

## ABSTRACT

Lon protease belongs to the AAA + protein family (ATPases associated with various cellular activities) and is conserved among all domains of life. Lon protease degrades denatured proteins during proteotoxic stress and is also responsible for the degradation of proteins involved in key metabolic processes in a cell. Tight regulation of Lon protease activity is essential for maintaining proteostasis in a cell. Lon protein consists of three functional domains: N-terminal, ATPase and peptidase domain. The ATPase and peptidase domains have been extensively described, while the N-domain is still not well understood. It has been shown that the *Escherichia coli* Lon protein (*EcLon*) can exist in two oligomeric states: hexamer and double hexamer (dodecamer). So far, the biological significance of the formation of these two types of quaternary structures of Lon is still enigmatic. Whether and how the N-terminal domain of Lon is involved in dodecamer assembly as well as the importance of the N-terminal domain for Lon protein activity is also unknown.

The purpose of my doctoral thesis was to answer the question about the role of the N-terminal domain and quaternary structure change in *E. coli* Lon protein activity.

In the first stage, I purified the wild-type form of the *EcLon* protein, the Lon E240K point mutant described in the literature as a stable dodecamer, and I also designed purified the *EcLon* protein mutants lacking 46, 160, 176 and 300 N-terminal amino acids. My *in vitro* experiments have shown that the N-domain participates in the creation and stabilization of the quaternary structure of Lon. By using *in vivo* test methods, I showed that N-domain is necessary to regulate the cellular response to UV stress, and thus to its survival under stress conditions. *In vitro* experiments with the use of native substrates and a non-physiological substrate showed that the amino acids located at the beginning of the N-domain are necessary in the recognition and binding of the substrate and its proteolysis. I also demonstrated that N-domain, through its participation in the formation of the quaternary protease structure, and interaction within dodecamer influences the ATPase activity of the protein. Experiments have shown that the Lon N-terminal domain is essential for efficient ATP hydrolysis.