INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

The caseinolytic proteases (Clps) highly conserved large oligomeric protein complexes found in prokaryotes and eukaryotes. These complexes are generally involved in maintaining cellular homeostasis as well as virulence regulation. In this study, we aimed at characterizing the Clps of pathogenic *Leptospira in vitro* that might play an essential role in bacterial survival and virulence. Bioinformatic analysis of the genome of the sequenced strains of *Leptospira interrogans* shows the presence of the clp system that includes *clpA*, *clpB*, *clpC*, *clpX*, two isoforms of *clpP*- *clpP1* and *clpP2*, *clpS*, *clpY*, and *clpQ*. Based on the *in silico* information, we selected the highly conserved caseinolytic protease P (ClpPs)- ClpP1 and ClpP2 and the cognate ATPase chaperone ClpX for further studies. We have initially characterized the leptopiral ClpP isoforms along with ClpX by various biochemical and biophysical studies. The ClpP isoforms- LepClpP1 and LepClpP2 were in themselves inactive against small peptide substrates but were rendered functionally active when they were mixed. The LepClpP isoforms mixture showed optimum activity at a stoichiometric ratio of 1:1, suggesting a heterocomplex formation. We show that this heterocomplex is a tetradecameric structure and is hypothesized to form two stacked heptamer rings. We have also characterized the ATPase chaperone LepClpX and found that the LepClpP heterocomplex can only degrade larger protein substrates exclusively in the presence of LepClpX in an energy-dependent manner. Nevertheless, the pure LepClpP1 and LepClpP2 could not be stimulated by LepClpX and ATP to degrade the large protein substrates. Notably, on mutating the serine residue of the catalytic triad of either LepClpP1 or LepClpP2, the heterocomplex became inactive against peptides or protein substrates. Further investigation on the acyldepsipeptide antibiotic (ADEP) mediated activation of LepClpP1P2 heterocomplex revealed that the chemoactivation of ClpP is conditional on the duration of the self-compartmentalization of each of the LepClpP isoforms. We propose a second interaction site of ADEP in the LepClpP heterocomplex, hinted at by the allosteric activation of the LepClpP1P2^{S97A}, an otherwise inactive complex. Antibiotic ADEP also hampered the growth of *L. interrogans in vitro* and elongated the morphology of the spirochete. ClpPs, being conserved in most prokaryotes, including pathogen *L. interrogans*, has shown to be a promising drug target.

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