TTHA0252 is a conserved hypothetical protein that belongs to the β-CASP family, within the metallo-β-lactamase superfamily. Archetypal metallo β-lactamases degrade β-lactam antibiotics, whereas the β-CASP family proteins degrade nucleic acids. Recently, it was reported that two β-CASP family proteins from *Bacillus subtilis* are functional homologues of *E. coli* RNase E. RNase E is a key enzyme for mRNA degradation in *E. coli* but no homolog is found in most bacteria. To reveal the biological role of this novel RNase family with a β-CASP fold in RNA metabolism, we currently address the structure and function of TTHA0252 from *Thermus thermophilus* HB8. We have determined the crystal structure of TTHA0252, which represents the first report of the tertiary structure of a β-CASP family protein (1). TTHA0252 comprises two separate domains: a metallo-β-lactamase domain and a clamp domain. The active site of the enzyme is located in a cleft between the two domains. The width of the cleft (10 Å) suggests that TTHA0252 can recognize a single-stranded region, but not a double-stranded region (diameter of 20 Å), of RNA as substrate. The active site of TTHA0252 comprises two zinc ions and seven conserved residues which are similar to those of other β-lactamases. A sulfate ion was also observed near the active site. Since the position of the sulfate ion, appears to mimic the 5'-terminal phosphate group of the substrate, we predicted TTHA0252 to have 5' to 3' exonuclease activity. TTHA0252 actually showed single-strand-specific 5' to 3' exonuclease activity to various oligonucleotides. The effects of mutations of active site residues are also discussed.

(1) Ishikawa, H., et al. (2006) *J. Biochem.* **140**(4), 535-542

Keywords: ribonuclease, protein structure, protein X-ray crystallography

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Structural basis for different substrate specificities of two ADP-ribose pyrophosphatase

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ADP-ribose (ADPR) is one of the main substrates of Nudix proteins. Among the eight Nudix proteins of *Thermus thermophilus* HB8, we previously determined the crystal structure of Ndx4, an ADPR pyrophosphatase (ADPRase)[1)]. In this study we show that Ndx2 of *T. thermophilus* also preferentially hydrolyzes ADPR and FAD, and have determined its crystal structure. We have determined the structures of Ndx2 alone, and in complex with Mg^{2+}, with Mg^{2+} and AMP, and with Mg^{2+} and a nonhydrolyzable ADPR analogue[2)]. Although Ndx2 recognizes the AMP moiety in a manner similar to other ADPRases, it recognizes the terminal ribose in a distinct manner. The residues responsible for recognition of the substrate in Ndx2 are not conserved among ADPRases. This may reflect the diversity in substrate specificity among ADPRases. Based on these results, we propose the classification of ADPRases into two types: ADPRase-I enzymes, which exhibit high specificity for ADPR; and ADPRase-II enzymes, which exhibit low specificity for ADPR. In the active site of the ternary complexes, three Mg^{2+} ions are coordinated to the side chains of conserved glutamate residues and water molecules. Substitution of Glu90 and Glu94 with glutamine suggests that these residues are essential for catalysis. These results suggest that ADPRase-I and ADPRase-II enzymes have nearly identical catalytic mechanisms[3)] but different mechanisms of substrate recognition.

[1)] Yoshiba, S. *et al.* (2004) *J. Biol. Chem.* **279**(35), 37163-37174
[2)] Wakamatsu, T. *et al.* (2008) *J. Bacteriol.* **190**(3), 1108-1117
[3)] Ooga, T. *et al.* (2005) *Biochemistry* **44**(26), 9320-9329

Keywords: hydrolase, proteins structure, protein X-ray crystallography

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Dimerization is important for the GTPase activity of chloroplast translocon components

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Arabidopsis Toc33 (atToc33) is a GTPase and a member of the Toc (translocon at the outer-envelope membrane of chloroplasts) complex that associates with precursor proteins during protein import into chloroplasts. By inference from the crystal structure of psToc34, a homologue in pea, the arginine at residue 130 (Arg130) has been implicated in formation of the atToc33 dimer and inter-molecular GTPase activation within the dimer. Here we report the crystal structure at 3.2 Å resolution of an atToc33 mutant, atToc33(R130A), in which Arg130 was mutated to alanine. Both in solution and in crystals, atToc33(R130A) was present in its monomeric form. In contrast, both wild-type atToc33 and another pea Toc GTPase homologue, pea Toc159 (psToc159), were able to form dimers in solution. Dimeric atToc33 and psToc159 had significantly higher GTPase activity than monomeric atToc33, psToc159 and atToc33(R130A). Molecular modeling using the structures of psToc34 and atToc33(R130A) suggests that, in an architectural dimer of atToc33, Arg130 from one monomer interacts with the ・-phosphate of GDP and several other amino acids of the other monomer. These results indicate that Arg130 is critical for dimer formation, which is itself important for GTPase activity. Activation of GTPase activity by dimer formation is likely to be a critical regulatory step in protein import into chloroplasts.

Keywords: atToc33, GTPase activity, psToc159

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Crystal structure of RuBisCO-like protein from *Bacillus subtilis*

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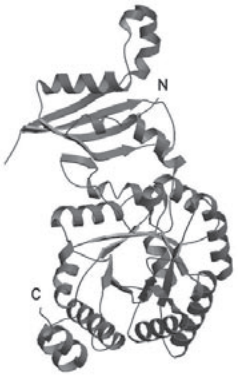
Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes the addition of gaseous CO_2 into ribulose 1,5-bisphosphate (RuBP). RuBisCOs are classified into four forms based on sequence similarity: forms I, II and III are bona fide RuBisCOs, and form IV is called the RuBisCO-like protein (RLP). RLP lacks several residues involved in the substrate binding, and it does not catalyze RuBP-dependent CO_2 fixation in vitro. We have demonstrated that RLP from *Bacillus subtilis* (BsRLP) catalyzed the 2,3-diketo-5-methylthiopentyl-1-phosphate enolase reaction as the fourth step in the methionine salvage pathway. The crystal structure of BsRLP in the apo form was determined at 2.3 Å resolution. The structural comparison with other homologous proteins reveals that an induced fit of Lys150 to the active site plays a crucial role in the substrate recognition. Two loops of RLP, which corresponds to the catalytic flexible loops (60's loop and Loop6) in RuBisCO, are structurally ordered in the apo (open) and substrate binding (closed) conformations, respectively. Thus, the structural analysis sheds light on its functional evolution to RuBisCO.



Keywords: crystal structure, enzyme evolution, enzyme function

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Molecular mechanism of the redox-dependent interaction between ferredoxin reductase and ferredoxin

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Redox-dependent affinity regulation is critical to fast and efficient electron transfer (ET) between ET proteins. The molecular mechanism of the affinity regulation, however, remains elusive due to the lack of tertiary structures of the ET proteins in every redox state relevant to the ET reaction. BphA4 and BphA3 are, respectively, an FAD-containing NADH-dependent ferredoxin reductase and a Rieske-type [2Fe-2S] ferredoxin from a biphenyl dioxygenase BphA derived from *Acidovorax* sp. strain KKS102. Our biochemical study showed that the reduction of the FAD in BphA4 increases the affinity between BphA3 and BphA4 approximately 20-fold. In order to reveal the molecular mechanism of this redox-dependent affinity regulation, we determined the crystal structure of BphA4 in oxidized, hydroquinone, semiquinone, and reoxidized forms; the crystal structure of BphA3 in oxidized and reduced forms; and the crystal structure of the ET complex of BphA3 and BphA4 (Senda, M *et al.* J. Mol. Biol. (2007)). A comparative analysis of the seven crystal structures obtained revealed that the conformational changes of BphA4 upon reduction of FAD are required for the formation of the high-affinity BphA3-binding site in BphA4. The complex formation between BphA3 and BphA4 induces a conformational change of His66(A3), forming an ET pathway composed of Trp320(A4) and His66(A3). After accepting an electron, BphA3 is likely to undergo a flip of the peptide bond between Gly46 and Glu47. This peptide flip seems to break the electrostatic interaction between Glu47(A3) and Arg327(A4), inducing the dissociation of BphA3 from BphA4. The interplay of ET and induced conformational changes seems to be critical to the sequential reaction of the ET from NADH to BphA3.

Keywords: electron transfer mechanism, macromolecular interactions, flavoproteins

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Crystal structures of GAR transformylase 1 (PurN) from *A. aeolicus*, *S. toebii* and *G. kaustophilus*

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Glycinamide ribonucleotide (GAR) transformylase 1, PurN, catalyzes the 3rd reaction in the purine nucleotide biosynthetic pathway; a formyl transfer from 10-formyl tetrahydrofolate (10fTHF) to β-GAR. This enzyme is one of the targets of anti-cancer drug and it is important to elucidate the structural insight of the reaction catalyzed by PurN. The crystal structures of PurN from *E. coli* and human have been determined and are subjected to the structure-based drug design. Here, we determined crystal structures of PurN from three thermophilic eubacteria, *Aquifex aeolicus* VF5, *Symbiobacterium toebii* and *Geobacillus kaustophilus* HTA426. The space group, maximum resolution and *R*-value (free *R*-value) for each structure are as follows: *A. aeolicus* (2YWR), $P4_12_12$, 1.77 Å, 19.2% (22.8%), *S. toebii* (2YZP), $P3_121$, 2.07 Å, 20.8% (24.6%), *G. kaustophilus*, $C222_1$, 1.85 Å, 20.0% (22.6%). Multiple alignments of the five sequences corresponding to *A. aeolicus*, *S. toebii*, *G. kaustophilus*, *E. coli* and human shows that the three proteins studied here have an extra residue at the N-terminus. Overall structure of the three PurN are similar to each other and the conformation of the three conserved residues in the catalytic center, Asn107, His109, Asp145 for the three protein are well overlapped to Asn106, His108, Asp144 for *E. coli* (1C2T, 1C3E, 1CDE) and human (1MEO, 1NJS, 1RBM, 1RBQ, 1RBY, 1RBZ, 1RC0, 1RC1, 1ZLX, 1ZLY). Detail of structural analysis as well as the molecular dynamics analysis will be presented.

Keywords: nucleoside metabolism, transformylase, thermophilic proteins

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Structural basis for natural lactonase and promiscuous phosphotriesterase activities

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