TTHA0252 is a conserved hypothetical protein that belongs to the \( \beta\)-CASP family, within the metallo-\( \beta\)-lactamase superfamily. Archetypal metallo \( \beta\)-lactamases degrade \( \beta\)-lactam antibiotics, whereas the \( \beta\)-CASP family proteins degrade nucleic acids. Recently, it was reported that two \( \beta\)-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 \( \beta\)-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 \( \beta\)-CASP family protein (1). TTHA0252 comprises two separate domains: a metallo-\( \beta\)-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 \( \beta\)-lactamases. A sulfite ion was also observed near the active site. Since the position of the sulfite 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 sites residues are also discussed.

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. 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 but different mechanisms of substrate recognition.

Keywords: ribonuclease, protein 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 γ-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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