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