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ARTICLE

Resonance assignments of a putative PilT N-terminus domain protein SSO1118 from hyperthermophilic archaeon *Sulfolobus solfataricus* P2

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Abstract PilT N-terminus (PIN) domains exist broadly in all three kingdoms of life, but the functions are not clear for most of them. Archaea species often encode multiple PIN domain-containing proteins, and the signaling and stress response roles have been proposed for these proteins. Some PIN domain proteins possess nuclease activities, which were proposed to be important in toxin-antitoxin stress response, nonsense-mediated mRNA decay, or RNA interference. SSO1118 from hyperthermophilic archaeon *Sulfolobus solfataricus* P2 is a putative PIN domain protein with low homology to other known PIN domain proteins. Here we report the NMR resonance assignments of SSO1118 for further structural determination and functional studies. The secondary structures predicted from the assigned chemical shifts consist with those of archaeal PIN domain proteins.

Keywords PIN domain · *Sulfolobus* · NMR assignments · Secondary structure · Archaea

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Biological context

The first PilT N-terminus (PIN) domain was identified in the N-terminus of a bacterial protein PilT which was involved in the biosynthesis of type IV pili (Wall and Kaiser 1999). PIN domain-containing proteins, in stand-alone versions as well as fusions with other domains, are present in all three domains of life (Makarova et al. 1999; Glavan et al. 2006; Bunker et al. 2008). In eukaryotic cells, PIN domains play roles in nonsense-mediated mRNA decay (NMD) and RNA interference (RNAi) through the postulated ribonuclease activities (Clissold and Ponting 2000). Nuclease activities were confirmed in several PIN domain-containing proteins (Glavan et al. 2006; Fatica et al. 2004; Daines et al. 2007). Bioinformatics analysis indicated that Archaea species often encode multiple PIN domain-containing proteins with low sequence homology, and a role in signaling was proposed for PIN domains (Makarova et al. 1999). PIN domain proteins in mycobacteria and archaea were also proposed to be the toxic components of toxin-antitoxin operons for stress response, while the toxic activity came from their nuclease activity (Bunker et al. 2008; Gerdes et al. 2005; Arcus et al. 2005). However, the physiological roles of PIN domain-containing proteins are still not clear, especially for the multiple archaeal stand-alone PIN domain proteins.

SSO1118 from hyperthermophilic archaeon *Sulfolobus solfataricus* P2 was annotated as a hypothetical protein conserved in *Sulfolobales*. The results of BLAST to search for homologues in UniProt or NCBI non-redundant database were mostly the hypothetical proteins in *Sulfolobales* with more than 40% identity to SSO1118. Besides, several putative PIN domain proteins showed homology with SSO1118 with a low identity, such as TON_1538 from

Thermococcus onnurineus NA1 (23% identity in 106 residues), APE0276 from *Aeropyrum pernix* K1 (52% identity in 35 residues), and B3TBD2 from the uncultured marine crenarchaeote HF4000_APKG8O8 (41% identity in 41 residues). These results suggested that SSO1118 might be a putative PIN domain protein. Here, we report the backbone and side chain NMR assignments of SSO1118. The secondary structure order predicted from the assigned backbone chemical shifts is in agreement with PIN domain proteins. The chemical shift assignments and predicted secondary structures provide the basis for future structural and functional studies by NMR spectroscopy.

Methods and experiments

The gene with ID 3543 in the *Sulfolobus* Database (Brügger 2007) encoding SSO1118 was amplified by PCR from *Sulfolobus solfataricus* P2 genomic DNA using the forward and reverse primers 5′-CGCCATATGTTTGCAGTTATC TCGC-3′ and 5′-CGCCTCGAGAGATGATGCTAAAAGC TC-3′, respectively. The PCR product was cloned into the vector pET30a between the *Nde* I and *Xho* I sites resulting a plasmid pET30a-SSO1118. The protein product of this construct contains an additional C-terminal His-tag (LEHHHHHH) to facilitate the protein purification.

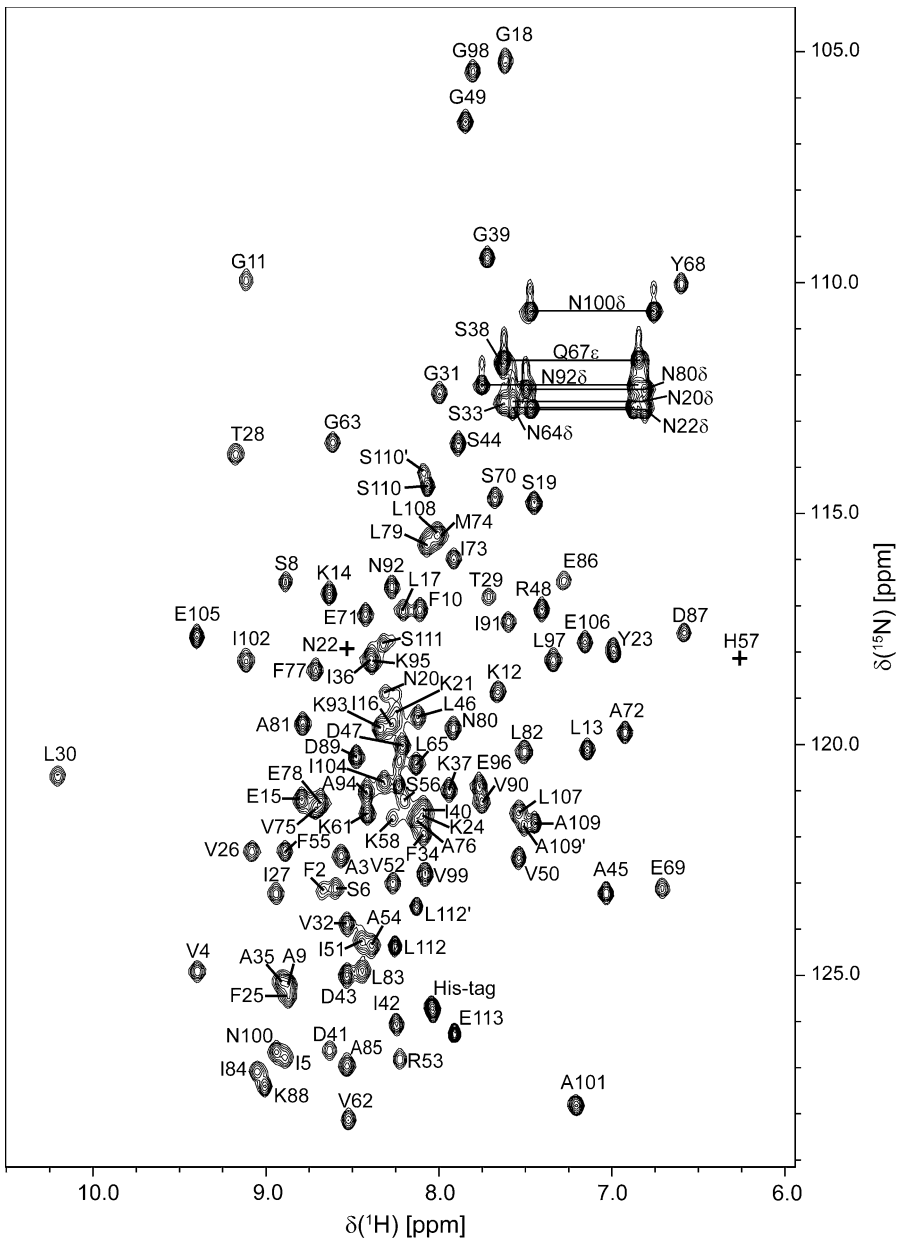

Fig. 1 2D ^{1}H-^{15}N HSQC spectrum of SSO1118 at 298 K. Assignments are indicated by one-letter amino acid code and the sequence number. Signals from N22 and H57 are under the plot contour level and their positions are indicated by crosses. Residues 109–113 show two sets of peaks, which may be caused by the C-terminal His-tag

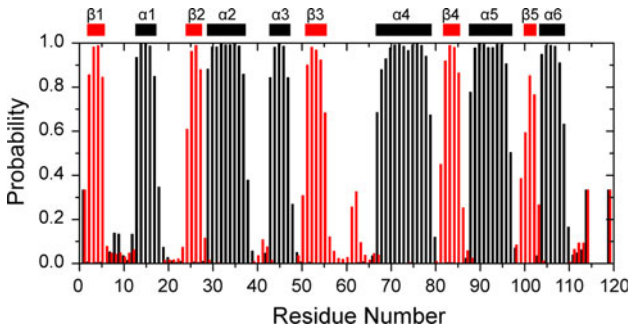

Fig. 2 The secondary structures of SSO1118 predicted by the software TALOS+ (Shen et al. 2009). The predicted α-helix and β-sheet probabilities of each residue are plotted in black and red, respectively. The secondary structure elements indicated on the top of the figure, were obtained with the criterion that the probability >0.5 for three or more continuous residues

The pET30a-SSO1118 plasmid was transformed into *Escherichia coli* Rosetta(DE3) cells for the protein expression. The cells were cultured overnight in 20 ml LB media, and then amplified into 1 l M9 minimal media. When the optical density at 600 nm (OD_{600}) of the cells reached 0.8, the protein expression was induced by addition of 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h. The cells were harvested by centrifugation at 4800 g, 4°C for 30 min. The cell pellets were resuspended in 30 ml buffer A (50 mM Tris–HCl, pH 8.0, 200 mM NaCl) and then frozen in −20°C overnight. The resuspended cell pellets were thawed, and then lysed by sonication. After centrifugation at 30700 g for 30 min, the supernatants were applied on a Chelating Sepharose Fast Flow (GE Healthcare) column. SSO1118 were eluted with buffer A containing 300 mM imidazole. The elute was concentrated to 2 ml using Amicon Ultra-15 centrifugal filter units (3 kDa NMWL) (Millipore), and further purified by gel filtration chromatography using a Superdex 75 column (GE Healthcare) pre-equilibrated in 50 mM potassium phosphate buffer at pH 7.0. Fractions containing purified SSO1118 were collected and concentrated. The protein concentration was determined by the UV absorption at 280 nm with a theoretical molar extinction coefficient 2560 $M^{-1}cm^{-1}$.

Uniformly ^{15}N- and $^{15}N/^{13}C$-labeled proteins were obtained by growth in M9 minimal media containing $^{15}NH_4Cl$ and [^{13}C]-glucose as the sole nitrogen and carbon sources, respectively. NMR samples consisted of ~0.8 mM SSO1118 in 90% H_2O/10% D_2O containing 50 mM potassium phosphate buffer (pH 7.0), 0.02% (w/v) NaN_3, 0.02% (w/v) sodium 2,2-dimethylsilapentane-5-sulfonate (DSS). All NMR experiments were performed at 298 K on a Bruker DMX 600 spectrometer equipped with a z-gradient triple-resonance cryoprobe. Backbone and side-chain resonance assignments were derived from 2D ^1H–^{15}N HSQC and ^1H–^{13}C HSQC, 3D ^1H–^{13}C–^{15}N HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, HBHA(CBCA)(CO)NH, HBHA(CBCA)NH, H(C)CH–TOCSY, and (H)CCH–TOCSY (Sattler et al. 1999). The aromatic side chain resonance assignments were obtained from 2D ^1H–^{13}C HSQC and 3D ^1H–^{13}C NOESY–HSQC spectra recorded for the aromatic region. The backbone and side chain chemical assignments were verified further by 3D ^1H–^{15}N NOESY–HSQC and 3D ^1H–^{13}C NOESY–HSQC spectra. The mixing times for ^{13}C–^{13}C TOCSY and ^1H–^1H NOESY experiments were 12 and 150 ms, respectively. All NMR spectra were processed and analyzed using the software Felix (Accelrys Inc.). Proton chemical shifts were referenced to the internal DSS, and ^{15}N and ^{13}C chemical shifts were referenced indirectly (Markley et al. 1998).

Assignments and data deposition

The recombinant protein SSO1118 contains 119 residues including 8 additional C-terminal residues derived from the His-tag (LEHHHHHH). Backbone resonance assignments were obtained for nearly all residues (Fig. 1), with the exception of all proline ^{15}N chemical shifts, as well as the ^{15}N and ^1HN resonances for Met1, Asn64, Gln66, and the C-terminal histidine residues. More than 95% side chain assignments for residues 1–113, including the aliphatic and aromatic residues, were obtained. Although the backbone ^{15}N and ^1HN resonances of Asn64, Gln66 were not assigned, the resonances for the side chain NH_2 groups of them were assigned using 3D CBCA(CO)NH and HBHA(CBCA)(CO)NH spectra. The chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 17298.

The secondary structures of SSO1118 were predicted from the assigned chemical shifts using the software TALOS+ (Shen et al. 2009). Results indicate that SSO1118 contains six α-helices and five β-strands in order of β1-α1-β2-α2-α3-β3-α4-β4-α5-β5-α6 (Fig. 2). The predicted secondary structures consist with those of archaeal PIN domain proteins (Arcus et al. 2004; Levin et al. 2004; Bunker et al. 2008), suggesting that SSO1118 is a novel PIN domain protein. The possible PIN domain structure and nuclease activity of SSO1118 will be assessed by the future structure determination and functional studies.

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