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Molecular Recognition of Tryptophan tRNA by Tryptophanyl-tRNA Synthetase from *Aeropyrum pernix* K1

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The identity elements of transfer RNA are the molecular basis for recognition by each cognate aminoacyl-tRNA synthetase. In the archaea system, the tryptophan tRNA identity has not been determined in detail. To investigate the molecular recognition mechanism of tryptophan tRNA by tryptophanyl-tRNA synthetase (TrpRS) from the hyperthermophilic and aerobic archaeon, *Aeropyrum pernix* K1, various mutant transcripts of tryptophan tRNA prepared by an *in vitro* transcription system were examined by overexpression of *A. pernix* TrpRS. Substitution of the discriminator base, A73, impaired tryptophan incorporation activity. Changing the G1–C72 base pair to other base pairs also decreased the aminoacylation activity. Substitutions of anticodon CCA revealed that the C34 and C35 mutants dramatically reduced aminoacylation with tryptophan, but the A36 mutants had the same activity as the wild type. The results indicate that the anticodon nucleotides C34, C35, discriminator base A73 and G1–C72 base pair are major recognition sites for *A. pernix* TrpRS.

Key words: tRNA identity, tryptophan tRNA, tryptophanyl-tRNA synthetase, archaea, *Aeropyrum pernix* K1.

Abbreviations: ARS, aminoacyl-tRNA synthetase; DTT, dithiothreitol; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; IPTG, isopropyl-β-D(–)-thiogalactopyranoside; OD_{600}, optical density at 600 nm; PMSF, phenylmethylsulphonyl fluoride; TrpRS, tryptophanyl-tRNA synthetase; WT, wild type.

For accurate translation during protein biosynthesis, the aminoacyl-tRNA synthetase (ARS) must distinguish the cognate tRNA and an amino acid, and catalyse an aminoacylation reaction to each 3′ terminal adenosine residue of the tRNA. Therefore, specific recognition of the amino acid and tRNA by ARS is an extremely important and essential mechanism.

To distinguish the cognate tRNA from a pool of various tRNAs consisting of similar L-shaped tertiary structures, it was found that ARS generally recognizes a relatively small number of nucleotides of each tRNA including anticodon bases and the discriminator base, N73, together with the base pair(s) of the acceptor stem (*1*). Although the tRNA recognition sites for ARS have been widely investigated in various bacteria and eukaryotes including *Escherichia coli*, *Saccharomyces cerevisiae* and *Homo sapiens*, the tRNA recognition sites remain unknown for ARS from archaea, which are classified as the third domain (*2–4*).

Recently, proteins have been produced with new features resulting from the introduction of non-natural amino acids mediated by an artificial tRNA such as a suppressor tRNA or a four-base anticodon tRNA (*5–7*). In the design of a new functional protein, an ARS having a catalytic property in which a non-natural amino acid is charged to artificial tRNA is a very important genetic engineering enzyme.

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Tryptophanyl-tRNA synthetase (TrpRS) is a relatively small enzyme consisting of an α_2 subunit. Structurally, TrpRS belongs to the class Ic ARS family, and its catalytic domain is built around a Rossmann fold (*8*). The Rossmann fold has two conserved sequences, HIGH and KMSKS, participating in ATP binding (*9*, *10*). All Class I synthetases charges the amino acid with the 2′-OH group of the 3′-terminal ribose moiety of tRNA (*11*).

In *E. coli*, the recognition site of $tRNA^{Trp}$ for TrpRS is reported to comprise the anticodon CCA, the discriminator base G73 and three base pairs A1–U72, G2–C71 and G3–C70 of the acceptor stem end (*12*, *13*). However, in archaea and eukaryotes, the base pair in the acceptor stem ends of $tRNA^{Trp}$ are G1–C72. In the case of *S. cerevisiae* TrpRS, neither this end base pair of the acceptor stem nor the discriminator base is recognized, and the major recognition elements were only the first and second bases of the anticodon, C34 and C35 (*14*). Therefore, we clarified the recognition mechanism of $tRNA^{Trp}$ by archaeal TrpRS to gain insight into the molecular evolution of TrpRS among the three domains, eubacteria, eukarya and archaea.

In this study, we investigated the molecular recognition of $tRNA^{Trp}$ by overexpressed TrpRS from the extreme hyperthermophilic and aerobic crenarchaeon, *Aeropyrum pernix* K1 by using various *in vitro* mutant transcripts of $tRNA^{Trp}$. *Aeropyrum pernix* was the first crenarchaeota whose complete genome sequence was determined (*15*, *16*).

MATERIALS AND METHODS

Materials—Oligodeoxynucleotides were purchased from Proligo and Texas Genomix Japan. The high-level expression and purification of T7 RNA polymerase have been described previously (*17*, *18*). The overexpression vector pET30a (+)-*A. pernix* TrpRS was constructed in our laboratory. Cibacron Blue 3GA was obtained from Sigma. [3-^{14}C] L-Tryptophan (48 mCi/mmol) was purchased from Moravek Biochemicals. All other chemicals and enzymes were obtained from Nacalai Tesque, Wako Pure Chemical Industries, TaKaRa Bio, Toyobo, New England Biolabs, Promega and Sigma.

Cloning and In Vitro *Transcription of Wild Type and Mutant tRNA^{Trp}*—The tRNA gene was constructed using the overlap PCR method with oligodeoxynucleotides pair (*19*, *20*). Synthetic DNA oligomers carrying the T7 promoter sequence upstream of the tRNA genes were ligated into pGEM-T Easy vector (Promega) and transformed into *E. coli* strain JM109 (TaKaRa). These DNA sequences were confirmed by DNA sequencing. Each template gene was amplified by PCR. Transcripts of the tRNA genes were prepared in a reaction mixture containing 40 mM Tris–HCl (pH 8.0), 20 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 1 mM spermidine, 5 mg/ml bovine serum albumin, 2.5 mM NTPs, 25 mM GMP, 0.5 mg/ml template DNA and 2 mg/ml T7 RNA polymerase. Transcripts initiated with a C, A and U were prepared in a mixture containing 25 mM CMP, AMP and UMP instead of 25 mM GMP, respectively. The reaction was performed at 37°C for 8 h. The transcripts were purified by 12% polyacrylamide gel electrophoresis under the denaturing condition. Prior to the aminoacylation assay, the transcripts were heated for refolding at 85°C for 5 min and slowly cooled to room temperature (*21*).

Cloning and Preparation of TrpRS—The *A. pernix trpS* gene was cloned into pET30a vector (+) (Novagene). The expression vector encoding *A. pernix* TrpRS gene was transformed into *E. coli* BL21-CodonPlus (DE3)-RIL (Stratagene). The cells were cultured in LB medium supplemented with 20 μM kanamycin at 37°C until the $OD_{600} = 0.6$. *A. pernix* TrpRS expression was then induced by 1 mM isopropyl-β-D(–)-thiogalactopyranoside (IPTG) for 3 h. TrpRS was purified by heat treatment at 90°C for 60 min and affinity chromatography using Cibacron Blue 3GA column (Sigma) as described previously (*22*). The purified *A. pernix* TrpRS was stored in Buffer [10 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 5 mM 2-mercaptoethanol and 2 mM PMSF] containing 50% glycerol at −30°C.

Tryptophanylation Assay and Determination of Kinetic Parameters—Time courses of the aminoacylation reaction were carried out at 60°C. The reaction mixture (40 μl) contained 100 mM HEPES–NaOH (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 2 mM ATP, 40 μM [3-^{14}C] L-tryptophan, 2.5 μM tRNA transcripts and 0.1 μM *A. pernix* TrpRS. An aliquot (8 μl) of the reaction mixture was spotted on Whatman 3MM filter paper, which had been infiltrated 5% trichloroacetic acid (TCA). The filter papers were then washed twice with 5% TCA for 15 min each, and the radioactivity of insoluble fractions was measured in a liquid scintillation counter (*23*).

Tryptophanylation reactions to determine the kinetic parameters were carried out at 60°C in 40 μl reaction mixtures containing 100 mM HEPES–NaOH (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 2 mM ATP, 40 μM [3-^{14}C] L-tryptophan, 0.5–66.5 μM tRNA transcripts and 0.08–3.57 μM *A. pernix* TrpRS. The initial rates of the tryptophanylation reactions were determined using different concentrations of tRNA transcripts for a given concentration of *A. pernix* TrpRS. Kinetic parameters for tryptophanylation were calculated by Lineweaver–Burk plots and represented as the results of at least three independent experiments (*24*).

RESULTS

Cross-Species Aminoacylation by A. pernix TrpRS—To determine the recognition sites in tRNA^{Trp} for *A. pernix* TrpRS, we measured tryptophanylation activities with the tRNA^{Trp} transcripts that have different structures from *A. pernix* tRNA^{Trp}. We used *S. cerevisiae* as a eukaryote, *E. coli* as a eubacteria and *A. pernix* tRNA^{Trp} as an archaeon for cross-species aminoacylation experiments (Fig. 1). The wild-type *S. cerevisiae* and *E. coli* tRNA^{Trp} sequence were determined previously (Fig. 1B and C), however, the sequence of the wild-type mature *A. pernix* tRNA^{Trp} was not clear. Therefore, we deduced the *A. pernix* tRNA^{Trp} sequence by RT-PCR using a purified *A. pernix* tRNA^{Trp} (Fig. 1A). As a result of the cross-species tryptophanylation, we determined that *S. cerevisiae* tRNA^{Trp} is a poor substrate for *A. pernix* TrpRS, with an ~7-fold decrease in aminoacylation efficiency as compared with *A. pernix* tRNA^{Trp}. On the other hand, tryptophanylation activity of *E. coli* tRNA^{Trp} by *A. pernix* TrpRS was not observed (Fig. 2).

Identity Elements in the Acceptor Stem and the Discriminator Base—From the results of the cross-species aminoacylation studies, the major recognition sites of tRNA^{Trp} for *A. pernix* TrpRS were expected to exist in the acceptor helix including the discriminator base, which has a different structure to three species of tRNA^{Trp}. To investigate the role of acceptor stem in recognition of tRNA^{Trp} by *A. pernix* TrpRS, many tRNA^{Trp} variants with mutations at these positions were assayed for their *in vitro* tryptophan incorporation ability with *A. pernix* TrpRS (Table 1). The discriminator base mutation A73G decreased aminoacylation efficiency by 125-fold, but A73 substituted by U and C resulted in only 11- and 4-fold loss of activities, respectively (Table 1). The aminoacylation ability of *A. pernix* TrpRS was almost lost when the G1–C72 base pair in the end of the acceptor stem was substituted. The aminoacylation efficiency decreased to 150- and 220-fold by the base pair substitutions A1–U72 and C1–G72, respectively, and the mutation to the U1–A72 base pair caused decreases of more than 600-fold (Table 1). Moreover, the G2–C71 base pair mutants (C2–G71, A2–U71 and U2–A71) caused only moderate decreases in tryptophanylation efficiency by *A. pernix* TrpRS (11–14-fold). However, although the k_{cat} and K_M values of these G2–C72 mutants varied their k_{cat}/K_M values were almost equal (Table 1). Furthermore, when the monophosphate group at the 5′ end of tRNA^{Trp} was substituted for a triphosphate group

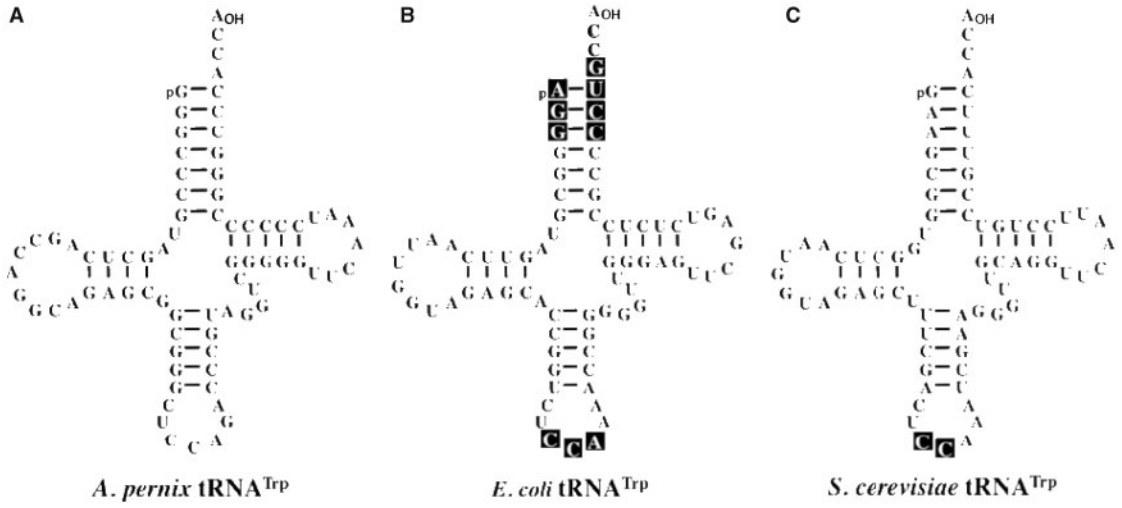

Fig. 1. **Secondary structure and identity elements of tRNA^{Trp}s.** Cloverleaf structure of archaeal *A. pernix* (A), bacterial *E. coli* (B) and eukaryotic *S. cerevisiae* tRNA^{Trp} transcripts (C). The highlighted bases are the recognition nucleotides that have been reported previously.

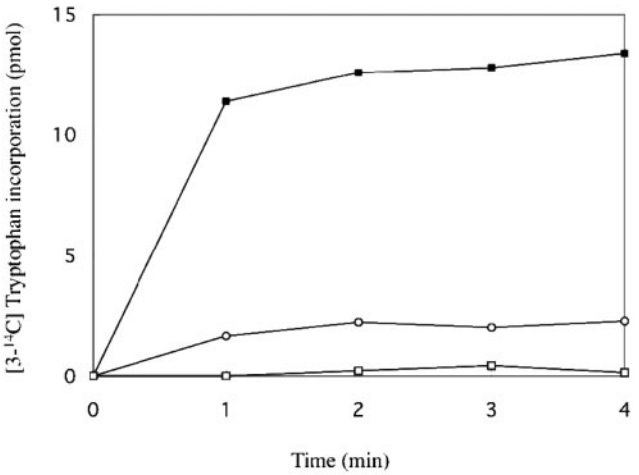

Fig. 2. **Cross-species tryptophan incorporation activity of *A. pernix* TrpRS.** Time course for *in vitro* tryptophanylation of *A. pernix* (closed square), *S. cerevisiae* (open circle) or *E. coli* tRNA^{Trp} transcripts (open square) were assayed with [3-^{14}C] L-tryptophan.

or dephosphorylated, the aminoacylation efficiency decreased 34- and 25-fold, respectively (Table 1).

Recognition of the Anticodon Triplet—Extensive studies of tRNA recognition elements have revealed that for most tRNAs, the anticodon triplets are very important for aminoacylation of the tRNA by the cognate ARS (*1*). Therefore, we performed a base substitution approach for the anticodon nucleotides to elucidate the recognition mechanism of *A. pernix* TrpRS. As a result, the aminoacylation efficiency by *A. pernix* TrpRS disappeared dramatically when the first and second bases of the anticodon triplet, C34 and C35, were substituted (Table 1). However, the remarkable decrease of tryptophanylation activity was not seen for mutants of the third anticodon nucleotide, the A36 mutants. The A36G mutant displayed only about a 5-fold increase in K_M and a 4-fold decrease in k_{cat}, but this decrease in k_{cat} hardly decreases compared with the wild type when substituting A36 for C and U their K_M increased similarly to A36G (Table 1).

Tryptophanylation Activity of A. pernix tRNA^{Arg} Mutants—The A36 base of the anticodon triplet is a weak recognition element for *A. pernix* TrpRS (Table 1). Therefore, *A. pernix* TrpRS could recognize tRNA^{Arg}(CCG), tRNA^{Arg}(CCU) and tRNA^{Gly}(CCC) as well as tRNA^{Trp}(CCA) and there is a possibility of mis-acylation with tryptophan. Especially, *A. pernix* tRNA^{Arg}(CCU) has a similar secondary structure to that of *A. pernix* tRNA^{Trp} there are only 10 bases differing between them over their whole base sequence, and the anticodon is misrecognized by *A. pernix* TrpRS (Fig. 3). Therefore, we predicted that the danger of mis-aminoacylation was very high. However, they are surely distinguished in nature. Therefore, we performed base switching between *A. pernix* tRNA^{Trp} and tRNA^{Arg} to study the recognition mechanism how *A. pernix* TrpRS removed *A. pernix* tRNA^{Arg}. As a result, the k_{cat}/K_M value of the U36, G73 double mutant tRNA^{Trp} was shown to have a k_{cat}/K_M almost the same as that of the wild-type *A. pernix* tRNA^{Arg} (Table 1).

DISCUSSION

The recognition of tRNA by the corresponding ARS is dependent on various elements and interactions. The three-dimensional structure of tRNA, base-specific nucleotide-protein contacts with ARS and factors such as the conformational changes of tRNA caused as a result of the interaction, reflect the accuracy of the recognition mechanism of tRNA. The recognition of tRNA by base-specific interaction is the most basic and important of the recognition process of all ARS, whose recognition mechanism has been reported (*1*). On the other hand, aminoacylated suppressor tRNA or four base anticodon tRNAs are used for the creation of new functional proteins by

Table 1. **Kinetic parameters for aminoacylation of tRNA^{Trp} and tRNA^{Arg} mutants by *A. pernix* TrpRS.**

tRNA	K_M (μM)	k_{cat} (10^{-1} s^{-1})	k_{cat}/K_M (μM^{-1}/s^{-1})	Relative activity[a]
Wild-type	1.2 ± 0.23	160 ± 12	$1.4 \times 10^{-1} \pm 0.20 \times 10^{-1}$	1
G1U–C72A	21 ± 3.0	4.6 ± 0.51	$2.2 \times 10^{-4} \pm 0.097 \times 10^{-4}$	1.6×10^{-3}
G1A–C72U	25 ± 1.3	23 ± 0.61	$9.3 \times 10^{-4} \pm 0.27 \times 10^{-4}$	6.8×10^{-3}
G1C–G72G	5.5 ± 0.40	3.4 ± 0.14	$6.1 \times 10^{-4} \pm 0.22 \times 10^{-4}$	4.5×10^{-3}
G2U–C71A	5.8 ± 1.1	72 ± 10	$1.3 \times 10^{-3} \pm 0.55 \times 10^{-3}$	9.2×10^{-2}
G2A–C71U	15 ± 2.2	190 ± 19	$1.3 \times 10^{-3} \pm 0.53 \times 10^{-3}$	9.2×10^{-2}
G2C–G71G	1.9 ± 0.095	17 ± 0.85	$9.4 \times 10^{-3} \pm 0.91 \times 10^{-3}$	6.9×10^{-2}
A73G	5.8 ± 0.98	6.2 ± 0.30	$1.1 \times 10^{-3} \pm 0.13 \times 10^{-3}$	8.0×10^{-3}
A73U	5.1 ± 1.1	61 ± 7.4	$1.2 \times 10^{-2} \pm 0.17 \times 10^{-2}$	9.1×10^{-2}
A73C	1.7 ± 0.41	55 ± 7.3	$3.4 \times 10^{-2} \pm 0.38 \times 10^{-2}$	2.5×10^{-1}
pppG1	12 ± 3.0	45 ± 4.9	$4.0 \times 10^{-3} \pm 0.59 \times 10^{-3}$	2.9×10^{-2}
HOG1	6.0 ± 0.67	32 ± 0.74	$5.4 \times 10^{-3} \pm 0.52 \times 10^{-3}$	4.0×10^{-2}
C34G	38 ± 6.7	1.3 ± 0.21	$3.3 \times 10^{-5} \pm 0.035 \times 10^{-5}$	2.4×10^{-4}
C34U	48 ± 9.4	1.1 ± 0.18	$2.4 \times 10^{-5} \pm 0.10 \times 10^{-5}$	1.8×10^{-4}
C34A	60 ± 7.2	1.7 ± 0.21	$2.8 \times 10^{-5} \pm 0.096 \times 10^{-5}$	2.1×10^{-4}
C35G	38 ± 6.0	0.79 ± 0.060	$2.1 \times 10^{-5} \pm 0.20 \times 10^{-5}$	1.5×10^{-4}
C35U	21 ± 2.4	4.7 ± 0.39	$2.2 \times 10^{-4} \pm 0.067 \times 10^{-4}$	1.6×10^{-3}
C35A	14 ± 1.3	1.5 ± 0.061	$1.1 \times 10^{-4} \pm 0.10 \times 10^{-4}$	7.9×10^{-4}
A36G	5.3 ± 0.82	44 ± 2.0	$8.4 \times 10^{-3} \pm 0.83 \times 10^{-3}$	6.2×10^{-2}
A36U	5.8 ± 0.91	210 ± 12	$3.6 \times 10^{-2} \pm 0.36 \times 10^{-2}$	2.7×10^{-1}
A36C	5.9 ± 0.14	110 ± 2.3	$1.8 \times 10^{-2} \pm 0.029 \times 10^{-2}$	1.4×10^{-1}
A36U, A73G	9.8 ± 1.2	2.7 ± 0.38	$2.8 \times 10^{-4} \pm 0.76 \times 10^{-4}$	2.1×10^{-3}
A. pernix tRNA^{Arg}	2.1 ± 0.27	1.6 ± 0.066	$7.8 \times 10^{-4} \pm 0.70 \times 10^{-4}$	5.7×10^{-3}

Values of K_M, k_{cat} and k_{cat}/K_M are expressed as mean ± SD.
[a]Relative activity refers to the loss in aminoacylation efficiency relative to that of the wild type. Relative activity is calculated as the ratio [k_{cat}(wild type)/K_M(wild type)]/[k_{cat}(mutant)/K_M(mutant)].

introducing non-natural amino acid. The ARS, which is able to identify these artificial tRNAs and catalyse the addition of the non-natural amino acid, is very useful as a genetic engineering tool. Therefore, it is very important to elucidate the recognition mechanism of the tRNA by ARS.

In the case of *A. pernix* tRNA^{Trp}, we determined the recognition elements by measuring the aminoacylation activities of various *A. pernix* tRNA^{Trp} mutant transcripts by overexpressed *A. pernix* TrpRS. To clarify the recognized sites of *A. pernix* tRNA^{Trp}, cross-species tryptophanylation reactions of the tRNA^{Trp} from *E. coli* and *S. cerevisiae* were examined by *A. pernix* TrpRS (Figs 1 and 2). As a result, tryptophan-accepting activity of both tRNA^{Trp}s decreased significantly (Fig. 2). These results suggested that apart from the anticodon nucleotides that are common to all three kinds of tRNA, the recognition sites for *A. pernix* TrpRS were different. Therefore, we determined the kinetic parameters of *A. pernix* tRNA^{Trp} mutants, which had substitutions of the variant nucleotides in the acceptor stem that had been revealed as the most important recognition sites in many tRNA identity studies. The different structures of the three kinds of tRNA^{Trp} are shown in Fig. 1. As a result, we suggest that the first base pair of the acceptor stem G1–C72, which is preserved in archaeal tRNA^{Trp}, is a major recognition element, whereas the second base pair G2–C71 is a comparatively minor identity element for *A. pernix* TrpRS (Table 1). Aminoacylation efficiency decreased dramatically only when the discriminator base A73, which is conserved in archaea and eukaryotic tRNA^{Trp}, is substituted to the G base that is conserved in bacterial tRNA^{Trp}s. It is thought that the aminoacylation efficiency decreased in the cross-species tryptophanylation, because these recognition elements are different nucleotides between *E. coli* and *S. cerevisiae* tRNA^{Trp}. *E. coli* tRNA^{Trp} has a different structure from that of *A. pernix* tRNA^{Trp} in the A1–U72 base pair and discriminator base G73, and the A2–U71 base pair as for yeast tRNA^{Trp} is also different (Fig. 1 and Table 1). Interestingly, aminoacylation efficiency largely deteriorated upon dephosphorylation or triphosphorylation of the 5′ end monophosphate group of tRNA^{Trp} (Table 1). These results suggest that the monophosphate of the 5′ end may also contribute to interaction with *A. pernix* TrpRS.

In the anticodon, which most ARSs recognized, it became clear the C bases in position 34 and 35 were major recognition elements. In contrast, the disappearance of aminoacylation efficiency was not seen for mutants of A36. When A36 was changed to a pyrimidine base, especially to U, a slight decrease of aminoacylation activity was observed compared with the wild-type tRNA^{Trp} (Table 1). CCU is the anticodon of tRNA^{Arg}. Moreover, the secondary structure of *A. pernix* tRNA^{Arg} is very similar to that of tRNA^{Trp} (Fig. 3). Therefore, we demonstrated that these two tRNAs were distinguished by *A. pernix* TrpRS in a transplantation experiment. The A36U and A73G double mutant of tRNA^{Trp} showed tryptophanylation efficiency almost equal to wild-type *A. pernix* tRNA^{Arg}. Therefore, it seems likely that *A. pernix* TrpRS distinguishes these bases at the position of 36 and 73 (Fig. 3). However, we hypothesize that the interaction of *A. pernix* tRNA^{Arg} with *A. pernix* TrpRS happens relatively easily in the anticodon, because base N36 of the anticodon is a minor recognition element.

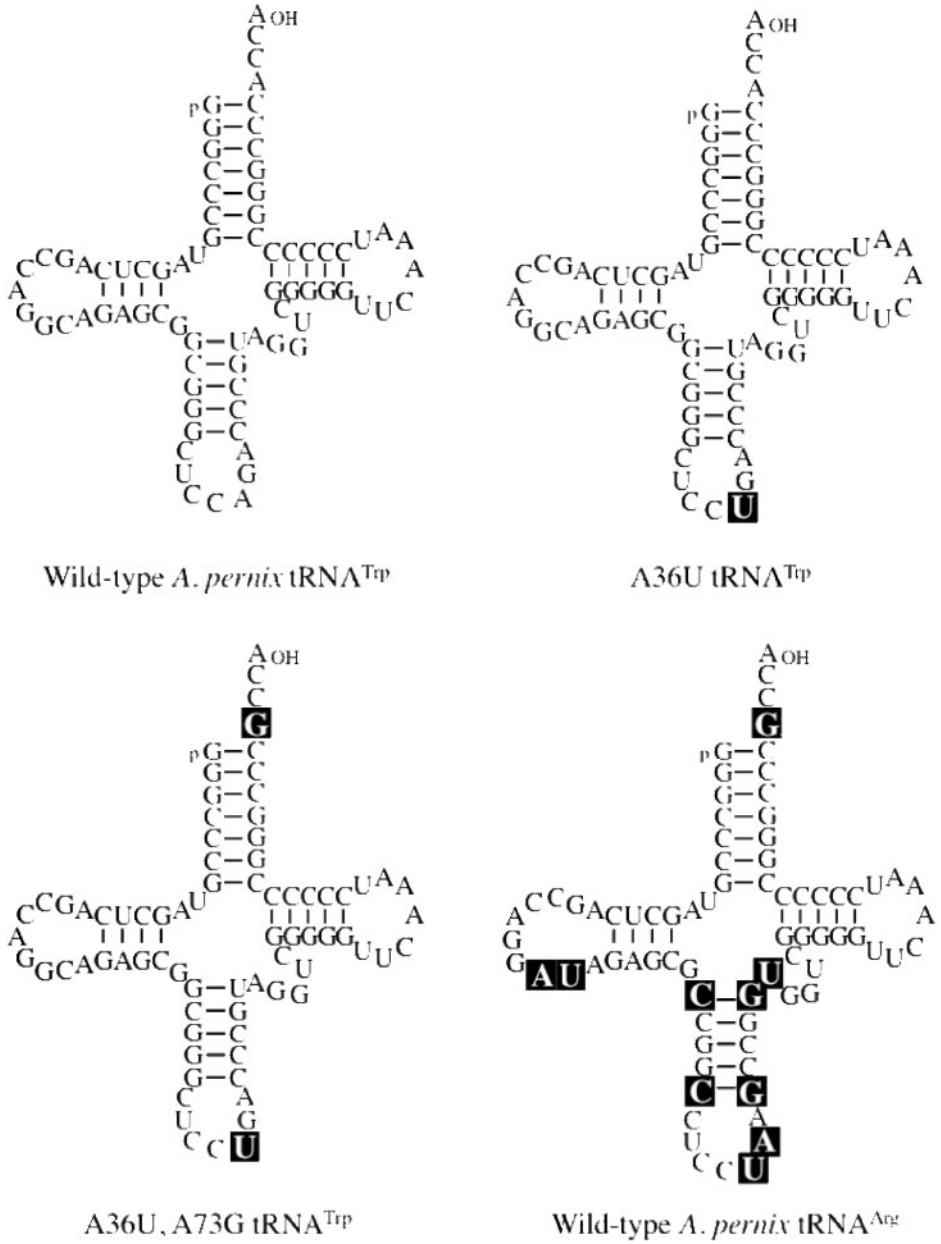

Fig. 3. **Cloverleaf structures of wild-type *A. pernix* tRNA^{Trp} and tRNA^{Arg} and the tRNA^{Trp} mutants studied.** In the mutants, highlighted nucleotides are the recognition elements and are different from the wild-type *A. pernix* tRNA^{Trp}.

Therefore, we suggest that a negative selection performed in the acceptor stem end binding sites of *A. pernix* TrpRS sifts the other tRNA, which is not excluded from recognition of the anticodon, to improve the selectivity of the tRNA is. It is thought that the accurate selection of the tRNA is aided by a contribution of the discriminator base A73 to a negative selection, preventing mis-aminoacylation of *A. pernix* tRNA^{Arg}.

The archaeal *A. pernix* tRNA^{Trp} identity elements that we clarified are supported by results of the cocrystal structure analysis with human TrpRS and tRNA^{Trp}. The homology of *A. pernix* TrpRS and human TrpRS is about 30% overall, which is not too high, but the amino acid residues which interact with the acceptor stem of the tRNA are conserved, showing ~65% homology. A hydrogen bond network forms between the positive charge of Arg318 and the carboxyl group of Asp99 in the human TrpRS and the N1 position of discriminator base A73 of tRNA^{Trp} (*25*, *26*). In addition, the carbonyl group in the peptide bond of Lys102 and the N6 position of A73 also interact by a hydrogen bond. In the *A. pernix* TrpRS, Arg318 and Asp99 residues are conserved as Arg223 and Asp16, respectively, although human Lys102 is substituted as Arg21 that has the same positive charge. Therefore, we assumed that these amino-acid residues of *A. pernix* TrpRS interact with discriminator base A73 of *A. pernix* tRNA^{Trp} in a similar way to human TrpRS. When A73 is substituted into G, aminoacylation

efficiency decreased remarkably. This result will reflect the change of an electronic character of N1 and N6 of the purine base, and the hydrogen bond cannot be formed. The base preference A > C > U > G of discriminator base A73 in the activity by *A. pernix* TrpRS is also reported for human TrpRS (*27*). The monophosphate group of the tRNA at the 5′ end forms a hydrogen bond with Lys311 of human TrpRS. It is suggested that a similar interaction occurs through the phosphate group in *A. pernix* TrpRS Arg241 that has the same positive charge as human Lys311.

Due to the low conservation of the amino-acid sequence of TrpRSs between human and *A. pernix*, a structural analysis of the anticodon-binding domain is very difficult. The X-ray crystal structure analysis of bacterial and eukaryotic TrpRS has already been solved (*28–30*), however, the crystal structure analysis of archaeal TrpRS has not been reported yet. Therefore, it is difficult to examine the recognition mechanism that we propose here from a structural viewpoint. Therefore, we must elucidate the nucleotide–protein interaction of $tRNA^{Trp}$ and TrpRS by X-ray crystal structure analysis of *A. pernix* TrpRS.

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CONFLICT OF INTEREST

None declared.

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