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Extended Abstract

Journal of Computational Methods in Molecular Design, 2021 **ISSN 2231-3176**
https://www.scholarsresearchlibrary.com/journals/journal-of-computational-methods-in-molecular-design/

Deciphering the Structural Basis of Translocator-Chaperone Interaction of Type III Secretion System-A Key to Drug Design Against Pathogenic Yersinia enterocolitica

Abhishek Basu, Department of Molecular Biology and Biotechnology
E-mail: abasu4@rediffmail.com

Yersinia enterocolitica is an opportunistic pathogen which causes enteric diseases like gastroenteritis and mesenteric adenitis in immune-compromised individuals. The gastrointestinal phase of Y. enterocolitica infection is mediated by Yersinia secretion apparatus - Yersinia secretion protein (Ysa-Ysp) Type III Secretion System (T3SS). Enhanced virulence of Y. enterocolitica Biovar 1B is attributed to the activation of Ysa-Ysp T3SS, which is further regulated by the formation of functional injectisome. YspB and YspC are hydrophobic translocator proteins which are responsible for the formation of functional translocon at the tip of the needle complex. These translocators are sequestered in the bacterial cytoplasm by their cognate chaperone SycB. SycB plays the dual role of a class II chaperone and a regulator of Ysa-Ysp T3SS. Homology model of SycB depicts a structure with a concave core formed by tetratricopeptide repeats (TPRs) and a flexible N-terminal helix. Deletion mutants of SycB showed that the N-terminal helix of SycB is responsible for its dimerization, which is further corroborated by molecular docking analysis. The dimeric state of SycB dissociates during the interaction with YspC due to steric hindrance. It forms a 1:1 heterodimeric YspC-SycB complex as confirmed by size-exclusion chromatography, chemical cross-linking and molecular docking studies. FRET analysis indicated that the tyrosine residues present in first two TPRs of SycB is responsible for its interaction with YspC. Deletion mutants of SycB possessing the first two TPR regions interacted with YspC, as depicted by the YspC-SycB interaction model. YspC is a unique minor translocator protein having monomeric form with high stability and rigid tertiary structure unlike any other translocator proteins. It shows structural alteration in the complex form with SycB as shown by spectroscopic data and proteolytic digestion. YspC has a Y-shaped three dimensional structure and SycB completely localizes within the fork formed by the two arms of Y-shaped YspC. Like other major translocator proteins YspB possesses a highly helical structure and transmembrane helices required for its translocation through the narrow conduit of the needle and its insertion within the host cell plasma membrane. Being a translocator protein it has to interact with chaperones and other translocators, which is evident from the existence of intramolecular coiled-coil regions in YspB structure. The YspB model depicted a star-shaped structure with alpha helices interspersed by random coil regions. The inner concave core of SycB forms the interface of interaction with YspB. This interaction is polar or ionic in nature and mediated by the first two TPRs of SycB. Therefore, simultaneous binding of YspB and YspC to SycB is not possible due to the common interaction domains. ConSurf analysis predicted that the evolutionarily conserved residues are mostly present in the regions of YspB involved in interaction with SycB. Exposure of translocator proteins to the extra-cellular milieu makes them potential drug targets. Therefore, elucidation of the three dimensional structure of translocators would enable us to determine precise antigenic epitopes for drug targeting. Structural analysis and understanding the mechanism of interaction between translocators and chaperones would be beneficial in designing peptide drugs to deregulate the Ysa-Ysp T3SS and attenuate the virulence of Yersinia enterocolitica. Pathogenic Yersinia species cause human diseases ranging from relatively mild intestinal disease for Yersinia pseudotuberculosis and Yersinia enterocolitica to bubonic plague for Yersinia pestis (Perry and Fetherston, 1997). Despite the differences in disease, virulence of these Yersinia species requires a conserved type III secretion system (T3SS) that has become a well-established model system for this form of protein secretion. Though first described in Yersinia, type III secretion is a conserved virulence factor amongst many human pathogens such as enteropathogenic Escherichia coli (EPEC), enterohemorrhagic Escherichia coli (EHEC), Salmonella sp., Pseudomonas aeruginosa, Shigella flexneri, and Chlamydia sp., which collectively cause significant healthcare costs annually. The T3SS has been described as a molecular syringe that delivers cytotoxic effectors into host cells. Because this virulence mechanism is conserved in so many pathogenic organisms, it makes an attractive target for new therapeutics. Interfering with effective delivery of effectors could have substantial consequences on disease pathology and, therefore, it is important to understand how bacteria sense cell contact in order to activate the T3SS and how both the fidelity and kinetics of effector delivery is coordinated. In the Yersinia, genes of the T3SS are located on a 70 kb virulence plasmid, and the expression of these genes in vitro is controlled primarily by temperature and calcium concentration, a phenomenon referred to as the low calcium response (LCR). At ambient temperature, T3SS genes are not expressed. However, upon transfer of Yersinia cultures from 26°C to 37°C in the presence of millimolar calcium, conditions representing the mammalian host, T3SS genes are expressed at low levels and the injectisome is built. Chelating calcium from the medium in vitro causes the bacteria to undergo growth cessation and triggers massive up-regulation of T3SS gene expression along with secretion of T3SS substrates, known as Yops and growth cessation is thought to be overcome by additional environmental signals.

Bottom Note: *This work is partly presented at EuroSciCon conference on Protein, Proteomics and Computational Biology*
December 06-07, 2018 Amsterdam, Netherlands