Poster Presentation

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Substrate discrimination by size-exclusion in the intramembrane protease RseP

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Regulated intramembrane proteolysis (RIP), wherein a target membrane protein is specifically cleaved within the transmembrane region, is now accepted as a form of cellular signaling. As a result of proteolysis, a soluble portion of the target membrane protein is liberated to act as a signaling molecule. RIP is catalyzed by intramembrane-cleaving proteases, which are now classified into site-2 protease, rhomboid and γ-secretase/SPP families based on the mechanism of catalysis. E. coli possesses a site-2 protease homolog RseP, which is implicated in the extracytoplasmic stress response. RseP cleaves a membrane-spanning anti-σE protein RseA to release σE from the membrane, where truncation of the C-terminal periplasmic part of RseA by a membrane-anchored protease DegS triggers the action of RseP. Hence, there must be some mechanism by which RseP senses the DegS-cleavage of RseA. RseP possesses two tandemly-arranged PDZ domains (PDZ tandem) in the periplasmic region, which have been suggested to be involved in the regulation of cleavage. Although PDZ domains generally recognize the C-terminal sequence of a ligand, most of the previous works suggested that the RseP PDZ domains are involved in the suppression of the intramembrane cleavage of RseA. In this study, we determined the 3D structure of the PDZ tandem by X-ray crystallography and SAXS and showed that the two PDZ domains are arranged in an overall "clam-like" configuration to constitute a "pocket-like" structure. Sequence analysis suggested that the PDZ tandem would lie just above the active center sequestrated within the membrane. Furthermore, chemical modification demonstrated that the interior of the pocket is inaccessible to a bulky reagent in the full-length RseP. Taken together, we have made a proposal that RseP accommodates the truncated RseA into the active center by a steric size-exclusion mechanism through the PDZ tandem, rather than by recognition of a specific sequence/motif of RseA.

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