

Structural analysis on Salmonella effector SseK3-UDP complex

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Salmonellae are Gram-negative bacterial pathogens that infect a wide variety of animals. However, the manifestation of the disease varies between hosts. *Salmonella enterica* is a common food borne human pathogen with *S. Typhimurium* causing gastroenteritis and *S. Typhi* causing typhoid fever. *S. Typhimurium* uses the type III secretion system (T3SS) to deliver virulent effectors into the host to promote intracellular growth and replication. A group of T3SS effectors known as the Salmonella secreted effector K (SseK 1, 2, and 3) was identified based on their amino acid sequence similarity to the NleB effector protein present in pathogenic *Escherichia coli* strains. NleB functions as a glycosyltransferase that transfers a N-Acetylglucosamine moiety to an arginine residue *via* an N-glycosidic linkage. The known targets are FAS-associated death domain protein (FADD), death domain of TRADD and RIPK1. This modification blocks death receptor signaling and apoptosis.

Here we report the structure of SseK3 in complex with uridine diphosphate (UDP). SseK3 was shown to modify TRADD weakly but not FADD. All members of the NleB/SseK family share a conserved DXD motif that is known to bind Mn²⁺ and/or coordinate the sugar donor in other GT-A glycosyltransferases. Effector SseK3 shows the classical GT-A glycosyltransferase fold with several α -helices flanking a twisted β -sheet in addition to an α -helical insertion. The crystals were grown in the presence of UDP N-acetylglucosamine, a known sugar donor for NleB family, yet clear density is only observed for UDP but not N-acetylglucosamine. Atom O2 on UDP forms a hydrogen bond with the backbone amide of Phe⁵³, while the N3 atom forms a hydrogen bond with the backbone carbonyl oxygen of Phe⁵³. In addition, the uracil ring of UDP forms pi-stacking interaction with Trp⁵² and Phe¹⁹⁰. The backbone carbonyl oxygen of Gln⁵¹ and hydroxyl group of Tyr²²⁴ form hydrogen bonds with the ribose C2 hydroxyl while the C3 hydroxyl forms hydrogen bond with the backbone amide of Ala²²⁷. Effector SseK3 is the first member of the NleB/SseK family to be structurally characterized, information gained from this study can be used for homology modeling to further our understanding on this family of virulent effectors.