STRUCTURE OF CLOSTRIDIUM ABSONUM PHOSPHOLIPASE C AT 2.5Å

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The α toxin of *Clostridium perfringens* (the first toxin identified with phospholipase C activity) has previously been shown to be the main causative agent in gas gangrene. *C.absonum* PLC, whilst maintaining 60% sequence identity when compared to C.perfringens α toxin, has differing characteristics regarding phospholipid specificity and haemolytic activity. We report on the cloning, expression, characterisation and structure solution of this C.absonum PLC, and attempt to shed more light on the specificity and activity of this important family of cytotoxic PLCs. Differences between *C.absonum* PLC and *C.perfringens* α-toxin are apparent, including major differences in the conformation in the functionally important 60-90s loop. This loop acts as a lid and is involved in the change between active and inactive forms. The proposed active site of *C.absonum* PLC structure is more open and exposed than that of the previously identified open form of *C.perfringens* α-toxin. Differences in specificity between *C.perfringens* α toxin and *C.absonum* PLC for various phospholipid types have also been discovered, and we will explain these differences using the structural information obtained from the C.absonum and other related toxin structures.

Keywords: BACTERIAL TOXIN GAS GANGRENE CLOSTRIDIUM ABSONUM

TOWARDS THE STRUCTURE DETERMINATION OF P30: AN AUTOTRANSPORTER DOMAIN

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Bordetella Pertussis, the etiological agent of whooping cough, has several virulence factors which belong to the autotransporter protein family. Autotransporter proteins are found in a number of bacteria and they are defined by a conserved C-terminal domain of approximately 30 kDa which is proposed to enable translocation to the cell surface by forming a β barrel in the outer membrane through which the mature protein can pass. The best characterised member of the B. pertussis autotransporter family is P93 which is cleaved to form mature pertactin, P69, and an autotransporter domain, P30. P69 remains cell associated and functions as an adhesin. The P30 domain remains in the outer membrane as a major component. The high resolution structure of P69 has been determined. Little is known about the structure or function of autotransporter domain. We have expressed P30 in *E. coli* and have been able to purify and refold large amounts of P30 from inclusion bodies. We have also been able to target expressed P30 into the outer membrane of E.coli. Crystallization trials on the refolded P30 and purification of the membrane associated P30 are ongoing. The crystal structure of the P30 domain will complete the model of P93 protein and will provide a model for autotransporters for other relevant bacteria.

Keywords: MEMBRANE PROTEIN, AUTOTRANSPORTER, INFECTION

STRUCTURE OF THE von WILLEBRAND FACTOR A1 DOMAIN IN COMPLEX WITH THE SNAKE TOXIN, BOTROCETIN

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von Willebrand Factor (vWF) is a plasma multidomain glycoprotein that plays a crucial role in primary hemostasis. The adhesive properties of vWF are tightly regulated *in vivo*, such that plasma vWF does not normally interact with circulating platelets. At sites of vascular injury, however, vWF becomes activated through poorly understood conformational change triggered by shear forces, and then binds to the platelet receptor glycoprotein Ib (GpIb) via its A1 domain. *In vitro*, the binding can be induced by gain-of-function mutations at a regulatory site of the A1 and by complex formation between the A1 and exogenous modulators including the snake venom protein, botrocetin. Botrocetin forms a tight binary complex with the A1, causing high affinity for GpIb-binding. To understand the structural basis of affinity modulation in vWF, we have determined the crystal structure of a gain-of-function mutant A1 (I546V) and its complex with botrocetin. The uncomplexed mutant A1 crystallized isomorphously with the wild-type one, and its overall structure is very similar to the wild-type one. We observed small conformational changes in the A1 on complex formation that switch it back towards the wild-type (low affinity) conformation. Botrocetin binds to a surface on the A1 domain adjacent to the GpIb-binding surface and does not seem to switch the A1 conformation into a high affinity state. We suggest that botrocetin increases affinity for GpIb by augmenting the binding surface on the A1 domain rather than through allosteric control.

Keywords: VON WILLEBRAND FACTOR GAIN-OF-FUNCTION PLATELET ADHESION

CRYSTAL STRUCTURE OF HUMAN DJ-1, A SPERMATOGENESIS RELATED PROTEIN

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DJ-1 was identified as a novel oncogene that transforms mouse NIH3T3 cells in cooperation with activated ras. DJ-1 functions as a positive regulator of Androgen Receptor (AR)-dependent transcription by binding to PIASx α which downregurates AR-dependent transcription. Also, DJ-1 is reported to be decreased by exposure of the rat to sperm toxicants such as ornidazole. Furthermore, DJ-1 was found as a novel circulating tumor antigen in breast cancer. Here, we determined the structure of human DJ-1 by X-ray crystallography using the method of multiple isomorphous replacement (MIR). The final model at 1.95 Å resolution was refined to an R-factor of 17.1 %(Rfree=19.4 %). DJ-1 has the Rossmann-like fold and exists as a dimer in the asymmetric unit. Its dimer interface is formed with six α helices and one intermolecular β sheet. A search with the program DALI revealed extensive similarity between the three-dimensional structure of DJ-1 and protease I from Pyrococcus horikoshii. The search also revealed that DJ-1 has a similar topology with anthranilate synthase (Sulfolobus solfataricus) and gmp synthetase (*E.coli*). These proteins have catalytic triad similar to that of cysteine-proteases. Since DJ-1 also has the cysteine residue conserved in the catalytic triad, DJ-1 has a potential to function as a cysteine protease by itself or by forming a complex with some factors under physiological conditions.

Keywords: SPERMATOGENESIS PROTEASE DIMER