

Poster Sessions

A thermostable organic-solvent-tolerant Lipase 42 (L42) was successfully crystallized under the condition of 0.1M MES monohydrate, 0.1M NaH₂PO₄, 0.1M KH₂PO₄ and 2.0M NaCl using counter-diffusion and sitting-drop vapor diffusion technique. Remarkably after data processing, counter-diffusion technique shows an enhancement of resolution at 2.0Å [1] as compared to sitting-drop method with only 2.4Å. There are several advantages of counter-diffusion technique; it promotes slower and mild diffusion of protein and precipitant.

As a conclusion, a better L42 lipase resolution was achieved by counter-diffusion technique. Thus, by obtaining a better model of protein, deeper understanding on protein-solvent interaction could be obtained

[1] M.S. Khusaini, R.N.Z.R.A Rahman, M.S.M. Ali, T.C. Leow, A.B. Salleh, M. Basri. *Acta Cryst.* **2011** F67, 401-403.

Keywords: counter diffusion, vapour diffusion, organic solvent tolerant lipase

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A component of the xanthomonadaceae T4SS Combines a VirB7 motif with a N0 Domain

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Type IV secretion systems (T4SS) are used by Gram-negative bacteria to translocate protein and DNA substrates across the cell envelope and into target cells. Translocation across the outer membrane is achieved via a ringed tetradecameric outer membrane complex made up of a small VirB7 lipoprotein (normally 30 to 45 residues in the mature form) and the C-terminal domains of the VirB9 and VirB10 subunits [1]. Several species from the genera of *Xanthomonas* phytopathogens possess an uncharacterized type IV secretion system with some distinguishing features, one of which is an unusually large VirB7 subunit (118 residues in the mature form).

Here, we report the NMR and X-ray structures of the VirB7 subunit from *Xanthomonas citri* subsp. *citri* (VirB7_{XAC2622}) and its interaction with VirB9 [2]. The solution structure showed that VirB7_{XAC2622} has an unfolded N-terminus and a unique C-terminal domain whose topology is strikingly similar to that of N0 domains found in proteins from different systems involved in transport across the bacterial outer membrane. We submitted the globular N0 domain of VirB7_{XAC2622} to crystallization trials, that resulted in large plates which belong to space group C222₁ and diffracted up to 1.0 Å. Molecular replacement was performed using the solution structure of the globular domain as the search model, resulting, after refinement, in a model with R_{work} of 13.0% and R_{free} of 15.2%.

NMR solution studies showed that residues 27-41 of the disordered flexible N-terminal region of VirB7_{XAC2622} interacts specifically with the VirB9 C-terminal domain, resulting in a significant reduction in the conformational freedom of both regions. We show that VirB7_{XAC2622} oligomerizes through interactions involving conserved residues in the N0 domain and residues 42-49 within the flexible N-terminal region and that these homotropic interactions can persist in the presence of heterotropic interactions with VirB9. Finally, we propose that VirB7_{XAC2622} oligomerization is compatible with the core complex structure in a manner such that the N0 domains form an extra layer on the perimeter of the tetradecameric ring.

[1] V. Chandran, R. Fronzes, S. Duquerroy, N. Cronin, J. Navaza, G. Waksman, *Nature* **2009**, 462, 1011-1015. [2] D.P. Souza, M.O. Andrade, C.E. Alvarez-Martinez, G.M. Arantes, C.S. Farah, R.K. Salinas, *PLoS Pathogens* **2011**, Accepted for publication.

Keywords: structural biology, protein NMR, type IV secretion system

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The structure of a bacterial esterase essential for NO-stress response. Rafael M Couñago,

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Nitrosative stress is an important factor in host-pathogen interactions. *Neisseria gonorrhoeae* (ng) and *N. meningitidis* (nm) are closely related human pathogens that can pose significant health problems. Both bacteria display a series of mechanisms to cope with oxidative and nitrosative stress, which can come from the bacteria's own metabolism and the host's microenvironment, including the host's innate immune response. Previous work [1] has identified Esterase D (EstD) as essential for protection of *N. gonorrhoea* against nitrosative stress in vitro and for its intracellular survival in human primary cervical epithelial cells. EstD is a carboxylic ester hydrolase and is part of the NmlR regulon, which is upregulated during nitrosative stress and present in a number of pathogenic bacteria.

Here we present the structure of EstD from *N. meningitidis* (97% sequence identity to ngEstD) at 1.3 Å resolution. The enzyme displays the characteristic α/β hydrolase family fold and a catalytic triad formed by Ser, Asp and His residues. The identity of active site residues was further confirmed by mutagenesis studies. The structure of mnEstD also reveals that the "gate-keeper" Cys-54 residue is found at the enzyme's active site entrance. In the eukaryotic counterparts of Neisserial EstDs, oxidation of this residue by glutathione has been shown to modulate the enzyme's activity. Likewise, we show that wild-type mnEstD can be reversibly inhibited via oxidation of Cys-54 by glutathione and that an engineered Ala-54 mnEstD mutant is insensitive to glutathione challenge. Although common in eukaryotes, modulation of protein function by glutathione oxidation has been shown for only a handful of proteins in prokaryotes.

Ongoing experiments in *Neisseria* are to complement our biochemical and structural analysis of EstD and will provide a better understanding of how EstD's function protects these pathogens against nitrosative stress and the host's innate immune response.

[1] Potter et al., *J. Infect. Diseases* **2009**, 200, 273-278.

Keywords: bacterial, esterase, crystallography

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Crystal structure of Clostridium perfringens Delta toxin and a model of its pore-form

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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 beta-pore 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.

[1] M. Manich, O. Knapp, M. Gibert, E. Maier, C. Jolivet-Reynaud, B. Geny, R. Benz, M.R. Popoff, *PLoS One* **2008**, *3*, e3764. [2] J. Huyet, M. Gilbert, M.R. Popoff, A.K. Basak, *Acta Crystallogr Sect F Struct Biol Cryst Commun*, **2011**, *67*, 369-371.

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 host-pathogen 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 multi-wavelength 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 β-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*