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The mechanism for biogenesis of the modified nucleoside found in archaeal tRNA^{Ile}

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Research aims

In most archaea, the wobble cytidine (C34) of the AUA-codon specific isoleucine tRNA (tRNA^{Ile2}) is modified to 2-agmatinylcytidine (agm^{2}C), an agmatine-conjugated cytidine derivative[1] (Fig. 1a). The precursor form of tRNA^{Ile2} with unmodified C34 deciphers the methionine AUG codon, since tRNA^{Ile2} bears the CAU anticodon sequence that also appears in tRNA^{Met}. In contrast, tRNA^{Ile2} with the agm^{2}C modification precisely deciphers the isoleucine AUA codon, instead of AUG. Thus, the agm^{2}C modification switches the codon specificity from AUG to AUA. The formation of agm^{2}C is catalyzed by tRNA^{Ile}-agm^{2}C synthetase (TiaS), using ATP and agmatine as substrates[1]. Although TiaS catalyzes agm^{2}C formation in an ATP-dependent manner, a clear ATP-binding motif is not apparent in the primary structure. Therefore, the mechanism for agm^{2}C formation has remained obscure. In this study, to elucidate the mechanisms of the ATP-dependent agm^{2}C formation by TiaS, we determined the crystal structures of the *Archaeoglobus fulgidus* TiaS–tRNA^{Ile2} complex[2,3].

Methods

The recombinant *A. fulgidus* TiaS was produced in *E. coli* strain C41(DE3), and then purified. *A. fulgidus* tRNA^{Ile2} was transcribed *in vitro* using T7 RNA polymerase, and then purified. We prepared co-crystals of the TiaS-tRNA^{Ile2}-ATP ternary complex and TiaS-tRNA^{Ile2}-AMPcPP-agmatine quaternary complex. The X-ray diffraction data were collected at the beam-line BL-17A of Photon Factory. We first determined the TiaS-tRNA^{Ile2}-ATP ternary complex structure by the single-wavelength anomalous dispersion method, at a resolution of 2.9 Å. Subsequently, the structure of the TiaS-tRNA^{Ile2}-AMPcPP-agmatine quaternary complex was determined at 3.1 Å resolution, by molecular replacement method.

Results

The TiaS-tRNA^{Ile2}-ATP ternary complex structure revealed that TiaS is folded into four structural domains, comprising the TCK domain, FL domain, OB domain, and ZR domain (Fig. 2). ATP is bound to the TCK domain. The biochemical study showed that TiaS first hydrolyzes the ATP α-β phosphate bond, and then phosphorylates C34 with the γ-phosphate[4] (Fig. 1b). The invariant Asp8, Asp9, and Asp11 residues are located in the vicinity of the triphosphate. The D8A, D9A, and D11A mutations abolished the ATP-hydrolysis and agmatine-incorporating activities. Since ATP hydrolysis is requisite for subsequent C34 phosphorylation by TiaS, these results strongly suggest that the

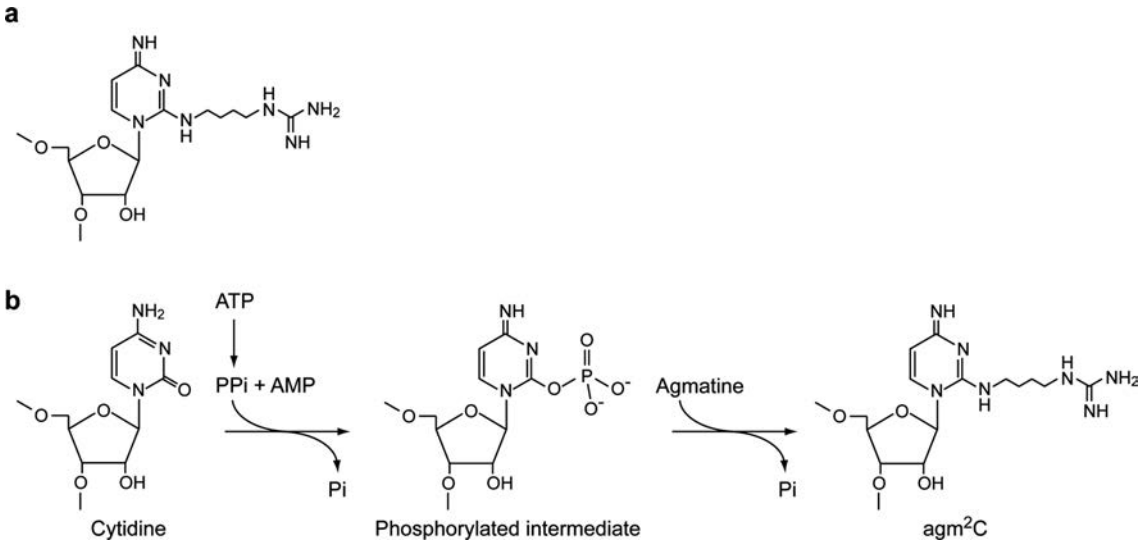

Figure 1. Chemical structure and reaction scheme for the formation of 2-agmatinylcytidine (agm^{2}C)

TCK domain functions as the catalytic module to phosphorylate C34.

In addition to the ternary complex, we also determined the TiaS-tRNA^{Ile2}-AMPcPP-agmatine quaternary complex structure. A comparison between the ternary and quaternary complexes revealed structural differences, especially in the tRNA anticodon region (Fig. 3). In the ternary complex, C34 is located at the pocket in the FL domain, in which the ATP γ-phosphate is too far away from C34 (10 Å) for the phosphorylation reaction to occur. In contrast, C34 in the quaternary complex is kept out of this pocket (because of the presence of agmatine in this pocket), and is situated near the γ-phosphate of AMPcPP (3.1 Å). Therefore, it is suggested that the quaternary complex adopts a productive conformation, relative to that of the ternary complex, with respect to the phosphorylation reaction.

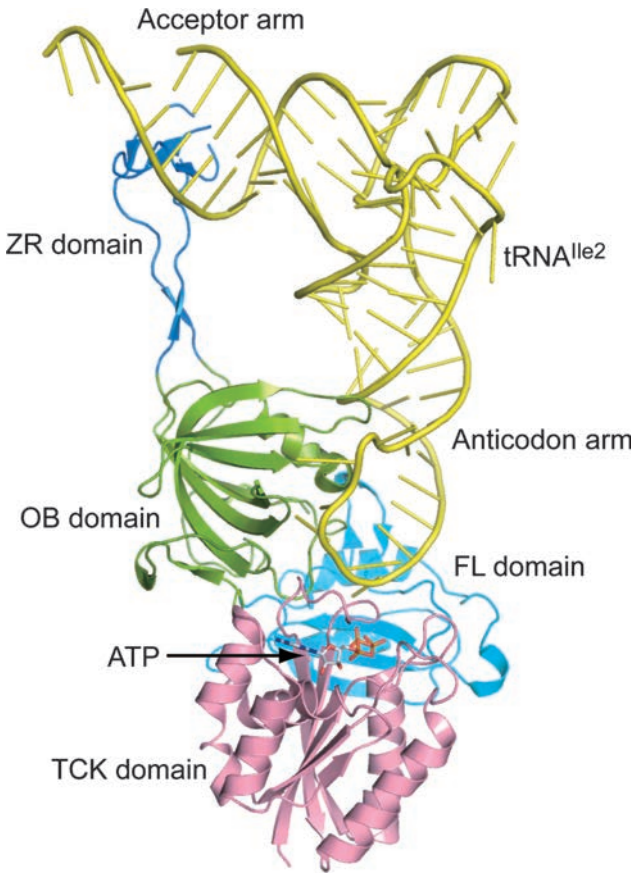

Figure 2. Crystal structure of the TiaS-tRNA^{Ile2}-ATP ternary complex

Conclusion

Based on (i) a structural comparison between the ternary and quaternary complexes, (ii) the estimated cellular concentrations of tRNA^{Ile2} and agmatine, and (iii) the TiaS K_m values for agmatine and tRNA^{Ile2}, it is suggested that TiaS have already bound agmatine prior to the interaction with tRNA^{Ile2}, and after tRNA binding, C34 could be directly recognized near the ATP γ-phosphate by an induced-fit mechanism. In contrast, if the cellular concentration of agmatine is insufficient for the modification reaction, then C34 could be trapped in the pocket of the FL domain, far away from the ATP-binding site. Since TiaS has an intrinsic basal ATP-hydrolysis activity[4], regardless of the presence or absence of the tRNA, segregation of C34 from the ATP γ-phosphate is requisite to prevent the accumulation of a phosphorylated C34 intermediate (pC34). In this case, the subsequent agmatine supply to the enzyme causes the structural transition, especially of the tRNA anticodon region, to relocate C34 adjacent to the ATP γ-phosphate for the phosphorylation. In either case, the presence of agmatine in TiaS is important for properly locating C34 to the active site, for activation by phosphorylation. Once C34 is phosphorylated, the anticodon region might become restructured to place pC34 in the vicinity of agmatine, so the agmatine amino group can perform the nucleophilic attack on the C2 carbon of C34, to form agm^{2}C. The present study suggests that the ternary and quaternary complex structures represent the C34-trapping and pre-phosphorylation states, respectively.

References

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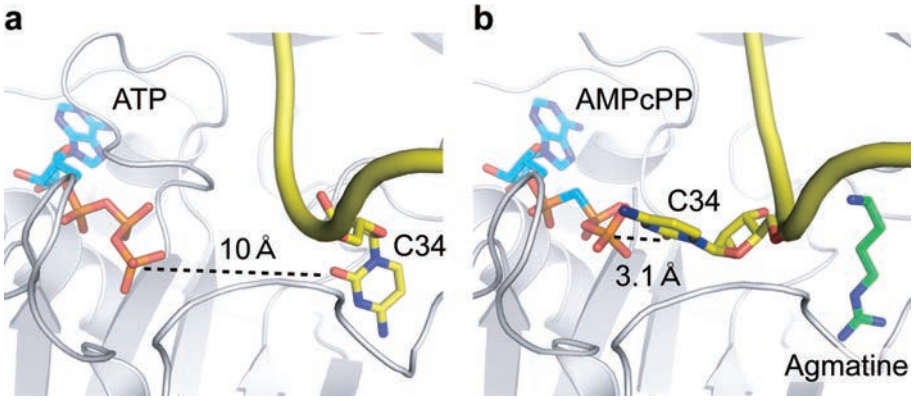

Figure 3. Structural comparison of the active sites in the ternary (a) and quaternary (b) complexes