Conformational and Solvent Entropy Contributions to the Thermal Response of Nucleic Acid-Based Nanothermometers

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With device miniaturization comes the need to measure temperature changes on molecular scales. Recent experiments show that thermoresponsive devices may be constructed based on the temperature-dependence of the relative populations of left- and right-handed nucleic acid helical conformations: upon an increase in temperature, particular sequences of DNA oligonucleotide duplexes in high salt conditions switch from a left-handed (Z) form to a right-handed (B) one, while RNA responds inversely by switching from a right- (A) to a left-handed (Z) form. We use existing temperature-dependent circular dichroism experimental data [Tashiro and Sugiyama, 2005] and a two-state model to extract the entropic contribution to the free energy difference between left- and right-handed form. Then, to address the microscopic origin of the inverse temperature response of RNA and DNA, we perform all-atom molecular dynamics simulations from which we compute both configurational nucleic acid and solvent entropies for a number of RNA and DNA systems; because the ionic conditions in the experiments are outside the physiological range, we cover a wider landscape of sequence, salt conditions, and helical direction. Calculations reveal a complex interplay between configurational, water, and ionic entropies, which, combined with the sequence-dependence, rationalize the experimentally observed transitions from A- to Z-RNA and Z- to B-DNA in high salt concentrations and provide insight that may aid future developments of the use of nucleic acids oligomers for thermal sensing at the nanoscale in physiological conditions.

1. Introduction

There exists substantial excitement in the development of methods to control the mechanics of molecular-scale devices made of nucleic acids by triggering conformational changes as a result of various stimuli.[1−3] Given the increasing miniaturization brought about by nanotechnology, and given the fundamental difference between physicochemical properties in the macroscopic and the nanoscopic regimes, there is also substantial interest (but significant challenge) in accurately measuring temperature changes over nanometer scales in diverse fields spanning from nanofluidics, to computer chip design, to hyperthermal cancer treatment (for a review, see, for example, ref 4).

When the motion-inducing stimuli are thermal, measuring the ensuing conformational movement has been proposed as a means to detect local changes in temperature on the nanometer scale. This would qualify such devices as molecular "nanothermometers", lending them useful, for example, in mapping temperature differences within single cells. In a set of recently reported fluorescence experiments, Tashiro and Sugiyama[5,6] have shown that, by taking advantage of the characteristic properties of nucleic acids in salt solutions, nanothermometers may be constructed on the basis of the temperature-dependent preference of DNA and RNA oligonucleotide duplexes to be in either left-handed (Z-DNA or Z-RNA) or right-handed (B-DNA or A-RNA) forms. In these experiments, a fluorescent adenine analogue that is sensitive to the handedness of helicity was inserted into an alternating purine−pyrimidine sequence: d(GCGCGC-Ap-CGCGCG) (DNA1)/(CGCGCGTGCGCGC) (DNA2), with uracil replacing thymine in RNA helices. These C-G repeat sequences are known to adopt Z-form structures under appropriate salt conditions.[7]

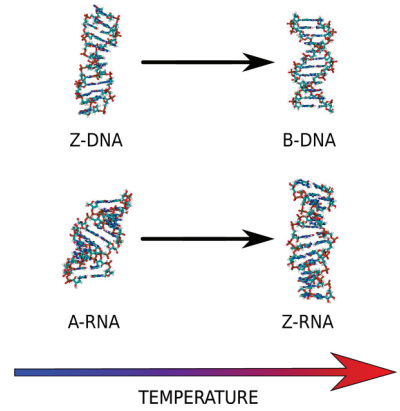

Figure 1. Experimental findings for C-G repeat sequences in high salt. As temperature increases from 20 to 45 C, DNA transitions from a left-handed Z-DNA to a right-handed B-DNA state, while the RNA oligomer undergoes an inverted response of transitioning from a right-handed A-RNA helix to a left-handed Z-RNA form. The four conformations depicted are snapshots of respective equilibrated structures from our simulations.

In high ionic conditions, upon increasing the temperature, the RNA transitioned from A- to Z-form (as gauged from the increase in the fluorescence of the base), while the DNA transitioned from Z- to B-form (reflected by a decrease in fluorescence) as shown in Figure 1. This experimental feat brings promise for the design of devices that, with an appropriate composition of RNA and DNA duplexes, could accurately measure temperatures at the nanoscale. Before that, however,

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in addition to the general proof of principle put forth by the experiments, more needs to be known in detail about the cause of the apparent relative free energy shift and about the contributions of solute, solvent, and sequence to it.

It has become clear, from recent experimental and theoretical studies (see ref 8 for a review), that water molecules and ions within complex nucleic acid structures can display long residency times, and assist drug binding and catalytic reactions. For example, a molecular dynamics (MD) study showed that the hydration shell was affected by the helical conformation (A- or B-form), by the sequence, and by the ionic concentration of the environment.[9] It is therefore expected that these considerations should play a role in conformational switching between B, Z, or A forms. For example, the ability of canonical B-DNA to undergo switching to left-handed Z-DNA depends strongly on salt and sequence.[7,10] While for the B to Z transition the electrostatic effect of the solvent including ions is well calibrated,[11] less understood is the contribution of the entropy change upon switching. A key factor in the relative stabilization of Z- and B-form DNA is described by the observation that the closest approach distance between phosphates on opposites strands decreases from 11.7 Å in B-DNA to 7.7 Å in Z-DNA.[12] It is thus not surprising that a high salt concentration screens this electrostatic repulsion and helps to stabilize Z-DNA.[10] Using nonlinear Poisson−Boltzmann calculations, Misra and Honig[11] analyzed the electrostatic contributions to the B to Z transition in DNA and have concluded that electrostatic forces favor Z-DNA, and that the dominant role in the relative stabilization of B-DNA is, by consequence, dictated by nonelectrostatic forces, out of which entropy was suggested to be significant. Moreover, while DNA B−Z switching is well studied and simulated,[13] relatively less is known about RNA switching. It is known that the stabilization of Z-RNA tends to require an even higher salt concentration and temperature than Z-DNA, potentially due to the increased energy required to force pyrimidine residues into a C2′-endo puckering conformation.[14]

Assuming a weak temperature dependence of the ΔU and ΔS components of the relative free energy $\Delta F = \Delta U - T\Delta S$ between left and right handed forms, a shift in the temperature T drastically affects the entropy change component, $T\Delta S$, which is obviously the primary factor that can change the sign of ΔF, a sign change that is responsible for the conformational switching activity exhibited by the oligomers studied experimentally. However, a detailed understanding of (i) the microscopic origin of this temperature-induced switching activity and (ii) its properties (or even its existence) for other sequences or external conditions is lacking, despite the potential these systems exhibit for nanoscale thermometric technology. To investigate these questions, we have performed a series of MD simulations on RNA and DNA duplexes and calculated their conformational entropy (i.e., the entropy pertaining to the macromolecular solute), as well as their solvent and their ionic entropies. By comparing entropies of different helical structures with different sequences in various ionic conditions spanning not only the experimental but also physiologically relevant conditions, we will show that conformational, solvent, and ionic entropies play, concertedly, an important role in explaining the thermodynamic basis for these devices. As will be seen, the difference in macromolecular flexibility as reflected by the conformational entropies may only partially account for the sequence dependence and ionic conditions required by these experiments, thus an inclusion of other entropic components is required for a full description of the thermodynamics of switching.

While conformational entropy (the solute entropy) can be calculated relatively satisfyingly in the quasiharmonic approximation,[15−17] the solvent and ionic entropies have traditionally been more difficult to compute because of the large volume of conformational space available to solvent molecules in comparison with that sampled in MD simulations. However, a recently developed method of permutation reduction that exploits the permutation symmetry of the solvent molecules goes a long way toward lessening this complication and toward providing reasonable estimates for these values;[18] we employ it herein.

The outline of the paper is as follows. As noted above, it is apparent that entropy drives the temperature-induced switching for both DNA and RNA. The total entropy change between the left- and right-handed helices may be partitioned into $\Delta S = \Delta S_{NA} + \Delta S_{water} + \Delta S_{ion} + C$, where the correction term C, which, due to coupling between configurational nucleic acid (NA), water, and ionic terms, has been shown to be negligible for systems with little volume change[19,20] and has thus been disregarded herein. We first compute the enthalpic and entropic differences between left- and right-handed helices from the experimental data presented in ref 6. We then present the results of 80 independent MD simulations, each 2 ns long, on solvated RNA and DNA helices with a variety of sequences, ionic concentrations and helical forms. From the simulations, we compute the entropic components of the systems and show that the overall entropy difference responsible for the observed transitions is dependent on the balance between solvent and configurational entropies. We conclude with a discussion of how these results fit into our overall understanding of DNA and RNA structure and flexibility available from existing data, and of what thermodynamic driving forces must be considered in nucleic acid-based nanothermometer design developments.

2. Entropy Changes from Temperature Dependent Data. In the experiments of Tashiro and Sugiyama, it is the fluorescence signal that translates into an ability to use the oligomers as thermal switches. The fluorescence of the adenine analogue is much stronger in Z-DNA than in B-DNA, because the continuous π-stacks in B-DNA cause efficient quenching, in contrast to the discrete pattern of four-base stacks in Z-DNA with diminished quenching. This fluorescence is the on−off signal reporting the dominant helical form that the oligonucleotides assume. However, in addition to the fluorescence data, the authors also report circular dichroism spectroscopic measurements. While the CD data is not directly useful for thermometry, the relative population of Z versus B (or A) form can be inferred from the change in the circular dichroism absorption spectrum at peak wavelengths for a left- versus right-handed helix. Data presented in Figure 3b and the Supporting Information of ref 6 provide the fraction p_Z of Z-form RNA and DNA at temperature intervals from 20 −45 C. If one assumes a two-state system (that is, a coexistence between A- and Z-RNA, or, respectively, between B- and Z-DNA only), then the ratio of the number of each type of nucleic acid molecules in each state is proportional to their Boltzmann weighted free energy difference. By further assuming that the entropic and enthalpic differences between the states remain constant over this temperature range, the following must hold:

$$k_B \ln\left(\frac{1 - p_Z}{p_Z}\right) = -\frac{\Delta U}{T} + \Delta S \qquad (1)$$

where ΔS and ΔU are the entropic and enthalpic difference ascribed to the transition from right to left handed helices (which

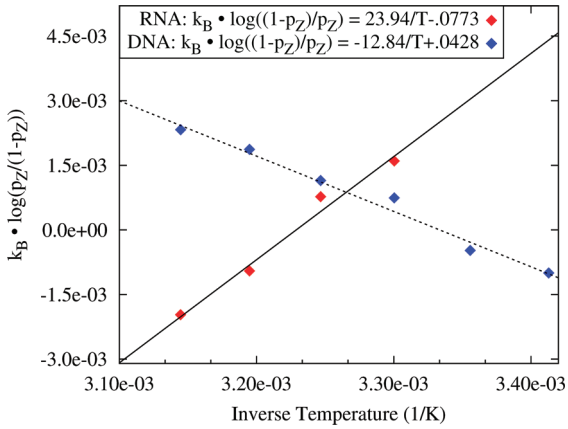

Figure 2. Determination of entropic and enthalpic differences between left and right-handed nucleic acid helices C-G repeat duplexes from experiments. The ratio of left-handed Z-form to A- or B-form helices is related to the free energy difference between the states by a Boltzmann relation, $(1 - p_Z)/p_Z = \exp[-(\Delta U - T\Delta S)/k_BT]$ (assuming a two state model), yielding a linear relationship between $k_B \log((1 - p_Z)/p_Z)$ and $1/T$. A linear fit to data presented in ref 6 indicates enthalpic differences of $\Delta U = -23.94$ and 12.84 kcal/mol and entropic ones of $-T\Delta S = 22.65$ and -12.54 kcal/mol (at 293 K) for RNA and DNA, respectively.

henceforth will be the convention used throughout our paper). It should be noted that eq 1 is the integrand of the van't Hoff equation employed in calorimetry studies, which relates the enthalpic difference of a reaction to the ratio of equilibrium constants over a range of temperatures. Indeed, if one is interested solely in this ratio, then the entropic component, ΔS is irrelevant. However, in a bistable system in which the equilibrium switches from favoring one state relative to the other upon a temperature change, the entropy is of prime importance. Its value determines whether or not it is possible for the equilibrium to flip from favoring one state over the other. To clarify, let us assume such a two-state case for the right−left-handed nucleic acid equilibrium, R ⇌ L, for which $K = p_L/p_R$, with p denoting populations. The dependence of K on temperature as embodied in the thermodynamic relationship of van't Hoff equation has no ΔS dependence. For example, if $\Delta U < 0$ (exothermic), then K decreases with increasing temperature. However, consider the same example of a negative $\Delta U < 0$ with two subcases: (i) If $\Delta S > 0$, then $\Delta F = \Delta U - T\Delta S$ at the initial, lower T (and at subsequent Ts) will be negative, which means $K > 1$, so the right-to-left temperature-induced switching cannot occur. Increasing the temperature will simply populate more and more the left-handed state. (ii) If $\Delta S < 0$, it can be (if its absolute value is sufficiently large) that $\Delta F > 0$ at low T, but becomes negative at high T, so T-induced switching can occur. That is, while the van't Hoff equation describes the variation of K with T, the absolute value of K (to see it switching above and below unity) is dictated by the values of the entropy.

A linear fit of the available experimental data is plotted in Figure 2 (with RNA data points at 293 and 298 K excluded due to their poor fit). From it we determine an experimental entropic difference $-T\Delta S$ of 22.6 kcal/mol for RNA and −12.5 kcal/mol for DNA (at 293 K). The quality of the linear fit serves to validate our choice of a two-state model and indicates that alternative nucleic acid forms (such as melted) are minimally represented in vitro.

3. Computational Methods. We have set up 16 distinct nucleic acid duplexes, all in water boxes and simulated with periodic boundary conditions and constant pressure and temperature through the Nosé−Hoover algorithm[21−23] with the program CHARMM,[24] using version 27 of the nucleic acid force field parameters.[25,26] For each nucleic acid form (i.e., A, B, or Z), half of the simulations were performed with a mixed sequence $(GCGAAATTTGCG)_2$, with uracil replacing thymine in RNA molecules. The other half were done with the actual C·G sequence used in experiments $(CGCGCGCGCGCG)_2$. While the latter sequence (the CG repeat sequence) has a propensity to form Z structures, the former one (i.e., the mixed sequence) does not, and was selected to calibrate the ability of the simulation to estimate correctly thermodynamic stability. Of the total number of simulations with both the mixed and C·G sequences, half were performed with a right-handed helix (A-RNA or B-DNA) and half in the Z form. Furthermore, half of the simulations were performed with just enough sodium counterions to neutralize the charge of the system, while the other half included a number of sodium and chloride ions that created an approximately 4 M NaCl environment (with excess sodium to neutralize the system). A 4 M NaCl environment was used for direct comparison to the DNA data presented by Tashiro and Sugiyama (see Figure 4 of ref 6) and was maintained for the RNA simulations for consistency. As with the choice of sequences, while the 4 M concentration creates conditions for Z propensity, the low-sodium (charge neutralizing) condition does not, and was chosen for validation and reference. In summary, a span of nucleic acid type, helix conformation, sequence, and ionic condition was simulated. Each system was minimized extensively and equilibrated for 200 ps with gradual removal of initially applied harmonic restraints on the nucleic acid heavy atoms. As production runs, for each of the 16 systems, five 2-ns equilibrium simulations were calculated, yielding a total of 80 2-ns simulations.

Systems varied slightly in size but had between 9000 and 9950 atoms with dimensions of approximately $60 \times 40 \times 40$ Å ^{3}. Short-range electrostatics were truncated at 12 Å with a switching function beginning at 10 Å, while long-range electrostatics were calculated with the particle mesh Ewald method[27] with a grid spacing of 1.0 Å and a sixth-order B-spline used for interpolation. The TIP3P water model was used[28] and a 2 fs time step was employed with SHAKE[29] to constrain bonds containing hydrogens.

Conformational entropies for the nucleic acid molecules were calculated by the use of quasiharmonic frequency analysis,[16,17] a method in which the eigenvalues λ_i of the mass weighted covariance matrix of atomic fluctuations are calculated to determine the quasiharmonic frequencies, $\omega_i = (k_BT/\lambda_i)^{1/2}$, which are then used to calculate the absolute value of conformational entropy via

$$S = k_B \sum_i^{3n-6} \frac{\hbar\omega_i/k_BT}{e^{\hbar\omega_i/k_BT} - 1} - \ln(1 - e^{-\hbar\omega_i/k_BT}) \qquad (2)$$

Frequencies were calculated from the nucleic acid's fluctuations beginning at 500 ps of production-run simulation time (to allow sufficient convergence of the covariance matrix of the fluctuations) and ending at each subsequent 100 ps interval until the end of the simulation time; as such, entropies $S(t)$ were determined from increasingly long trajectories of duration $t =$ 0.1, 0.2, ..., 1.5 ns. The limit of entropy at infinite sampling time S_∞ was extrapolated from the time-dependent entropies at the time points described above by using the formula[30,31]

$$S(t) = S_\infty - \frac{\alpha}{t^\gamma} \quad (3)$$

where α and γ were chosen to best fit the data. The mean conformational entropies at infinite sampling time were calculated and are shown in Tables 1 and 2, with the error bars representing the standard deviations between each of the five simulations for each set of conditions.

The driving force for the transitions is, however, the *change* in free energy between the right- and left-handed helical states, and so the relative entropy components of the free energy change as a result of the sum of conformational, solvent, and ionic entropies (as well as from just the conformational part) at 293 K are shown in Table 3.

Recently, a method of permutation reduction in which water molecules are "relabeled" to speculate permutation symmetry and, as a result, to enhance the convergence of the water covariance matrix was developed. This method aims to minimize the difference between a permuted frame of a trajectory and a reference structure. That is, a permutation (or "relabeling") π_i on a frame $x(t_i)$ is chosen so that the difference between the new frame and a reference structure x_0 is minimized (cf. eq 3 in ref 18):

$$\min(|\pi_i \cdot x(t_i) - x_0|^2) \quad (4)$$

This method was applied to both the water and ionic species (in high salt simulations) with code provided by the authors. Following this, quasiharmonic analysis was performed and entropies at infinite sampling were calculated as described before (with the exception of time points <1 ns being excluded from eq 3). For water in all the various systems, entropy values were calculated only for those molecules with an average oxygen position over the first 100 ps less than 3 Å away from a nucleic acid heavy atom (although all were permuted), as this minimized the sum of the variances from water entropies. For the ionic contribution, all ions in the system were used, with permutations and entropies from sodium and chloride ions calculated separately and combined for presentation.

On the topic of the accuracy of the force field used, it is of relevance to compare to studies of related nucleic acid conformational changes. An example concerns the simulations of the A−B transition utilizing the CHARMM27 force field, which reproduce well *structural* experimental data. For example, Reddy et al.[32] showed that the current CHARMM force field reliably produces a B-like helix in solution. Banavali and Roux[33] performed two-dimensional umbrella sampling to examine the thermodynamics of the A−B transition and presented free energy profiles that exhibited minima near the B- and α-helical forms. On the other hand, accurate *themodynamic* experimental data are lacking. It is difficult to experimentally measure the free energy difference between A- and B-DNA in aqueous solution because the structural difference between the two forms (unlike the difference between Z- and B-DNA) is difficult to resolve. This obviously impedes comparison with the free energies computed in simulations. Nevertheless, without the input of crystal structures of either helix, simulations show propensities for the correct high-resolution crystal structures expected under the corresponding solution conditions, providing for validation of the nucleic acid force fields.

4. Results

RNA. Switching experiments suggest Z-RNA to be entropically favored over A-RNA in the case of their chosen C·G sequence in high ionic environments. Conformational entropy calculations (Table 1) are clearly in accord with this: C·G Z-RNA dodecamers in low and high salt concentrations show a conformational entropic stabilization of 23−29 kcal/mol. Mixed-sequence RNA molecules show an inverted response, i.e., A-RNA conformations are entropically favored in low salt, whereas in high salt the two forms have almost identical conformational entropies.

Entropy differences of the solvent shells are large and overwhelm conformational entropies in low ionic environments (see Figure 3a) such that the total entropic value of the system favors the Z form in the case of a mixed sequence and the A form in C·G sequences (Table 3). High ionic environments tend to suppress solvent entropy differences, and in the C·G simulations ionic and solvent entropies nearly negate one another, resulting in conformational entropy dominating the entropic differences between left- and right-handed systems, with a total entropic difference of 30.0 kcal/mol (compared to the 22.6 kcal/mol value derived from experimental data).

DNA. Comparisons between entropies for DNA and RNA systems show interesting differences. While conformational entropy differences in RNA are largely sequence dependent, DNA conformational entropy differences show a high correlation with ionic environment and little sequence dependence (see Table 2). Absolute conformational entropies do show a decrease of 0.094 ± 0.024 kcal/mol/K for C·G sequences relative to mixed ones, possibly a result of more Watson−Crick hydrogen bonds in C·G sequences serving to stabilize the dodecamer and thus reducing motion. The presence of ions dampens conformational entropies in both sequences; however, the reduction for Z-DNA molecules is over twice as large as for B-DNA. Nevertheless, in all situations, left-handed helices are strongly favored by conformational entropy over right-handed ones.

Similar to results on RNA molecules, solvent and ionic entropies tend to oppose the stabilization effect of conformational entropy differences, except for the mixed sequence in high salt environment, where a slight reinforcement of the conformational entropy difference is observed (Figure 3b). Mixed sequences in low salt environments show an almost complete cancellation of entropy differences between Z and B form, whereas with the C·G sequence the solvent difference is much less in low ionic environment and there is still a preference for Z form. The only situation where a B form is entropically preferable concerns the C·G high ionic concentration simulations, in which both the solvent and ionic entropies combine to overcompensate for the conformational entropy favoring the Z form. Given the notoriously noisy estimates of entropy available from simulations, the difference of −28.2 kcal/mol compares favorably to the value of −12.5 kcal/mol we calculated from experimental data.

5. Concluding Discussion

The results presented here serve to explain the thermal switching seen between left and right handed nucleic acid devices as a complex interplay between conformational, solvent, and ionic entropy contributions. In the RNA case, sequence dictates the direction of conformational entropy preference, whereas an increase in ionic concentration tends to flip solvent entropy from opposing to reinforcing the direction favored by conformational entropy. For DNA, the strength of the ionic environment is the determining factor for the magnitude of the conformational entropy difference (which always favors the Z form) while solvent and ionic entropies tend to favor the B form. In the case of a C·G sequence in high salt, solvent and ionic

TABLE 1: RNA Absolute Conformational Entropy Values in Units of kcal·mol^{-1}·K^{-1}

	mixed sequence		C·G sequence	
	A-RNA	Z-RNA	A-RNA	Z-RNA
0 M [NaCl]	2.419 ± 0.069	2.319 ± 0.044	2.333 ± 0.054	2.413 ± 0.023
4 M [NaCl]	2.298 ± 0.027	2.282 ± 0.036	2.202 ± 0.034	2.302 ± 0.035

TABLE 2: DNA Absolute Conformational Entropy Values in Units of kcal·mol^{-1}·K^{-1}

	mixed sequence		C·G sequence	
	B-DNA	Z-DNA	B-DNA	Z-DNA
0 M [NaCl]	2.345 ± 0.009	2.584 ± 0.109	2.258 ± 0.033	2.518 ± 0.040
4 M [NaCl]	2.278 ± 0.020	2.447 ± 0.042	2.177 ± 0.062	2.315 ± 0.038

TABLE 3: Total Entropy Change, $-T\Delta S$, and, in Parentheses, Conformational Entropy Change, $-T\Delta S_{NA}$, of Transition from Left- to Right-Handed Forms in Units of kcal/mol at $T = 293$ K[a]

	Z→A-RNA		Z→B-DNA	
	mixed sequence	C·G sequence	mixed sequence	C·G sequence
0 M [NaCl]	30.1 ± 93.0	−12.2 ± 81.4	−2.1 ± 85.4	32.6 ± 87.8
	(−28.4 ± 20.8)	(23.3 ± 21.4)	(70.0 ± 27.8)	(76.2 ± 13.8)
4 M [NaCl]	−3.5 ± 26.9	30.0 ± 30.9	56.5 ± 36.1	−28.2 ± 61.6
	(−4.7 ± 12.6)	(29.2 ± 12.9)	(49.4 ± 12.0)	(40.4 ± 20.2)

[a] The large errors in the total entropy relative to the errors in the conformational entropies stem from the notorious difficulty in computing water entropy.

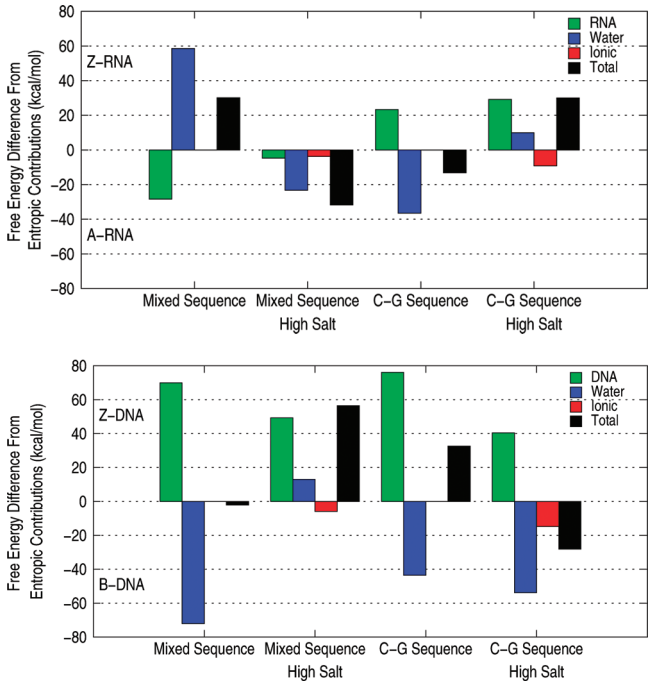

Figure 3. Entropic contributions to switching between left- and right-handed helices for (**a**) RNA and (**b**) DNA. Entropy component $-T\Delta S$ of free energy differences, at 293 K, for nucleic acid (green), water (blue), salt ions (red, only in high salt simulations), and their sum (black). Upon increasing temperature, left-handed helices are favored by positive values of this entropy component, while those favoring right-handed ones are negative.

entropies are enough to overcome the conformational entropy and favor the B form. For both RNA and DNA C·G sequences in high salt environments, our calculations are in agreement with experiments, which indicate an entropic preference for Z-RNA and B-DNA (by detecting a switching to these forms as the temperature increases). A study of high-resolution crystal structures showed that water molecules uniquely hydrate Z-DNA with an alternating C·G sequence.[34] Thus, with this in mind, it is not surprising that an entropic solvent difference would exist between forms of nucleic acid sequences.

The method of permutation reduction used here shows promise as a tool in calculations of solvent entropies. Absolute entropies calculated from this method corresponded to $T\Delta S$ free energies ranging from −5.30 to −6.21 kcal/mol/molecule, similar to values calculated from another recent theoretical scheme based on velocity and rotational autocorrelation methods that predicted entropies of individual water molecules surrounding DNA to be between −6.41 and −7.27 kcal/mol.[35] The large number of solvent molecules results in the sum of water entropies being much higher than those for the nucleic acid sequences, causing large error bars when differences are taken between systems. These errors were substantially reduced by examining only a subset of the solvent (those within 3 Å of the macromolecule). However, they are still quite large in some calculations (as can be seen in the difference between the errors in conformational and total entropy differences in Table 3). These uncertainties are not unique to this study and reflect the inherent difficulty in computing entropy accurately. Similar difficulties exist resolving the entropic components of free energy differences in MD simulations by other means, such as thermodynamic integration, thermodynamic perturbation, or nonequilibrium theorems, for which entropy change estimates involve, just like here, small differences of large averages.[36−38]

The error bars point toward limitations in the permutation reduction methods, and water entropy calculation results should be taken qualitatively, rather than quantitatively. It is, however, reassuring to note that the sign and magnitude of these values agree with what one would expect from a combination of only conformational entropies (which show much lower errors) and experimental results. Despite these errors, it is the sign of the difference that is meaningful and provides insight into nucleic acid equilibrium properties. While an exact value of the entropy change is not available from our simulations, assuming normal error distributions, we find a 67% probability for negative sign even in the worst case error (CG DNA sequence in high salt, see Table 3).

A connection can be made with comparative studies of the conformational flexibility of DNA and RNA. Nilsson and co-workers, and, independently, MacKerell and co-workers reported results from MD simulations indicating different structural

fluctuations of duplex RNA relative to DNA, leading to increased base pair opening events on the nanosecond time scale in the former (being favored by as much as 12 kcal/mol for a single opening).[39,40] There exists some controversy over the relative flexibilities of A-RNA and B-DNA helices. Results from NMR and some MD simulations suggest that B-DNA is more flexible than A-RNA,[41−43] whereas other MD studies point toward B-DNA as possessing a higher rigidity.[44] One interesting study pointed out that both scenarios may be true, in that, while DNA is more disordered on the local scale, these fluctuations tend to remain local and that the concept of overall nucleic acid stiffness may depend on the helical coordinate under consideration such that RNA is more mobile on a global scale.[45,46] We note that when comparing the A-RNA to B-DNA simulations presented here for dodecamers, the RNA consistently has a higher conformational entropy; however, the magnitude of that difference depends on the system being studied. For example, in the mixed sequence/low salt conditions, A-RNA has an entropy 0.079 kcal/mol/K higher than that of B-DNA in a similar simulation, but this difference may drop by a factor of 4 when immersed in a high-salt environment. It is evident that the conformational entropy of left- and right-handed nucleic acid helices plays an important role in their thermal transitions, and that the driving force resulting from these differences can explain their sequence and salt dependence when they are used as nanothermometers devices. These calculations are akin to MD simulations on proteins, which demonstrate that the temperature dependence of ionic conditions is related to amino acid sequence,[47] and further demonstrate the complex balance between local structures, ionic conditions, and conformational entropy in biomolecules.

It is of interest to comment on an early calculation of B- versus Z-form conformational entropy by Irikura et al.,[48] which had indicated, for a $(CGCGCG)_2$ hexamer, an opposite result than that for the $(CGCGCGCGCG)_2$ dodecamer studied here. Using normal mode decomposition (i.e., a purely harmonic analysis by diagonalization of the Hessian matrix instead of the covariance matrix of atomic fluctuations used in quasiharmonic analysis), the Irikura et al. study showed a higher configurational entropy for the B-form compared to the Z-form hexamer. When we used normal-mode analysis for our dodecamer CG sequence equilibrated in high salt concentration, B- and Z-DNA molecules showed a similar result to the normal mode study on the hexamer when a $4r$ distance-dependent dielectric function was used. However, when we performed the normal mode calculations with a generalized Born implicit solvation model,[49] the configurational entropy switched to favoring the Z form (as in the quasiharmonic results in explicit water). While both normal modes and implicit solvation are approximations, the differences in the results provide further evidence that solvent conditions have a significant effect on the entropy difference between helical states.

Another early computational study addressed the issue of the stability of A•T basepairs relative to C•G ones in B- and Z-DNA hexamers.[50] In that work, free energy perturbations were performed to mutate bases, both in the B- and Z- helices. Results indicated that, for a system in solution with neutralizing ions, there is an additional 3 kcal/mol energy change required to mutate a C•G base into an A•T one in the Z form relative to the B form, and that there is a cooperative effect in mutating multiple bases. While not directly comparable, results presented in Tables 2 and 3 show that, in the low ionic case, the mutation of six basepairs results in a lessening of the "Z-phobicity" by 34.7 kcal/mol due to entropic effects. It is not surprising that, due to factors such as sampling length, force fields, and system configurations, there is quantitative discrepancy. For example, in their study, results were derived from 40 ps of simulation time, an insufficient length for sampling the rearrangement of solvent molecules near the DNA surface. Indeed, our results indicate that the conformational entropy, which converges much quicker, has an entropic Z-phobicity of only 6.2 kcal/mol. Nevertheless, our results compare quite well in a qualitative sense to those from Dang et al. in that mutation of DNA bases in low ionic conditions from A•T to C•G favors Z-DNA structures.

In summary, we have performed an extensive analysis of the entropic components of left- and right-handed DNA and RNA helices in multiple systems of varying ionic concentrations and nucleic acid sequences. For the system for which experimental data exists (the high salt, C•G sequence), our results compare favorably with experimental data and serve to explain the thermoresponsive nature of these oligomers. Comparisons can also be made between the A-RNA and B-DNA systems and add to the discussion on the different flexibilities of the two molecules. The concentration of salt used in the experiments is outside the physiological role; this tempers any excitement of using them to measure temperature in cellular environments. However, it may be possible to design modifications to the duplexes that would allow them to access left-handed conformations in lower salt conditions. The variability of our computed entropy changes for the variety of simulations for the mixed sequence and in low−high salt serves to indicate a complex dependence on salt and sequence, and that will have to be accounted for and finely tuned by any future development of nanothermometers based on nucleic acid oligomers. For example, the fact that Z-DNA can be stabilized effectively by negatively supercoiled DNA[7] can be speculated by suggesting negatively supercoiled DNA minicircles that can be used in physiological environments for thermal nanoscale sensing in the cell.

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References and Notes

(1) Yan, H.; Zhang, X. P.; Shen, Z. Y.; Seeman, N. C. A robust DNA mechanical device controlled by hybridization topology. *Nature* **2002**, *415*, 62–65.

(2) Mao, C. D.; Sun, W. Q.; Shen, Z. Y.; Seeman, N. C. A nanomechanical device based on the B−Z transition of DNA. *Nature* **1999**, *397*, 144–146.

(3) Yurke, B.; Turberfield, A. J.; Mills, A. P.; Simmel, F. C.; Neumann, J. L. A DNA-fuelled molecular machine made of DNA. *Nature* **2000**, *406*, 605–608.

(4) Lee, J.; Kotov, N. A. Thermometer design at the nanoscale. *Nano Today* **2007**, *2*, 48–51.

(5) Tashiro, R.; Sugiyama, H. A nanothermometer based on the different π stackings of B- and Z-DNA. *Angew. Chem., Int. Ed.* **2003**, *42*, 6018–6020.

(6) Tashiro, R.; Sugiyama, H. Biomolecule-based switching devices that respond inversely to thermal stimuli. *J. Am. Chem. Soc.* **2005**, *127*, 2094–2097.

(7) Rich, A.; Nordheim, A.; Wang, A. H. J. The chemistry and biology of left-handed Z-DNA. *Annu. Rev. Biochem.* **1984**, *53*, 791–846.

(8) Auffinger, P.; Hashem, Y. Nucleic acid solvation: from outside to insight. *Curr. Opin. Struct. Biol.* **2007**, *17*, 325–333.

(9) Feig, M.; Pettitt, B. M. A molecular simulation picture of DNA hydration around A- and B-DNA. *Biopolymers* **1998**, *48*, 199–209.

(10) Pohl, F. M.; Jovin, T. M. Salt-induced cooperative conformational change of a synthetic DNA - Equilibrium and kinetic studies with poly(dG-dC). *J. Mol. Biol.* **1972**, *67*, 375–396.

(11) Misra, V. K.; Honig, B. The electrostatic contribution to the B to Z transition of DNA. *Biochemistry* **1996**, *35*, 1115–1124.

(12) Wang, A. H. J.; Quigley, G. J.; Kolpak, F. J.; Vandermarel, G.; Vanboom, J. H.; Rich, A. Left-handed double helical DNA - Variations in the backbone conformation. *Science* **1981**, *211*, 171–176.

(13) Kastenholz, M. A.; Schwartz, T. U.; Hunenberger, P. H. The transition between the B and Z conformations of DNA investigated by targeted molecular dynamics simulations with explicit solvation. *Biophys. J.* **2006**, *91*, 2976–2990.

(14) Teng, M. K.; Liaw, Y. C.; Vandermarel, G. A.; Vanboom, J. H.; Wang, A. H. J. Effects of the O2′ hydroxyl group on Z-DNA conformation - Structure of Z-RNA and (araC)-[Z-DNA]. *Biochemistry* **1989**, *28*, 4923–4928.

(15) Karplus, M.; Kushick, J. N. Method for estimating the configurational entropy of macromolecules. *Macromolecules* **1981**, *14* (2), 325–332.

(16) Brooks, B. R.; Janezic, D.; Karplus, M. Harmonic-analysis of large systems. 1. Methodology. *J. Comput. Chem.* **1995**, *16*, 1522–1542.

(17) Andricioaei, I.; Karplus, M. On the calculation of entropy from covariance matrices of the atomic fluctuations. *J. Chem. Phys.* **2001**, *115*, 6289–6292.

(18) Reinhard, F.; Grubmuller, H. Estimation of absolute solvent and solvation shell entropies via permutation reduction. *J. Chem. Phys.* **2007**, *126*, 014102.

(19) McMillan, W. G.; Mayer, J. E. The statistical thermodynamics of multicomponent systems. *J. Chem. Phys.* **1945**, *13*, 276–305.

(20) Soumpasis, D. M. Statistical-mechanics of the B→Z transition of DNA - Contribution of diffuse ionic interactions. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5116–5120.

(21) Nosé, S. A unified formulation of the constant temperature molecular-dynamics methods. *J. Chem. Phys.* **1984**, *81*, 511–519.

(22) Hoover, W. G. Canonical dynamics - Equilibrium phase-space distributions. *Phys. Rev. A* **1985**, *31*, 1695–1697.

(23) Nose, S.; Klein, M. L. Constant pressure molecular-dynamics for molecular-systems. *Mol. Phys.* **1983**, *50*, 1055–1076.

(24) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM - A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983**, *4*, 187–217.

(25) Foloppe, N.; MacKerell, A. D. All-atom empirical force field for nucleic acids: I. Parameter optimization based on small molecule and condensed phase macromolecular target data. *J. Comput. Chem.* **2000**, *21*, 86–104.

(26) MacKerell, A. D.; Banavali, N.; Foloppe, N. Development and current status of the CHARMM force field for nucleic acids. *Biopolymers* **2000**, *56*, 257–265.

(27) Essmann, U.; Perera, L.; Berkowitz, M. L.; Dar-den, T.; Lee, H.; Pedersen, L. G. A smooth particle mesh Ewald method. *J. Chem. Phys.* **1995**, *103*, 8577–8593.

(28) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **1983**, *79*, 926–935.

(29) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. Numerical integration of the Cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. *J. Comput. Phys.* **1977**, *23*, 327–341.

(30) Harris, S. A.; Gavathiotis, E.; Searle, M. S.; Orozco, M.; Laughton, C. A. Cooperativity in drug-DNA recognition: A molecular dynamics study. *J. Am. Chem. Soc.* **2001**, *123*, 12658–12663.

(31) Harris, S. A.; Laughton, C. A. A simple physical description of DNA dynamics: Quasi-harmonic analysis as a route to the configurational entropy. *J. Phys.: Condens. Matter* **2007**, 19.

(32) Reddy, S. Y.; Leclerc, F.; Karplus, M. DNA polymorphism: A comparison of force fields for nucleic acids. *Biophys. J.* **2003**, *84*, 1421–1449.

(33) Banavali, N. K.; Roux, B. Free energy landscape of A-DNA to B-DNA conversion in aqueous solution. *J. Am. Chem. Soc.* **2005**, *127*, 6866–6876.

(34) Gessner, R. V.; Quigley, G. J.; Egli, M. Comparative-studies of high-resolution Z-DNA crystal-structures. 1. Common hydration patterns of alternating dC-dG. *J. Mol. Biol.* **1994**, *236*, 1154–1168.

(35) Jana, B.; Pal, S.; Maiti, P. K.; Lin, S. T.; Hynes, J. T.; Bagchi, B. Entropy of water in the hydration layer of major and minor grooves of DNA. *J. Phys. Chem. B* **2006**, *110*, 19611–19618.

(36) Fleischman, S. H.; Brooks, C. L. Thermodynamics of aqueous solvation - Solution properties of alcohols and alkanes. *J. Chem. Phys.* **1987**, *87*, 3029–3037.

(37) Lu, N.; Kofke, D. A.; Woolf, T. B. Staging is more important than perturbation method for computation of enthalpy and entropy changes in complex systems. *J. Phys. Chem. B* **2003**, *107*, 5598–5611.

(38) Nummela, J.; Yassin, F.; Andricioaei, I. Entropy-enthalpy decomposition from nonequilibrium work trajectories. *J. Chem. Phys.* **2008**, *128*, 024104.

(39) Hart, K.; Nystrom, B.; Ohman, M.; Nilsson, L. Molecular dynamics simulations and free energy calculations of base flipping in dsRNA. *RNA-A Publication of The RNA Society* **2005**, *11*, 609–618.

(40) Banavali, N. K.; MacKerell, A. D. Free energy and structural pathways of base flipping in a DNA GCGC containing sequence. *J. Mol. Biol.* **2002**, *319*, 141–160.

(41) Fujiwara, T.; Shindo, H. P-31 nuclear magnetic-resonance of highly oriented DNA fibers 0.2. Molecular motions in hydrated DNA. *Biochemistry* **1985**, *24*, 896–902.

(42) Cheatham, T. E.; Kollman, P. A. Molecular dynamics simulations highlight the structural differences among DNA:DNA, RNA:RNA, and DNA:RNA hybrid duplexes. *J. Am. Chem. Soc.* **1997**, *119*, 4805–4825.

(43) Auffinger, P.; Westhof, E. Water and ion binding around RNA and DNA (C, G) oligomers. *J. Mol. Biol.* **2000**, *300*, 1113–1131.

(44) Pan, Y. P.; MacKerell, A. D. Altered structural fluctuations in duplex RNA versus DNA: A conformational switch involving base pair opening. *Nucleic Acids Res.* **2003**, *31*, 7131–7140.

(45) Noy, A.; Perez, A.; Lankas, F.; Luque, F. J.; Orozco, M. Relative flexibility of DNA and RNA: A molecular dynamics study. *J. Mol. Biol.* **2004**, *343*, 627–638.

(46) Perez, A.; Noy, A.; Lankas, F.; Luque, F. J.; Orozco, M. The relative flexibility of B-DNA and A-RNA duplexes: Database analysis. *Nucleic Acids Res.* **2004**, *32*, 6144–6151.

(47) Ma, L.; Cui, Q. The temperature dependence of salt-protein association is sequence specific. *Biochemistry* **2006**, *45*, 14466–14472.

(48) Irikura, K. K.; Tidor, B.; Brooks, B. R.; Karplus, M. Transition from B-DNA to Z-DNA - Contribution of internal fluctuations to the configurational entropy difference. *Science* **1985**, *229*, 571–572.

(49) Lee, M. S.; Salsbury, F. R.; Brooks, C. L. Novel generalized Born methods. *J. Chem. Phys.* **2002**, *116*, 10606–10614.

(50) Dang, L. X.; Pearlman, D. A.; Kollman, P. A. Why do A·T base pairs inhibit Z-DNA for-mation. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4630–4634.

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