Molecular recognition of proline tRNA by prolyl-tRNA synthetase from hyperthermophilic archaean, *Aeropyrum pernix* K1

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**ABSTRACT**

To investigate the recognition mechanism of tRNAPro by prolyl-tRNA synthetase from hyperthermophilic archaean, *Aeropyrum pernix* K1, various tRNAPro transcripts were prepared by *in vitro* transcription system. These transcripts were aminoacylated with proline by overexpressed *A. pernix* prolyl-tRNA synthetase. From prolylation experiments, recognition elements of *A. pernix* tRNAPro were determined to be G35 and G36 of anticodon, discriminator base A73, and G1-C72 base pair at acceptor stem end.

**INTRODUCTION**

Correct recognition of tRNA by aminoacyl-tRNA synthetase (ARS) is essential to maintain accurate translation. To distinguish the cognate tRNA from a pool of various tRNAs consisting of a similar L-shaped tertiary structure, it was found that the ARS recognized a relatively small number of nucleotides of tRNA including anticodon bases and the discriminator base together with the base pair(s) of the acceptor stem (1). One ARS at least exist toward each of twenty amino acids except for particular organisms that possess a dual amino acid-specific ARS or the system of tRNA-dependent amino acid synthesis (2-4). Although the tRNA identity elements have been widely investigated in eubacterial system including *Escherichia coli*, little is known about tRNA recognition sites for ARS from archaea, which is classified as a third kingdom (5-7).

Prolyl-tRNA synthetase (ProRS) belong to Class II ARS that their catalytic domain consist of antiparallel β sheets with conserved three domains. From sequence alignment based on the crystal structure analysis of *Thermus thermophilus* ProRS, this enzyme is divided into two groups, prokaryote-like and eukaryote/archaea-like type (8). Moreover, archaean *Methanococcus jannaschii* ProRS is two amino acid-specific active enzyme, ProCysRS that can synthesize both prolyl-tRNAPro and cysteinyl-tRNAcys (3). It was reported that *E. coli* tRNAPro identity elements were G35 and G36 of the anticodon, discriminator base A73, and G72 in the acceptor stem (9, 10). To investigate the recognition sites of tRNAPro for ProRS from an aerobic and hyperthermophilic crenarchaeon, *Aeropyrum pernix* K1 (11,12), we examined *in vitro* prolylation experiments by using various *A. pernix* tRNAPro mutants.

**MATERIALS AND METHODS**

*A. pernix* ProRS was prepared by cloning and overexpression in *E. coli*. ProRS gene was amplified by PCR from *A. pernix* genomic DNA and the PCR product was cloned into plasmid pET30 and pET28. These vectors were transformed into *E. coli* strain BL21 (DE3). The recombinant ProRS was purified by Ni2+-NTA affinity chromatography. Substrate *A. pernix* tRNAPro variants were prepared by *in vitro* transcription with T7 RNA polymerase. For a detection of aminoacylation reaction, [14C]-proline was used to prolylation assay.

**RESULTS AND DISCUSSION**

In this *in vitro* mutation study, it is focused on the anticodon and the acceptor region as possible major recognition elements of tRNAPro by *A. pernix* ProRS because discriminator base A73 is conserved within eubacterial and archaeal tRNAPro and a G1-C72 base pair of acceptor stem end is common to all tRNAPro's in eukaryote and archaea. Substitutions of the second
position G35 and the third position G36 of the anticodon to the other nucleotides led to a remarkable decrease of proline charging activity by expressed *A. pernix* ProRS. Mutation at the discriminator base A73 caused a great reduction in activity. Major identity elements of *E. coli* tRNA\(^{\text{Pro}}\) were determined to be G35 and G36 at the anticodon loop, discriminator base A73, and G72 of a C1-G72 base pair in the acceptor stem end (9,10). So we examined G1-C72 base pair at acceptor stem end as a possible recognition site by *A. pernix* ProRS. Mutations from G1-C72 base pair to another sets of base pairs led to the almost complete loss of prolylation activity. Almost all tRNAs have this G1-C72 base pair at the acceptor stem end. In case of *E. coli* system, only tRNA\(^{\text{Thr}}\) has a G1-C72 base pair as the recognition site for threonyl-tRNA synthetase (13). These results indicate that difference of recognition site between *A. pernix* and *E. coli* tRNA\(^{\text{Pro}}\) by each ProRS is the nucleotides of position 1 and 72. In fact, cross-species prolylation reaction between *A. pernix* and *E. coli* was not occurred. In *vitro* mutant transcript of *E. coli* tRNA\(^{\text{Pro}}\) having a G1-C72 base pair showed increased proline charging activity by *A. pernix* ProRS. These experimental evidences suggest that the difference of recognition system within *A. pernix* and *E. coli* ProRS may caused to change of nucleotides at position 1 and 72 of tRNA\(^{\text{Pro}}\) during evolution.

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