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

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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.

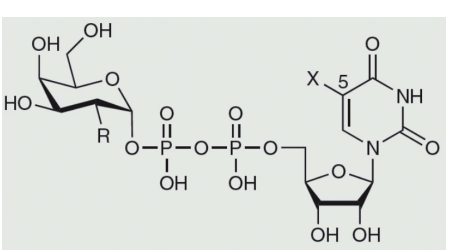

Figure 1

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Keywords: inhibitor, glycosyltranserase, structural dynamics

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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.

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Protonation states of key residues observed during *in situ* ADPRase reaction

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ADP-ribose pyrophosphatase (ADPRase), an enzyme classified into the Nudix family, catalyses the hydrolysis reaction of ADP-Ribose (ADPR) to AMP and ribose-5'-phospholus (R5'P) in the presence of divalent metal cations such as Mn^{2+}, Zn^{2+}, and Mg^{2+}. Crystal structures of ADPRases from four species, human, *M. tuberculosis*, *E. coli*, and *Thermus thermophilus* HB8 (*Tt*) have been studied in parallel as well as ones of point-mutants and their reaction kinetics in solution. However, the reaction mechanism has been under discussion. This is the first presentation referring to protonation states of key residues based on *in situ* observation of *Tt*ADPRase hydrolysis reaction in the crystalline-state at atomic resolutions around 1.0 Å.

We collected X-ray diffraction data from the *apo*-form crystal of *Tt*ADPRase at an ultra-high resolution of 0.91 Å at BL38B1 of SPring-8, Japan. Crystals of ADPR-ADPRase binary complex were prepared by soaking the *apo*-form crystals into ADPR solution over night at room temperature. The ADPR hydrolysis reaction did not progress in the binary complex crystals because of absence of divalent metal cations. The hydrolysis reaction in crystals was triggered by soaking the binary complex crystals into an ADPR solution including Mn^{2+} at room temperature and stopped at different reaction times by flash-cooling with N_2 gas stream at 90K. In total, diffraction data sets were collected from 7 crystals of Mn^{2+}-ADPR-ADPRase ternary complex, and each crystal structure was solved independently at resolutions beyond 1.3 Å. In order to discuss the protonation states for key residues of ADPRase hydrolysis reaction, we compared two C-O bond lengths of Glu and Asp carboxyl side chains with considering their standard deviation after the unrestrained anisotropic refinement with *SHELX-97* as Ahmed discussed previously [1].

The time-resolved crystallographic analysis revealed; (i) the first Mn^{2+}, introduced into the reaction cavity of ADPRase and coordinated by Glu86, changed ADPR conformation to the intermediate form, (ii) the second Mn^{2+} altered Glu82 conformation to the active form, and bound the intermediate ADPR, the active Glu82, and a water molecule, (iii) Glu82 may deprotonate this water molecule to a hydroxide ion in the coordination sphere of the second Mn^{2+}, and (iv) the hydroxide ion may attack to the α-phosphorus atom of ADPR to cleave the pyrophosphate bond. Glu86 and Glu82 are highly-conserved residues among the Nudix family proteins and considered as the most important key residues. Glu86 was in an equilibrium state between protonated and deprotonated ones throughout the reaction, but Glu82 may be deprotonated before the ADPR hydrolysis and be protonated after that. Glu85, the highly-conserved residue as well as Glu82 and Glu86, changed the protonation states also from deprotonated to protonated ones along the reaction time. Glu85 may receive a proton from the nucleophilic water molecule via Glu82 through a hydrogen bond network. In the conference, the results obtained for Glu73, Glu77, and Asp107 will also be presented and discussed.

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Keywords: time resolved crystallography, ultra high resolution, proton transfer

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Versatile loops in mycocypins inhibit three protease families

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Mycocypins, clitocypin and macrocypins are cysteine protease inhibitors isolated from the mushrooms Clitocybe nebularis and Macrolepiota procera. Lack of sequence homology to other families of protease inhibitors suggested that mycocypins inhibit their target cysteine protease by a unique mechanism and that a novel fold may be found. The crystal structures of the complex of clitocypin with the papain-like cysteine protease cathepsin V and of macrocypin and clitocypin alone have revealed yet another motif of binding to papain like-cysteine proteases, which in a yet unrevealed way, occludes the catalytic residue. The binding is associated with a peptide-bond flip of glycine that occurs prior to or concurrently with the inhibitor docking. Mycocypins possess a beta-trefoil fold, the hallmark of Kunitz type inhibitors. It is a tree-like structure with 2 loops in the root region, a stem comprising a six-stranded beta-barrel, and two layers of loops (6+3) in the crown region. The two loops that bind to cysteine cathepsins belong to the lower layer of the crown loops, while a single loop from the crown region can inhibit trypsin or asparaginyl endopeptidase, as demonstrated by site directed mutagenesis. These loops present a versatile surface with the potential to bind to additional classes of proteases. When appropriately engineered, they could provide the basis for possible exploitation in crop protection

Keywords: cathepsins, mycocypins, inhibition

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Identification and structural studies of novel bacterial chitinases

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Bacterium *Clostridium paraputrificum* J4, inhabiting the human gastrointestinal tract, produces a spectrum of extracellular enzymes including several types capable of hydrolysis of chitin at various levels of degradation steps. Endo- and exochitinase as well as β-N-acetylglucosaminidase activities have been detected in the extracellular extracts of this bacterium grown on a chitin-containing medium [1]. Catalytic digestion of chitin supports the anaerobic bacterium by supply of the final product of the degradation cascade – monosaccharides and can also substantially enhance human immunity by attacking pathogenic fungi with chitin-containing cell walls.

In the frame of a complex study we have identified a chitinase B homologous to chitinase B from *C. paraputrificum* M-21 [2], and an endochitinase and an exochitinase with molecular weights 61.2 and 62.7 kDa, respectively, as well as other chitinolytic enzymes produced