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Translational Entropy and DNA Duplex Stability

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ABSTRACT Investigation of folding/unfolding DNA duplexes of various size and composition by superprecise calorimetry has revised several long-held beliefs concerning the forces responsible for the formation of the double helix. It was established that: 1) the enthalpy and the entropy of duplex unfolding are temperature dependent, increasing with temperature rise and having the same heat capacity increment for CG and AT pairs; 2) the enthalpy of AT melting is greater than that of the CG pair, so the stabilizing effect of the CG pair in comparison with AT results not from its larger enthalpic contribution (as expected from its extra hydrogen bond), but from the larger entropic contribution of the AT pair that results from its ability to fix ordered water in the minor groove and release it upon duplex unfolding; 3) the translation entropy, resulting from the appearance of a new kinetic unit on duplex dissociation, determines the dependence of duplex stability on its length and its concentration (it is an order-of-magnitude smaller than predicted from the statistical mechanics of gases and is fully expressed by the stoichiometric correction term); 4) changes in duplex stability on reshuffling the sequence (the "nearest-neighbor effect") result from the immobilized water molecules fixed by AT pairs in the minor groove; and 5) the evaluated thermodynamic components permit a quantitative expression of DNA duplex stability.

INTRODUCTION

Although more than half a century has passed since it was recognized that the DNA double helix is formed from two complementary strands and the sequence of AT and CG basepairs carries genetic information, the forces stabilizing this molecular construction are still under discussion, as are attempts to predict stabilities of DNA duplexes in practical applications.

Originally it was supposed that an essential role in maintaining the double helix is played by hydrogen bonding between basepairs: two between AT and three between CG (1). This was supported by the observation that the stability of the DNA double helix rises with an increase in the CG content (2). Subsequent studies of thermal unfolding synthetic DNA duplexes using various physical methods led to the conclusion that the enthalpic and entropic contribution of CG basepairs significantly exceeds those of AT and both are temperature independent, i.e., unfolding of the duplex proceeds without any heat capacity increment (3–7). However, later detailed investigation of dissociation/association of DNA duplexes of various length and composition by highly precise differential scanning calorimetry and isothermal titration calorimetry (i.e., nano-DSC and nano-ITC (8)) showed that the enthalpy of dissociation/association of the DNA duplex is temperature dependent (i.e., proceeds with a heat capacity increment and, moreover, the enthalpic and entropic contribution of the AT pair significantly exceeds that of CG (9,10)). This is illustrated in Fig. 1, showing that although the thermal stability of the duplexes containing AT basepairs are lower than CG duplexes of the same size, as expected, their heats of melting are larger. Plotting the heat capacities of CG duplexes of various length, expressed per basepair, against their melting temperatures showed that the specific heat of duplex melting increases with a rise in the melting temperature: the slope of this dependence (*inset* in Fig. 2) represents the heat capacity increment on duplex unfolding, which amounts to (0.13 ± 0.04) kJ/K•mol-bp.

One might be surprised that duplex thermostability increases with the number of basepairs. If the enthalpy and entropy of duplex formation are additive functions, the duplex unfolding temperature should not depend on its size. As shown in Fig. 2, the enthalpy of unfolding the all-CG duplexes is indeed a linear function of length. It appears therefore that the entropy of duplex unfolding cannot be an additive function. Indeed, although the conformational entropy also increases linearly with the number of basepairs in the CG duplexes, the total entropy additionally includes the translation entropy term that results from the appearance of a new kinetic unit on dissociation of the strands and that does not depend on the number of bases nor on the temperature.

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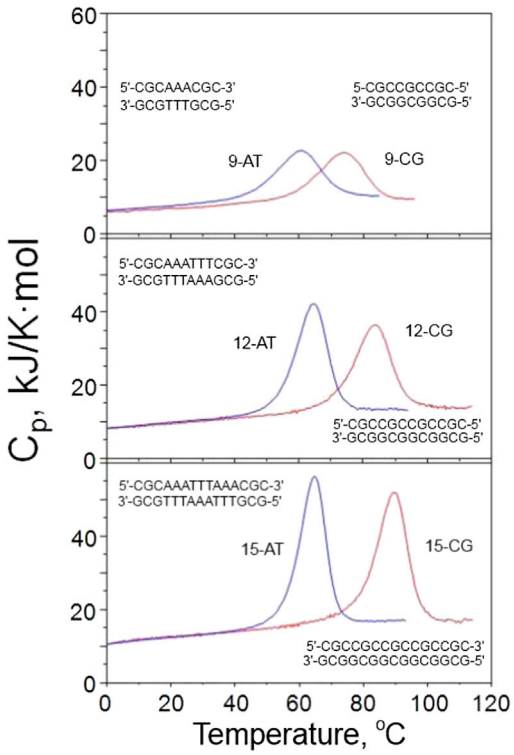

FIGURE 1 Comparison of the partial molar heat capacities of 9-, 12-, and 15-basepair CG duplexes (in *red*) and the same length duplexes having AT pairs in the central region (in *blue*). All measurements are at the identical duplex concentration of 283 μM in 150 mM NaCl, 5 mM Na-Phosphate, pH 7.4. Reproduced from (10). To see this figure in color, go online.

Translational entropy

According to the original proposal by Gurney (11), the translational entropy is expressed by the cratic term, δS^{cratic}, which is just the entropy of mixing the additional kinetic unit that appears upon complex dissociation with the solvent. This cratic entropy is assumed to be independent of the solution composition and the molecular weight of the solute. For the formation of a dimer in 1 M standard aqueous solution (55 molar water), $\delta S^{\text{cratic}} = R\ln(1/55) = -8.02$ cal/K•mol $= -33.3$ J/K•mol. However, this cratic entropy later became the target of severe criticism by physicists as being physically ungrounded.

Assuming that the translational entropies of macromolecules in aqueous solution do not differ from those of small molecules in the gaseous phase and can be calculated by the simple Sackur-Tetrode equation, Finkelstein and Janin (12) found that the translational entropy of dissociating a typical dimeric protein at 300 K is 180–230 J/K•mol, depending on the molecular weight of the protein. According to these authors, the rotational entropy increase is of the same order of magnitude. Therefore, the total value of ($\Delta S^{\text{trans}} + \Delta S^{\text{rot}}$) amounts to 400 J/K•mol, with a positive sign for the dissociation of a dimer and a negative sign for its association. Very similar values for the entropy effects of dimer dissociation were obtained by Tidor and Karplus (13) using the statistical-thermodynamic approach of Chandler and Pratt (14). According to these authors, dimerization of insulin should result in a decrease of the translational entropy by 180 J/K•mol and a decrease of the rotational entropy by 200 J/K•mol, but this should be accompanied by an increase of the vibrational entropy by 110 J/K•mol; thus, the overall change in the external entropy (i.e., the entropy not associated with changes in conformation or hydration) upon dimerization of insulin should amount to $\Delta S^{\text{trans}} = 270$ J/K•mol. Translation entropy values in the range from 300 to 400 J/K•mol have been widely used by many authors in the thermodynamic analysis of forming protein/protein and protein/DNA complexes (see e.g., (15–18)). However, early calorimetric studies of unfolding an S-S cross-linked and non-cross-linked dimeric globular protein and also an α-helical coiled-coil in aqueous solution showed that the translation entropy appears much lower than suggested by the statistical mechanics of gases (19,20). The question is then: what is the translation entropy of DNA duplex dissociation? Without knowing its magnitude, it is not possible to properly predict the stabilities of DNA duplexes.

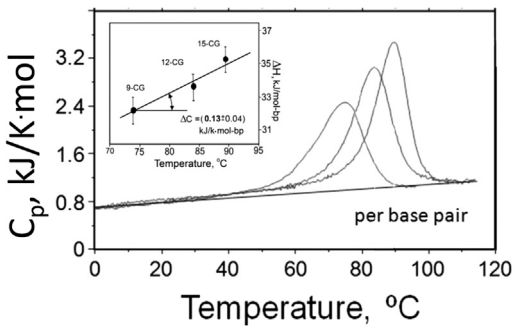

FIGURE 2 The partial heat capacities of the three CG duplexes in 150 mM NaCl, 5 mM Na-phosphate, pH 7.4, calculated per mole of basepair. (*Inset*) Given here is the dependence of the excess enthalpy on the transition temperature, the slope of which gives the heat capacity increment (from (10)).

MATERIALS AND METHODS

See the Supporting Material for Materials and Methods.

RESULTS AND DISCUSSION

The entropy of DNA duplex dissociation

Consider two cases: the 15- and 9-basepair CG duplexes (seen in Figs. 1 and 2) unfold cooperatively at temperatures 362.7 and 347.2 K, with enthalpies of 408 and 223 kJ/mol (see Table 1 of (10)). Thus, the total entropies of their unfolding at their transition temperatures are

$$\Delta S_t^{\mathrm{tot}}(15CG) = \frac{408\ \mathrm{kJ/mol}}{362.7\ \mathrm{K}} = 1125\ \mathrm{J/K \cdot mol}, \quad (1)$$

and

$$\Delta S_t^{\mathrm{tot}}(9CG) = \frac{223\ \mathrm{kJ/mol}}{347.2\ \mathrm{K}} = 642\ \mathrm{J/K \cdot mol}. \quad (2)$$

Extrapolating these entropies to the standard temperature of 25°C (using $\Delta C_p = 0.13$ kJ/K•mol-bp; (10)) and expressing the total entropy as the sum of the conformational and translational components, we have

$$\begin{aligned} \Delta S_{25}^{15CG} &= 15\Delta S_{25}^{\mathrm{conf}}(CG) + \Delta S^{\mathrm{trans}} \\ &= \frac{\Delta H_t}{T_t} - 15 \times \Delta C_p \ln\left(\frac{363}{298}\right) + \Delta S^{\mathrm{trans}} \\ &= 743\ \mathrm{J/K \cdot mol} + \Delta S^{\mathrm{trans}}, \end{aligned} \quad (3)$$

$$\begin{aligned} \Delta S_{25}^{9CG} &= 9\Delta S_{25}^{\mathrm{conf}}(CG) + \Delta S^{\mathrm{trans}} \\ &= \frac{\Delta H_t}{T_t} - 9 \times \Delta C_p \ln\left(\frac{347}{298}\right) + \Delta S^{\mathrm{trans}} \\ &= 464\ \mathrm{J/K \cdot mol} + \Delta S^{\mathrm{trans}}. \end{aligned} \quad (4)$$

Bearing in mind that both experiments were carried out at the same duplex concentration, i.e., the translational entropies are the same for both cases and assuming the conformational entropies are additive like the enthalpies, subtracting one from the other and dividing by the difference in the number of basepairs, we obtain

$$\Delta_9^{15} S_{25}^{\mathrm{conf}}/6 = (46.5 \pm 3.0)\mathrm{J/K \cdot mol\text{-}bp}. \quad (5)$$

With an accurate value of the conformational entropy of a CG pair in hand, the translational entropy is best evaluated by analyzing the dependence of duplex thermostability (the melting temperature, T_t) on the number of basepairs. Bearing in mind that the heat capacity increment on duplex dissociation, ΔC_p, does not depend on temperature (10), the transition temperature can be expressed by the straightforward equation

$$T_t = \frac{\left[\Delta H_{25}^{CG} + \Delta C_p\left(T_t^1 - 298\right)\right] \times n^{CG}}{\left[\Delta S_{25}^{CG} + \Delta C_p \ln\left(T_t/298\right)\right] \times n^{CG} + \Delta S^{\mathrm{trans}}}. \quad (6)$$

The magnitudes of the total enthalpy and entropy of the CG pair at 25°C are 26.5 kJ/mol-bp and 64.0 J/K•mol-bp, respectively, as given in Table 3 of (10). In Eq. 6, these are corrected to T_t using $\Delta C_p = 0.13$ kJ/K•mol-bp.

From Eq. 6, we have for $\Delta S^{\mathrm{trans}}$

$$\begin{aligned} \Delta S^{\mathrm{trans}} &= \left[\Delta H_{25}^{CG} + \Delta C_p \times (T_t - 298) \times n^{CG}\right]/T_t \\ &\quad - \left[\Delta S_{25}^{CG} + \Delta C_p \times \ln\left(\frac{T_t}{298}\right)\right] \times n^{CG}. \end{aligned} \quad (7)$$

The derived values of $\Delta S^{\mathrm{trans}}$ are very sensitive to the magnitude of the conformational entropy, ΔS_{25}^{CG}, which comes from calorimetrical measurements carrying significant error: Table 1 therefore shows $\Delta S^{\mathrm{trans}}$ calculated for the three all-CG duplexes using several values of the conformational entropy close to 46.5 J/K•mol-bp. However, the translational entropy should not depend on the number of basepairs in the duplexes, nor on the conformational entropy of the bases: a requirement realized for the considered three CG duplexes at a conformational entropy value of 44.6 J/K•mol-bp—and for which the translational entropy is calculated to be $\Delta S^{\mathrm{trans}} = (73.2 \pm 0.5)$ J/K•mol. This analysis therefore permits optimization of both the conformational entropy of a CG pair and also gives the translational entropy for dissociation of the duplexes under these conditions.

TABLE 1 Using the 9-, 12-, and 15-CG Basepair DNA Duplex Data To Calculate Translational Entropies, $\Delta S^{\mathrm{trans}}$, for Variable Magnitudes of the Conformational Entropy of a CG Pair, ΔS^{conf}, at 25°C Lying within the Experimental Error of 46.5 ± 3.0 J/K•mol-bp

$\Delta S_{CG}^{\mathrm{conf}}$	9-CG	12-CG	15-CG
J/K•mol-bp	$\Delta S^{\mathrm{trans}}$ J/K•mol	$\Delta S^{\mathrm{trans}}$ J/K•mol	$\Delta S^{\mathrm{trans}}$ J/K•mol
44.0	78.4	80.5	82.3
44.5[a]	73.9[a]	74.5[a]	74.8[a]
44.7[a]	72.1[a]	72.1[a]	71.8[a]
45.0	69.4	68.5	67.3
45.3	66.7	64.9	62.8
45.5	64.9	62.5	59.8
45.8	62.2	58.9	55.3
46.0	60.4	56.5	52.3
46.3	57.6	52.9	47.8
46.5	55.9	50.5	31.4

[a]Closest fit to a constant value of $\Delta S^{\mathrm{trans}}$.

It is notable that the translational entropy thus obtained for separation of the DNA strands is at least five times smaller than that derived by statistical mechanics for the dissociation of dimeric macromolecules in the gas phase (12–16) and also differs from the cratic entropy value proposed by Gurney (11). However, it is essentially identical to the stoichiometric correction term used when considering the entropy of heterodimer dissociation:

$$\Delta S(T_t) = \frac{\Delta H_t^{\mathrm{coop}}}{T_t} + R \ln\left(\frac{[\mathrm{N_o}]}{2}\right), \quad (8)$$

where $[\mathrm{N_o}] = N_o/N_{\mathrm{st}}$ is the dimensionless initial concentration of the complex and $R = 8.31$ J/K•mol is the universal gas constant (8). At the DNA concentration of 283 μM used in many of our experiments, this correction term amounts to 73.7 J/K•mol, a magnitude corresponding accurately to the above calorimetrically determined value of the translational entropy $\Delta S^{\mathrm{trans}} = (73.2 \pm 0.5)$ J/K•mol. It thus appears that the translation entropy is fully expressed by the stoichiometric correction term. It is important to note that the translational entropy does not include the hydration effects associated with unfolding the DNA duplex; these are included in the conformational entropy term.

TABLE 2 Enthalpic and Entropic Contributions of the AT Basepair to DNA Duplex Stabilization

DNA Duplex	T_t °C	ΔH_t^{coop} kJ/mol	ΔH_{25}^{coop} kJ/mol	ΔH_{25}^{AT} kJ/mol-bp	ΔS_{25}^{coop} J/K•mol	ΔS_{25}^{AT} J/K•mol-bp
5′-CGCAAACGC-3′ 3′-GCGTTTGCG-5′	60.4	251	210		621	
5′-CGCAAAAAACGC-3′ 3′-GCGTTTTTTGCG-5′	63.0	360	301	30.3	884	87.7
5′-CGCAAATTTCGC-3′ 3′-GCGTTTAAAGCG-5′	64.5	350	288	26.0	844	74.3
5′-CGCTTTAAACGC-3′ 3′-GCGAAATTTGCG-5′	60.8	327	271	20.3	801	60.0
5′-CGCATATATCGC-3′ 3′-GCGTATATAGCG-5′	60.3	326	271	20.3	802	60.3
5′-CGCAAATTTAAACGC-3′ 3′-GCGTTTAAATTTGCG-5′	64.8	440	362	25.3	1058	72.8
5′-CGCAAAAAAAAACGC-3′ 3′-GCGTTTTTTTTTGCG-5′	65.1	443	365	25.8	1063	73.7
Averaged				25 ± 3		72 ± 10

Results in this table were obtained by subtracting the 9-bp duplex (*top row*) from those of the longer six duplexes. All measurements were at a duplex concentration of 283 μM in 0.15 mM NaCl, pH 7.4. Original data was from (10). ΔH_t^{coop} represents the total enthalpy of the cooperative transition at the dissociation temperature T_t and ΔH_{25}^{coop} is its magnitude corrected to 25°C. ΔH_{25}^{AT} is the enthalpy of a single AT pair at 25°C. The corresponding entropies are denoted by ΔS_{25}^{coop} and ΔS_{25}^{AT}.

Contributions of AT basepairs to duplex stabilization

The enthalpic and entropic contributions of the AT basepairs in duplexes containing AT runs—flanked by CGC/GCG triplets for thermal reinforcement and the avoidance of end-effects (see Table 2)—were estimated by first extrapolating the measured enthalpies of all the AT-containing duplexes to the standard temperature of 25°C. The enthalpy of the smallest, the 9-bp AT duplex, consisting of 6CG and 3AT basepairs (210 kJ/mol), was then subtracted from the enthalpies of each of the longer AT duplexes and the result divided by the difference in the number of their AT pairs—giving the enthalpic contribution of a single AT pair at 25°C, as seen in column 5 of Table 2. The same procedure was adopted with the entropies: subtracting the cooperative entropy of the 9-bp AT duplex from that of the longer duplexes gives the conformational entropy of an AT basepair (Table 2, *last column*). The most notable feature of the AT pairs is that their enthalpic, and particularly their entropic, contributions are substantially larger than those of the CG basepair (summarized in Table 3).

Prediction of DNA duplex stability

The conformational and translational entropies obtained, together with the enthalpies, can then be used for estimating the expected melting temperatures of the considered duplexes. For example, using Eq. 6 and the parameters specifying the contributions of the CG basepairs, one can calculate the expected melting temperatures for the duplexes consisting only of CG basepairs. The main obstacle in using this equation is that the quantity to be obtained, T_t, is also included in the right-hand side. The equation can, however, be solved by stepwise consecutive iterations. This can be done bearing in mind that melting of the DNA duplexes in 0.15 NaCl solutions takes place at temperatures between 50 and 95°C, depending on their sequence. Therefore, to a first approximation, one can take $T_t^1 = 75°C = 348$ K, i.e., 50 K above the standard temperature. The value of T_t obtained on this assumption can then be used for a second approximation. Usually the second iteration gives a value of T_t, which is close to that experimentally observed—as seen for the 9-, 12-, and 15-CG duplexes (Table 4). As seen in Table 4, increasing the number of CG basepairs from 9 to 25 leads to the duplex melting temperature

TABLE 3 Optimized Contributions of the CG and AT Basepairs to the Enthalpy, Entropy, and Heat Capacity Increment of Double Helical DNA Dissociation at 25°C

Basepair	ΔC_p kJ/K•mol-bp	ΔS^{trans} J/K•mol	ΔH^{coop} kJ/mol-bp	ΔS^{conf} J/K•mol-bp
CG	0.13 ± 0.01	$R\ln(2/[No])$[a]	18.8 ± 0.3	44.7 ± 0.2
AT			25 ± 3	72 ± 10

Data from this analysis and from (10) were all obtained at a duplex concentration of 283 μM in 0.15 mM NaCl, pH 7.4.

[a]ΔS^{trans} (403 μM) = 70.7 J/K•mol; ΔS^{trans} (337 μM) = 72.2 J/K•mol; ΔS^{trans} (283 μM) = 73.7 J/K•mol; ΔS^{trans} (214 μM) = 76.0 J/K•mol; ΔS^{trans} (107 μM) = 81.7 J/K•mol; ΔS^{trans} (87 μM) = 83.4 J/K•mol; and ΔS^{trans} (40 μM) = 90.0 J/K•mol.

increasing by 25 K, a change entirely due to inclusion of the translational contribution in the total entropy.

To determine the transition temperature of DNA duplexes containing not only CG basepairs but also AT basepairs and not necessarily at the concentration of 283 μM used in many of our experiments but at any other [N_o], one has to expand Eq. 6 to include AT pairs (see Table 4) and solve it by consecutive iterations using Eq. 9:

$$T_t = \frac{\left[\Delta H_{25}^{AT} + \Delta C_p \times (348 - 298)\right] \times n^{AT} + \left[\Delta H_{25}^{CG} + \Delta C_p (348 - 298)\right] \times n^{CG}}{\left[\Delta S_{25}^{AT} + \Delta C_p \ln\left(\frac{298 + 50}{298}\right)\right] \times n^{AT} + \left[\Delta S_{25}^{CG} + \Delta C_p \ln\left(\frac{298 + 50}{298}\right)\right] \times n^{CG} + R \ln\left(\frac{2}{N_o}\right)}. \quad (9)$$

TABLE 4 The Melting Temperatures of Various DNA Duplexes Calculated Using the Data Given in Table 3 for CG and AT Pairs

DNA Duplexes	Composition	References	Concentration μM	T_t °C Experiment	T_t °C Calculated
5′-CGCCGCCGC-3′ 3′-GCGGCGGCG-5′	9-CG	(10)	283	74.0	73.5
5′-CGCCGCCGCCGC-3′ 3′-GCGGCGGCGGCG-5′	12-CG	(10)	283	83.6	83.1
5′-CGCCGCCGCCGCCGC-3′ 3′-GCGGCGGCGGCGGCG-5′	15-CG	(10)	283	89.5	89.3
5′-CGCCGCCGCCGCCGCCGCCG-3′ 3′-GCGGCGGCGGCGGCGGCGGC-5′	20-CG	N/A[a]	283	N/A	94.2
5′-CGCCGCCGCCGCCGCCGCCGCGCGC-3′ 3′-GCGGCGGCGGCGGCGGCGGCGCGCG-5′	25-CG	N/A[a]	283	N/A	98.7
5′-CGC**AAA**CGC-3′ 3′-GCG**TTT**GCG-5′	6CG, 3AT	(10)	283	60.4	60.3
5′-CGC**AAATTT**CGC-3′ 3′-GCG**TTTAAA**GCG-5′	6CG, 6AT	(10)	283	64.5	62.1
5′-CGC**AAAAAA**CGC-3′ 3′-GCG**TTTTTT**GCG-5′	6CG, 6AT	(10)	283	63.0	62.1
5′-CGC**TTTAAA**CGC-3′ 3′-GCG**AAATTT**GCG-5′	6CG, 6AT	(10)	283	60.8	62.1
5′-CGC**ATATAT**CGC-3′ 3′-GCG**TATATA**GCG-5′	6CG, 6AT	(10)	283	60.3	62.1
5′-CGC**AAATTTAAA**CGC-3′ 3′-GCG**TTTAAATTT**GCG-5′	6CG, 9AT	(10)	283	64.8	63.3
5′-CGC**AAAAAAAAA**CGC-3′ 3′-GCG**TTTTTTTTT**GCG-5′	6CG, 9AT	(10)	283	65.1	63.3
5′-CGC**AGAGAGAGA**CGC-3′ 3′-GCG**TCTCTCTCT**GCG-5′	10CG, 5AT	N/A[b]	283	72.2	73.6
5′-CGC**ACACACACA**CGC-3′ 3′-GCG**TGTGTGTGT**GCG-5′	10CG, 5AT	N/A[b]	283	75.9	73.6
5′-CG**AACAAT**CG-3′ 3′-GC**TTGTTA**GC-5′	5CG, 5AT	(9)	214	51.3	57.1
5′-CG**AACAAT**CG-3′ 3′-GC**TTGTTA**GC-5′	5CG, 5AT	(9)	107	49.0	53.8
5′-GCG**AACAAT**CGG-3′ 3′-CGC**TTGTTA**GCC-5′	7CG, 5AT	(9)	403	64.8	67.3
5′-GCG**AACAAT**CGG-3′ 3′-CGC**TTGTTA**GCC-5′	7CG, 5AT	(9)	87	60.6	61.5

Experimental data from (9,10) are given where available.
[a]Calculated T_t values only.
[b]Previously unpublished experimental data from our laboratory.

The enthalpy, entropy, and heat capacity increment on dissociation of AT and CG basepairs used in the calculation of melting temperatures are all given in Table 3. Calculated values of T_t for duplexes of various compositions and concentrations are compared with experimentally determined melting temperatures in Table 4.

Considering Table 4, one can see that the correspondence between the calculated and experimentally determined melting temperatures is much better for duplexes consisting only of CG basepairs, for which the deviation between the predicted and measured melting temperatures is within ± 0.3 K, whereas for the duplexes containing AT basepairs, it is one order larger. Because for duplexes containing AT pairs the melting temperatures were determined using different sequences (see Table 2), it appears that the enthalpic and entropic contributions of the AT basepair are sequence dependent.

The fact that the stability of a DNA duplex depends not only on the composition of AT and CG basepairs forming the duplex, but also on their arrangement, was first noted by Tinoco et al. (21) and is usually explained by the effects of nearest-neighbor interactions. However, the nature of these nearest-neighbor interactions has so far been quite obscure. It now appears that this is an AT effect and the physical basis for this must be the unique ability of AT pairs to fix waters by binding the polar groups of A and T bases in the minor groove of DNA (10).

SUPPORTING MATERIAL

Supporting Materials and Methods are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(17)31210-9.

AUTHOR CONTRIBUTIONS

Both P.L.P. and C.C.-R analyzed data and wrote the manuscript.

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