Structures of *C.perfringens* α-toxin mutant, T74I, that affects its Activity

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The α-toxin of *Clostridium perfringens* is the major virulence determinant produced by the bacterium associated with gas gangrene in man. The toxin is a Zn^{+2} dependent, Ca^{+2} activated phospholipase C (PLC), is haemolytic and able to interact with membrane-packed phospholipids. The ability to interact with eukaryotic cell membranes distinguishes the α-toxin from related enzymes, such as *C. bifermentans* and *B. cereus* PLC.

Several crystal structures of this enzyme from different Clostridial strains and sources have showed the structure is composed of two, an α-helical (N-terminal) and a β-sandwich (C-terminal) domains.

A site directed mutagensis study revealed that the substitution of a single residue, Thr74 with Ile (T74I), resulted in the loss of haemolytic, phospholipase C and the sphingomyelinase activities by 1/250 fold to that of wild enzyme. We have determined the crystal structure of T74I mutant in two different crystal forms, $C222_1$ and $P4_32_12$ to1.9Å and 3.2Å resolution respectively. The crystal contains a monomer, in $C222_1$ structure, and a trimer in $P4_32_12$ structure, in the asymmetric unit of their unit cell. The overall topologies of the mutant structures are very similar, but have conformational differences in the mutant containing 60-90 loop, which is one of the proposed membrane interacting loops. We will compare the structures of T74I mutant with other α-toxin structures and relate the differences to the loss/reduction of its haemolytic, phospholipase C and sphingomyelinase activity.

Keywords: bacterial toxins, crystal structures, site-directed mutant