum for changes of a control parameter. The two phases are simultaneously in equilibrium for the set of control parameters at which the two minima are degenerate, i.e., at the same value of free energy. The plot in control variable space where this happens defines the coexistence curve of the transition as a function of the control variables.

In a second-order transition, only one minimum is present. The shift to second-order behavior in this version of Landau theory occurs when the two minima needed for a first-order transition merge leaving only one minimum. That minimum can shift from one value of the order parameter to another defining a second-order phase change but the change is continuous. Since the value of the order parameter determines the phase, the change in phase is also continuous as is the free energy and the entropy. The continuity of the change of these factors then becomes the experimental criterion used to determine order of the transition. The two phases are not simultaneously present except when they are indistinguishable at the critical point. An analysis of a second-order transition between two phases cannot be described in terms of two curves representing the free energy of the two phases as a function of a control variable, because two curves representing two local minima do not exist, and a crossing of curves cannot be used to specify the location of the transition. The analysis has to be in terms of the change of the character of the phase along one free energy curve.

The connection between the description based on fluctuations and that based on two minima going to one is noted by the fact that fluctuations effecting the order parameter can take the system from one minima to another. If the mean fluctuations in order parameter are larger than the separations between minima, the formulation should mix minima to achieve a broad merged effective minimum. Large fluctuations therefore lead to a single minimum and second-order transitions, infinite size fluctuations necessarily lead to second-order transitions. In most cases the definition of a second-order transition as one in which the correlation length of the fluctuation goes to infinity is identical to the Landau definition of a second-order transition as one in which only one local minimum in the free energy exists and the change in order parameter is continuous.

The order parameter is found by minimizing the free energy in order parameter space. For a transition to a ferromagnetic state, the order parameter can be the magnetization.

For a liquid to gas transition, the order parameter can be the particle density. In the Landau first-order transition, there is always a discontinuous change in the value of the order parameter as the system jumps from one minimum at one value of order parameter to the minimum at a different value of order parameter. The first-order transition between liquid and gas displays a discontinuous change in density, the appropriate order parameter. At the critical point (second-order end of the coexistence curve), no discontinuous change in density occurs. There are discontinuities in a number of derivative thermodynamic elements such as the specific heat.

Supercooling occurs when the system is in a local minimum that is not the global minimum but does not undergo a fluctuation large enough to cause the system to jump to the global minimum. For a second-order transition, there is only one minimum; the change in order parameter is continuous, and the order parameter follows the continuous movement of the location of the single minimum in the configuration space.

In practice the value of the Landau approach is that it concentrates on that parameter, the order parameter, which best describes the transition and is the variable for which the analysis is simplest. A second advantage of the Landau approach is that one can study the dynamics of the transition as an expansion about some fixed point as a function of the order parameter. It is a clean expansion without having to evaluate free energies with respect to some absolute value of free energy. The free energy differences are plotted on the same scale with respect to some common zero for all of the phase space. The determination of free energy contributions from factors that do not contribute in any way to the transition but that contribute to the absolute value of the free energy can be safely ignored. The free energy difference is essentially expanded in terms of the order parameter about some floating common value.

In a first-order transition, each phase of the system is in a well defined local minimum and is an ordinary point in the dynamics of that phase. The fluctuations are not large unless one is near the critical point close to the end of the coexistence curve. The transition is then a change from one ordinary point to another of different phase. The difference in enthalpy between the two phases has to be supplied by a latent heat that in principal is a delta function in temperature at the transition temperature rather than in a specific heat anomaly that pumps up the fluctuations. No large build-up in fluctuations occur at ordinary points far from the critical point and the specific heat is well behaved on both sides of the transition. On approaching a second-order critical point from the more ordered phase, i.e., low temperature side, large fluctuations build up. These increase the free energy and bring about an increase in specific heat to power this increase. The transition occurs at the critical point without additional latent heat as the entropies of the two phases including the fluctuational contribution in the more ordered phase are equal at the transition point and bring about the continuous transition. This is so because the fluctuations of the higher temperature phase have already filled the volume. One sees an anomalous rise in heat capacity that begins on approach to the critical temperature from the low temperature side but ends abruptly at the critical temperature. This specific heat anomaly is often mistaken for a latent heat. The observed excess specific heat is in principal broader than a latent heat and more asymmetrical rising more slowly at the low temperature side and being cut off abruptly at the high temperature end. A recent very accurate observation of the specific heat at the DNA melting transition looks quite broad, has the expected asymmetry, and looks very much more like a specific heat anomaly than a latent heat (Chairs and Sturtevant, 1988).

SECOND-ORDER THERMODYNAMIC LIMITING LAW

If one defines the integral of the excess heat as Q then the free energy can be written as

$$G_1(T_M - \delta_1) + Q = G_2(T_M + \delta_2)$$

where T_M is the melting temperature, and δ_1 is a temperature shift large enough to ensure that G_1 is at a temperature below the onset of fluctuations on the low temperature side of the transition, which also means that it is below the onset of the specific heat anomaly. δ_2 is an infinitesimal temperature shift just large enough to ensure that G_2 is unambiguously in the high temperature phase. If Q is then measured at a specific salt concentration c_1 then the equation holds with G_1, G_2, and Q all at c_1.

If in addition δ_1 and δ_2 are small then one gets a thermodynamic relation which is like that obtained for the first-order limiting law derivation except it is a result of the continuity of G across a transition rather than an equality in G at a first-order transition. It also has an excess heat that is from a specific heat anomaly rather than a latent heat.

The equation can be used at different salt concentrations by assuming that G_1 and G_2 have contributions from both structural elements and polyelectrolyte effects. It is clear that one wants G_1 at $T_M - \delta_1$ to properly evaluate the polyelectrolyte contribution based on Manning theory. In the region $T_M - \delta_1 < T < T_M$, the melting fluctuations in the helix are large. In the melted regions the charge density would be more like that of the single strand phase. The intercharge distance b used in Manning theory would be larger than that for the helix and the formulation of the polyelectrolyte contribution to the free energy would be somewhere between that of the helix and the single strand contributions. Only by evaluating G_1 at $T_M - \delta_1$ will the Manning formulation of the polyelectrolyte contribution to the free energy, which assumes a stable double helical conformation, be correct. Similarly only at or above $T_M + \delta_2$ will the polyelectrolyte contribution to the free energy G_2 be a pure case gotten by using single strand factors. With these substitutions and definitions Eq. 1 looks like the starting point of the earlier first-order limiting law derivation and a look alike limiting law can be reproduced.

TEMPERATURE DEPENDENCE OF THE EXCESS HEAT

In classical second-order Landau transition theory, the specific heat is

$$C_p = C_{0p} + a^2 T_M / 2C$$

at the critical point. C_{0p} is the specific heat of the more homogeneous phase, and a and C are parameters in the Landau expansion of the free energy. At temperatures above the critical point, the second term drops out and the specific heat falls to C_{0p}. There is a discontinuous shift in the specific heat at the transition. The second term that disappears is the anomalous part of the specific heat that is present only in the vicinity of the transition. The width of the critical region is narrow in temperature for systems with long range forces and is found to be proportional to T_M for one dimensional systems in at least some systems (Toulous and Pfuety, 1977). The area under the specific heat anomaly is then proportional to T_M^2. The approximations used define classical Landau theory.

DISCUSSION

That DNA undergoes critical transition melting, that is, it has a vanishing coexistence curve with its dissociated state is due to the one dimensional nature of the helix. Ideal one dimensional systems with short range interactions are so susceptible to fluctuations that they are incapable of cooperative transitions. DNA is not an ideal one dimensional system in that a break in a bond does not separate the helix into two independent parts, but it still has an excitation spectrum that is determined by the one dimensional character of the helix. DNA is one dimensional enough that large fluctuations arise that disrupt the two local minima needed for a coexistence curve. DNA is soft with respect to transverse displacements that stretch and disrupt the H-bonds and determine base disruption. Essentially such displacements are not opposed by stiff valence bonds. This is easily seen to be the case by examining the normal mode spectrum of the helix (Girirajan et al., 1989). There are a large number of low frequency modes associated with transverse displacements. Low frequency spectra is also observed experimentally associated with transverse motion (Lindsay and Powell, 1983). In particular the H-bond breathing modes work against a soft H-bond force constant and move large mass transversely against weakly coupled solvent. This mode is centered about 85 cm^{-1} (Urabe and Tominaga, 1982). The low frequency modes give rise to large amplitude displacements in thermal equilibrium and, therefore, large thermal fluctuations associated with melting. The ease of inducing large fluctuations cause the system to easily reach critical behavior associated with second-order transitions. This tendency for one dimensional systems to have low frequency modes and large fluctuations can also be reached by considerations based on analysis referred to as fractal dimension analysis.

No direct measurement of critical exponents for the melting of repeat sequence DNA seems to have been done. The T_M^2 dependence of the excess heat does indicate that the system is displaying classical critical behavior and could be expected to display classical critical exponents. Not all systems display such exponents (Toulous and Pfuety, 1977), and the implication that the melting of DNA does argues that they must have long range interactions possibly helped by the fact that DNA is not an ideal one dimensional system in that it has finite width. All one dimensional theories including the Ising-like Helix-coil theories had to introduce some form of long range interaction to get any kind of transition (Poland and Scheraga, 1966; Fisher, 1966; Azbel, 1974; Wartell and Benight, 1985). The observation of a narrow specific heat anomaly also implies long range forces (Toulous and Pfuety, 1977). The two likely sources of these long range interactions are loop-closing entropy effects that were included in the Ising models (Poland and Scheraga, 1966; Fisher, 1966) and Coulomb interactions.

Loop-closing entropy effects have been found to have an effective range that can be fit by a power law that falls off as $r^{-(3/2+\epsilon)}$ (Poland and Scheraga, 1966; Fisher, 1966). The parameter ϵ is due to excluded volume effects and is expected to be small (Fisher, 1966). The Coulomb interaction is usually thought of as falling off exponentially due to shielding by counterions. The shielding is certainly effective under static conditions but not necessarily under dynamic conditions. During fluctuations, which induce melted regions, the linear charge density of the helix fluctuates from that of the intact helix to that of the single strands. This would necessitate a rearrangement of the counterion structure. The frequency associated with the onset of the fluctuation is that of the interbase breathing motion that is at 85 cm^{-1} whereas the relaxation frequency of the ion structure is less than a megahertz. It is unlikely that the ion rearrangement can follow the fluctuations in real time and the breakdown of the shielding leads to enhanced long range interactions in the presence of the fluctuations. The unshielded Coulomb interaction associated with the fluctuations would then approach a power range r^{-1}. It is the interaction range associated with the fluctuations that effects the critical behavior.

The region of classical behavior is determined as a function of two parameters (Toulous and Pfuety, 1977): d, the dimension and σ, which is related to the range of forces involved. The long range power law interactions are expressed as a function of distance r by

$$V(r) = r^{-(d+\sigma)}$$

The dimension d in this case is the number of dimensions that the system extends to very large distance and the helix dimension for this analysis is one. For a one dimensional system $\sigma < ½$ falls into the classical long range regime. For values of σ where $½ < \sigma < 1$ in a one dimensional system, the system is in the nontrivial long range regime (Toulous and Pfuety, 1977) that retains some classical features. Both of the long range interactions considered would cause the helix to fall into the long range regime very close or into the classical long range regime.

Classical critical behavior is often called mean field behavior. Mean field theories only give rise to classical critical behavior and mean field theories are accurate approximations under conditions where classical critical behavior occurs. MSPA calculations of helix melting (Chen and Prohofsky, 1993a, b, c) are mean field calculations and can be expected to give good results for the second-order melting transition of the helix. In mean field calculations the order parameter appears in the interaction potential. The best known example is in the ferromagnetic case. There the interaction potential between a magnetic atom and the internal magnetic field is μM where μ is the magnetic moment of the atom and M, the magnetization. M is, however, also the order parameter for the ferromagnetic transition. In cooperative MSPA applied to DNA, the potential between atoms is $(1 - P)\phi x^2$ (Chen and Prohofsky, 1993a, b, c) where P is the open bond probability, ϕ is the MSPA intact effective force constant, and x is the interatomic displacement from equilibrium. Note that $(1 - P)$ is also the order parameter for the disruption of that bond. The MSPA approach also deals properly with the dimensionality of the helix, which is another important element in the theory of transitions. The true microscopic conformation of the helix is used in that approach along with long range interactions. The probe of the onset of fluctuational breakdown of the helical structure as conducted in the MSPA analysis should be a particularly appropriate approach of the melting of the helix.

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