peak wavelength of gold, and processed with HKL2000. Phasing, model building and refinement were conducted using PHENIX, REFMAC5, COOT and other programs of CCP4. Electron densities for the catalytic domain of the enzyme were clearly obtained, but those for the linker and PHB-binding domains were not, in spite that there is remaining space for these domains to be packed in the crystal lattice.

The overall structure of the catalytic domain represented an α/β hydrolase fold which is often observed for the large superfamily of esterases including lipases and carboxypeptidases. A cervice was formed on the surface of the protein, at the bottom of which are located catalytic triad residues Ser-166, Asp-241 and His-300. The structure was compared with that of a fungal single-domain depolymerase with circular permuted polypeptide connectivity [1]. These two enzymes differ in the product composition. The main product is dimer of *R*-3-hydroxybutyrate for the bacterial enzyme, whereas it is monomer for the fungal enzyme. Structures of loop regions around the active site were different between the two enzymes. This may differentiate the mode of interactions with monomer units of the substrate polymer for these enzymes, which may explain the difference in the product composition.

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Keywords: biodegradable polyester, esterase, crystallography

MS93.P50

Acta Cryst. (2011) A**67,** C786

Intermediates along the NAD$^+$ cyclisation reaction pathway of ADP-ribosyl cyclase

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Calcium is a ubiquitous, versatile intracellular signal regulating numerous cellular processes [1]. The versatility of Ca^{2+} signalling emerges from the use of an extensive repertoire of signalling components known as a Ca^{2+} signalling toolkit that can be assembled in combinations to create signals with different spatial and temporal profiles. Mobilisation of intracellular Ca^{2+} stores into the cytoplasm is mediated through three structurally divergent messengers, one of them being cyclic ADP-ribose (cADPR) [2].

cADPR acts on the ryanodine receptor to elicit Ca^{2+} release. it is ubiquitous across species and is an endogenous modulator of Ca^{2+}-induced Ca^{2+} release thereby regulating a wide range of physiological processes [3]. cADPR is a cyclic nucleotide converted from NAD$^+$ by multifunctional enzymes of the ADP-ribosyl cyclase family [4]. *Aplysia* ADP-ribosyl cyclase (*Aplysia* cyclase) was the first enzyme identified to catalyse the cyclisation of NAD$^+$ to cADPR, with the release of nicotinamide . *Aplysia* cyclase is a 30kDa protein initially isolated from *Aplysia* ovotestis [5]. It is also found to be present in the neurons of the *Aplysia* buccal ganglion, where production of cADPR can enhance the evoked synaptic transmission [6]. Recently, it has been shown that *Aplysia* cyclase translocates from the cytosol into the nucleus upon depolarisation of *Aplysia* neurons, providing a mechanism for selective and specific activation of the nuclear Ca^{2+} store in neurons offering versatility for the neurons to respond to a wide range of stimuli [7].

Cyclisation of NAD$^+$ into cADPR involves a two step reaction, the elimination of the nicotinamide ring and the cyclisation of the intermediate resulting in the covalent attachment of the adenine ring to the anomeric carbon of the terminal ribose. Cyclisation of NAD$^+$ results in the linkage of the adenine and terminal ribose moieties established via the N1-position of the adenine ring, while cyclisation of NAD$^+$ analogues resulted in the covalent attachment to the terminal ribose via the N7 position of the purine ring of these analogues [8].

In this study, we have determined the structures of wildtype *Aplysia* cyclase complexed with its substrates, NAD$^+$ and NGD$^+$, and its products cADPR and cGDPR. In addition, we were also able to capture the reaction intermediates of the cyclisation reaction either by controlling the soaking time of the substrate or with the use of substrate analogues. Taken together, we are able to obtain snapshots of the cyclisation process of the dinucleotide resulting in either N1 or N7 linkage of the purine ring to the terminal ribose.

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Keywords: enzyme, catalysis, mechanism

MS93.P51

Acta Cryst. (2011) A**67,** C786-C787

Sulfolobus solfataricus adenine phosphoribosyltransferase

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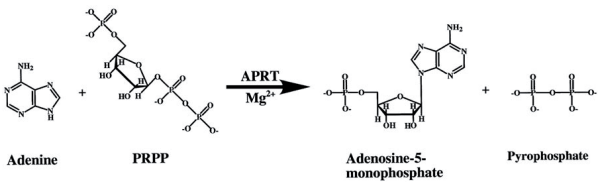
Phosphoribosyltransferases (PRTases) are a group of enzymes that catalyze the formation of nucleotide 5´-monophosphates as essential precursors in the synthesis DNA or RNA. **PRTases** all use a common substrate, 5-phosphoribosyl-α-1-pyrophosphate (PRPP), and in the presence of Mg^{2+} they transfer nucleobases to C1 of the ribose 5´-phosphate moiety of PRPP to form nucleotide 5´-monophosphates under the release of pyrophosphate ($P_2O_7^{4-}$). PRTases are involved in both the *de novo* biosynthesis of nucleotides, e.g. orotate PRTase (OPRTase) and in the salvage pathways, e.g. uracil PRTase (UPRTase), adenine PRTase (APRTase) and hypoxantine-guanine-xanthine PRTases (HGXPRTases; often with mixed specificity). PRTases share a common domain fold (type 1) which defines the specificity for PRPP and a variable domain for recognition of the various nucleobases.



Adenine PRTase structures are known for eukaryotes and bacteria, and details of their active sites have been mapped out. **APRTase** from the **thermophile archaean *Sulfolobus solfataricus*** is an enzyme with unexpected properties: The substrate binding order is reversed with adenine binding first followed by PRPP. It has a double pH optimum and is potently inhibited by AMP and ADP. Phosphate PO_4^{3-} seems to facilitate adenine binding.

Sequentially, ***Ss*APRTase** does not resemble other APRTases but merely HGXPRTases from eukaryotes, bacteria and archaea. The closest sequence homologues in PDB (1nul [1] & 1vdm [2]) used for molecular replacement share about 31 % sequence identity for a 143/210 residue stretch of the sequence. The remaining sequence does

not resemble any known structure. *Ss*APRTase is to our knowledge the first archaean APRTase to be structurally characterized.

We will present dimeric $P6_1$ structures of "apo" *Ss*APRTase (with PO_4^{3-}) together with the complexes *Ss*APRTase:AMP (product) and *Ss*APRTase:ADP (inhibitor) based on ESRF (Grenoble) synchrotron data to about 2.4 Å resolution. The current work concentrates on obtaining substrate complexes of *Ss*APRTase.

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Keywords: nucleotide, metabolism, enzyme

MS93.P52

Acta Cryst. (2011) A**67,** C787

UDP-Sugar derivatives reveal novel dynamic features of blood group glycosyltransferases

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Glycosyltransferases (GTs) are carbohydrate-active enzymes with essential roles in numerous fundamental biological processes such as cellular adhesion, cell signaling, carcinogenesis and cell wall biosynthesis in human pathogens. These enzymes therefore underpin human health and disease and thus inhibitors of GTs are highly sought after as small molecular tools for chemical biology and as lead compounds for drug discovery. Previously, reported ground-state GT donor or acceptor analogues often possess only limited inhibitory potency and the design is complicated by the complex reaction mechanism.

Recently, new and potent GT inhibitors were developed by structural modification of the UDP-galactose donor at position 5 of the uracil base (figure 1) [1]. Initially, we solved the crystal structure of a representative GT with the most potent UDP-sugar derivative bound. The complex structure reveals that the derivative binds in the active site in a similar manner as the natural donor, but almost completely abolishes sugar transfer by locking the target enzyme in a catalytically inactive conformation [2]. This unique mode of inhibition for GTs seems to be generally applicable to other enzymes in this family. Interestingly, new structures of other similar UDP-sugar derived inhibitors bound to the active site including binding of acceptor reveal striking adaptive dynamics of the enzyme and provide an explanation for its ability to remain slightly active. In addition, the new structures provide an explanation for the dependence of inhibitory potency on the subsituent structure. Finally, by modifying a UDP-GalNAc we have for the first time obtained a structure with an intact UDP-GalNAc in the binding site of human blood group GTs. This structure reveals important aspects of the specificity of the enzymes responsible for creating blood type A and B. These results demonstrate the dynamics of the GTs and provide a template for the development of a new class of allosteric GT inhibitors.

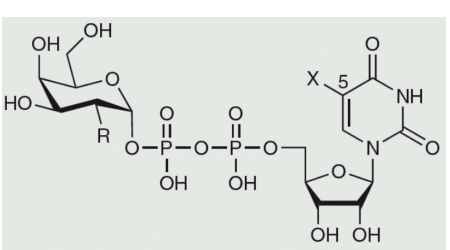

Figure 1

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Keywords: inhibitor, glycosyltranserase, structural dynamics

MS93.P53

Acta Cryst. (2011) A**67,** C787-C788

The 1.3 Å structure of copper nitrite reductase from thermophilic denitrifer

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Denitrification is known as anaerobic respiration in which nitrogenous compounds (NO_2^- or NO_3^-) are used as terminal electron acceptors. Copper-containing nitrite reductase (CuNIR) catalyzes the one electron reduction of nitrite to nitric oxide (NO), which is the key step in the denitrification pathway. This enzyme folds a homo-trimeric structure, having two copper binding sites per a monomeric unit. The type 1 copper (T1Cu) relays an electron from the electron-donor to the type 2 copper site (T2Cu). The T2Cu is located at the interface of adjacent monomers and coordinated by a water molecule and three histidine residues, serving as the active site for nitrite reduction. Though a wide variety of microorganisms is involved in denitrification, little attention has been paid to denitrifying extremophiles. The genome of thermophilic *Geobacillus kaustophilus* HTA426 contains denitrification genes including *nirK* encoding CuNIR (*Gk*NIR). The sequence alignment analysis indicated *Gk*NIR shares low sequence identity with other typical CuNIRs. It is essential for understanding the structure-function relationship of the thermophilic CuNIRs in depth to determine the structure of *Gk*NIR. Recently, we have achieved crystallization of *Gk*NIR and performed the preliminary X-ray diffraction analysis [1]. Here, we describe structure analysis of *Gk*NIR at 1.3 Å resolution.

There are main characteristics of *Gk*NIR in two loops (tower loop [2] and extra loop regions) and the N-terminal region. In *Gk*NIR the seventeen-residue deletion in the tower loop results in the shortest tower loop in all the CuNIR structures already solved. An extra loop composed of eleven residues of the downstream of the tower loop is unique to *Gk*NIR. In all the known CuNIRs, a surface Glu forms a hydrogen bond with His ligand for T1Cu and this hydrogen bond provides direct surface accessibility to the T1Cu. However, in *Gk*NIR His95 for T1Cu forms a hydrogen bond with Asp89 positioned on the random coil containing His95. Moreover, Asp89 forms a hydrogen bond with His21 on the N-terminal α-helix. The N-terminal α-helix is positioned in the vicinity of the tower loop. In the known CuNIRs, the tower loop is located near the T1Cu site and constructs the docking surface for the electron donor, cytochrome c_{551} [3]. Superposition of the crystal structure of *Gk*NIR to the known crystal structure of the electron-transfer complex for CuNIR with its electron donor cytochrome c_{551} suggests that the N-terminal α-helix structure may be involved in the protein-protein interaction during formation of electron transfer complex. These characteristic structures found in 1.3 Å *Gk*NIR structure suggest evolutionary diversity of CuNIR and play a key role in functioning in the particular environment where *G. kaustophilus* inhabits.

Figure. Structure of a monomeric unit of *Gk*NIR. The α-helix surrounded by dashed line is the unique N-terminal α-helix and two copper ions are showed as black spheres.