

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

## P04.19.402

*Acta Cryst.* (2008). A64, C356

### 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  $\beta$ TAD 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

## P04.19.403

*Acta Cryst.* (2008). A64, C356

### Okadaic acid, a conformational study in the solid state

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Okadaic 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 search of a new cytotoxic compound, OA was subsequently characterized as the main agent responsible for diarrhetic 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

## P04.19.404

*Acta Cryst.* (2008). A64, C356

### 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-RNase1 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