is a key player in the formation of large complexes on gene promoters leading to chromatin remodelling and gene activation. CARM1 has now been shown to methylate a large variety of proteins which are all vital to gene expression. CARM1 contains 608 amino acids in human and its architecture has been schematically divided into three domains. CARM1 is built around a catalytic core domain that is well conserved in sequence (and therefore in structure) among all PRMTs members. CARM1 possesses two unique additional domains attached, respectively, at the NH_2-terminal and at the COOH-terminal end of the PRMT active site. Both NH_2-terminal domain and COOH-terminal domain have been shown to be required for the coactivator function of human CARM1. We have solved six crystal structures corresponding to three isolated modules of CARM1. Five crystal structures of the CARM1 catalytic module, two free, two cofactor and one inhibitor bound forms have revealed large structural modifications and shown that the NH_2-terminal and the COOH-terminal end of CARM1 catalytic module contain molecular switches that may inspire how CARM1 regulates its biological activities by protein-protein interactions. Full detailed analysis of the structures will be presented.

Keywords: epigenetic, protein ariginine methyltransferase, CARM1

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Crystal structure of Histo-aspartic protease from *Plasmodium falciparum*

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Histo-aspartic protease (HAP) from *Plasmodium falciparum* is a promising new target for the development of anti-malarial drugs. The sequence of HAP reveals an overall similarity to aspartic proteases, but crucial replacement by histidine of one of the two catalytic aspartates, Asp32, combined with several more changes of the catalytically important residues in the active site area, indicated a possible novel mechanism of action. The structures of the recombinant HAP, as apoenzyme and a complex with pepstatin A, were solved at 2.5 and 3.3 Å resolution, respectively. In the apoenzyme crystals HAP forms a unique dimer, which has not been observed in any known aspartic proteases. The interactions between the monomers in a very tight dimer of HAP affect the conformations of two flexible loops, the functionally important “flap” (residues 77-88) and its structural equivalent in the C-terminal domain (237-249), as well as the orientation of the helix 225-235. The flap in the apoenzyme was found in an open conformation. Unexpectedly, the active site contains a zinc ion tightly bound to His38 and Asp218 from one monomer, and to Glu282 from the other monomer, with the coordination resembling its counterparts in metalloproteases. Although the mode of binding of pepstatin A in the active site of HAP is different than in other pepsin-like aspartic proteases, the presence of the inhibitor questions the previously proposed hypothesis that HAP is a serine protease. The flap is closed in the structure of the complex and Lys82, present at the tip of the flap, interacts with the inhibitor. The novel features of the active site of HAP should allow designing specific inhibitors that could be developed into antimalarial drugs.

Keywords: aspartic proteases, malaria, enzyme mechanism

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Structural and mutational studies of anthocyanin malonyltransferases

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The BAHD family is a class of acyl-CoA-dependent acyltransferases that are involved in plant secondary metabolism and show a diverse range of specificities for acyl acceptors. Anthocyanin acyltransferases make up an important class of the BAHD family and catalyze the acylation of anthocyanins that are responsible for most of the red-to-blue colors of flowers. Here, we describe crystallographic and mutational studies of three similar anthocyanin malonyltransferases from red chrysanthemum petals: anthocyanidin 3-O-glucoside-6’’-O-malonyltransferase (Dm3MaT1), anthocyanidin 3-O-glucoside-3’’, 6’’-O-dimalonyltransferase (Dm3MaT2), and a homolog (Dm3MaT3). Mutational analyses revealed that seven amino acid residues in the N- and C-terminal regions are important for the differential acyl-acceptor specificity between Dm3MaT1 and Dm3MaT2. Crystallographic studies of Dm3MaT3 provided the first structure of a BAHD member, complexed with acyl-CoA, showing the detailed interactions between the enzyme and acyl-CoA molecules. The structure, combined with the results of mutational analyses, allowed us to identify the acyl-acceptor binding site of anthocyanin malonyltransferases, which is structurally different from the corresponding portion of vinorine synthase, another BAHD member, thus permitting the diversity of the acyl-acceptor specificity of BAHD family to be understood.

Keywords: BAHD family, acyltransferase, crystal structure

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Reaction pathway of ADP-ribose pyrophosphatase, revealed by time-resolved X-ray crystallography

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ADP-ribose pyrophosphatase (ADPRase) is a member of nudix family proteins that metabolize many kinds of nucleotide diphosphates. ADPRase catalyzes the divalent metal ion-dependent hydrolysis of ADP-ribose (ADPR) to AMP and ribose 5’ -phosphate. Crystal structures of ADPRases from three organisms including *Thermus thermophilus* (*Tt*) HB8 have already been reported. The structures are very similar with each other, but two kinds of different reaction mechanisms are proposed based on 3D structures of ternary complexes with metal ions and ADPR or ADPR analogue. In order to reveal the real mechanism, we traced the reaction pathway of *Tt*-ADPRase by time-resolved X-ray crystallography. ADPRase crystals were soaked first into ADPR solution at pH 4.6 for one

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