# Characteristic anticodon sequences of major tRNA species from an extreme thermophile, *Thermus thermophilus* HB8

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Major species of $tRNA^{Glu}$, $tRNA^{Lys}$, $tRNA^{Val}$, $tRNA^{Ala}$, $tRNA^{Ser}$ and $tRNA^{Arg}$ were purified from *Thermus thermophilus* HB8, and their anticodon sequences were found to be CUC, CUU, CAC, GGC, GGA and CCG, respectively, with unmodified G or C as the first letter. These anticodons exactly correspond to the codons as used most frequently among synonymous codons in the structural genes of extreme thermophiles. The stable complexes of these anticodons (in particular, GGN or CCN) with their corresponding codons probably contribute to the correct protein biosynthesis in extreme thermophiles at high temperature.

*tRNA* *Anticodon* *Codon usage* (Thermus thermophilus *HB8*) *Thermophile*

## 1. INTRODUCTION

*Thermus thermophilus* HB8 is an extreme thermophile that grows at high temperature (50–85°C). As for the protein synthesis system of this thermophile, we have studied the structures and functions of thermostable components, including tRNA [1–6] and aminoacyl-tRNA synthetases [7,8]. We have determined the nucleotide sequences of $tRNA_{f1}^{Met}$, $tRNA_{f2}^{Met}$ and $tRNA_{1}^{Ile}$ species from *T. thermophilus* HB8 and found, in each species, 2-thioribothymidine ($s^2T$) in position 54 [3,6]. Such a post-transcriptional modification of T to $s^2T$ raises the melting temperature of *T. thermophilus* $tRNA_{1}^{Ile}$ species [6]. We have found that the thermostability of *T. thermophilus* tRNA species is primarily due to the steric effect of the bulky 2-thiocarbonyl group of $s^2T(54)$ [2,5].

Here we have isolated a number of tRNA species from *T. thermophilus* and analyzed the sequences, in particular in the anticodon region, of six major tRNA species. The first letters of the anticodons of these major tRNA species are found to be unmodified G or C, in contrast with the cases of the major tRNA species from *E. coli* [9]. Furthermore, in the anticodons of major $tRNA^{Ala}$, $tRNA^{Ser}$ and $tRNA^{Arg}$ species, the first letter (G or C) is found to be the same as the second letter (G or C). The anticodons of such major tRNA species from *T. thermophilus* probably form more stable complexes with corresponding codons of mRNA, as compared with the anticodons of other isoacceptor tRNA species.

## 2. MATERIALS AND METHODS

*T. thermophilus* HB8 was cultured at 65°C. Crude tRNA was prepared according to Zubay [10] and was fractionated by chromatography on columns of DEAE-Sephadex A-50, BD-cellulose and Sepharose 4B [4,11]. Each of the major tRNA species was purified to homogeneity by two-

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dimensional polyacrylamide gel (10% and 20%) electrophoresis [12] and finally by 20% polyacrylamide gel electrophoresis in the presence of 7 M urea. The aminoacylation of tRNA was assayed at 65°C using aminoacyl-tRNA synthetases from *T. thermophilus* HB8 [7].

## 3. RESULTS AND DISCUSSION

### 3.1. *Purification*

Crude preparation of *T. thermophilus* tRNA (73000 $A_{260}$ units) was fractionated first by chromatography on a DEAE-Sephadex A-50 column and amino acid accepting activity was assayed for each of 15 amino acid species (fig.1). Fraction I (nos 311–326) contained the major peaks of $tRNA^{Val}$ and $tRNA^{Glu}$, while fraction II (nos 384–410) contained the major peaks of $tRNA^{Ala}$, $tRNA^{Ser}$, $tRNA^{Arg}$ and $tRNA^{Lys}$. These tRNA species were collected and purified from the major peaks in each step of successive column chromatography. In contrast, the minor $tRNA^{Ala}$ peak in fraction nos 350–375 is much less abundant than the major peak in fraction II. Thus, each of the six tRNA species as purified in this study is most abundant among isoacceptor tRNA species.

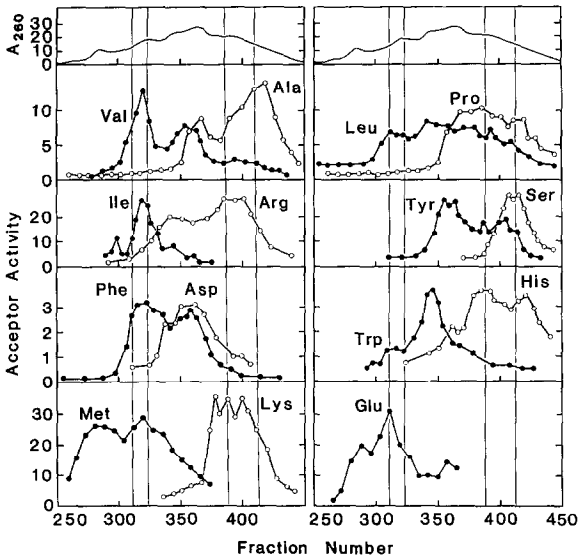


Fig.1. Fractionation of *T. thermophilus* tRNA species. Crude preparation of tRNA (73000 $A_{260}$ units) was applied to a DEAE-Sephadex A-50 column (4.2 × 150 cm). Elution was performed with 20 l of a linear gradient of $MgCl_2$ (from 8 to 16 mM) and NaCl (from 375 to 525 mM) in 20 mM Tris-HCl buffer (pH 7.5). Fractions of 20 ml were collected. Amino acid accepting activity (in $10^2$ cpm/disc) was assayed as described [7].

### 3.2. *Nucleotide sequence*

The nucleotide sequences of 6 major tRNA species purified from *T. thermophilus* were analyzed by the post-labeling method [13]. Anticodon loops (positions 32–38 in accord with [9]) were readily identified, as shown in fig.2, from the location in the cloverleaf model (the complete se-

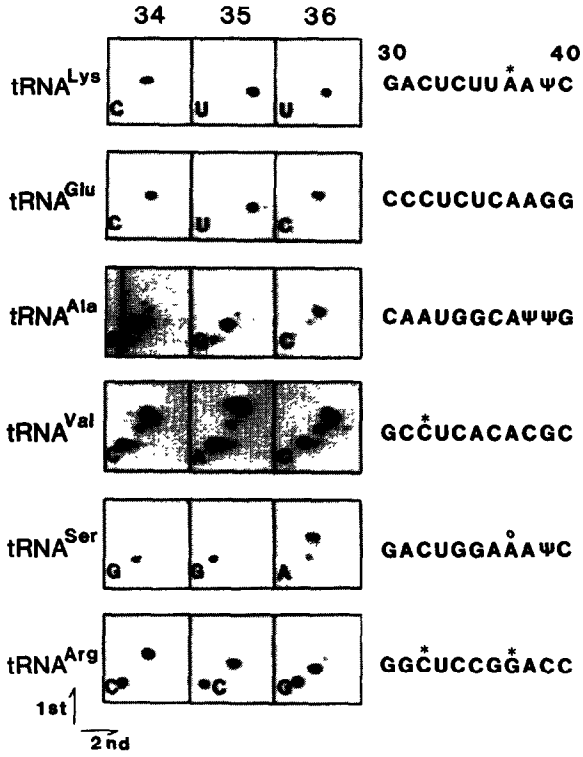


Fig.2. Autoradiograms of two-dimensional thin-layer chromatography of 5′-$^{32}$P-labeled mononucleotides from positions 34–36 (left) and nucleotides in positions 30–40 (right) of major tRNA species from *T. thermophilus* HB8. Ψ, pseudouridine; Ĉ, 2′-*O*-methylcytidine (Cm); Å, 2-methylthio-$N^6$-(threoninocarbonyl)adenosine ($ms^2t^6A$); Å, 2-methylthio-$N^6$-($\Delta^2$-isopentenyl)-adenosine ($ms^2i^6A$); Ĝ, 1-methylguanosine ($m^1G$). The solvent systems for two-dimensional thin-layer chromatography were isobutyric acid/concentrated ammonia/water (50:1:29, v/v) for the first dimension, and 2-propanol/concentrated HCl/water (70:15:15, v/v) for the second dimension [12].

quences of these tRNA species will be reported separately). Thus, the anticodon sequences (positions 34–36) were determined as CUU ($tRNA^{Lys}$) and CUC ($tRNA^{Glu}$) for the amino acids of the two-codon family, CAC ($tRNA^{Val}$) and GGC ($tRNA^{Ala}$) for the four-codon family, and CCG ($tRNA^{Arg}$) and GGA ($tRNA^{Ser}$) for the six-codon family (fig.2). Further, in the major $tRNA^{Arg}$ species, the third letter of anticodon (CCG) is G rather than U. Thus for each amino acid, the G + C content in the anticodon of the major tRNA species from *T. thermophilus* was found to be highest among those of possible synonymous anticodons.

### 3.3. *First letter of anticodon and codon usage*

In all these major tRNA species from *T. thermophilus*, the first letters of anticodons are now found to be unmodified guanosine or cytidine. In contrast, in the major *E. coli* tRNA species for the 6 amino acids the first letters of anticodons are inosine or modified uridine [9]. The modifications of uridine in the first position of anticodon have been found to play important roles in the correct and efficient recognition of codons [14]. However, in *T. thermophilus* cells at high temperature, the correct recognition of codon is probably favored by the formation of G·C pairs (three hydrogen bonds) between the first letter of anticodon and the third letter of codon.

The most abundant tRNA species among isoacceptors in *E. coli* have been found to correspond to the codons as used most frequently in the structural genes for abundant proteins [15]. However, in the gene for 3-isopropylmalate dehydrogenase (IPMDH) of *T. thermophilus* HB8, the codon usage is significantly different from that in *E. coli*, and codons terminating in G or C are almost exclusively used (90% of amino acid codons) [16]. Such a remarkable tendency in the codon usage has also been found in the gene of L-lactate dehydrogenase from another extreme thermophile, *T. caldophilus* GK24 (Kunai, K. et al., personal communication). Accordingly, at high temperature, G as the first letter of the anticodon probably recognizes C much more efficiently than U in the third letter of codon.

The anticodons of major tRNA species as determined in this study exactly correspond to the codons which are used most frequently in the IPMDH gene of *T. thermophilus* [16]. Thus, the fractions of codons as recognized by major tRNA species are 100% (AAG) for Lys and 90% (GAG) for Glu (two-codon family), 65% (GCC) for Ala and 65% (GUG) for Val (four-codon family), and 50% (UCC) for Ser and 40% for Arg (six-codon family).

### 3.4. *Correlation of first and second letters of anticodon*

Furthermore, in major tRNA species from *T. thermophilus*, a correlation between the first letter (G or C) and the second letter of anticodon is found, in this study, for the tRNA species specific to amino acids of the four-codon family and the six-codon family. For example, the major $tRNA^{Ala}$ has the anticodon GGC rather than CGC, $tRNA^{Ser}$ has GGA rather than CGA or GCU, and $tRNA^{Arg}$ has CCG rather than GCG. Thus, GG and CC sequences (rather than CG and GC sequences, respectively) are used as the first and second letters of major tRNA species. It is important here to recall that, in double-stranded RNA helices, $\overset{GC}{GC}$ is more stable than $\overset{GC}{CG}$ or $\overset{CG}{GC}$ [17]. All these results suggest that the anticodon-codon complexes are the most stable for the tRNA species with anticodons GGN or CCN than for other isoacceptor tRNA species. This is consistent with the finding for the IPMDH gene [16] that the fractions of Pro codon CCC (to be recognized by the anticodon GGG) and Gly codon GGG (to be recognized by the anticodon CCC) are predominant among synonymous codons. Such stable codon-anticodon interactions are probably required for the correct biosynthesis of abundant proteins in extreme thermophiles at high temperature.

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