day. The ADPR complex crystals were then transferred into Zn ion solution for starting hydrolysis. After eight kinds of reaction times from 3 min to 1 hr, each crystal was cryo-trapped in N_2 gas stream at 100 K and diffraction data was collected independently over 1.6 Å resolution. The crystals were all highly isomorphous to the initial ADPR complex one. Nine structures were refined by REFMAC in the CCP4 program suits to the final *R*-values less than 20 %. Results clearly show that (i) ADPR in the reaction cavity changes its conformation to a reaction intermediate within 10-15 min, (ii) a water molecule coordinated to the Zn ion at the M-I site is activated to hydroxide (OH^-) by Glu82, and (iii) the OH^- anion attacks the α -phosphorus atom of ADPR in the inline geometry to the removing oxygen between α-P and β-P. The real pathway revealed is different from both of the mechanisms previously proposed. It is indicated once again that reaction mechanisms based on 3D crystallography should be proofed by the time-resolved analysis.

Keywords: time-resolved crystallography, ADP-ribose pyrophosphatase, Zn ion soaking

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Ligand-induced conformational change of D-alanine:D-alanine ligase from *Thermus thermophilus* HB8

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D-Ala:D-Ala ligase (Ddl) catalyzes the synthesis of the dipeptide D-Ala:D-Ala from two D-Ala molecules. The D-Ala:D-Ala is incorporated into a peptidoglycan precursor. After the synthesis of a peptidoglycan strand is completed, the penultimate D-Ala of the D-Ala:D-Ala terminus on one strand is cross-linked to an amine group on an adjacent strand to produce the bacterial cell wall. Ddl is thus an essential enzyme for cell wall biosynthesis and an important target for the development of new antibiotics. Here, we report the ligand-induced conformational change playing a critical role in ligation catalysis, based on the three-dimensional structure of Ddl from Thermus thermophilus HB8. The structure of free Dsl has been determined at 2.3Å resolution by means of a multiple wavelength anomalous diffraction (MAD) phasing method, and those of the complexes with D-Ala and/or ADP have been determined by molecular replacement at 1.9 ~ 2.2Å resolution using the coordinates of the free Ddl. The tertiary structure of Ddl was divided into three $\alpha + \beta$ domains (N-terminal, center, and C-terminal domains), and the ATP-binding site was found between the β-sheets of the center and C-terminal domains. Structural comparison of free Ddl with the D-Ala complex revealed that no significant change in the overall conformation occurs on binding of the D-Ala. On the other hand, the structure of the complex with ADP showed a marked conformational change around the loop consisting of residues 217 to 235, which is involved in the active site formation in Ddl from E.coli. In this paper, we will discuss the reaction mechanism of Ddl in detail using several X-ray structures for complexes with ligands and the results of kinetic analysis.

Keywords: crystal structures, peptidoglycan biosynthesis, dipeptides

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Structure of wild type Plk1 kinase domain in complex with a selective DARPin

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As a key regulator of mitosis, the Ser/Thr protein Polo-like kinase-1 (Plk-1) is a well validated drug target in cancer therapy. In order to enable structure-guided drug design, we set out to determine the crystal structure of the kinase domain of Plk-1. Using a multi-parallel cloning and expression approach, we identified a set of length variants which could be expressed in large amounts from insect cells and which could be purified to high purity. However, all attempts to crystallize these constructs failed. Crystals were ultimately obtained by generating designed ankyrin repeat proteins (DARPins) selective for Plk-1 and by using them for co-crystallization. Here we present the first crystal structure of the kinase domain of wild-type apo Plk-1, in complex with DARPin 3H10, underlining the power of selective DARpins as crystallization tools. The structure was refined to 2.3 Å; resolution and shows the active conformation of Plk-1. It broadens the basis for modeling and cocrystallization studies for drug design. The binding epitope of 3H10 is rich in arginine, glutamine and lysine residues, suggesting that the DARPin enabled crystallization by masking a surface patch which is unfavorable for crystal contact formation. Based on the packing observed in the crystal, a truncated DARPin variant was designed which indeed showed improved binding characteristics.

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Keywords: enzymatic proteins, drug design, crystallization process of protein molecules

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Crystallization of rice BGlu1 β-glucosidase E176Q mutant with oligosaccharide substrates

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Rice BGlu1 beta-glucosidase hydrolyzes β-1,3- and β-1,4- linked oligosaccharides and belongs to glycosyl hydrolase family 1. The catalytic mechanisms of family members involve two glutamate

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