by the bacterium. Purification from the supernatants of the crude bacterial extracts by a combination of ultrafiltration, chromatography on EDTA-modified DOWEX-1x2 anion exchanger and size exclusion chromatography (Superdex 200) leads to separation of the 62.7 kDa enzyme. N-terminal sequencing of the first 30 residues confirms the enzyme to be a chitinase with the closest sequence homologue a chitinase D–like enzyme from *Bacillus thuringiensis* serovar *finitimus* YBT-020 [3], even if the overall domain organisation differs and has not been observed as yet.

Quality of the 62.7 kDa exochitinase samples has reached the level suitable for structural studies. The first crystallisation experiments and further sequencing and characterisation have been performed.

The project is supported by the Czech Science Foundation, project no. 310/09/1407.

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Keywords: enzyme, structure-function studies, chitinase

MS93.P57

Acta Cryst. (2011) A**67,** C789

Structural insights into autoactivation mechanism of p21-activated protein kinase

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The p21-activated kinases (PAKs) are serine/threonine protein kinases defined by their interaction with the small G proteins, and play important roles in diverse cellular processes including cytoskeletal dynamics, growth/apoptotic signal transduction and regulation of transcription factors. All PAKs contain an N-terminal regulatory domain and a C-terminal kinase domain. Full activation of PAKs requires autophosphorylation of a critical threonine/serine (Thr423 in PAK1) located in the activation loop of kinase domain. A large body of experimental evidence shows that phosphorylation of PAK1 Thr423 is a trans-autophosphorylation reaction that is wholly dependent on dimerization of PAK1 (i.e. between two identical kinase molecules). Here, we report the crystal structures of phosphorylated and unphosphorylated PAK1 kinase domain. The phosphorylated PAK1 kinase domain has the conformation typical of all active protein kinases. Interestingly, the structure of unphosphorylated PAK1 kinase domain reveals an unusual dimeric arrangement expected in an authentic enzyme-substrate complex, in which the activation loop of the putative 'substrate' is projected into the active site of 'enzyme'. The 'enzyme' is bound to AMP-PNP and has an active conformation, whereas 'substrate' is empty and adopts an inactive conformation. Thus, the structure of asymmetric homodimer mimics a trans-autophosphorylation complex, and suggests that the unphosphorylated PAK1 could dynamically adopt both the active and inactive conformations in solution.

Keywords: p21-activated kinase, pre-existing equilibrium, trans-autophosphorylation

MS93.P58

Acta Cryst. (2011) A**67,** C789

Site directed mutagenesis to the rescue: unravelling conformational changes of Inositol 1,3,4,5,6 pentakisphosphate 2-kinase

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Inositol polyphosphates are a wide group of second messengers, involved in key cellular events. In particular, Inositol Pentakisphosphate 2-Kinase (IP5 2-K) converts inositol pentakisphosphate (IP5) to inositol hexakisphosphate or phytic acid (IP6). IP5 2-K is the unique enzyme that phosphorylates the axial position (2-OH) of the inositide ring. IP6 is present in all eukaryote cells and plays an essential role in processes such as lymphocyte development or apoptosis. In addition, IP6 tends to accumulate in plant seeds, what may have detrimental effects in human health and environment. Briefly, phytic acid is a potent chelator agent, contributing to malnutrition in populations where the diet is grain-based. We are undertaken structural studies by X-ray Crystallography to understand the mechanism underlying the function and regulation of this key enzyme in cell biology.

We have very recently crystallized and solved the first structure of an IP5 2-K from *A. thaliana*, in complex with substrates (IP5, IP5 plus AMP-PNP) and products (IP6, IP6 plus ADP) [1], [2]. The enzyme presents an αβfold, being divided in two lobes, a N lobe, conserved from protein kinases, and a C-terminal lobe. The N lobe and some residues from the C lobe are implicated in the nucleotide binding Also, a big part of this C-terminal lobe (the CIP-lobe) forms a novel structural region to bind the inositol phosphate. Despite all this knowledge, many obscure aspects remains around the catalytic mechanism and conformational changes of the enzyme. In order to elucidate this questions, and after a deep analysis of existing structural information, we are combining X-ray crystallography with other techniques, principally site directed mutagenesis. This approach has been very successful to improve the crystallizability of our samples as well as to gain insights into the no bonded (apo) and AMP-PNP bonded forms of IP5 2-K. All these findings represent an important tool to design inhibitors for the enzyme, what have potential applications in biomedicine and animal feed staff industry, for example in designing crops with low phytate levels.

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Keywords: crystallization, enzyme, kinase

MS93.P59

Acta Cryst. (2011) A**67,** C789-C790

Structural basis of methylglucose lipopolysaccharide biosynthesis in mycobacteria

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The 6-O-methylglucosyl-containing lipopolysaccharides (MGLPs) and the 3-O-methylmannosylcontaining polysaccharides (MMPs) are two unusual polymethylated polysaccharides (PMPS) produced by mycobacteria. Both PMPS localize to the cytoplasm, where they have been proposed to regulate fatty-acid biosynthesis owing to their ability to form stable 1:1 complexes with long-chain fatty acids and acyl-coenzyme A derivatives. In sequestering the products of fatty-acyl synthase I (FAS I), PMPS are thought to facilitate the release of the neo-synthesized chains from the enzyme, thereby not only reopening active sites essential for enzyme turnover but also terminating their elongation. In addition, PMPS have been proposed to serve as general fatty-acyl carriers, the role of which would be to facilitate the further processing of very long and insoluble fatty-acyl CoAs, including mycolic acids, by increasing the tolerance of mycobacteria to high cytoplasmic concentrations of these products while protecting them from degradation. The glucosyl-3-phosphoglycerate synthase (GpgS), is a retaining a-glucosyltransferase that initiates the biosynthetic pathway of the MGLPs in mycobacteria. The enzyme transfers a Glc*p* moiety from UDP-Glc to the 3 position of the phosphoglycerate to form glucosyl-3-phosphoglycerate. Here we report new crystal structures of the *apo* and UDP complex forms of GpgS from *Mycobacterium tuberculosis* at 2.6 and 3.0 Å resolution respectively. The overall structure shows the two-domain organization typical of GT-A GTs. We propose a plausible model for donor and acceptor substrates recognition and catalysis. The implications of this model for the comprehension of the early steps of MGLPs biosynthesis and the catalytic mechanism of other members of the GT-A family are discussed.

Keywords: Mycobacteria, glucosyl-3-phosphoglycerate synthase (GpgS)

MS93.P60

Acta Cryst. (2011) A**67,** C790

Crystal structure of cyclophilin-a enzyme from *Azotobacter vinelandii*

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The Peptidyl-Prolyl Isomerases (Cyclophilins, FKBPs and Parvulins) catalyze the cis-trans isomerization of peptide bonds preceding prolyl residues, therefore accelerating protein folding [1]. Cyclophilins (CyPs) have been established as a model system in enzymology in terms of extensive efforts of understanding the mechanism of enzyme catalysis in full depth [2]. *Azotobacter vinelandii* is a well known agricultural, aerobic, soil-dwelling bacterium, which fixes atmospheric nitrogen, converting it to ammonia, which is the most ingestible form of the element for the plants. There are two known cyclophilins in *A. vinelandii*: cytoplasmic AvCyPA and periplasmic AvCyPB.

The crystal structure of the cytoplasmic cyclophilin A was determined by molecular replacement at 1.7 Å resolution. In addition, the crystal structure of the protein complexed with the synthetic tetrapeptide succinyl-Ala-Phe-Pro-Phe-p-nitroanilide (sucAFPFpNA) was determined at 2.0 Å resolution. The tetrapeptide sucAFPFpNA was used as a substrate for an assay that confirmed that *A. vinelandii* AvCyPA possesses PPIase activity. The tetrapeptide is bound as a proline cis-isomer and adopts different conformations from those observed in other related structures. Comparisons between the uncomplexed and complexed structures as well as other CypA structures provides additional insights about structure-function relationships of this enzyme. Also structural studies for PPIAses from a new organism may complement existing studies and help achieve a better understanding of the link between sequence variation and enzymatic function.

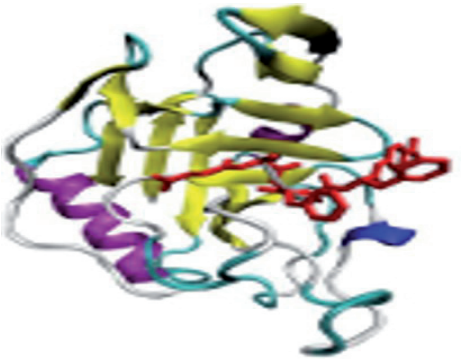

Figure 1: The complex between AvCyPA and synthetic peptide sucAFPFpNA solved at 2.0Å resolution

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Keywords: peptidyl-prolyl isomerase, azotobacter vinelandii, catalysis

MS93.P61

Acta Cryst. (2011) A**67,** C790-C791

Crystal structure analysis of the genetic encoded photosensi-tizer KillerRed

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Several methods have been developed to elucidate the protein functions and interactions in living cells up to the present. Among them, methods for losing protein functions derived meaningful data from biochemical and cellular biological experiments. Chromophore-assisted light inactivation (CALI) is one of promising techniques to inactivate target proteins in living cells [1]. In CALI, chromophore molecules are used as photosensitizer, which produce highly reactive free radicals including reactive oxygen species (ROS) by irradiation of intense light. ROS have short lifetime, therefore the damage radius is limited to approximately 3-4 nm [2]. This indicates that inactivation of the protein(s) is limited in short timescales and very small regions, where the inactivation light is exposed. So far some fluorescent small molecules such as malachite green and fluorescein were used as photosensitizer for CALI applications. These photosensitizers should exogenously introduce into living specimen, which is the bottleneck of developing versatile application of CALI. KillerRed is the first genetically encoded photosensitizer, which has notable phototoxicity. KillerRed is developed by protein engineering from the hydrozoan chromoprotein anm2CP, a homolog of GFP [3].

For the farther development of KillerRed, we determined the crystal structure of KillerRed to understand the structural basis for its phototoxicity. The crystal structure of KillerRed was solved by S-SAD at 2.8Å resolution. The data sets were collected using the loopless data-collection method [4] with chromium Kα X-rays. The overall structure of KillerRed was 11-stranded β-barrel with an internal α-helix passing through inside of the barrel, which is characteristic of the fluorescent protein family. The chromophore formed by the autocatalytic cyclization