

Structural Analysis of *Clostridium difficile* Toxins A and B Using a Hybrid Approach

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C. difficile is a gram-positive, spore-forming anaerobe that infects the colon, causing a range of disorders including diarrhea, pseudomembranous colitis, and toxic megacolon (1, 2). While *C. difficile* does not generally infect healthy individuals, it poses a threat to individuals with a depleted natural intestinal flora. Thus, patients undergoing antibiotic treatment are most at risk of acquiring *C. difficile* associated disease (CDAD). *C. difficile* is encountered most often in nursing home and hospital settings, where there are large populations of infected and at risk individuals. Kyne *et al.* estimated that in 1999, the financial burden of CDAD on the US healthcare system was \$1.1 billion per year (4). Since that time, there has been a dramatic increase in both the prevalence and the severity of *C. difficile* infections due to the emergence of the hypervirulent strain NAP1/027 (5, 6). The emergence of this new strain underscores the significance of *C. difficile* as one of the most threatening nosocomial pathogens.

Clostridium difficile toxins A and B are the main cause of pseudomembranous colitis and hospital-acquired diarrhea. Both members of a family of large clostridial toxins (LCT), the structure and mechanism of these proteins have been poorly characterized. TcdA and TcdB are central targets in the prevention and treatment of *C. difficile*-associated disease. As such, it is essential that we gain a structural understanding of how the toxins are organized and their structures change to gain entry into host cells. TcdA and B contain four functional domains that are associated with host-receptor binding, membrane pore formation, autoprocessing, and the enzymatic modification of host Rho-family proteins.

TcdA and TcdB were purified from *C. difficile* culture supernatants and shown to be active in a cell-rounding assay. Toxin was adsorbed to carbon-coated glow-discharged grids, stained with uranyl formate. TcdB particles were clearly more heterogeneous than the TcdA toxin; however, analysis of ~2,500 TcdB particles and ~3,000 TcdA particles into class averages show that these two toxins share a similar structural organization. A 3D reconstruction of TcdA was calculated to ~24 Å resolution using the random conical tilt approach (Fig. 1B). The molecule has a 'pincher-like' head and two tails, long and short. Expression of recombinant fragments indicates that the 'pincher-like' head corresponds to the delivery domain while the long tail corresponds to the receptor-binding domain. Inducing the auto-proteolytic processing event resulted in removal of the short tail, revealing that this domain corresponds to the glucosyltransferase domain. The location of the autoprotease domain within the map of TcdA was not experimentally determined, but the structure is anticipated to be located so that the C-terminus of the glucosyltransferase domain can be cleaved in the active site of the protease. The TcdB glucosyltransferase and TcdA autoprocessing domain X-ray crystal structures along with a model of the TcdA receptor-binding domain were manually docked into the experimental EM density (Fig. 1A).

To understand the structural basis for pore formation within the endosome, we analyzed TcdA particles at low pH. TcdA was applied to a carbon-coated, glow-discharged grid, washed with

a pH 4.5 buffer, and then stained with uranyl formate. The 3D structure of TcdA at low pH shows a molecule that has undergone a major conformational change from the structure observed at neutral pH (Fig. 1C). Although the conformation of the binding domain has not changed, the low pH form is more elongated due to an extension of the head domain. The low pH structure of TcdA has a volume that corresponds to a MW of ~240 kDa and is thus considerably smaller than the structure of native TcdA whose volume corresponds to a MW of ~320 kDa. This difference may be due to greater flexibility and/or partial unfolding of the glucosyltransferase domain at low pH.

References

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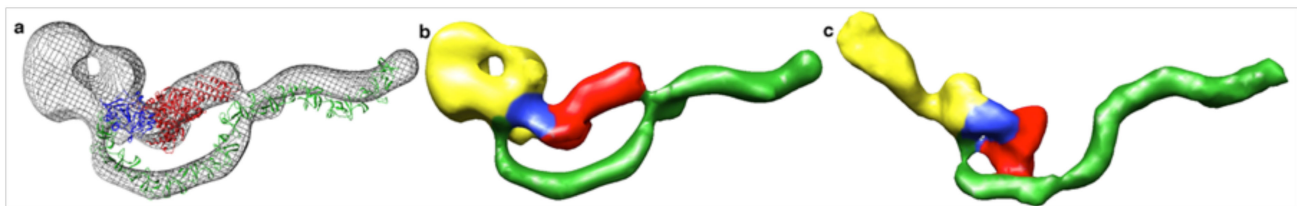


FIG. 1. The 3D structure of TcdA has been obtained at neutral and low pH by electron microscopy. a) The structural models for the glucosyltransferase (red), autoprotease (blue), and receptor-binding (green) domains have been docked into the neutral pH density, but at present, no structural models for the delivery domain exist. b) The structure at neutral pH reveals a pincher-like head domain (yellow) and two tails, short and long. Colored as in Fig. 1a. c) The structure at the low pH of the endosome reveals significant change in the TcdA head region