Structure and Functional Characterization of Thermostable Direct Hemolysin from Grimontia Hollisae

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Single crystal X-ray diffraction is the technique of choice for studying the interactions of macromolecules with proteins by determining their three-dimensional structures. The use of crystal structures of therapeutically relevant drug targets in pharmaceutical research has significantly increased over the last decade. The application of structure-based drug design has resulted in several marketed drugs and is now an established discipline in most pharmaceutical companies. To collect and process the X-ray diffraction data into usable structural information and to use three-dimensional protein structure is a basis for drug discovery.

Thermostable direct hemolysin, (TDH), a pore-forming toxin, has been recognized as a virulence factor in Vibrio pathogenesis. It has a variety of activities, including hemolytic activity, cytotoxicity, cardiotoxicity, mouse lethality, and enterotoxicity. TDH from Grimontia hollisae was crystallized using the hanging-drop vapour-diffusion method. According to X-ray diffraction data, the unit cell belongs to space group $P2_12_12$ with unit-cell parameters a = 60.902, b = 105.343, c = 112.688. A full diffraction data set for wild-type TDH has been measured to 2.0 Å resolution on flash-frozen crystals using synchrotron radiation. A fully exchanged selenomethionyl TDH derivative (containing 3 Se atoms per TDH molecule) was also prepared and crystallized in an isomorphous crystal form, providing full selenium MAD data at three wavelengths and enabling phase solution and structure determination (Fig. 1) [1; 2; 3]. Preliminary analysis indicated the presence of four TDH molecules in the asymmetric unit, with 49.0% solvent content.

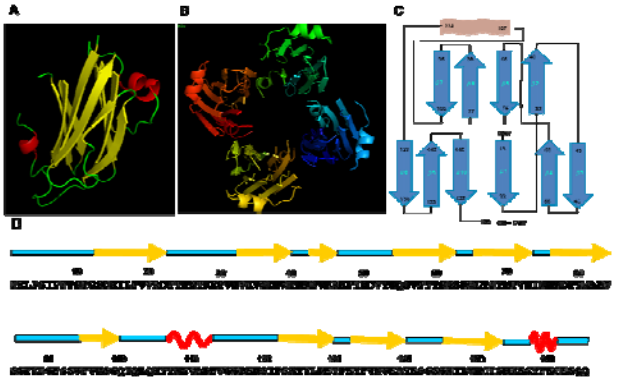

Fig. 1: Overall structure and folding of TDH. (A) A ribbon representation of the TDH monomer. Secondary structure elements of β strands are shown in yellow, elements of α helices are shown in red, and the loop regions are shown in green. (B) Top view of a ribbon representation of the crystallographic TDH tetramer. The individual subunits are colored differently to highlight their organization. (C) A topological diagram of the TDH structure. β strands are labeled in the order of their appearance from the N to the C terminus. The first residue of each secondary structure element is marked with its sequence ID. (D) The sequence of TDH, with annotated secondary structure elements on the top. Data were collected at SPXF beamline BL13B1 and BL13C1 at NSRRC (Taiwan, R. O. C.) using ADSC Quantum-315 CCD, ADSC Quantum-210 CCD detector and processing by HKL2000 software (Z. Otwinowski and W. Minor).

References

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