Interestingly, however, PerCR does not show the specific targeting when introduced into the cells with a protein transfection reagent. To resolve the structural basis for peroxisomal localization of PerCR, we have determined the crystal structure of PerCR at 1.5 A resolution [1]. The structure revealed that the C-terminal PTS1 of each subunit of PerCR was involved in intersubunit interactions and was buried in the interior of the tetrameric molecule. These data indicate that the monomeric form of PerCR whose C-terminal PTS1 is exposed will be recognized by the PTS1 receptor Pex5p in the cytosol and then, is targeted into the peroxisome and thereby forms tetramer.
[1] Tanaka *et al.*, *Structure* **16**, 388-397 (2008).

Keywords: carbonyl reductase, PTS1, SDR

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Advances in the structural elucidation of *Clostridium difficile* toxin B using SAXS and MX techniques

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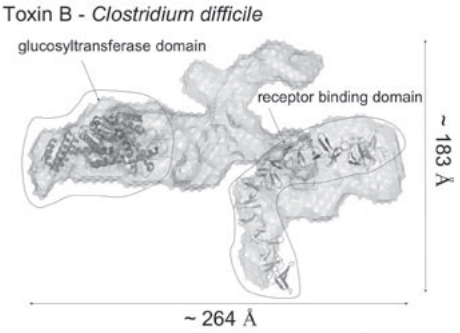
Clostridium difficile is an anaerobic bacterium that is present in the gut of up to 3% of healthy adults and 66% of infants. *C. difficile* can however cause serious gastrointestinal disease, ranging from severe diarrhoea to pseudomembranous colitis. Disease is particularly evident in elderly patients who have undergone antibiotic therapy. Two toxins: A and B [1], can be produced by *C. difficile*. These toxins are members of the Large Clostridial Cytotoxin family and are high molecular weight glucosyltransferases (toxin A: 308 kDa; toxin B: 270 kDa). These two toxins exert their cytopathic action from within the cytosol after receptor-mediated endocytosis. In the growing effort to fully understand the mechanism of action of these toxins, we are carrying out their structural characterization by macromolecular crystallography and SAXS techniques. Current progress will be presented, including the first low-resolution SAXS structure obtained for toxin B and a high-resolution structure of the receptor binding domain [2].



High resolution structure of the recombinant glucosyltransferase domain (Reisert, D.J., *et al.* (2005) *J. Mol. Biol.* 351: 973-981) and model of the receptor binding domain of *C. difficile* toxin B fitted into the low resolution SAXS structure of the full length native toxin.

1. von Eichel-Streiber, *et al.* (1996). Trends Microbiol. 4(10) : 375-82.
2. David Albesa-Jove, *et al. in preparation*.

Keywords: Clostridium difficile, SAXS: Small Angle X-ray Scattering, MX: Macromolecular Crystallography

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Activities and structure of beta toxin

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Beta toxin is a virulence factor of Staphylococcus aureus that catalyzes the cleavage of sphingomyelin(SM) in biological membranes to ceramide and phosphorylcholine causing lysis of erythrocytes. Crystals of beta toxin were found to be fully merohedrally twinned. The structure was solved via molecular replacement using SmcL (SMase C from Listeria ivanovii) as the search model and refined to 2.4 Å resolution. Beta toxin belongs to α/β protein family and is arranged in a 4-layer sandwich. Assays of native and structure suggested site-directed mutants of beta toxin demonstrate that the lysing of sheep erythrocytes and the killing of proliferating human lymphocytes is linked to the SMase activity of beta toxin. These data are the first to show a direct effect upon human tissue and provide a rationale for the importance of beta toxin in virulence. A C-terminal β hairpin has been proposed to penetrate the lipid bilayer and aid in substrate binding and positioning. Our analysis shows this involved in the observed twinning. Three variations of the β hairpin were created, crystallized and solved via molecular replacement and refined. The β hairpin mutations did not significantly perturb the structure of beta toxin, but do affect toxicity towards human cells. A partial lipid was found in one of the structures. SM has been co-crystallized with Beta toxin, and the structure solved and refined to 1.65 Å resolution. The β hairpin has an important role in the SMase activity and cytotoxicity. Current experiments are aimed at elucidating the role of the β hairpin using liposome disruption assays and co-crystallization of the mutants with SM.
[1]www.cdc.gov/ [2]Huseby et al. J Bac, 2007. [3]Openshaw et al. JBC, 2005.

Keywords: sphingomyelinase, toxin, staphlococcus aureus

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Structure and function of C-terminal catalytic region of *Pasteurella multocida* toxin

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Pasteurella multocida toxin (PMT) is one of virulence factors responsible for the pathogenesis in some *Pasteurellosis*. We determined the crystal structure of the C-terminal region of PMT (C-PMT), which carries an intracellularly active moiety. The overall structure of C-PMT displays a Trojan horse structure, composed of three domains arranged in feet, body and head subunits with each linker loops, which were designated C1, C2, and C3 domains from the N- to C-terminus, respectively. The C1 domain showing marked similarity in steric structure to the N-terminal domain of Clostridium difficile toxin B, was found to lead the toxin molecule to the plasma membrane. We found in the C3 domain the Cys-His-Asp catalytic triad that is organized only when the Cys is released from a disulfide

bond. The steric alignment of the triad corresponded well to that of papain or other enzymes carrying the Cys-His-Asp triad. Our results indicate that PMT is an enzyme toxin carrying the cysteine protease-like catalytic triad dependent on the redox state, and functions on the cytoplasmic face of the plasma membrane of target cells
Kitadokoro et.al., *Proc Natl Acad Sci USA*, 103, 5139-44, (2007).

Keywords: bacterial toxins, bacterial pathogenesis, hydrolase

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Structural basis of actin recognition and ADP-ribosylation by *Clostridium perfringens* iota-toxin

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The ADP-ribosylating toxins (ADPRTs) produced by pathogenic bacteria modify intracellular protein and affect eukaryotic cell function. Actin-specific ADPRTs (including Clostridium perfringens iota-toxin and Clostridium botulinum C2 toxin) ADP-ribosylate G-actin at Arg177, leading to disorganization of the cytoskeleton and cell death. Although the structures of many actin-specific ADPRTs are available, the mechanisms underlying actin recognition and selective ADP-ribosylation of Arg177 remain unknown. Here we report the crystal structure of actin-Ia, which is an enzymatic component of iota-toxin, in complex with the non-hydrolyzable NAD analog βTAD at 2.8 Å resolution. The structure indicates that Ia recognizes actin via five loops around NAD: loop-I (Tyr60-Tyr62 in the N-domain), loop-II (active-site loop), loop-III, loop-IV (PN loop), and loop-V (ADP-ribosylating turn-turn loop). We used site-directed mutagenesis to confirm that loop-I on the N-domain and loop-II are essential for the ADP-ribosyltransferase activity. Furthermore we revealed that Glu378 on the EXE loop is in close proximity to Arg177 in actin, and proposed that the ADP-ribosylation of Arg177 proceeds by an SN1 reaction via first an oxocarbenium ion intermediate and second a cationic intermediate, by alleviating the strained conformation of the first oxocarbenium ion. Our results suggest a common reaction mechanism for not only actin-specific ADPRT but also other types of ADPRT. In this meaning, this study is a milestone in research of ADP-ribosylating enzymes.
[1] Tsuge H.,et al., *J Mol Biol* (2003) 325:471-483 [2] Tsuge H., Nagahama M., Oda M., Iwamoto S., Utsunomiya H., Marquez VE., Katunuma N., Nishizawa M.,Sakurai J., *PNAS* (2008) In press.

Keywords: protein toxins, actin, protein-protein interactions

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Okadaic acid,a conformational study in the solid state

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Okadai acid (OA) , a polyether produced by dinoflagellates of the genus Prorocentrum and Dinophysis, has had an extraordinary impact upon different life science areas such as human health, seafood control analysis, pharmacology, natural product chemistry, fishery industry economics, etc., promoting new developments in all these areas. Originally isolated from a marine sponge as a result of a screening program undertaken by a pharmaceutical company in searh of a new cytotoxic compound, OA was subsequently characterized as the main agent responsible for diarrethic shellfish poisoning, as well as being a potent tumor promoter. The latter discovery was a decisive due to unlocking the secret of its mechanism of action, which involves selective inhibition of serine/treonine protein phosphatases (PPs).Therefore, OA is now recognized as the first member of the “okadaic acid class” of PPs inhibitors, a remarkably different panel of metabolites that have become valuable tools for studying the cellular roles of different PPs.We report here the crystal and molecular structure of OA and its conformational properties in the solid state.

Keywords: marine toxin, polyether, dinoflagellates

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X-ray diffraction studies of two dimeric variants of human pancreatic ribonuclease

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Ribonucleases catalyze RNA cleavage reactions. A specialized class of RNases shows a high cytotoxicity toward tumor cell lines, critically dependent on the ability of these molecules to evade the action of the cytosolic ribonuclease inhibitor (RI). Here, we report the X-ray structure of two dimeric variants of RNase1: HHP2-RNase1 and des(16-20)RNase1. HHP2-RNase1 is a covalent dimeric protein, cytotoxic for several malignant cell lines, in which RNase1 has been engineered to reproduce the sequence of bovine seminal ribonuclease helix-II and to eliminate a negative charge on the surface [1]. Des(16-20)RNase1 is a highly stable domain swapped dimer constituted by chains in which five residues in the loop linking the N-terminal helix of RNase1 to the rest of the protein have been deleted [2]. The analysis of HHP2-RNase crystals indicates structural details which can explain the high antitumor activity of the protein. The structure of des(16-20)RNase1 shows a tetrameric association of two swapped dimers, that suggests a pathway of large-scale oligomerization of the protein. [1] Piccoli et al., PNAS 96, 7768 (1999) [2] Russo et al. Biochemistry 39, 3585 (2000) Grant Sponsor:FIRB RBNE03B8KK

Keywords: ribonuclease, antitumour compounds, oligomers