Crystallography

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The Active Site of a Lon Protease from *Methanococcus jannaschii* Distinctly Differs from the Canonical Catalytic Dyad of Lon Proteases

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Introduction

ATP-dependent Lon proteases catalyze the degradation of various regulatory proteins and abnormal proteins within cells. *Methanococcus jannaschii* Lon (Mj-Lon) is a homologue of *Escherichia coli* Lon (Ec-Lon) but has two transmembrane helices within its N-terminal ATPase domain. Sequence comparisons suggest that Lon contains a catalytic Ser-Lys dyad, and the first crystal structure of the proteolytic domain from Ec-Lon confirmed the presence of a catalytic Ser-Lys dyad within a unique structural fold, distinct from that of the classical serine proteases. In the present study, however, we found that the Mj-Lon proteolytic domain employs a unique catalytic Ser-Lys-Asp triad. Extensive sequence alignment and comparison of the structures of their proteolytic domains clearly indicate that Lon proteases can be classified into two groups depending on the configuration of the catalytic residues in the active site, as represented by Ec-Lon and Mj-Lon.

Results and Discussion

Description of the Structure

The proteolytic domain of Mj-Lon shares 29% identity and 49% similarity with the proteolytic domain of Ec-Lon over 193 amino acids. In the present study, we determined the crystal structure of the proteolytic domain of Mj-Lon (residues 456-649) using multiwavelength anomalous dispersion and refined it to 1.9-Å resolution.

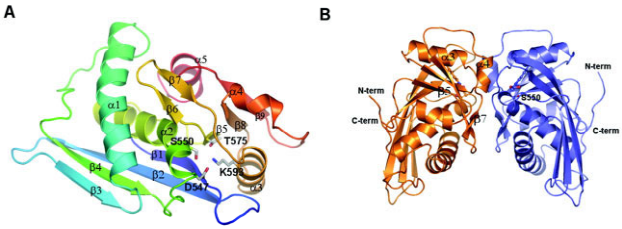

Figure 1. Structure of the proteolytic domain of *M. jannaschii* Lon. A) Ribbon diagram with the catalytic residues shown in ball-and-stick B) Dimeric structure of Mj-Lon proteolytic domain

The structure of the proteolytic domain consists of five α-helices and nine β-strands. The N-terminal β1 strand and antiparallel β2 strand form a long β-hairpin loop. The parallel β3 and β4 strands, which are connected by the longest helix (α1), form the first large β-sheet with the β1 and β2 strands. The subsequent helix α2 is kinked at Ser-550, which is a catalytic residue in this enzyme. (Figure 1).

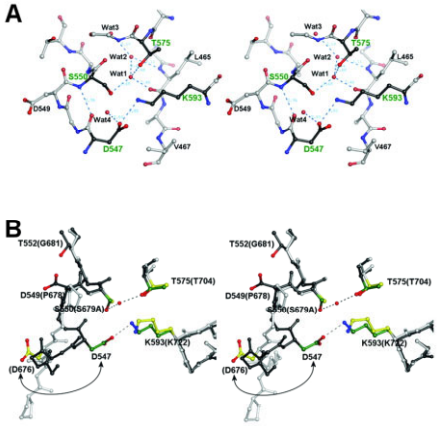

Figure 2. A) Active site of *M. jannaschii* Lon protease. B) Mj-Lon is colored in gray and Ec-Lon is colored in white

Active Site

The structure of the active site shows that Mj-Lon employs a pseudocatalytic triad comprised of Ser-550, Lys-593, and Asp-547. Asp-547 and the catalytic residue Ser-550 are located in the same face of helix α2, oriented toward Lys-593 in helix α3. Superposition of the structures of the Ec- and Mj-Lon proteolytic domains shows that Ser-550 and Lys-593 share almost identical positions in the two enzymes, but Mj-Lon has an additional residue, Asp-547, that is located in the N-terminal end of helix α2 and interacts with the catalytic residues. The carboxyl group of Asp-547 is located at the first turn of helix α2 and makes a salt bridge with Lys-593 and a hydrogen bond with a water molecule. In Ec-Lon, the Asp residue is also conserved at the sequence level, but it is exposed to the solvent and not involved with the active site residues because the segment corresponding to the N-terminal end of helix α2 is a β-strand, which puts the Asp residue at a position distant from the active site (Figure 2).

References

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