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Denaturation of Drew–Dickerson DNA in a High Salt Concentration Medium: Molecular Dynamics Simulations

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We have performed molecular dynamics simulation on B-DNA duplex (CGCGAATTGCGC) at different temperatures. The DNA was immerged in a salt-water medium with 1 M NaCl concentration to investigate salt effect on the denaturation process. At each temperature, configurational entropy is estimated using the covariance matrix of atom-positional fluctuations, from which the melting temperature (T_m) was found to be 349 K. The calculated configuration entropy for different bases shows that the melting process involves more peeling (including fraying from the ends) conformations, and therefore the untwisting of the duplex and peeling states form the transition state of the denaturation process. There is a narrow minor groove in the AATT sequence that becomes wider by increasing temperature which disappears at high temperatures, especially above the melting temperature. We have also calculated the fraction of denatured base pairs, *f*-curve, from which T_m was found to be 340 K, close to experimental value of 341 K. We found that DNA at high salt concentrations has few hydrogen bonds even at temperatures higher than the T_m. Our calculations show the fact that adding salt leads to increase of T_m and stabilization of DNA. © 2011 Wiley Periodicals, Inc. J Comput Chem 32: 3354–3361, 2011

Keywords: DNA · configurational entropy · melting temperature · molecular dynamics · fraction of denatured base pairs

Introduction

The main role of DNA molecules is the long-term storage of information to construct other components of cells, such as proteins and RNA molecules.

The study of the structure of DNA is important for understanding some phenomena, such as replication, transcription, and breathing of DNA.[1] In such phenomena, the DNA is separated into two single strands, which occurs when the hydrogen bonds between two strands are broken. One feature of the DNA that has attracted noticeable attention is its thermal denaturation or melting, that is the transition from the native double helix B-DNA to its melted form, in which two strands spontaneously separate on heating,[2] because it provides an example of a one-dimensional phase transition. Experiments show this transition is very sharp, which suggests that it could be a first order.[3] The knowledge of denaturation process is important to understand the DNA replication, manipulation, and interactions involving DNA double helix stability.

Entropy is a key property to the understanding of a wide variety of physical, chemical, and biochemical phenomena.[4–6] It is a thermodynamic property that measures the order of a system. For soft-matter systems, such as polymers, proteins, and nucleic acids, the entropy plays an important role in the thermodynamic stability of conformational states.[7] The total entropy of a molecule in solution can be separated into two parts, a solvent entropy associated with solvent motions, and a solute, or configurational entropy associated with solute motions.[8] Changes in the entropy of solute are thought to make a substantial contribution to the important biochemical processes, such as the protein folding and molecular association. For example, the Irikura et al. showed that for the Z and B forms of DNA, their configurational entropy difference is important in stabilizing of the B form, compared with the Z form. The difference in the stabilization provides the evidence that the solvent conditions (including salt concentration, salt ions, temperature, and water) have a significant effect on the entropy difference between the two mentioned forms.[9] Therefore, the solvent conditions in fact influence on the configurational entropy of solute. As a consequence, methods of quantifying the configurational entropy could help explain how the biomolecules function, and methods of controlling it could be valuable for molecular design.

Entropy calculations are notoriously difficult because the entropy is a measure of the overall extent of phase space accessible to a molecular system.[10] Using experimental calorimetric studies, only the total change in entropy associated with a given process is directly accessible.[11] It has become possible to estimate changes in the configurational entropy from NMR data, this approach yields remarkable insights.[12] The possibility to estimate the configurational entropy from molecular dynamics (MD) trajectories was first proposed by Karplus and Kushick under a quasi-harmonic assumption.[13] In this approach, the distribution of the various degrees of freedom is assumed to have a multivariate Gaussian form. It was applied to bound (nondiffusive) systems on the basis of internal coordinates (such as the bond lengths, bond angles, and dihedral angles). But this approach has a number of drawbacks, for example, the use of internal coordinates complicates the computational implementation of the method.[6] Some years later,

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Schlitter introduced a heuristic formula in terms of Cartesian coordinates instead of internal ones.[14] The Schlitter's formula computes an approximate upper bound for the absolute entropy of a molecule from simulation trajectory.[6] Andricioaei and Karplus revised the quasiharmonic approach to enable the use of Cartesian coordinates.[15] It was later shown that the formulations which was proposed by Schlitter and by Andricioaei and Karplus, resulted in very similar entropy estimates.[16,17] The Schlitter's approach is computationally less expensive, and also it allows not only the calculation of the configurational entropy of the entire chain but also the calculation of the configurational entropy for different subsets of atoms or degrees of freedom. For such advantages, this approach is applied in this work. This method was successfully tested for biomolecular simulations of peptide folding[18,19] and also applied to simulate protein on the molten globule states.[20] Dolenc et al. considered only the entropy change due to the change in ligand flexibility for calculation of configurational entropy change of Netropsin and Distamycin on DNA minor groove binding.[21] Hsu et al. calculated the configurational entropy changes of HIV-1 Env gp120, its receptor CD4, and their complex based on three different sets of atoms.[22] Baron et al. calculated configurational entropy to compare atomic-level and coarse-grained models for liquid hydrocarbons.[17] The procedure outlined by Schlitter, which is based on Cartesian coordinates was implemented and tested on simulations of reversible peptide folding in methanol at different temperatures.[23] Baron et al. evaluated the configurational entropy of lipid tails in pure and hydrated lipid bilayers.[24] We have recently reported a melting temperature based on the configurational entropy and hydrogen bonding energy for Drew–Dickerson DNA but in the pure water medium.[25]

Determining the melting temperature, T_m, of an oligo is essential for many applications, such as polymerase chain reaction, capture assays, mutagenesis, hybridization, and sequencing. The exact T_m of DNA can be determined only by empirical means. The common experimental method to determine T_m is to slowly heat a sample of buffer solution containing the DNA duplex, while recording its absorbance at a wavelength of 260 nm. As the absorbance of two single strands is higher than that of the same strands forming a double helix, the cooperative melting of a DNA duplex can be monitored as an increase in absorption with temperature.[26]

In many cases, the T_m of a DNA or RNA duplex can be determined within a theoretical model. The simplest equation for T_m is the Wallace rule that only considers the number of occurrences of each nucleotide in the DNA.[27]

Another familiar equation to predict T_m of DNA which is generally used for the oligos with more than 50 bases is the GC ratio method, where the GC ratio is the number of G or C nucleotides divided by the total length of the DNA.[28]

The nearest-neighbor method is considered to be one of the most accurate predictions for T_m. This equation takes into account the actual sequence of DNA, whereas the other equations consider only the base composition of DNA.[29–31] This method takes into account both the enthalpy of the pair formation between two nucleotides and the stacking effect between the nearest-neighbored nucleotide pairs. In many cases, however, a computational approach is not feasible or accurate, for instance, when the nonstandard buffer conditions are used, or two DNA strands are mismatched or contain the secondary structure, extensively. In these cases, experimental determination of T_m is necessary. The melting temperature for short duplex DNAs is usually calculated using the nearest-neighbor formula that typically employs 1 M monovalent cation as a reference buffer. The estimated T_m obtained from this calculation will be the most accurate value if the used buffer is similar to that originally used in experiment, in which the nearest-neighbor thermodynamic parameters have already been determined. The melting temperature is then scaled (i.e., for the salt correction) from the reference condition to a buffer with the desired composition. Significant progress has been made to improve the nearest-neighbor and next-nearest-neighbor parameters that are used to predict T_m for the DNA and RNA duplex oligomers.[32–35]

Owczarzy et al. reviewed different equations used to predict T_m of the DNA and compared them. Their analysis demonstrated that the usage of these equations can lead to large errors in the predicted value of T_m ($>10^\circ$C). They published a new equation to scale T_m when the monovalent cation concentration changes,[36] and presented a more accurate empirical formulas for prediction of T_m in the buffers containing magnesium and monovalent ions.[37] As far as we know, the configurational entropy and also the fraction of denatured base pairs, *f*-curve, obtained by using MD simulation have not been employed to determine T_m of DNA.

The aim of this study is to investigate the configurational entropy for atoms participating in the interstrand hydrogen bonding of DNA, the configurational entropy per particle for each base pair, and the fraction of denatured base pairs of a 12-base-pair (bp) segment of DNA at different temperatures to obtain the melting temperature (T_m) and the pathway of DNA melting. Simulations have been performed on a B-form sequence d(CGCGAATTCGCG) which is known as Drew–Dickerson oligomer. It has been extensively studied both structurally and theoretically,[38] because containing the underlined EcoRI binding site (CGCGAATTCGCG) and the central AATT sequence constitutes a feature (called spine of hydration) stabilizing B-form DNA.[39] We use an approach which is based on the covariance matrix of atomic mass-weighted fluctuations, because it allows the calculation of the configurational entropy for different subsets of atoms or degrees of freedom. Finally we shall compare the melting temperature (T_m) and shape of transition curves obtained from the configurational entropy, and *f*-curve, to see the impact of different interactions on the denaturation process. Also we investigate high salt concentration effect on DNA melting, as well as DNA melting pathway.

Computational Procedures

MD simulations have been carried out in a hexagonal cell with the periodic boundary conditions imposed in the *xy*-plane and *z*-direction. Simulations were performed on a B form sequence

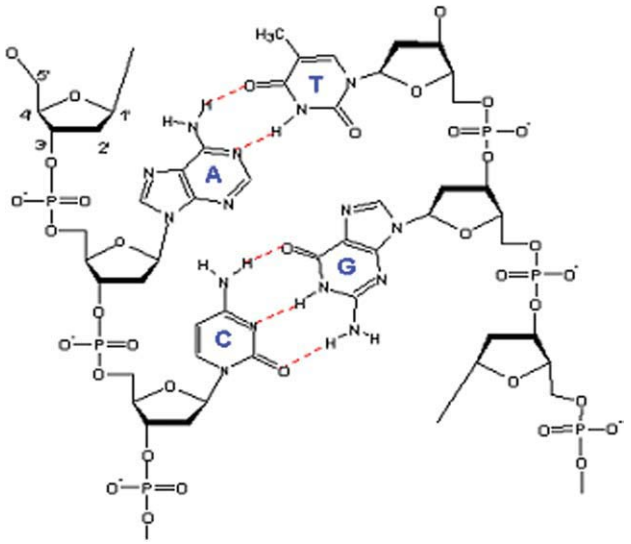

Figure 1. A portion of primary structure of DNA, only with two base pairs. Hydrogen bonds between the pairs of adenine, thymine, guanine, and cytosine are shown by the red-dash lines.

d(CGCGAATTCGCG) employing from the Protein Data Bank crystal structures (1bna.pdb).[40] This DNA is solvated with 3558 transferable intermolecular potential 3 point water molecules which allows for a 10 Å-shell of water surrounding a solute molecule. The system has been neutralized by adding 45 sodium ions and 23 chloride ions, corresponds to 1 M NaCl, which is the total ions concentration. The MD simulation has been carried out at seven different temperatures, within the range of 280–400 K with the 20 K intervals, in the Isothermal-Isobaric (*NPT*) ensemble. The simulation was performed for 50 ns at 280, 360, 380, and 400 K; but for 7 ns at 300, 320, and 340 K. An integration time step of 2 fs was used. Each MD simulation was carried out at constant temperature and pressure (1 atm) using the Langevin dynamics. Long range electrostatic interactions were treated using the particle mesh Ewald approach and the cut-off distance for the van der Waals interactions was 12 Å. All simulations were performed using version 27 of the CHARMM force field[41,42] and the MD program NAMD 2.6.[43] All hydrogen bonds were constrained during the MD simulations using the SHAKE algorithm.[44] The cell dimensions were 42.5 × 46.9 × 63.9 $Å^3$. Each system contained 11,500 atoms, including the ions.

Entropy calculation

Configurational entropy calculations were performed following the formulation by Schlitter,[14] which provides an approximate[17] upper bound to the absolute entropy *S*:

$$S < S_{\text{Schiltter}} = \frac{1}{2} k_B \ln \det\left[\underline{\mathbf{1}} + \frac{k_B T e^2}{\hbar} \underline{\mathbf{M}}\sigma\right] \quad (1)$$

where k_B is Boltzmann factor, *T* is the absolute temperature, *e* is Euler's number, $\hbar$ is Planck's constant divided by 2π, $\underline{\mathbf{M}}$ is a 3*N*-dimensional diagonal matrix containing *N* atomic masses of the solute atoms for which the entropy is calculated, $\underline{\mathbf{1}}$ is the unitary matrix, and $\underline{\sigma}$ is the covariance matrix of atom-positional fluctuations with the elements:

$$\sigma_{ij} = \left\langle (x_i - \langle x_i \rangle)(x_j - \langle xj \rangle) \right\rangle \quad (2)$$

where x_i is the Cartesian coordinate of atom *i*, considered in the entropy calculation. The application of quasi-harmonic analysis in terms of Cartesian coordinates requires the removal of the overall (center of mass) translational motion from the sampled configurations (because the analysis assumes a bound system).[6] In principle, the removal of the overall rotational motion is not required, but it is recommended in practice.[6,19] In practice, the overall translation and rotation from sampled configurations is commonly performed by atom-positional least-squares fitting of successive structures along a trajectory onto a common reference structure.[45] The subsets of atoms or coordinate system that used for fitting influence on the final entropy estimates,[21] and the optimal coordinate system will be the one leading to the lowest estimate.[6] Unfortunately, there is currently no systematic procedure for determining this optimal coordinate system. Schäfer et al. calculated entropy for a beta-heptapeptide in methanol at different temperatures.[19] As the rigid body motion is not removed automatically during the simulation, three different fitting procedures were studied: (1) no fit, all rigid body (translation and overall rotation) was kept. (2) A translational fit, so translation is removed. (3) A least-square fit on the positions of all atoms,

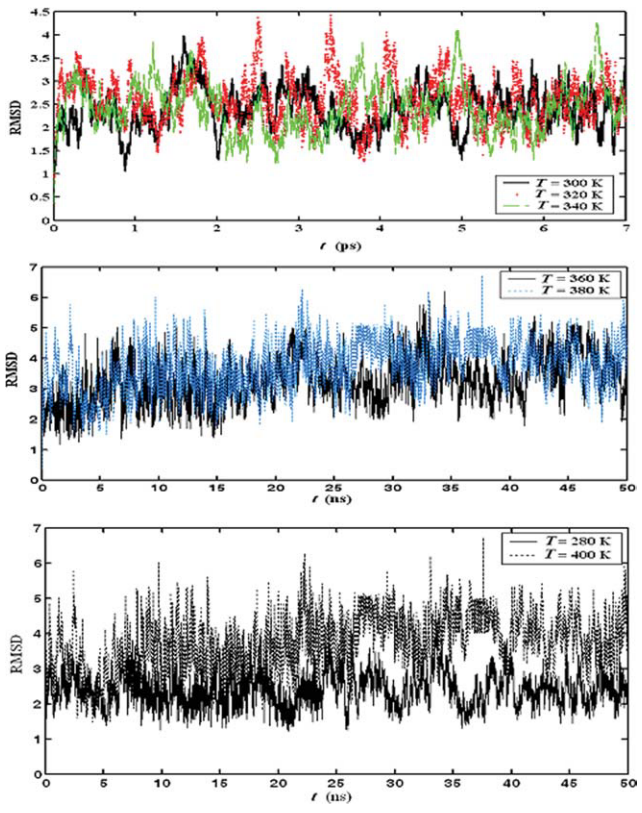

Figure 2. Atom-positional RMSD (for all atoms in the DNA structure) of trajectory structures from the initial structures at the given absolute temperatures.

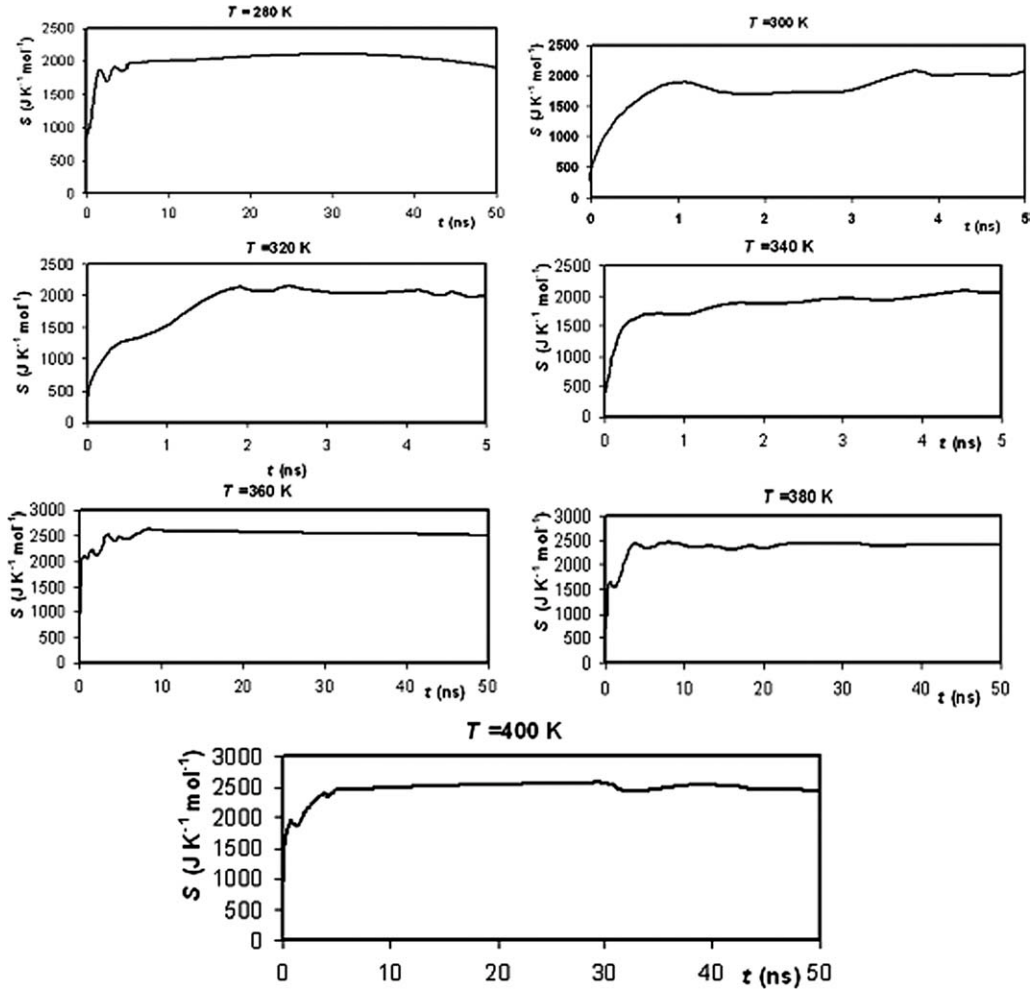

Figure 3. Calculated configurational entropy for those atoms participating in the H-bondings between two strands of Dickerson DNA in a salt-water medium.

so translational and rotational motions are removed. The difference between the entropy calculated without fitting and that calculated with the translational fit yields the translational entropy contribution.[19] But in this work, we do not apply a least-squares fitting in the calculation of the configurational entropy, because: (1) we are going to calculate the transition curve from entropy data so if all rigid body (translation and overall rotation) is kept in one data it will be repeated in the others, as well. (2) Translational and rotation motions are important in the DNA melting, and therefore have considerable contributions in entropy. (3) The choice of subsets of atoms for fitting procedure influences on the entropy estimate.

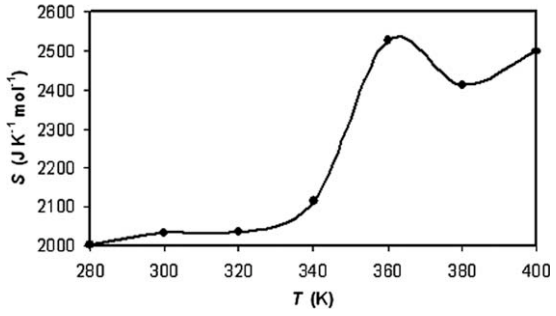

Figure 4. The calculated configurational entropy versus T to estimate the melting temperature obtained from simulations. The simulation data are fitted by a cubic constrained spline with $R^2 = 1.00$.

Entropy calculations are performed on trajectory structures saved every 1 ps. Figure 1 illustrates a portion of primary structure of DNA, only with two base pairs. Four different bases in DNA are shown in this figure. Subsets of atoms that are used in the calculation of the configurational entropy are those directly participate in hydrogen bonding in 5-GCGTTAACGC-3 sequence, for example, O, N, and N atoms in Guanine, excluding hydrogen atoms due to having high fluctuation and are poorly approximated by harmonic oscillator. The backbone and the terminal base pairs exhibit large movements, partially as a result of their extensive contact with the surrounding solvent molecules; therefore they are excluded, as well.

Results

The structural stability in MD simulations is often characterized by the root mean square displacement (RMSD) of atomic positions from a suitable reference structure. The calculated RMSDs

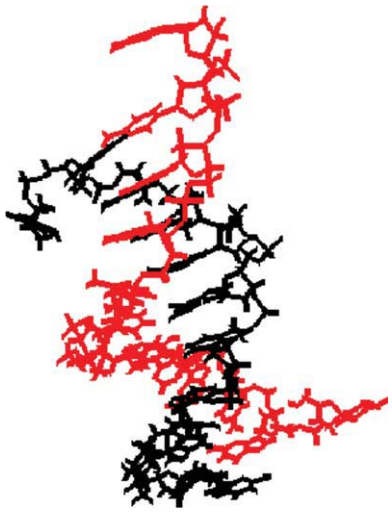

Figure 5. A snapshot of the peeling conformation at 360 K.

at some temperatures are presented in Figure 2. On the basis of Figure 2, we consider the initial time of 2000 ps (pico second) needed to reach the equilibrium state. As shown in Figure 2, it is obvious that the RMSD increases with temperature. The simulation was performed for 50 ns at 280, 360, 380, and 400 K, however, the performance was done only for 7 ns at 300, 320, and 340 K, because of the fact that the system reaches to the equilibrium, quickly. Also, unlike other alternative temperatures, between $T = 340$ and $T = 360$ K, a gap in RMSD can be seen in the last steps of the simulation.

We have calculated the entropy on the basis of the approach described in the previous section. Figure 3 shows the configurational entropy for the DNA segment at some different temperatures. The fluctuations in entropy are indicative of the exploration of phase space by the DNA; each jump corresponds to a new region of phase space.[19] Amino acids are flexible, so their tumbling time is generally long, like any other macromolecules. We observed many base pairs break and reform, so fluctuation (or exploration of the phase space) in the configurational entropy is high. The entropy usually increases within the temperature range of 280–400 K, but the increase within 340–360 K is significantly higher than that for the other ranges. The convergence of entropy can be easily seen in Figure 3, after some period of time. We have shown the equilibrium entropy at each temperature with a point in Figure 4, but the line is simply drawn to guide the eye, which is fitted by a mathematical method (spline estimation and cubic constrained algorithm with the coefficient of determination $R^2 = 1.00$). Each point is obtained from the converged value of entropy given in Figure 3. This is a transition curve which resembles a first order phase transition curve. Inflection point of this curve turns out to be at 349 K, which is the melting temperature of the DNA segment used in the simulations. We have put the segment in a salt-water medium of 1 M NaCl. The melting temperature experimentally measured for Dickerson DNA is 341 K[46] in the presence of 1 M NaCl. Solvent and counterions play a major role in stabilizing the double helix and in determining its overall conformation. DNA has a polyionic nature, and increasing the counterion concentration increases its melting temperature.[47] The trend of the entropy with temperature is generally correct: entropy increases with temperature. The calculated entropy at 380 K is slightly lower than that at 360 K, which can be attributed to the peeling conformational states (Fig. 5). Wong and Pettitt found that when peeling and untwisting states do not occur, the DNA is able to search for various possible Watson–Crick complements. This searching is stopped when the system forms non-native stacking or peeling states.[48] In our simulation, the peeling states seen at 360 K are more significant than that at 380 and 400 K. For instance, the average RMSD value for CYT1 at 360 K is 2.96, whereas at 380 K and 400 K are 1.96 and 2.64, respectively. Therefore, we may conclude that the untwisting and peeling of the duplex forms the transition state of the denature process.[48]

By using only a subset of the atoms of a molecule in the calculation of the covariance matrix of atom-positional fluctuations, it is possible to calculate the entropy of each base pair of DNA. In Figure 6, the entropy per particle for different base pairs of Drew–Dickerson oligomer is shown. Owing to the fact that adenine, guanine, thymine, and cytosine have different number of atoms, so to make a reasonable comparison, the calculated entropy of each base pair is divided by its corresponding number of atoms. It is observed that the entropy for the base pairs at the ends of helix is more than the remaining pairs (in the bottom of Fig. 6, the order of base pairs is shown by the numbers). So the terminal base pairs exhibit large movements, partially as a result of their extensive contact with the surrounding solvent molecules and also DNA proceeds to denature from the ends. By increasing temperature, the entropy increases for all base pairs. However, above the melting temperature, this trend does not follow for four base pairs from the CYT3 end (the nucleotide sequence of DNA by numbering can also be seen in bottom of Fig. 6) at 360 K, in other words, the entropy per atom of DNA bases at the CYT3 end is higher than that at 380 and 400 K. As shown before in Figure 4, the entropy at 360 K is larger than that at 380 and 400 K. As shown before in Figure 5, the untwisting of the duplex and

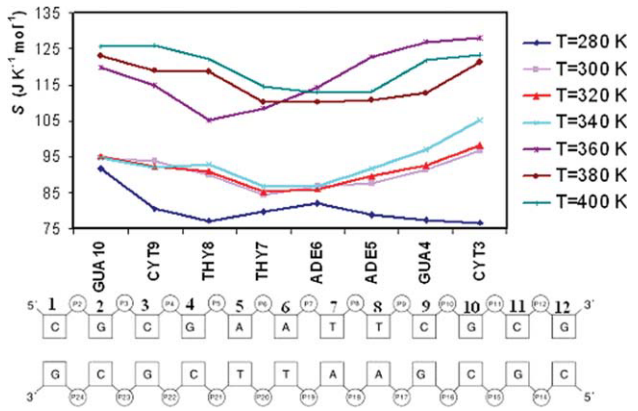

Figure 6. The configurational entropy per atom for different base pairs of the DNA strand at the given temperatures. The sequences of base pairs of the Drew–Dickerson oligomer which is specified by the numbers are shown at the bottom.

peeling conformations are seen for two ends of the DNA at 360 K, hence the larger movements cause the increment of the configurational entropy. Based on such results, we may conclude that the untwisting of the duplex forms the transition state of the denaturing process.[48] The entropy in the middle of helix above the melting temperature is approximately constant, because the interstrand hydrogen bonds are broken. As the two strands become further apart, water molecules can penetrate interbase gap and it may energetically become more favorable to form H-bond with water molecules rather than between the strands,[49] which leads to an increase in entropy. Hamelberg et al.[50] reported, wherever cross-strand cation–phosphate oxygen pair interactions exist, that be seen narrowing of the minor groove width. In other words, the minor groove is most narrow at the site of ion interactions and in each case, the minor groove progressively widens away from the site of interaction. The narrowing of the minor groove width causes decrease in entropy. At 280 K, the entropy is almost same for all bases in the minor groove (GAATTC). So, at low temperatures there are ion–phosphate interactions in all sites of minor groove. But by increasing temperature, the entropy is the lowest at the middle of DNA sequence. But at high temperatures (380 and 400 K), this trend does not follow. Therefore, we can conclude that the ion–phosphate interactions are disrupted at high temperatures.

Intrahydrogen bonding of two strands

Both nonbonding stacking and hydrogen bonding take part in the helix stability. Regarding the stability of H-bond, two factors are important: the distance between donor–acceptor must be less than 3 Å and the angle of donor-H-acceptor must be approximately 160°.[51] At each temperature, we have calculated the average distance of donor–acceptor and average angle of donor-H-acceptor, then by applying the mentioned conditions, the fraction of denatured base pairs, *f*, is calculated. Such calculation has been done at seven different temperatures; the results are depicted in Figure 7. This curve is some what broader than its experimental counterpart, particularly in the shoulder regions of the curve, because in simulation there is some arbitrariness at the molecular level for definition of base pairing, which influences only the shape of the curve, and not the melting temperature.[52] Based on the *f*-curve, the melting temperature is found to be around 340 K, which is insignificantly different from 349 K, obtained from the configurational entropy (see Fig. 4). As seen in Figure 7, in high salt concentration, the fraction of denatured base pairs is lower than one even at 400 K. In other words, in high salt concentration, the stability of DNA increases and perhaps that is one reason for surviving a live tissue in a salt environmental for a long time.

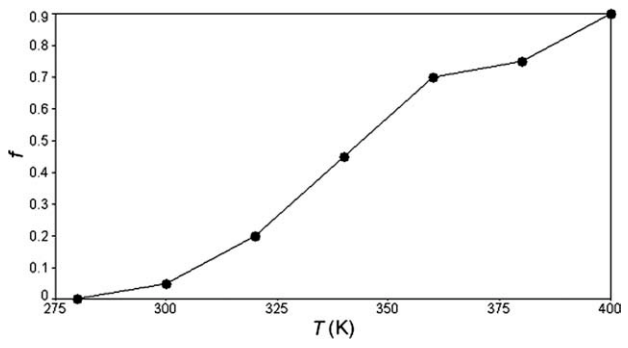

Figure 7. Fraction of the denatured bases (*f*) versus temperature. The points are connected to each other by spline estimation method and cubic constrained algorithm with $R^2 = 1.00$.

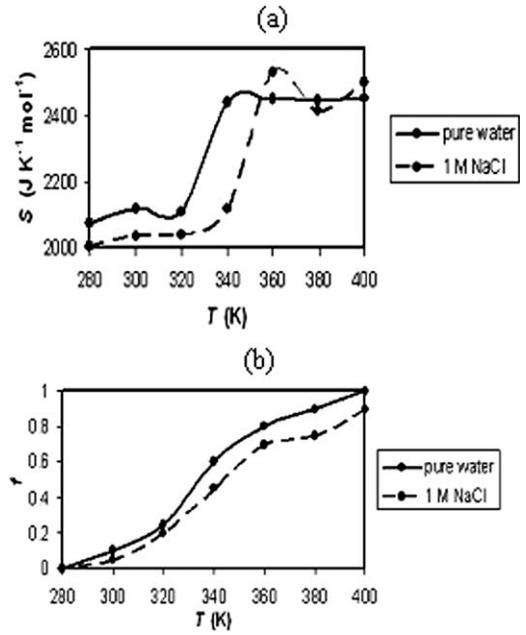

Figure 8. Comparison of a) configurational entropy and b) the fraction of denatured base pairs versus temperature in two different mediums. The data for the pure water medium are taken form Ref. [[25]].

Discussion

MD simulation was performed on the Drew–Dickerson oligomer with d(CGCGAATTCGCG) sequence and B-form structure in 1 M NaCl concentration. The simulations run in the *NPT* ensemble for 7 ns at 300, 320, and 340 K and for 50 ns at remaining temperatures within 280–400 K with the 20 K intervals. Then for each temperature, the configurational entropy was calculated by the Schlitter formulation (see Fig. 3). The transition curve is computed on the basis of configurational entropy (see Fig. 4). This curve shows a sharp slop at the transition temperature (at which 50% of interstrand hydrogen bonds break apart) and in this regard is similar to a first order phase transition. We found that the transition state of melting process involves the untwisting of DNA and peeling conformations (Fig. 5). This is in agreement with experimental results.[53] These configurational changes occur at a timescale much longer than that of the breaking and reforming of base pairs. The untwisting of DNA increases the configurational entropy, so we see a sharp slope in the transition curve of entropy. Schlitter's formulation is merely used for those atoms participating in interstrands hydrogen bonds.

The entropy per atom for some base pairs of a strand of the DNA is calculated (see Fig. 6). We have concluded that the base pairs at the end of DNA have more entropy, compared with the ones in the middle. That is as a result of their extensive contact with the surrounding solvent molecules, and also the DNA proceeds to denature from the ends. The Na^+–phosphate and base–water interactions are the dominating factors for producing a narrow minor groove of the AATT sequence.[50] The presence of monocations in the minor groove in the AATT sequence was observed theoretically[54] and also by NMR[55] and in X-ray studies.[56] A narrow minor groove limits motion of atoms in DNA bases, so the entropy reduces. We have found, by increasing temperature, the reduction of entropy in the middle of duplex toward its end, in such a way that at high temperatures (380 and 400 K), the entropy of the AATT sequence is not lower than the remaining portion of DNA. Such a behavior may be due to ion–phosphate interactions. At 280 K, the entropy for the AATT sequence is almost same with the rest, because of the narrowing of duplex in this sequence. But by increasing temperature especially above the melting temperature, the ion–phosphate interactions disrupted, hence the configurational entropy increases.

We have used the distance of A–D < 3 Å and angle of A–H–D $< 160^\circ$ criteria for the hydrogen bonding to obtain the fraction of denatured base pairs (f), f-curve, see Figure 7. The f-curve has a jump at the transition temperature which is relatively broader than that of the entropy curve. The transition temperature obtained from the f-curve and the entropy curve was found to be 340 K and 349 K, respectively. The experimental data for melting temperature of Drew–Dickerson oligomer in 1 M NaCl is 341 K. So, the T_m obtained from the hydrogen bond breaking is closer to experimental value. As shown in Figure 7, in high salt concentration (1 M NaCl) even at high temperature (400 K), the hydrogen bond breaking is not fully done (the value of f is 0.9 at this temperature). Also, according to an experimental observation, the Drew–Dickerson oligomer exhibits no clear melting curve in high salt concentrations.[57] So, DNA may be stable in the high salt concentrations, even at high temperatures.

Experimental data for the melting point of Drew–Dickerson oligomer is 341 K which is close to 340 K, obtained from the f-curve. The interstrand interactions are disrupted by the melting. Both nonbonding stacking and hydrogen bonding take part in the helix stability. Therefore, both mentioned interactions are weakened by the melting. In the calculation of the configurational entropy, shown in Fig. 4, both nonbonded stacking and hydrogen bonding interactions are taken into account. For this reason, the temperature-jump at the transition is sharp. But in calculation of f-curve in which the stacking interactions are excluded, the sharpness of the curve is much smoother than those observed experimentally. So, the hydrogen bonds mainly determine the melting temperature, whereas, the nonbonded stacking interactions influence on the sharpness of entropy at the transition temperature of the DNA.

In Figure 8, the configurational entropy and fraction of denatured base pairs (f) at different temperatures, both in pure water medium (from previous work[25]) and in the presence of 1 M NaCl, are compared. As shown in these figures, we may conclude that the hydrogen bonds in the pure water medium break apart more quickly than in the salt medium, by raising temperature. The configurational entropy is higher in water medium, therefore, the Columbic interactions stabilizes the DNA. Hence, the melting temperature is raised by adding ions.

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