



Letter to the Editor: ^1H , ^{15}N , and ^{13}C resonance assignments and secondary structure of the Ssh10b from hyperthermophilic archaeon *Sulfolobus shibatae*

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Biological context

The families of small, abundant, and basic DNA binding proteins in thermoacidophilic archaea of the genus *Sulfolobus* were first characterized by Reinhardt and colleagues in the 1980s (Kimura et al., 1984). These proteins can be classified into three groups, 7, 8, and 10 kDa, according to their molecular weights. Two members of the 7 kDa proteins, Sso7d from *Sulfolobus solfataricus* and Sac7d from *Sulfolobus acidocaldarius*, have been under extensive studies. However, little is known about the 8 and 10 kDa groups. Sac10b is one of the two 10 kDa proteins from *Sulfolobus acidocaldarius*, yet no three-dimensional structure has been reported for this protein so far. Forterre et al. identified a *Sulfolobus shibatae* gene, denoted as ssh10b, which encodes a protein homologous to Sac10b (Forterre et al., 1999). Moreover, Ssh10b has at least one homologue in each of all the archaeal species whose genomes have been completely sequenced, and these homologues form the Sac10b family. However, neither in bacteria nor in eukarya can be found the Ssh10b homologous. Given the ubiquity of the members of the Sac10b family, they are presumed to be physiologically important to these organisms.

Xue et al. (2000) isolated a protein encoded by gene ssh10b from hyperthermophilic archaeon *Sulfolobus shibatae*. This highly basic protein consists of 97 amino acid residues, and most interestingly, affects DNA topology in a temperature-dependent fashion that has not been reported for any other DNA binding

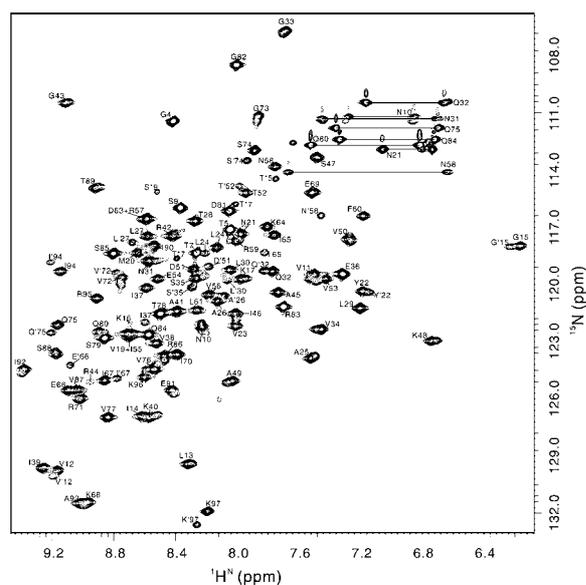
proteins (Xue et al., 2000). Insight into the structural basis by NMR spectroscopy may help us to understand the mechanism of the temperature dependence of the interaction of Ssh10b with DNA.

Methods and experiments

The gene encoding Ssh10b was chemically synthesized and cloned into expression vector pET-11a. ^{15}N - or $^{15}\text{N}/^{13}\text{C}$ - uniformly labeled recombinant Ssh10b was overexpressed in *E. coli* strain BL21(DE3) in soluble form and purified. The purity of the recombinant Ssh10b was checked by SDS-PAGE to be better than 95%. Samples for NMR measurements were prepared by dissolving about 8.4 mg ^{15}N labeled or $^{13}\text{C}/^{15}\text{N}$ double labeled protein in 500 μL of 90% $\text{H}_2\text{O}/10\%\text{D}_2\text{O}$ containing 20 mM deuterated acetate buffer, pH 4.8, 50 μM NaN_3 , and 20 mM KCl. All NMR experiments were carried out at 310 K on a Bruker Avance DMX 600 spectrometer equipped with a triple resonance probe and actively shielded three-axis gradient. Proton chemical shifts were referenced relative to those of internal DSS at 0 ppm. The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the corresponding consensus Ξ ratios. The NMR experiments performed included 2D ^1H - ^{15}N HSQC, CT ^1H - ^{13}C HSQC, 3D HNCA, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO and ^{15}N -edited NOESY for backbone assignment, HBHA(CO)NH, HBHANH, C(CO)NH and HCCH-TOCSY for side chain assignment. Amino acid type selective experiments for Gly (Schubert et al., 1999), Ser (Schubert

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A



B

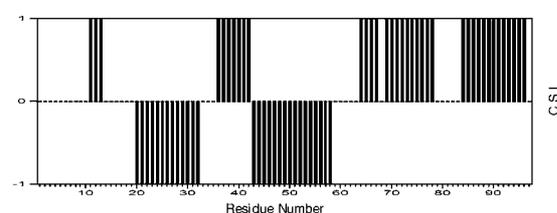


Figure 1. (A) A 600 MHz 2D ^1H - ^{15}N HSQC spectrum of recombinant Ssh10b at 310 K. The resonance assignments of the H form of the Ssh10b are indicated with the one-letter amino acid code and residue number. Side-chain NH_2 resonances of Asn and Gln are connected by horizontal lines. The amino acid codes with a prime indicate cross peaks for the L form of the Ssh10b, while the amino acid codes with a star indicate cross peaks caused by proline isomerization (B) CSI consensus plot for the H form of the Ssh10b, determined using $^1\text{H}^\alpha$, $^{13}\text{C}^\alpha$, $^{13}\text{C}^\beta$ and $^{13}\text{C}'$ chemical shifts values.

et al., 2001a), and Lys (Schubert et al., 2001b) were carried out to assist in backbone assignment while a set of Pro selective experiments (Schubert et al., 2000) were used to determine the sequential-specific assignments of proline residues and to obtain the chemical shifts of proline nitrogen. Side chain NH_2 frequencies of Asn and Gln were identified from 3D HNCACB and 2D ^1H - ^{15}N HSQC based on side chain ^{13}C assignments. All the spectra were processed and analyzed using FELIX 98.0 from Accelrys Inc.

Extent of assignments and data deposition

We observed coexistence of two forms of Ssh10b at temperatures from 283 K to 320 K, with one dominating at lower temperature (denoted L form) and the other (denoted H form) at higher temperature. The NMR data is consistent with the observation of temperature-dependant interaction of Ssh10b and DNA (Xue et al., 2000). At 310 K, the 2D ^1H - ^{15}N HSQC spectrum (Figure 1A) shows good dispersion of amide proton and nitrogen resonances of the protein. $^{13}\text{C}^\alpha$, $^1\text{H}^\alpha$, $^{13}\text{C}'$ and $^{13}\text{C}^\beta$, $^1\text{H}^\beta$ (if available) assignments were obtained for all residues of the H form except the two N-terminal amino acid residues. $^1\text{H}^N$ assignments were identified for 97% of the non-proline residues except Met1-Ser3 while ^{15}N assignments were identified for 96% of all residues except Met1-Ser3, and Pro 62. The side chain NH_2 of all the Asn and Gln residues of the H form were also assigned. Nearly complete assignment were achieved for other side-chain ^1H and ^{13}C resonances. The secondary structure of the Ssh10b monomer was deduced from the consensus chemical shift index (Figure 1B). It appears that the NMR deduced α -helix content (29.9%) of Ssh10b is lower than that derived from circular dichroism (>51%, Xue et al., 2000). The chemical shifts of the ^1H , ^{15}N and ^{13}C of the H form at 310 K have been deposited in the BioMagResBank with the entry number 5226.

Acknowledgements

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