Nucleic Acids Research, 1996, Vol. 24, No. 3 405–410

The discriminator bases G73 in human tRNA^{Ser} and A73 in tRNA^{Leu} have significantly different roles in the recognition of aminoacyl-tRNA synthetases

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Received November 6, 1995; Revised and Accepted December 8, 1995

ABSTRACT

The recognition of human tRNA^{Leu} or tRNA^{Ser} by cognate aminoacyl-tRNA synthetases has distinct requirements. Only one base change (A73→G) in tRNA^{Leu} is required to generate an efficient serine acceptor *in vitro*, whereas several changes in three structural domains (the acceptor stem, DHU loop and long extra arm) of tRNA^{Ser} are necessary in order to produce a leucine acceptor. Hence, the molecular basis for the discrimination between human tRNA^{Ser} and tRNA^{Leu} by the seryl-tRNA synthetase depends almost exclusively on a highly specific recognition of the discriminator base G73. In order to elucidate the specific role of the functional groups of this base in discrimination, tRNA^{Ser} constructs were made which contain the artificial base analogues 2-aminopurine riboside or inosine at the discriminator position 73. Aminoacylation of these constructs by a HeLa S100 extract showed that molecules with 2-aminopurine riboside, but not with inosine, in position 73 could be serylated at low efficiency. However, the 2-aminopurine riboside and the inosine derivatives of tRNA^{Ser} were equally efficient competitive inhibitors of serylation, whereas tRNAs^{Ser} with any other natural base at position 73 did not competitively inhibit serylation of tRNA^{Ser}. This was in contrast to leucylation of tRNA^{Leu}, where tRNA^{Leu} transcripts with any other nucleotide in the discriminator position acted as strong competitive inhibitors. These results suggest that the discriminator bases in human tRNA^{Ser} and tRNA^{Leu} play completely different roles in recognition of the tRNAs by their cognate aminoacyl-tRNA synthetases.

INTRODUCTION

All tRNAs in any family and most tRNAs which accept chemically similar amino acids have the same residue at position 73, the discriminator base (1). It is the only variable unpaired nucleotide near the site of aminoacylation except for tRNA^{His} in archaebacteria, eubacteria or yeast mitochondria (2) and it has been shown that this nucleotide contributes to the identity of most tRNAs (3–6). Furthermore, the discriminator base plays a central and important role in the recognition of all human tRNAs with a long extra arm (class II tRNAs) by their cognate aminoacyl-tRNA synthetases because: (i) replacement of the leucine-specific discriminator base in human tRNA^{Leu} by serine-specific G73 alone is sufficient for an identity switch to serine acceptance and for the complete loss of leucine identity (7); (ii) the reverse identity switch also requires the discriminator base and, in addition, several changes in three structural domains of tRNA^{Ser} to generate an efficient leucine acceptor (8); (iii) the long extra arm and the discriminator base G73 are the major identity elements for the serylation of human tRNA^{Ser} (9) and tRNA^{Sec} (10).

There are only a few reports that altered tRNA aminoacylation can be caused by single changes of amino, keto, hydroxyl or other chemical groups. They refer mainly to *Escherichia coli* tRNA^{Gln} (11) and *E.coli* tRNA^{Ala} (12,13). The 2-amino group of guanosines in positions 2, 3 and 10 of tRNA^{Gln} is important for specific recognition by glutaminyl-tRNA synthetase (GlnRS) (11). Substitution of guanosine residues with inosine allowed the identification of an unpaired 2-amino group of G3 essential for recognition by alanyl-tRNA synthetase (AlaRS) in the minor groove of the RNA (12). Individual substitutions by 2′-deoxy or by 2′-*O*-methyl nucleotides have pinpointed three particular 2′-hydroxyl groups (C69, C71 and C72) in the RNA minor groove which may contact AlaRS (13). In another case Pütz *et al.* (14) have shown that a single methyl group in m^{1}G37 in tRNA^{Asp} of yeast contributes to tRNA identity by preventing mischarging of tRNA^{Asp} by yeast arginyl-tRNA synthetase.

An approach to understanding the function of the discriminator base and to characterizing essential functional elements within the discriminator base is to exchange the base or to introduce modifications of individual functional groups. Here we have studied the role of G73 in the interaction of serine-specific tRNAs with its cognate aminoacyl-tRNA synthetase (SerRS) by deletion of single functional groups in this base. Therefore, the discriminator base in human tRNA^{Ser} was changed from G to 2-aminopurine riboside (2-AP) or to inosine (I). These altered tRNAs were used to study their roles as substrates for aminoacylation with regard to both catalytic and binding activity. Furthermore, tRNAs^{Ser} with A, C or U and tRNAs^{Leu} with C or G in position 73, which

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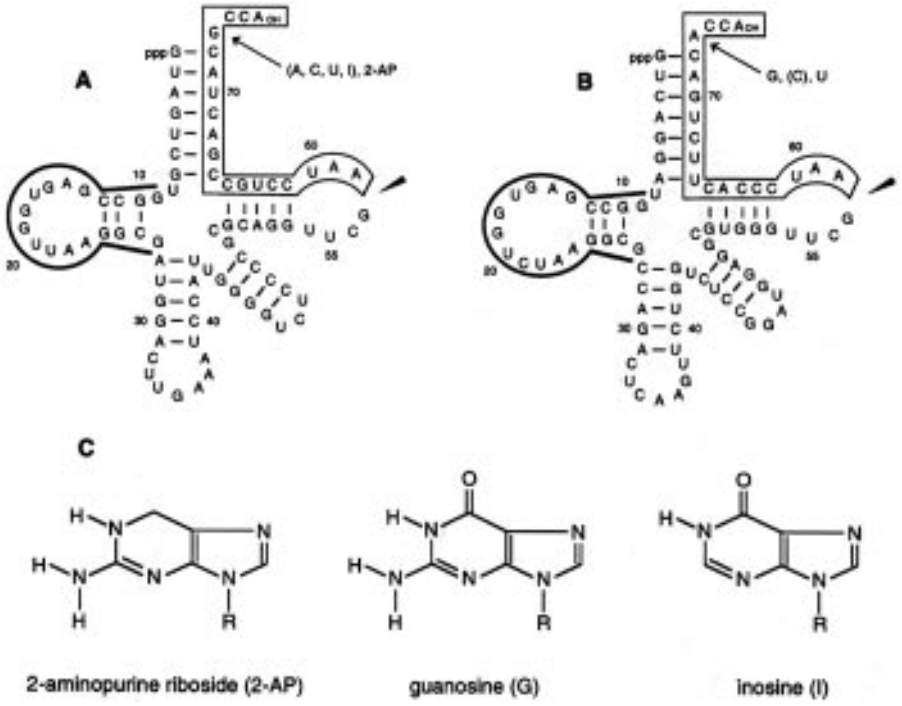

Figure 1. Structure of *in vitro* synthesized human $tRNA^{Ser}$, human $tRNA^{Leu}$ and their derivatives. Arrows indicate substitutions in the discriminator position 73; those in brackets are not substrates for aminoacylation (Table 1). Boxed RNA 3′-sequences were chemically synthesized. The 5′-moieties were prepared by T7 RNA polymerase transcription. Arrowheads identify the site of covalent joining of the two halves with T4 RNA ligase. Sequences characterized by bold lines correspond to the base pairing region of the complementary oligodeoxynucleotides added to the ligation assay. (**A**) $tRNA^{Ser}$ with a UGA anticodon (18). Discriminator base substitutions are A73, C73, U73, 2-AP73 and I73 (2-AP = 2-aminopurine riboside; I = inosine). (**B**) $tRNA^{Leu}$ with a CAA anticodon (19). The discriminator base A73 is replaced by G, C and U respectively. (**C**) Structures of 2-aminopurine riboside (2-AP), guanosine (G) and inosine (I).

do not serve as substrates for aminoacylation (7,8), were tested as potential competitive inhibitors for serylation and leucylation respectively.

MATERIALS AND METHODS

Enzymes and reagents

T7 RNA polymerase was prepared as described by Zawadzki and Gross (15) from an overproducing strain of *E.coli* kindly provided by Dr W. Studier. HeLa cytoplasmic S100 extract was prepared and dialysed as described by Dignam *et al.* (16). Preincubation studies with this extract did not reveal any measurable degradation even of low amounts of tRNAs within 20 min. All other enzymes were obtained from commercial suppliers. [3-^{3}H]Serine (1.07 TBq/mmol) and [4,5-^{3}H]leucine (1.9 TBq/mmol) were purchased from Amersham-Buchler (Braunschweig, Germany) and DuPont de Nemours (Bad Homburg, Germany) respectively. [α-^{32}P]dATP and [γ-^{32}P]ATP were obtained from Hartmann (Braunschweig, Germany). Tetrabutylammonium fluoride (1.1 M) in tetrahydrofuran was obtained from Aldrich (Steinheim, Germany). Oligoribonucleotides were prepared on an Applied Biosystems 380B DNA synthesizer by Drs T. Tuschl and F. Eckstein (Göttingen). They were base-deprotected and purified as described by Tuschl *et al.* (17).

Bacterial strains and plasmids

Escherichia coli JM109 was used as host for propagation of plasmid pUC19 and its derivatives.

Construction of tDNA clones

The templates coding for $tRNA^{Ser}$ with a UGA anticodon (18) and $tRNA^{Leu}$ with a CAA anticodon (19), including the T7 promoter and the *Bst*NI recognition site, were assembled from synthetic oligodeoxynucleotides and cloned into pUC19. All subclones derived from these genes were constructed using appropriate synthetic oligodeoxynucleotides as polymerase chain reaction primers and were cloned into pUC19. The sequences of all constructs were confirmed by dideoxy sequencing (20).

Preparation of tDNA transcripts

Transcription of *Bst*BI- and *Bst*NI-linearized tDNA templates and tRNA 5′-fragments (sequence from position 1 to 57, Fig. 1A and B) with T7 RNA polymerase yielded unmodified tRNAs, which were used for aminoacylation studies, and fragments for ligation reactions, respectively (Fig. 1). The conditions for transcription with T7 RNA polymerase were as described by Achsel and Gross (9).

The nucleoside at the 3′-end of tRNA fragments transcribed with T7 RNA polymerase was determined by 3′-end-labelling the tRNA fragment with 1.85 MBq [5′-^{32}P]pCp and T4 RNA ligase. The radioactive fragments were purified by 15% polyacrylamide (8 M urea) gel electrophoresis and ~168 Bq of labelled tRNA fragment was then digested to completion with 0.05 U RNase T2 in 20 µl 5 mM NH_4OAc, pH 4.6, at 37°C for at least 5 h. Nucleoside 3′-monophosphates were identified by thin layer chromatography according to Nishimura (21).

Ligation of inosine- and 2-aminopurine riboside-containing oligoribonucleotides with T4 RNA ligase

Synthetic inosine (I73)-, 2-aminopurine riboside (2-AP73)- and G73-containing 19mer ribonucleotides spanning the sequence from position 58 to 76 of $tRNA^{Ser}$ (Fig. 1A) and a $tRNA^{Leu}$-specific oligoribonucleotide of the same length (Fig. 1B) with the discriminator base A73 were chemically synthesized (17). These chemically synthesized donor oligoribonucleotides (identified by boxed sequences in Fig. 1A and B) were phosphorylated at their 5′-ends with T4 polynucleotide kinase according to the supplier's instructions. For the ligation reaction the 5′-phosphorylated donor oligoribonucleotide (17.5 µM, 3-fold molar excess), the 3′-OH acceptor tRNA fragment (5.8 µM), the ligation buffer (10×, 10 µl) and 100 U T4 RNA ligase (Boehringer) were combined in a final volume of 100 µl and incubated at 37°C for 2 h. For efficient ligation of the serine-specific tRNA fragments an oligodeoxynucleotide (29 µM, 5-fold molar excess) pairing to the DHU loop and stem domain of the tRNA 5′-fragment was added (Fig. 1A). The reaction was then carried out as mentioned above. Product and substrate were separated by electrophoresis in a denaturing 8 M urea gel of 10% polyacrylamide for preparative separations or 20% gels for analytical purposes.

In vitro aminoacylation

All tRNAs were heated for 4 min at 68°C in 5 mM $MgCl_2$ and allowed to cool slowly to room temperature. Leucine-specific aminoacylation was at 37°C in 36 µl containing 20 mM imidazole–HCl, pH 7.5, 75 mM KCl, 6 mM $MgCl_2$, 5 mM ATP, 0.5 mM CTP, 0.5 mM DTT, 3.3% (v/v) dialysed HeLa cytoplasmic S100 extract (16) (8.1 mg protein/ml) and 5 µM leucine, which included 0.38 µM [4,5-^{3}H]leucine (1.9 TBq/mmol). tRNA concentrations ranged from 1.0 to 10 µM. Serine-specific aminoacylation was also performed at 37°C in 36 µl of a reaction mixture containing 20 mM imidazole–HCl, pH 7.5, 150 mM KCl, 10 mM $MgCl_2$, 5 mM ATP, 0.5 mM CTP, 0.5 mM DTT, 3.3% (v/v) HeLa cytoplasmic S100 extract (8.1 mg protein/ml) and 5 µM serine, which included 0.7 µM [3-^{3}H]serine (1.07 TBq/mmol). tRNA concentrations ranged from 0.4 to 4µM. Samples (6 µl) of the reaction mixture were transferred onto pieces of Whatman 3MM paper which were washed with 10% trichloroacetic acid, twice in 5% trichloroacetic acid and twice in ethanol to remove free [^{3}H]amino acids. Radiolabelled aminoacyl-tRNA was then measured by liquid scintillation counting.

Competitive inhibition of aminoacylation

Aminoacylations were carried out as mentioned above. tRNA transcripts serving as potential inhibitors were added to reaction mixtures in 10-fold molar excess (4 and 10 µM respectively) with regard to $tRNA^{Ser}$ or $tRNA^{Leu}$. An increase in the concentrations of $tRNA^{Ser}$ or $tRNA^{Leu}$ without changing the concentration of the inhibitors leads to abolition of the inhibition, indicating a competitive interaction at the active centre of the aminoacyl-tRNA synthetase.

RESULTS

Design of $tRNA^{Ser}$ molecules with modified nucleosides at the discriminator position 73

T4 RNA ligase was used to connect specific tRNA fragments to construct a mature tRNA with modified nucleosides at the discriminator position 73. One tRNA fragment was based on either $tRNA^{Ser}$- or $tRNA^{Leu}$-specific sequence from position 1 of the 5′-site of the acceptor stem and ending at position 57 with the universal, highly conserved sequence 5′-UUCG$_{OH}$-3′ (Fig. 1A and B). These fragments were transcribed by T7 RNA polymerase from corresponding DNA templates. Analysis of the last nucleoside at the 3′-site of these tRNA fragments showed that the 3′-ends were always correct, ending with residue G (not shown). The fragments from position 58 to the 3′-end were chemically synthesized oligoribonucleotides (19mers) containing residue G or the modified purines inosine or 2-aminopurine riboside at the discriminator position for construction of human $tRNA^{Ser}$ and its derivatives and adenosine for generation of $tRNA^{Leu}$ (Fig. 1). The acceptor stem and TΨC stem domains of each fragment were annealed to each other (Fig. 1A and B). Covalent joining of the two tRNA halves with T4 RNA ligase was facilitated and the generation of by-products was avoided because the two fragments were partially hydrogen bonded and the sites to be ligated were in proximal neighbourhood.

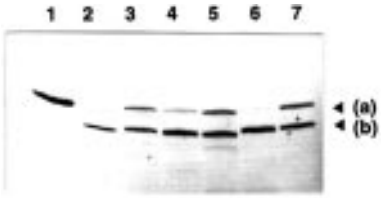

Figure 2. Separation of substrates and ligation products of $tRNA^{Ser}$ and $tRNA^{Leu}$ on a 20% denaturing polyacrylamide (8 M urea) gel stained with toluidine blue. Lane 1, human $tRNA^{Ser}$ transcribed with T7 RNA polymerase; lane 2, $tRNA^{Ser}$ 5′-fragment transcribed with T7 RNA polymerase as used for the ligation reaction; lanes 3 and 5, ligation assay of serine-specific tRNA 5′- and 3′-fragments in the presence of the additional hybridizing oligodeoxynucleotide (Fig. 1A); lane 4, the same reaction as in lanes 3 and 5 but without addition of the oligodeoxynucleotide; lane 6, ligation assay of leucine-specific tRNA 5′- and 3′-fragments in the presence of the additional hybridizing oligodeoxynucleotide (Fig. 1B); lane 7, ligation of leucine-specific tRNA fragments without addition of the oligodeoxynucleotide. (a) mature tRNA; (b) tRNA 5′-fragment generated by T7 RNA polymerase transcription.

The use of an oligodeoxynucleotide hybridizing to the DHU domain can dramatically facilitate ligation in the TΨC loop

Figure 2 shows ligation products and substrates of $tRNA^{Ser}$ and $tRNA^{Leu}$ separated in a 20% denaturing polyacrylamide (8 M urea) gel and stained with toluidine blue. Lanes 3–5 show the products of ligation reactions used for construction of human $tRNA^{Ser}$. The reaction mixture shown in lanes 3 and 5 contained an additional oligodeoxynucleotide, pairing to the DHU stem and loop domain of the longer tRNA fragment that had been transcribed with T7 RNA polymerase (Fig. 1A). These reactions yielded substantial amounts of the ligated product, which co-migrated with $tRNA^{Ser}$ (lane 1). When the reaction mixture did not contain the additional pairing oligodeoxynucleotide little or no ligation product was produced (lane 4). Therefore, for efficient ligation an oligodeoxynucleotide masking the complete DHU stem and loop domain of the $tRNA^{Ser}$ 5′-fragment must be added, possibly to prevent an unfavourable tertiary structure. In contrast, addition of a corresponding oligodeoxynucleotide to the ligation reaction mixture containing the two $tRNA^{Leu}$ halves inhibited ligation

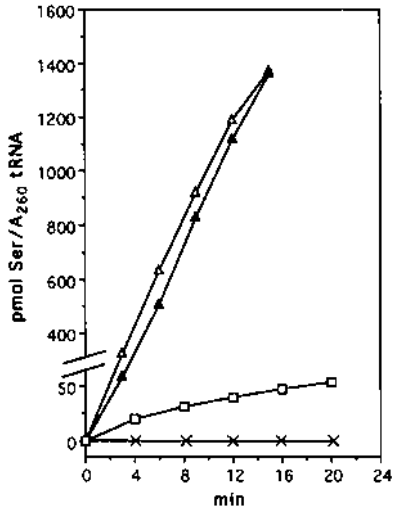

Figure 3. Kinetics of serylation of human $tRNA^{Ser}$ in HeLa cytoplasmic S100 extract. $tRNA^{Ser}$ (▲) was generated by complete transcription with T7 RNA polymerase; $tRNA^{Ser}$ (Δ), $tRNA^{Ser}$2-AP73 (□) and $tRNA^{Ser}$I73 (×) were generated by covalent joining of two tRNA fragments (Fig. 1A) with T4 RNA ligase. tRNA concentrations of 1.0 µM were used. The coupling yields of inosine and 2-aminopurine riboside phosphoramidites monitored by trityl group release were measured to be >99% (17). It is unknown whether the low rate of $tRNA^{Ser}$2-AP73 serylation is caused by slow aminoacylation or by competition between aminoacylation and rapid hydrolysis of the ester bond.

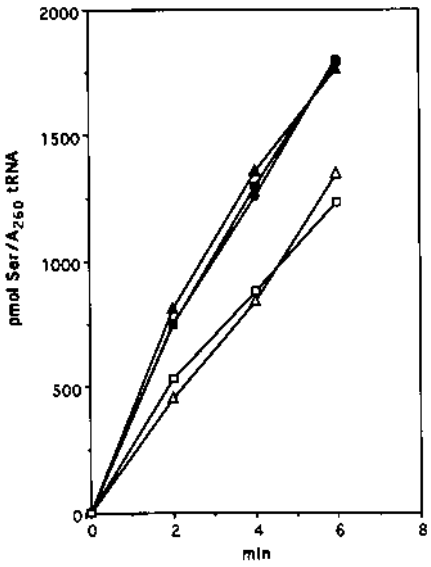

Figure 4. Inhibition assay for $tRNA^{Ser}$ serylation in HeLa cytoplasmic S100 extract. $tRNA^{Ser}$ (▲) was used at a concentration of 0.4 µM. $tRNA^{Leu}$ (■), $tRNA^{Ser}$A73, $tRNA^{Ser}$C73 and $tRNA^{Ser}$U73 (●), $tRNA^{Ser}$I73 (Δ), which by themselves are not substrates for serylation, and $tRNA^{Ser}$2-AP73 (□) were added as potential competitive inhibitors in 10-fold molar excess (4.0 µM). It was not possible to determine apparent K_m and K_i values due to the low amounts of $tRNA^{Ser}$2-AP73 and $tRNA^{Ser}$I73 available.

(Fig. 2, lane 6), whereas the reaction mixture without the oligodeoxynucleotide yielded complete $tRNA^{Leu}$ (Fig. 2, lane 7). The sequences of leucine- and serine-specific tRNA fragments at the site of covalent joining are identical (Fig. 1A and B). In all reactions formation of other than the major product was rare. When the $tRNA^{Leu}$ halves were annealed by hydrogen bonding the structure formed could be leucylated, although only to a small extent (not shown), which suggests that a correct $tRNA^{Leu}$-like tertiary structure had formed. In contrast, the product of annealing the halves of $tRNA^{Ser}$ could not be serylated. This suggests that intramolecular tertiary interactions in $tRNA^{Leu}$ and $tRNA^{Ser}$, such as those between DHU and TΨC loop nucleotides, are different in both tRNAs.

2-Aminopurine riboside but not inosine in the discriminator position 73 allows serylation with low efficiency

The serine-specific discriminator base G (Fig. 1C) contains two exocyclic functional groups which can potentially form hydrogen bonds with SerRS: (i) the NH_2 group at position two of the purine ring; (ii) the O^6-keto group. To study the functions and roles of these chemical groups either the amino or the keto group was deleted. Analogues of guanosine such as inosine and 2-aminopurine riboside were incorporated (Fig. 1C). The guanosine analogue inosine lacks the exocyclic 2-amino group, whereas in 2-aminopurine riboside the O^6-keto function was deleted. Four different kind of tRNA species were constructed by ligation with T4 RNA ligase: $tRNA^{Leu}$, $tRNA^{Ser}$, $tRNA^{Ser}$ with the modified nucleoside 2-aminopurine riboside at the discriminator base position 73 ($tRNA^{Ser}$2-AP73) and $tRNA^{Ser}$ with inosine at position 73 ($tRNA^{Ser}$I73). Complete $tRNA^{Ser}$ transcript as well as $tRNA^{Ser}$ produced by ligation can be aminoacylated with the same efficiency (Fig. 3). Corresponding results were obtained for the $tRNA^{Leu}$ transcript and for ligated $tRNA^{Leu}$ (not shown). $tRNA^{Ser}$I73 cannot be charged with serine, whereas $tRNA^{Ser}$2-AP73 can be serylated, albeit weakly (Fig. 3). $tRNA^{Ser}$ with any other natural base at position 73 cannot be aminoacylated by SerRS (Table 1; ref. 7). Thus the exocyclic 2-amino group of G73 is absolutely required for serylation.

Both $tRNA^{Ser}$2-AP73 and $tRNA^{Ser}$I73 competitively inhibit aminoacylation of human $tRNA^{Ser}$

The fact that $tRNA^{Ser}$2-AP73 is weakly serylated and $tRNA^{Ser}$I73 is not raised the question whether the latter nevertheless interacts with SerRS. For this purpose human $tRNA^{Ser}$ was aminoacylated in the presence of $tRNA^{Ser}$I73, $tRNA^{Ser}$2-AP73 or $tRNA^{Ser}$ transcripts which contained the discriminator base A, C or U ($tRNA^{Ser}$A73, $tRNA^{Ser}$C73 or $tRNA^{Ser}$U73) or $tRNA^{Leu}$ as potential inhibitors. These tRNAs were added in 10-fold molar excess with regard to the $tRNA^{Ser}$ transcript (Fig. 4). Similar competitive inhibition by $tRNA^{Ser}$2-AP73 and $tRNA^{Ser}$I73 was determined, however, no inhibition of serylation could be observed in the presence of $tRNA^{Ser}$A73, $tRNA^{Ser}$C73, $tRNA^{Ser}$U73 or $tRNA^{Leu}$ (Table 1 and Fig. 4). These results show that at least one guanine-specific group (O^6 or NH_2[2]) is needed in the purine ring for interaction with SerRS.

Table 1. Serylation and leucylation of human $tRNA^{Ser}$, $tRNA^{Leu}$ and of their discriminator base variants and inhibition of aminoacylation of human $tRNA^{Ser}$ and $tRNA^{Leu}$

tRNA	Serylation	Leucylation	Inhibition of serylation[a] of $tRNA^{Ser}$	Inhibition of leucylation[a] of $tRNA^{Leu}$
$tRNA^{Ser}$	++	–	/	(+)
$tRNA^{Ser}A73$	–	–	–	(+)
$tRNA^{Ser}C73$	–	–	–	/
$tRNA^{Ser}U73$	–	–	–	/
$tRNA^{Ser}2–AP73$[b]	(+)	/	+	/
$tRNA^{Ser}I73$[c]	–	/	+	/
$tRNA^{Leu}$	–	++	–	/
$tRNA^{Leu}C73$	–	–	/	+
$tRNA^{Leu}G73$	+	–	n.d[d]	+
$tRNA^{Leu}U73$	–	(+)	/	n.d[d]
pre-$tRNA^{Tyr}$	/	/	/	–

Symbols ++, +, (+) and – indicate aminoacylation and inhibition efficiencies respectively (see Figs 3–5; refs 6,7).
[a]Potential competitive inhibitors of aminoacylation were added in 10-fold molar excess with respect to $tRNA^{Ser}$ or $tRNA^{Leu}$.
[b]2-AP, 2-aminopurine riboside.
[c]I, inosine.
[d]Not done because the competitor tRNA is a substrate for aminoacylation.

The discriminator base A73 does not play a dominant role in interaction with leucyl-tRNA synthetase

Modified $tRNA^{Leu}$ transcripts were added to the standard aminoacylation assay in up to 10-fold molar excess with regard to $tRNA^{Leu}$. $tRNA^{Leu}$ transcripts containing residues C and G at position 73 ($tRNA^{Leu}C73$ and $tRNA^{Leu}G73$), human $tRNA^{Ser}$, $tRNA^{Ser}A73$ or a pre-$tRNA^{Tyr}$ from *Arabidopsis thaliana* with an intron were used as potential inhibitors. Addition of pre-$tRNA^{Tyr}$ had no effect on leucylation efficiency and of $tRNA^{Ser}$ and $tRNA^{Ser}A73$ had little effect (Table 1 and Fig. 5). However, addition of either $tRNA^{Leu}G73$ or $tRNA^{Leu}C73$ strongly decreased leucylation of $tRNA^{Leu}$ (Table 1 and Fig. 5), $tRNA^{Leu}C73$ having a greater effect than $tRNA^{Leu}G73$ (Fig. 5). These results demonstrate that the discriminator base of human $tRNA^{Leu}$ does not play a significant role in interaction with leucyl-tRNA synthetase (LeuRS) and suggest that a correct $tRNA^{Leu}$-specific tertiary structure is much more important.

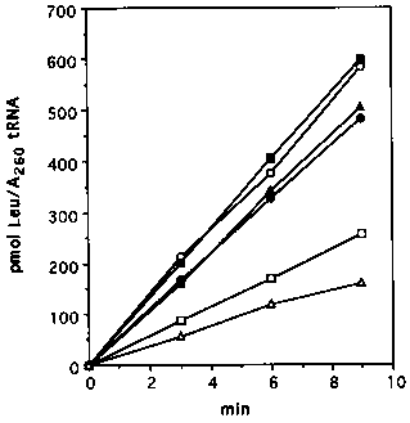

Figure 5. Inhibition assay for $tRNA^{Leu}$ leucylation in HeLa cytoplasmic S100 extract. $tRNA^{Leu}$ (■) was used at a concentration of 1.0 µM. $tRNA^{Ser}$ (▲), $tRNA^{Ser}A73$ (●), $tRNA^{Leu}G73$ (□), $tRNA^{Leu}C73$ (△) and a $tRNA^{Tyr}$ from *A.thaliana* with an intron (○), which by themselves are not substrates for leucylation, were used as potential competitive inhibitors in 10-fold molar excess (10.0 µM).

DISCUSSION

We have achieved a remarkable advance in knowledge about the different modes of interaction of $tRNA^{Ser}$ and $tRNA^{Leu}$ with their cognate synthetases by the incorporation of different base analogues into the discriminator position 73 of these tRNAs. Both functional groups, NH_2[2] and O^6, of the human $tRNA^{Ser}$ discriminator base G73 are essential for binding to SerRS. However, 2-aminopurine riboside, but not inosine, in the discriminator position 73 allowed serylation, which indicates the importance of the exocyclic 2-amino group of G73 in the catalytic step, possibly for a correct positioning of the CCA 3′-end at the catalytic site of SerRS. $tRNAs^{Ser}$ with any other natural base in position 73 did not act as competitive inhibitors and hence do not interact with SerRS. X-ray analyses of the crystallographic structures of SerRS–$tRNA^{Ser}$ complexes of *Thermus thermophilus* and *E.coli* unfortunately do not reveal any detailed data about the four single-stranded nucleotides at the 3′-end of these tRNAs (22,23).

The roles of the discriminator bases in human $tRNA^{Leu}$ and $tRNA^{Ser}$ are clearly different. Not only is $tRNA^{Leu}$ with U73 leucylated with low efficiency (8,9), in contrast to similarly modified $tRNA^{Ser}$, but $tRNA^{Leu}$ molecules with C73 or G73 strongly inhibit leucylation of human $tRNA^{Leu}$, whereas $tRNA^{Ser}$ and $tRNA^{Ser}A73$ are only weak inhibitors. This indicates the necessity of a $tRNA^{Leu}$-like tertiary structure, which seems to be more important than the correct discriminator base for binding to LeuRS. The absence of any effect of pre-$tRNA^{Tyr}$ on LeuRS (Fig. 5) indicates that non-specific inhibition did not occur under

our conditions. It should be noted that all data were obtained with impure enzyme fractions (HeLa cytoplasmic S100 extract), as is usual for many studies with human enzymes, with the advantage of a general *in vivo*-like competition between all aminoacyl-tRNA synthetases.

There is no evidence to indicate whether the functional groups of the serine-specific discriminator base G interact directly with SerRS or whether they cause a structural change in the first base pair of the acceptor stem to achieve an efficient interaction with the cognate synthetase. It has been demonstrated recently that the discriminator base plays a crucial role in influencing tRNA structure. Lee *et al.* (24) have shown that exchange of the discriminator base from A73 to either C73 or U73 makes the C in the C1:G72 base pair of mutant *E.coli* initiator tRNAs more reactive toward the single-strand-specific reagent sodium bisulfite. The activity of the enzyme Met-tRNA transformylase toward these tRNAs is also consistent with destabilization of the C1:G72 base pair. A more detailed picture is given by NMR studies of variant acceptor stem hairpins of initiator $tRNA^{fMet}$, which clearly demonstrated that a discriminator base change (A73→U) affected the conformation at the 3′-CCA end (25). However, it is not known if these influences of the discriminator bases A or U are only specific for $tRNA^{fMet}$, which has an unpaired terminal base pair C1:A72 in the wild-type form.

The discriminator base G73 has been proposed to act as a determinant of $tRNA^{Gln}$ identity (26–30). Base substitutions with pyrimidines at this position were harmful to recognition of the cognate enzyme, whereas substitution with A73 had little effect on aminoacylation. Rould *et al.* (26) have shown that in the GlnRS–$tRNA^{Gln}$ complex the exocyclic 2-amino group of G73 forms a hydrogen bond with the phosphate oxygen of the adjacent nucleotide at position 72. This interaction stabilizes a hairpin turn at the 3′-end, which positions the acceptor stem for aminoacylation. In this context it is noteworthy that both an acceptor stem microhelix^{Gln}, which is aminoacylated by GlnRS, and its variant with C73 as discriminator base, which is not aminoacylated by GlnRS, can act as competitive inhibitors of $tRNA^{Gln}$ aminoacylation (31). However, the role of the discriminator base in the yeast AspRS–$tRNA^{Asp}$ interaction differs in that the enzyme contacts the discriminator base directly (32,33). In contrast to GlnRS, AspRS approaches the tRNA from the major groove side of the helical amino acid acceptor stem and an undistorted helical conformation of the acceptor stem is maintained up to C74. In the case of an alanine minihelix the nature of the discriminator base appears to be a critical determinant of the transition state for the reaction of bound alanyl adenylate with RNA (34).

It is not known in which, or if in either, of these ways human SerRS interacts with $tRNA^{Ser}$. SerRS differs from all other aminoacyl-tRNA synthetases in an exceptional flexibility in tRNA substrate recognition and interaction, as for example in the efficient serylation of the three very different substrates $tRNA^{Sec}$, $tRNA^{Ser}$ and $tRNA^{Leu}$G73 (7). Discrimination between both $tRNA^{Sec}$ and $tRNA^{Ser}$ and the only other structurally similar tRNA, $tRNA^{Leu}$, with a long extra arm, is therefore predominantly enforced by the discriminator base.

ACKNOWLEDGEMENTS

We thank Dr F. Eckstein and especially Dr T. Tuschl for the synthesis of modified nucleosides and their phosphoramidites as well as for the preparation of chemically synthesized oligoribonucleotides, R. Amberg for stimulating discussions and Drs H. Beier and M. A. Mayo for critical reading of the manuscript. This work was supported by Deutsche Forschungsgemeinschaft (SFB 165) and Fonds der Chemischen Industrie.

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