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Closing the Lid on the Mono-ADP-ribosylating Reaction Mechanism by Bacterial Toxins. R. Jorgensen, X. Wang, X. Liu, R. Merrill, Molecular and Cellular Biology, Univ. of Guelph, Guelph, ON N1G 2W1 Canada.

The bacteria causing diphtheria, cholera and other human diseases secrete mono-ADP-ribosylating toxins that modify proteins in the target host eukaryotic cell. Recently, we have solved four 3 Å crystal structures of a catalytically active complex of the enzyme domain of Exotoxin A (ETA) and its substrate, elongation factor 2 (eEF2), which have led to a breakthrough in the understanding of the reaction mechanism of this family of deadly toxins. The target residue in eEF2, a modified histidine, diphthamide, spans across a cleft in the complex and faces the two phosphates of the NAD⁺ analogue, βTAD. This suggests that the diphthamide is involved in NAD⁺ cleavage and is interacting with the proposed oxacarbenium intermediate during the nucleophilic substitution reaction. Notably, the βTAD phosphates mimic the phosphate backbone of two highly conserved nucleotides in the 18S rRNA, thereby achieving universal recognition of eEF2 by ETA. In addition, we have new data showing that there are catalytic residues located within an active-site loop of the toxin. We propose that this loop is in an open conformation in the protein complex structures and during transition-state closes in on the active site partly as a cover for solvents and partly to help stabilize the oxacarbenium ion.

