Clostridium perfringens is a gram-positive bacterium, which causes a wide range of diseases in both humans and animals, by producing a large number of toxins. C. perfringens bacteria are classified into five toxinotypes (A-E) depending on their ability to produce the major toxins: Alpha-, Beta-, Epsilon- and Enterotoxin. Delta toxin is one of the three hemolysins produced by C. perfringens type C and possibly type B strains. Delta toxin was shown to be cytotoxic to cells containing  $G_{M2}$  in their membrane. Delta toxin is also expected to heptamerise and form pores in the lipid bilayer of host cell membranes [1]. Nevertheless, its exact mode of action remains to be clarified. In order to further characterize this toxin at the molecular level, we have determined its crystal structure.

The fully active recombinant *C. perfringens* Delta toxin has been over-expressed as a his-tagged protein in *E. Coli* BL21 and purified by affinity chromatography. Delta toxin crystallizes in two crystal forms. The tetrahedral bipyramidal crystals diffracted weakly, whereas the needle shape crystals diffracted up to 2.4 Å [2]. We determined the crystal structure by molecular replacement using *Staphylococcus aureus* leukocidin F (LukF), with which Delta toxin shares 30% sequence identity.

C. perfringens Delta toxin in our crystals is monomeric and folds as an elongated molecule. It is composed of mainly beta-sheets organized into three domains. There are three Zn, located in the upper part of the protein, and three glycerol molecules, located in the lower part, bound to the toxin. Delta toxin's fold is closely related to S. aureus alpha hemolysin and the leukocidins. The superposition of Delta toxin structure with the phospholipid-bound LukF structure revealed that the glycerol molecules in Delta toxin and phospholipids in LukF structure are accommodated in the same hydrophobic clefts. Interestingly, from the only structure of the detergent-solubilized heptamer of S. aureus alpha hemolysin available so far, these hydrophobic patches on the surface of the protein correspond to where the protein latches on to the membrane. This result confirms the on-going hypothesis that Delta toxin shares the same pore-forming mechanism as the betapore forming toxins of the S. aureus family, and the structure we have determined is the water-soluble form of Delta toxin. On the basis of their structural homologies, we constructed a model of the Delta toxin heptameric form.

The structure of *C. perfringens* Delta toxin, which is the first *C. perfringens* hemolysin structure ever reported, provides new highlights in our understanding of *C. perfringens* mechanism of action and pathogenesis.

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#### Keywords: toxin, bacterial, pathology

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## Structure of L. pneumophila NTPDase, A functional homolog of eukaryotic NTPDases

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Many pathogenic bacteria have sophisticated mechanisms to interfere with the mammalian immune response. These include the disruption of host extracellular ATP levels that, in humans, is tightly regulated by the nucleoside triphosphate diphosphohydrolase family (NTPDases). NTPDases are found almost exclusively in eukaryotes, the notable exception being their presence in some pathogenic prokaryotes. To address the function of bacterial NTPDases, we describe the structures of an NTPDase from the pathogen Legionella pneumophila (Lpg1905/Lp1NTPDase) in its apo state and in complex with the ATP analog AMPPNP and the subtype-specific NTPDase inhibitor ARL 67156. Lp1NTPDase is structurally and catalytically related to eukaryotic NTPDases. The structure also provides a basis for NTPDase-specific inhibition. Furthermore, we demonstrate that the activity of Lp1NTPDase correlates directly with intracellular replication of Legionella within macrophages. Collectively, these findings provide insight into the mechanism of this enzyme and highlight its role in hostpathogen interactions.

#### Keywords: NTPDase, L. pneumophila, pathogenesis

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# The adhesin domain of the multidomain protein Epf from *Streptococcus pyogenes*

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The Gram-positive bacterium *Streptococcus pyogenes* is a strictly human pathogen and infects primarily epithelia of the human throat and skin. To be able to adhere to and colonise these host epithelia, *S. pyogenes* employs an arsenal of cell wall-anchored adhesins. We have previously shown that the multidomain protein Epf from *S. pyogenes* is an adhesin important for binding to human epithelial cells. However, the mode of action of Epf is unknown and there is no sequence similarity between the domains of Epf and any protein of known structure or function. We identified the N-terminal domain of Epf as the mediator of adhesion. Sequence analysis shows that this N-terminal domain represents the tip of Epf followed by 16 C-terminal repeat domains that are likely to form a long stalk ending with the cell wall anchor.

Here, we report the crystal structure of the N-terminal domain of Epf. We solved this structure to a resolution of 1.6 Å, using multiwavelength anomalous dispersion methods on a selenomethionine derivative. The *R* value is 15.1 % ( $R_{free}$ =18.1 %). The N-terminal domain of Epf forms two  $\beta$ -sandwich subdomains, one of which has a fibronectin type III-like fold.

Surprisingly, the other subdomain, located at the very N-terminus of Epf, shows structural similarities to carbohydrate-binding modules (CBMs), which usually occur as domains of carbohydrate-modifying enzymes. The most closely related CBMs are those of laminarases and xylanases. As Epf appears not to possess an enzymatic domain, we hypothesise that it uses its carbohydrate-binding module to bind to glycans on the surface of human epithelia. Currently, we are testing this hypothesis and are investigating carbohydrates that may be targets of Epf.

Keywords: adhesion, bacterial, Streptococcus pyogenes