Thermodynamics of dT−dT Base Pair Mismatching in Linear DNA Duplexes and Three-Arm DNA Junctions†

Min Zhong,‡ Luis A. Marky,* and Neville R. Kallenbach*

Department of Chemistry, New York University, New York, New York 10003

Donald W. Kupke

Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908

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ABSTRACT: We have used a combination of magnetic-suspension densimetry and calorimetry to derive complete thermodynamic profiles, including volume changes, for the formation of linear DNA duplexes and three-arm branched DNA junctions, from their component strands, with and without dT−dT mismatches. The formation of each type of complex at 20 °C is accompanied by a favorable free energy, with a favorable enthalpy term partially compensated by an unfavorable entropy. Formation is associated also with net uptake of water molecules. Using the formation of the fully-paired linear duplex or three-arm junction as reference states, we can establish a thermodynamic cycle in which the contribution of the single-strand species cancels. From this cycle, we determine that substitution of dA for dT has a differential free energy of $\Delta\Delta G°$ of +2.4 kcal mol^{-1} for mismatched duplex and +2.0 kcal mol^{-1} (on the average) for the mismatched junction. These unfavorable differential free energies result from an unfavorable enthalpy, partially compensated by a favorable entropy, and a negative $\Delta\Delta V$. The free energies in the two cases have signs opposed to those of $\Delta\Delta V$, a situation that implicates hydration changes in creating the mismatch. When the $\Delta\Delta V$ terms are normalized by the total number of base pairs involved, the immobilization of structural water molecules (and/or substitution of electrostricted for hydrophobic water molecules) is about 7 times greater for junctions than duplexes. This is consistent with more extensive hydrophobic hydration of branched DNA structures than of duplexes.

Physical or chemical damage affects the integrity of the duplex structure of DNA, resulting in formation of non-Watson−Crick base pair mismatches, unpaired bases (bulges), base modification, and/or strand scission (Kornberg, 1980). Incomplete or erroneous repair of such lesions is associated with mutagenesis (Friedberg et al., 1995). A complete understanding of the mechanisms of mutations has to include detailed analysis of the structure of duplexes containing different lesions as well as a detailed description of the thermodynamics of these lesions.

A variety of mismatches in several local base pair stack environments have been investigated structurally and thermodynamically. Tinoco's group has characterized dT·dT and dA·dA mismatches flanked by dC·dG base pairs (Arnold et al., 1987); the dT·dT mismatch is intercalated in the helix causing limited distortion to the helix backbone while the dA·dA mismatch must tilt the helix axis in order to reduce the overlap of the N6 amino groups with no evidence of hydrogen bonding. The conformation of dG·dA mismatches is highly dependent on the flanking sequences (Cheng et al., 1992). The predominant conformation is G(anti)·A(anti), the most stable being the one flanked by pyr·pur and pur·pyr base pairs. Pyr·pyr mismatches flanked by dC·dG base pairs are stacked inside the helix, each pyrimidine base with an *anti* glycosidic bond conformation.

There are two reports that deal with the effect of mismatches on the thermodynamic stability of the host DNA. One is the effect of mismatches in the vicinity of a dAT/dAT base pair stack (Aboul-ela et al., 1985), and the second set concerns mismatches in a dTA/dAT base pair stack environment (Gaffney & Jones, 1989). The following trends have been observed: the most stable are G−G, T−G, and A−G, while A−A mismatches are always destabilizing. For these and other mismatches, the effect on stability of the host duplex depends on the flanking nucleotide sequences. Specifically, a T−T mismatch is more destabilizing in a dAT/dAT background than in dTA/dTA or dCG/dGC (Rentzeperis and Marky, unpublished results). In each of the above cases, stabilization of one mismatch relative to a second is enthalpic in origin. Overall, the magnitudes of the destabilization in free energy are comparable, while the average enthalpies depend on the control molecules: for a single mismatch values range from +15.6 kcal/mol (Aboul-ela et al., 1985) to +11.4 kcal/mol (Gaffney & Jones, 1989). These differences may be explained in terms of the local base pair stacking environment into which the mismatch inserts, the relative position of the mismatch with respect to the ends of a short helix, and the overall thermodynamic stability of the host duplex.

The conformational manifold of duplex DNA includes a variety of conformations outside the classical B helix that

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* Authors to whom correspondence should be addressed.

‡ Present address: Department of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20892.

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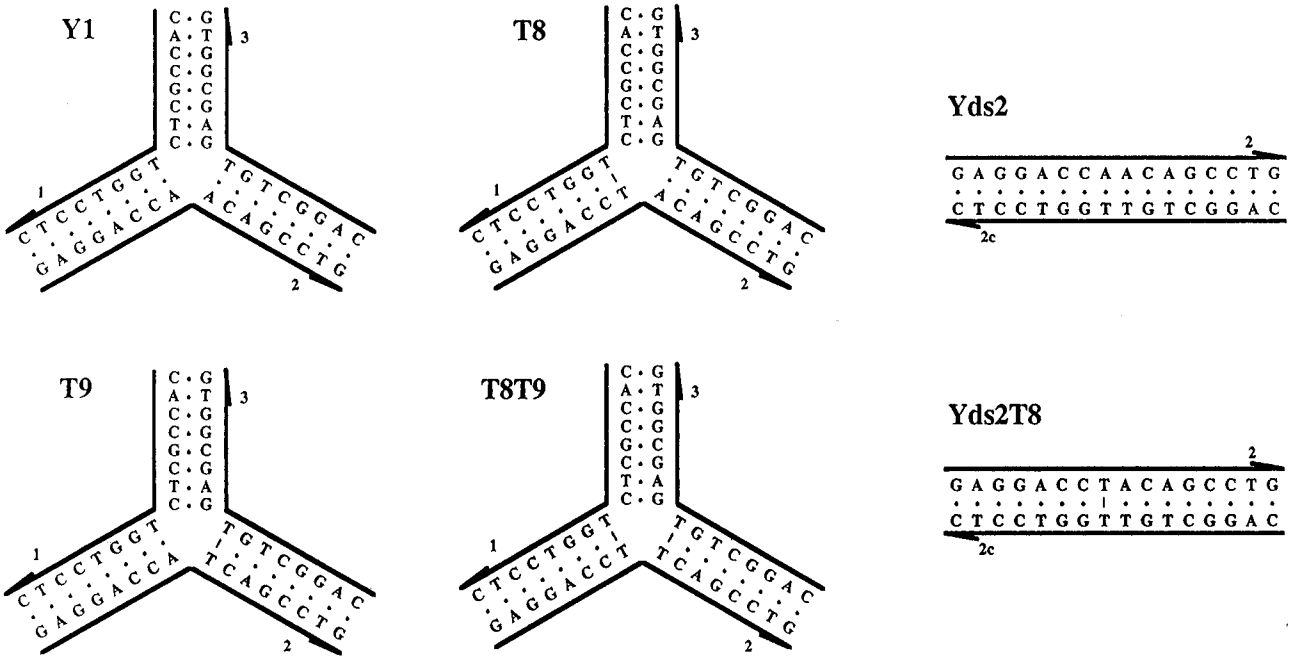

FIGURE 1: Sequences and schematic representations of the DNA molecules used in this investigation. Individual strands are designated as Y_{101}, Y_{102}, and Y_{103} in Y_1, for example, counterclockwise from 12 o'clock. Letters following these indicate the strands with mismatches, e.g., Y_{102T8}.

have functional implications. Sequences which favor bending of the helix axis can profoundly influence the interaction between transcription factors and RNA polymerase (Wu & Crothers, 1984). Branched structures created by the intersection of three or four duplexes can arise transiently in DNA as a result of recombination, as in Holliday junctions (Kallenbach & Zhong, 1994), or as intermediates in the process of repair of damaged sequences. Three-arm branched DNA structures at the ends of parvovirus DNA, formed by palindromic repeats, play an essential role in initiation of viral replication (Corsini et al., 1996). Finally, replication or recombination of DNA sequences containing multiple simple-tandem repeats probably involves branched intermediates, as detected by HMG protein binding (Zhao et al., 1996). It is therefore of interest to understand the structural and thermodynamic properties of both linear and branched states in DNA.

The thermodynamics of branch formation in immobile four-arm DNA junctions has been investigated by scanning calorimetry and by use of an equilibrium gel mobility assay (Lu et al., 1992). Formation of the four-arm branch from two DNA duplexes is an unfavorable process, in which the enthalpy and entropic terms ($T\Delta S$) are both positive, hence partially compensate (Lu et al., 1992). In contrast to elementary base-pairing interactions, there is a large apparent ΔC_p term associated with branch formation, such that branching which is only slightly unfavorable at 20 °C becomes strongly unfavorable at temperatures near 50 °C. The resulting picture of branch formation in a duplex indicates a loss of favorable enthalpy originating from reduced base stacking interactions, with associated large-scale changes in counterion distribution, hydration, or solvation as additional negatively charged strands come together.

The X-shaped structure of a four-arm branched junction in the presence of Mg^{2+} (Cooper & Hagerman, 1987; 1989; von Kitzing et al., 1990) entails close apposition of the two duplexes comprising the arms of the X (Churchill et al., 1988; von Kitzing et al., 1990). Timsit and Moras (1991) have described a groove−backbone interaction between adjacent DNA duplexes in a tetragonally packed crystal form of DNA. The interaction they observe is "insertion" of the backbone of one duplex into the groove of a second, as would happen near the branch in a four-arm structure, and they proposed that such an interaction could mediate formation of branches. While the geometry in a three-arm junction differs from that of four-arm species, the two share the problem of apposing charged strands closely in space, with the associated ionic and hydration changes this requires. In both cases, there are potentially larger-scale changes in ion binding and hydration relative to duplex formation. We show here that this is the case for three-arm junction formation, and that base mismatches at the branch are associated with much larger volume changes than in duplexes, when normalized per base pair present.

We present here also an analysis of the thermodynamics and particularly the solvation changes that result from substituting a canonical dA·dT base pair for a dT·dT mismatch in the context of a 16mer duplex and a three-arm DNA junction, using a combination of magnetic-suspension densimetry, differential scanning calorimetry, and isothermal titration calorimetry. The volume change on formation of each of these DNA complexes has been measured by mixing appropriate sets of complementary strands (see Figure 1). The formation of each mismatched molecule from the fully-paired duplex is accompanied by an unfavorable free energy resulting from the partial compensation of exothermic enthalpies and unfavorable entropies and uptake of water molecules. By setting up appropriate thermodynamic cycles, we find that substitution of dA for dT, to form dT·dT mismatches, in these molecules yields $\Delta\Delta G°$ values of +2.4 kcal mol^{-1} and +2.0 kcal mol^{-1} (on the average) for creating a mismatch in a duplex and three arm junction, respectively. These positive free energy terms are accompanied by

negative differential volume changes, $\Delta\Delta V$. Based on previous correlations (Zieba et al., 1991; Marky et al., 1996), this is consistent with greater inmmobilization of structural water molecules (and/or substitution of electrostricted for hydrophobic water molecules) in the branched structures than in duplexes.

MATERIALS AND METHODS

Materials. Deoxyoligonucleotides used in this study were synthesized on an automated DNA synthesizer, deprotected by routine phosphoramidite procedures (Caruthers, 1982), and purified by ion exchange HPLC. The concentration of strands in stock solutions was determined spectrophotometrically at 260 nm and 80 °C, using the nearest-neighbor values of Cantor et al. (1970). The formation of the DNA complexes shown in Figure 1 in an appropriate buffer were confirmed by native polyacrylamide gel electrophoresis as described previously (Zhong et al., 1993). All measurements were carried out in a buffer containing 20 mM sodium cacodylate (pH 7.0), 100 mM NaCl, and 1 mM $MgCl_2$.

Magnetic Suspension Densimetry. The volume change, ΔV, that accompanies the formation of each complex was determined by measuring the density on weighed samples in a magnetic-suspension densimeter that has been described previously (Gillies & Kupke, 1988). The ΔV value is calculated by measuring the mass and the equilibrium density of solutions before and after mixing; the observed change in volume, Δv, upon mixing i complementary strands to form a particular DNA helical structure is given by

$$\Delta v = m_{\mathrm{mix}}/\rho_{\mathrm{mix}} - \Sigma(m_i/\rho_i),\ i = 2 \text{ or } 3 \qquad (1)$$

where m is the mass in grams and ρ is the density of the solutions in grams per milliliter. The density of each sample is obtained by relating the measured voltage to the straight-line calibration equation of voltage versus density of KCl solutions of known density. The independent density values do not need to be of high absolute accuracy since it is their differences which are required for Δv. Since the masses are identical on both sides of the minus sign, small weighing errors do not contribute significantly to the value of Δv. With repetitive samples the density is measured with a precision of better than 5×10^{-6} g/mL. The value of Δv in milliliters is then reduced to that per mole of the limiting reagent to give ΔV. To ensure that the complexes are formed completely, weighed duplex samples were heated to 55 °C and cooled to room temperature in tightly closed 0.4 mL polyethylene tubes to prevent evaporation. The temperature was held at 20 ± 0.001 °C in a specially constructed bath. In a typical experiment, equal volumes of solutions of each strand were mixed to give 400 μL of the final solution to allow for rinsings and duplicate measurements; two or more such mixtures were prepared routinely, and the mean value was taken as the one reported for ΔV. In these experiments the concentrations of the individual strands were ~3 mM in phosphate and were mixed at equimolar ratios. Thus, any contributions from solute−solute interactions to the ΔV were assumed to be negligible.

Titration Calorimetry. The measurement of the heat of mixing complementary strands and the corresponding dilution heats was carried out using the Omega titration calorimeter (Microcal Inc., Northampton, MA). A detailed description of this instrument has been presented elsewhere (Wiseman et al., 1989). For DNA duplexes, solutions of one strand were used to titrate the complementary strand to form each duplex. For three-arm DNA junctions, pairs of strands were first titrated to determine heats for forming one arm of the junction, and then the complemetary third strands were used to titrate the two-stranded complexes to form three-arm junctions. A 100 μL syringe was used for the titrant; mixing was effected by stirring this syringe at 400 rpm. Typically, *ca.* 6.5 μM samples of DNA in 1.4 mL of buffer were titrated using 5−15 injections of 5−8 μL each of samples of the complementary strand at 100 μM in the same buffer solution at temperatures of 10, 15, and 20 °C, respectively. Determination of the temperature dependence of the enthalpy change allows us to determine the apparent heat capacity change (ΔC_p). The instrument was calibrated by means of a standard electric pulse.

Table 1: Enthalpies of Y_1 Formation at 20 °C (in kcal/mol)

titrations	ΔH_1[a]	ΔH_2[b]	$\Delta H_{\mathrm{total}}$[c]
$Y_{103} + (Y_{101} + Y_{102}) = Y_1$	−43.8	−48.2	−92.0
$Y_{102} + (Y_{101} + Y_{103}) = Y_1$	−47.5 (−46.3)	−44.5	−92.0
$Y_{101} + (Y_{102} + Y_{103}) = Y_1$	−33.0	−60.5	−93.5

[a] ΔH_1 is the enthapy change due to formation of complex in parentheses. [b] ΔH_2 is the second titration resulting in formation of a three arm junction. In each experiment the titrant is listed first. [c] The sum of two titrations. Titrations were carried out at 20 ± 0.2 °C. In this particular case total enthalpy values of the forming three-arm DNA junction Y_1 do not depend on the titrating schemes.

The isothermal titration calorimetry (ITC) measurements were designed to obtain primarily the enthalpy of forming each complex and their stoichiometries. The enthalpy is obtained by averaging the heats of the initial four to five injections or in some cases by deconvolution of the resulting calorimetric isotherms, representing total heat vs total titrant (or strand) concentration using a three-parameter fit for enthalpy change ΔH, stoichiometry, and binding constant (K). These three parameters are obtained interactively using standard Marquardt algorithms. We estimate that either procedure gives enthalpy values to an error of ±3%, and this is the error for the parameters other than ΔV reported in Tables 1 and 2. The ΔV values are conservatively estimated to be within ±5%, allowing for fluctuations in temperature and the accuracy of concentration measurements (Gillies & Kupke, 1988). It should be noted that the accurate range for determining binding constants (K) using the Omega calorimeter extends only to *ca.* 10^5 due to the concentrations required (the upper limit of detection for K is $1/C_{\mathrm{strands}}$). Thus, isothermal calorimetry with the present instrument is not optimal for accurate determination of association constants for formation of the DNA complexes of this study, which are estimated to be ~10^6−10^7. Instead, we calculate the free energy change $\Delta G(T)$ for DNA complex formation by using the value from DSC experiments (Zhong et al., 1992), corrected for the unfavorable contribution of base−base stacking interactions of the single strands, as described previously (Rentzeperis et al., 1994; Marky et al., 1996):

$$\Delta G(T) = \Delta G_{\mathrm{DSC}}(T_{\mathrm{m}})\Delta H(T)/\Delta H(T_{\mathrm{m}}) \qquad (2)$$

where $\Delta H(T)$ is the enthalpy change for formation of DNA complex at temperature T derived from the isothermal titration calorimetry (ITC) measurement. $\Delta H(T_{\mathrm{m}})$ is the enthalpy value measured from differential scanning calo-

Table 2: Complete Thermodynamic Profiles for Forming DNA Complexes at 20 °C

DNA complex	ΔC_p[a] (kcal/mol K)	ΔG[b] (kcal/mol)	ΔH[c] (kcal/mol)	$\Delta\Delta H$[d] (kcal/mol)	$-T\Delta S$[e] (kcal/mol)	ΔV (mL/mol)	$\Delta\Delta V$[f] (mL/mol)
Y_1	−3.7	−8.9	−92.5	−	83.6	−80	−
$Y_{102\mathbf{T8}} + (Y_{101} + Y_{103}) = \mathbf{T8}$	−2.8	−7.1	−79.0	13.5	71.9	−283	−203
$Y_{102\mathbf{T9}} + (Y_{101} + Y_{103}) = \mathbf{T9}$	−3.0	−6.9	−78.0	14.5	71.1	−250	−170
$Y_{102\mathbf{T8T9}} + (Y_{101} + Y_{103}) = \mathbf{T8T9}$	−2.1	−6.7	−70.0	22.5	63.3	−341	−261
Y_{ds2}	−2.4	−10.4	−70.0	−	59.6	−99	−
$Y_{ds2\mathbf{T8}}$	−1.9	−8.0	−61.0	9.0	53.0	−118	−19

[a] ΔC_p values are calculated for the formation of three-arm DNA junctions by linear least-squares fitting of the ΔH versus temperature (10, 15, and 20 °C). [b] These values are calculated from equation $\Delta G(T) = \Delta G_{DSC} \cdot \Delta H(T)/\Delta H(T_m)$, assuming that there is no entropy change involved in the single-strand base stacking interaction. $\Delta H(T_m)$ is the enthalpy change for formation of DNA complex measured by differential scanning calorimetry (DSC). ΔG_{DSC} is the free energy change for formation of DNA complex at 20 °C calculated from $\Delta G_{DSC} = \Delta H(T_m) - T\Delta S(T_m)$. [c] ΔH values at 20 °C are calculated from the equation $\Delta H(T) = \int \Delta C_p \, dT$, with 3% experimental errors. [d] The $\Delta\Delta H$ values are relative to the corresponding enthalpies for Y_1 or Y_{ds2}. [e] $T\Delta S = \Delta H(T) - \Delta G(T)$. [f] The $\Delta\Delta V$ values are relative to the volume changes for Y_1 or Y_{ds2}.

rimetry (DSC). $\Delta G_{DSC}(T)$ is the free energy change for formation of DNA complex in DSC measurements derived from the Gibbs equation (Zhong et al., 1993). The $T\Delta S$ terms for ITC can be calculated from the relationships $T\Delta S = \Delta H(T) - \Delta G(T)$.

RESULTS AND DISCUSSION

Duplex and Junction Formation from Their Component Single Strands Results in Uptake of Water Molecules. Using a magnetic suspension densimeter, we have measured directly the change in volume at 20 °C associated with the formation of each molecule from mixing appropriate sets of complementary strands. The volume change that acompanies such a reaction reflects the differences in molar volume of water around the solutes relative to bulk water. The results are listed in Table 2, each entry representing an average of at least two determinations. The negative ΔV values in this table indicate that the formation of each duplex and Y junction is accompanied by an uptake of water molecules; the different ΔV values imply that the mismatched molecules differ from their respective control molecules significantly in their degree of hydration. In formation of a DNA complex from mixing its component strands, two dominant but opposing influences need to be taken into account: (i) the higher immobilization of structural water by the single strands due to the greater exposure of nucleotides to solvent; and (ii) the higher immobilization of electrostricted water by the complex due to its higher charge density. Do the observed differences in hydration correspond to an uptake of electrostricted water or structural water or to a substitution of one kind of water molecules for the other? An answer to this question can be given if the measured ΔV values are correlated with standard thermodynamic profiles for the same reactions of complex formation. This is because heat is released in the immobilization of electrostricted water (Gasan et al., 1990) while for the release of structural water this energetic contribution is nearly zero or slightly positive. The hypothesis is that structuring water to improve packing around hydrophobic groups eliminates void spaces, in contrast to electrostriction which compresses the water dipoles (Conway, 1981). At the same time, this reorganization of water molecules increases the order of the system, yielding an unfavorable entropy term.

Evaluating Isothermal Titration Calorimetry Schemes. The heat of duplex formation is determined directly by titrating a single strand with its complementary strand, or vice-versa; however, to evaluate the effects of different titration schemes on the measured heats of forming three-stranded complexes, three titration calorimetry measurements were carried out in which the three-arm DNA junction Y_1 forms at 20 °C:

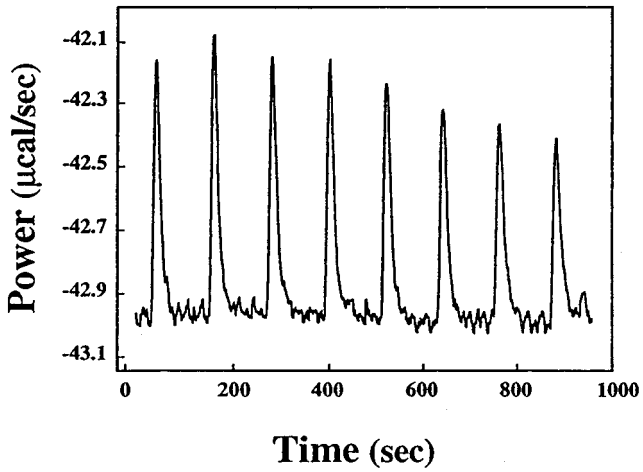

FIGURE 2: Typical calorimetric titration curve of strand Y_{103} with strand Y_{101} (forming one arm of the Y_1 junction). Each peak corresponds to an 8 μL injection of Y_{101} solution (104.8 μM in strands) to 1.4 mL of Y_{103} in the cell (6.5 μM).

$$Y_{103} + (Y_{101} + Y_{102}) = Y_1 \quad (3)$$

$$Y_{102} + (Y_{101} + Y_{103}) = Y_1 \quad (4)$$

$$Y_{101} + (Y_{102} + Y_{103}) = Y_1 \quad (5)$$

where the titrant is listed first. A typical titration curve obtained with the Omega unit is shown in Figure 2; here, strand Y_{101} is added to strand Y_{103} to form one arm of the Y_1 junction. Prior to saturation, the heats released in the initial injections for a particular type of binding site are independent of the total concentration of added ligand and can be used directly to estimate the molar binding enthalpy (ΔH). After correction for the heat of dilution of the titrant, measured in a separate experiment, ΔH is calculated by averaging the first three to four injections. As shown in Table 1, the total enthalpy values of forming a three-arm DNA junction Y_1 do not depend on the titrating schemes, even though the enthalpies for forming the intermediate species vary significantly. Formation of the three-arm DNA junction Y_1 results in a 92.5 ± 2.8 kcal/mol enthalpy change at 20 °C.

The significant observation in these titrations is that we observe exothermic enthalpies for the formation of each

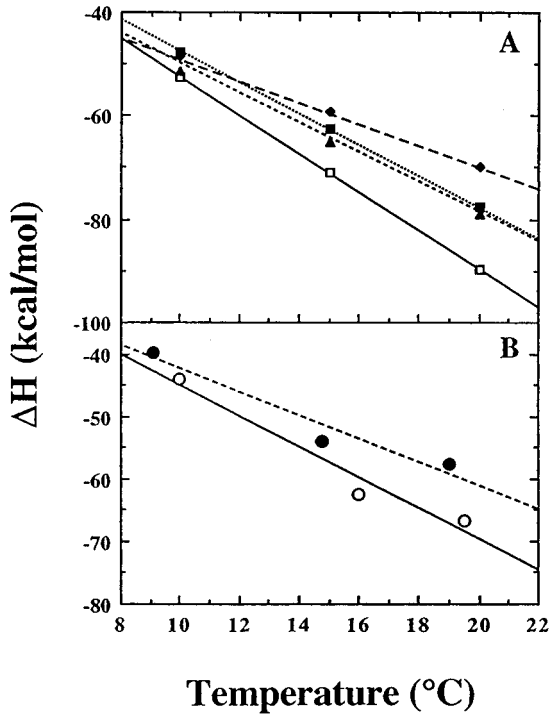

FIGURE 3: Plots of enthalpy change versus temperature. The data points are shown with linear least-squares fitting described by the following equations: (A) formation of three-arm DNA junction Y_1 (□), $\Delta H = -3.7(T) - 15.45$; **T8** (▲), $\Delta H = -2.76(T) - 23.77$; **T9** (■), $\Delta H = -2.99(T) - 17.74$; **T8T9** (◆), $\Delta H = -2.13(T) - 27.3$; and (B) formation of DNA duplex Y_{ds2} (○), $\Delta H = -2.44(T) - 20.73$; $Y_{ds2\mathbf{T8}}$ (●), $\Delta H = -1.86(T) - 23.95$.

helix, corresponding to exothermic contributions from formation of base pair stacks, hydrogen bonding, and uptake of water molecules. These terms override the endothermic contribution from disrupting base−base stacking interactions of the single strands; the differential uptake of cations normally has a negligible contribution to the enthalpy. The overall magnitude of these enthalpies depends on the nature of the structure formed.

Similar titrations at three different temperatures (from 10 to 20 °C) yield the heat at each temperature. The lower the temperature, the lower the heat that is evolved. This indicates that the unfavorable heat contribution from single-stranded base stacking interactions is larger at lower temperatures, yielding an apparent heat capacity effect (see Figure 3A and B).

Complete Thermodynamic Profiles for the Formation of Duplex and Junctions. Complete thermodynamic profiles obtained from isothermal titration calorimetry and volumetric measurements at 20 °C, as well as standard thermodynamic profiles that were reported earlier from differential scanning calorimetry (Zhong et al., 1993), are presented in Table 2. By complete profiles, we mean specification of the complete set of parameters $\Delta G°$, $\Delta H°$, and $\Delta C_p°$, $\Delta S°$, and ΔV for the formation of the linear or branched structures. For proper comparisons, the latter parameters have been extrapolated to 20 °C assuming that in these profiles the helical and coil states have similar heat capacity differences, as has been described in previous reports (Rentzeperis et al., 1994; Marky et al.,1996). At 20 °C, very similar favorable free energies values are obtained for the formation of each molecule; we attribute this to the characteristic partial compensation of exothermic enthalpies and unfavorable entropies. However, differences are seen in the magnitude of the enthalpy and entropy terms that give rise to the overall observed free energy change. The unfavorable entropy values are consistent with the volume change measurements which indicate an increase in order (decrease in entropy) due to immobilization of water molecules in the formation of each molecule. Other unfavorable contributions to the entropy include the uptake of counterions, due to the higher charge density of each complex, and the ordering associated with the molecularity of the reactions. Comparison of these sets of thermodynamic parameters with the corresponding DSC profiles (Zhong et al., 1993) reveals differences between the enthalpy and free energy terms, which allow us to estimate entropy differences. For instance, the enthalpy changes of 51.0 kcal mol^{-1} (Y_{ds2}) and 63.5 kcal mol^{-1} (for Y_1) can be explained in terms of the additional contribution of base stacking interactions in the single strands. These are enhanced at lower temperatures (Zieba et al., 1991; Rentzeperis et al., 1994; Marky et al., 1996), and can be observed directly in UV melts below temperatures of about 30 °C.

Differential Thermodynamic Profiles. If the thermodynamic parameters for the formation of the $Y_{ds\mathbf{T8}}$ duplex are subtracted from the corresponding parameters for forming the Y_{ds2} duplex, reactions 6 and 7 below, the contributions from the single strands cancel out almost exactly (since there is a single base difference out of 32), and the resulting differential thermodynamic profiles apply to reaction (8) below:

$$Y_{ds2} \rightarrow Y_{102} + Y_{102c} \qquad (6)$$

$$Y_{102\mathbf{T8}} + Y_{102c} \rightarrow Y_{ds2\mathbf{T8}} \qquad (7)$$

$$Y_{ds2} \rightarrow Y_{ds2\mathbf{T8}} \qquad (8)$$

Reaction 8 corresponds to substitution of a dA·dT base pair for a dT·dT mismatch within a duplex of 16 base pairs. For reaction 8, we calculate a $\Delta\Delta G°$ of $+2.4 \pm 0.15$ kcal per mol of duplex, the resultant of an unfavorable $\Delta\Delta H$ of +9.0 kcal per mol of duplex and a favorable $\Delta(T\Delta S)$ of +7.0 kcal per mol of duplex. The positive $\Delta\Delta G$ value in reaction 8 reflects the higher stability of the fully-paired duplex. The similar signs of the $\Delta\Delta H$ and $\Delta(T\Delta S)$ terms again contribute to a partial compensation. Since the contributions of base pair stacking and base pairing cancel in reaction 8, this result suggests the participation of water molecules, as has been indicated previously (Lumry & Rajender, 1970; Marky & Kupke, 1989; Rentzeperis et al., 1992, 1993; Marky et al., 1996). This compensation effect is accompanied by a $\Delta\Delta V$ of -19 ± 1.2 mL mol^{-1}. Therefore, a Δn_{water} term needs to be included on the left hand side of eq 8, which corresponds to a change in the uptake of water molecules. Furthermore, the opposite signs between $\Delta\Delta V$ and the differential free energy suggest that the substitution of a dA for a dT, to yield a mismatched $Y_{ds2\mathbf{T8}}$ duplex, actually immobilizes more structural water molecules (Zieba et al., 1991; Marky et al., 1996). Note that if the sign of $\Delta\Delta V$ were positive, a release of electrostricted water molecules would be indicated, in order to be consistent with the energy needed to remove electrostricted water molecules, which also contributes to the disorder of the system (favorable entropy). Thus it is likely that these differential terms correspond to a local substitution of electrostricted water for hydrophobic water molecules.

Equivalent thermodynamic cycles can be set up for the strands of junctions in order to evaluate the effect of dT·dT mismatches in three-arm junctions, Y_1. The differential profiles show trends similar to those of the linear duplexes. However, the differential hydration effects are greater in the mismatched junctions and stronger still in the double-mismatched junction. This may reflect structural differences in the initial states, increased flexibility of the final states, or both. Other possibilities include additional exposure of nonpolar groups to the solvent arising from structural differences (bending, etc., see below).

Comparison with Earlier Thermodynamic Measurements. Creating a mismatch in a DNA duplex destabilizes the host molecule by loss of base pair stacking interactions and/or weakening of H-bonding, as reflected in the unfavorable enthalpy terms. The magnitude of the effect depends on the neighboring sequence, the length of the molecule, and the nature of the mismatch (GG, GT, or GA are more stable), and, as we show here also, on the presence of a branch.

The thermodynamics of three-arm branching have been investigated by Ladbury et al. (1994). Analyzing cycles similar to the ones reported here, they report a large heat capacity effect, −1.6 kcal/mol, consistent with earlier investigations and the results we obtain here. They used ITC values directly to determine the free energy changes, in contrast to the procedure we use here. The enthalpy value obtained for formation from single strands of the junction they studied, containing a bulge of two adenines at the branch, is −84.5 kcal/mol at 25 °C. This is intermediate between the value we obtain here for Y_1, −92.5 kcal/mol at 20 °C, and the value for the junctions containing mismatches at the branch in Table 2. Hence we believe the results of these studies are consistent in the enthalpy values determined.

CONCLUSION

This work presents the first complete thermodynamic profile for formation of three-arm DNA junctions, with and without dT−dT mismatches at the branch. The interest in these species is that they arise naturally at the ends of otherwise single-stranded parvovirus genomes [see Corsini et al. (1996)], where they are essential for DNA replication and packaging. Three-arm branched structures arise also as intermediates in replication or repair of long simple sequence repeats in DNA (Wells, 1996; Zhao et al., 1996). The thermodynamics of these structures is thus of current interest.

The structure in three-arm DNA junctions has been considered from two different viewpoints. Studies by Guo et al. (1990) of the angular deformation in three arm junctions were interpreted as indicating a dynamic or flexible structure, giving rise to an asymmetry that had not been detected in similar experiments by Lilley's group (Duckett & Lilley, 1990). A second interpretation, namely, that the geometry of three-arm junctions is pyrimidal rather than planar, has been proposed (Shlyakhtenko et al., 1994a,b). Either model can account for the asymmetry in strand reactivity in these molecules and seemingly also for the formation of tight circles upon ligating sticky-ended versions as well. The presence of alternative structural states has been detected in ^{1}H NMR studies of three-arm branched DNA models (Rosen & Patel, 1993), supporting the earlier view of conformational mobility. However, at present, neither model can be excluded definitively, and the two may not be completely inconsistent.

The most striking effect we observe here is the exceptionally large hydration changes accompanying branch formation, with or without a mismatch at the branch. We attribute this large change to ionic and solvation effects at the branch site, which has a unique electrostatic environment detectible also by selective cleavage by metal ion probes (Lu et al., 1990). Perturbation of the ionic and solvation environment of duplexes near a branch would be expected from models such as that of Timsit and Moras (1991), as we have noted above. If we think of mismatch formation as a probe reaction for the state of the DNA surface, then the hydration of duplex and junction appear to be different, although we cannot specify the mechanism from thermodynamic measurements alone. In the case of forming a duplex and then introducing a mismatch within the duplex, the thermodynamic directions are opposite: $\Delta G°$ for the former process is negative, that for the latter is positive. In both cases, the free energy is opposite in sign to the $\Delta\Delta V$, that is, hydration changes are involved in the compensation between enthalpy and entropy terms. Thus arguments asserting that the general enthalpy−entropy compensation in DNA strand pairing originates from loss of internal rotations within the single strands seem to be incomplete at best (Searle & Williams, 1993).Conversely models that emphasize a fundamental role of solvation in determining the thermodynamics of DNA structure are supported by our results (Petruska & Goodman, 1995). This is true both for the process of forming a branched DNA structure as well as modifying the structure by introducing base pair mismatches.

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