

## Abstract

Lon belongs to a family of ATP-dependent proteases that are involved in protein quality control. Bacterial Lon protease is considered the most important factor engaged in proteins degradation. Its activity is important in a number of cellular processes, including adaptation to stress conditions, cell division and differentiation, DNA replication. Our research group has shown that Lon protease from *Escherichia coli* specifically degrades TrfA protein (replication initiation protein of plasmid RK2) engaged in nucleoprotein complex with DNA. Moreover, Lon itself interacts with DNA. The presence of both factors, TrfA and DNA, effectively stimulates the ATPase and proteolytic activities of Lon. To date, however, it is still unknown how DNA affects the degradation of substrates by Lon and DNA-binding sites within the Lon structure remain unidentified.

The main aim of the project was to search for DNA-binding sites within Lon protease from *E. coli*. Three truncated variants of Lon protease were constructed and purified, each containing only a single full-length domain. The DNA-binding ability was identified for the ATPase domain of Lon. On the basis of this result and our model of *E. coli* Lon oligomer, the positively-charged patches over the surface of the Lon ATPase domain were proposed to serve as the binding site for DNA. By means of site-directed mutagenesis, full-length Lon mutants containing substitutions that change the charge from positive to negative on the surface of the selected region were constructed, purified and tested for DNA-binding ability. Lon variants that exhibited severe impairment in nucleoprotein complex formation with DNA were further analyzed for proteolytic activity towards specific substrates. The results revealed that the loss of Lon ability to bind DNA does not impair degradation of Lon substrates that do not form nucleoprotein complexes, however, it abolishes degradation of Lon substrates interacting with DNA, e.g. TrfA. Further analyses concerned the interaction of the Lon variants with the substrates, the ATPase activity assay and the phenotypic effects of Lon defectiveness in interaction with DNA.

By means of biophysical techniques and electron microscopy, the structure of Lon protease and the factors affecting the formation of nucleoprotein complexes were analyzed. The obtained results demonstrated dodecamers as a major oligomeric state of the Lon particles population and the influence of  $Mg^{2+}$  ions and DNA conformation on Lon-DNA interaction. Based on the results, a hypothetical model was proposed for the structural-functional relation of Lon protease dependent on interaction with nucleic acids.