Heat Capacity Effects on the Melting of DNA. 1. General Aspects

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ABSTRACT In this paper we analyze published data on ΔH and ΔS values for the DNA melting transition under various conditions. We show that there is a significant heat capacity increase ΔC_p associated with DNA melting, in the range of 40–100 cal/mol K per base pair. This is larger than the transition entropy per base pair, $\Delta S^0 \approx 25$ cal/mol K. The ratio of $\Delta C_p/\Delta S^0$ determines the importance of heat capacity effects on melting. For DNA this ratio is 2–4, larger than for many proteins. We discuss how ΔC_p values can be extracted from experimental data on the dependence of ΔH and ΔS on the melting temperature T_m. We consider studies of DNA melting as a function of ionic strength and show that while polyelectrolyte theory provides a good description of the dependence of T_m on salt, electrostatics alone cannot explain the accompanying strong variation of ΔH and ΔS. While T_m is only weakly affected by ΔC_p, its dependence on one parameter (e.g., salt) as a function of another (e.g., DNA composition) is determined by ΔC_p. We show how this accounts for the stronger stabilization of AT relative to GC base pairs with increasing ionic strength. We analyze the source of discrepancies in ΔH as determined by calorimetry and van't Hoff analysis and discuss ways of analyzing data that yield valid van't Hoff ΔH. Finally, we define a standard state for DNA melting, the temperature at which thermal contributions to ΔH and ΔS vanish, by analyzing experimental data over a broad range of stabilities.

INTRODUCTION

DNA melting is the process of separating the two strands wound in a double helix into two single strands. Conceptually, this phenomenon is similar to the much-studied protein unfolding. In both cases the macromolecule cooperatively loses its secondary structure and exposes a large fraction of its internal surface to aqueous solution. The analogy goes further, because in both cases the macromolecular secondary structure has marginal stability under physiological conditions. This means that the transition free energy is so small that even moderate changes in environmental conditions can shift the equilibrium between the two states. This marginal macromolecular stability does not imply that the interactions that hold the double helix or a native protein together are weak. Rather, it is the result of the compensation of two large quantities: a favorable enthalpy ΔH and unfavorable entropy ΔS.

The stability of a protein is not only determined by direct interactions between its atoms or the conformational freedom of its backbone and side chains. Another very important factor is the increase in hydrophobicity as the buried amino acid side chains are exposed to water in the unfolded state. Hydrophobicity is not really an interaction; rather it is the ability of nonpolar residues to affect the structure and fluctuations of the aqueous solvent. This is most characteristically manifested in a large increase in the heat capacity ΔC_p of the system, resulting in a strong temperature dependence of the transition enthalpy and entropy. At small deviations from a standard temperature, thermal contributions to ΔH and $T\Delta S$ cancel each other, when summed to give the free energy ΔG. But over a broader temperature range, the different functional forms of the enthalpy and entropy can result in a significant contribution of the heat capacity to the overall transition free energy. It can sometimes dominate the stability of proteins and can even lead to the striking phenomenon of cold denaturation (Franks, 1995; Makhatadze and Privalov, 1994).

Although they are commonplace in protein denaturation, heat capacity effects are rarely considered important in DNA thermal denaturation. In fact, it is usually assumed that ΔH and ΔS for DNA melting are essentially independent of the temperature (Breslauer et al., 1986; Grosberg and Khokhlov, 1994; Klump, 1988; Privalov et al., 1969). The reason for this assumption, which we show below to be erroneous, is primarily historical. Differential scanning calorimeters in the 1970s and 1980s were not able to detect ΔC_p directly in the differential C_p versus T melting curves, on a background of sloping baselines and significant noise. Moreover, the ΔC_p value, if any, constituted less than 1% of its overall change in the course of the melting transition, making it unsurprising that it escaped quantitation. Therefore it was assumed that, being below the detection level, ΔC_p was not important for the transition thermodynamics. At the same time, when ΔH and ΔS values obtained in the same studies were presented as a function of the transition temperature, a significant positive ΔC_p became apparent.

In this paper we analyze published data on ΔH and ΔS values for the DNA melting transition under various conditions. We show that there is a significant heat capacity increase associated with DNA melting, in the range of 40–100 cal/mol K per base pair. "Significant" means that ΔC_p is larger than the transition entropy per base pair, $\Delta S^0 \approx 25$ cal/mol K. On a mass basis, ΔC_p is less for DNA

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than for proteins (Sturtevant, 1977), but it is the ratio of ΔC_p to ΔS^0 that determines the importance of heat capacity effects on melting. For DNA this ratio is 2–4, larger than for many proteins. Whether this means that hydrophobic effects are important in DNA melting, or whether there is some other explanation for the large heat capacity change, remains to be seen.

The structure of this paper is as follows. In the next section we discuss how ΔC_p values can be extracted from experimental data on the dependence of ΔH and ΔS on the melting temperature T_m. In the third section we consider studies of DNA melting as a function of ionic strength. We show that while polyelectrolyte theory provides a good description of the dependence of T_m on salt, electrostatics alone cannot explain the accompanying strong variation of ΔH and ΔS. In the fourth section we show that while T_m is only weakly affected by ΔC_p, its dependence on one parameter (e.g., salt) as a function of another (e.g., DNA composition) is determined by ΔC_p. This explains the stronger stabilization of AT relative to GC base pairs with increasing ionic strength. In the fifth section we address the question of whether ΔH and ΔS as determined by calorimetry and van't Hoff analysis are equivalent and assess the relative error in determination of ΔH in the latter. In the sixth section we attempt to find the "standard state" for DNA melting, i.e., the temperature at which thermal contributions to ΔH and ΔS vanish, by analyzing experimental data over a broad range of stabilities. Our summary and conclusions are presented in the seventh section.

In the accompanying paper (Rouzina and Bloomfield, 1999) we apply this analysis to the extensive compilations of data on the thermal melting of oligonucleotides of defined sequence, the aim of which has been to tabulate standard values of ΔG, ΔH, and ΔS for various nearest-neighbor base pairs. While the free energies determined in various studies generally agree with each other, there is little consensus on the enthalpy and entropy values. The most striking differences are between the results obtained on oligomeric versus polymeric DNA. We show that no special concepts are needed to explain these results, if the temperature dependence of the thermodynamic parameters is properly taken into account.

THERMODYNAMICS OF DNA MELTING WITH HEAT CAPACITY CHANGE

Basic formulation

The changes in free energy ΔG, enthalpy ΔH, entropy ΔS, and heat capacity ΔC_p are related to temperature T and to each other by the familiar equations

$$\Delta G = \Delta H - T\Delta S, \tag{1}$$

$$\Delta H(T) = \Delta H^0 + \delta H + \Delta C_p(T - T^0), \tag{2}$$

$$\Delta S(T) = \Delta S^0 + \delta S + \Delta C_p \ln(T/T^0), \tag{3}$$

where $T^0 = \Delta H^0/\Delta S^0$ is the melting temperature in the reference state. The quantities denoted by δ refer to perturbations from standard conditions, such as might be introduced by a change in salt concentration.

These equations can conveniently be written in terms of reduced variables:

$$\Delta H = \Delta H^0[1 + \gamma_H + \alpha t], \tag{4}$$

$$\Delta S = \Delta S^0[1 + \gamma_S + \alpha \ln(1 + t)], \tag{5}$$

where

$$\alpha = \Delta C_p/\Delta S^0, \tag{6}$$

$$t = (T - T^0)/T^0, \tag{7}$$

and

$$\gamma_H = \delta H/\Delta H^0, \quad \gamma_S = \delta S/\Delta S^0. \tag{8}$$

In practice the relative deviations from standard conditions of t, γ_H, and γ_S are all much less than 1. The smallness of these quantities leads to the expansion for the free energy to leading order in the reduced variables,

$$\Delta G = \Delta H^0[\gamma_H - \gamma_S - (1 + \gamma_S)t - (\alpha/2)t^2]. \tag{9}$$

The melting temperature, determined by the condition $\Delta G = 0$, is

$$t_m = t_m^0\left(1 - \frac{\alpha/2}{1 + \gamma_S}\, t_m^0\right), \tag{10}$$

where

$$t_m^0 = \frac{\gamma_H - \gamma_S}{1 + \gamma_S} \quad \text{or} \quad T_m^0 = T^0(1 + \gamma_H - \gamma_S) \tag{11}$$

is the melting temperature if $\Delta C_p = 0$, or $\alpha = 0$. Equations 10 and 11 are correct to first order in small quantities.

The key feature of these equations is that ΔH and ΔS vary to first order as αt, which, because $\alpha > 1$, is a stronger temperature variation than that of ΔG or T_m, which vary only as the smaller quantities t or γ. Examples of such behavior from several experimental studies on DNA melting, in which equilibrium was altered by varying the ionic strength I, are presented in Fig. 1. It is clear that the moderate (~10%) variation in free energy, as compared to the large (~40%) variation in enthalpy and entropy, is the result of mutual compensation of significant heat capacity contributions.

Determination of ΔC_p and ΔS^0 from experimental data

General thermodynamic principles indicate that ΔC_p at T_m should be given equivalently by either of the two slopes $\partial\Delta H/\partial T_m$ or $\partial\Delta S/\partial \ln T_m$. However, when the data of Fig. 1 are plotted as a function of T_m, as in Fig. 2, the enthalpy

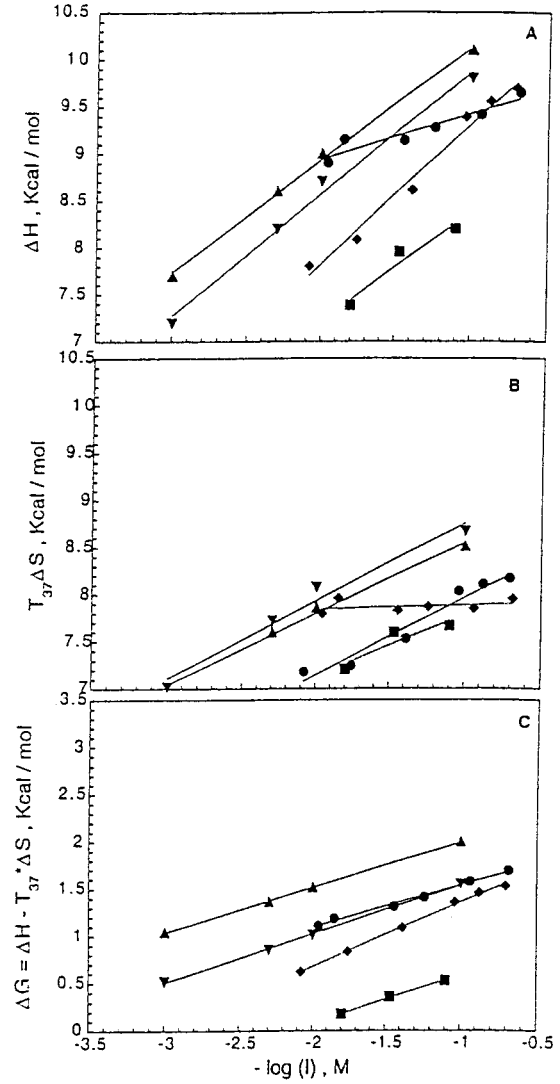

FIGURE 1 Thermodynamic parameters for melting of polymeric B-DNA double helix. Enthalpy $\Delta H(T_m)$ (*A*), entropic component of the transition free energy $T_{37}\Delta S(T_m)$ (*B*), free energy of melting $\Delta G = \Delta H(T_m) - T_{37}\Delta S(T_m)$ (*C*), corresponding to physiological temperature $T = 37°C = 310$ K, as a function of solution ionic strength, *I*, in monovalent salt. $\Delta H(T_m)$ and $\Delta S(T_m)$ were obtained by calorimetry in all studies except for Karapetian et al. (1990), where differential ligand binding method was employed. ●, Calf thymus DNA, (42% GC), in Na_2SO_4, at pH 6.8 (Gruenwedel, 1974). ■, Poly(A+U) and poly(A+2U) in Na^+ and K^+ salts, at pH 7 (Krakauer and Sturtevant, 1968). ▲ and ▼, *C. perfringens* 31% GC and *M. lysodeictikus* 71% GC DNA, respectively, in NaCl at pH 7 (Karapetian et al., 1990). ◆, T2 DNA, 35% GC, in NaCl, at pH 7 (Privalov et al., 1969). Note similar scales but the different ranges of the panels. Clearly the experimental ΔG values vary significantly from one study to another. This can be related to different DNA sequences and slightly different solution conditions. Independently of these differences variation of ΔG with salt in each case was less then 1 kcal/mol, while it was $\leq$3 kcal/mol for ΔH and $\leq$2 kcal/mol for $T_{37}\Delta S(T_m)$.

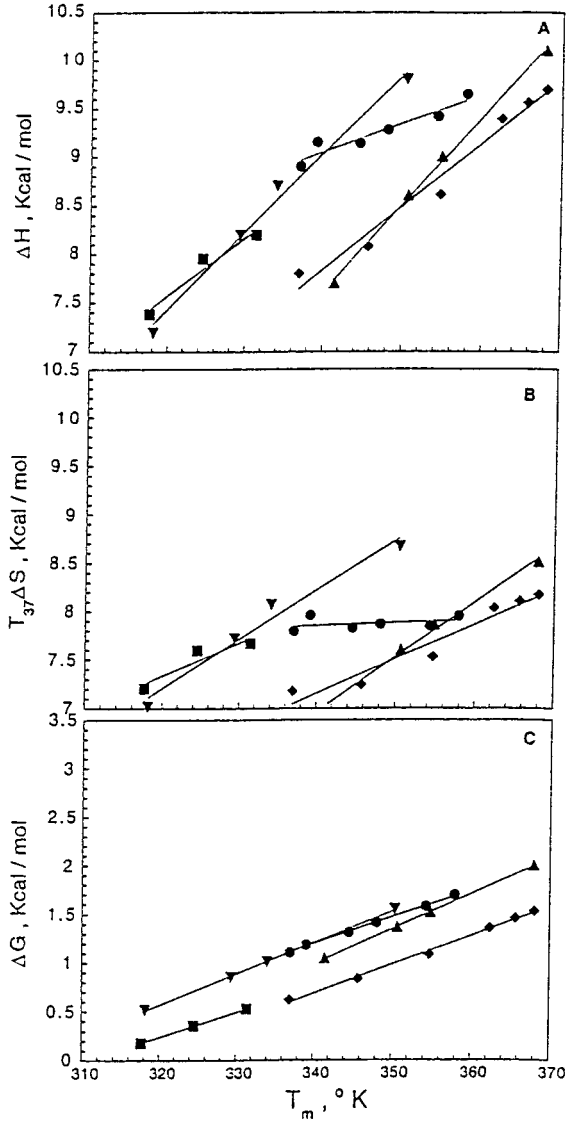

FIGURE 2 The same data and symbols as in Fig. 1, but presented as a function of melting temperature T_m, rather than solution ionic strength. Note significantly stronger ΔH as compared to $T_{37}\Delta S$ dependence. The $\Delta G(37)$ dependence is even weaker and is quite similar for all of the cases studied.

slope is always larger (see Table 1). From Eqs. 2 and 3, we obtain

$$\frac{\partial \Delta H}{\partial T_m} = \frac{\partial \delta H}{\partial T_m} + \Delta C_p \qquad (12)$$

and

$$\frac{\partial \Delta S}{\partial \ln T_m} = \frac{\partial \delta S}{\partial \ln T_m} + \Delta C_p. \qquad (13)$$

Thus the experimental slopes have contributions from the thermal dependence of the perturbations δH and δS, as well as from the common ΔC_p.

We can relate the two slopes by noting that at T_m, $\Delta G(T_m) = 0$, and this remains the case if solution conditions and temperature are adjusted simultaneously to remain at $\Delta G = 0$, so that

$$\frac{\partial \Delta G(T_m)}{\partial T_m} = \frac{\partial \Delta H(T_m)}{\partial T_m} - \frac{\partial \Delta S(T_m)}{\partial \ln T_m} - \Delta S = 0 \qquad (14)$$

TABLE 1 Experimental slopes and derived ΔS^0 and ΔC_p parameters (in cal/mol K per base pair) for melting of various genomic DNAs

DNA	$\partial H/\partial T_m$	$\partial S/\partial \ln T_m$	ΔS^0	ΔC_p	Reference
ct, 42%GC	65.3	40.4	24.9	55.3	Gruenwedel (1974)
poly(AU)	59.5	35.5	24.0	49.9	Krakauer and Sturtevant (1968)
ct, pH 7	41.0	21.0	20.0	33.0	Shiao and Sturtevant (1973)
T_2, 35%GC	30.0	3.2	26.8	19.3	Privalov et al. (1969)
C. perf., 31%GC	80.5	55.2	25.3	70.4	Karapetian et al. (1990)
M. lys., 71%GC	90.3	65.2	25.1	80.3	Karapetian et al. (1990)

DNA stability and T_m were altered by varying the ionic strength between 0.001 and 1 M in pH 7 buffers.

or

$$\frac{\partial \Delta H}{\partial T_m} - \frac{\partial \Delta S}{\partial \ln T_m} = \Delta S^0 + \delta S + \Delta C_p \frac{T_m - T^0}{T^0} = \Delta S. \quad (15)$$

The difference between the two slopes always equals the transition entropy. The individual slopes contain contributions from ΔC_p as well as from ΔS, the latter being partitioned between the slopes in the same proportion p by which the perturbation δG is partitioned between enthalpy and entropy: $\Delta H = p\Delta G$, $T\Delta S = -(1 - p)\Delta G$. That is,

$$\frac{\partial \Delta H(T_m)}{\partial T_m} = p\Delta S + \Delta C_p,$$
$$\frac{\partial \Delta S(T_m)}{\partial \ln T_m} = -(1 - p)\Delta S + \Delta C_p. \quad (16)$$

If the perturbation is purely enthalpic, e.g., in the case of DNA composition change, then $p = 1$ and the apparent enthalpy slope is larger than ΔC_p. On the other hand, if the perturbation is purely entropic, e.g., in the case of variation of oligomer strand concentration, then $\delta H = 0$, $p = 0$, and the enthalpy slope reflects only the heat capacity change. Thus the accurate determination of ΔC_p from experimental enthalpy and entropy slopes requires knowledge of p, i.e., how the perturbation is partitioned into its components. Unfortunately, because there are only two slopes and three unknowns (ΔS, ΔC_p, and p), this partitioning cannot be performed uniquely. An estimate of ΔC_p must then be obtained as the arithmetic average of the enthalpy and entropy slopes; this estimate will be most adequate if the slopes are close to each other.

A number of experimental results for the slopes and the derived ΔS and ΔC_p are summarized in Table 1, in which T_m was manipulated by varying the salt concentration. We see from this table that all of the entropies are close to $\Delta S^0 = 25 \pm 2$ cal/mol K. (It is interesting that this is very close to the value estimated from the number of degrees of freedom frozen upon double helix formation (Cantor and Schimmel, 1980; DeVoe and Tinoco, 1962; Klump, 1988).) The heat capacity changes are in the range $\Delta C_p \approx 30$–100 cal/mol K, so that α (Eq. 6) is generally in the range of 2–4.

In contrast, data obtained for low concentrations of short oligomeric DNA (Table 2) shows values of $\Delta S \leq 5$ cal/mol K. That is, the enthalpy and entropy slopes have absolute magnitudes of 70–100 cal/mol K per base pair but are different by less than 5%. In this case the small difference between the two slopes comes from the large negative contribution to the melting entropy due to the low T_m of oligomeric relative to polymeric DNA (see the third term in the middle equality of Eq. 15).

Knowledge of the actual partitioning of the perturbation between enthalpy and entropy requires a physical model. In the next section we study the particular case of perturbation by varying the ionic strength.

IONIC STRENGTH DEPENDENCE OF DNA MELTING

An understanding of the effect of ionic strength on DNA melting temperature has been one of the major accomplishments of polyelectrolyte theory. However, as we shall show in this section, although polyelectrolyte theory accounts very well for the dependence of ΔG and T_m on ionic strength I, it can explain almost nothing about the dependence of ΔH and ΔS. We couch our treatment, for the sake of simplicity, in terms of counterion condensation theory (Manning, 1975, 1978), but the same results emerge from various applications of Poisson-Boltzmann theory (Bond et al., 1994; Frank-Kamenetskii et al., 1987).

In counterion condensation theory, the polyelectrolyte is modeled as a cylindrical rod with length per unit charge b. A key parameter that characterizes the electrostatic behavior is the ratio ξ of b to the Bjerrum length l_B:

$$\xi = l_B/b, \quad (17)$$

where

$$l_B = \frac{e^2}{\epsilon k_B T}, \quad (18)$$

TABLE 2 Temperature dependence of oligomer melting

Sequence	ΔC_p
CCGC	98
ACCGGp	81
CCGGUp	73
ACCGGUp	75
CCGGAp	55

Petersheim and Turner (1983). In all cases, $\Delta S \leq 5$ cal/mol K.

e is the electron charge, ϵ is the solvent dielectric constant, and k_B is the Boltzmann constant. Because of the different linear charge densities of helix and coil, they territorially bind different numbers of counterions, so that upon the helix-coil transition a number Δn of counterions is released per DNA phosphate charge. This leads to the free energy difference between helix (H) and coil (C),

$$\delta G^{el} = k_B T \Delta n \ln(I), \tag{19}$$

where

$$\Delta n = \frac{1}{\xi_C} - \frac{1}{\xi_H}. \tag{20}$$

Equation 19 is strictly valid only in the limit of zero ionic strength, but for polyelectrolytes with the high charge density of DNA it holds up to at least 0.1 M (Rouzina and Bloomfield, 1996). If this equation is substituted into Eq. 11, it results in the expression for the dependence of T_m on I,

$$T_m = T^0\left(1 + \frac{R\Delta n \ln(I)}{\Delta S^0}\right), \tag{21}$$

where we have converted to molar quantities by replacing k_B with the gas constant R. Using the values $b_H = 0.17$ nm, $b_C = 0.34$ nm, and $l_B = 0.714$ nm, we find that $\Delta n = 0.24$. Substituting $\Delta S^0 = 25$ cal/mol K we get $T_m = T^0[1 + 0.044 \log(I)]$, which is essentially identical to the $T_m = 377.5[1 + 0.045 \log(I)]$ obtained from a summary of experimental results for DNAs of normal composition (Blake and Delcourt, 1998).

To obtain the polyelectrolyte contributions to the enthalpy and entropy, we differentiate δG^{el} with respect to temperature according to the standard equations:

$$\delta H^{el} = \frac{\partial(\delta G^{el}/T)}{\partial(1/T)}, \quad \delta S^{el} = -\frac{\partial(\delta G^{el})}{\partial T}. \tag{22}$$

When δG^{el} (Eq. 19) is divided by T, the only remaining T dependence is in Δn through the dependence on T of l_B. But l_B (Eq. 18) varies as the reciprocal of ϵT, and it is commonly assumed that ϵ is inversely proportional to T. If this were the case, $\delta G^{el}/T$ would be independent of T, leading to $\delta H^{el} = 0$ and $\delta S^{el} = -\delta G^{el}/T$. This is, in fact, the common assumption for DNA and other polyelectrolytes—that electrostatic effects are purely entropic.

However, accurate measurements of ϵ for water over a wide range of T (Eisenberg and Kauzmann, 1964; Weast and Astle, 1979) yield the more complex behavior

$$\epsilon(T) = \epsilon^*(T^*/T)^\nu, \tag{23}$$

where $\epsilon^* = 78.54$ at $T = 298$ K and $\nu = 1.4$. Manipulation of the above equations then yields

$$\delta H^{el} = (\nu - 1)\delta G^{el}, \quad T\delta S^{el} = -(2 - \nu)\delta G^{el}. \tag{24}$$

Thus for helix-coil transitions in DNA, $\delta H^{el} \approx 0.4\delta G^{el}$ and $T\delta S^{el} \approx 0.6\delta G^{el}$, where $\delta G^{el} \approx 163 \log(I)$ cal/mol per base pair at room temperature.

Values of δG^{el}, δH^{el}, and $T\delta S^{el}$ calculated in this way are plotted in Fig. 3 *B* and may be compared with typical experimental data (Gruenwedel, 1974) in Fig. 3 *A*. The experimental values were obtained by subtracting from the total thermodynamic quantities ΔG, ΔH, and $T\Delta S$ their values at $I = 1$ M, a procedure that yields the salt-dependent part of the thermodynamic parameters. It is evident that the calculated and experimental values of δG^{el} are in close agreement. However, the experimental dependence of ΔH on salt is much stronger than that calculated, while the salt dependence of $T\Delta S$ even has a slope of opposite sign.

This discrepancy becomes even more pronounced when the data are replotted as a function of melting temperature, as is done in Fig. 4, using Eq. 21 with $T^0 = 377.5$ K. The root of the discrepancy is made clear by substituting $p = \nu - 1$ into Eq. 16. If $\Delta C_p = 0$, the purely polyelectrolyte components of the slopes in Fig. 4 would be 10 cal/mol K for δH^{el} and -15 cal/mol K for $T\delta S^{el}$, while the experimental values are 65.2 and 40.4 cal/mol K, respectively. Clearly, the difference is due to a heat capacity change of nonpolyelectrolyte origin of substantial magnitude, ~55 cal/mol K. Analyzing other data in a similar way (Table 1) similarly yields nonelectrostatic contributions to ΔC_p of 30–100 cal/

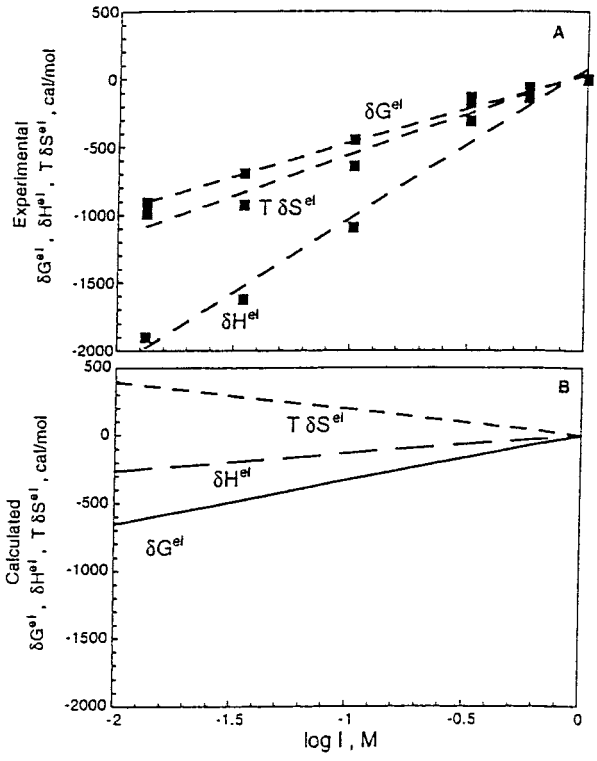

FIGURE 3 Electrostatic contribution to thermodynamic parameters of DNA melting as a function of ionic strength, I. (*A*) Experimental (–■–) points (Gruenwedel, 1974) were obtained as a difference between the measured values of thermodynamic parameters at the given salt and at $I = 1$ M. (*B*) Theoretical (——) dependencies are calculated as described in the text with $\Delta C_p = 55$ cal/mol K. Note that the polyelectrolyte theory predicts a much weaker $\delta H(T_m)$ dependence and the wrong sign of the slope of $\delta S(T_m)$ dependence as compared to the experimental behavior of these quantities.

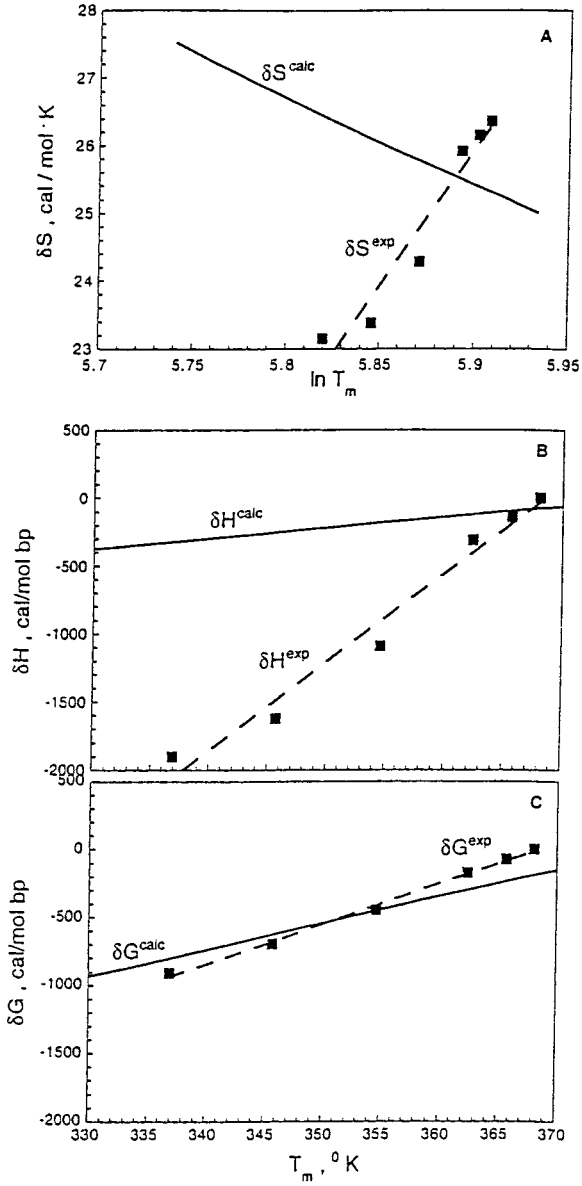

FIGURE 4 Electrostatic contribution to thermodynamic parameters of DNA melting as a function of T_m. These are the same data as in Fig. 3, but replotted using the relationship between T_m and log(I) given by Eqs. 3–9 with T^0 = 377.5 K. Note the inability of polyelectrolyte theory to reproduce the experimental behavior of the enthalpic and entropic components of the transition free energy.

mol K. Thus there is clear evidence from ionic strength variation experiments of a significant increase in the heat capacity of DNA upon melting.

Using Eqs. 23 and 24, we find that the general polyelectrolyte contribution ΔC_p is

$$\Delta C_p^{el} = \frac{\partial \delta H^{el}}{\partial T} = \frac{\partial \delta S^{el}}{\partial \ln T} = (\nu - 1)(2 - \nu)\frac{\delta G^{el}}{T}. \quad (25)$$

With the parameters for aqueous DNA solutions given above, this yields ΔC_p^{el} = 0.55 cal/mol K, which is small compared to ΔS^0 even in low salt, so that the polyelectrolyte contribution to the heat capacity cannot have a significant effect on the thermodynamics of DNA melting.

BASE COMPOSITION DEPENDENCE OF DNA MELTING

Our discussion of heat capacity effects on DNA melting can be used to gain insight into the base composition dependence of T_m. It is well known that GC-rich DNA melts at a higher temperature than AT-rich DNA, because GC base pairs have three hydrogen bonds, while AT base pairs have only two. It is less well known, and not well understood, that this difference in stability becomes smaller with increasing ionic strength.

An early survey led to the empirical equation for the dependence of T_m on mole fraction X_{GC} and ionic strength I (Frank-Kamenetskii, 1971),

$$T_m = 355.4 + 36.0X_{GC} + (18.3 - 7.04X_{GC})\log(I), \quad (26)$$

and a more recent summary (Blake and Delcourt, 1998) gave an only moderately different equation

$$T_m = 360.31 + 34.47X_{GC} + (20.15 - 6.52X_{GC})\log(I). \quad (27)$$

From Eq. 27 we calculate, for example, that the difference in T_m between d(GC) and pure d(AT) is 65°C in 10^{-5} M salt, but only 35°C in 1 M salt.

If we try to understand this result using the simple polyelectrolyte result (Eq. 21), we must conclude that either the change in the number of counterions bound, Δn, or the intrinsic entropy of melting, ΔS^0, is different for AT and GC base pairs. However, neither of these seems a very satisfactory explanation.

The assumption that Δn differs for different base pairs is commonly made (Blake and Delcourt, 1998; Santalucia, 1998) and is interpreted as a difference in linear charge density of the various bases in the melted form (see Eq. 20). This interpretation has even been extended to obtain information on the structure of the single-stranded DNA as a function of composition (Korolev et al., 1994). However, the polyelectrolyte effect reflects only the charge spacing averaged over a distance greater than the Debye length, which can include tens or hundreds of base pairs at low salt. There is no reason to expect that the charge spacing will be a simple arithmetic average of the spacings for different types of base pairs, because the secondary structure of single-stranded DNA, if any, should have some cooperativity. Attempts to encompass the fine-grained structure of single-stranded DNA are beyond the scope of mean-field polyelectrolyte theory.

The assumption that ΔS^0 differs significantly among base pairs also is problematic. The basic physical assumption that the two kinds of base pairs differ primarily in their binding enthalpy, not their entropy, is supported by a large body of experimental data obtained primarily on polymeric DNA (Blake and Delcourt, 1998; Grosberg and Khokhlov,

1994). On the other hand, some thermodynamic data, obtained primarily on oligomeric DNA (see Santalucia, 1998, and references therein), showed significant variations in the entropies of melting from one nearest-neighbor base pair to another. Differences between the oligomeric and polymeric DNA melting parameters will be discussed subsequently. Here we show that the intrinsic entropies of melting that enter Eq. 21 are the same for AT and GC pairs, but their thermal contributions, $\Delta C_p(T_m - T^0)$, can be different, because of the strong dependence of T_m on the type of base pair. It is the heat capacity change that is responsible for the behavior summarized in Eqs. 26 and 27.

Equation 21 for T_m is equivalent to Eq. 11, which neglects the heat capacity contribution contained in the more complete Eq. 10. This zeroth-order approximation is sufficient for the estimate of the average salt dependence, but it is inadequate when finer details, such as the composition dependence of the slope, are considered. Taking into account the complete expression, we obtain

$$\frac{\partial T_m}{\partial \log I} = 2.3\,\frac{RT^0\Delta n}{\Delta S^0}\,\frac{\partial t_m}{\partial \gamma_S}, \tag{28}$$

where we assumed that the polyelectrolyte perturbation is primarily entropic, i.e.,

$$\gamma_S = 2.3\,\frac{R\Delta n}{\Delta S^0}\log(I). \tag{29}$$

According to Eqs. 10 and 11,

$$\begin{aligned}\frac{\partial t_m}{\partial \gamma_S} &= \frac{\partial t_m^0}{\partial \gamma_S}\left(1 - \frac{\alpha}{1+\gamma_S}\,t_m^0\right) \\ &= -\frac{1+\gamma_H}{(1+\gamma_S)^2}\left[1 - \alpha\,\frac{\gamma_H - \gamma_S}{(1+\gamma_S)^2}\right].\end{aligned} \tag{30}$$

In the case $\gamma_S \ll 1$, appropriate for the whole experimental range of interest, this derivative is

$$\left(\frac{\partial t_m}{\partial \gamma_S}\right)_{\gamma_S \to 0} = -(1+\gamma_H)(1-\alpha\gamma_H) \approx -1 + \gamma_H(\alpha - 1), \tag{31}$$

where we have neglected the term $\alpha\gamma_H^2$ because of the smallness of γ_H. The right-hand side of Eq. 31 should be compared to its value $-1 - \gamma_H$ for the case $\alpha = 0$. We see that, although the magnitude of the slope remains near unity, the heat capacity effect when $\alpha > 1$ changes the sign of the variation of $\partial t_m/\partial \gamma_S$ with DNA composition.

We can cast our results in the form of Eqs. 26 and 27 by assuming that the enthalpic perturbation is linear in X_{GC}:

$$\gamma_H = \gamma_H^0(X_{GC} - 1). \tag{32}$$

Substituting this into Eq. 11 and evaluating at $I = 1$ M where $\gamma_S \approx 0$, we obtain

$$T_m(X_{GC}, I = 1\text{ M}) = T^0[1 + \gamma_H^0(X_{GC} - 1)]. \tag{33}$$

Comparing this with Eq. 26 gives $T^0 = 391.4$ K and $\gamma_H^0 = 0.092$, while comparison with Eq. 27 gives $T^0 = 394.8$ K and $\gamma_H^0 = 0.087$. These values for polymeric DNA are strikingly similar to those obtained by analyzing extensive data on oligomers (Santalucia, 1998) ($T^0 = 397.5$ K and $\gamma_H^0 = 0.123$), a strong confirmation of the basic correctness of the approach. Furthermore, all three estimates of T^0 are very close to the value of 397 K obtained from the fit to the completely different data analyzed by Petrushka and Goodman (1995).

The result $\gamma_H \approx 0.1$ is important both because it confirms the internal consistency of our treatment and because it enables an estimate of the real "chemical" enthalpy difference between GC and AT base pairs: $\delta H = \Delta H^0\gamma_H^0 = 9.93 \times 0.1 \approx 1$ kcal/mol. This value is considerably smaller than the energy of a single hydrogen bond. It reflects the fact that the hydrogen bond is not completely lost upon melting, but rather is replaced by a weaker bond with water.

Combining Eqs. 28, 31, and 33, we predict the slope:

$$\frac{\partial T_m}{\partial \log(I)} = 2.3\,\frac{RT^0\Delta n}{\Delta S^0}\,[1 - \gamma_H^0(X_{GC} - 1)(\alpha - 1)], \tag{34}$$

which can be compared to experiment. Using the already determined values of the parameters γ_H^0, T^0, and ΔS^0, we estimate $\Delta n = 0.36$ and $\alpha = 4.02$ from Eq. 26, and $\Delta n = 0.37$ and $\alpha = 3.81$ from Eq. 27. Both estimates for Δn are comparable to, but somewhat larger than, the simplest polyelectrolyte value 0.24. The fitted value of $\alpha \approx 3$–4 agrees very well with the direct calorimetric measurement $\alpha \approx 2$–4. This is convincing evidence that the stronger salt dependence of the melting temperature of AT sequences relative to GC is due to the heat capacity-induced increase of the transition entropy.

The lesson to be drawn from this result extends beyond the context in which it was derived. While T_m itself is only weakly perturbed by the heat capacity correction, its derivative with respect to salt as a function of DNA composition is determined by $\alpha > 1$. This should also be the case for any two parameters that affect T_m, e.g., the salt dependence of T_m as a function of cosolvent concentration or concentration of some ligand. The general relationships of this section, especially Eq. 30, allow one to calculate the derivative of T_m with respect to any parameter, given the dependence of the perturbation free energy components γ_H and γ_S on that parameter.

RECONCILING VAN'T HOFF AND CALORIMETRIC ENTHALPIES

Enthalpies of reaction may be measured by calorimetry, which monitors the heat of the reaction directly, or by van't Hoff analysis of the temperature dependence of the free energy of the reaction according to the general expression

$$\Delta H_{vH} = \frac{\partial(\Delta G/T)}{\partial(1/T)}. \tag{35}$$

The van't Hoff method is convenient and can be implemented in many different ways. For DNA melting, any experiment that monitors the fraction of melted base pairs as a function of temperature is sufficient to obtain ΔH_{vH}.

However, when ΔH_{vH} values from such analyses are compared to the calorimetric values ΔH_{cal}, they often disagree with each other. This has been conventionally attributed to the different experimental conditions used in such studies. The development of highly precise microcalorimeters has made it possible to perform both calorimetric and van't Hoff measurements within a single experiment (Liu and Sturtevant, 1995, 1997; Naghibi et al., 1995). When the authors of these studies performed direct comparisons of the ΔH_{vH} and ΔH_{cal} values for a large number of different reactions, they found that the two quantities never agreed and that the discrepancies between them often approached 100%. No reasonable interpretation of this observation was suggested, except for "unaccounted participants in the reaction."

Our analysis in terms of heat capacity effects, which contribute substantially to ΔH and ΔS individually but largely cancel out in ΔG, gives insight into this discrepancy. If the precise T dependence of ΔG is known, then the derivative, Eq. 35, should yield a temperature-dependent enthalpy similar to the calorimetric value. But in practice, any appreciable experimental noise in ΔG significantly obscures the weak curvature of its T dependence and leads to an incorrect apparent ΔH_{vH}. The relative error in this quantity should be on the order of αt_m, which can easily reach ~50% because of the large $\alpha = 2–4$. The same conclusion was reached in a recent Monte Carlo study (Chaires, 1997).

In fact, reasonable temperature-dependent enthalpies can be obtained by van't Hoff analysis. A good example is a study of melting of short DNA oligomers (Petersheim and Turner, 1983), which depends strongly on the concentration of single strands in solution, C_t. An extra $-RT \ln(C_t)$ of binding entropy per mole of oligomer results in the variation of melting temperature with C_t:

$$\frac{1}{T_m} = -\frac{R \ln(C_t)}{\Delta H} + \frac{\Delta S}{\Delta H}. \tag{36}$$

The slope of a plot of $1/T_m$ versus $\ln(C_t)$ yields ΔH_{vH} of melting per oligomer.

A complementary way to measure the same quantity is to perform traditional van't Hoff analysis of the DNA optical melting curves. This procedure, performed at several values of C_t, yields ΔH and ΔS as a function of C_t. When these measured ΔH and ΔS values are plotted as a function of T_m rather than C_t, the strong temperature dependence of both quantities becomes apparent. The slopes $\partial \Delta H/\partial T_m$ and $\partial \Delta S/\partial \ln T_m$ are similar to each other and close to the calorimetrically measured ΔC_p, which ranges between 52 and 95 cal/mol K in different oligomers. This is in close agreement with the ΔC_p values obtained in studies of polymeric DNA melting as a function of solution ionic strength (Table 1).

Despite this clear evidence of significant heat capacity effects, temperature-dependent ΔH and ΔS values are conventionally averaged and compared with the corresponding quantities obtained by data fitting to Eq. 36. These two determinations of average ΔH and ΔS are generally in good agreement, providing proof of the ability of the van't Hoff analysis to capture the thermal variation of ΔH and ΔS in a consistent way. Ironically, this is a standard way to produce ΔH and ΔS values, which are then reported as temperature-independent parameters (Breslauer et al., 1986; Krug et al., 1976; Owczarzy et al., 1997; Plum et al., 1995; Santalucia, 1998).

DEFINING THE STANDARD STATE FOR DNA MELTING

We have seen that DNA melting enthalpy and entropy are strongly influenced by solvent, DNA composition, and temperature. To be able to compare data from different studies, and to come to definite conclusions about the influence of a particular parameter, we need to define the standard state. At first it might seem that it could be chosen arbitrarily, e.g., as some particular set of conditions for which the most data are available. Then the enthalpy and entropy components will be arbitrarily split into the standard parts ΔH^0 and ΔS^0, and the perturbations δH and δS.

However, the presence of the heat capacity change ΔC_p introduces a temperature dependence into both components. If the T dependencies were linear, then shifting the reference temperature T^0 would simply result in redefining the standard values ΔH^0 and ΔS^0. But because of the different functional dependences of ΔH and ΔS on T_m (Eqs. 2 and 3), this is not true. Instead, changing the reference temperature by the relative amount Δt results in a relative variation of ΔS^0 on the order $\sim \alpha t \Delta t$, which depends on temperature and can be significant for $\alpha > 1$, $t \approx 1$, $\Delta t \approx 1$.

Strictly speaking, thermal contributions to ΔH and ΔS are both zero only at a single temperature T^0, which is the true standard temperature for melting. This hypothetical temperature can be determined from experimental data for ΔH and ΔS if these quantities are available over a wide range of stabilities, so that the nonlinearity of ΔS with T_m becomes apparent. This is the case for the data analyzed by Petrushka and Goodman (1995), who found strong nonlinear correlations between experimental values of ΔH and ΔS for regular base-pair doublets and a number of mismatched and modified base pairs. Some of these noncanonical base-pair doublets had very low or even negative stabilities; i.e., they decreased the stability of the normal oligomer. This provided the required broad range of stabilities of individual base pairs.

The authors fitted ΔS versus ΔH data at $I = 1$ M to the phenomenological expression

$$\Delta S = \frac{\Delta H}{T^* + \Delta H/\beta'}, \tag{37}$$

with the constants $T^* = 273$ K and $\beta = 80$ cal/mol K. (We have changed the original notation of Petrushka and Goodman (1995).) No physical interpretation of this equation was given, but it was noted that Eq. 37 is equivalent to the temperature dependence of the enthalpy,

$$\Delta H = \beta(T_m - T^*). \tag{38}$$

This is equivalent to the pair of equations

$$\begin{aligned} \Delta H &= \Delta H^0 + (\Delta S^0 + \Delta C_p)(T_m - T^0), \\ \Delta S &= \Delta S^0 + \Delta C_p \ln(T_m/T^0) \end{aligned} \tag{39}$$

if

$$\beta = \Delta S^0 + \Delta C_p \quad \text{and} \quad T^0 = T^* \frac{\Delta S^0 + \Delta C_p}{\Delta C_p}. \tag{40}$$

Taking the standard value $\Delta S^0 = 25$ cal/mol K, we find

$$\begin{aligned} &\Delta C_p = 55 \text{ cal/mol K}, \; T^0 = 397 \text{ K}, \\ &\Delta H^0 = \Delta S^0/T^0 = 9927 \text{ cal/mol}, \end{aligned} \tag{41}$$

in good agreement with other fits of these parameters in previous sections. The fit with parameters of Eq. 41 in Fig. 5 is at least as good as the original empirical fit (Petrushka and Goodman, 1995), and is obviously different from a simple linear dependence in the region of maximum stability.

It is worth noting that individual fits of ΔS versus T_m and ΔH versus T_m according to Eq. 39 are not nearly as good as for the ΔH versus ΔS plot. This reflects contributions to ΔH and ΔS for different nearest-neighbor base pairs that are not correlated with the nearest-neighbor doublet identity, but are induced by random solution variations. Nevertheless, because the contributions are always of thermal origin, they are always coupled through ΔC_p, as in Eq. 39.

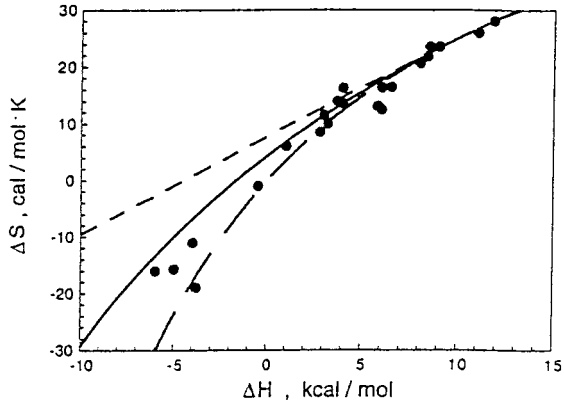

FIGURE 5 Entropy-enthalpy correlation in DNA melting transition. ●, Experimental data (Petrushka and Goodman, 1995) for 10 standard and several modified NN doublets. - - - -, Linear approximation from the high-stability region; ——, Empirical fit by the authors Petrushka and Goodman (1995); – – –, Our fit, assuming a temperature-independent heat capacity increase $\Delta C_p = 55$ cal/mol K and standard values $\Delta S^0 = 25$ cal/mol K and $T^0 = 397$ K.

We will not speculate here on the physical origin of T^0. We simply note that this is a very high temperature, above the boiling point of water and thus not normally accessible, which is needed as a parameter for the analysis of experimental data in the regular temperature range. Its formal meaning is that temperature at which the thermal contributions to ΔH and ΔS vanish.

Except for the "true" standard temperature T^0, the other conditions for the standard state can be chosen at our convenience. Setting the standard $T_m = T^0$ gets rid of thermal components and fixes the ratio $T^0 = (\Delta H^0 + \delta H)/(\Delta S^0 + \delta S)$. Then, choosing $\delta S = 0$, for example, for the case of polymeric DNA in 1 M salt, with $\Delta S^0 = 25$ cal/mol K, fixes the value of $\Delta H^0 = 9927$ cal/mol, which is characteristic of the most stable GC base pair. Therefore the standard state we have chosen corresponds to polymeric d(GC) in 1 M aqueous salt solution.

SUMMARY AND CONCLUSIONS

Analysis of a large body of experimental data suggests that there is a significant heat capacity increase, $\Delta C_p \approx 30$–100 cal/mol K per base pair associated with DNA melting. Being larger than the standard entropy of DNA melting $\Delta S^0 = 25$ cal/mol K per base pair, ΔC_p dominates changes in transition enthalpy and entropy induced by any variation in solution conditions. The heat capacity effect on the transition free energy and melting temperature is less pronounced, but it is responsible for a number of subtle phenomena. In particular, the heat capacity change determines the variation of T_m with one parameter (e.g., solution ionic strength) as a function of another (e.g., DNA composition). This accounts for the stronger stabilization by salt of AT relative to GC base pairs.

Any heat capacity effects on DNA melting thermodynamics can be analyzed with the help of the general expressions in Eqs. 1–8. The new feature we have introduced is an explicit account of the perturbation enthalpy δH and entropy δS, which allows direct linking between T_m, external parameters, and ΔC_p.

We have discussed practical aspects of the determination of ΔC_p from experimental data and have shown that the experimental slopes $\partial\Delta H/\partial T_m$ and $\partial\Delta S/\partial \ln T_m$ always contain both entropic and heat capacity contributions and differ from each other by the value of ΔS. Appreciation of the strong temperature dependence of ΔH and ΔS helps to interpret the apparent differences in enthalpy values obtained in van't Hoff and calorimetric experiments. Even though ΔH and ΔS are often statistically coupled (Krug et al., 1976; Owczarzy et al., 1997; Plum et al., 1995), because ΔS is determined as $\Delta H/T_m$, T_m is measured independently, and so the coupling should indeed reflect the coupled thermal contributions to both quantities, which cancel out in T_m.

In the particular case of the effect of salt on double-helix stability, we have used polyelectrolyte theory to analytically calculate the enthalpy, entropy, and heat capacity changes in

the course of DNA melting. We have shown that the enthalpic part of the polyelectrolyte free energy of the transition comes from the peculiar temperature behavior of the dielectric constant of water. The calculated polyelectrolyte heat capacity change is too small to account for the experimental temperature dependence of ΔH and ΔS of DNA melting.

In the accompanying paper (Rouzina and Bloomfield, 1999), we apply this general treatment to a critical analysis of the melting thermodynamics of oligonucleotides of defined sequence, where we show that it resolves a number of apparent inconsistencies between data from various sources.

After this paper was submitted for publication, a paper by Breslauer and co-workers (Chalikian et al., 1999) appeared that provides remarkable confirmation of the ideas proposed here. By direct, high-precision calorimetric measurements of helix-coil transitions of five polymeric duplexes, they found positive heat capacity changes with an average value $\Delta C_p = 64.6 \pm 21.4$ cal/deg-mol. This is in striking agreement with the values reported in this paper.

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