Competition of aminoacyl-tRNA synthetases for tRNA ensures the accuracy of aminoacylation

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Received January 17, 1992; Revised and Accepted March 12, 1992

ABSTRACT

The accuracy of protein biosynthesis rests on the high fidelity with which aminoacyl-tRNA synthetases discriminate between tRNAs. Correct aminoacylation depends not only on identity elements (nucleotides in certain positions) in tRNA (1), but also on competition between different synthetases for a given tRNA (2). Here we describe in vivo and in vitro experiments which demonstrate how variations in the levels of synthetases and tRNA affect the accuracy of aminoacylation. We show in vivo that concurrent overexpression of Escherichia coli tyrosyl-tRNA synthetase abolishes misacylation of supF tRNA^Glu with glutamine in vivo by overproduced glutamyl-tRNA synthetase. In an in vitro competition assay, we have confirmed that the overproduction mischarging phenomenon observed in vivo is due to competition between the synthetases at the level of aminoacylation. Likewise, we have been able to examine the role competition plays in the identity of a non-suppressor tRNA of ambiguous identity, tRNA^Glu. Finally, with this assay, we show that the identity of a tRNA and the accuracy with which it is recognized depend on the relative affinities of the synthetases for the tRNA. The in vitro competition assay represents a general method of obtaining qualitative information on tRNA identity in a competitive environment (usually only found in vivo) during a defined step in protein biosynthesis, aminoacylation. In addition, we show that the discriminator base (position 73) and the first base of the anticodon are important for recognition by E. coli tyrosyl-tRNA synthetase.

INTRODUCTION

The fidelity of the translation step in protein biosynthesis is dependent on the ability of an aminoacyl-tRNA synthetase to discriminate with remarkable selectivity amongst many structurally similar tRNAs. At least two factors are important in determining the accuracy of aminoacylation: (i) 'identity elements' in tRNA denote nucleotides in certain positions crucial for positive interactions with the protein or negative interactions preventing non-cognate association (reviewed in 1), and (ii) the occurrence in vivo of competition between aminoacyl-tRNA synthetases for a particular tRNA which may have ambiguous identity (reviewed in 3).

Genetic (1,4–6), biochemical (7–10) and biophysical approaches (11–13) are being employed to determine the nature of tRNA identity for many acceptor RNAs chiefly in E. coli. For the tRNAs used in this study, glutamine (14) and tyrosine (15, reviewed in 16) identity elements have been shown to be located in the anticodon, the discriminator base in position 73 (17), the acceptor helix (for glutamine) and in the extra arm (for tyrosine).

Transfer RNA molecules are structurally very similar and have affinity for both cognate and non-cognate synthetases (e.g., 18,19). As expected, competition between aminoacyl-tRNA synthetases has been shown to be crucial to the accuracy of protein biosynthesis, presumably by affecting the accuracy of the aminoacylation process directly (2,20–22). This phenomenon may be a particularly important factor in the aminoacylation of tRNAs with ambiguous identity (2,22). All conclusions about tRNA identity derived from in vivo approaches already include the effect of competition (1,6,23–25) although for detailed studies variations in concentrations of tRNA and synthetases should be made (22). There are however several limitations to the in vivo systems as tRNA identity can only be studied in a suppressor background: in the case of amber suppressors, there is an inherent bias toward recognition by GlnRS and LysRS (reviewed in 1), while in the case of the missense suppression initiation assays (23–27), 'initiation competence' is required for translation. Furthermore, the results of the in vivo assays reflect the participation of the tRNAs in all the steps of protein biosynthesis, not just aminoacylation-tRNA formation. Finally, the in vivo assays are extremely sensitive. Although attempts have been made to correlate the kinetic parameters obtained in vitro with the results of in vivo Dihydrofolate Reductase (DHFR) experiments (22), the competition phenomenon has not been directly examined by in vitro aminoacylation using purified components.

Here we describe an in vitro competition system which allows us to assess the effect of competition on the accuracy of aminoacylation of in vivo made suppressor as well as non-suppressor tRNAs. The assay also permits the examination of the relative affinities of various synthetases for a given tRNA.
The competition phenomenon appears to be particularly relevant for the E. coli GlnRS system which we have chosen to study. Not only is the in vivo ratio of GlnRS:tRNA\(^{\text{Glu}}\) approximately 1:1 (28), but also it has been demonstrated that the level of GlnRS influences the accuracy of translation in vivo (2). The tRNAs chosen for these experiments (Fig. 1) are supF tRNA\(^{\text{Tr}}\) which was shown to be mischarged by GlnRS when GlnRS was overproduced (2) and tRNA\(^{\text{Glu}}\), as the amber suppressor derived from tRNA\(^{\text{Glu}}\) was shown to be mischarged in vivo by glutamine (reviewed in 1).

**MATERIALS AND METHODS**

**General**

Purified E. coli tRNAs (with the specific acceptor activity) were purchased from Subrident RNA in the case of tRNA\(^{\text{lu}}\) (1450 pmol/\(\mu\)g) and tRNA\(^{\text{Tr}}\) (1500 pmol/\(\mu\)g), while tRNA\(^{\text{In}}\) (1500 pmol/\(\mu\)g) was prepared as described (29). Highly purified E. coli aminocyl-tRNA synthetases (of the specific activity) were obtained as follows: GlnRS (886 nmol/mg·min) was purified as described (30); GluRS (1005 nmol/mg·min) and MetRS were the gifts of U. Michelsen and R. Basavappa, respectively. [\(^{14}\)C]Amino acids and enzymes used for cloning and mutagenesis were obtained commercially. Aminocyl-tRNA synthetase purifications were carried out at 4°C unless otherwise noted. Media for bacterial growth were standard (31).

**Strains and plasmids**

E. coli strains used in this study include: BT32 (F\(^{-}\), lacZ\(_{1000}\), metE\(_{33}\), trpE\(_{33}\), str\(^{+}\), rpsL\(_{5}\), bubC\(_{10}\), supF\(_{1}\), BL21(DE3) (33), CJ236 and a \(\lambda\) derivative of DH1. The plasmids used for overproducing GlnRS and TyrRS, pRS11 and pBR7277T (gift of D. Barker), respectively, have been described previously (34). Plasmid pJ1A11 (gift of J. Arnez) is derived from pUC19 and contains an EcoRI BamHI fragment with an artificial supF tRNA\(^{\text{Tr}}\)G73 gene constructed with the T7 promoter positioned such that transcription starts with G1 of the mature tRNA. The supF tRNA\(^{\text{Tr}}\)G73 gene (in plasmid pJ7E, a construct analogous to pJ1A11) was obtained by site-directed mutagenesis (35) from supF tRNA\(^{\text{Tr}}\)G73.

**Preparation of supF tRNA\(^{\text{Tr}}\)G73 and supF tRNA\(^{\text{Tr}}\)G73 by in vivo overexpression**

E. coli strain BL21(DE3) was transformed with the plasmid pJ7E or pJ1A11. Induction of T7 RNA polymerase was as described (33) with the exception that ampicillin was boosted at the time of IPTG induction. The cells (from 750 ml cultures) were collected by centrifugation and resuspended in 10 ml of 10 mM magnesium acetate, 10 mM Tris, pH 7.5. An equal volume of water-saturated phenol was added and the mixture shaken gently at room temperature for 20 min. After a low-speed centrifugation, the aqueous phase was removed and the phenol layer re-extracted with TE, pH 7.5. The aqueous phases from the two extractions were then combined, re-extracted with phenol and then ethanol precipitated. The RNA was resuspended in 10 ml Tris, pH 9.0 and incubated at 37°C for 30 min to deacylate the tRNA. The deacylated RNA was re-precipitated and resuspended in water. After a low-speed spin (to remove any insoluble material) the supernatant was applied to 25 ml DEAE-cellulose which had been pre-equilibrated with 0.2 M NaCl. Bound tRNA was eluted with 1.0 M NaCl and ethanol precipitated. Approximately 2 mg of crude tRNA was loaded on a 40 cm \(\times\) 20 cm \(\times\) 1 mm polyacrylamide (8%) gel containing 7M urea and the electrophoresis carried out until the xylene cyanol marker had traveled 30 cm. This gave good separation of short extra arm tRNAs from those with long extra arms. The supF tRNA\(^{\text{Tr}}\)G73 containing band was excised and eluted (in 0.5 M Tris, pH 7.0, 0.1% SDS, 0.1 mM EDTA, 1 mM MgCl\(_2\)) by shaking at room temperature after a rapid freeze-thaw. The eluent was then passed over a G-50 column. Levels of overexpression of supF obtained ranged from 30-40% of total tRNA following induction. When the supF tRNA\(^{\text{Tr}}\)G73 preparation was charged in vitro with tyrosine a plateau of 151 pmol/A\(_{260}\) was reached very quickly. As nucleotide changes in position 73 are detrimental to tyrosylation (see below) we estimate that there is a maximum of 10% wild-type tRNA\(^{\text{Tr}}\) contamination.

**Purification of TyrRS**

E. coli strain DH1 \(\lambda^{+}\)/pBR7277T was grown in LB medium containing ampicillin (0.1 mg/ml) to an A\(_{500}\)\(=\)1.0. The ampicillin was boosted halfway through the growth. The cells were harvested and washed with buffer A (20 mM potassium phosphate, pH 7.0, 10 mM 2-mercaptoethanol). The cells were re-pelleted, resuspended in a total of 90 ml of buffer A and then disrupted by sonication. The mixture was centrifuged at 15,000 g for 30 min and the resulting supernatant was centrifuged for 1 hr at 100,000 g. The final supernatant (S-100) was dialyzed against buffer A containing 10% glycerol and then applied to a 50 ml DEAE-cellulose column (pre-equilibrated with buffer A). After elution with a 0-250 mM KCl gradient in buffer A, the fractions which contained TyrRS activity were pooled and dialyzed against buffer B (20 mM potassium phosphate, pH 7.8, 10 mM 2-mercaptoethanol) containing 20% glycerol. This fraction was loaded on a 50 ml DEAE-A50 Sephadex column (pre-equilibrated in buffer B) and eluted with a 100-250 mM KCl gradient in buffer B. The fractions with TyrRS activity were pooled and dialyzed against buffer C (10 mM KCl, 20 mM potassium phosphate, pH 7.0, 10 mM 2-mercaptoethanol). The pooled sample was then loaded on a hydroxyapatite Ultrogel FPLC column and eluted at room temperature with a gradient of 60-180 mM potassium phosphate, pH 7.0. This column separated TyrRS activity from the remaining GlnRS activity. The TyrRS preparation appeared as one band (0.03 \(\mu\)g applied) on a silver-stained SDS-PAGE gel and had a specific activity of 1467 nmol/mg·min.

**In vivo competition assays**

E. coli strain BT32 was transformed first with pRS11 (a pACYC184 derivative carrying the gene encoding GlnRS) and then with either pBR7277T (a pBR322 derivative containing the gene encoding TyrRS) or with pBR322. The strain BT232/pRS11/pBR7277T grows very slowly. Due to the instability of this strain, it was necessary to maintain double antibiotic selection to prevent segregation (observed on MacConkey lactose plates). Four individual transformants of both BT32/pRS11/pBR7277T and BT32/pRS11/pBR322 were used to inoculate minimal A medium supplemented with ampicillin (5.0 \(\mu\)g/ml), chloramphenicol (3.4 \(\mu\)g/ml) and casamino acids (0.2%). The cultures were grown overnight at 37°C and diluted 1:10 into fresh medium supplemented as above and containing 1 mM IPTG (t=0). The cell densities of all of the cultures at the time of induction (t=0) were comparable. At two hr intervals, a 1.0 ml sample was removed for A\(_{600}\) determination and then split into duplicate 0.5 ml samples. These were added to 0.5 ml...
Z buffer for β-galactosidase assays. Each point (in Fig. 2) is an average of four independent isolates and differences in A$_{600}$ were taken into account in calculating the β-galactosidase units (31). The level of TyrRS overproduction was assayed by comparing TyrRS activity in mini-protein preparations (36) of strains BT32/pRS11/pBRTyrTS and BT32/pRS11/pBR322.

**Aminoacylation assays**

Reaction mixtures (60 μl) contained 100 mM sodium cacodylate, pH 7.0, 10 mM magnesium acetate, 2–4 mM ATP, and $^{[14]}$C-amino acid (specific activities varied from 22–720 cpm/pmol). In all cases, ATP and the amino acid were at or near saturating concentrations. For the *in vitro* competition experiments, cold amino acid specific for the competing synthetase was added at saturating or near saturating concentrations (600 μM Glu and 120 μM Tyr). Enzymes were diluted in enzyme dilution buffer (50 mM sodium cacodylate, pH 7.0, 20 mM 2-mercaptoethanol, 10% (v/v) glycerol and 1mg/ml bovine serum albumin) and the amount added did not exceed 10% of the reaction volume. Incubation was at 37°C for the appropriate times. The reaction mixture or an aliquot was spotted onto 3MM filters (in case of charging with $^{[14]}$C-Tyr the filters were pre-soaked in 1.0–2.5 mM $^{[14]}$C-Tyr to prevent non-specific binding of the radioactive free amino acid), washed in 5% TCA several times, soaked in 95% ethanol, dried and analyzed by liquid scintillation counting.

**RESULTS**

**In vivo competition**

We wished to provide further support for the idea that the accuracy of aminoacylation *in vivo* is critically dependent on the relative concentrations of tRNAs and their cognate aminoacyl-tRNA synthetases (2,21,22) and that the amount of free, uncomplexed tRNA or enzyme is crucial for tRNA to be misacylated. For the *in vivo* studies in *E. coli*, we employed a system which takes advantage of the *lacZ* amber mutation in the β-galactosidase gene; active enzyme can be formed by glutamine but not by tyrosine insertion at the site of the amber mutation. Thus, strain BT32 (lacZ*$_{1000}$*, supF) is phenotypically Lac−, unless supF tRNA$^{{	ext{Tyr}}}$ is misacylated with glutamine (32).

Earlier experiments had shown that supF tRNA$^{{	ext{Tyr}}}$ was mischarged by GlnRS when this enzyme was overproduced and that this effect could be abolished when the cognate substrate, tRNA$^{{	ext{Gln}}}$, was overexpressed concurrently (2). We then asked if overproduction mischarging of supF tRNA$^{{	ext{Tyr}}}$ by GlnRS could be abolished if TyrRS was concurrently overproduced, as in this case supF tRNA$^{{	ext{Tyr}}}$ would be complexed with TyrRS.

The experiment made use of two strains; BT32/pRS11/pBRTyrTS and BT32/pRS11/pBR322. The first strain contains two plasmids overexpressing independently GlnRS (pRSl 1) and TyrRS (pBRTyrTS). The latter strain overexpresses only GlnRS. The results (Fig. 2, top) demonstrate that when GlnRS alone is overproduced, β-galactosidase activity, and thus the amount of mischarging, increases with cell density. However, concurrent overproduction of TyrRS gives rise to a much slower growing strain (note the difference in the growth curves for Fig. 2 top and bottom) and effectively abolishes mischarging of supF tRNA$^{{	ext{Tyr}}}$ (Fig. 2, bottom). The β-galactosidase activity, which is approximately the same in both strains at the beginning of the experiment, is about 80-fold lower in the strain overproducing both GlnRS and TyrRS after 8 hr (see different scales in top and bottom panel of Fig. 2) and actually decreased with increasing cell density. Assays of the mini-protein extracts (36) from these strains (grown for 18 hr) showed that GlnRS and TyrRS are overproduced 30- and 10-fold, respectively. Thus, a 10-fold overproduction of TyrRS is enough to abolish mischarging of supF tRNA$^{{	ext{Tyr}}}$ caused by a 30-fold elevated GlnRS level.

**Aminoacylation**

Since the *in vivo* misacylation assay is based on functional nonsense suppression which involves all the steps of protein biosynthesis, the *in vivo* competition experiments do not allow us to attribute the decrease in mischarging directly to the effect competition has on the accuracy of aminoacylation. However, the fact that the increase in accuracy of translation occurs when overexpression of either the cognate tRNA (tRNA$^{{	ext{Gln}}}$) for the mischarging enzyme (2) or the cognate enzyme (TyrRS) for the mischarged tRNA (supF tRNA$^{{	ext{Tyr}}}$) is concurrent with the overproduction of GlnRS (Fig. 2) argues that it is the accuracy of the aminoacylation event itself that is enhanced. In order to show specifically that the accuracy of aminoacylation is dependent...
shows that while tRNA\textsubscript{Glu} can be mischarged it is 1
The mischarging with glutamine of the three tRNA\textsuperscript{acceptors}
Tyr\textsubscript{73} as compared with wild-type tRNA\textsubscript{Tyr} charging with tyrosine. The reduced affinity of TyrRS for
supF discriminator base (position 73) of the suppressor tRNA abolishes
Tyr\textsubscript{Tyr}G73 (Fig. 1). As Table I shows, the
\textsuperscript{73}tRNA\textsuperscript{species} is evident and that each change (Q34 — C in the
anticodon are involved in the tRNA recognition by TyrRS. In
this in more detail we determined the glutaminylation and/or
in vitro (37) could be mischarged by GlnRS
T7 RNA polymerase transcription). In our experiments with TyrRS, we found that TyrRS competes
\textsuperscript{in vitro} competition assays
We wanted to test whether the effects of the competition by
synthetases for tRNA observed \textit{in vivo} could be duplicated \textit{in vitro}. Thus, two \textit{in vitro} competition experiments were carried
out; one in which GluRS was used to prevent Gln-tRNA\textsubscript{Glu} formation, and the other employing TyrRS to block Gln-
tRNA\textsubscript{Tyr} formation. For this purpose, aminoacylation of tRNA\textsubscript{Glu} and the tRNA\textsubscript{Tyr} species by GluRS with glutamine was examined in the presence and absence of the cognate aminoacyl-
tRNA synthetase (Fig. 3 and 4).
GluRS competes extremely well with GluRS for tRNA\textsubscript{Glu} and
could abolish almost all observable mischarging so long as sufficient quantities of GluRS were added (Fig. 3). All observable
mischarging was abolished when 70 nM GluRS competed with 2 \mu M GlnRS for 5 \mu M tRNA\textsubscript{Glu} (Fig. 3A). Most likely, the
small amount of residual charging observed under these conditions is due to contaminating tRNA\textsubscript{Glu} in the commercial
tRNA\textsubscript{Glu} preparation.
Is the increase in accuracy of aminoacylation due to competition
a specific effect, \textit{i.e.}, must the competing synthetase be the
cognate one for the mischarged tRNA? We chose MetRS, which
does not charge tRNA\textsubscript{Glu} (data not shown), as the non-specific
competitor. As can be seen in Fig. 3, mischarging of tRNA\textsubscript{Glu} was unaltered in the presence of MetRS. Clearly, a competing
synthetase must have significant affinity for the particular tRNA
species. In our experiments with TyrRS, we found that TyrRS competes
effectively with GluRS for supF tRNA\textsubscript{Tyr}A73 (Fig. 3) under the
conditions employed. However, this applies only to the real 'dual
TyrRS and GluRS can decrease the levels of mischarging by the competition occurs at the aminoacylation step and that both enzyme which determines the accuracy with which aminoacyl-tRNA formation occurs.

\[ \text{Tyr}^{\text{supF}} \text{1} \] as well as free tRNA cognate synthetase, TyrRS. Thus, intracellularly, it is the amount sequestering the mischarged \( \text{Tyr}^{\text{supF}} \text{tRNA}_{y} \), it can also be abolished by GlnRS upon addition of TyRS could be seen in the assays done in the absence of MetRS (data not shown).

It is interesting to note the relative ability of TyrRS and GluRS to compete with GlnRS for their respective substrates. While a 75% decrease in glutaminyltRNA formation of tRNA\(^{\text{Glu}}\) is observed in the presence of >720-fold molar excess of GlnRS to GluRS, a 60% decrease is observed with a 2-fold molar excess of GlnRS to TyrRS. Presumably, GluRS competes more effectively than TyrRS because GluRS has a higher affinity for tRNA\(^{\text{Glu}}\) than TyrRS for \( \text{supF} \) tRNA\(^{\text{Ty}}\).


tRNA identity' \( \text{supF} \) tRNA\(^{\text{Ty}}\)A73 (Table I); the glutaminyltRNA of \( \text{supF} \) tRNA\(^{\text{Ty}}\)G73 could not be suppressed by TyrRS under the experimental conditions used. This was expected from the apparent inability of TyrRS to recognize and aminoacylate \( \text{supF} \) tRNA\(^{\text{Ty}}\)G73 in vitro (Table I). In these experiments, MetRS was added concurrently with GlnRS to all the assays in order to rule out the possibility of non-specific competition. The same decrease (60%) in mischarging of \( \text{supF} \) tRNA\(^{\text{Ty}}\) by GlnRS upon addition of TyRS could be seen in the assays done in the absence of MetRS (data not shown).

It is interesting to note the relative ability of TyrRS and GluRS to compete with GlnRS for their respective substrates. While a 75% decrease in glutaminyltRNA formation of tRNA\(^{\text{Glu}}\) is observed in the presence of >720-fold molar excess of GlnRS to GluRS, a 60% decrease is observed with a 2-fold molar excess of GlnRS to TyrRS. Presumably, GluRS competes more effectively than TyrRS because GluRS has a higher affinity for tRNA\(^{\text{Glu}}\) than TyrRS for \( \text{supF} \) tRNA\(^{\text{Ty}}\).

**DISCUSSION**

The fact that elevated levels of TyrRS in vivo can abolish the mischarging of \( \text{supF} \) tRNA\(^{\text{Ty}}\)A73 by overproduced GlnRS have confirmed that the balance of the amounts of aminoacyl-tRNA synthetase and tRNA is critical for faithful protein translation. Not only can the overproduction mischarging phenomenon be abolished by complexation of the GlnRS by overexpressing its cognate substrate tRNA\(^{\text{Glu}}\), it can also be abolished by sequestering the mischarged \( \text{supF} \) tRNA\(^{\text{Ty}}\) by overexpressing its cognate synthetase, TyrRS. Thus, intracellularly, it is the amount of free (uncomplexed) substrate, \( \text{supF} \) tRNA\(^{\text{Ty}}\), as well as free enzyme which determines the accuracy with which aminoacyl-tRNA formation occurs.

The in vitro competition experiments demonstrate directly that the competition occurs at the aminoacylation step and that both TyrRS and GluRS can decrease the levels of mischarging by GlnRS of \( \text{supF} \) tRNA\(^{\text{Ty}}\) and tRNA\(^{\text{Glu}}\) respectively. However, it appears that the relative affinities of the synthetases, as well as their relative levels, may play a role in the accuracy of aminoacyl-tRNA formation. GlnRS has a greater affinity for tRNA\(^{\text{Glu}}\) and thus can compete more effectively against GluRS for tRNA\(^{\text{Glu}}\) than TyrRS can for \( \text{supF} \) tRNA\(^{\text{Ty}}\). Likewise, TyrRS cannot compete with GlnRS for the discriminator mutant of \( \text{supF} \) tRNA\(^{\text{Ty}}\) (\( \text{supF} \) tRNA\(^{\text{Ty}}\)G73), for which it has little or no apparent affinity. However, it is expected that TyrRS would be as effective a competitor for wild-type tRNA\(^{\text{Ty}}\) as GluRS is for tRNA\(^{\text{Glu}}\). The competition effect is a specific one in that an unrelated synthetase, MetRS, cannot compete with GlnRS for any of the mischarged tRNAs tested. Although we have not directly tested whether an editing activity of GlnRS and/or TyrRS accounted for the observed decrease in the mischarging of their respective cognate tRNAs by GluRS, we consider this unlikely as neither of the synthetases in question has been demonstrated to have a proofreading activity (reviewed in 40). In fact, it has been shown that TyrRS compensates for its lack of editing activity by employing very specific mechanisms for recognition and activation of tyrosine (41) and GluRS, like GluRS, has a very high \( K_M \) for its cognate amino acid (37,42,43).

Unlike the standard in vivo methods used to study tRNA identity, in vitro competition can be applied to non-suppressor tRNAs (thus eliminating any bias toward recognition of the suppressor anticodons by a specific subset of synthetases) and allows one to examine the aminoacylation event exclusively. The in vitro competition technique does not require additional purified enzymes or substrates beyond those needed for current in vitro methods. Thus, it could be used to examine the effect of mutations, even anticodon mutations, on tRNA identity, e.g., the identity of tRNA\(^{\text{Ty}}\)-G73 (anticodon QUA) could be compared with that of \( \text{supF} \) tRNA\(^{\text{Ty}}\)G73 (anticodon CUA). In addition, those anticodon mutants which do not confer methionine or other 'initiation-competent' amino acid identity and thus are

**Figure 3.** Addition of GluRS diminishes in vitro mischarging of tRNA\(^{\text{Glu}}\) by GlnRS. The concentration of tRNA\(^{\text{Glu}}\) was 5.0 \( \mu \text{M} \), while the concentration of GlnRS was 2.0 \( \mu \text{M} \). The specific activity of the \([\text{\textsuperscript{14}}\text{C}]\)glutamine was 22 cpm/pmol. GluRS, and MetRS when present, was added simultaneously with GlnRS in the following amounts: (0) 0 pmol, A) 4.23 pmol, B) 0.85 pmol, C) 0.17 pmol and MetRS 0.67 pmol.

**Figure 4.** Effect of TyrRS addition on in vitro mischarging by GlnRS of tRNA\(^{\text{Ty}}\) species. Both of the \( \text{supF} \) tRNA\(^{\text{Ty}}\) species were present at 1.0 \( \mu \text{M} \) concentrations, which was a two-fold molar excess of tRNA over GlnRS. In all cases, when TyrRS (0) 0 pmol, TyrRS 17.0 pmol) was present, it was added simultaneously with the GlnRS and MetRS. The measured specific activities of the \([\text{\textsuperscript{14}}\text{C}]\)glutamine varied slightly between experiments (77–92 cpm/pmol).
not assayable in the initiation missense suppression assays (25,27) could be analyzed in this way. Finally, mutants of aminoacyl synthetases could also be examined in the in vitro competition system.

The aminoacylation results for the three tRNA<sub>Tyr</sub> species demonstrate that the discriminator base and the first base of the anticodon are important for recognition by E. coli tyrosyl-tRNA synthetase (Table I). This was already noted for the discriminator position (15,38,39), while the second position of the anticodon has also been shown to be important for tyrosine charging (15).

ACKNOWLEDGEMENTS

We thank Kelley Rogers and Sergey Beresten for helpful scientific discussions. This work was supported by a grant from NIH.

REFERENCES


2852 Nucleic Acids Research, Vol. 20, No. 11