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The Structural Modularity and Inherent Dynamics of the DegP Protease together with its Intertwined Role in the Bacterial Periplasm

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Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 14 januari 2022 kl. 9:00 i hörsal Hamberger, Institutionen för kemi och molekylärbiologi, Medicinargatan 16, Göteborg.

ISBN: 978-91-8009-576-1 (PRINT)

ISBN: 978-91-8009-577-8 (PDF)

Available online at <http://hdl.handle.net/2077/69931>



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Abstract

The protein quality control machinery is a delicate and integrated network of molecular tools working together to fold or remove unwanted proteins from the cell. A distinct set of “gatekeepers” are involved in this process including molecular chaperones, proteases, oxidoreductases, transferases, and many others in both eukaryotic and prokaryotic organisms. Whereas eukaryotes use a more advanced and multi-layered protein machinery, prokaryotes are more adapted to respond to sudden stresses *via* straight, simple pathways. As a member of the gram-negative bacteria, *E. coli* has evolutionarily adapted to have a rich periplasm that protects it against external and internal dangers.

One of the periplasmic “gatekeepers” is the homo-oligomeric DegP-protease that plays a crucial role in the biogenesis and degradation of β -barrel outer-membrane proteins within the periplasmic space. The DegP protein consists of a protease and two regulatory PDZ domains, that control and modulate DegPs association from a 300 kDa hexamer to a 1 MDa cage complex. DegP is activated under heat shock conditions and is well characterized on both the genetic as well as the biochemical level, however, its structural transitions, loop configurations that govern active site regulation as well as dynamical details, remain poorly understood. The aim of my thesis was to delineate the origin of molecular changes of DegP at the atomic level along with DegP's role in the periplasm by using biochemistry and advanced solution NMR techniques.

In this thesis, I characterized an induced temperature switch of DegP that is controlled by transmission from hexamer to trimer at elevated temperatures *via* a stabilizing methionine-aromatic motif in the regulatory PDZ domains. Furthermore, fine-tuned dynamics of the protease domain exposed inherent relaxation of allosteric residues within the protease core as well as the extent of inhibitory LA loop motions. Along assigning for the first time DegP individual domains by solution by NMR spectroscopy approaches, I started out to study full-length DegP by solid-state NMR. Finally, we characterized novel interactions between periplasmic chaperone Skp and Deg proteases. We explicitly observed the degradation of the periplasmic chaperone Skp monomer by both DegP and its homologue DegQ in *in vitro* experiments, revealing potentially a novel layer of regulation in *E. coli* protein quality control. Altogether, I managed to overcome the challenging DegP size for NMR spectroscopy and described its structural and dynamic properties to a level of detail previously not possible

Keywords: serine protease, DegP, nuclear magnetic resonance, dynamics, *E. coli*, protein quality control, chaperone, HtrA