Enthalpy of the B-to-Z Conformational Transition of a DNA Oligonucleotide Determined by Isothermal Titration Calorimetry

Jaime M. Ferreira and Richard D. Sheardy
Department of Chemistry and Biochemistry, Seton Hall University, South Orange, New Jersey 07079

ABSTRACT The influence of high concentrations of Na^+ or $[Co(NH_3)_6]^{3+}$ on the conformation of two related DNA oligomers was investigated by circular dichroism spectropolarimetry (CD), isothermal titration calorimetry (ITC), and differential scanning calorimetry (DSC). As revealed by CD, DNA oligomers, $(dC\text{-}dG)_4$ and $(dm^5C\text{-}dG)_4$, both form right-handed double helical structures (B-DNA) in standard phosphate buffer with 115 mM Na^+ at 25°C. However, at 2.0 M Na^+ or 200 μM $[Co(NH_3)_6]^{3+}$, $(dm^5C\text{-}dG)_4$ assumes a left-handed double helical structure (Z-DNA), whereas the unmethylated $(dC\text{-}dG)_4$ analog remains right-handed under those conditions. ITC was then used to determine the enthalpy change upon increasing the concentration of either Na^+ or $[Co(NH_3)_6]^{3+}$ for both DNA oligomers at 25°C. The titration with Na^+ resulted in endothermic isotherms with $(dm^5C\text{-}dG)_4$ being more endothermic than $(dC\text{-}dG)_4$ by 700 cal/mol basepair. In contrast, titration with $[Co(NH_3)_6]^{3+}$ resulted in exothermic isotherms with $(dC\text{-}dG)_4$ being more exothermic than $(dm^5C\text{-}dG)_4$ by 720 cal/mol basepair. We attribute the enthalpy difference to the conformational transition from B-form DNA to Z-form DNA for $(dm^5C\text{-}dG)_4$, a transition which does not occur for the unmethylated $(dC\text{-}dG)_4$. The value of ~700 cal/mol basepair for the enthalpy of the B-Z transition compares favorably with previously published results obtained by different techniques. DSC was used to monitor the duplex to single strand transitions for both oligomers under the different concentrations. These results indicated that methylation of the cytidine destabilizes $(dm^5C\text{-}dG)_4$ relative to $(dC\text{-}dG)_4$. Coupling the DSC data with the ITC data allowed construction of a thermodynamic cycle which gives insight into the influence of both temperature and ionic strength on the heat content of the two DNA systems studied. Further, this study reveals the utility of using ITC for determinations of transition enthalpies with the appropriate choice of control.

INTRODUCTION

In 1953, Watson and Crick (1) were the first to propose a right-handed double-helical conformation for DNA, now known as B-DNA from x-ray diffraction studies. Since then, it has been shown that under certain physiological conditions, double-helical DNA is highly polymorphic. In fact, nearly twenty other slight variations of DNA conformations exist, such as A-DNA, C-DNA, and Z-DNA. Perhaps the most radical change in conformation occurs when the B-DNA double helix undergoes a conformational transition to a left-handed conformation with the elimination of the major groove apparent in B-DNA. Pohl and Jovin (2) were the first to propose the existence of a left-handed double helix by observing an inverted circular dichroism spectrum of poly(dC-dG) under high salt concentrations. In 1979, Rich et al. (3) determined the structure of an alternating (GC) oligomer in the presence of Mg^{+2} and $[Co(NH_3)_6]^{3+}$ by x-ray crystallography to be a left-handed helical structure, which maintains its Watson-Crick basepairing. Due to the zig-zag nature of the sugar-phosphate backbone, this conformation was designated as Z-DNA. Behe and Felsenfeld (4) studied the effects on the B-DNA to Z-DNA transition upon methylation of the cytidine residues at the 5 position in poly(dG-dC) using circular dichroism. They found that the transition occurred at a much lower ionic strength for poly $(dm^5C\text{-}dG)$ than for the unmethylated polymer, poly(dC-dG), when using Na^+, Mg^{2+}, or trivalent cobalt hexamine $[Co(NH_3)_6]^{3+}$ as the Z-conformation inducer.

The importance in understanding the B-to-Z transition has led our group and others to study the thermodynamics involved in the transition. The Na^+ induced B-to-Z transition of poly(dC-dG) as reported by Pohl and Jovin (2) was found to be independent of temperature over the range of 30–50°C. Their van't Hoff analysis yielded, under their conditions, an enthalpy near zero (0.0 ± 1 kcal/mol basepair) for the B-to-Z transition. On the contrary, Chaires and Sturtevant (5) reported a reversible thermally-driven B-to-Z transition for poly($m^5dG\text{-}dC$) upon raising the temperature from 18 to 50°C and determined an enthalpy of $\Delta H = 0.61 \pm 0.07$ kcal/mol basepair by differential scanning calorimetry.

Our research entails observing conformational changes and their thermodynamic parameters associated with two self-complementary eight-basepair DNA oligomers, known as Z8A, $(dC\text{-}dG)_4$, and Z8M, $(dm^5C\text{-}dG)_4$. Both double-stranded DNA oligomers are studied under low and high concentrations of Na^+ and $[Co(NH_3)_6]^{3+}$ to observe any conformational differences. Circular dichroism (CD) studies were used to determine the conformation of each oligomer under all conditions and isothermal titration calorimetry (ITC) was used to determine the enthalpy of the B-to-Z conformational transition of Z8M using Z8A as a control. Using differential scanning calorimetry (DSC) to determine

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Address reprint requests to Richard D. Sheardy at his present address, Dept. of Chemistry and Physics, Texas Woman's University, PO Box 425859, Denton, TX 76204. E-mail: rsheardy@twu.edu.

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the enthalpy of the duplex to single strand transition under various solution conditions allowed the construction of a thermodynamic cycle.

MATERIAL AND METHODS

DNA oligomer synthesis and preparation

DNA oligonucleotides were synthesized on a 1.0 μm scale using the phosphoramidite method on an Applied Biosystems (Foster City, CA) 380B DNA Synthesizer with purification by trityl selective RPHPLC on cartridges supplied from Glen Research (POLY-PAK II^{TM}) as previously described (6,7). The β-cyanoethylphosphoramidites and other DNA synthesis reagents were obtained from Glen Research, Inc. After detritylation, the purified DNA oligomers were lyophilized to dryness, reconstituted in water and subjected to exhaustive dialysis versus water and then lyophilized to dryness. Purity and size analysis of the oligomers was accomplished through a combination of analytical RPHPLC and/or denaturing polyacrylamide gel electrophoresis. The $[Co(NH_3)_6]Cl_3$ was obtained from Kodak (Rochester, NY) and used without further purification. The lyophilized samples were reconstituted in a 10 mM phosphate buffer (pH 7.0), 0.1 mM EDTA with NaCl or $[Co(NH_3)_6]Cl_3$ complex added to vary their concentrations. Samples were then heated to 90°C for 2 min followed by slowly cooling and equilibration for 48 h at 4°C.

Ultraviolet/visible spectroscopy

DNA concentrations were determined with a Varian CARY 100E (Varian, Mulgrave, Victoria, Australia) ultraviolet/visible spectrophotometer interfaced to Windows-based computer with Cary/Varian WinUVBio version 2.0 software. This spectrometer was equipped with a Peltier thermoelectric heating/cooling block, a multicell-transport device and a nitrogen-purged sample compartment. The DNA concentrations were determined by spectrophotometric absorbance using extinction coefficients, ε (L mol^{-1} cm^{-1} in basepairs), of 13,000 for Z8A and 13,620 for Z8M at 255 nm.

Circular dichroism spectropolarimetry

The conformation of each oligomer in the presence of low and high concentrations of Na^+ or $[Co(NH_3)_6]^{3+}$ was determined using an AVIV 60DS circular dichroism spectropolarimeter equipped with a multiple cell turret and Peltier heating/cooling device. The CD spectrum of each DNA oligomer ([DNA] = 0.5 – 1.0 μM) in standard phosphate buffer with 115 mM Na^+, 2.0 M Na^+ or 115 mM Na^+ with 200 μM $[Co(NH_3)_6]^{3+}$ were determined. All CD data, reported as the average of triplicate scans, were blank corrected and analyzed with Aviv DOS-based software and exported into a graph-plotting software.

Isothermal titration calorimetry

Isothermal Titration Calorimetry measurements were carried out using the isothermal titration module of CSC Model 4200 ITC (Calorimetry Sciences Lindon, UT) interfaced to a Gateway 2000 Pentium II MMX PC. CSC Run, Bindwork and Origin 4.0 software were used for data acquisition and analysis. The calorimeter was calibrated using the Tris base reaction with HCl, for which $\Delta H = -11.38$ kcal/mol for acid neutralization. Each experiment was set up such that 10 μl of 800 μM $[Co(NH_3)_6]Cl_3$ or 10 μl of 4 M NaCl was titrated into the sample cell containing either Z8A or Z8M at 115 μM duplex for up to a total of 25 injections.

Each titration experiment was performed so that the unitless parameter C ($C = K_bM_t(0)$, where K_b is the binding constant and $M_t(0)$ is the initial DNA concentration) had values between 1 and 200. The lag time between subsequent injections allowed equilibration such that the heat exchange between the sample and reference cell was no more than ± 5 μW.

The heat absorbed or released due to the addition of aliquots of the salt solution to the DNA solution was measured by a thermoelectric device between the sample and reference cells. The heat associated with each injection was observed as a peak which corresponds to the power required to maintain the sample and the reference cells at identical temperatures. The peaks produced over the course of a titration can be converted to heat output per injection by integration and correcting for cell volume and sample concentration. The heat released for the ith injection, $\Delta Q(i)$, is given by:

$$\Delta Q(1) = \Delta Q(i) + dV_i/2V[Q(i) + Q(I-1)] - Q(i-1), \quad (1)$$

where dV_i is the volume of salt titrant added to the DNA solution and V is the cell volume. The total heat absorbed or released (Q_t) was fit by a non-linear least-square minimization method to the total DNA concentration (X_t) using the following equation:

$$Q_t = nM_t\Delta H_bV\{1 + X_t/nM_t + 1/nK_bM_t - [(1 + X_t/nM_t + 1/nK_bM_t)^2 - 4X_t/nM_t]^{1/2}\}/2, \quad (2)$$

where n is the number of binding sites per monomer. Control experiments were carried out to determine the contributions to the enthalpy from the heat of dilution for both the Na^+ and $[Co(NH_3)_6]^+$ into buffer or water alone. The net enthalpy for each injection was determined by subtraction of the component heats of dilution.

Differential scanning calorimeter

All heat capacity measurements were made with a Nano-II power-compensation differential scanning calorimeter from Calorimetric Sciences (Lindon, Utah). The calorimeter was interfaced directly to a Windows-based computer with ''DSCRun'' version 2.1.1 software (Calorimetric Sciences) for both data collection and DSC control. The reference solution for the reference side of the calorimetric measurement was derived from the buffer used to equilibrate the sample. Calorimetric measurements were performed as the average of two independent samples. Each DNA ([DNA] = 1.0 mM in basepairs in standard phosphate buffer plus added salts) sample was thermally scanned from 20°C to 95°C at 0.5°C/min over a minimum of 4 forward and 4 reverse scans. The samples were equilibrated for ten minutes at the upper (reverse scan) or lower (forward scan) set points between thermal scans. The raw thermographic data were blank corrected and integrated with ''CpCalc'' version 2.1 (Calorimetric Sciences) software. For most samples, the nature of the pretransition and posttransition baselines enabled integration of the calorimetric enthalpy with the use of a linear baseline.

RESULTS AND DISCUSSION

Previous work from this lab (6,7) has shown that the duplex formed from $(dm^5C - dG)_4$ is a right-handed double helical structure in 115 mM Na^+ but undergoes a conformation transition to a left-handed double helical structure upon the addition of Na^+ or $[Co(NH_3)_6]^{3+}$. This DNA oligomer, designated here as Z8M, served as a control sequence for studies focusing on the formation of B-Z junctions in 16 basepair oligonucleotides (8–11). Fig. 1 (*lower panel*) shows the CD spectra of this oligonucleotide under different conditions. The CD spectrum under conditions of 115 mM Na^+ is indicative of a right-handed conformation (i.e., B-DNA) with the characteristic peak at 280 nm and trough at

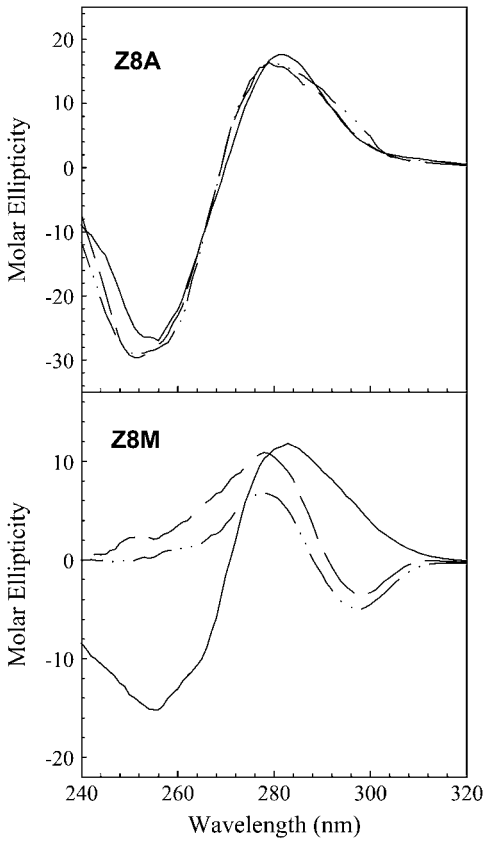


FIGURE 1 CD spectra at 25°C of Z8A and Z8M ([DNA] = 5.4×10^{-5} M in basepairs) in the presence of 10 mM phosphate buffer with a), 115 mM Na^+ (*solid*), b), 2.0 M NaCl (*dash*), or c), 115 mM Na^+ with 200 μM $[Co(NH_3)_6]^{3+}$ (*dash-dot-dot*) at pH 7. Molar ellipticities are reported in terms of basepair concentration.

255 nm. However, in the presence of either 2 M Na^+ or 200 μM $[Co(NH_3)_6]^{3+}$, each CD spectrum is characterized by a trough at ~295 nm and a peak at ~278 nm, characteristic of a left-handed conformation (i.e., Z-DNA). However, the unmethylated analog, Z8A, remains in a right-handed conformation under these same conditions (Fig. 1, *upper panel*). Hence, Z8A does not undergo the B-to-Z transition in the presence of 2 M Na^+ nor 200 μM $[Co(NH_3)_6]^{3+}$. Behe and Felsenfeld (4) demonstrated that methylation of the cytidine residues dramatically enhances the facility of the B-to-Z transition in the alternating GC polymer as evidenced by much lower concentrations of Z-inducing agent required to attain the transition midpoint. For short oligomers, the number and order of GC repeats is also crucial for the ease of the transition (12,13). Thus, the observation of the transition for Z8M and not for Z8A at the concentrations of Z-inducer used (i.e., 2 M Na^+ or 200 μM $[Co(NH_3)_6]^{3+}$) is consistent with these findings. At concentrations >4 M Na^+, Z8A did appear to undergo the B-to-Z transition; however, even at concentrations >200 mM $[Co(NH_3)_6]^{3+}$, the oligomer did not undergo the transition (data not shown).

Several different groups (2,5,14–20) have reported the enthalpy of the B-to-Z transition obtained by a variety of experimental techniques. One of the main goals of this project was to determine the enthalpy of the transition using ITC based on the following premise. We have demonstrated by the CD studies above that Z8M will undergo the B-to-Z transition using either Na^+ or $[Co(NH_3)_6]^{3+}$ but Z8A does not undergo the transition at [Na^+] <4.0 M or cobalt hexammine concentrations <200 mM. Hence, any enthalpy differences between Z8A and Z8M in the thermograms obtained from their titrations with these inducers must be due to conformational transitions.

Examination of the raw calorimetric data in the upper panels of Fig. 2 reveal that the titrations of both Z8A and Z8M with Na^+ are endothermic, whereas those with

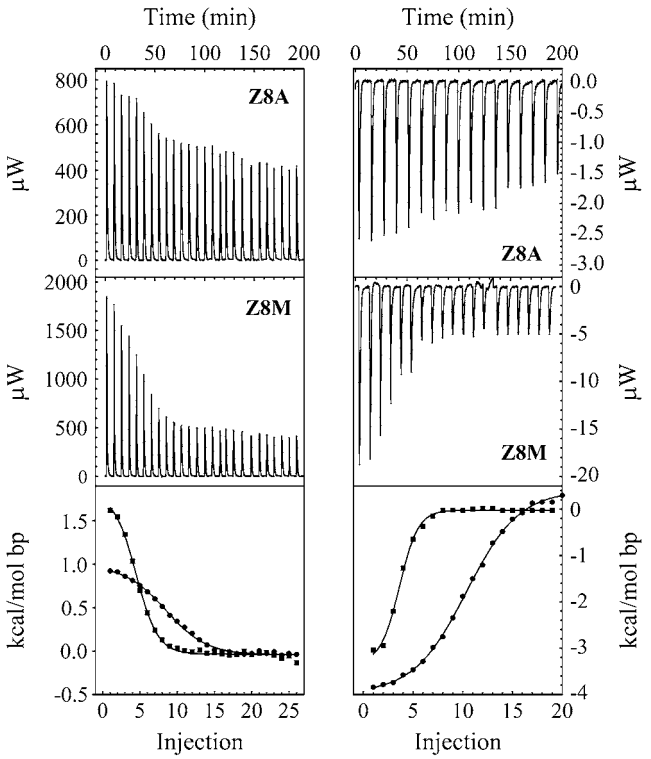


FIGURE 2 ITC profiles for the titration of Z8A or Z8M (concentration of DNA = 1.0 mM in basepairs) with 4.0 M NaCl (*left panels*) or 800 μM $[Co(NH_3)_6]^{3+}$ (*right panels*) at 25°C. Raw calorimetric titration data for the injections (10 μl/injection) are shown. The integrated results of the data after subtraction of the heats of dilution are represented in the lower panels for Z8A (*circles*) and Z8M (*squares*) and the solid lines represents the best least-square fits of the integrated data. For the titrations with NaCl, DNA oligomer and NaCl solutions were prepared in 10 mM phosphate buffer, 115 mM Na^+, pH 7.0 with 0.1 mM EDTA. For the titrations with $[Co(NH_3)_6]^{3+}$, all solutions were prepared in pure water with 115 mM Na^+ adjusted to pH 7.0 to preclude any $[Co(NH_3)_6]^{3+}$ - buffer phosphate interactions as per Matulis et al. (21). The CD spectra of Z8A and Z8M under these conditions were identical to those of Fig. 1. The number of injections for each titration was such that the final concentrations of Na^+ or $[Co(NH_3)_6]^{3+}$ were 2.0 M and 200 μM, respectively, to match those concentrations for the Z DNA CD spectra of Fig. 1.

TABLE 1 Fitting parameters for the isotherms of the titrations of Z8A and Z8M with Na^+ or $[Co(NH_3)_6]^{3+}$ at 25°C

Inducer	$K_{b,Z8A}$	n_{Z8A}	$K_{b,Z8M}$	n_{Z8M}
Na^+	0.28 ± 0.04 (× 10^2)	5.8 ± 0.4	2.0 ± 0.03 (× 10^2)	5.3 ± 0.2
$[Co(NH_3)_6]^{3+}$	0.90 ± 0.05 (× 10^5)	5.0 ± 0.3	1.0 ± 0.06 (× 10^5)	5.1 ± 0.3

$[Co(NH_3)_6]^{3+}$ are exothermic at 25°C, as are the respective heats of dilution (data not shown). Subtraction of the heats of dilution from the respective raw data, followed by integration results in the isotherms shown in the lower panels of Fig. 2. Titration of Z8A with either Na^+ or $[Co(NH_3)_6]^{3+}$ results in a fairly broad isotherm whereas the titration of Z8M with either inducer appears to have a sharp, cooperative transition. In addition, titration of Z8M with Na^+ is more endothermic than titration of Z8A, whereas titration of Z8A with $[Co(NH_3)_6]^{3+}$ is more exothermic than titration of Z8M. The fitting parameters for these isotherms, K_b and n, are given in Table 1 and the resultant enthalpies ($\Delta H_{obs,Z8A}$ and $\Delta H_{obs,Z8M}$) for these titrations in Table 2.

Careful examination of the isotherms in the lower panels of Fig. 2 reveal not only sigmoidal transitions for Z8M but also for Z8A. The CD data in Fig. 1 clearly show that Z8M undergoes the B-to-Z transition upon the addition of Na^+ or $[Co(NH_3)_6]^{3+}$ and as previously reported (6,7). Our previously published work also reported that the Na^+ induced transition for Z8M is best described as three state (via deconvolution of the CD titration data). We have also determined by the same approach that the $[Co(NH_3)_6]^{3+}$ induced B-to-Z transition is also three state (data not shown). The formation of the intermediate state is likely due to dehydration before the conformational transition. The apparent thermodynamic transition observed here with Z8A most likely corresponds to the formation of a semidehydrated state. Hence, a transition for Z8A is observed calorimetrically that is not observed optically.

Regardless of any type of transition, increasing the concentration of Na^+ in a DNA solution should be enthalpically unfavorable as observed here due to the release of water from the duplex. Increasing the concentration of $[Co(NH_3)_6]^{3+}$ will also induce the release of water from the duplex. However, this enthalpically unfavorable process apparently is compensated by the enthalpically favorable binding of the $[Co(NH_3)_6]^{3+}$ to the DNA backbone as indicated by the ITC determined enthalpy.

Although the B-to-Z transition is three state, we are only interested in the initial and final states. Thus, we assume that the difference in total enthalpy between Z8A and Z8M upon titration with either Na^+ or $[Co(NH_3)_6]^{3+}$ is the transition enthalpy. In other words,

$$\Delta H_{obs} = \Delta H_{pe} + \Delta H_{bz}, \quad (3)$$

where ΔH_{obs} is the observed calorimetric enthalpy for Z8M, ΔH_{bz} is the enthalpy for the transition itself and ΔH_{pe} is the enthalpy for all other possible processes such as uptake or loss of Na^+, uptake or loss of water, and the binding of $[Co(NH_3)_6]^{3+}$ when titrating with that inducer. The assumption is that ΔH_{pe} is the same for Z8A and Z8M. In other words,

$$\Delta H_{bz} = \Delta H_{obs,Z8M} - \Delta H_{obs,Z8A}. \quad (4)$$

As seen in Table 2, the enthalpy of the transition is 700 cal/mol basepair when inducing with Na^+ and 720 cal/mol basepair when inducing with $[Co(NH_3)_6]^{3+}$. Adding validity to our approach is the comparison of our results of previously published work (Table 3). Our transition enthalpy of ~0.70 kcal/mol basepair obtained at 25°C using either Na^+ or $[Co(NH_3)_6]^{3+}$ compares quite favorably with the results of Chaires and Sturtevant (5) and is close in agreement in sign and magnitude with the other studies, although obtained by different techniques (i.e., ITC versus CD, DSC, or NMR).

Even though similar enthalpies are observed for both the Na^+ and $[Co(NH_3)_6]^{3+}$ induced B-to-Z transitions, it must be kept in mind that the mechanism for the transitions are different for the two inducers used. In the case of Na^+, the transition is due to dehydration of the DNA by high

TABLE 2 Experimentally determined enthalpies of the titrations of Z8A and Z8M with Na^+ or $[Co(NH_3)_6]^{3+}$ at 25°C and the enthalpy of the B-to-Z transition

Inducer	$\Delta H_{obs,Z8A}$ (kcal/mol bp)	$\Delta H_{obs,Z8M}$ (kcal/mol bp)	ΔH_{BZ}
Na^+	0.92 ± 0.10	1.62 ± 0.1	0.70 ± 0.1
$[Co(NH_3)_6]^{3+}$	−3.85 ± 0.2	−3.13 ± 0.2	0.72 ± 0.2

The enthalpy of the B-to-Z transition, ΔH_{BZ}, was determined using Eq. 4 and is given in terms of the DNA concentration in basepairs.

TABLE 3 Comparison of different techniques used to determine the enthalpy of the B-to-Z transition

Sequence	ΔH_{BZ} (kcal/mol bp)	Technique	Group
Poly $d(C-G)$	0.00 ± 1	CD	Pohl and Jovin (2)
Poly $d(5mC-G)$	0.61 ± 0.07	DSC	Chaires and Sturtevant (5)
Poly $d(C-G)$	2.02 ± 0.2	DSC	Chaires and Sturtevant (20)
Poly $d(C-G)$	2.0 ± 0.2	DSC	Klump et al. (18)
d5mCdGdCdGd5mCdG	1.3 ± 0.2	NMR	Tran-Dinh et al. (19)
$(d5mC-dG)_4$	0.70 ± 0.04	ITC	This work

concentrations of Na^+. However, in the case of $[Co(NH_3)_6]^{3+}$, the transition is induced by disruption of water bound to the DNA by the electrostatic binding of the cobalt complex ultimately leading to dehydration. Hence, the values of K_b and n in Table 1 should not be construed as a true binding constant and site size in the case of Na^+ since a binding event is not occurring with this cation. Nonetheless, the values of K_b and n for $[Co(NH_3)_6]^{3+}$ do have physical significance since binding is occurring with this cation. It is further noteworthy that the binding constant and site size are essentially identical for both oligos and, thus, are independent of any conformational transition.

Regardless of the inducer used, the B-to-Z transition is accompanied by the release of both water, as noted above, and Na^+, due to the lower charge density of Z-DNA, from the duplex into the bulk solution. Such release is entropically favorable. We have previously shown that the free energy for the Na^+ induced B-to-Z transition for Z8M can be calculated for any $[Na^+]$. As noted above, SVD (singular value decomposition) of the CD spectra obtained in the titration of Z8M with Na^+ indicated that the transition from B-to-Z is best described as three state:

$$B \leftrightarrow I \leftrightarrow Z.$$

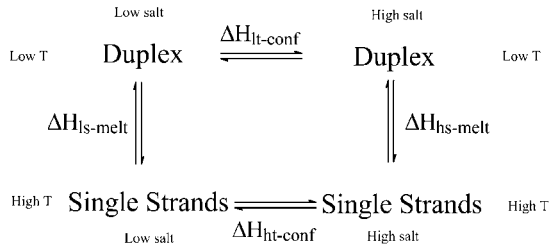
The free energy for each transition can be calculated from the respective equilibria constants, which are obtainable from the fit of the data in the fraction of transition versus concentration of cation plot. The total free energy of the B-to-Z transition is simply the sum of the free energies for the individual steps (6). Analysis of the transition profile induced by $[Co(NH_3)_6]^{3+}$ also indicates a three state transition.

Using the titration end point of 2.0 M Na^+, the free energy of the B-to-Z transition is calculated as -2.6 kcal/mol bp. Upon application of the Gibbs relationship, $T\Delta S = \Delta H - \Delta G$, we obtain the $T\Delta S$ contribution of $+3.5$ kcal/mol bp at 298K. Proceeding in a similar fashion, one obtains the free energy of transition for Z8M induced by $[Co(NH_3)_6]^{3+}$ to be -4.3 kcal/mol bp at 200 μM $[Co(NH_3)_6]^{3+}$ (the end point of the titration) giving a $T\Delta S$ contribution of -5.0 kcal/mol bp at 298K. Thus, the B-to-Z transition is entropically driven for both Na^+ and $[Co(NH_3)_6]^{3+}$ induced transitions. The complete thermodynamic values for the B-to-Z transition are reported in Table 4.

Consider the thermodynamic cycle in Scheme 1. With an appropriately chosen DNA oligomer, such as Z8A or Z8M, one can determine enthalpies for the duplex to single strand transition (i.e., low T to high T) using classical techniques

TABLE 4 Complete thermodynamic profile for the B-to-Z transition of Z8M induced with Na^+ or $[Co(NH_3)_6]^{3+}$ at 298K

Inducer	ΔG (kcal/mol bp)	ΔH (kcal/mol bp)	$-T\Delta S$ (kcal/mol bp)
Na^+ (2.0 M)	−2.8	+0.70	−3.5
$[Co(NH_3)_6]^{3+}$ (200 μM)	−4.3	+0.72	−5.0



SCHEME 1 Simple thermodynamic cycle depicting different states for an appropriately chosen duplex, such as Z8A or Z8M. In this scheme: 1), Low T is 25°C and high T is 95°C; 2), low salt is 115 mM Na^+ (standard phosphate buffer) and high salt is either 2.0 M Na^+ or 115 mM Na^+ with 200 μM $[Co(NH_3)_6]^{3+}$; 3), $\Delta H_{ls\text{-}melt}$ and $\Delta H_{hs\text{-}melt}$ are the enthalpies of the duplex to single strand transition under low salt or high salt conditions, respectively; and, 4), $\Delta H_{lt\text{-}conf}$ and $\Delta H_{ht\text{-}conf}$ are the enthalpies of any conformational transition (or change in physical state) induced by increasing the ionic strength of the media at low temperature or high temperature, respectively.

such as optical melting studies or DSC. Further, as demonstrated above, and one can determine the enthalpy associated for the isothermal transition from low salt to high salt using ITC. In this particular case, we observe the enthalpy upon increasing the salt concentration at low temperature whereby the oligomers are in the duplexed state. Obtaining such data from three sides of the cycle allows for determination of the fourth by difference. Hence, we can determine the enthalpy difference upon increasing the salt concentration at high temperatures whereby the oligos assume the single stranded conformation.

The enthalpies for the duplex to single strand transitions at different salt conditions have been determined using DSC. A typical DSC thermogram for Z8A in 4.0 M NaCl is shown in Fig. 3 and the experimentally determined enthalpies for all

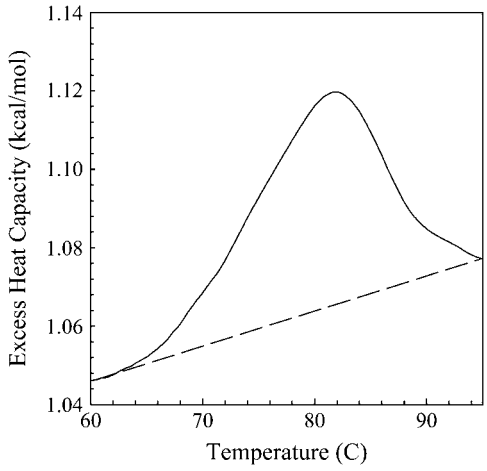


FIGURE 3 Typical DSC thermogram for Z8A at 4.0 M Na^+ in 10 mM phosphate Buffer, 0.1 mM EDTA, pH 7.0, and a scan rate of 0.5°C min^{-1}. The solid line represents the data and the dashed line is the base line. Determination of the peak areas gave the enthalpy values listed in Table 4.

systems are reported in Table 5 as $\Delta H_{\text{ls-melt}}$ for the low salt transitions and $\Delta H_{\text{hs-melt}}$ for the high salt transitions. The enthalpies for isothermally changing the conditions from low salt to high salt are designated as $\Delta H_{\text{lt-conf}}$ for the low temperature transitions (experimentally determined) and $\Delta H_{\text{ht-conf}}$ (calculated by difference) for the high temperature transitions. Several observations can be made from the data in Table 5. First, methylation of the cytidine base enthalpically destabilizes the duplex. This destabilization can be attributed to the presence of the methyl group in the major groove giving rise to a less hydrated groove. Second, both duplexes are more enthalpically stable in the higher salt concentrations, as expected (22). Finally, the change in the state of the system from low salt to high salt is more endothermic for Z8M than for Z8A when using just Na^+, regardless of temperature. However, when using $[Co(NH_3)_6]^{3+}$, the change in the state of the system from low salt to high salt is less exothermic for Z8M than for Z8A at low temperature but more exothermic at high temperature. Thus, the binding of the cobalt complex is influencing the thermodynamic state of both the duplex and single strands in different ways. Finally, the last column in Table 5 indicates the difference in enthalpy between the low and high salt conditions at temperatures where the oligos are single stranded. Thus, the application of the thermodynamic cycle allows determination of enthalpy differences that may not be directly obtainable experimentally.

In summary, we have determined the enthalpy of the B-to-Z conformational transition using Isothermal Titration Calorimetry. Our results compare favorably with those obtained from other groups using different techniques. This work demonstrates that ITC can be used to monitor conformational transitions that may not be observed spectroscopically.

TABLE 5 Enthalpy values for the thermodynamic cycle

Oligomer	$\Delta H_{\text{ls-melt}}$ (kcal/mol bp)	$\Delta H_{\text{hs-melt}}$ (kcal/mol bp)	$\Delta H_{\text{lt-conf}}$ (kcal/mol bp)	$\Delta H_{\text{ht-conf}}$ (kcal/mol bp)
		Na^+		
Z8A	8.7	10.6	0.92	2.8
Z8M	5.2	8.1	1.6	4.5
		$[Co(NH_3)_6]^{3+}$		
Z8A	8.7	11.0	-3.85	-1.6
Z8M	5.2	5.9	-3.13	-2.4

For the values reported above: $\Delta H_{\text{ls-melt}}$ is the enthalpy for the duplex to single strand transition for the DNA prepared in standard phosphate buffer under low salt conditions (i.e., 115 mM Na^+); $\Delta H_{\text{hs-melt}}$ is the enthalpy for the duplex to single strand transition for the DNA prepared in standard phosphate buffer under high salt conditions (i.e., 2.0 M Na^+ or 115 mM Na^+ with 200 μM $[Co(NH_3)_6]^{3+}$); $\Delta H_{\text{lt-conf}}$ is the enthalpy for transition from low salt to high salt at low temperature (i.e., 25°C); and, $\Delta H_{\text{ht-conf}}$ is the enthalpy for transition from low salt to high salt at high temperature (i.e., 95°C). The enthalpies for the duplex to single strand transitions were determined by DSC; the $\Delta H_{\text{lt-conf}}$ values were determined by ITC; and the values for $\Delta H_{\text{ht-conf}}$ were determined by difference: $\Delta H_{\text{ht-conf}} = (\Delta H_{\text{hs-melt}} + \Delta H_{\text{lt-conf}}) - \Delta H_{\text{ls-melt}}$. All DSC values are estimated to be ±5%.

In addition, with the selection of an appropriate control, ITC perhaps is the most direct method of determining conformational transition enthalpies. Coupled with DSC studies for the duplex to single strand transition, we can construct a thermodynamic cycle with gives insight into how both temperature and ionic strength influence the heat content of our systems.

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