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Enthalpies of DNA melting in the presence of osmolytes

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Abstract

The melting of DNA in the presence of osmolytes has been studied with the intention of obtaining information about how base pair stability is affected by changes in solution conditions. In previous investigations, the melting enthalpies were assumed to be constant as osmolalities change, but no systematic evaluation of whether this condition is true has been offered. This paper presents calorimetric data on the melting of two synthetic DNA samples in the presence of a number of common osmolytes. Poly(dAdT)·poly(dTdA) and poly(dGdC)·poly(dCdG) melting have been examined by differential scanning calorimetry in solutions containing ethylene glycol, glycerol, sucrose, urea, betaine, PEG 200 and PEG 1450 at increasing osmolalities. The results show small, but significant changes in the enthalpy of melting of the two polynucleotides that are different, depending on the structure of the cosolvent. The polyols, ethylene glycol, glycerol, PEG 200 and also urea all show decreases in melting enthalpy, while betaine and sucrose display increases with increasing concentration of cosolvent. The large stabilizing PEG 1450 shows no change within the experimental errors. Using concepts relating to preferential interactions of the cosolvents with the DNA base pairs, it is possible to interpret some of the observed changes in the thermodynamic properties of melting. The results indicate that there is strong entropy–enthalpy compensation upon melting base pairs, but entropy increases dominate to cause the decreases in stability with increased cosolvent concentration. Excess hydration parameters are evaluated and their magnitudes discussed in terms of changes in cosolvent interactions with the DNA base pairs.

1. Introduction

The stability of DNA duplex in solution is influenced by the presence of molecules and ions in the surrounding medium. Since these ions and molecules can mediate processes in which DNA is involved in the cell, considerable attention has been given to understanding the thermodynamics of nonspecific interactions between solution additives and DNA. Of particular importance is the competition between water and small cosolvent components, such as common osmolytes or ions, for the space around the duplex or single strand state of the DNA in which influence is exerted on the properties of the macromolecule. Studies of preferential interactions of the cosolvent and water with DNA and proteins have revealed a variety of effects on stability depending on the structure and charge of the additives [1–11]. In addition, the size of the cosolvent can have a strong influence on the stability of duplex and triplex forms of DNA and a variety of proteins through excluded volume effects [12–16].

A number of studies have focused on the evaluation of preferential interactions involving DNA. Felitsky et al. and Hong et al. [6,7] have used direct measurement of osmotic properties of cosolvent solutions in the presence and absence of DNA to determine preferential interaction parameters. The measurement of the effects of additives on the melting transition temperature of DNA duplex provides data for the net effect of transformation from duplex to single strand and thus gives directly the influence of cosolvents on stability [13,15–20]. DNA condensation has been demonstrated in the presence of osmotic stressors using X-ray diffraction methods, and the results interpreted in terms of hydration changes resulting from the presence of the cosolvents [21]. Changes in the hydration parameters of DNA during drug binding have been investigated by measuring the binding constants in the presence of cosolvents [22–25]. Several studies

Abbreviations: Poly(dAdT), poly(dAdT)·poly(dTdA); Poly(dGdC), poly(dGdC)·poly(dCdG); PEG, polyethylene glycol; DSC, differential scanning calorimetry.

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have addressed hydration effects associated with the binding of proteins to duplex DNA [26–28].

The analysis of data in these studies has relied on examination of how the free energy of melting or a specific association process involving DNA changes with cosolvent concentration or water activity as the medium changes [2–4]. Different specific approaches have been used that are variations on the preferential interactions theme, but are tied together with the common problem that is a consequence of the Gibbs–Duhem relationship, i.e., that changes in the chemical potentials of reactants and products in a medium which is changing as a consequence of cosolvent concentration being altered is also changing water activity with cosolvent composition [5]. Thus, the effects of water activity changes and cosolvent concentration are linked in any consequence they may have on macromolecular behavior. Whether one chooses to examine cosolvent preferential interactions with DNA or hydration effects, the final result is that the thermodynamic parameters obtained contain contributions from both terms. In addition, in the most general approach contributions relating to the size of the cosolvent should be considered, since there are experimentally observed consequences of excluded volume in the behavior of macromolecules [13–15]. Recently, new analytical approaches have been reported that address the issue of size [9–11].

Conscious of the above complications relating to analysis of results of experimental observation on DNA stability in mixed solvents, we have chosen to examine one other issue for which there is little information available. In studies of DNA melting, it has been assumed that the enthalpy of melting of the DNA duplex is the same in cosolvent solutions as in cosolvent-free buffers [13,17]. Given that there are likely to be not only changes in the state of hydration of both duplex and single strand forms, but also cosolvent interactions that could influence either or both states of the DNA, and thus the enthalpy of melting of the duplex, it seemed worthwhile to measure the enthalpies of melting in solutions typically used in studies of the influence of cosolvents on hydration and preferential interactions. To this end, we have measured the enthalpies of melting of the synthetic DNAs, poly (dAdT)·poly(dTdA) and poly(dGdC)·poly(dCdG), in solutions of varying osmolality containing the following list of cosolvents: ethylene glycol, glycerol, urea, betaine, sucrose, PEG 200 and PEG 1450. The measurements are done by scanning calorimetric measurement of the thermal transitions so that transition temperatures as well as enthalpies can be obtained directly from the transition curves. Osmotic pressures of the solutions are measured, which provide data for correlation of the changing water activity with melting temperature and enthalpy. The data are analyzed by examination of hydration parameters derived from the osmotic changes in the solutions as cosolvent composition changes.

2. Experimental procedures and data analysis

2.1. Materials

The poly(dAdT)·poly(dTdA) and poly(dGdC)·poly(dCdG) were obtained from Amersham Biosciences, Piscataway, NJ, and were used without purification. All cosolvents were from Sigma-Aldrich Chemical Co., St. Louis, MO, and were of the highest grade possible. Buffer components were Reagent Grade and also from Sigma-Aldrich.

2.2. Solution preparations

Solutions of DNA duplex were made up in BPE buffer containing 6 mM disodium phosphate, 2 mM monosodium phosphate, 1 mM disodium ethylenediaminetetraacetic acid, at pH = 7.0. Cosolvent solutions were prepared in BPE buffer at concentrations in the range of 4–16% wt/vol cosolvent. Because the melting temperatures for the two alternating polynucleotides are widely separated (43 and 97 °C) and are quite sharp, calorimetric measurements were made on mixtures of the two. Solutions for the melting experiments were prepared by mixing 0.25 ml each of about 1 mM nucleotide of poly (dAdT)poly(dTdA) and poly(dGdC)poly(dCdG) in BPE. Concentrations of the solutions were determined from UV absorption at 262 nm, using molar absorptivities per nucleotide of 6600 and 8125 $l\ mol^{-1}\ cm^{-1}$ for p(dAdT) and p(dGdC), respectively. The DNA mixtures were then dialyzed against 500 ml of cosolvent solution for a minimum of 24 h in Pierce Slide-A-Lyzer dialysis cassettes of 0.5 ml capacity (Cole-Parmer, Inc., Vernon Hills, IL). After completion of the dialysis procedure, the osmolalities of the dialyzate and the sample solutions were measured and found to coincide to within a few percent, which is approximately the precision of the osmolality measurements with the Wescor Model 5520 Vapor Phase Osmometer (Wescor, Inc., Provo, UT) used in determining the osmolality of the solutions. Also, the final total concentrations of the sample solutions was determined by UV absorption at 262 nm and scaled where necessary from the original concentration ratios used to make up the dialysis solutions.

2.3. Calorimetric measurements and analysis

Differential scanning calorimetry measurements were carried out with the MicroCal Capillary DSC fitted with an auto sampler from MicroCal, Inc., Northampton, MA. Sample solutions were placed in the autosampler in a 96-well plate fitted with rubber covers. The capillary DSC requires about 0.4 ml of solution in the capillary in order to get the appropriate volume of sample (0.133 ml) in the thermal sensing area. The instrument was set up to do initially 6 water–water scans, followed by for each sample a scan of the cosolvent (dialyzate) in both sample and reference cells, and then a scan of the DNA sample in the corresponding cosolvent solution referenced against cosolvent. Control of the sampling and calorimetric scans was accomplished with the software provided by MicroCal.

Using the automated protocols described above, and with the instrument running 24 h/day, it was possible to measure almost 100 melting transitions for these DNA samples in less than a week's time.

After completion of the calorimetric scans, the entire data set was transferred to data treatment software for extraction of the transition information. First, the reference scans were subtracted from the DNA scans to get reference-corrected data (see Fig. 1A).

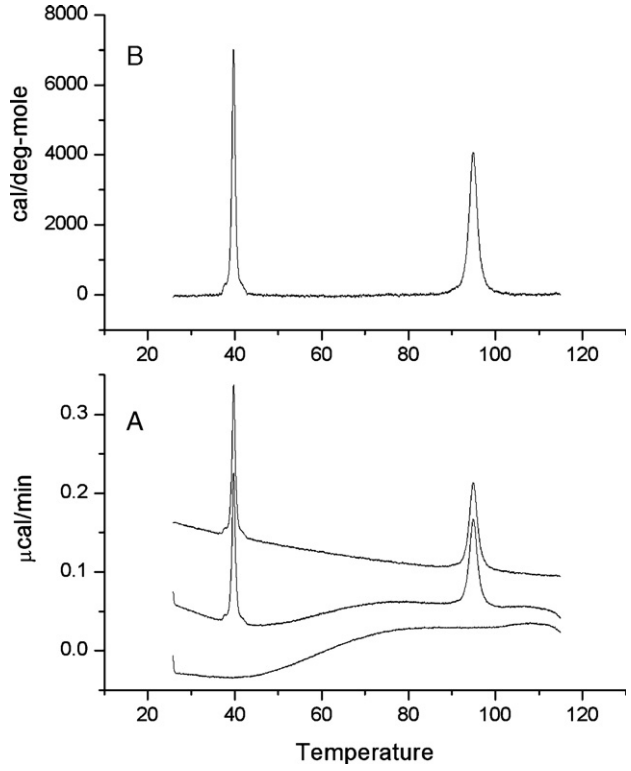

Fig. 1. (A) Raw DSC experimental data for 12% w/v glycerol. Lower curve is reference curve of cosolvent solution vs. cosolvent. Middle curve is the raw DNA sample scan, and the upper curve is the result of subtracting the reference from the DNA scan. (B) Final baseline corrected curve of the data in (A). The lower temperature peak is for p(dAdT) and the upper is p(dGdC).

Then, in order to normalize the data sets to zero baseline, a software generated baseline was subtracted from the reference-corrected scans, yielding transitions such as those shown in Fig. 1B. The individual transitions for p(dAdT) and p(dGdC) were then integrated to get areas and, from knowledge of the solution concentrations, the enthalpies of melting were determined. The T_m values were determined from the maxima in the excess heat capacity curves.

2.4. Data analysis

In order to analyze the effects that changes in the enthalpy of melting have on the analysis of hydration parameters for the DNA, the following formalism is used, based on the approach used by Parsegian et al. [5]. For the melting of the DNA duplex strands,

Duplex→2 single strands

The equilibrium constant for melting in the absence or presence of a cosolvent at a suitable reference temperature can be expressed, assuming no change in heat capacity for melting, in terms of the Gibbs–Helmholtz equation:

$$\ln K^{i}(T_r) = -\frac{\Delta H^{i}_m}{R}\left[\frac{1}{T_r}-\frac{1}{T^{i}_m}\right] \quad (1)$$

$K^o(T_r)$ is the equilibrium constant in aqueous buffer at the reference temperature and $K^c(T_r)$ would correspond to the case in the presence of cosolvent. Thus, the change in the equilibrium constant of melting when transferring from aqueous buffer to a cosolvent solution of a specific osmolality is:

$$\ln\frac{K^c(T_r)}{K^o(T_r)} = \left[\frac{\Delta H^c_m}{RT^c_m}-\frac{\Delta H^o_m}{RT^o_m}\right]-\frac{1}{RT_r}\left[\Delta H^c_m-\Delta H^o_m\right] \quad (2)$$

And the change in free energy of melting for transfer from buffer to cosolvent is:

$$\Delta\Delta G_m(T_r) = -RT_r\cdot\ln\left(\frac{K^c(T_r)}{K^o(T_r)}\right) \quad (3)$$

The reference temperature is often 25 °C, but for DNA melting experiments the reference is more often the melting temperature in buffer, since the assumption of zero heat capacity change can be problematic in extrapolating from the melting temperature to 25 °C. If T_r is the melting temperature in buffer in the absence of cosolvent, T^o_m, then Eq. (2) simplifies to:

$$\ln\left(\frac{K^c}{K^o}\right) = \frac{\Delta H^c_m}{R}\left[\frac{1}{T^c_m}-\frac{1}{T^o_m}\right] \quad (4)$$

Keep in mind that in this equation both ΔH^c_m and T^c_m can change with the cosolvent concentration.

In order to get information relating to the effects of water activity on the equilibrium constant, it is necessary to evaluate the change in the excess (or deficit) water associated with the reacting macromolecule. In this case, the DNA duplex is surrounded by an environment of water and cosolvent in a particular ratio, n_w/n_c, in the bulk solution. If we define N_w/N_c as the ratio in the immediate vicinity of the macromolecule, the excess (or deficit) water associated with the DNA is determined from the equation:

$$N_{ew} = N_w\left(1-\frac{n_w}{n_c}\cdot\frac{N_c}{N_w}\right) \quad (5)$$

A similar equation applies to the single strand, so that upon melting of the DNA there is a change in the excess number of water molecules associated with thermal denaturation of the duplex.

$$\Delta N^{DS}_{ew} = N^{S}_{ew}-N^{D}_{ew} \quad (6)$$

The change in the free energy of melting with respect to change in the chemical potential of water gives access to ΔN^{DS}_{ew} through a measurable osmotic property of the cosolvent solution, the osmolality (osm).

$$\frac{d\Delta G_m}{d\mu_w} = -\Delta N^{DS}_{ew} = -\frac{55.55}{RT_r}\frac{d\Delta G_m}{d(osm)} \quad (7)$$

Thus, a plot of free energy of melting in various cosolvent solutions vs. the osmolality of the solution provides a way to evaluate the change in excess water upon melting of the DNA strands. In practical terms, the desired results can be obtained from plots of $\ln(K^c/K^o)$ vs. osmolality, since

$$-55.55\frac{d\left(\ln\left(\frac{K^c}{K^o}\right)\right.}{d(osm)} = \Delta N^{DS}_{ew} \quad (8)$$

A useful way to look at ΔN_{ew} is by rearranging Eq. (5), and recognizing that n_w/n_c will be about the same for both duplex and single strand forms of the DNA. Then, one can write:

$$\Delta N_{ew}^{DS} = \Delta N_w - \frac{n_w}{n_c}\left[N_c^S - N_c^D\right] \quad (9)$$

There are several consequences that emerge from this correlation. First, if the cosolvent is excluded from the vicinity of the macromolecule in both states, then ΔN_w reflects the change in number of thermodynamically unique waters when the melting of DNA occurs. A more likely situation is that both ΔN_w and ΔN_c contribute some magnitude to the overall change in excess water, ΔN_{ew}. Since most small cosolvents destabilize DNA duplex, causing a lowering of the melting transition temperature, or an increase in K^c, then ΔN_{ew} must be negative for typical osmolytes. Negative values of ΔN_{ew} can arise from having ΔN_w be negative, but the second term on the right of Eq. (9) can make a negative contribution if there is preferential interaction of cosolvent that is greater in the single strand than in duplex DNA. We will examine the results of these studies in the above contexts.

3. Results

Fig. 1 shows typical results for the calorimetric scans, in this case for the melting of p(dAdT) and p(dGdC) in 12% wt/vol glycerol solution, starting with a raw scan, and showing the effects of applying the reference and baseline corrections to the experimental curves. Because the thermal transitions are narrow and cooperative, there is little difficulty in carrying out the necessary subtractions and calculations for the individual peaks. Fig. 2 shows the results for the solutions containing from 4% to 16% wt/vol glycerol. The data for the other cosolvent solutions were similar except for the p(dGdC) sample in urea solutions. For reasons that are not totally clear, there were sudden dramatic changes in the baseline when the melting point (near 98 °C) occurred. This behavior is not observed for the other cosolvents,

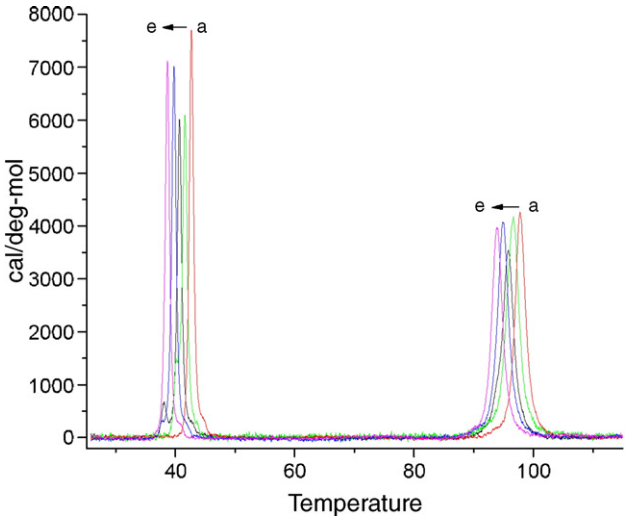

Fig. 2. DSC scans of glycerol solutions of p(dAdT) and p(dGdC). The data are in order a→e for BPE buffer, 4%, 8%, 12% and 16% w/v glycerol in BPE.

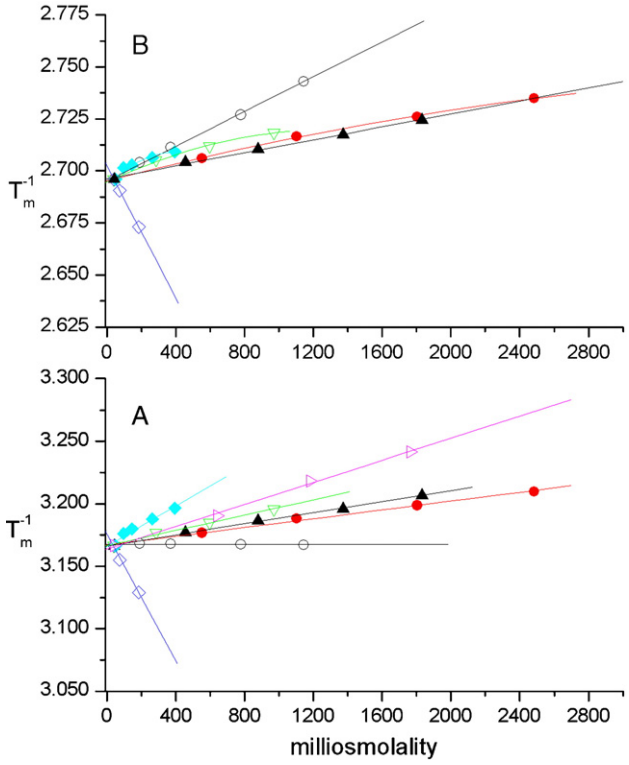

Fig. 3. Plots of inverse melting temperature for p(dAdT) (panel A) and p(dGdC) (panel B) vs. osmolality for various cosolvents. (closed circle) Ethylene glycol, (closed triangle) glycerol, (open inverted triangle) PEG 200, (open diamond) PEG 1450, (open circle) betaine, (closed diamond) sucrose, (right open triangle) urea.

so may be related to decomposition of the urea at high temperature, or because of a specific interaction of urea with the single strand form of the p(dGdC).

In order to show the transition temperature changes with cosolvent composition, Fig. 3 plots the inverse melting temperature vs. osmolality of the solutions. The lower panel shows the data for p(dAdT) and the upper for p(dGdC) samples. Most cases studied show positive slopes and thus indicate a destabilizing effect with increasing cosolvent osmolality on both alternating polynucleotides. The exceptions are for the larger PEG 1450 polymer, which gives a negative slope, and for betaine with p(dAdT) which shows virtually a zero slope, indicating no net effect on the melting transition. The majority of these plots are linear for the p(dAdT) cases, while several of the correlations show slight curvature for p(dGdC), particularly in sucrose and PEG 200 solutions. The curves in Fig. 3 are frequently used to evaluate ΔN_{ew} when it is assumed that there is no change in enthalpy of melting with solution composition [13]. It is clear from these plots that there are some differences in the slopes, meaning that the excess water value is influenced by cosolvent interactions as the transformation from duplex to single strand occurs. Table 1 contains values of ΔN_{ew} per base pair calculated from the limiting slopes of lines in Fig. 3, as well as those calculated from Eq. (8) described in Section 2.4 in which account is taken of variations of the enthalpy of melting, presented next.

The calorimetric enthalpies of melting are shown in Fig. 4 for the cases studied. Fig. 4A shows data for ethylene

Table 1
Values of ΔN_{ew} for melting of DNA

Cosolvent	Poly(dAdT) [a]		Poly(dGdC) [a]		V_c [b]
	$-\Delta N$	$-\Delta N_{(o)}$	$-\Delta N$	$-\Delta N_{(o)}$	
Eth glycol	5.0±0.2	4.2±0.2	7.2±0.4	5.3±0.3	55
Glycerol	5.9±0.3	5.3±0.2	6.0±0.5	5.2±0.2	69
Betaine	0±0.2	0±0.2	15.7±0.5	14.0±0.3	98
Sucrose	27±8	19±2	27±6	11±2	211
Urea	11±2	11±0.5	–	–	43
PEG 200	9.7±0.9	7.3±0.5	10.2±0.9	7.9±0.9	169
PEG 1450	−61±8	−60±6	−54±7	−50±6	1212

[a] Values of ΔN are excess water parameters determined using Eq. (8) and with enthalpy values varying. $\Delta N_{(o)}$ are values determined assuming enthalpy constant and using the value observed in BPE buffer. Uncertainties are based on calculated error in the limiting slope of each plot.

[b] V_c is the partial molar volume of the cosolvent in ml/mol.

glycol, glycerol, PEG 200 and urea for the two polynucleotides. (p(dGdC), upper, and p(dAdT), lower). For the AT copolymer, the curves all show slight decrease in melting enthalpy with increasing cosolvent content. For p(dGdC) melts, ethylene glycol and glycerol enthalpies show slight decreases, while for PEG 200 the values are essentially the same as in buffer. Fig. 4B shows the enthalpy data for betaine, sucrose and PEG 1450. Here there are substantial increases in the melting enthalpies for both sucrose and betaine, while the PEG 1450 data show little change from the buffer value. (There is rather large scatter in the p(dGdC) data.) We estimate precision of measurement, based on standard deviations of fits to enthalpy vs. osmolality plots, to be about ±6% for p(dGdC) and ±4% for p(dAdT).

Because the enthalpies do show some variation with osmolality, it is informative to calculate ΔN_{ew} using Eq. (8) to compare the excess water values with those obtained by assuming no change in enthalpy that are tabulated in Table 1. Figs. 5–7 are plots of $\ln(K^c/K^o)$ vs. osmolality from which ΔN_{ew} can be calculated, and the results are presented in Table 1. The curves in Figs. 5–7 are generally nonlinear; thus, the slope values for $\ln(K^c/K^o)$ vary slightly with osmolality. We adopt the strategy of using the slopes of the lines at zero osmolality based on fitting the curves to a second order polynomial. The solid curves are the fitted curves to the data using the polynomial equation, except for the betaine and PEG 1450 plots, which are considered linear.

Several trends emerge from the data in Table 1. First, there is generally a difference between the hydration parameters calculated with the enthalpies varying as observed experimentally, compared with those for which the enthalpies were considered constant. An approximate 20% larger value in ΔN_{ew} is obtained in most cases when using the exact enthalpy for each cosolvent solution. The exceptions to this trend are the parameters for sucrose for which there are rather significantly smaller values when assuming enthalpy constant in the calculations. Another observation showing up in the data in Table 1 is that the nucleotide sequence, whether dAdT or dGdC, does not seem to make large differences in the values of the hydration parameter. A glaring exception is the case for betaine, for which the ΔN_{ew} is zero for the dAdT sequence, while the value is near −16 for p(dGdC). For the other cosolvent solutions, the differences are within the

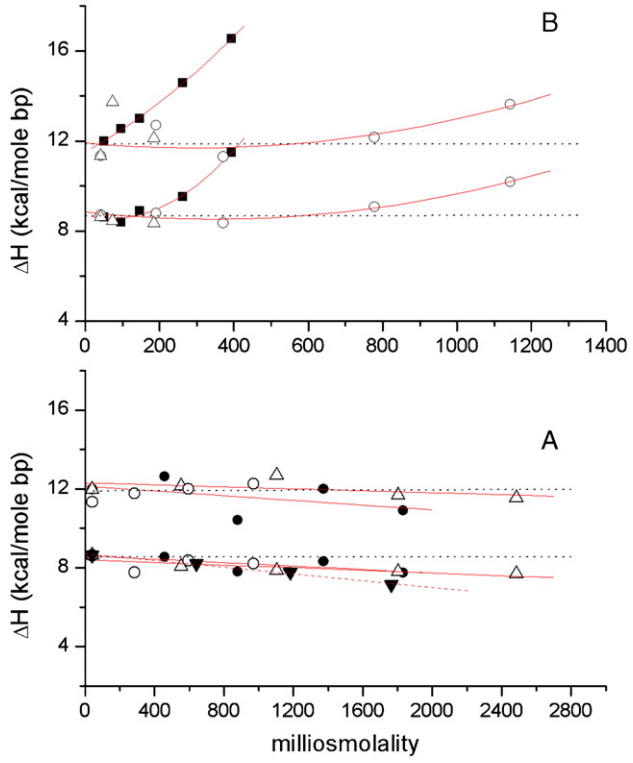

Fig. 4. Enthalpies of melting of p(dAdT) and p(dGdC) vs. osmolality for various cosolvents. (Panel A) Ethylene glycol (open triangle), glycerol (closed circle), PEG 200 (open circle) and urea (inverted closed triangle). (Panel B) Betaine (open circles), sucrose (closed squares) and PEG 1450 (open triangles).

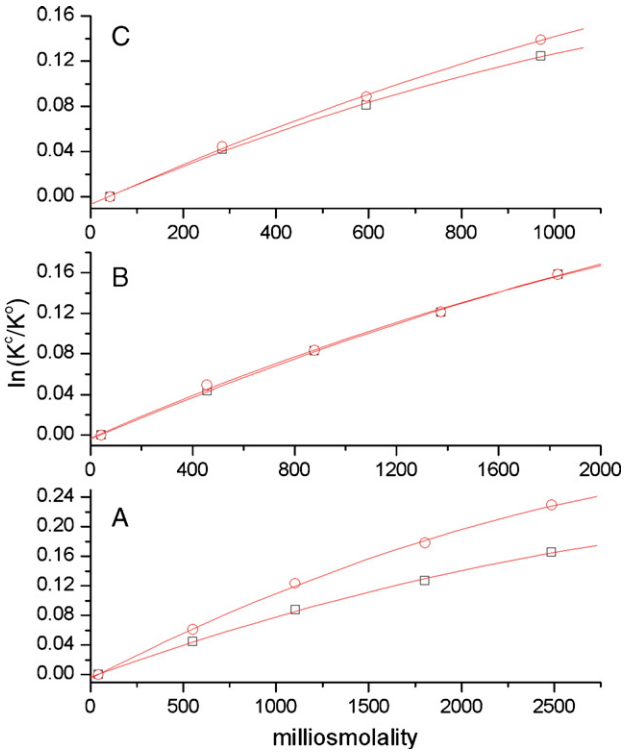

Fig. 5. Plots of $\ln(K^c/K^o)$ vs. osmolality for ethylene glycol (A), glycerol (B) and PEG 200 (C) solutions containing p(dAdT) (squares) and p(dGdC) (circles).

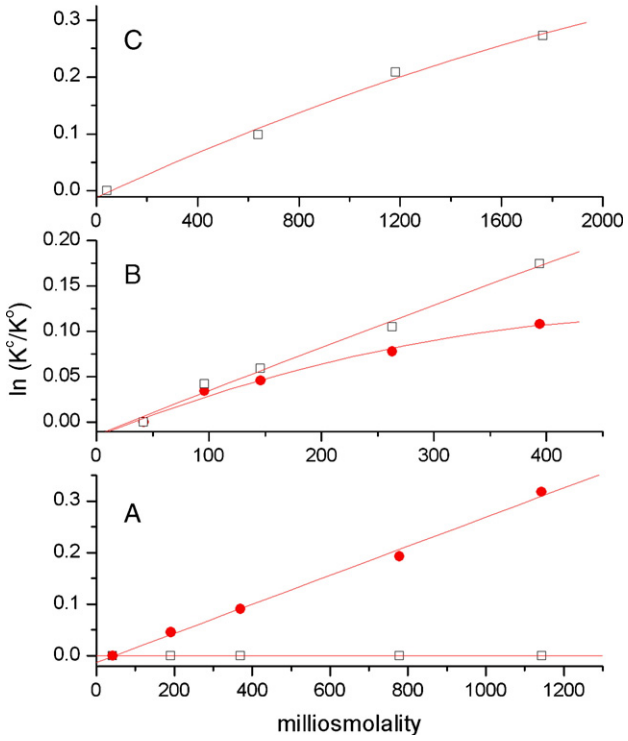

Fig. 6. Plots of $\ln(K^c/K^o)$ vs. osmolality for betaine (A), sucrose (B) and urea (C) solutions containing p(dAdT) (squares) and p(dGdC) (circles).

experimental error (with the possible exception of ethylene glycol). Other significant issues in the data relate to the chemical identity of the cosolvent and its size. The large polyethylene glycol homolog (PEG 1450) is definitely revealing the effects of large size with the hydration parameter being positive, and showing stabilization of the duplex rather than, as with the smaller cosolvents, causing lowering of the melting temperature. In the melting of the poly(dA)–poly(dT) duplex in the presence of various polyethylene glycol derivatives, it has been observed that, when the PEG molecular weights are above about 1000, there is stabilization of the duplex, while lower molecular weight compounds cause melting point lowering [13,33,35]. That trend seems evident here, since the PEG 200 is destabilizing while the PEG 1450 is stabilizing.

There are differences in ΔN_{ew} among the various small cosolvents that also have been observed for other processes involving DNA [3,5] The smaller polyols, such as ethylene glycol, glycerol or PEG 200, have hydration parameters between 5 and 10, while cosolvents, sucrose or betaine, show numbers in the range of 15–25. There is weak dependence on molecular volume in these data; however, as will be discussed below, we prefer to analyze these results in terms of specific interactions of cosolvent with the DNA.

4. Discussion

4.1. Origins of the enthalpy and stability variations

The source of the small contributions to the enthalpy of melting of DNA base pairs as cosolvent concentration changes can be interpreted as a result of recognized changes in solvent accessible surface area upon transition from duplex to single strand state. The best estimates of SASA upon melting of a DNA base pair yield increases of about 200 $Å^2$, or in terms of water sites about 20 water molecules [6,7]. This number represents a maximum change in the number of water molecules in the vicinity of the base pair, assuming complete exclusion of the cosolvent from the DNA surface and a single water layer around the newly exposed space. The realistic situation is that the net change is a result of both accumulation of cosolvent in the vicinity of the macromolecule and changes in associated water as cosolvent fraction increases. The sources of alterations in stability and the energetics of interactions then lie in part from these nonspecific environmental changes around the DNA in duplex and single strand forms [37,38]. In addition, there is the possibility that water molecules can occupy sites that are not on the surface, but are in the interstices and crevices of the macromolecule, particularly in the duplex form. These water molecules can be released upon melting of the duplex, thus causing the transition temperature to have additional sensitivity to changes in water activity as cosolvent concentration increases [4].

It is useful to look first at situations for which we have some knowledge about preferential interactions involving DNA melting. Felitsky et al. and Hong et al. have analyzed osmotic measurements on solutions of DNA containing betaine or urea in order to get information on preferential interactions [6,7]. Their results suggest that betaine is excluded from duplex DNA surface, so that any interaction that would alter the enthalpy of melting will likely result from changes in hydration and/or cosolvent contact in the single strand state. The data suggest that there is accumulation of betaine relative to bulk concentration near the single strand, which would mean less water than expected and more betaine. The enthalpy of melting in betaine solutions does not change much until the osmolality is near 0.75 osmolal, but then increases, more endothermic for both p(dAdT) and p(dGdC) (see Fig. 4). This behavior is interesting,

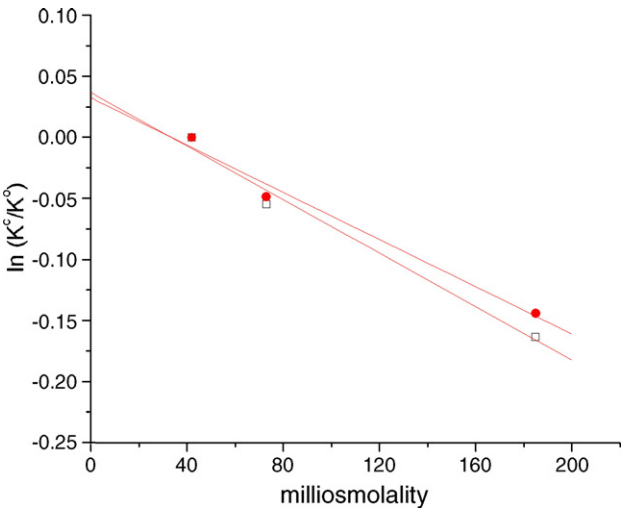

Fig. 7. Plots of $\ln(K^c/K^o)$ vs. osmolality for PEG 1450 solutions containing p(dAdT) (squares) and p(dGdC) (circles).

given that, although the melting temperature of p(dGdC) decreases with increasing betaine concentration, the p(dAdT) polynucleotide stability does not change. A constant melting temperature of the AT polymer is in agreement with the work of Rees et al. [18], who explain the isostabilization of various DNAs of different GC content by betaine and several tetraalkylammonium ions as due to association of the solute in the major groove of the AT duplex. The work of Hong et al. [7] suggests that there is slight exclusion of betaine from AT pairs in single strand and sizeable exclusion around the duplex. The enthalpy data here seem most consistent with the idea that there is some interaction with duplex p(dAdT) that is compensated by similar interactions in single strand, leading to no net change in melting temperature. At higher osmolalities, the duplex interaction begins to dominate slightly, producing endothermic contributions that increase melting enthalpy. Because stability is not affected much by these enthalpic increases, there must be corresponding entropy increases as the concentration of betaine changes. In the case of the p(dGdC) melting enthalpy, there again is an endothermic increase at higher betaine content, but in this case the stability of the duplex is reduced quite substantially per mole of added betaine. The nature of the interaction of betaine with GC base pairs seems to be different from AT pairs, producing a drop in stability, lowering the melting point. This behavior could imply that there is less interaction of betaine with GC pairs in duplex form, or more interaction in the single strand state. It appears that there is a rather delicate balance of interactions in the case of betaine that determine the overall behavior of DNA base pairs.

Preferential interactions in the case of urea and the polyols are somewhat easier to interpret. Urea shows little excess accumulation in duplex DNA and modest accumulation in the single strand state [7]. So, the decrease in enthalpy of melting in this case must be a result of an exothermic contribution as urea accumulates at the newly exposed surface in the single strand state. Urea is a known denaturant of DNA and proteins, and the results here show decreased stability as urea concentration increases. There is some evidence that urea can specifically interact with the polar groups of the exposed bases in single strand DNA [7].

The polyols, such as ethylene glycol, glycerol and PEG 200, all cause the enthalpy of melting to decrease slightly with increasing osmolality. Again newly exposed sites in single strand base pairs provide potential for accumulation of polyol, which can contribute exothermically to the enthalpy and to a decrease in stability. The sucrose data are quite distinctive from the other polyol cosolvents. There are significant *increases* in the melting enthalpy at low osmolalities, and the stabilities of both p(dGdC) and p(dAdT) duplexes are reduced as concentration of sucrose increases. In fact, the values of ΔN_{ew} for sucrose are the most negative in Table 1, meaning that per osmolal these solutions show the greatest destabilizing effect of all cosolvents studied. This effect on DNA thermal stability has been observed for other sugars [19,20], but on the other hand, sucrose is known to stabilize protein native structures [29–31]. The protein behavior has been interpreted as a result of increases in the surface tension of water in the presence of sucrose, so for protein unfolding the increased surface area for the denatured state leads to a shift to the native structure [30]. In the absence of information about specific preferential interactions of DNA with sucrose, we point out that the origins of the instability are a result not only of the large endothermic enthalpic contributions, but because there is a significantly larger entropic increase upon melting. Table 2 shows calculated values for the thermodynamics of melting of AT and GC base pairs in 0.5 osmolal cosolvent for the various cases studied. For most cosolvents, there are relatively small enthalpy changes from the value in buffer, and there is clear entropy–enthalpy compensation, so that the small stability differences caused by the various cosolvent media result from small increases in the entropy of melting. Sucrose shows somewhat larger enthalpic and entropic effects, suggesting that, if there is accumulation of the cosolvent in the duplex form, upon melting there is significant reorganization of both water and cosolvent to produce large entropic compensation.

Table 2 and Figs. 3 and 4 demonstrate also the striking differences with PEG 1450 as cosolvent, which shows a stabilizing of duplex state with increased concentration. This enhancement of stability by the larger PEG cosolvents has been observed both for proteins and DNA [13,29,33]. The interpretations given for these effects have generally focused on the large excluded volumes in solutions of PEG copolymers, which cause crowding in the mixtures, even at low osmolalities [35]. The crowding makes it more difficult for melting because of the larger covolumes of the single strand state of DNA base pairs. The enthalpies of melting are in this case larger than the entropies of melting and are significantly dominant. Melting in a crowded environment thus appears enthalpically determined in the case of DNA.

4.2. Origins of variations in ΔN_{ew} for DNA melting

The data presented in this paper indicate that melting of duplex DNA in the presence of typical osmolytes is dependent on both changes in water activity and cosolvent identity. The plots of $\ln(K^c/K^o)$ vs. osmolality in Figs. 5–7, indicate in some cases there is curvature in the correlations that, according to Eq. (9), must mean that either or both ΔN_w and ΔN_c are varying with composition in the vicinity of DNA when melting occurs. And the fact that ΔN_{ew} is variant from one cosolvent to another

Table 2
Thermodynamic data for the melting of DNA base pairs in various osmolytes (at 0.5 osmolality)

Osmolyte	AT base pairs			GC base pairs		
	ΔH	$T_m\Delta S$	ΔG	ΔH	$T_m\Delta S$	ΔG
Ethylene glycol	8170	8190	− 20	12,040	12,080	− 40
Glycerol	8280	8310	− 30	11,500	11,540	− 40
PEG 200	8230	8280	− 50	11,870	11,920	− 50
PEG 1450	7820	7500	+ 320	12,520	12,190	+ 330
Sucrose	13,480	13,630	− 150	17,850	17,930	− 80
Betaine	9020	9020	0	12,210	12,300	− 90
Urea	8170	8210	− 40	–	–	–

All values are in calories per mole base pairs and were calculated from fitted curves to the experimental data.

must mean that there is accumulation or exclusion of cosolvent depending on the extent of cosolvent interaction in the vicinity of the DNA base pairs. A number of studies have indicated that when there is significant change in surface area involving a macromolecule, such as DNA or protein, then one might expect more sensitivity to cosolvent contacts [3,4,7,34]. Clearly, Eq. (9) shows that changes in ΔN_{ew} are quite sensitive to ΔN_c, since its value is multiplied by the mole ratio of water to cosolvent in the bulk phase. Thus, for a 1 molal solution each unit change in ΔN_c contributes −55 to the value of the excess (or deficit) water. This feature was pointed out for the case of protein unfolding by Timasheff et al. [4] and means that, as cosolvent concentration changes, there will be corresponding changes associated with cosolvent intrusion into the space near the macromolecular states. It does appear that at lower values of osmolality the correlations become closer to linear, which means that ΔN_w and ΔN_c become constant with changing osmolality [3,5].

It is useful to examine Eq. (9) in a bit more detail with the purpose of analyzing the consequences of exchange of cosolvent in the water layer surrounding the DNA. Supposing we assume an associated monolayer of water in each state of the DNA and that when osmolytes exchange, water will be replaced by cosolvent molecules [32,36–38]. Our assumption of a water monolayer is arbitrary, but provides a kind of baseline for comparison. If the layer is deeper, the numbers will be different, but the trends in the values of cosolvent exchange should be the same. One can write the relationship for excess (or deficit) water for the duplex form, N_{ew}^{dup}, in an expanded form:

$$N_{ew}^{dup} = N_w^d(o) - N_c^d[\Delta N_w^d(ex)] - (n_w/n_c)N_c^d \quad (10)$$

$N_w^d(o)$ is the number of water molecules in the immediate vicinity of the base pair surface (~34 molecules based on available area)[7], N_c^d is the number of cosolvent molecules that exchange in the local domain of the base pair, and $\Delta N_w^d(ex)$ represents the number of water molecules displaced from the local domain when a cosolvent occupies space near the base pair [36]. (n_w/n_c) is the bulk mole ratio of water to cosolvent. A similar equation can be written for the single strand state of the base pairs.

$$N_{ew}^{ss} = N_w^{ss}(o) - N_c^{ss}[\Delta N_w^{ss}(ex)] - (n_w/n_c)N_c^{ss} \quad (11)$$

Here $N_w^{ss}(o)$ is around 54 molecules [7], again based on a monolayer of water surrounding the single strand pair; the other terms are analogous to those in Eq. (10). It is informative to look at the consequences of using these equations to examine values of $\Delta N_{ew} = N_{ew}^{ss} - N_{ew}^{dup}$, which can be compared with experimental values. If we take a solution of 1 osmolal, which corresponds to about 1.8 mol% of cosolvent or 8–10% wt/vol, and $n_w/n_c = 55$, and first assume that there is no exclusion or accumulation in the two states, one can calculate N_c^d and N_c^{ss} based on the number of water molecules near the surface of DNA and on the mole ratio that will correspond to random accumulation of osmolyte in the local domain, which is in proportion to the bulk composition. In order to get some numbers for $\Delta N_w(ex)$, we assume that the water exchange number is in direct proportion to the ratio of partial molar volumes of cosolvent to water. In the case for which there is random distribution, the value of ΔN_{ew} will be zero, no net accumulation or exclusion. In Table 3 we show some calculated values for N_c^d and N_c^{ss}, based on no accumulation or exclusion. The excess water values are different for each osmolyte because of the differences in size of the cosolvents, but N_c^d falls in the range of 0.56±0.03, while N_c^{ss} values average about 0.89±0.05. Thus, based on surface area considerations, there would be about one cosolvent per two base pairs in the duplex and approximately one per base pair in the single strand state. There is an increase of about 0.33 cosolvent molecules upon melting of the base pair. Thus, in Eq. (9), the term $(n_w/n_c)\cdot\Delta N_c$ contributes about −18.

The experimental values of the excess water parameter also are presented in Table 3, using the average values for AT and GC base pairs for ΔN_{ew}. From these experimental numbers, it is possible to calculate a value of ΔN_c for each cosolvent using the values of water exchange parameters, shown in Table 3, and the original number of waters estimated to be around duplex and single strand pairs (34 and 54, respectively). The numbers in

Table 3
Calculated values of ΔN_c using Eqs. (10) and (11)

Cosolvent	Volume ratio [a]	Experimental [b] ΔN_{ew}	Calculated [c] N_c [d](o)	Calculated [c] N_c^{ss}(o)	Calculated [d] ΔN_c(o)	Experimental [e] ΔN_c	Calculated [f] ΔN_w
Ethylene glycol	3.1	− 6.1	0.59	0.93	0.34	0.45	18.6
Glycerol	3.8	− 6.0	0.58	0.92	0.34	0.44	18.3
PEG 200	9.4	− 10	0.53	0.84	0.31	0.47	15.6
Urea	2.4	− 11	0.59	0.94	0.35	0.54	18.7
Sucrose	11.7	− 27	0.51	0.81	0.30	0.70	11.8
Betaine	5.4	− 15	0.56	0.89	0.33	0.56	17.0

[a] Volume ratio of cosolvent to water, thus the number of water molecules exchanged when cosolvent enters domain of DNA.
[b] Experimental values of change in excess water from Table 1, and using an average value for AT and GC base pairs, except for betaine.
[c] Calculated number of molecules of cosolvent in local area of duplex and single strand base pairs, based on a bulk concentration of 1 molal, and assuming no excess accumulation or exclusion from the DNA in either state.
[d] Change in number of molecules of cosolvent on melting, assuming no accumulation or exclusion.
[e] Change in number of molecules of cosolvent, based on experimental ΔN_{ew}. The values were calculated using the relationship: $\Delta N_{ew} = [20 - \Delta N_w(ex)\cdot\Delta N_c] - 55\cdot\Delta N_c$.
[f] Change in associated water calculated from $[20 - \Delta N_w(ex)\cdot\Delta N_c]$.

column 7 in Table 3 are the values of ΔN_c determined from the experimental values. The experimental change in number of cosolvent molecules in the local area around the DNA is larger than that for the situation where there is no net accumulation or exclusion, implying that there obviously is exclusion or accumulation. The changes for polyols and urea, combined with the fact that the enthalpies of melting decrease, as shown above, suggest that the values of ΔN_c of 0.45 to 0.50, are due in part because of accumulation in the single strand state. A 10% increase in the accumulation from just random distribution in the single strand would almost account for the changes in ΔN_c. Of course, there are an infinite number of possibilities that could describe the changes, but given that we suspect accumulation in single strand state for these cases with negative enthalpic trends in melting does provide reasonable justification for the observed increase in ΔN_c. The larger changes in ΔN_c for sucrose and betaine again show that these cosolvents are special cases. For betaine, an approximate 40% exclusion in the duplex state as has been suggested [7], and a small (10%) increase for the single strand number would increase ΔN_c to 0.63, compared with the observed 0.56. For sucrose, an over doubling of the value ΔN_c almost requires significant exclusion in duplex form and a large accumulation in single strand. However, the rather large endothermic contribution to melting enthalpies for this case confuses the interpretation of changes. The thermodynamic data in Table 2 for sucrose again suggest that significant entropic effects are responsible for the large changes in the excess water parameter on melting of DNA in the presence of sucrose. Finally, we have not included the PEG 1450 cosolvent for the obvious reason that excluded volume effects appear so dominant in this case, that the hydration numbers would have little meaning.

What emerges from the numbers in Table 3 is that even in these dilute solutions there are changes in accumulated cosolvent that significantly change the values of the excess water parameter, ΔN_{ew}. In solutions that are less than 2 mol% or around 5% by volume of cosolvent, it is important to recognize that the magnitude of ΔN_{ew} contains important contributions from the interchange with cosolvent as well as changes in extent of hydration of the macromolecule, even though the majority of the solvent in the local domain of the base pairs is water. The last column in Table 3 contains values of ΔN_w, the change in associated water molecules calculated from the magnitude of exchanged cosolvent in each case. Thus, to assume that ΔN_{ew} simply represents the changes in state of hydration of the DNA base pairs would be suspect in the case of DNA melting. Although the calculation exercise described above requires assumptions about the original hydration numbers of the base pairs, changes in those numbers only affect the absolute values of the derived parameters. The general trends are the same; namely, that cosolvent interactions play a major role in the magnitudes of the observed dependence of DNA melting on osmotic conditions.

4.3. Summary

This study provides several conclusions about DNA melting in the presence of osmolytes. (i) There are small, but significant, changes in the enthalpy of melting of DNA base pairs with increased osmolality that are different for the various osmolytes studied. (ii) These changes in enthalpy have a small (~20%) effect on derived excess water parameters when compared with values that assume constant enthalpy. (iii) There is relatively little difference between the enthalpy effects for AT and GC base pairs, except for the special case of betaine. (iv) The enthalpy changes for several of the osmolytes studied can be rationalized in terms of expected changes in preferential interactions between osmolytes, water and DNA base pairs. (v) There is strong enthalpy–entropy compensation in the melting of DNA, with entropy effects providing the small driving force necessary for changes in stability with osmolyte concentration. (vi) The role of small cosolvent exchanges in the local domain of the base pairs plays a large role in determining the magnitudes of observed changes in excess water parameters upon melting of the base pairs.

5. Postscript

The authors are honored and privileged to have known Julian, and to have had the opportunity to work in his laboratory. One aspect of our study would have been of great interest to Julian, the rate of data collection. While two of us (CHS and JBC) were at Yale around 1980, the two DSC instruments in Julian's laboratory were running day and night, operating in three shifts. Julian commanded the daytime shift, and others in the laboratory (B.Z. "Babs" Chowdhry, Gert Lipke, Karl Muller, Adam Dahliel) jockeyed for the remaining slots, with the midnight to 8 a.m. slot being, obviously, the least desirable. Evening social life revolved around cleaning, equilibration and run cycles of the calorimeters. Babs, in particular, had rather precise timing of those events, along with exact transit times to a number of New Haven bars and pizza parlors. He could, with remarkable precision, check his watch, gulp the remnants of a beer, and make it back to the lab at the exact moment necessary to start a run or to start cleaning the DSC in preparation for the next run.

That situation, however, was a great improvement over pace of data collection in the laboratory even a few years earlier using a DSC built in 1967, one of the few then available for studies of biopolymers. As Julian described in his *Recollections*[1]: "We made much use of our home-built **DSC** for several years, despite the fact that to cool it down after a scan we had to fill the thermostatic bath with ice and water, which meant that we were effectively limited to one experiment per day."

Julian would surely have been amazed and amused at the ability to do 10–20 DSC runs a day on samples of only a few hundred microliters using the MicroCal Cap-DSC. He just as surely would have questioned the capacity of men (and women) to keep pace with the machine to properly analyze and understand the data. "I don't know how the hell he got that baseline" was a typical concern expressed by Julian when

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examining thermograms. The concern remains, multiplied by a factor of 10.

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