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Letter to the Editor: Assignments of the ^{1}H,^{13}C, and ^{15}N resonances of the substrate-binding SSD domain from Lon protease

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Biological context

How proteases recognize their targets is an important biological problem because protein degradation is irreversible. Lon (La) protease is an ATP-dependent serine protease with homologs in bacteria, yeast and higher eukaryotes (for a review, see Gottesman, 1996). In bacteria, Lon is a heat shock protein, which degrades abnormal proteins and a number of specific protein substrates, including SulA, RcsA, CcdA, and the λN protein. In both yeast and humans, Lon is a mitochondrial enzyme, which has been implicated in abnormal protein degradation. Lon also functions as a chaperone, apparently mediating the insertion of specific proteins into membranes and the disassembly of specific protein complexes.

The molecular details of substrate recognition by Lon protease are unknown. Recently, however, *Escherichia coli* Lon was shown to contain a 'sensor and substrate discrimination' (SSD) domain that folds as a monomer and recognizes known Lon substrates in a manner that parallels proteolytic specificity observed *in vivo* (Smith et al., 1999). The Lon SSD domain is homologous to substrate recognition domains in the Clp/Hsp100 family of ATPases (Smith et al., 1999, Levchenko et al., 1997) including ClpY (HslU) whose crystal structure is known (Bochtler et al., 2000; Sousa et al., 2000). No high-resolution structural information has been reported for Lon. As a prelude to studies of structure of the Lon SSD domain and its substrate recognition, we report here the nearly complete proton, carbon and nitrogen NMR assignments and the predicted secondary structure of this domain.

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Methods and experiments

The Lon SSD domain, consisting of Lon protease residues 489–588 of the intact protein, was expressed in *E. coli* strain BL21(DE3) and purified by ion-exchange chromatography on QHR sepharose (Amersham Pharmacia Biotech, Inc.) and gel-filtration chromatography on Superdex 75 10/60 (Amersham Pharmacia Biotech Inc.). The uniformly $^{15}N/^{13}C$ enriched protein was produced by growth in M9 minimal medium with $^{13}C_6$-glucose and $^{15}NH_4Cl$ as the sole carbon and nitrogen sources. For NMR spectroscopy, 3 mM protein was prepared in 50 mM d_4 acetate (pH 5.3) containing 20 mM NaCl, 0.5 mM DTT, 10% D_2O and 3 mM TSP.

NMR spectra were acquired at 298K on Bruker DMX600 and Varian Unity*Inova* 750 spectrometers equipped with pulse field gradient accessories. Specific sequential resonance assignments were obtained from triple resonance 3D NMR spectroscopy, including HNCACB, CBCA(CO)NH, HNCO, HCC(CO)NH, CC(CO)NH, HBHA(CO)NH, and HCCH-TOCSY experiments (reviewed in Sattler et al., 1999). ^{1}H chemical shifts were referenced to TMSP at 0.00 ppm, and ^{13}C and ^{15}N chemical shifts were calculated from the ^{1}H frequency (Wishart et al., 1995). All spectra were processed using the NMRpipe package version 1.7 (Delaglio et al., 1995) and analyzed using nmrDraw 1.7 and nmrView 4.1.3 with the Berkeley Distribution Patch on a Redhat Linux 6.2 PC.

Based on the Lon SSD $^{1}H\alpha$, $^{1}H\beta$, $^{13}C\alpha$, $^{13}C\beta$, ^{13}CO, ^{15}N, and ^{1}HN chemical shift values, the data-

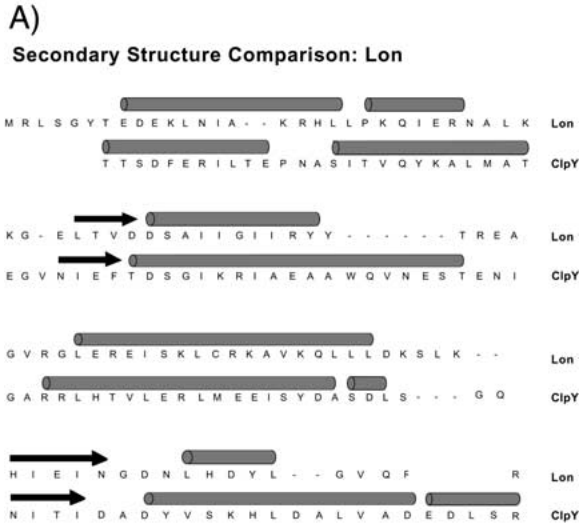

Figure 1A. Secondary structures predicted for the Lon SSD domain by TALOS and observed in the structure of the ClpY SSD domain (Bochtler et al., 2000; Sousa et al., 2000). No secondary structure is reported for Lon SSD where TALOS made ambiguous predictions or where only an isolated residue was found to adopt secondary structure.

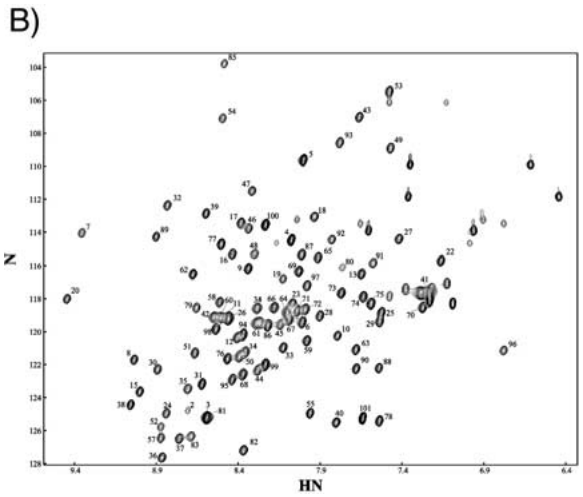

Figure 1B. Annotated ^{1}H-^{15}N HSQC spectrum of the Lon SSD domain. Residue 2 in the Lon SSD domain corresponds to residue 489 in the full-length protein sequence.

base system TALOS (Cornilescu et al., 1999) predicted 5 α-helices and 6 β-strands. The helices and 2 longest β-strands are located at positions similar to those for secondary structural elements in the homologous ClpY SSD (Figure 1A) (Bochtler et al., 2000; Sousa et al., 2000). Hence, it is likely that the SSD domains of Lon and ClpY adopt roughly similar folds.

Extent of assignments and data deposition

From the ^{1}H-^{15}N HSQC spectrum (Figure 1B), the ^{1}H and ^{15}N resonances of the backbone amides for 98 of 100 non-Pro residues were assigned. The unassigned residues were Met1 and Gly56. From the triple resonance experiments, further backbone and non-aromatic side-chain assignments were made to the following extents: 97% of ^{13}Cα, ^{1}Hα, ^{13}Cβ, ^{1}Hβ; 95% of ^{13}CO, ^{13}Cγ and ^{1}Hγ; 93% of ^{13}Cδ and ^{1}Hδ; and 80% of ^{13}Cε and ^{1}Hε resonances. No side chain data was available for Leu20, Arg55 or Arg101.

The assigned ^{1}H, ^{15}N, and ^{13}C chemical shifts of Lon SSD have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number BMRB-4523.

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