Two Conformations of Archaeal Ssh10b

THE ORIGIN OF ITS TEMPERATURE-DEPENDENT INTERACTION WITH DNA*

Qi Cui‡‡, Yufeng Tong‡‡, Hong Xue‡, Li Huang‡, Yingang Feng‡, and Jinfeng Wang‡

From the National Laboratory of Biomacromolecules, Center for Molecular Biology, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, 100101 Beijing, China and State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, 100080 Beijing, China

The DNA-binding protein Ssh10b from the hyperthermophilic archaeon Sulfolobus shibatae is a member of the Sac10b family, which has been speculated to be involved in the organization of the chromosomal DNA in Archaea. Ssh10b affects the DNA topology in a temperature-dependent fashion that has not been reported for any other DNA-binding proteins. Heteronuclear NMR and site-directed mutagenesis were used to analyze the structural basis of the temperature-dependent Ssh10b-DNA interaction. The data analysis indicates that two forms of Ssh10b homodimers co-exist in solution, and the slow cis-trans isomerization of the Leu61-Pro62 peptide bond is the key factor responsible for the conformational heterogeneity of the Ssh10b homodimer. The T-form dimer, with the Leu61-Pro62 bond in the trans conformation, dominates at higher temperature, whereas population of the C-form dimer, with the bond in the cis conformation, increases on decreasing the temperature. The two forms of the Ssh10b dimer show the same DNA binding site but have different conformational features that are responsible for the temperature-dependent nature of the Ssh10b-DNA interaction.

Proteins of the Sac10b family are highly conserved among thermophilic and hyperthermophilic Archaea, and homologous sequences have also been identified in eukaryal proteins from higher plants, protists, and vertebrates (1–3). The members of this family have been postulated to play a role in chromosomal organization in Archaea since the initial isolation of Sac10b from the hyperthermophile Sulfolobus acidocaldarius in the mid-1980s. Sac10b exists as a dimer of two 10-kDa subunits in solution and binds to DNA nonspecifically (4, 5). Electron microscopic studies have shown that Sac10b binds to DNA cooperatively and forms different protein-DNA complexes depending on protein/DNA ratios but does not induce DNA supercoiling or compact DNA (6).

Recently, Bell et al. (2) discovered that Alba (also named Sas10b, a member of Sac10b family) forms a specific complex with a Sir2 homolog in Sulfolobus solfataricus cell extracts. They found that Sir2 is in the presence of NAD⁺ can regulate the DNA binding affinity of Alba by deacetylation of Lys⁶⁶ of the protein. More recently, the crystal structure of Alba has been solved. Interestingly, the protein shares structural homology to the C-terminal domain of the Escherichia coli translation factor IF3 and the N-terminal DNA binding domain of Dnase I. A model for the Alba-DNA interaction has been proposed (7).

Ssh10b, another member of the Sac10b family, was isolated from Sulfolobus shibatae (1). The protein is highly abundant and basic and binds double-stranded DNA without apparent sequence specificity. Gel retardation assays have shown that Ssh10b has two modes of DNA binding with distinctly different binding densities. In the low binding density mode, Ssh10b exhibits a binding size of ~12 bp of DNA, whereas in the high binding density mode, the protein appears to bind shorter stretches of DNA. Interestingly, Ssh10b affects DNA topology in a temperature-dependent fashion; it is capable of significantly constraining DNA in negative supercoils at temperatures higher than 318 K, but this ability is drastically reduced at 298 K (1). A previous NMR study revealed the co-existence of two forms of Ssh10b dimers at temperatures between 283 and 320 K, with one dominating at lower temperatures and the other at higher temperatures (8). However, the structural basis for the conformational heterogeneity of the Ssh10b dimer and the temperature dependence of the interaction of Ssh10b with DNA remained to be clarified.

In the present study, we investigated the heterogeneous conformations of Ssh10b and the structural factors influencing the interaction of Ssh10b with DNA by heteronuclear NMR spectroscopy. We found that the cis-trans isomerization of the Leu⁶¹-Pro⁶² peptide bond of Ssh10b is the primary determinant of the conformational heterogeneity of the Ssh10b dimer. We also found that the equilibrium between the cis- and trans-forms of Ssh10b is sensitive to temperature. Our data suggest that the effect of temperature on the capacity of the protein to constrain negative DNA supercoils is related to the temperature-dependent conversion between the two Ssh10b conformations.

EXPERIMENTAL PROCEDURES

Expression and Purification of Ssh10b and Its Mutants—Ssh10b was produced from a synthetic gene with codon usage optimized for expression in E. coli. The gene was created from 12 overlapping oligonucleotides primers that were ligated and then cloned into the EcoRI/HindIII sites of vector pET22b. The genes of Δ8 (deletion of the N-terminal eight residues), Δ8P18A (Pro⁸ replaced by Ala of the Δ8 mutant), and P62A (Pro⁶² replaced by Ala) mutants of Ssh10b were obtained by primer-directed mutagenesis. Each gene was cloned into expression vector pET11c, and the products were used to transform E. coli BL21(DE3) cells. The transformed cultures were grown at 37 °C in 1 liter of LB broth containing 50 mg/liter ampicillin until A₆₀₀ was 0.8–1.0, and expression was induced for 2 h by adding isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 mM. The harvested cells were resuspended in 20 ml of buffer containing 30 mM potassium phosphate, pH 6.6, 0.1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride and sonicated. The lysate was centrifuged at 150,000 × g for 2.5 h at 4 °C and then the supernatant was heated for 20 min at 80 °C to precipitate the E. coli proteins. After centrifugation, the supernatant

* This work was supported in part by the National Natural Science Foundation of China Grants 39823001, 39270301, 39925001, and 30020100. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Contributed equally to this work.

Received for publication, August 4, 2003, and in revised form, September 29, 2003
Published, JBC Papers in Press, September 30, 2003, DOI 10.1074/jbc.M308510200

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.
was applied to a Resource-S column. Bound proteins were eluted within a 50-mM KC1 gradient (0 to 0.75 M). Fractions containing Ssh10b proteins were pooled, dialyzed against distilled de-ionized water, and finally lyophilized. The purity of the proteins was confirmed by SDS-PAGE to be more than 95% and by matrix-assisted laser desorption ionization time-of-flight to be free of nucleic acid contaminants.

NMR Sample Preparation—15N or 13C singly labeled and 15N/13C doubly labeled Ssh10b proteins were expressed in E. coli strain BL21(DE3) grown in M9 minimal medium using 15NH4Cl and/or 13C-glucose as the sole nitrogen and carbon sources. All protein samples for NMR measurements were dissolved in 500 μM of 90% H2O/10% D2O containing 20 mM deuterated acetate buffer, pH 4, 50 μM of NaN3, 1 μM 2,2-dimethyl-2-silapentanesulfonic acid and 20 mM KC1 to a final protein concentration of about 1 mM, unless otherwise indicated. The sample for determination of the dimer interface of Ssh10b was 15N/13C asymmetrically labeled.

NMR Spectroscopy—All NMR experiments were carried out on a Bruker DMAX 600 spectrometer equipped with a triple resonance probe and an actively shielded three-axis gradient unit. The experimental temperature was set to 310 K except for the temperature-dependent experiments. 1H chemical shifts were referenced to the internal standard 2,2-dimethyl-2-silapentane sulfonic acid at 0 ppm. 15N and 13C chemical shifts were calculated indirectly using the corresponding consensus δ (ratio).

Although the assignment of the T-form Ssh10b has already been published (8), assignment of the remaining resolved resonances of the C-form Ssh10b was achieved by further exploring existing spectra: 3D 1H-13C-15N HNCA, HN(CA)CO, CBCA(CO)NH, HN(CA)CO, and HNCA experiments for backbone assignments and HBHA(CBCA)NH, HBHA(CBCA)(CO), and C(CO)NH, as well as 2D 1H-13C HCCl-TOCSY experiments for side chain assignments. Most of the backbone assignments (8) were confirmed by comparison with Ssh10b; the remaining ambiguities were resolved with an HNCA experiment. A 3D NOESY-H 15C-HMQC experiment was carried out to distinguish the cis and trans conformations of the X-prolyl bond of Ssh10b. Conformational exchange of Ssh10b was monitored using a series of 2D 1H-15N correlation experiments for simultaneous measurement of the temperature-dependent chemical shifts and deuterium relaxation rates at exchange delays of 12, 52, 152, 302, 452, 552, 652, 752, 902, 1102, 1202, and 1602 ms (10). The temperature dependence of 1H-15N HSQC spectra of Ssh10b was measured at temperatures ranging from 283 to 330 K at increments of 3.5 K. To determine residues involved in the dimer interface, a 2D NOESY-H 15N-HSQC experiment (11) was carried out on the asymmetrically labeled Ssh10b sample, and 15N was allowed to evolve in the indirect dimension. The mixing time of the experiment was 150 ms. 2D intensity modulated HSQC (12) was used to measure the 3JNHN correlations for 15N singly labeled proteins. The delay for J correlation evolution was set to 30 ms. Two 2D 1H-15N HSQC experiments were also used for exploring the binding behavior of Ssh10b with synthesized 16-bp double-strand DNA fragments (5'-GGCGAG-CGCCCTGTCGCC). All NMR data were processed and analyzed using Felix 98 (Accelrys Inc.). The data points in each indirect dimension were usually doubly by linear prediction and zero-filled. A 90 to 60° shifted square sine bell apodization was used for all dimensions prior to Fourier transformation.

Electrophoretic Mobility Shift Assay (EMSA)—A 108-bp double-stranded DNA fragment (5'-CGACGGTGTAAACAGAGCGGCTAGTCG-AAGCTTGGTCAAGTACCAGCCGTCGAC-3') was prepared (8) and labeled with [32P]ATP. The labeled fragment was incubated with Ssh10b or [P62A]Ssh10b at various mass ratios for 5 min at temperatures of 298 or 330 K. The ligating reactions were then performed as described previously (15). T4 DNA ligase (3 Weiss units) and Pfu DNA ligase (4 Weiss units) were used for the reactions carried out at 298 and 330 K, respectively. After the ligation reaction, the samples were analyzed by 1.4% agarose electrophoresis in 0.5× Tris-Phosphate-EDTA (13).

RESULTS

Co-existence of Two Ssh10b Homodimers with Different Conformations—As shown in the previous study, two sets of cross-peaks (for simplicity denoted as “doublets” hereafter) are observed for most residues of Ssh10b in the 2D 1H-15N HSQC spectrum (Fig. 1A) (8). The signal intensities of the resonance doublets are not equal, with one signal stronger than the other in all doublets. Although the results of chemical cross-linking (1) and the related crystal structure (7) suggest that Ssh10b is a dimer, the resonance doublets with unequal signal intensities (Fig. 1A) cannot be attributed to an asymmetric arrangement of the two monomeric units of the Ssh10b molecule under the sample conditions used in the NMR experiments, as in this case the intensities of the two resonance signals in the doublet should be equal. The chemical shift differences between the higher (δH) and lower (δL) intensity resonance signals in the doublets in Fig. 1A were calculated as the weighted differences between the 15N (δN = δH - δL) and 1H (δN = δH - δL) values, according to ∆δ = (δH - 0.17 δL)(2) (Fig. 2) (16). Although both monomer and dimer crystal forms of Alba have been obtained (7), the ∆δ values make it clear that the Ssh10b molecule does not exist as a mixture of dimeric and monomeric forms in solution. If this were the case, then the resonance doublets should be observed only for residues located at the dimer interface. The cross-peaks shown in Fig. 3 correspond to the residues at the Ssh10b dimer interface, detected by X-nucleus edited NOESY. When mapped to the sequence, the data indicate that helix αC2, a portion near the C-terminal of strand β3, and the N-terminal part of strand β4 are involved in the dimeric surface (Fig. 4A). However, ∆δ values (Fig. 2) reveal that the residues of the N-terminal part of strand β4 give only “singlet” signals, whereas residues in helix αL, and strands β3 and β4, which are distant from the interface, generate doublet signals (Fig. 4B). The line widths for each pair of doublets are the same. In addition, the ratios of the signal intensities of the doublets are independent of the concentrations (0.05–1.5 mM) of Ssh10b as revealed by NMR experiments (data not shown). These therefore also exclude the possibility of an oligomerization equilibrium. Because Ssh10b is dimeric as confirmed by size-exclusion chromatography (data not shown), it was considered whether two forms of Ssh10b dimer might co-exist in solution. The form with higher signal intensity in doublets was assigned as the T-form and the other with lower signal intensity as the C-form. No ‘multiplets’ other than doublets were observed for most residues of Ssh10b (Fig. 1A), consistent with both the T-form and the C-form as homodimers, with the monomeric subunits arranged symmetrically in each dimer.

The main-chain torsion angle φ is closely related to the backbone conformation of proteins and can be calculated from the J1H,NH scalar coupling constants. The J-coupling constants of Ssh10b were measured by 2D intensity modulated HSQC (12). The differences between the J1H,NH value for the T-form and for the C-form (∆J1H,NH) at each residue position is shown in Fig. 2. Residues with an absolute ∆J1H,NH value greater than 1 Hz are found in segments spanning the whole molecule: the N-terminal region, the loop linking strand β1 and helix α1, helices αL and αR, and the C-terminal of strand β4. This suggested that the main-chain conformations are different in the two forms of the Ssh10b dimer.

Cis and Trans Conformations of Ssh10b—Fig. 5 shows portions of a 1H-15N heteronuclear chemical exchange spectrum at an exchange delay of 1.3 s (10). Two categories of doublets,
Residues Thr5, Thr7, and Ser9 gave exchange cross-peaks (Fig. 5A) when the exchange delays were set in the range of 0.1 to 1.6 s. However, the remaining doublets shown in Fig. 1A did not give any exchange cross-peaks at the same exchange delays but gave a result like that shown in Fig. 5B for residue Lys97. Thr5-Pro6-Thr7-Pro8-Ser9 is an unstructured N-terminal segment of Ssh10b as determined by the chemical shift index (8). The appearances of the exchange cross-peaks of Thr5, Thr7, and Ser9 are clearly because of the cis-trans isomerization of the X-prolyl bonds in this segment. Thr7 is between Pro6 and Pro8 and therefore showed two minor peaks in Fig. 1A and four exchange cross-peaks in Fig. 5A. The intensity ratios of the major auto-peaks to the minor ones were about 11:1 at 310 K, a value much higher than that for the remaining doublets in Fig. 1A. Therefore, the remaining doublets were not caused by the cis-trans isomerization of Thr5-Pro6 or Thr7-Pro8 peptide bonds. This was confirmed by the 2D 1H-15N HSQC spectrum of [P62A]Ssh10b, in which all resonance doublets remained the same as those in Fig. 1A, except for the absence of the cross-peaks for residues Gly4, Thr5, Thr7, Ser9, Met10, and Val11, because the N-terminal eight residues are truncated in [P62A]Ssh10b molecule.

Ssh10b contains four prolines at positions 6, 8, 18, and 62 in the sequence. The 2D 1H-15N HSQC spectrum of [P62A]Ssh10b was almost the same as that of [P62A]Ssh10b except for the appearance of a new cross-peak for Ala62 (data not shown). Thus, replacement of the proline by alanine at position 18 did not influence the conformational heterogeneity of the Ssh10b dimer.

Pro62 is located in the loop linking helix a2 and strand b3 (8). The P62A mutant Ssh10b ([P62A]Ssh10b) was confirmed to be a dimer in solution by diffusion measurements using the BPP-LED pulse sequence (18, 19) designed for determining the molecule size (data not shown). The 2D 1H-15N HSQC spectrum of [P62A]Ssh10b is shown in Fig. 1B. Only a single set of cross-peaks was observed for all residues except the residues Gly4, Thr5, Thr7, Ser9, and Asn10 of [P62A]Ssh10b. Substitution of Pro62 by Ala62 eliminated the cis-trans isomerization, so that the mutant Ssh10b dimer was found in a single conformational state. Overlay of Fig. 1, B and A shows that the cross-peaks of [P62A]Ssh10b can be mapped to the cross-peaks of the T-form of Ssh10b. This indicates that the T-form of the Ssh10b homodimer adopts the same conformation as that of the [P62A]Ssh10b homodimer.

Fig. 6A shows 1H-1H slices at the 13C frequencies of Leu61 and Pro62 extracted from the 3D NOESY-1H,13C-HMQC (lower strip) and the 3D HCCH-TOCSY (upper strip) spectra for the T-form Ssh10b dimer, whereas Fig. 6B shows those for the C-form Ssh10b dimer. Two inter-residue NOE cross-peaks between 1Hα of Leu61 and 1Hα of Pro62 could be observed in the 3D NOESY-HMQC strip for the T-form Ssh10b dimer (Fig. 6A). The appearance of these two NOEs characterizes the trans conformation of the Leu61-Pro62 peptide bond in the T-form Ssh10b dimer. In the strips corresponding to the C-form Ssh10b dimer (Fig. 6B), only one NOE cross-peak between the 1Hα resonances of Leu61 and Pro62 was observed. This NOE thus provides conclusive evidence that the Leu61-Pro62 peptide bond in the C-form homodimer of Ssh10b is in a cis conformation. The above experimental results show that the global conformational heterogeneity of Ssh10b is the result of the cis-trans transformation of the Leu61-Pro62 peptide bond of...
Conformations of Ssh10b and Its Binding with DNA

FIG. 2. Differences of chemical shifts (Δδ) and J-coupling constants (Δ3JNH) of the resonance doublets at 310 K and the changes in the 1HN chemical shifts versus residue number of Ssh10b. Values of Δδ(C→T) were obtained from experiments with the sample of Ssh10b at different temperatures. Values of Δ3J(DNA)18 and Δδ1H(DNA)18 were obtained from the experiments with a sample of Ssh10b in complex with DNA at 318 K. Secondary structural elements of Ssh10b are labeled at the top of the figure. Filled circles below the secondary structural elements indicate the residues involved in the dimer interface. At the bottom of the figure, the black bar indicates the DNA binding regions.

FIG. 3. Residues involved in the dimer interface of Ssh10b. The assigned cross-peaks are labeled with the one-letter amino acid code and residue number.

obtained by measuring the intensities of the corresponding cross-peaks for both conformations over the temperature range 283 to 330 K (Fig. 7). The ratio of C-form to T-form was around 0.26 at 318 K and greater than 0.6 below 298 K. Clearly, the T-form of the Ssh10b dimer is dominant, although the population of the C-form increases on decreasing the temperature.

The 1H chemical shifts of all resolved cross-peaks in the 2D 1H-15N HSQC spectra were measured at different temperatures for the T-form and the C-form Ssh10b dimers. The differences between the 1H chemical shifts at 298 K (δ298) and at 318 K (δ318) for the T-form (ΔδT = δ298 - δ318) and the C-form (ΔδC = δ298 - δ318) of a Ssh10b sample containing a high concentration of salt (200 mM KCl), and ΔδT = δ318 - δ298 and ΔδC = δ320 - δ318 of a Ssh10b sample containing a low concentration of salt (20 mM KCl), were obtained (data not shown). On increasing the temperature, the 1H chemical shifts of the majority of the cross-peaks were shifted upfield (+Δδ) for both the T-form and the C-form Ssh10b dimers. However, this was not the case for residues Ala25, Leu26, Val53, Arg57, and Leu61 of the C-form of Ssh10b in the sample containing 20 mM KCl and for residues Asn29 and Asp63 of the C-form of Ssh10b in the sample containing 200 mM KCl, which all showed downfield shifts (-Δδ) on increasing the temperature.

The extent of upfield movements of the 1H chemical shifts (+Δδ) varied for the residues in the Ssh10b dimer that generated resonance doublets. The differences between the changes in the 1H chemical shifts of the resonances for the T-form and for the C-form of Ssh10b (ΔδT,C = ΔδT - ΔδC), obtained from the data of the Ssh10b sample containing 200 mM KCl at temperature of 298 and 318 K, are shown in Fig. 2 and also mapped onto 3D structure (Fig. 4C). For residues Tyr22, Val23, Ala26, Ala29, and Leu27, located in helix α1, and residues Lys48, Asp63, Val64, Gly73, Arg75, and Asn85, located in helix α2, the ΔδT,C values are larger than +0.01 ppm. Residues Val34, located in the turn between helix α1 and strand β2, and Asp63, in the loop linking helix α2 and strand β3, also showed -ΔδT,C (>0.01 ppm). However, negative ΔδT,C with absolute values larger than 0.01 ppm were observed for residues Ser25 and Ile37 at the N terminus of strand β3, residues Lys64, Gly65, Gly73, Ser74, and Gln75 in strand β3, and residues Ile71, Ile74, Arg75, Lys88, and Lys93 at the C terminus of strand β3 (Fig. 2). Thus, the upfield shifts of the 1H resonances of the residues in helices α1 and α2 of the T-form were larger than those of the
C-form on increasing the temperature. However, for residues involved in formation of the $\beta_2$-$\beta_4$-$\beta_5$ antiparallel $\beta$-sheet, the upfield shifts of the $^1$H$_N$ resonances of the T-form were smaller than those of the C-form when the temperature was increased. This provides further evidence for conformational differences, in particular different strength of hydrogen bonding, between the two forms of the Ssh10b dimers.

**NMR Spectral Features of the dsDNA-Ssh10b Complex at Different Temperatures**—The 2D $^1$H-$^1$H HSQC spectra of Ssh10b and the dsDNA-Ssh10b complex in sodium acetate buffer, pH 5.5, containing 200 mM KCl were recorded at temperatures of 318 and 298 K for a Ssh10b-DNA ratio of 1:3 (spectra not shown). The $^1$H$_N$ chemical shifts of all resolved cross-peaks in the 2D $^1$H-$^1$H HSQC spectra of Ssh10b (black) and of Ssh10b in complex with DNA (red) were measured. The differences of $\Delta\delta$ (DNA) from 0 of the cross-peaks ($\Delta\delta$ (DNA) = $\delta$(DNA) - $\delta$) were obtained for both the T-form ($\Delta\delta_T$(DNA)) and the C-form ($\Delta\delta_C$(DNA)) Ssh10b molecules at 318 K ($\Delta\delta_T$(DNA)$_{318}$ = $\delta_T$(DNA)$_{318}$ - $\delta_T$(DNA)$_{298}$). The $\Delta\delta_T$(DNA)$_{318}$ and $\Delta\delta_T$(DNA)$_{298}$ values provided information about the location of DNA binding sites and the local conformational changes of the Ssh10b dimer induced by binding of DNA.

Compare the values of $\Delta\delta_T$(DNA)$_{318}$ and $\Delta\delta_T$(DNA)$_{298}$ with $\Delta\delta_C$(DNA)$_{318}$ and $\Delta\delta_C$(DNA)$_{298}$, respectively, and the differences in the $^1$H$_N$ chemical shift perturbations by DNA binding between two forms of Ssh10b dimer can be noticed. The residues showing the observable differences are indicated in the 3D structure (Fig. 4D). The differences were found mainly in two helices and in three $\beta$ strands. However, upon interaction with DNA, $^1$H$_N$ resonances of the residues in the segments, Ala$_{11}$-Ser$_{17}$ and Val$_{76}$-Ile$_{90}$, still remain as singlet. It seems that DNA binding produces similar conformational changes to these two polypeptide segments in the two forms of the Ssh10b molecule, although different changes in the $^1$H$_N$ resonances between the two forms of the Ssh10b dimer are observed in other regions of the polypeptide chain of the molecule.

**Temperature-dependent Interaction of DNA with Ssh10b and [P62A]Ssh10b**—The interaction of DNA with the Ssh10b dimer has been found to be temperature-dependent (1). To further investigate the temperature-dependent features of DNA binding, EMSA and nick closure assays were performed on both Ssh10b and the P62A-mutant Ssh10b ([P62A]Ssh10b) under identical experimental conditions.

In the EMSA assay, a $^{32}$P-labeled 108-bp dsDNA fragment (0.5–1 ng) was mixed with different amounts of Ssh10b or [P62A]Ssh10b and analyzed by gel electrophoresis at 293 and 320 K. The bands show the distribution of the products of the DNA-protein interaction by complex size (Fig. 8). The apparent $K_d$ was...
about 0.04 μM estimated by the amount of protein required to retard 50% of the DNA. The cooperative binding of Ssh10b to DNA was observed; therefore the ratio of protein:DNA influenced the migration pattern of the DNA-protein complexes in EMSA. The DNA-Ssh10b and DNA-[P62A]Ssh10b complexes showed similar migration patterns at 293 and 320 K when the concentration of the protein was lower, such as 0.04 μM (lane 1) or 0.08 μM (lane 2). However, when the protein concentration was higher, such as 0.16 or 0.32 μM, the nature of cooperative binding of Ssh10b to DNA makes the migration patterns of the DNA-Ssh10b complexes at 320 K totally different from those at 293 K, but resemble those of the DNA-[P62A]Ssh10b complexes at both temperatures (lanes 3 and 4). Therefore, the mode of binding of the Ssh10b dimer to DNA in the complex at 293 K must be different from that at 320 K or in the DNA-[P62A]Ssh10b complex at either temperature.

Nick closure assays were carried out to detect the capabilities of the proteins to constrain DNA in supercoils at 298 or 330 K. In the nick closure assay, a single-nick plasmid pUC18 was ligated in the absence and presence of the proteins. The results for different protein:DNA ratios is shown in Fig. 9. The assay in the absence of protein (lane 1 in Fig. 9) was performed as a control. At 298 K, addition of Ssh10b to the reaction mixture produced only a weak CCC (covalently closed circular plasmid) band with a high supercoil density at a protein:DNA mass ratio of 2:1 (Fig. 9, upper left panel). However, when the protein concentration was higher, such as 0.16 or 0.32 μM, the nature of cooperative binding of Ssh10b to DNA makes the migration patterns of the DNA-Ssh10b complexes at 320 K totally different from those at 293 K, but resemble those of the DNA-[P62A]Ssh10b complexes at both temperatures (lanes 3 and 4). Therefore, the mode of binding of the Ssh10b dimer to DNA in the complex at 293 K must be different from that at 320 K or in the DNA-[P62A]Ssh10b complex at either temperature.

Nick closure assays were carried out to detect the capabilities of the proteins to constrain DNA in supercoils at 298 or 330 K. In the nick closure assay, a single-nick plasmid pUC18 was ligated in the absence and presence of the proteins. The results for different protein:DNA ratios is shown in Fig. 9. The assay in the absence of protein (lane 1 in Fig. 9) was performed as a control. At 298 K, addition of Ssh10b to the reaction mixture produced only a weak CCC (covalently closed circular plasmid) band with a high supercoil density at a protein:DNA mass ratio of 2:1 (Fig. 9, upper left panel). However, when the protein concentration was higher, such as 0.16 or 0.32 μM, the nature of cooperative binding of Ssh10b to DNA makes the migration patterns of the DNA-Ssh10b complexes at 320 K totally different from those at 293 K, but resemble those of the DNA-[P62A]Ssh10b complexes at both temperatures (lanes 3 and 4). Therefore, the mode of binding of the Ssh10b dimer to DNA in the complex at 293 K must be different from that at 320 K or in the DNA-[P62A]Ssh10b complex at either temperature.

Nick closure assays were carried out to detect the capabilities of the proteins to constrain DNA in supercoils at 298 or 330 K. In the nick closure assay, a single-nick plasmid pUC18 was ligated in the absence and presence of the proteins. The results for different protein:DNA ratios is shown in Fig. 9. The assay in the absence of protein (lane 1 in Fig. 9) was performed as a control. At 298 K, addition of Ssh10b to the reaction mixture produced only a weak CCC (covalently closed circular plasmid) band with a high supercoil density at a protein:DNA mass ratio of 2:1 (Fig. 9, upper left panel). However, when the protein concentration was higher, such as 0.16 or 0.32 μM, the nature of cooperative binding of Ssh10b to DNA makes the migration patterns of the DNA-Ssh10b complexes at 320 K totally different from those at 293 K, but resemble those of the DNA-[P62A]Ssh10b complexes at both temperatures (lanes 3 and 4). Therefore, the mode of binding of the Ssh10b dimer to DNA in the complex at 293 K must be different from that at 320 K or in the DNA-[P62A]Ssh10b complex at either temperature.

Nick closure assays were carried out to detect the capabilities of the proteins to constrain DNA in supercoils at 298 or 330 K. In the nick closure assay, a single-nick plasmid pUC18 was ligated in the absence and presence of the proteins. The results for different protein:DNA ratios is shown in Fig. 9. The assay in the absence of protein (lane 1 in Fig. 9) was performed as a control. At 298 K, addition of Ssh10b to the reaction mixture produced only a weak CCC (covalently closed circular plasmid) band with a high supercoil density at a protein:DNA mass ratio of 2:1 (Fig. 9, upper left panel). However, when the protein concentration was higher, such as 0.16 or 0.32 μM, the nature of cooperative binding of Ssh10b to DNA makes the migration patterns of the DNA-Ssh10b complexes at 320 K totally different from those at 293 K, but resemble those of the DNA-[P62A]Ssh10b complexes at both temperatures (lanes 3 and 4). Therefore, the mode of binding of the Ssh10b dimer to DNA in the complex at 293 K must be different from that at 320 K or in the DNA-[P62A]Ssh10b complex at either temperature.

Nick closure assays were carried out to detect the capabilities of the proteins to constrain DNA in supercoils at 298 or 330 K. In the nick closure assay, a single-nick plasmid pUC18 was ligated in the absence and presence of the proteins. The results for different protein:DNA ratios is shown in Fig. 9. The assay in the absence of protein (lane 1 in Fig. 9) was performed as a control. At 298 K, addition of Ssh10b to the reaction mixture produced only a weak CCC (covalently closed circular plasmid) band with a high supercoil density at a protein:DNA mass ratio of 2:1 (Fig. 9, upper left panel). However, when the protein concentration was higher, such as 0.16 or 0.32 μM, the nature of cooperative binding of Ssh10b to DNA makes the migration patterns of the DNA-Ssh10b complexes at 320 K totally different from those at 293 K, but resemble those of the DNA-[P62A]Ssh10b complexes at both temperatures (lanes 3 and 4). Therefore, the mode of binding of the Ssh10b dimer to DNA in the complex at 293 K must be different from that at 320 K or in the DNA-[P62A]Ssh10b complex at either temperature.
topology of DNA are similar at high temperature (330 K) but different at low temperature (298 K).

The results of EMSA and nick closure assays indicate that [P62A]Ssh10b, existing in a single conform trans in solution, shows the same features upon interaction with DNA at both high and low temperatures. Therefore, the temperature-dependent nature of the interaction of Ssh10b with DNA correlates with the two conformations of the Ssh10b dimer.

**DISCUSSION**

**Different Conformational Features of the T-form and C-form Ssh10b Homodimer**—The conformational features of a protein are strongly influenced by factors that affect the strength of intramolecular hydrogen bonds. Changes in the chemical shifts of $^1$H$_N$ resonances are a sensitive indicator of changes in the strength of hydrogen bonding. The chemical shifts of $^1$H$_N$ resonances are affected by hydrogen bond acceptors, particularly carbonyl groups (20, 21). Wagner and co-workers (22, 23) demonstrated that $^1$H$_N$ chemical shifts depend on the inverse third power of the distance between the $^1$H$_N$ and the hydrogen bond acceptor. In the case of hydrogen bonding with C=O, large downfield shifts are observed for strongly hydrogen-bonded amide protons. Within protein secondary structure, the $^1$H$_N$ of the ith residue forms a hydrogen bond with the C=O of the $(i+4)$th residue in an $\alpha$-helix, and in an antiparallel $\beta$-sheet, the $^1$H$_N$ and C=O of a residue in one $\beta$ strand forms hydrogen bonds with the C=O and the $^1$H$_N$ of a residue in the opposite strand. When the temperature is increased, the thermal fluctuations of an $\alpha$-helix or a $\beta$-sheet are enlarged, and the average distance between the $^1$H$_N$ and the C=O increases. As a consequence of weakened hydrogen bonding, the chemical shifts of the $^1$H$_N$ resonances will tend to move upfield.

The $^1$H$_N$ resonances of almost all cross-peaks in the 2D $^1$H-$^15$N HSQC spectrum of the Ssh10b molecule were shifted upfield on increasing the temperature. This is thus consistent with a general weakening of the hydrogen bonding in both the T-form and the C-form of the Ssh10b dimer on increasing the temperature. However, the temperature-dependent shifts of the $^1$H$_N$ resonances were different in size for the T-form ($\Delta \delta_T$) and the C-form ($\Delta \delta_C$). This then suggests a difference between the hydrogen bonding strengths within the secondary structure of the two forms of the Ssh10b dimer. The values of $\Delta \delta_T - \Delta \delta_C$ for each residue are shown in Fig. 2. On increasing the temperature, the residues in helices $\alpha_1$ and $\alpha_2$ show larger upfield shifts of the $^1$H$_N$ resonances for the T-form ($\Delta \delta_T$), whereas the residues involved in the antiparallel $\beta$-sheet show larger upfield shifts for the C-form ($\Delta \delta_C$) (Fig. 2). In the antiparallel $\beta$-sheet formed by $\beta_2$-$\beta_4$, strands, the $^1$H$_N$ and C=O groups of residues Ile$^{94}$, Arg$^{35}$, Lys$^{96}$, and Lys$^{37}$ form hydrogen bonds with C=O and $^1$H$_N$ of Ile$^{97}$, Glu$^{66}$, Ser$^{36}$, and Lys$^{37}$, respectively. Residues Ile$^{94}$, Arg$^{35}$, Lys$^{96}$, and Lys$^{37}$ are located in the C-terminal of strand $\beta_4$. Residues Leu$^{97}$ and Ser$^{36}$ are located in the N-terminal region of strand $\beta_4$, and Glu$^{66}$ and Lys$^{64}$ are located in the N-terminal region of strand $\beta_3$. Thus, on increasing the temperature, the shortening of the hydrogen bond distances in the portion of antiparallel $\beta$-sheet near to the C-terminal of the Ssh10b molecule is greater for the C-form than that for the T-form Ssh10b dimer. Conversely, the shortening of the hydrogen bond distances in the $\alpha$-helices is greater for the T-form. These results suggest that the spatial packing of the residues is tighter in the $\alpha$-helices and looser in the portion of the antiparallel $\beta$-sheet near the C-terminal of the molecule for the C-form than that for the T-form of Ssh10b molecule.

Further support for different temperature-dependent features of the secondary structure of the T-form and the C-form of the Ssh10b dimer was from the observation that for a few $^1$H$_N$ resonances, the direction of the temperature-dependent shift is different in the two forms. The $^1$H$_N$ resonances of residues Ala$^{25}$, Leu$^{48}$, Val$^{53}$, Arg$^{57}$, and Leu$^{61}$ for the sample containing 20 mM KCl, and of residues Asp$^{58}$ and Asp$^{63}$ for the sample containing 200 mM KCl, shifted downfield for the C-form and upfield for the T-form of Ssh10b molecule. Residues Ala$^{25}$, Leu$^{48}$, Val$^{53}$, Arg$^{57}$, and Asn$^{36}$ are located in $\alpha$-helices. Leu$^{61}$ and Asp$^{63}$ are the nearest neighbors of Pro$^{62}$, located in the loop linking helix $\alpha_2$ and strand $\beta_3$. The magnitudes of downfield shifts of the $^1$H$_N$ resonances of these residues upon increasing temperature were in the range 0.6–2.8 ppm/K. In addition, the $\Delta^3J_{N\alpha}$ values for residues Leu$^{24}$, Leu$^{27}$, Lys$^{48}$, Val$^{53}$, and Glu$^{66}$ were all greater than $\pm 1$ Hz (Fig. 2), corresponding to a change in backbone torsion angle, $\Delta \phi > \pm 10^\circ$, between the two forms of Ssh10b molecule. Residues Ala$^{25}$ and Asn$^{58}$ also showed small variations of $\Delta^3J_{N\alpha}$ (Fig. 2). Thus, differences in the hydrogen bonding strengths in the secondary structure of the two forms correlate with main-chain conformational differences between the T-form and the C-form Ssh10b molecule.

**DNA Binding Sites on the T-form and the C-form ssh10b Dimers**—Proteins bind in the major or minor grooves of DNA and some protein structures have contacts with DNA in both grooves simultaneously (24). In the model proposed for the DNA-Alba complex (7), the Alba dimer interacts simultaneously with a major groove and the two flanking minor grooves of the DNA. Residues Lys$^{16}$, Lys$^{17}$, and Arg$^{42}$ in the central “belly” of Alba dimer are involved in DNA binding at the major groove, and the $\beta$-hairpin of Alba interacts with the minor grooves.

Ssh10b lacks the first three N-terminal amino acid residues of Sso10b but is otherwise identical in sequence. In fact, the protein used to solve Alba crystal structure is identical with Ssh10b (7). Thus, Ssh10b is supposed to have the same DNA binding sites shown by the DNA-Alba complex. Examination of the interaction of the Ssh10b dimer with DNA in solution by NMR spectroscopy revealed the location of the DNA binding sites on both the T-form and the C-form Ssh10b molecule. The DNA binding regions of the
T-form and C-form Ssh10b dimers at 318 K are apparent from the DNA-induced changes in chemical shift values, \( \Delta \delta_{298}^{C}(DA_{\alpha 18}) \) and \( \Delta \delta_{298}^{T}(DA_{\alpha 18}) \) (Fig. 2). Three regions of the Ssh10b molecule can be identified as involved in DNA binding: Val17-Ile69, a region spanning the C-terminal of strand \( \beta_3 \), the N-terminal of strand \( \beta_3 \), and the \( \beta \)-turn between them. Inspection of the amino acid sequence of the Ssh10b molecule indicates that a group of polar residues, Ser4, Gln5, Thr18, Ser27, Gln36, Gln43, Ser45, Ser46, and Thr80, is clustered in this region. The \( \beta \)-turn also contains a positively charged residue, Arg91. Positively charged and polar residues complement the negative charges on the DNA (24). Therefore, it is reasonable to conclude that the \( \beta \)-turn region of the Ssh10b dimer binds in the minor grooves of dsDNA as suggested by the DNA-Alba dimer complex (7).

The other two regions, Leu13-Asn21 and Ala41-Ser47, include the loop linking residues, Leu13-Asn21 and Ala41-Ser47, which form a cluster of positively charged residues in the Ssh10b molecule. This cluster is about 22 Å distant from the \( \beta \)-turn in the DNA-Alba dimer complex, and a similar distance was observed in the DNA-Ssh10b dimer complex. Therefore, it is reasonable to conclude that the two forms of the Ssh10b dimer interact with DNA at both 298 and 318 K, deduced from the fact that \( \Delta \delta_{298}^{C}(DA_{\alpha 18}) \) and \( \Delta \delta_{298}^{T}(DA_{\alpha 18}) \) show similar histograms to that of \( \Delta \delta_{298}^{C}(DA_{\alpha 18}) \) and \( \Delta \delta_{298}^{T}(DA_{\alpha 18}) \) (Fig. 2). In addition, very weak signals were observed for residues Lys16, Arg42, and Arg44, which are positively charged on the DNA-Ssh10b dimer and to the different conformational changes on binding of DNA. Such differences between the T-form and the C-form Ssh10b dimer with DNA allows the following model to be proposed: the relative spatial positions of the \( \beta \)-turn and the belly of the Ssh10b dimer in the C-form may deviate from that in the T-form Ssh10b molecule. This deviation may increase when DNA interacts with the Ssh10b dimer. Because the DNA-Ssh10b dimer interfaces remain the same for the T-form and the C-form and have similar main-chain conformational features, the DNA may have to undergo some adjustment in conformation to facilitate binding with the two forms of the Ssh10b dimer. Consequently, the conformations of DNA and the Ssh10b dimer in the DNA-T-form complex may help to constrain DNA in supercoils but not in the DNA-C-form complex. The results of EMSA and nick closure assays with [P62A]Ssh10b (see Fig. 8, right two panels and Fig. 9, lower two panels) show that the T-form, which is favored at high temperature, is the form of Ssh10b that causes DNA to adopt a supercoiled formation. This then provides direct evidence that the co-existence of two forms of the Ssh10b dimer is the origin of the temperature-dependent interaction of the Ssh10b dimer with DNA.

**Acknowledgment**—We thank Professor Sarah Perrett for critically reading the manuscript.

**REFERENCES**

1. Xue, H., Guo, R., Wen, Y., Liu, D., and Huang, L. (2000) *J. Bacteriol.* 182, 3929–3933

2. Bell, S. D., Botting, C. H., Wardleworth, B. N., Jackson, S. P., and White, M. F. (2002) *Science* 296, 148–151

3. Ferré-Pèire, P., Confalonieri, F., and Knapp, S. (1999) *Mol. Microbiol.* 32, 669–670

4. Dijk, J., and Reinhardt, R. (1999) *In Biochemical Chromatography* (Gualerzi, C. O., and Pon, C. L., eds) Springer-Verlag, New York

5. Grote, M., Dijk, J., and Reinhardt, R. (1986) *Biochem. Biophys. Acta* 873, 405–413

6. Lorz, R., Grote, M., Dijk, J., Reinhardt, R., and Dobrinski, B. (1986) *EMBO J.* 5, 3715–3719

7. Wardleworth, B. N., Russell, R. J., Bell, S. D., Taylor, G. L., and White, M. F. (2000) *EMBO J.* 19, 4654–4662

8. Tong, Y., Cui, P., Peng, Y., Huang, L., and Wang, J. (2002) *J. Biol. Chem.* 277, 385–386

9. Markley, J. L., Bax, A., Arata, Y., Hilbers, C. W., Kaptein, R., Sykes, B. D., Wright, P. E., and Wuthrich, K. (1998) *J. Biomol. NMR* 12, 1–23

10. Parrow, N. A., Zhang, O., Forman-Kay, J. D., and Kay, L. E. (1994) *J. Biomol. NMR* 4, 727–734

11. Muhendrarn, D. R., Xu, G. Y., and Kay, L. E. (1993) *J. Biomol. NMR* 3, 463–470

12. Perlman, P., Kinelplained, I., Annali, A., and Heikkinen, S. (2000) *J. Biomol. NMR* 16, 259–257

13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd Ed., Vol. 3, p. B.23, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

14. Clark, D. J., and Pihlidi, G. (1991) *EMBO J.* 10, 397–395

15. Mai, V. Q., Chen, X., Hong, R., and Huang, L. (1998) *J. Bacteriol.* 180, 2560–2563

16. Cao, K., Nagata, K., Horuchi, M., Ehsis, R., Hasuda, T., Yuzawa, S., Nishida, M., Hanataka, H., and Inagaki, F. (2002) *J. Biol. Chem.* 277, 37–46

17. Olson, W. K., Gorin, A. A., Lu, X. J., Hock, L. M., and Zhurkin, V. B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 11163–11168

18. Jones, J. A., Wilkins, D. K., Smith, L. J., and Dobson, C. M. (1999) *J. Biomol. NMR* 19, 199–203

19. Wilkins, D. K., Grimshaw, S. B., Receveur, V., Dobson, C. M., Jones, J. A., and Smith, L. J. (1999) *Biochemistry* 38, 16424–16431

20. Baxter, N. J., and Williamson, M. P. (1997) *J. Biomol. NMR* 9, 359–369

21. Gortz, T., and Oleskiew, J. (2001) *J. Biomol. NMR* 21, 249–261

22. Paridi, A., Wagner, G., and Wuthrich, K. (1983) *Eur. J. Biochem.* 137, 445–454

23. Wagner, G., Paridi, A., and Wuthrich, K. (1983) *J. Am. Chem. Soc.* 105, 5948–5949

24. Jones, S., van Heyningen, P., Berman, H. M., and Thornton, J. M. (1999) *Science* 287, 877–896

25. Fabo, C. O., and Sauer, R. T. (1992) *Annu. Rev. Biochem.* 61, 1053–1095

26. Schultz, S. C., Shields, G. C., and Steitz, T. A. (1991) *Science* 253, 1001–1007

27. Winkler, F. K., Banner, D. W., Oefner, C., Tsernoglou, D., Brown, R. S., Heathman, S. P., Bryan, R. K., Martin, P. D., Petrusas, K., and Wilson, K. S. (1993) *EMBO J.* 12, 1751–1759

Q. Cui, Y. Tong, and J. Wang, unpublished data.