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Functional details of human HtrA2 protease studied by NMR spectroscopy

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Abstract

Cells rely on an array of cellular machineries in the protein quality control system (PQC) to maintain the health of the collective proteome. The HtrA family of serine proteases are found in all kingdoms of life and function in the PQC by degrading damaged and aggregated proteins as well as by acting as molecular chaperones. The human mitochondrial serine protease HtrA2 functions as a pro-apoptotic agent in addition to its protease function in the mitochondrial PQC where it targets a diverse set of proteins including presenilins, α-synuclein and amyloid-β42. Dysfunction of HtrA2 is associated to multiple diseases including Parkinson's disease, Alzheimer's disease and multiple types of cancer. As such, HtrA2 is an important target of study and the structural details of the HtrA2 functional cycle remain incomplete to date. In this thesis, I have focused on detailing the allosteric activation of HtrA2 as well as characterizing the interaction between HtrA2 and its natural substrates XIAP and α-synuclein by using solution nuclear magnetic resonance (NMR) spectroscopy as my main method of choice. I showed that HtrA2 protease activity can be modulated by divalent cations binding to the HtrA2-PDZ domain, providing novel insight into the link between how metal dyshomeostasis can influence the PQC and subsequently lead to disease. I further detailed the allosteric activation pathway of HtrA2 and show for the first time that also the amino-terminal helix of HtrA2, harboring a motif critical for interaction with inhibitor of apoptosis (IAP) proteins, is affected by allosteric activation. My characterization of the interaction between HtrA2 and XIAP shows previously unreported weak interactions between XIAP and the HtrA2-PDZ domain and reveals that while mutations in the IAP-binding motif of HtrA2 drastically diminishes the interaction with XIAP, it does not alter the proteolytic efficiency of HtrA2 towards XIAP but hampers the ability to stimulate the HtrA2 proteolytic activity by use of divalent cations. Further, I show that HtrA2 can degrade monomeric α-synuclein and Tau isoforms Tau-39 and Tau-40. In conclusion, my results provide novel insights into the structural details of the HtrA2 functional cycle in atomical resolution and widens our understanding of the role of HtrA2 in apoptotic cell regulation and in the progression of neurological disease.

Keywords: HtrA2, nuclear magnetic resonance, protein quality control, neurodegenerative disease