Abstract

Title: Structural and Functional Study of Type Three Secretion System Proteins from *Yersinia enterocolitica*

Index No: 215/16/Life Sc./25

The Type Three Secretion System (T3SS) is a nanomachine used by most gram-negative pathogenic bacteria to secrete effector toxins directly into the cytoplasm of their host. These effectors perturb their host cellular pathway in such a way that the pathogen can escape the host immune system. Recent studies have provided a greater insight into the structure and function of T3SS. Such T3SS bears a highly conserved AAA+ ATPase as its component which is necessary for their efficient functioning. The details about, how these ATPases function and regulate T3SS is still not clear. In *Yersinia enterocolitica* YsaN is an ATPase associated with the T3SS and is necessary for their virulence. *In-vitro* study of YsaN through enzyme kinetics, biochemical and biophysical methods reveal that YsaN functions as higher order oligomer (probably dodecamer) as its most active form. This study also suggests that YsaN oligomerization requires active catalysis of ATP. YsaN forms hexamers as well as higher-order oligomers depending on the substrate concentration. Overall YsaN is an oligomerization-activated ATPase of *Y. enterocolitica* T3SS and shows a two-step cooperative kinetics mechanism. Secretion of effectors through the T3SS needle requires prior interaction of the effector chaperone complex with the ATPase complex present at the export gate of T3SS. The nature of such interaction is still not properly understood. To understand this interaction structural information on the effector, chaperone, and effector-chaperone complex is required. This study also presents the purification and crystallization of YopE (effector) and SycE (chaperone). YopE binds with SycE dimer forming a heterotrimeric complex. Due to the degrading nature of YopE, the crystallization of the YopE-SycE complex was difficult. Whereas SycE forms a dimer in solution and the structure of SycE is highly stable.

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