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Crystal structure of bacterial cytotoxic necrotizing factor CNFy reveals molecular building blocks for intoxication

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Cytotoxic Necrotizing Factors (CNFs) are single-chain AB-toxins and important virulence factors in pathogens such as uropathogenic *E. coli* (UPEC) or enteropathogenic *Yersinia* species. Their cytotoxic effect is based on the constitutive activation of small GTPases (Rho family) by deamidation of a glutamine residue in the switch II region, leading to cytoskeletal alterations and finally death of the host cell. Several steps are required for the toxin to fulfill its task, such as receptor binding, endocytosis and yet unknown structural changes in the B-part in order to translocate the catalytically active, toxic A-part through the endosomal membrane into the cytosol. So far, a crystal structure that could provide insight into these processes is still lacking.
Here we report on the crystal structure of full-length CNFy from *Yersinia pseudotuberculosis* at a resolution of 2.7 Å. The structure was solved by molecular replacement with the existing model of the C-terminal catalytic domain and the structure of the B-part, where the phases of the latter were obtained by single anomalous dispersion experiments in advance. The full-length AB-toxin comprises 1014 amino acid residues. While the two C-terminal domains (485 residues) are forming the A-part, the N-terminal B-part (529 residues) is consisting of 3 individual domains, of which all possess novel folds. From cell-biology experiments, the receptor-binding and translocation functions could be assigned to the first three domains although their mechanism(s) and the receptor of the host cell remain still unknown. The fourth domain shows structural similarity to ADP-ribosyl transferases but no similar function in CNFy could yet be detected.
Additionally, we also determined the structure of the two-domain A-part - as it should be released into the cytoplasm - alone (1.8 Å). The two domains show a different orientation towards each other than in the structure of the full-length toxin, which could hint towards activation of the catalytic domain upon release of the A-part.
Our first crystal structure of a full-length CNF-toxin lays the groundwork for further studies of the complex mechanism of this important bacterial virulence factor. CNF-toxins might not only be promising targets for future development of anti-infective drugs, the B-part might even have the potential to be exploited as delivery vehicle for large and complex drugs.

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