Poster

Structural basis for the differential toxicity of cholera toxin and heat-labile enterotoxin

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Vibrio cholerae and enterotoxigenic *Escherichia coli* (ETEC) are two bacterial pathogens responsible for millions of diarrhea cases each year. These pathogens release two similar AB_5 toxins that are directly responsible for the severe diarrhea: the cholera toxin (CT) and *E. coli* heat-labile enterotoxin (LT), respectively. They consist of a catalytically active A1 subunit, an A2 linker, and a pentamer of cell-binding B-subunits [1]. Both toxins bind to the same GM1 surface receptor on the host cells and have similar levels of enzymatic activity, yet CT is more potent than LT, making cholera the more severe disease. The difference in toxicity has been attributed to structural differences near the C-terminus of the A2 linker (amino acid residues 226-236) [2], but the underlying molecular mechanism remains unknown. Recently, we showed that toxin disassembly by protein disulfide isomerase (PDI), which is a key event in the intoxication process, is more efficient for CT than for LT [3]. We hypothesized that the difference in toxin disassembly is related to the positioning of the A1 subunit relative to the B-pentamer [3] (Fig. 1).

Here, we determined the crystal structures of two cholera toxin variants where either one (D229E) or four (D229E, I230V, T232I, H233Y) amino acid residues in the critical A2 linker sequence were substituted for the residues present in LTA2 (Figure 1; colored residues within the pores of the grey pentamers). In order to obtain sufficient amounts of protein for crystallization, we established a protocol for production of the toxins in *Vibrio natriegens*, which improved the yield by at least 10-fold [4]. The toxin crystals diffracted to 1.6-2.1 Å resolution. The results from the structural analysis and cell biology will be presented here.

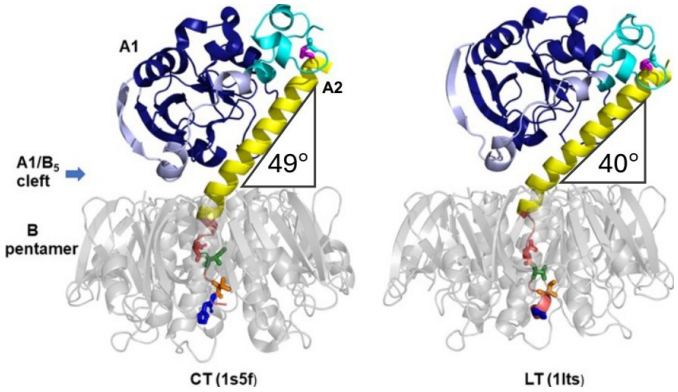

Figure 1. Structures of wild-type CT and LT [3]. For the two toxins, the angle of the A1 subunit relative to the B-pentamer differs by 9 degrees.

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[3] Serrano A, Guyette J. L., Heim J. B., Taylor, M., Cherubin, P., Krengel, U., *et al*. (2022) *Sci. Rep*., **12**, 34.
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