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SycB localizes within the arms of Y-shaped unique translocator YspC by an interaction mediated by the TPR regions of SycB

Abhishek Basu, Manali Biswas and Debjani Mandal

Department of Molecular Biology and Biotechnology, Sripat Singh College, Jiaganj, Murshidabad, Under University of Kalyani, West Bengal, INDIA

Presenting author: abasu4@rediffmail.com

Abstract

Introduction: *Yersinia enterocolitica* is an opportunistic pathogen which causes enteric diseases like gastrointestinal 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). Certain serotypes of *Y. enterocolitica* Biovar 1B exhibit enhanced virulence due to presence of Ysa-Ysp T3SS. Translocator proteins form the translocation apparatus (translocon) at the tip of the needle complex of the T3SS. YspC is a minor hydrophobic translocator protein which is sequestered in the bacterial cytoplasm by its cognate chaperone SycB. SycB plays the dual role of a class II chaperone and a regulator of Ysa-Ysp T3SS.

Experiments and key results findings: 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 hindrances. And 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 also depicted by the YspC-SycB interaction model. YspC is a unique translocator protein with high stability, rigid tertiary structure and monomeric form unlike any other translocator proteins. It shows structural alteration in the complex formed 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. This structural analysis and mechanism of interaction between YspC and SycB would be potentially beneficial for designing drugs to deregulate the Ysa-Ysp T3SS and attenuate the virulence of *Yersinia enterocolitica*.

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