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ARTICLE

^{1}H, ^{13}C and ^{15}N resonance assignments of α-domain for *Bacillus subtilis* Lon protease

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Abstract The small α-domain of Lon protease is thought to carry the substrate-recognition, nucleotide-binding, and DNA-binding sites. Here we report the complete resonance assignment of the α-domain for *Bacillus subtilis* Lon protease (Bs-Lon α-domain).

Keywords AAA^{+} superfamily · α-Domain · Lon protease · SSD domain

Biological context

The superfamily of ATPases associated with diverse cellular activities (AAA^{+}) is responsible for various cellular processes such as proteolysis, DNA replication and membrane fusion (Ogura and Wilkinson 2001). The Lon protease, belonging to one of the ATP-dependent proteases of AAA^{+} superfamily and found in all species, is mainly responsible for degrading misfolded or damaged proteins to control the cellular protein quality. Lon protease plays its role in protein degradation as a ring-shaped homo-oligomer, and each subunit consists of three distinct functional domains: an N-terminal domain, a central ATPase domain, and a C-terminal proteolytic domain.

The central ATPase domain is an AAA^{+} module, with a structural core composed of two structural sub-domains: a larger sub-domain at the N-terminal responsible for nucleotide binding (RecA-like α/β-domain) and a smaller helical sub-domain at the C-terminal (α-domain) (Lupas and Martin 2002). Being a part of AAA^{+} module, the amino-acid sequences of α-domains are highly diverse among the AAA^{+} superfamily while their topology folds tend to be similar. Besides for participation in nucleotide binding and oligomerization with α/β-domain on the adjacent subunit, the small α-domains have been investigated to carry the substrate-recognition site, thus α-domains are also termed as the sensor- and substrate-discrimination (SSD) domain (Smith et al. 1999). Interestingly, the α-domain of Lon protease from *Brevibacillus thermoruber* has been demonstrated that it is responsible for DNA binding activity (Lee et al. 2004). Recently, we found that the α-domain of *Bacillus subtilis* Lon protease (Bs-Lon α-domain) also possesses DNA binding activity. To date, NMR resonance assignment and X-ray structure of *E. coli* Lon α-domain, which shares sequence identity of 53.1% with Bs-Lon α-domain, have been reported (Smith et al. 2001; Botos et al. 2004). To further gain insight into the structure-function relationships, especially DNA binding characteristic, we analyzed the NMR data of Bs-Lon α-domain to establish its structural information. Here we

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present the complete ^{1}H, ^{13}C and ^{15}N resonance assignments of α-domain for Bs-Lon α-domain.

Methods and experiments

The recombinant Bs-Lon α-domain (residues 490–604) uniformly labeled with ^{15}N or $^{15}N/^{13}C$, containing an extra Met residue at the N-terminus and the LEHHHHHH tag residues at the C-terminus, was overexpressed by vector pET-21a(+) in *E. coli* strain BL21(DE3). Cells were grown in MOPS medium supplemented with $^{15}NH_4Cl$ and/or ^{13}C-glucose at 37°C, and ampicillin (50 μg ml^{-1}) was added for plasmid selection. Medium was inoculated (1:20) with an overnight culture and incubated until the culture reached an OD_{600} of 0.6. Protein expression was induced by addition of 1.0 mM IPTG. The cells were harvested by centrifugation after 3 h and resuspended in buffer containing 50 mM sodium phosphate (pH 8.0), 300 mM NaCl, and 5% glycerol. Cells were disrupted using an M-110S microfluidizer (Microfluidics). The cell lysate was centrifuged at 50,000×*g* for 30 min at 4°C, and the supernatant was applied to nickel-nitrilotriacetic acid affinity resin. The column was washed with 5 mM imidazole in 50 mM sodium phosphate (pH 8.0), 300 mM NaCl, and 5% glycerol to remove contaminants and then eluted with the same buffer containing 120 mM imidazole. For further purifying the obtained protein, gel filtration chromatography was applied by using a Superdex75 XK 16/60 column (Amersham Biosciences) equilibrated with buffer containing 50 mM sodium phosphate (pH 7.0) with 100 mM NaCl and eluted at a flow rate of 0.8 ml min^{-1}. The purity of the eluted protein was shown to be greater than 95% on a Coomassie-blue-stained SDS-polyacrylamide gel.

All NMR spectra were acquired at 298 K and 310 K on a Bruker AVANCE 600 equipped with a QXI probe or an AVANCE 800 equipped with a z-gradient TXI cryoprobe. The sample (1.5 mM protein in 0.35 ml) was prepared in 50 mM sodium phosphate (pH 5.8) containing 100 mM NaCl, 50 mM Arg, 50 mM Glu, and 5 mM DTT in 90% H_2O/10% D_2O in a Shigemi NMR tube. All heteronuclear NMR spectra of the Bs-Lon α-domain were obtained as described previously (Kay 1995). Assignment of the main-chain ^{15}N, $^{1}H^{N}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$ chemical shifts was based on NHCACB, CBCA(CO)NH, HNCO, and HN(CA)CO spectra. Assignment of side-chain resonances was based on the ^{1}H-^{15}N TOCSY-HSQC, ^{1}H-^{13}C HCCH-TOCSY, C(CO)NH, and HBHA(CO)NH spectra. 2-D ^{1}H-^{15}N HSQC and 3-D NOESY-HSQC spectra were used to assign side-chain amide resonances of Asn and Gln residues. Aromatic resonances were assigned using 2-D ^{1}H-^{13}C HSQC, TOCSY, and NOESY spectra. DSS was used as the external chemical shift standard at 0.00 ppm, and the ^{15}N and ^{13}C chemical shifts were indirectly referenced using the consensus Ξ ratios of the zero-point frequencies at 310 K (Wishart et al. 1995). All spectra were processed using XWINNMR (Bruker) or using the NMRPipe software package (Delaglio et al. 1995) and analyzed using NMRView (Johnson and Blevins 1994). Linear prediction was used in the ^{13}C and ^{15}N dimensions to improve the digital resolution.

Assignment and data deposition

The NMR resonances of all backbone ^{15}N, $^{1}H^{N}$, $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C'$, and side-chain $^{13}C^{\beta}$ were completely assigned, with the exception of the $^{1}H^{N}$ resonances of Ala^{538}, Glu^{562}, and Lys^{564}, presumably because of the fast exchange rates with H_2O, which did not allow cross peaks to be detected. A 2D ^{1}H-^{15}N HSQC spectrum with assignment annotations was shown in Fig. 1. The amide protons of Ser^{542} and Glu^{570} were detected in the downfield regions at 11.56 and 10.81 ppm, respectively, and the side-chain ^{15}N and amide protons of the Gln and Asn residues were located within very broad regions. A consensus chemical shift index (CSI) generated using $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$ chemical shifts revealed that the secondary structure of the Bs-Lon α-domain is primarily composed of four α-helices and two β-strands. The chemical shifts of Bs-Lon α-domain at pH 5.8 and 310 K have been deposited into the BioMagResBank

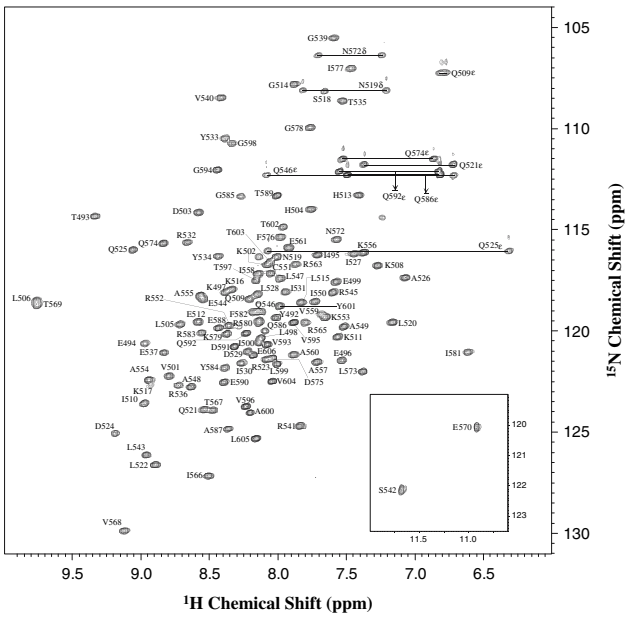

Fig. 1 ^{1}H–^{15}N HSQC spectrum of Bs-Lon α-domain (residues 490–604) at 310 K and pH 5.8. Side-chain NH_2 resonances of Asn and Gln are connected by horizontal lines. Inset: The assignments of unusual $^{1}H^{N}$ shifts of Ser^{542} and Glu^{570}

(http://www.bmrb.wisc.edu) under accession number BMRB-6615.

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