Title:	Structure-function studies on Vibrio parahaemolyticus thermostable direct hemolysin
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Abstract:	Pore-forming toxins (PFTs) are typically produced as water-soluble monomers, which upon interacting with target cells assemble into transmembrane oligomeric pores. Vibrio parahaemolyticus thermostable direct hemolysin (TDH) is an atypical PFT that exists as a tetramer in solution, prior to membrane binding. The TDH structure highlights a core β-sandwich domain similar to those found in the eukaryotic actinoporin family of PFTs. However, the TDH structure harbors an extended C-terminal region (CTR) that is not documented in the actinoporins. This CTR remains tethered to the β-sandwich domain through an intra-molecular disulphide bond. Part of the CTR is positioned at the interprotomer interface in the TDH tetramer. Here we show that the truncation, as well as mutation, of the CTR compromise tetrameric assembly, and the membrane-damaging activity of TDH. Our study also reveals that intra-protomer disulphide bond formation during the folding/assembly process of TDH restrains the CTR to mediate its participation in the formation of inter-protomer contact, thus facilitating TDH oligomerization. However, once tetramerization is achieved, disruption of the disulphide bond does not affect oligomeric assembly. Our study provides critical insights regarding the regulation of the oligomerization mechanism of TDH, which has not been previously documented in the PFT family.
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