Comparisons of the Structures of Isolated Proteolytic Domains of Lon Proteases

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Crystal structure of the proteolytic domain of *A. fulgidus* B-type Lon protease (*Af*LonB) revealed significant differences in the conformation of the active site compared to two other known Lon P-domains, from *E. coli* (*Ec*LonA) and *M. jannaschii* **(***Mj*LonB) [1], despite the similarity of the overall fold. The differences in the interactions of the catalytic residues in the active sites of *Af*LonB and the other two Lon proteases are primarily connected to the variable conformational state of the segment that precedes catalytic Ser509. It appears that in isolated P-domains of single chain Lon proteases this segment does not have a stable conformation that could maintain proper structure of the active site. Other ATP-dependent proteases with known structures, such as HslUV or ClpAP, are two-chain enzymes, and in their independently-folded proteolytic subunits the catalytic residues are in appropriate positions. We suggest that the interactions with other domains (the ATPase domain in particular), as well as ligand binding, might lead to rearrangements in Lon P-domain active sites. Full-length *Af*LonB is proteolytically active in an ATP-dependent manner, whereas all individually purified wild-type and mutant P-domains are inactive. These results suggest that the structure of the active site in the P-domain of *Af*LonB represents an inactive state of enzyme. This raises the possibility that the surprising differences between the catalytic mechanisms of A and B type Lon proteases [1] might be artifacts, since the structure of the P-domain of *Mj*LonB could similarly represent an inactive state of that enzyme.

[1] ImY. J., et al., *J. Biol. Chem.*, 2004, **279**, 53451.

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