residues acting as catalytic acid/base and nucleophile, respectively. In order to investigate the specific glucose binding sites for oligosaccharide substrates, the putative acid/base was mutated and the mutated enzyme was crystallized with substrates. The rice BGlu1 with its acid/base (E176) mutated to glutamine (E176Q) or aspartate (E176D) was co-crystallized with specific substrates, including cellobiose, cellotriose, cellotetraose, cellopentaose and cellohexaose by hanging drop vapor diffusion with microseeding or the mutant crystals were soaked with laminaribiose substrate and 2-deoxy-2-fluoroglucoside inhibitor (G2F). The electron density was clearly visible only for cellotetraose, cellopentaose, laminaribiose and G2F in the active site of the E176Q mutant with diffraction to 1.95, 1.80, 1.35 and 1.75 Å resolution for the mutant enzyme with cellotetraose, cellopentaose, laminaribiose and G2F, respectively. The mutant crystals with substrates were found to belong to space group $P2_12_12_1$, and were isomorphous with wild type BGlu1 crystals.

Keywords: beta-glucosidase, rice, oligosaccharides

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Barley alkenal hydrogenase, a trans-2-nonenal processing enzyme

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Plants degrade linoleic and alpha-linolenic acid to a range of fatty acid derived signaling, regulatory and plant defense related molecules termed oxylipins. The degradation proceeds through the lipoxygenase pathway, a vastly branched pathway generating a multitude of products [1]. One product of this pathway is trans-2-nonenal [2], an α,β-unsaturated aldehyde, which can be further oxidized to the cytotoxic compound 4-hydroxy-2-nonenal [3,4]. Unfortunately, trans-2-nonenal has a very low taste threshold [5], and its presence or release in processed food results in a characteristic and unpleasant cardboard flavor [6]. Barley alkenal hydrogenase isozyme 1 (ALH1) is a reductase catalyzing the hydrogenation of the carbon-carbon double bond in α,β-unsaturated aldehydes and the enzyme has the capacity of reducing trans-2-nonenal to nonanal [7], which has a 150 times higher taste threshold [5]. ALH1 has been found in extracts of germinating barley kernels, and it might be one of the enzymes involved in regulating aldehyde levels and composition. The structure of barley ALH1 has been determined, and the structural analysis and the comparison to the structure of Arabidopsis thaliana AtDBR1 [8], the 11 ALH isozymes identified in A. thaliana and the 4 identified in rice are discussed with respect to substrate specificity.

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Keywords: hydrogenase, off-flavor, trans-2-nonenal

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Crystallization and preliminary X-ray analysis of phosphoribulokinase from *Synechococcus* sp.PCC 7942 cycle

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The Calvin cycle is a series of biochemical reactions that takes place in the stroma of chloroplasts. The one of key thiol enzyme of the Calvin cycle, phosphoribulokinase (PRK) is known to be redox-regulated in higher plants. Under light condition, the enzyme is reduced and activated via redox cascade, while it is oxidized and inactivated under a dark forming disulfide bonds. On the other hand, in cyanobacteria such as Synechococcus sp. PCC 7942 (S.7942), PRK does not seem to be regulated via redox cascade by light irradiation although it conserves the essential cysteine residues for redox regulation. Indeed, it is not inactivated by active oxygen such as H_2O_2, unlike the enzyme in higher plants. The cyanobacteria-specific regulatory mechanism of PRK is still unclear, because only one crystal structure has been avaliable for Rhodobactor. Instead, PRK have recently shown to be regulated by forming a supramolecular complex with the peptide CP12 and GAPDH in higher plants as well as Synechococcus spiecies, which suggests the novel regulatory mechanism in photosynthetic organisms. However, the molecular mechanism is also unclear, since no three-dimensional structures have been available for CP12 and PRK/CP12/GAPDH complex. As the first step, we focused on S.7942 PRK, which is not susceptible to the redox regulation. We have succeeded in crystallization of S.7942 PRK and obtaining X-ray diffraction data with a maximum resolution of 3.5Å. To collect higher resolution data, refinement of crystallization condition of PRK is in progress. Currently, crystallizations of PRK/CP12/GAPDH complexes are also under way.

Keywords: X-ray analysis, kinases, photosynthesis

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Toward a joint X-ray/neutron refinement of the cysteine peptidase papain: The 300K X-ray structure

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Cysteine peptidases (CPs) comprise the ~20 families of peptidases dependent on a cysteine residue at the active site. The best known family of CPs is C1; enzymes of this family appear in all protozoa, plants, and animals. While many principles of CP activity have been thoroughly studied, the molecular basis for CP substrate hydrolysis

remains poorly understood. The enzyme used in this study is papain, a member of the C1 family and the archetypal CP. Papain is obtained from papaya fruits; its utility in tenderizing meat has been known for thousands of years. Papain finds immunological utility in the cleavage of immunoglobulins into Fc and FAB fragments, and medical use in the treatment of stings and chronic wounds. Earlier work reported the crystallization of papain from ethanol/methanol solutions, revealing that papain comprises 2 major structural domains. Papain activation is believed to depend on the formation of a thiolate-imidazolium pair between residues Cys25 and His159 at the cleft between domains. In this study we have obtained crystals from a new aqueous condition containing PEG, buffer, and sodium thiosulfate. In this condition, papain crystallizes in a low-solvent-content unit cell. A 1.60 Å X-ray data set was collected at 300 K in 4 h on a copper-source diffractometer. Results show that a thiosulfate moiety is bound to the active site cysteine, Cys25. Efforts to optimize crystal size for ultra-high resolution X-ray diffraction and neutron diffraction data collection are ongoing. By locating the hydrogen atoms at the active site, we hope to determine the protonation state of His159 and obtain a clearer picture of papain activation and substrate hydrolysis.

Keywords: X-ray and neutron diffraction, proteases proteinases, agricultural natural products

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Crystal structures of *Streptococcus pneumoniae* penicillin-binding proteins acyl-enzyme complexes

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Penicillin-binding proteins (PBPs) are enzymes that catalyze the polymerization and cross-linking of peptidoglycan precursors during bacterial cell wall biosynthesis. β-lactam antibiotics inhibit transpeptidase and DD-carboxypeptidase activities of PBPs by acylating their active-site Ser. To envisage the biding of β-lactam antibiotics to PBPs, we determined a crystal structure of a trypsin-digested form of PBP 2X from *Streptococcus pneumoniae* strain R6 complexed with a cephalosporin antibiotic, cefditoren [1]. We also determined crystal structures of the trypsin-digested form of both PBPs 2X and 1A, each complexed with a carbapenem antibiotic, biapenem or tebipenem [2]. The structures of the acyl-enzyme complexes showed that the cephalosporin C3 side chain and the carbapenem C2 side chains form hydrophobic interactions with Trp374 and Thr526 of PBP 2X and with Trp411 and Thr543 of PBP 1A, however a conformational change of the Trp374 side chain of PBP 2X occurred only upon cefditoren binding. Although the structures studied here were products of inactivation reactions by β -lactams, these hydrophobic interactions are likely to play a role in drug binding upon acylation. There may be similar interactions in PBP 2B from *S. pneumoniae* because a crystal structure of PBP 2B showed that Trp429 and Thr605 occupy positions similar to those of the Trp and Thr residues in the active sites of PBPs 2X and 1A [3].

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Keywords: antibiotics, peptidoglycan biosynthesis, inhibitor interactions

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Crystal structure of a serine protease defines a novel family of secreted bacterial proteases

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Recently, a serine protease secreted by an alkaliphilic and moderately halophilic microbe AL20 belonging to the Nesterenkonia abyssinica family, has been purified and characterized. AL20 protease is optimally active at pH 10, 1.0 M NaCl and 343 K and it shows good stability at 323 K in the presence of EDTA and detergents. From sequence similarity search, AL20 protease has defined a novel protein family of bacterial secreted proteases. Combined with biochemical analyses and high resolution crystal structure determination, we have unambiguously characterized the AL20 protease and its sequence related family as a trypsin-like serine protease.

Keywords: Al20 protease, bacterial secreted proteases, trypsin-like

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Crystal structures of *Escherichia coli* γ -glutamyltrans -peptidase in complex with glutamine antagonists

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γ-Glutamyltranspeptidase (GGT) is a heterodimeric enzyme that catalyzes the transfer of the γ-glutamyl group in γ-glutamyl compounds such as glutathione and its S-conjugates either to water or to other amino acids and peptides. GGT is involved in a number of biological events such as drug resistance and metastasis of cancer cells by detoxification of xenobiotics. Azaserine and acivicin are classical and irreversible inhibitors of GGT, but their binding sites and the inhibition mechanisms remain to be defined. We have determined the crystal structures of GGT from *Escherichia coli* in complex with glutamine antagonists, azaserine and acivicin, at 1.65 Å resolution. Both inhibitors are bound to GGT at its substrate-binding pocket in a similar manner as observed previously with the γ-glutamyl-enzyme intermediate: they form a covalent bond with