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