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古細菌 tRNA メチル基転移酵素 aTrm56 の構造機能解析

Crystal structure and mutational study of archaeal tRNA-methylase for position 56 (aTrm56) from *Pyrococcus horikoshii*

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L-shaped tRNA tertially structure is stabilized by numerous posttranscriptional modifications. 2'-*O*-methylation of the cytidine residue at position 56 is an archaea-specific S-adenosyl-L-methionine-dependent modification, and considered to stabilize the tRNA structure. The enzymatic activity of the 2'-*O*-methylcytidine (Cm) formation at position 56 was first detected using the cell-free extract from *Pyrococcus furiosus* on yeast tRNA-Asp, but not on the T-stem/loop nor on yeast tRNA-Asp lacking D-stem/loop. Recently, it was discovered that the Cm formation is catalyzed by aTrm56 in most archaeal species and by a C/D sRNP in *Pyrobaculum aerophilum*. aTrm56 is the most distant member in the SpoU family, and conserves the signature motifs of the SpoU family only partly. The biochemical assay using the recombinant *Pyrococcus abyssi* aTrm56 protein revealed that tRNAs with low Tm values (55-60 °C) are efficiently and completely modified, whereas the tRNA with higher Tm (> 60 °C; transcribed *P. abyssi* tRNALeu(CAA)) is slowly and incompletely modified, indicating a partial melting of tRNA is required for the aTrm56 catalysis.

To gain a structural basis to understand the enzymatic activity of aTrm56, we determined the crystal structure of aTrm56 from *Pyrococcus horikoshii* complexed with S-adenosyl-L-methionine (AdoMet) at 2.48 Angstroms resolution. aTrm56 consists of the SPOUT methyltransferase domain, which contains a deep trefoil knot, and a unique C-terminal β-hairpin, and forms a dimer. AdoMet is bound in the deep trefoil knot in a similar way as other SpoU members. The C-terminal β-hairpin takes part in the dimerization, and is located near the active site of the adjacent subunit. Based on the structure, we carried out mutational studies to evaluate the roles of the conserved residues in the active site. We found an essential residue in a novel position. Moreover, biochemical assays in the presence or the absence of archaeosine tRNA-guanine transglycosylase revealed whether a Trm56 prefers the L-shaped tRNA or the lambda form as its substrate.

Reference, Kuratani M. *et al.* (2008) Crystal structure and mutational study of a unique SpoU family archaeal methylase that forms 2'-O-methylcytidine at position 56 of tRNA. *JMB*. 375:1064-1075.