Thermodynamic Stability of DNA Tandem Mismatches†

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Received April 26, 2004; Revised Manuscript Received May 30, 2004

ABSTRACT: The thermodynamics of nine hairpin DNAs were evaluated using UV-monitored melting curves and differential scanning calorimetry (DSC). Each DNA has the same five-base loop and a stem with 8−10 base pairs. Five of the DNAs have a tandem mismatch in the stem, while four have all base pairs. The tandem mismatches examined (ga/ga, aa/gc, ca/gc, ta/ac, and tc/tc) spanned the range of stability observed for this motif in a previous study of 28 tandem mismatches. UV-monitored melting curves were obtained in 1.0 M Na^+, 0.1 M Na^+, and 0.1 M Na^+ with 5 mM Mg^{2+}. DSC studies were conducted in 0.1 M Na^+. Transition T_m values were unchanged over a 50-fold range of strand concentration. Model-independent enthalpy changes ($\Delta H°$) evaluated by DSC were in good agreement (±8%) with enthalpy values determined by van't Hoff analyses of the melting curves in 0.1 M Na^+. The average heat capacity change (ΔC_p) associated with the hairpin to single strands transitions was estimated from plots of $\Delta H°$ and $\Delta S°$ with T_m and ln T_m, respectively, and from profiles of DSC curves. The average ΔC_p values (113 ± 9 and 42 ± 27 cal·K^{-1}·mol^{-1} of bp), were in the range of values reported in previous studies. Consideration of ΔC_p produced large changes in $\Delta H°$ and $\Delta S°$ extrapolated from the transition region to 37 °C and smaller but significant changes to free energies. The loop free energy of the five tandem mismatches at 37 °C varied over a range of ∼4 kcal·mol^{-1} for each solvent.

Accurate prediction of the stability of hybridized DNA strands is important to a number of molecular biology techniques such as polymerase chain reaction, Southern blotting, and DNA microarray diagnostics involving DNA hybridization. In each of these methods, a DNA strand hybridizes to a complementary DNA target site in the presence of a large number of other competing sites. Some of the competing DNA sites will have sequences similar to the target site. To determine conditions that maximize selective hybridization between a probe DNA and its target site and minimize hybridization to other sites, it is important to know the influence of mismatches (non-Watson−Crick base pairs) on DNA hybrid stability. Another instance where single or tandem mismatches may occur is in folded-back single-stranded sequences, which may occur in vivo (*5, 13*).

Thermodynamic parameters have been evaluated for a number of sequences with single base pair mismatches (*10*); however, relatively few studies have been published on the thermodynamics of tandem mismatches. Li and Agarwal (*7*) evaluated thermodynamic parameters for the ga/ga tandem mismatch surrounded by different closing base pairs. When flanked by a C·G and G·C this tandem mismatch, CgaG/CgaG, provides a sequence of unusual stability (*8*). In this paper lower case "a,t,c,g" designate mismatched bases and upper case letters are used for Watson−Crick base pairs. Ke and Wartell (*6*) employed temperature gradient gel electrophoresis (TGGE) to explore the relative stabilities of 28 tandem mismatches in DNA duplex fragments. The stabilities of the tandem mismatches were shown to be influenced by their sequence as well as the sequence of adjacent base pairs. The DNA transitions were examined in a gel buffer with 45 mM Tris Borate, formamide, and 2 mM Na^+. Although the TGGE method enabled the simultaneous comparison of a number of sequences, the stability parameters estimated by this method may not be comparable or scaleable to the 1 M Na^+ solvent used in most thermodynamic studies. To explore this question, thermodynamic parameters of tandem mismatches in hairpin DNA oligomers were evaluated by DNA denaturation in a 1.0 M Na^+ solvent and in solvents with 0.1 M Na^+, and 0.1 M Na^+ plus 5 mM Mg^{2+}.

The five tandem mismatches examined were selected from those previously studied by TGGE and spanned the highest to lowest stabilities evaluated (*6*). The sequences ga/ga, aa/gc, ca/gc, ta/ac, and tc/tc were embedded in a hairpin DNA structure with a five-base loop and eight base pairs in the stem (Figure 1). Four similar hairpin DNAs with all Watson−Crick base pairs in the stem were also examined. UV-absorbance monitored melting curves and differential scanning calorimetry (DSC)[1] were employed. Two van't Hoff methods were used to analyze the optical melting curves.

Our results showed that enthalpy values evaluated by DSC and the van't Hoff analyses of optical melting curves in 0.1 M Na^+ were generally in good agreement with each other (±8%). $\Delta H°$ values evaluated by the two van't Hoff analyses of UV-melting curves in 1 M Na^+ were within 6% of each other except for the hairpin DNAs with GpA steps where the difference was 12%. The larger deviations may indicate non-two-state characteristics in the transitions of these DNAs.

† This work was supported by funding from the GRA and a Dept of Ed. GAANN graduate fellowship to B.B.-P.

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[1] Abbreviations: nt, nucleotide; bp, base pair; DSC, differential scanning calorimetry; OD, optical density.

10.1021/bi049161w CCC: $27.50
Published on Web 07/10/2004

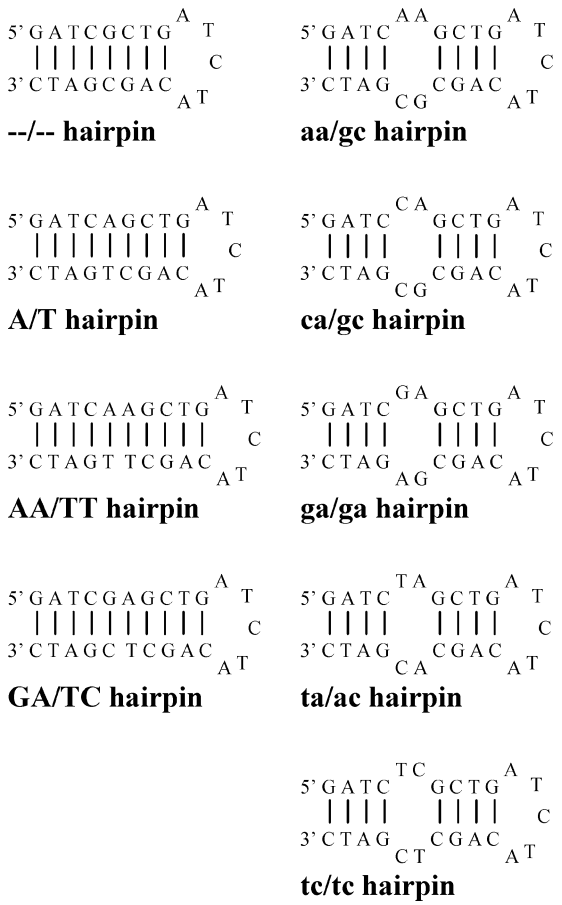

FIGURE 1: Sequence and schematic hairpin structure of the DNA molecules examined. Each DNA is named according to the nucleotides of the internal loop. The − −/− − molecule contains only eight base pairs in the hairpin stem. The A/T molecule contains one Watson−Crick bp at the location of the tandem mismatches in the other molecules.

The average heat capacity change (ΔC_p) associated with the DNA hairpin to single strand transitions was estimated from plots of enthalpy and entropy as a function of T_m and from the DSC transition profiles. Although the two approaches gave different values for ΔC_p (113 ± 9 and 42 ± 27 cal·K^{-1}·mol^{-1} of base pairs), both are in the range of values determined from previous work on two-stranded DNA duplexes (*2*, *4*, *11*, *16*). The loop free energy contribution of the five tandem mismatches at 37 °C in 1 M Na^+ ranged from −3.0 kcal·mol^{-1} for ga/ga to +1.1 kcal·mol^{-1} for ta/ac. A similar range of values was observed for the other two solvents.

EXPERIMENTAL PROCEDURES

Oligodeoxynucleotides. The oligodeoxynucleotides used in this study have the sequence 5′-GATCXYGCTGATC-TACAGCWZGATC-3′, where XZ and YW represent mismatched or complementary base pairs. In a few cases, one or both of the base pairs were omitted. The oligonucleotides were obtained commercially and purified using an Agilent Technologies Inc. 1100 series HPLC system with a Dionex DNAPac PA-100 column. A sodium perchlorate gradient was employed (7.5−145 mM). All oligonucleotides produced a single band on a 16.5 cm × 20 cm 20% polyacrylamide gel. Based on ethidium bromide staining sensitivity, they were estimated to be at least 95% pure. The hairpin DNA molecules in this work are referred to by the bases located at positions 5,6 and 21,22 (XY/WZ) in the above core sequence. The DNA hairpin designated − −/− − (Figure 1) has no bases in the positions represented by X, Y, W, and Z. The A/T DNA hairpin DNA contains one A·T base pair at the location of the tandem mismatches (Figure 1).

DNA Concentrations. The concentration of each oligonucleotide was determined by degrading the DNA with *Croatalus adamanteus* venom phosphodiesterase I (USB Corp or Worthington Biochemical Co) and measuring the resultant absorbance at 260 nm. A stock phosphodiesterase solution was made at a concentration of 1 mg/mL (31 units/mL) in a buffer containing 110 mM NaCl, 110 mM Tris-HCl, pH = 8.9, and 15 mM $MgCl_2$ and stored at 4 °C. The oligonucleotides at approximately 0.3 OD/mL were degraded in the same buffer at a final concentration of 0.5 units/mL phosphodiesterase. Each reaction was incubated at 25 °C until an absorbance plateau was assured (3−4 h). Oligonucleotide concentrations were determined from OD_{260} values and the extinction coefficients of the mononucleotides (*14*). Concentrations were also estimated using extinction coefficients calculated for single strands based on dinucleotide frequencies (*3*) and absorbance values of denatured oligomers measured at 90 °C. The latter approach produced concentrations similar to those determined by enzyme degradation for some oligonucleotides (±6%) but not others. For this reason, only concentration determined by phosphodiesterase degradation was utilized.

UV Melting Experiments. UV absorbance was employed to monitor the melting transitions of the hairpin molecules. The DNA was diluted to a concentration of approximately 0.45 OD/mL at 260 nm (approximately 1.8 μM strand) in either 1.0 M NaCl and 0.01 M MOPS (pH 7.0), 0.08 M NaCl and 0.01 M Na_2HPO_4 (pH 7.0), or the latter buffer with 5 mM $MgCl_2$. Prior to being loaded into the spectrophotometer, samples were denatured at 95 °C for 3 min, then slowly cooled to room temperature to allow annealing. Samples were placed in 1 cm path length quartz cuvettes. A Cary 100 spectrophotometer (Varian, Inc.) was used to measure absorbance as a function of temperature at 268 and 280 nm with a heating rate of 0.5 °C min^{-1}. Temperature was measured with a platinum resistance probe inserted into a solvent cell adjacent to the sample. Absorbance readings were taken every 0.1 °C over a range of 25−95 °C or 15−95 °C. Four or more melting transitions were obtained for each sample. Reverse transitions closely followed forward transitions.

The ga/ga, AA/TT, and − −/− − molecules, predicted to be among the most stable, were also prepared at concentrations of 0.8 and 40 μM in 1.0 M NaCl and 0.01 M MOPS, and melting curves were obtained. The 40 μM samples were denatured in a 2 mm path length cell. The midpoint temperature of the transitions, T_m, for these three molecules did not change significantly over the 50-fold concentration range. For the AA/TT hairpin, T_m values were 78.54 ± 0.19 and 78.04 ± 0.06 °C at 0.8 and 40 μM strand concentration, respectively. For the − −/− − molecule, the values were 78.70 ± 0.39 and 78.73 ± 0.38 °C, and for the ga/ga hairpin, the T_m's were 69.34 ± 0.23 and 69.75 ± 0.31 °C.

Calculation of Thermodynamic Parameters from UV Denaturation Curve. Absorbance vs temperature data obtained from the denaturation or "melting" curves were

expressed in terms of the fraction of molecules in the single-stranded state, θ_B, using the following equation (*15*):

$$\theta_B(T) = [A(T) - A_{pre}(T)]/[A_{post}(T) - A_{pre}(T)] \quad (1)$$

$A(T)$ is the absorbance of the sample at temperature T, and $A_{pre}(T)$ and $A_{post}(T)$ are the pretransition and posttransition linear baselines of the denaturation curves. The derivative melting curve was determined by dividing the increments $\Delta\theta_B(T)$ by the temperature increments, ΔT. SigmaPlot software was used to perform a 13-point Lowess smooth on the raw data and an 11-point smooth on the derivative curve.

Assuming the helix−coil transitions are two-state transitions, a van't Hoff analysis was used to evaluate the enthalpy and entropy at the T_m using the following equations (*9*):

$$\Delta H^\circ = 4RT_m^2(d\theta_B(T)/dT)_{T=T_m} \quad (2)$$

$$\Delta S^\circ = \Delta H^\circ/T_m \quad (3)$$

ΔH° and ΔS° are the enthalpy and entropy changes of the transition, R is the gas constant (1.987 cal·K^{-1}·mol^{-1}), and $(d\theta_B(T)/dT)_{T=T_m}$ is the height of the derivative melting curve at the T_m.

A second van't Hoff method was also used to determine ΔH° and ΔS° values averaged over the region of the transition. In this approach, a nonlinear least-squares regression (SigmaPlot) was employed to evaluate ΔH° and ΔS° pairs that best fit the shape of the DNA melting curves. Assuming a two-state monomolecular transition, the fraction of strands in the coiled state, $\theta_B(T)$, can be related to the enthalpy and entropy change through the equation (*9*)

$$-\Delta H^\circ + T\Delta S^\circ = RT \ln[(\theta_B(T))/(1 - \theta_B(T))] \quad (4)$$

For each transition curve, ΔH° and ΔS° that best fit eq 4 were determined. These values were generally very similar to those evaluated from eqs 2 and 3.

Differential Scanning Calorimetry. Calorimetry experiments on the hairpin DNAs were conducted with a Nano DSC series II differential scanning calorimeter (Calorimetry Sciences Corporation, American Fork, UT). The buffer used in the DSC measurements was 0.08 M NaCl and 0.01 M Na_2HPO_4, pH 7.0. Stock solutions of the DNA samples were diluted approximately 10:1 to a concentration of 100 μM strand. DNA and reference solutions were degassed for at least 30 min and run under three atmospheres of pressure. For each run, buffer−buffer scans were made from 15 to 95 °C four times to provide baselines for the sample−buffer scans. The heating rate was 1 °C min^{-1}. Each DNA sample was then heated and cooled four times over the same 15−95 °C range and at the same heating rate. A minimum of two separate runs using independent samples were made for each DNA.

The raw data was converted to apparent molar heat capacity vs temperature using the CpCalc software (Calorimetry Science Corporation). A buffer−buffer scan was subtracted from each sample−buffer scan, and a quadratic polynomial baseline was fit to the plot to obtain the excess molar heat capacity curve. The resultant data was exported to the SigmaPlot program for plotting. The enthalpy change of a transition was evaluated by integrating the area under the curve. T_m values were determined from the peak of the excess heat capacity curve. Standard deviations of the enthalpy change were based on multiple scans and runs for each DNA. To estimate the heat capacity change for a DNA hairpin transition, linear pre- and postbaselines were extrapolated to the T_m value, and the difference between them was measured.

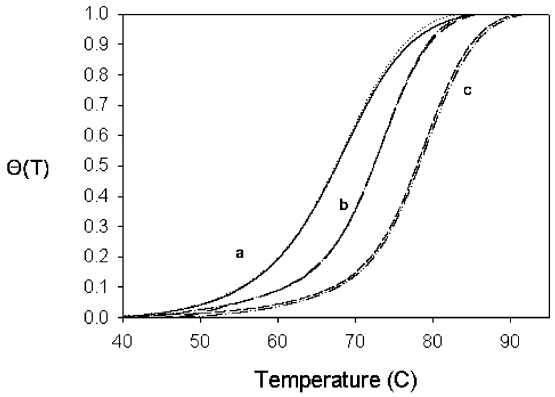

FIGURE 2: Normalized melting curves of three DNAs in 1M Na^+ solvent: (a) the solid line represents the denaturation curve of the aa/gc DNA, and the dotted line is the renaturation curve; (b) long dashed and dash−dot−dash lines are the denaturation and renaturation curves of the ga/ga DNA, and these two curves are shifted five degrees lower to prevent overlap with AA/TT curves; (c) short dash and the dash−dot−dot lines are the denaturation and renaturation curves of the AA/TT DNA.

It was noted that the melting temperatures of DNA samples measured by DSC were always higher than the DNA samples examined spectrophotometrically by 2.1 ± 0.6 °C. This was shown to be due to a difference in calibration of temperature probes. Denaturation of a single DNA hairpin sample was monitored by both DSC and UV absorbance, and the T_m value evaluated by DSC was shown to be 2.0 °C higher than the T_m determined by the UV melting curve. Since the temperature probe of the Cary 100 was shown to be within ±0.2 °C of a NIST-calibrated thermometer from 30 to 80 °C, we systematically reduced DSC T_m values by 2.0 °C and report these corrected values in Table 3.

RESULTS

Design of Oligodeoxynucleotides. Figure 1 shows the expected structures of the nine DNA molecules examined in this study. The DNA oligomers with tandem mismatches were designed to fold into hairpin molecules with a five base hairpin loop and a stem of eight Watson−Crick paired bases interrupted with two tandem mismatches. Control molecules had either eight paired bases in the stem, or one or two additional Watson−Crick pairs in place of the tandem mismatches.

Thermal Denaturation in 1 M Na^+. Figure 2 shows examples of UV monitored melting curves of a control DNA and two tandem mismatch DNAs in 1 M Na^+. Absorbance changes were normalized to a 0−1 scale and results are presented in terms of fraction of molecules in the single-stranded coil state. The transitions were highly reversible. The solid line and overlapping dotted line show the denaturation and renaturation transitions for the aa/gc hairpin DNA (curve "a"). The denaturation and renaturation transitions of the ga/ga DNA molecule are shown as curve "b" and are shifted 5 °C lower in the figure to prevent overlap with the transitions of the AA/TT hairpin DNA designated

Table 1: Thermodynamic Parameters Evaluated from DNA Transitions in 1.0 M Na^+[a]

DNA	T_m (°C) mfold	T_m (°C) (expt)	peak height $-\Delta H$ (kcal·mol^{-1})	curve fit $-\Delta H$ (kcal·mol^{-1})	peak height $-\Delta S$ (eu)	curve fit $-\Delta S$ (eu)	peak height $-\Delta G^*_{37}$ (kcal·mol^{-1})	curve fit $-\Delta G^*_{37}$ (kcal·mol^{-1})	ΔC_p-corrected $-\Delta G^\circ_{37}$ (kcal·mol^{-1})
− −/− −	81.4	77.7 ± 0.4	52.6 ± 0.4	52.7 ± 2.6	150 ± 3	150 ± 8	6.11 ± 0.15	6.22 ± 0.44	4.65 ± 0.50
A/T	80.7	78.1 ± 0.4	59.9 ± 1.7	58.6 ± 4.4	175 ± 14	167 ± 13	7.21 ± 0.63	6.68 ± 0.51	4.52 ± 0.43
AA/TT	80.9	79.2 ± 0.2	64.0 ± 1.1	59.7 ± 2.8	182 ± 3	170 ± 8	7.66 ± 0.12	6.95 ± 0.38	5.29 ± 0.57
GA/TC	84.3	82.6 ± 0.2	65.0 ± 1.6	56.4 ± 2.7	183 ± 5	159 ± 7	8.33 ± 0.22	7.05 ± 0.46	5.37 ± 0.64
ga/ga	62.3	77.7 ± 0.2	66.9 ± 1.4	58.8 ± 3.1	191 ± 4	168 ± 9	7.77 ± 0.17	6.57 ± 0.43	5.33 ± 0.63
aa/gc	61.7	68.5 ± 0.3	47.2 ± 2.4	46.7 ± 4.1	138 ± 7	137 ± 12	4.35 ± 0.21	4.19 ± 0.44	3.18 ± 0.35
ca/gc	60.0	62.9 ± 0.3	39.6 ± 1.4	41.4 ± 2.0	118 ± 4	123 ± 5	3.05 ± 0.13	3.32 ± 0.56	2.36 ± 0.24
ta/ac	62.3	58.7 ± 1.1	32.6 ± 0.3	33.0 ± 1.2	99 ± 1	100 ± 4	2.14 ± 0.09	1.93 ± 0.13	1.40 ± 0.15
tc/tc	60.0	54.9 ± 0.4	41.4 ± 0.8	41.5 ± 1.4	126 ± 3	127 ± 4	2.25 ± 0.05	2.19 ± 0.12	1.84 ± 0.21

[a] ΔG°_{37} was calculated after extrapolating the average ΔH and ΔS transition values to 37 °C using ΔC_p as described in text.

Table 2: Thermodynamic Parameters Evaluated from DNA Transitions in 5 mM Mg^{2+} and 0.1 M Na^+

DNA	T_m (°C)	peak height $-\Delta H$ (kcal·mol^{-1})	curve fit $-\Delta H$ (kcal·mol^{-1})	peak height $-\Delta S$ (eu)	curve fit $-\Delta S$ (eu)	peak height $-\Delta G^*_{37}$ (kcal·mol^{-1})	curve fit $-\Delta G^*_{37}$ (kcal·mol^{-1})	ΔC_p-corrected $-\Delta G^\circ_{37}$ (kcal·mol^{-1})
− −/− −	72.3 ± 0.1	56.0 ± 1.3	53.9 ± 2.9	162 ± 4	157 ± 8	5.73 ± 0.13	5.40 ± 0.31	4.36 ± 0.5
A/T	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
AA/TT	71.4 ± 0.1	66.5 ± 1.7	63.4 ± 3.5	193 ± 5	184 ± 10	6.64 ± 0.17	6.27 ± 0.36	5.15 ± 0.55
GA/TC	77.1 ± 0.2	67.8 ± 1.5	62.4 ± 1.9	194 ± 4	179 ± 5	7.76 ± 0.17	7.01 ± 0.23	5.47 ± 0.63
ga/ga	71.5 ± 0.3	65.3 ± 2.0	62.0 ± 4.7	190 ± 6	181 ± 14	6.54 ± 0.17	6.01 ± 0.50	4.78 ± 0.53
aa/gc	63.6 ± 0.5	53.4 ± 1.0	53.2 ± 2.0	161 ± 6	159 ± 6	4.30 ± 0.24	4.08 ± 0.18	2.87 ± 0.27
ca/gc	57.5 ± 0.3	41.8 ± 1.4	42.8 ± 2.4	127 ± 4	130 ± 8	2.59 ± 0.09	2.58 ± 0.15	1.96 ± 0.20
ta/ac	53.4 ± 0.8	35.6 ± 1.3	36.3 ± 1.3	109 ± 4	112 ± 4	1.78 ± 0.11	1.70 ± 0.06	1.36 ± 0.18
tc/tc	47.8 ± 0.4	38.2 ± 1.4	38.1 ± 2.5	119 ± 5	119 ± 8	1.29 ± 0.02	1.27 ± 0.11	1.10 ± 0.13

[a] Not determined.

as "c". The T_m of the AA/TT DNA was 79.2 °C, while the T_m of the ga/ga DNA was 77.7 °C.

Table 1 lists thermodynamic parameters of the nine DNAs evaluated from the 1 M Na^+ melting curves using the two van't Hoff methods described earlier. The ΔG^*_{37} values represent free energies calculated at 37 °C assuming that $\Delta H°$ and $\Delta S°$ evaluated in the transition region are independent of temperature. The ΔG°_{37} column lists the free energies assuming temperature dependent $\Delta H°$ and $\Delta S°$. The enthalpy and entropy change at 37 °C were calculated from the average $\Delta H°$ and $\Delta S°$ values evaluated at the T_m's by the two van't Hoff methods using the estimated heat capacity change of 75 cal·K^{-1}·mol^{-1} bp (described below).

$\Delta H°$ values obtained by the two van't Hoff methods were generally very similarl; however, two larger than expected differences were observed. The GA/TC and ga/ga hairpin DNAs gave the largest difference in $\Delta H°$ using the two methods (12.5%) compared to 6% or less for the other DNAs. A characteristic distinguishing these two molecules from the others is a GpA step at positions 5 and 6. The presence of this dinucleotide step may contribute to intermediate melting states in this solvent. We note that the average standard deviation for the peak height method was smaller than the average deviation for the curve fit method (1.2 vs 2.7 kcal·mol^{-1}). Thermodynamic parameters were not obtained by differential scanning calorimetry in 1 M Na^+ since the transitions occurred at a relatively high temperature and it was difficult to define posttransition baselines and obtain reliable parameters.

Table 1 lists T_m values of the hairpin DNAs calculated using the Mfold program (*18*) in 1 M Na^+. Values predicted by Mfold for the five tandem mismatch DNA hairpin molecules vary over a range significantly smaller than that experimentally observed. A comparison of the thermodynamic parameters used by this program for tandem mismatches with those determined from the current work is discussed later.

DNA Transitions in 0.1 M Na^+ and 5 mM Mg^{2+}. Although melting experiments are conventionally conducted in 1.0 M Na^+, intracellular monovalent cation concentration under common physiological conditions is generally lower (~0.2 M), and magnesium is present (*1*). To compare DNA stability parameters evaluated at 1 M Na^+ with parameters evaluated under more physiological conditions, the DNA hairpin transitions were examined in 0.1 M NaCl and 5 mM $MgCl_2$. Table 2 shows the enthalpy and entropy parameters for DNA hairpin formation evaluated by the peak height and the curve fit methods.

The two methods gave very similar results (±4%). The largest difference was seen for the GA/TC DNA (8%). The $\Delta H°$ values determined for the 0.1 M $Na^+$and 5 mM Mg^{2+} buffer were within 12% of the $\Delta H°$ values measured in 1.0 M Na^+ for each of the DNAs studied. As in Table 1, free energy values at 37 °C without and with temperature-corrected $\Delta H°$ and $\Delta S°$ values are given by ΔG^*_{37} and ΔG°_{37} respectively.

DNA Transition Thermodynamics in 0.1 M Na^+. Table 3 lists the results of the analysis of UV-monitored denaturation curves as well as DSC data in 0.1 M Na^+. The two van't Hoff methods for determining thermodynamic parameters produced results that were close to each other. The largest difference observed, for the A/T molecule, was still within experimental error.

Figure 3 shows examples of the molar heat capacity curves from DSC experiments in 0.1 M Na^+. Shown are curves for one duplex molecule (A) and three mismatch molecules (B−

Table 3: Thermodynamic Parameters Evaluated from DNA Transitions in 0.1 M Na^+ Determined by Analysis of UV Denaturation Curves and by DSC

	UV Denaturation							
DNA	T_m (°C)	peak height $-\Delta H$ (kcal·mol^{-1})	curve fit $-\Delta H$ (kcal·mol^{-1})	peak height $-\Delta S$ (eu)	curve fit $-\Delta S$ (eu)	peak height $-\Delta G^*_{37}$ (kcal·mol^{-1})	curve fit $-\Delta G^*_{37}$ (kcal·mol^{-1})	*Cp*-corrected $-\Delta G^\circ_{37}$ (kcal·mol^{-1})
− −/− −	71.0 ± 0.2	62.7 ± 1.7	61.7 ± 1.4	182 ± 5	180 ± 4	6.2 ± 0.18	6.0± 0.15	5.0 ± 0.16
A/T	69.1 ± 0.2	63.8 ± 1.6	57.8 ± 4.7	187 ± 5	170 ± 14	6.0 ± 0.14	5.2 ± 0.47	4.4 ± 0.24
AA/TT	68.3 ± 0.3	69.4 ± 1.8	68.3 ± 3.2	204 ± 5	200 ± 10	6.4 ± 0.17	6.20 ± 0.30	5.1 ± 0.22
GA/TC	73.6 ± 0.3	75.6 ± 3.3	75.7 ± 4.7	218 ± 10	219 ± 14	8.0 ± 0.36	7.9 ± 0.52	6.4 ± 0.41
ga/ga	68.9 ± 0.2	69.5 ± 0.9	66.7 ± 2.9	203 ± 3	196 ± 9	6.5 ± 0.06	6.1 ± 0.30	5.1 ± 0.15
aa/gc	58.0 ± 0.2	56.6 ± 1.6	56.0 ± 3.2	171 ± 5	169 ± 10	3.6 ± 0.12	3.5 ± 0.17	3.1 ± 0.14
ca/gc	50.8 ± 0.4	43.8 ± 2.0	46.6 ± 2.7	135 ± 6	144 ± 8	1.8 ± 0.14	2.1 ± 0.15	1.7 ± 0.14
ta/ac	45.4 ± 0.4	39.1 ± 1.6	40.4 ± 2.9	120 ± 5	127 ± 9	1.0 ± 0.08	1.2 ± 0.13	1.4 ± 0.10
tc/tc	45.4 ± 0.2	49.2 ± 0.5	48.6 ± 1.2	155 ± 2	153 ± 4	1.3 ± 0.03	1.3 ± 0.05	1.1 ± 0.04

	DSC				
DNA	T_m (°C)	$-\Delta H$ (kcal·mol^{-1})	$-\Delta S$ (eu)	$-\Delta G^*_{37}$ (kcal·mol^{-1})	C_p-corrected $-\Delta G^\circ_{37}$ (kcal·mol^{-1})
- -/- -	71.4 ± 0.5	55.5 ± 2.3	160 ± 7	5.88 ± 0.20	4.81 ± 0.49
A/T	68.7 ± 0.1	67.2 ± 1.1	196 ± 3	6.53 ± 0.13	5.40 ± 0.56
AA/TT	69.4 ± 0.1	64.3 ± 2.1	187 ± 6	6.42 ± 0.20	5.11 ± 0.53
GA/TC	73.5 ± 0.2	75.1 ± 4.8	215 ± 14	8.35 ± 0.48	6.92 ± 0.69
ga/ga	68.3 ± 0.2	62.2 ± 4.4	181 ± 13	6.04 ± 0.38	4.98 ± 0.50
aa/gc	58.2 ± 0.1	54.0 ± 1.9	162 ± 6	3.76 ± 0.17	3.24 ± 0.32
ca/gc	51.6 ± 0.1	43.3 ± 1.5	133 ± 4	2.26 ± 0.16	1.80 ± 0.18
ta/ac	46.2 ± 0.5	36.2 ± 3.2	113 ± 10	1.22 ± 0.16	1.07 ± 0.09
tc/tc	44.9 ± 0.4	39.6 ± 1.4	124 ± 4	1.20 ± 0.15	1.06 ± 0.10

D). The area under each curve was determined after fitting a quadratic polynomial baseline (dashed lines). This area along with the temperature value at the curve peak was used to obtain calorimetric thermodynamic parameters listed in Table 3. Figure 4 illustrates the difference between the van't Hoff and calorimetric enthalpies determined for the DNA molecules in 0.1 M Na^+. The van't Hoff values are represented by solid circles, while the calorimetric enthalpies are open circles. Gray shading shows a 15% range on either side of the van't Hoff values. Except for the tc/tc DNA, the calorimetric enthalpies fall well within 15% of the van't Hoff enthalpies. For the tc/tc molecule, the difference is 20%.

Estimating Heat Capacity Change from DNA Hairpin Transitions. Figure 3B shows linear baselines to the pre- and posttransition regions for a DSC curve of the tc/tc DNA hairpin and the extrapolated difference in heat capacity, ΔC_p, at the T_m. The value of ΔC_p is 280 cal·K^{-1}·mol^{-1}. Similar analyses of other DNA DSC curves produced small and positive ΔC_p values ranging from 250 to 960 cal·K^{-1}·mol^{-1} of oligomer. The value obtained for any given DNA was not, however, highly reproducible. Slight differences in buffer scans and the strong influence of subtracting buffer scans from sample scans on the pre- and posttransition slopes made accurate evaluation of ΔC_p difficult. Figure 3A, for example, illustrates a DSC curve of the AA/TT DNA where the heat capacity change between helix and coil states appears to be zero. Other DSC curves for this DNA gave ΔC_p values ranging from 190 to 550 cal·K^{-1}·mol^{-1} of oligomer. Averaging the ΔC_p values determined from 20 transitions of hairpin DNAs with 10 paired bases in the stem produced an average value of $\Delta C_p = 42 \pm 27$ cal·K^{-1}·mol^{-1} of bp.

Another method for estimating ΔC_p from the data is to plot the enthalpy and entropy changes of the DNA hairpin transitions as a function of the T_m and ln T_m values, respectively (*2*, *11*). Figure 5 shows the plot of the calorimetric ΔH data vs T_m for the DNA hairpins with 10 paired bases. The slope of the linear regression is 1214 ± 86 cal·K^{-1}·mol^{-1} of oligomer with an R^2 of 0.97. A plot of the entropy data produces a linear regression slope of 1040 ± 98 cal·K^{-1}·mol^{-1} of oligomer with a similar R^2. Rouzina and Bloomfield (*11*) derived equations relating the two slopes to ΔC_p and the average transition entropy, ΔS:

$$\partial \Delta H(T_m)/\partial T_m = p\Delta S + \Delta C_p \quad (5a)$$

$$\partial \Delta S(T_m)/\partial \ln T_m = -(1-p)\Delta S + \Delta C_p \quad (5b)$$

where p is the proportion ($0 < p < 1$) by which the perturbation giving rise to the change in enthalpy and entropy is partitioned between enthalpy and entropy; $\Delta H = p\Delta G$, and $T\Delta S = -(1-p)\Delta G$.

If the changes in the DNA hairpin sequence produce purely enthalpic perturbations, $p = 1$, and the slope evaluated from the entropy data equals ΔC_p . If p is uncertain, one may estimate ΔC_p as the arithmetic mean of the two slopes (*11*). Assuming the latter and dividing the heat capacity change by the 10 paired bases in the stem, one obtains $\Delta C_p = 113 \pm 9$ cal·K^{-1}·mol^{-1} of bp. This value and the one determined from the DSC curves are in the range of values obtained in previous studies on duplex DNAs (*2*, *11*, *16*). Given the experimental uncertainties in evaluating ΔC_p the average of these two values, $\Delta C_p \approx 75$ cal·K^{-1}·mol^{-1} of bp, was employed to calculate the temperature-dependent enthalpy and entropy changes from the T_m values to $T = 310$ K in Tables 1−3 using the equations

$$\Delta H(T_m) = \Delta H(T) + \Delta C_p(T_m - T) \quad (6a)$$

$$\Delta S(T_m) = \Delta S(T) + \Delta C_p \ln(T_m/T) \quad (6b)$$

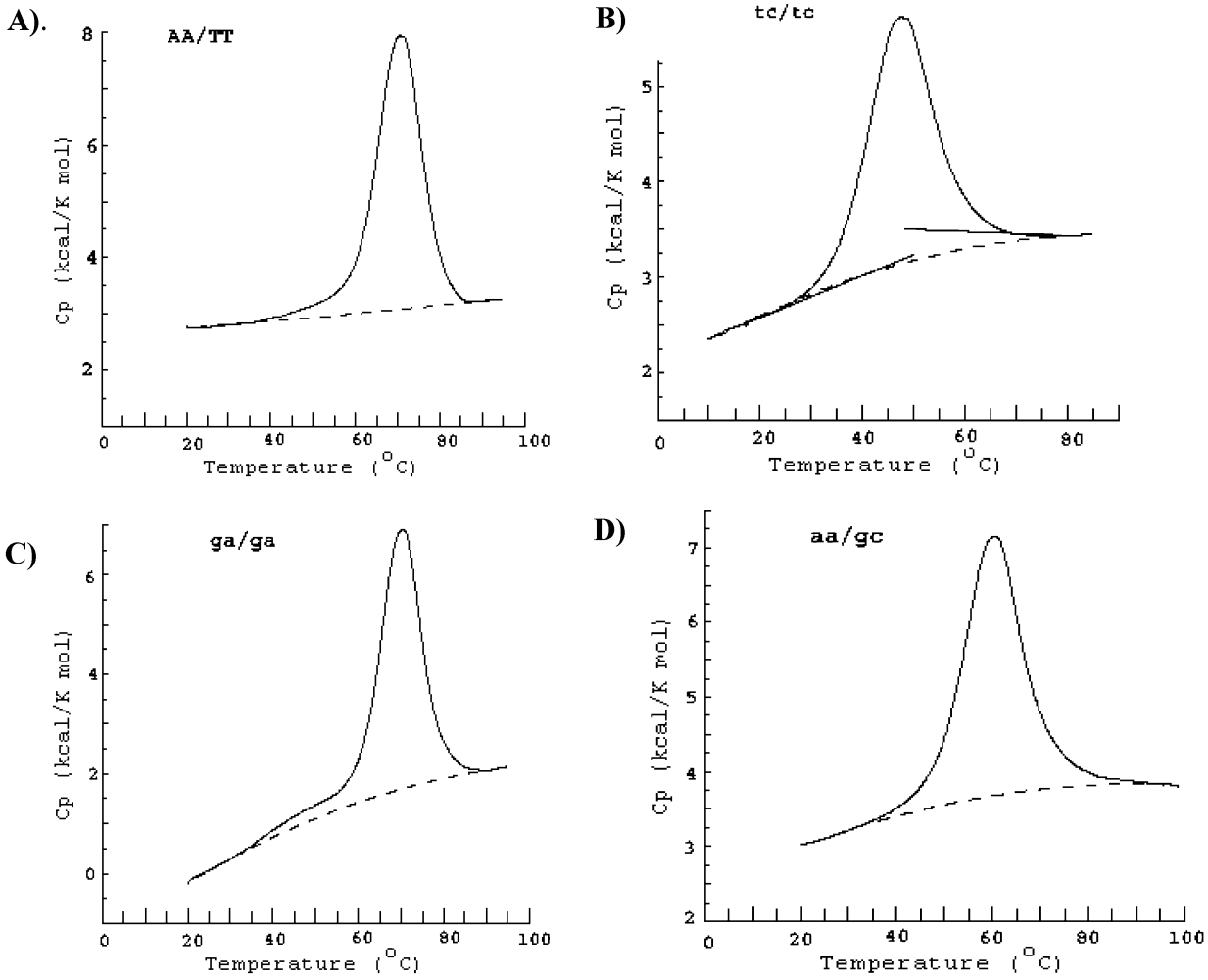

FIGURE 3: Representative molar heat capacity curves with baselines for the DNAs: (A) AA/TT; (B) tc/tc; (C) ga/ga; (D) aa/gc.

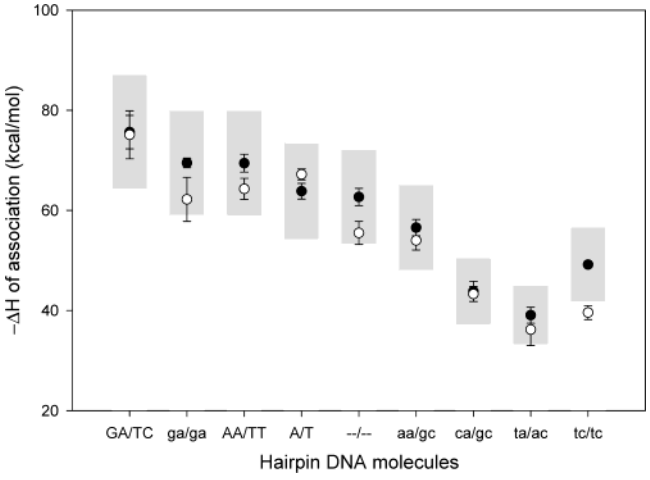

FIGURE 4: Comparison of measured van't Hoff and calorimetric enthalpy values for transitions in 0.1 M Na^+ solvent. Filled circles represent the van't Hoff values, while open circles represent the calorimetric data. Gray boxes show a 15% range on either side of each of the van't Hoff values, not including error bars.

Determination of Internal Loop Contribution. Table 4 lists the free energy of the tandem mismatch loops at 37 °C. The values were calculated using the equation

$$\Delta G_{\text{loop}} = \Delta G_{\text{hairpin with loop}} - (\Delta G_{\text{hairpin without loop}} - \Delta G_{\text{interrupted base pair}}) \quad (7)$$

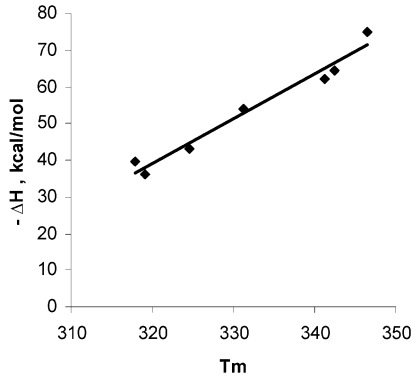

FIGURE 5: Plot of enthalpy change measured by DSC of DNA hairpins with 10 paired bases in stem vs their T_m values.

The last term of the equation ($\Delta G_{\text{hairpin without loop}} - \Delta G_{\text{interrupted base pair}}$) subtracts the energetic contribution of the parts of the DNA molecule not involved in the loop. The free energy of the −/− DNA molecule represents the "hairpin without the loop" and previously measured stacking free energy of the CG/CG doublet (*12*) was used for the interrupted base pair. The first column in Table 4 lists loop free energies (ΔG^*_{loop}) calculated from free energies that assumed temperature-independent enthalpy and entropy changes. The second column lists loop free energies ($\Delta G^\circ_{\text{loop}}$) calculated from enthalpy and entropy changes evaluated at 37 °C using eq 6 and the estimated heat capacity

Table 4: Loop Free Energies of Tandem Mismatches Evaluated for 1 M Na^+, 0.1 M Na^+ and 5 mM Mg^{2+}, and 0.1 M Na^+ Data Using Eq 7[a]

1 M Na^+

tandem mismatch	ΔG^*_{loop} (kcal·mol^{-1})	ΔG°_{loop} (kcal·mol^{-1})
ga/ga	−3.2	−3.0
aa/gc	−0.3	−0.7
ca/gc	0.8	0.1
ta/ac	2.0	1.1
tc/tc	1.8	0.7

0.1 M Na^+ and 5 mM Mg^{2+}

tandem mismatch	ΔG^*_{loop} (kcal·mol^{-1})	ΔG°_{loop} (kcal·mol^{-1})
ga/ga	−3.0	−2.6
aa/gc	−0.7	−0.2
ca/gc	1.0	0.6
ta/ac	1.8	1.0
tc/tc	2.3	1.3

0.1 M Na^+ (DSC Data)

tandem mismatch	ΔG^*_{loop} (kcal·mol^{-1})	ΔG°_{loop} (kcal·mol^{-1})
ga/ga	−2.1	−2.1
aa/gc	0.2	−0.3
ca/gc	1.7	1.1
ta/ac	2.75	1.85
tc/tc	2.8	1.8

[a] ΔG^*_{loop} are values determined assuming temperature-independent enthalpy and entropy changes, and ΔG°_{loop} are values determined after extrapolating enthalpy and entropy changes to 37 °C using average heat capacity change for hairpin to coil transition.

change, 75 cal·mol^{-1} of bp. The differences between the temperature-dependent and temperature-independent loop free energies are generally small. The range of values for the five tandem mismatches narrows after correcting for $\Delta C_p \neq 0$. The hierarchy of stability for the tandem mismatches based on T_m values or loop free energies was similar for the three solvents.

DISCUSSION

The results from this study indicate that the free energy contribution of a tandem mismatch sandwiched between GC base pairs depends on sequence and covers a range of ~4 kcal·mol^{-1}. Analysis of UV monitored denaturation curves and DSC for the 0.1 M Na^+ solvent indicate a loop free energy ranging from −2.1 to +1.9 kcal·mol^{-1} (Table 4). For the 1 M Na^+ solvent at 37 °C the loop free energies for ga/ga and aa/gc are stabilizing (−3.0 and −0.7 kcal·mol^{-1}) while the ca/gc, tc/tc, and ta/ac sequences are destabilizing (0.1, 0.7, and 1.1 kcal·mol^{-1}). We note that loop free energies employed in the Mfold program (*18*) for the five tandem mismatch loops range from 1.3 to 1.7 kcal·mol^{-1}. As mentioned earlier, the T_m values predicted by Mfold for the five tandem mismatch DNA hairpin molecules in 1 M Na^+ varied over a range significantly smaller than that observed experimentally (Table 1).

The order of stability of the five tandem mismatches based on the T_m values of the DNA hairpins was the same in the three solvents and was in agreement with the order of T_m values determined by TGGE (*6*). In the TGGE study, tandem mismatch loop free energies were evaluated relative to the sequence CAAG/CTTG. The $\delta(\Delta G)$ value for the tandem mismatches ga/ga, aa/gc, ca/gc, ta/ac, and tc/tc were −0.4, 1.4, 1.9, 2.5, and 3.0, respectively. From Table 1, corresponding values for 1 M Na^+ are −0.3, 2.3, 3.3, 4.2, and 3.6. Although the range of values is similar, the differences cannot be reconciled by a scaling factor and may be due to the different solution conditions employed in the two studies, assumptions made in the TGGE analysis, or both.

Loop free energy values from the current study may be used to improve stability predictions of DNA hybrids involving tandem mismatches in the context CXYG/CWZG. Since only six of the 78 possible tandem mismatches for this one symmetrical context were evaluated, parameters on other tandem mismatch sequences are needed. The combined use of TGGE to determine the relative stabilities of a large set of sequences and a more detailed thermodynamic analysis of a more limited set may provide the best approach. To a first approximation, loop free energies of tandem mismatches evaluated only by TGGE may be interpolated from their stability relative to sequences measured by both TGGE and a more detailed approach. Data on 50−100 tandem mismatches with different closing base pairs can be used to develop models to predict stabilities of all 1176 tandem mismatches (*17*).

Li and Agarwal (*7*) evaluated thermodynamic parameters governing the melting transitions of the hexamer DNA duplexes GCGAGC/GCTCGC and GCgaGC/GCgaGC in 1.0 M Na^+. The latter DNA contains tandem g−a mismatches with the same closing base pairs studied here. From their thermodynamic data and the stacking free energy of base pair doublets (*12*), we evaluated the loop free energy at 37 °C for the GgaC/GgaC segment using an equation analogous to eq 7. We note that Li and Agarwal evaluated enthalpy change using a van't Hoff analysis and assumed $\Delta C_p = 0$. Since the transition T_m's of the duplexes that they examined were close to 37 °C, correction for nonzero ΔC_p will have a small impact on the parameters determined at 37 °C. From their data, one obtains a loop free energy of $\Delta G^\circ_{loop} = -3.8$ kcal·mol^{-1}, 0.8 kcal·mol^{-1} more negative than the value determined in the current study. If we considered only the van't Hoff peak height data in evaluating the transition enthalpy of the ga/ga DNA hairpin molecule (Table 1), our ΔG°_{loop} value at 37 °C is −3.5 kcal·mol^{-1} in close agreement with the Li and Agarwal data.

ACKNOWLEDGMENT

We thank Gemma Ellis and Karissa McClinic for technical assistance in DNA purifications.

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BI049161W