Insights into the thermal stabilization and conformational transitions of DNA by hyperthermophile protein Sso7d: molecular dynamics simulations and MM-PBSA analysis

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In the assembly of DNA-protein complex, the DNA kinking plays an important role in nucleoprotein structures and gene regulation. Molecular dynamics (MD) simulations were performed on specific protein-DNA complexes in this study to investigate the stability and structural transitions of DNA depending on temperature. Furthermore, we introduced the molecular mechanics/Poisson–Boltzmann surface area (MM-PBSA) approach to analyze the interactions between DNA and protein in hyperthermophile. Focused on two specific Sso7d-DNA complexes (PDB codes: 1BNZ and 1BF4), we performed MD simulations at four temperatures (300, 360, 420, and 480 K) and MM-PBSA at 300 and 360 K to illustrate detailed information on the changes of DNA. Our results show that Sso7d stabilizes DNA duplex over a certain temperature range and DNA molecules undergo B-like to A-like form transitions in the binary complex with the temperature increasing, which are consistent with the experimental data. Our work will contribute to a better understanding of protein-DNA interaction.

Keywords: protein-DNA interaction; DNA kinking; molecular dynamics; MM-PBSA; thermal stability; structural transitions; hyperthermophile

Introduction

*Sulfolobus solfataricus*, an archaeabacterium which lives in volcanic hot springs, is a hyperthermophile (Baumann, Knapp, Lundback, Ladenstein, & Hard, 1994). It expresses large amounts of similar DNA-binding proteins, which are believed to play a significant role in protecting its DNA against thermal denaturation (Agha-Amiri & Klein, 1993; Baumann, Knapp, Lundback, Ladenstein, & Hard, 1994; Luscombe, Austin, Berman, & Thornton, 2000). Although the binding of sequence-general DNA-binding proteins to DNA is a fundamental question for understanding genome structure, structures of many protein-DNA complexes are not well understood (Gao et al., 1998; MacKerell & Nilsson, 2008). Sso7d, a 7kDa protein consisting of 63 amino acids, belongs to the family of small DNA-binding proteins isolated from *S. solfataricus* (Choli, Henning, Wittmann-Liebold, & Reinhardt, 1988). It is endowed with extreme thermal, acid, and chemical stability as well as DNA binding properties, which make the protein an attractive system for structural, thermodynamic, and DNA-binding studies (Bernini et al., 2011; Gao et al., 1998; Gera, Hussain, Wright, & Rao, 2011).

To date, the stabilization of DNA by hyperthermophilic proteins Sso7d has been investigated in extensive studies at elevated temperatures (Baumann, Knapp, Lundback, Ladenstein, & Hard, 1994; Hardy & Martin, 2008; Lopez-Garcia, Knapp, Ladenstein, & Forterre, 1998; Napoli et al., 2002; Priyakumar, 2012; Xu, Su, Chen, & Wang, 2011). Compared with a homolog hyperthermophilic proteins of Sac7d, which has been studied deeply (Priyakumar, 2012; Priyakumar, Harika, & Suresh, 2010), the protein Sso7d is more thermally stable with a denaturation temperature of 99 °C at PH 7 (Knapp et al., 1996). Sso7d binds strongly to DNA as monomers without sequence preference or cooperativity, and protects DNA from denaturation at the growth temperature of *S. solfataricus* (348–353 K) (Baumann, Knapp, Lundback, Ladenstein, & Hard, 1994; Gao et al., 1998; Lundback, Hansson, Knapp, Ladenstein, & Hard, 1998). The 3D structures of Sso7d-DNA complexes have been determined by X-ray crystallography (PDB codes: 1BNZ

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and 1BF4) (Gao et al., 1998). Those structures reveal that Sso7d binds DNA nonspecifically by partial intercalation of the hydrophobic side chains of Val26 and Met29, induces a sharp kink of about 60° in the DNA double helix and increases the melting of double stranded DNA (McAfee, Edmondson, Datta, Shriver, & Gupta, 1995); meanwhile, the DNA molecules in those complexes undergo conformational changes with respect to their backbone conformations (Dostal, Chen, Wang, & Welfle, 2004). Impressively, although Sso7d can significantly distort duplex DNA structures, the protein structure in the complexes is similar with that of the unbound Sso7d (Napoli et al., 2002). Previous studies also showed that Sso7d, the first protein with renaturation activity found in hyperthermophiles, promotes the renaturation of complement DNA strands at high temperatures, and then facilitates the stabilization of DNA duplexes (Guagliardi, Napoli, Rossi, & Ciaramella, 1997). However, it is not very clear to what extent the DNA structures in Sso7d-DNA complexes are changed, and their temperature dependence is not trivial.

Molecular dynamics (MD) simulations is a powerful tool to complement experimental results with detailed dynamical behavior of biomolecules (Lee, Jin, Han, & Kim, 2010; Nasiri, Bahrami, Zahedi, Moosavi-Movahedi, & Sattarahmy, 2010; Ren, Gao, Ge, & Li, 2009; Sklenovsky & Otyepka, 2010; Wiesner, Kriz, Kuca, Jun, & Koca, 2010). We studied intensively to achieve insight into the relative stability and structural transitions of DNA duplexes in the two Sso7d complexes. First, all-atom explicit water MD simulations were performed on five distinct systems (two complexes of Sso7d with different DNA sequences, two unbound DNA, and an unbound protein) from crystal structures at four different temperatures (300, 360, 420, and 480 K). Thereafter, the molecular mechanics/Poisson–Boltzmann surface area (MM-PBSA), a wildly used protocol in the study on the protein-DNA binding problem (Gorfe & Jelesarov, 2003; Habtemariam, Anisimov, & MacKerell, 2005; Jayaram, McConnell, Dixit, Das, & Beveridge, 2002; Lee, Kim, & Seok, 2010; Yang, Zhu, Wang, & Chen, 2011; Zhang & Schlick, 2006), was carried out to study the binding free energies and nonspecific recognition interactions of the binary Sso7d-DNA complexes. Finally, the decomposition of the total MM-PBSA free energies was performed on a per-residue basis in order to evaluate the contribution of key residues binding to DNA for the two complexes.

**Methods**

**MD simulations**

The starting structures for all MD simulations presented in this paper were taken from the X-ray crystal structures (Gao et al., 1998) of a AT-rich protein-DNA complex Sso7d-d(GTAATTAC)2 (PDB code: 1BNZ) and a GC-rich protein-DNA complex Sso7d-d (GCGTTGC+GCGAACGC) (PDB code: 1BF4) (Figure 1). Independent simulations were carried out for five systems (two protein-DNA complexes, two unbound DNA, and an unbound protein) derived from the complexes. Additional Na+ ions were added to neutralize individual system, and then each system was solvated in a truncated octahedron box of TIP3P water molecules under periodic boundary conditions. The distance was about 10 Å from the solute to the boundaries of the simulation box in all directions. The particle mesh Ewald method (Darden, York, & Pedersen, 1993) was taken to handle the long-range electro-
static interactions with a cut-off of 12 Å. The SHAKE algorithm (Ryckaert, Ciccotti, & Berendsen, 1977) was employed to all bonds involving hydrogen atoms, and the time step was set to 2 fs throughout.

The AMBER 10 software package (Case et al., 2008) and the ff03 force field (Duan et al., 2003) were employed to perform all of our MD simulations. Harmonic constraints of 50 kcal mol\(^{-1}\) Å\(^{-1}\) were placed on all protein/DNA atoms and ion positions during the 1000 steepest descent cycles and the 1000 conjugate gradient cycles. Then, these initial harmonic restraints were gradually reduced to zero in a series of progressive energy minimizations. After that, the whole systems were minimized using 5000 steps of steepest descent minimization followed by 4000 steps of conjugate gradient minimization with no harmonic restraints. Thereafter, the minimized systems were heated gradually to the final temperature (300, 360, 420, and 480 K), and then equilibrated for 500 ps in the absence of any restraints. The equilibration steps were carried out in constant volume. Finally, the equilibrated systems were subjected to MD simulations for 30 ns at four different temperatures under 1 atm. During the production runs, MD trajectories were collected every 2 ps for further analysis. For each of the two protein-DNA complexes at 360 K, two independent MD simulations were performed with different initial velocities. The VMD (Humphrey, Dalke, & Schulten, 1996) and PyMOL (Delano, 2002) software were used for visual inspection and generation of structural figures.

### Binding free energy and energy decomposition analysis

The MM-PBSA approach (Srinivasan, Cheatham, Cieplak, Kollman, & Case, 1998; Swanson, Henchman, & McCammon, 2004) implemented in AMBER 11 software package (Case et al., 2010) was employed to estimate the binding free energy in the two complex systems. Energetic post-processes of a single/triplet-trajectory analysis for each MM-PBSA computation were carried out by using the MM-PBSA module. For each of 1BNZ and 1BF4 complexes, the last 20 ns of the production dynamics stage were used for binding free energy calculations, specifically, 2000 snapshots of each system at 10 ps intervals for calculations of MM-PBSA. We computed the binding free energy (\(\Delta G_{\text{bind}}\)) of hyperthermophile protein Sso7d and DNA using the following equations:

\[
\Delta G_{\text{bind}} = \Delta G_{\text{complex}} - \Delta G_{\text{protein}} - \Delta G_{\text{DNA}} \tag{1}
\]

where \(\Delta G_{\text{complex}}, \Delta G_{\text{protein}},\) and \(\Delta G_{\text{DNA}}\) are the free energies of complex, protein and DNA molecules, respectively.

\[
\Delta G_{\text{x-complex, protein, DNA}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - T \Delta S \tag{2}
\]

where free energy (\(\Delta G_{\text{bind, complex, protein, DNA}}\)) of each state is estimated from gas-phase molecular mechanical energy \(\Delta E_{\text{MM}}\), solvation free energy \(\Delta G_{\text{solv}}\) and solute entropy \(S\), respectively (Gouda, Kuntz, Case, & Kollman, 2003). \(T\) is the temperature; the gas-phase molecular mechanical energy (\(\Delta E_{\text{MM}}\)) is the sum of electrostatic energy (\(E_{\text{ele}}\)), van der Waals interaction energy (\(E_{\text{vdw}}\)), and internal energy (\(E_{\text{int}}\)); the solvation free energy (\(\Delta G_{\text{solv}}\)) is computed as the sum of electrostatic solvation energy (\(G_{\text{pb}}\)) and nonelectrostatic solvation energy (\(G_{\text{nonp}}\)). The polar component (the electrostatic solvation energy) was computed using the Poisson–Boltzmann in Amber 11 (Bashford & Case, 2000; Luo, David, & Gilson, 2002). The dielectric constants were set to 1 and 80 for the solute and the surrounding solvent respectively in our calculations (Hou, Wang, Li, & Wang, 2011). The nonpolar contribution (the nonelectrostatic solvation energy) was defined by the equation:

\[
G_{\text{nonp}} = \gamma \text{SASA} + \beta. \tag{5}
\]

Here, \(\gamma\) and \(\beta\) are empirical constants and were set as 0.00542 kcal mol\(^{-1}\) Å\(^{-2}\) and 0.92 kcal mol\(^{-1}\), respectively; and SASA is the solvent accessible surface area estimated with a probe radius of 1.4 Å. The entropy \(S\) includes the translational, rotational, and vibrational entropy of the solute and was calculated with the Nmode module of AMBER 11. Since the entropy analysis in normal mode is extremely computationally expensive, we took 100 snapshots of the last 20 ns trajectory in the production dynamics stage at 200 ps interval.

In addition, the energy decomposition analysis was carried out with the MM-PBSA approach implemented in AMBER 11. The MM-PBSA energy decompositions by per-residue in the two complexes systems were also performed to address the contribution of each residue to the binding free energies. Per-residue free energy decompositions were performed on the van der Waals, electrostatic, polar solvation, and nonpolar solvation. The decomposition energies for each residue in the complexes, proteins, DNAs, and DELTAs were further broken down into backbone, side chain, and total contributions to their decomposition energies.

### Results and discussion

#### Structure and root mean-square deviations analysis

In order to assess the differences in the stabilities of the MD simulations, the average root mean-square deviations (RMSD) of the backbone atoms with respect to the starting structure over the last 20 ns in the simulations at dif-
Different temperatures are displayed in Table 1. In general, the mean RMSD values of protein in the binary complexes remain a stable value around 1.2 Å during the entire simulations at both 300 and 360 K. Such values indicate the protein is still quite stable at 360 K. This result is consistent with the experimental observation and computational studies for Sso7d protein (Merlino, Graziano, & Mazzarella, 2004; Priyakumar, Ramakrishna, Nagarjuna, & Reddy, 2010; Xu, Su, Chen, & Wang, 2011). At other two high temperatures (420 and 480 K), the maximum average RMSD value was only 3.8 Å indicating the protein does not undergo major structural transitions in the time scale of the simulations. The structural transitions of protein at 480 K are plotted in Figure 3 and will be discussed in some section later. However, with temperature increasing, the mean RMSD values of the binary complexes with respect to the X-ray crystal structures rise from 1.5 to 6.5 Å in both 1BNZ and 1BF4. Especially in the simulations at 360 K, the mean RMSD values of the two protein-DNA complexes display marginally larger increment with values of 3.5 and 2.5 Å in 1BNZ and 1BF4, respectively. Although the RMSD value exhibits marginally larger deviation at 360 K, the two protein-DNA complexes remain stable at the same temperature. But at the other two higher temperatures, 420 and 480 K, the maximum average RMSD reaches the high value of 4.0 and 6.5 Å, respectively, in the time scale of the simulations. In a word, the large deviations of the binary complexes may result from the instabilities of DNA double helix. Therefore, we focused on the behaviors of DNA in the complexes in comparison to the unbound DNA during the simulations.

To understand the variation of the RMSD in the binary complexes simulations better, we calculated the average RMSD of DNA molecule in the complexes. The time series of the RMSD values of the DNA molecules in MD simulations of DNA alone and protein-DNA complexes are given in Figure 2. In the binary complexes, the DNA molecules exhibit large deviations starting from 360 K. The mean RMSD values of the bound DNA with respect to the X-ray crystal structures rise from 1.6 to 3.9 Å in 1BNZ, and from 1.4 to 3.2 Å in 1BF4. This indicates that the changes in the structure of the DNA molecules give rise to the RMSD fluctuations of the binary complexes. Table 1 shows the RMSD of the bound DNA and the unbound DNA with respect to the canonical A- and B-DNA (Arnott & Hukins, 1972) to illustrate the influence of the protein Sso7d on bending of DNA. Because of the kink of DNA by intercalation of the side chains of Val26 and Met29 of Sso7d, the RMSD values of both the bound and the unbound DNA are reasonably larger compared with the values from the X-ray crystal structures. Judging from the change of RMSD values, we can make out that the bound DNA in the complexes is close to the canonical A-DNA than to the B-DNA, while the unbound DNA is vice versa. This is verified by calculation of pseudorotation angles of the sugar moieties in some section later.

Contact maps, which represent the averages of the inter-residue/nucleotide distances, can be used to analyze the interactions among inter and intramolecules in the protein-DNA complexes. The contact maps of the binary complexes at four different temperatures were calculated and plotted over the last 20 ns of the simulations in Fig-

| 1BNZ | 300 K | 360 K | 360 K | 420 K | 480 K |
|------|-------|-------|-------|-------|-------|
| Protein(alone) | 1.79 ± 0.20 | 1.81 ± 0.22 | 1.93 ± 0.27 | 3.32 ± 0.77 |
| Protein(complexed) | 1.30 ± 0.17 | 1.29 ± 0.22 | 1.53 ± 0.35 | 1.83 ± 0.48 | 3.25 ± 0.75 |
| Protein-DNA | 1.57 ± 0.23 | 3.43 ± 0.25 | 3.07 ± 0.67 | 4.48 ± 0.37 | 6.52 ± 1.19 |
| DNA(complexed)-xtal | 1.66 ± 0.39 | 3.96 ± 0.31 | 3.52 ± 0.86 | 6.42 ± 0.58 | 9.27 ± 1.97 |
| DNA(complexed)-B-form | 5.89 ± 0.32 | 6.20 ± 0.24 | 6.17 ± 0.32 | 7.91 ± 0.43 | 11.36 ± 1.91 |
| DNA(complexed)-A-form | 3.41 ± 0.21 | 5.63 ± 0.28 | 5.14 ± 0.90 | 7.67 ± 0.51 | 9.37 ± 1.56 |
| DNA(alone)-xtal | 4.55 ± 0.54 | 5.08 ± 0.86 | 7.11 ± 1.35 | 21.88 ± 11.75 |
| DNA(alone)-B-form | 3.86 ± 0.43 | 4.01 ± 0.65 | 7.19 ± 1.27 | 22.80 ± 12.37 |
| DNA(alone)-A-form | 5.80 ± 0.5 | 5.98 ± 0.61 | 7.65 ± 1.33 | 21.51 ± 11.80 |
| 1BF4 | | | | | |
| Protein(complexed) | 1.26 ± 0.14 | 1.19 ± 0.19 | 1.21 ± 0.19 | 1.30 ± 0.18 | 3.80 ± 1.19 |
| Protein-DNA | 1.44 ± 0.19 | 2.42 ± 0.26 | 2.56 ± 0.59 | 3.43 ± 0.45 | 6.47 ± 0.88 |
| DNA(complexed)-xtal | 1.40 ± 0.31 | 3.20 ± 0.44 | 2.90 ± 0.75 | 4.77 ± 0.73 | 6.83 ± 0.66 |
| DNA(complexed)-B-form | 4.93 ± 0.25 | 5.67 ± 0.28 | 5.55 ± 0.41 | 6.20 ± 0.51 | 7.68 ± 0.66 |
| DNA(complexed)-A-form | 3.92 ± 0.26 | 4.64 ± 0.33 | 4.50 ± 0.40 | 6.13 ± 0.54 | 7.35 ± 0.57 |
| DNA(alone)-xtal | 3.90 ± 0.56 | 3.67 ± 0.58 | 5.45 ± 0.65 | 10.46 ± 0.88 |
| DNA(alone)-B-form | 3.56 ± 0.41 | 3.47 ± 0.40 | 4.35 ± 0.51 | 11.10 ± 0.97 |
| DNA(alone)-A-form | 5.41 ± 0.51 | 5.32 ± 0.58 | 6.75 ± 0.67 | 10.12 ± 0.42 |
Figure 2. Time series of the RMSD values of the DNA molecules in MD simulations of DNA alone and protein-DNA complexes at four different temperatures.
ures S1 and S2. We found that the most impressive changes were the distances of the inter-nucleotide interaction in the DNA molecules in the two complexes with temperature increasing. On the other hand, the structures of protein in the two complexes were less affected during the four temperature simulations. It is consistent with our RMSD analyses above that the protein retains its initial structure at the temperature as high as 360 K. However, the contacts maps of DNA in 1BNZ differ drastically at the four individual temperatures (300, 360, 420, and 480 K), so do those maps in 1BF4. Most of the base pair distances of DNA increase to more than 12 Å at 480 K in the two protein-DNA complexes, indicating instability of the DNA double helix with temperature increased. However, the DNA double helix in the binary complexes is relatively stable at 300 and 360 K.

In addition, it’s pretty attractive and meaningful to compare the evolution of Sso7d bound with DNA at 480 K to those in unfolding studies for isolated Sso7d. Several three-dimensional structures at different times along with the unfolding processes of the studied proteins are shown in Figure 3. Of all the structural elements in the protein, the α-helix seems to be the most stable one within the time scale of the simulations. As shown in Figure 3(A), the unfolding process of the alone Sso7d begins with β-sheet at the N-terminal, and then β1–2 unfolds with β2 undergoes refolded. From the snapshot at 30 ns, it is found that the β3 strand went to unfolding states. The unfolding processes of the Sso7d in 1BNZ and 1BF4 complexes are given in Figure 3(B) and (C). Compared to the alone Sso7d protein, the unfolding of the Sso7d in 1BNZ complex evolved much more slowly which was indicated by the change of the RMSD. β1–2 did not start unfolding until the 30 ns of the evolution. Being similar with the alone Sso7d protein, the unfolding processes of the Sso7d in 1BF4 complex begin with β-sheet at the N-terminal, and then β1 unfolds with β2 undergoes refolded. The triple-strand β-sheet (β3-β4-β5) was very stable and stayed together during the time scale of the simulations, and the stability may be closely related to the interactions of β-sheet (β3-β4-β5) with DNA.

**Structure and stability of the DNA molecules**

All of the analyses in this section were performed by excluding the terminal base pairs of DNA to avoid end effects (the intermittent breaking of hydrogen bonds and the opening of the terminal base pair) (Leroy, Kochoyan, Huynh-Dinh, & Gueron, 1988; Marathe & Bansal, 2010; Nonin, Leroy, & Gueron, 1995).

**Structural deviations analysis**

The pyrimidine-purine N1–N3 base pair distances of the central six base pairs of both the bound and the unbound DNA strands are shown in Figures S3–S6 to illustrate the structural changes of DNA molecules. The criteria to Watson–Crick (WC) type base pairing is about 3.2 Å between N1 and N3 atoms, and the distance longer than 5 Å shows the disruption of base pair (Bueren-Calabuig, Giraudon, Galmarini, Egly, & Gago, 2011; Priyakumar et al., 2010). Figures S3–S6 illustrate the probability distributions of the N1–N3 distances at four different temperatures. Visual inspection of the DNA molecules structures from the MD simulations, along with N1–N3 base pair distances analysis, shows that the six WC base...
pairs remain unchanged in the Sso7d-DNA binary complexes at 300 K. In contrast, the three specific base pairs (T2A7 and A7T2 in the 1BNZ, and G7C2 in the 1BF4) in the two unbound DNA strands undergo partial base opening at 360 K, and the probabilities of the distance exhibit nonzero between N1 and N3 atoms beyond 5 Å. At 420 K, a higher temperature, almost all base pairs of the DNA undergo base opening. All base pairs of DNA molecules in the binary complexes are lost at a relative extreme temperature of 480 K. This reveals that Sso7d facilitates stabilization of the DNA over a certain temperature range. The result is similar to that of the studies on Sac7d-DNA complexes by MD simulations (Priyakumar et al., 2010). The percentage differences of N1-N3 base pair closed states are shown in Table 2. It illustrates that the WC base pairs are gradually destroyed with the increase in temperature. Most of the WC base pairs in the bound DNA duplexes are not disrupted at an even higher temperature of 420 K. This probably results from the annealing of DNA strands above its melting point promoted by the Sso7d protein (Baumann, Knapp, Lundback, Ladenstein, & Hard, 1994). Table 2 also reveals that the extent of base pair disruption is determined by the variation of DNA sequence and the binding of Sso7d.

Table S1 shows the interaction energies of all the atoms in a nucleotide pair on both the bound and unbound double DNA strands to illustrate the effect of the interaction energies of the base pairs on the extent of base pair disruptions. It is obvious that the interaction energies of all nucleotide pairs in 1BNZ and 1BF4 complexes simulations at 300 and 360 K are in consistent with theoretical values (Arora & Jayaram, 1998). This shows that those nucleotide pairs in the binary complexes are stable. As the interaction energies of AT nucleotide pairs are smaller than those of GC pairs in the binary complexes at the same temperature, the GC-rich DNA is relatively more stable than the AT-rich DNA. All WC base pairs are disrupted at 480 K, even though the DNA is bound to Sso7d. In order to illustrate the effect of Sso7d on the extent of base pairs disruption, the binding free energies of protein-DNA systems were computed using the MM-PBSA method. The binding free energies for the 1BNZ and 1BF4 complexes are estimated to be $-31.4$, $-23.7 \text{kcal/mol}$ at 300 K and $-34.6$, $-29.6 \text{kcal/mol}$ at 360 K, respectively, as presented in Table 3. The binding free energies at 360 K are larger than those at 300 K for the two complexes, which indicates the bindings between protein Sso7d and its target DNA at 360 K is stronger than those at 300 K in the two complexes. The results conform to those in previous experimental studies (Peters, Edmondson, & Shriver, 2004).

**B-like to A-like form transitions in DNA**

We used the pseudorotation angle ($\phi$) parameter (Cremer & Pople, 1975; Harvey & Prabhakaran, 1986; Saenger, 1984) to classify the sugar pucker conformation as follows: A-like sugars include the $\phi$ values from $30^°$ to $90^°$ and the B-like sugars include values between $90^°$ and $210^°$ (Tolstorukov, Jernigan, & Zhurkin, 2004). One end of the DNA duplex changes from the B-like form to A-like form at the kink junctions in order to accommodate itself into the Sso7d in the crystal structure of the binary complexes, and the other end of the DNA duplex maintains B-like form (Gao et al., 1998). In order to investigate the effect of protein binding and elevated temperatures on the conformation of DNA, we calculated the probability distributions of sugar pseudorotation.

Table 2. Percentage (%) of base pair closed states on DNA and protein-DNA complexes based on N1-N3 distances with a cut-off of 3.2 Å in the MD simulations.

|        | DNA (alone) | DNA (complexed) |        |
|--------|-------------|-----------------|--------|
|        | 300 K | 360 K | 420 K | 480 K | 300 K | 360 K | 360 K-1 | 420 K | 480 K |
| **1BNZ** |       |       |       |       |       |       |       |       |       |
| T2-A7  | 99.2  | 47.8  | 5.7   | 0.0   | 99.3  | 99.2  | 98.9   | 40.5  | 0.0   |
| A3-T6  | 99.9  | 96.1  | 3.2   | 0.0   | 99.9  | 99.5  | 99.3   | 69.0  | 0.0   |
| A4-T5  | 99.0  | 96.6  | 11.2  | 0.0   | 99.8  | 99.0  | 99.1   | 65.3  | 0.0   |
| T5-A4  | 99.1  | 96.8  | 9.3   | 0.0   | 99.8  | 98.8  | 98.2   | 74.7  | 1.1   |
| T6-A3  | 99.4  | 95.6  | 1.0   | 0.1   | 99.9  | 98.6  | 98.1   | 69.1  | 0.0   |
| A7-T2  | 99.9  | 56.8  | 0.9   | 0.0   | 99.9  | 97.8  | 97.7   | 0.0   | 0.1   |
| **1BF4** |       |       |       |       |       |       |       |       |       |
| C2-G7  | 100.0 | 96.3  | 31.8  | 1.2   | 100.0 | 100.0 | 99.9   | 99.9  | 3.7   |
| G3-C6  | 100.0 | 97.7  | 93.9  | 0.0   | 100.0 | 100.0 | 99.9   | 99.9  | 0.6   |
| T4-A5  | 100.0 | 92.2  | 94.3  | 0.0   | 99.7  | 98.7  | 99.0   | 98.6  | 0.7   |
| T5-A4  | 99.8  | 96.4  | 92.6  | 0.3   | 99.9  | 99.0  | 99.1   | 98.9  | 5.6   |
| C6-G3  | 100.0 | 97.4  | 91.7  | 4.1   | 100.0 | 100.0 | 99.5   | 98.2  | 5.4   |
| G7-C2  | 100.0 | 91.2  | 89.3  | 3.0   | 100.0 | 99.8  | 99.3   | 2.7   | 6.7   |
angles (shown in Table 4) for both DNA strands over the last 20 ns trajectory of the MD simulations. As shown in Table 4, the probability of A-like form conformation in the binary complexes at 360 K is higher than that at 300 K, while the B-like form conformation of the DNA exhibits the opposite trend. Neither the probability increase of A-like form conformation nor the probability decrease of B-like conformation is significant. Compared with the GC-rich sequence (1BF4), the variation in the probabilities of A-like or B-like form conformation is larger in the AT-rich sequence (1BNZ). The result is similar to that in the studies on Sac7d-DNA complexes by MD simulations (Priyakumar et al., 2010). The probability distributions of each deoxyribose ring pseudorotation angles are also given in Table 4 to obtain a better illustration of the structural transitions of nucleotides in DNA molecules. Data in Table 4 indicate that the nucleotides surrounding the interaction sites exhibit similar conformational behavior in the two complexes. The three nucleotides (D6a, D6b, and D6c) in the 1BNZ and the five nucleotides (D5b, D6a, D6b, D7a, and D7b) in the 1BF4, which adopt the B-like sugar conformations in the binary complexes, locate far away from the kinking sites. The three nucleotides (D4b, D5b, and D6a) in the 1BNZ and the two nucleotides (D3b and D4b) in the 1BF4, which sample the A-like sugar conformations, are close to the kinking sites. The impact of temperature on the structural transition of DNA is mainly contributed by D4a, D5a, and D6a in the 1BNZ as well as by D4b and D5a in the 1BF4.

Table 3. Percentage (%) of A-like (A) and B-like (B) conformations for the 12 individual nucleotides and the total 12 nucleotides in both complementary strands of the bound DNA at 300 and 360 K.

|        | 1BNZ       | 1BF4       |
|--------|------------|------------|
|        | 300 K      | 360 K      | 360 K-1    | 300 K      | 360 K      | 360 K-1    |
|        |            |            |            |            |            |            |
| A      | B          | A          | B          | A          | B          | A          |
| D2a    | 29.7       | 70.0       | 26.7       | 72.1       | 25.3       | 73.4       |
| D2b    | 0.1        | 99.7       | 4.1        | 94.3       | 3.7        | 93.4       |
| D3a    | 39.6       | 58.0       | 34.0       | 65.8       | 35.2       | 64.1       |
| D3b    | 0.7        | 98.3       | 0.5        | 90.9       | 0.5        | 90.3       |
| D4a    | 0.5        | 93.2       | 26.1       | 72.8       | 27.8       | 70.1       |
| D4b    | 93.8       | 4.8        | 93.5       | 5.6        | 92.9       | 6.3        |
| D5a    | 31.1       | 68.7       | 81.7       | 18.2       | 83.3       | 16.4       |
| D5b    | 89.8       | 9.7        | 85.8       | 14.1       | 83.5       | 15.3       |
| D6a    | 65.6       | 34.2       | 99.0       | 0.9        | 89.7       | 1.2        |
| D6b    | 10.2       | 89.2       | 5.0        | 93.1       | 6.7        | 92.5       |
| D7a    | 1.5        | 98.0       | 1.1        | 86.2       | 1.3        | 88.5       |
| D7b    | 7.6        | 92.2       | 1.2        | 97.3       | 1.7        | 96.8       |
| Total  | 34.2       | 64.5       | 40.2       | 57.6       | 42.4       | 56.1       |

Table 4. Binding free energies (kcal/mol) contributed by enthalpy and entropy at 300 K and 360 K from triplet-trajectory analysis for the two Protein-DNA complexes.

|        | 1BNZ       | 1BF4       |
|--------|------------|------------|
|        | 300 K      | 360 K      | 360 K-1    | 300 K      | 360 K      | 360 K-1    |
|        |            |            |            |            |            |            |
| E_{ele} | −2393.2    | −2481.8    | −2489.9    | −2295.5    | −2321.6    | −2322.9    |
| E_{edw} | −88.9      | −86.5      | −87.8      | −87.1      | −85.4      | −85.3      |
| G_{nonp} | −8.8       | −8.7       | −8.7       | −7.9       | −7.4       | −7.3       |
| G_{ph}  | 2426.8     | 2509.8     | 2509.7     | 2331.6     | 2351.7     | 2352.5     |
| ΔG_{np}^{a} | −97.7   | −95.1      | −96.5      | −95.0      | −92.8      | −92.6      |
| ΔG_{ph}^{a} | 33.6     | 28.0       | 28.8       | 36.1       | 30.1       | 29.6       |
| ΔF_{MM} | −2488.8    | −2581.5    | −2582.3    | −2389.6    | −2420.6    | −2420.9    |
| ΔG_{solv} | 2418.0    | 2501.1     | 2501.0     | 2323.7     | 2344.3     | 2345.2     |
| ΔH     | −70.8      | −80.4      | −81.3      | −65.9      | −76.3      | −75.7      |
| −TAS   | 39.4       | 45.8       | 45.8       | 42.3       | 46.7       | 47.5       |
| ΔG_{bind}^{c} | −31.4   | −34.6      | −35.5      | −23.7      | −29.6      | −28.2      |

aΔG_{np} = E_{ele} + G_{nonp}.

bΔG_{ph} = E_{edw} + G_{ph}.

cΔG_{bind} = ΔG_{np} + ΔG_{ph} + E_{int} − TAS.

Table 4. Percentage (%) of A-like (A) and B-like (B) conformations for the 12 individual nucleotides and the total 12 nucleotides in both complementary strands of the bound DNA at 300 and 360 K.
**Protein-DNA interactions**

The stability of binary complexes at 300 and 360 K allows us to analyze energetic and structural properties in detail. Binding free energies calculations for the two complexes were carried out using the MM-PBSA methodology and the single-/triplet-trajectory analysis. As the interior dielectric constant ($\varepsilon$) of the solute can change the final value of the polar energy, which can further affect the binding free energies in single-/triplet-trajectory analyses. The effect on the results of binding free energy by different interior dielectric constant ($\varepsilon$) of the solute was investigated. We selected different constants ($\varepsilon_{in} = 1$, $\varepsilon_{in} = 4$, and $\varepsilon_{in} = 8$) and calculated the binding free energies of the 1BNZ complex to verify the effects of interior dielectric constant on binding free energies. The computational results, shown in Table S2, can deviate substantially from the experimental results (Peters, Edmondson, & Shriver, 2004) with the increase of the interior dielectric constant ($\varepsilon$). Therefore, a low-solute dielectric constant ($\varepsilon_{in} = 1$) is selected in our computation of binding free energies. The binding free energies for both the 1BNZ and 1BF4 complexes reveal an opposite trend in single-trajectory analyses (given in Table S3), while those energies show a similar trend in triplet-trajectory analyses (shown in Table 3), compared with the experimental results (Peters, Edmondson, & Shriver, 2004). All energy items of the single trajectory, excluding the entropy changes and the binding free energies ($\Delta G_{\text{bind}}$), are produced to be similar with those of the triplet trajectory for the two complexes. These discrepancies probably stem from the large conformational changes of DNA, which are caused by the protein binding in 1BNZ and 1BF4 complexes. Because of these large conformational changes of DNA, the triplet-trajectory analysis method is an appropriate method for binding free energy calculations (Reyes & Kollman, 2000). Therefore, we preferred the triplet-trajectory analysis method, the results of which are listed in Table 3. We also analyzed the nonpolar energy ($\Delta G_{\text{np}}$) and the polar energy ($\Delta G_{\text{pb}}$) to understand the detailed information of binding energy. According to the energy components of the binding free energies listed in Table 3, the dominant contribution to the molecular mechanics energy changes $\Delta E_{\text{MM}}$ comes from the electrostatic energy changes $E_{\text{ele}}$. The electrostatic solvation free energies $G_{\text{pb}}$ provide important contributions to solvation free energy changes $\Delta G_{\text{solv}}$. However, the binding free energies $\Delta G_{\text{bind}}$ for the two individual systems are dominated by the $\Delta G_{\text{np}}$ component determined by the van der Waals energies $E_{\text{vdw}}$. Overall, the van der Waals energies and nonpolar/solvation interactions energies are beneficial to the binding of Sso7d and DNA in each of the two complexes. The entropy changes ($-T\Delta S$) impair protein-DNA binding in the binary complexes. The favorable electrostatic energy changes ($E_{\text{ele}}$) are completely screened by the unfavorable solvation energies ($G_{\text{pb}}$). The binding free energies for the 1BNZ and 1BF4 complexes have been analyzed in some section previously.

A much better understanding of the protein-DNA recognition was obtained via interaction energy analysis. Table S4 shows the interaction energies between protein Sso7d and the bases of each of the six nucleotide pairs, as well as that between the Sso7d and the backbone of the six individual nucleotide pairs. It indicates that the attractive interactions between Sso7d protein and the phosphate backbone are stronger than the corresponding interactions between the protein and the bases of nucleotides. The contributions from electrostatic and van der Waals energies to the calculation of interaction energies are listed in Table 3. The energy analysis, along with the visual inspection of both binary MD and crystal structures, indicates that the interaction energies of DNA and Sso7d are dominated by electrostatic energies between Sso7d and DNA phosphate backbone. This indicates that the Sso7d is a nonsequence specific DNA-binding protein, which is consistent with the results from experiments (Baumann, Knapp, Karshikoff, Ladenstein, & Hard, 1995; Lundback et al., 1998; Lundback & Hard, 1996).

The energy decomposition approach was performed on a per-residue basis in the two protein-DNA complexes to address the role of certain residues in protein-DNA binding. Tables S5 and S6 depict the decomposition of $\Delta G$ values of individual residues with $|\Delta G_{\text{bind}}| \geq 3.5 \text{ kcal mol}^{-1}$. Overall, the favorable interactions are mainly made by eight amino acids (Lys7, Tyr8, Lys9, Lys22, Trp24, Val26, Met29, and Arg43) in the binary complexes. As shown in Tables S5 and S6, the favorable electrostatic energies are screened by the unfavorable solvation energies. Polar interactions are found to facilitate the binding for three amino acids (Lys7, Lys9, and Lys22), which are involved in ionic interactions with the phosphates. Five amino acids (Tyr8, Trp24, Val26, Met29, and Arg43) are involved in the main binding attractions by van der Waals interactions. It is obvious that the energy decomposition results are consistent with the results in the two crystal structures analyses (1BNZ and 1BF4) (Gao et al., 1998).

**Conclusions**

We focused on the two specific Sso7d-DNA complexes (PDB codes: 1BNZ and 1BF4), and MD simulations were performed on those complexes at four different temperatures (300, 360, 420, and 480 K) to investigate the thermal stability and structural transitions of DNA. Simulations at 360 K show that both of the two Sso7d-DNA complexes are nearly unchanged in comparison with the experimental structures. At 360 K both of these
two complexes still maintain stable, however, slightly structural changes of DNA molecules were observed. And a small amount of base pairs start opening in the unbound DNA systems, indicating that Sso7d is helpful for stabilizing the DNA helical structures. In the simulations at 420 K, no more than 60% base pairs in the complex are open, while almost all base pairs of DNA undergo base opening in the unbound DNA, which further illustrates that Sso7d could stabilize DNA structures. We also performed the MD simulations at the extreme high temperature of 480 K, under which condition DNA undergoes denaturation completely even in the presence of the protein. On the whole, the protein Sso7d can stabilize the base pairs of DNA over a certain temperature range. In addition to the thermal stability, our studies also show that the DNA molecules undergo B-like to A-like form transitions in the binary complex with the temperature increasing, but the transitions only occur in a part of the nucleotides.

In addition, we introduced MM-PBSA approach to calculate the binding free energies in the two Sso7d-DNA complex systems. The results indicate that the binding free energies for the two systems are mainly driven by more favorable van der Waals and nonpolar/solvation interaction. Furthermore, the decompositions of MM-PBSA free energies show the extensive interactions between Sso7d with DNA phosphate backbones, the reason why nonspecific DNA-binding proteins Sso7d is proposed, and the decompositions illustrate that only eight residues (Lys7, Tyr8, Lys9, Lys22, Trp24, Val26, Met29, and Arg43) are critical to the binding to DNA in the binary complexes as well, which is consistent with the experimental results.

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