Biochimie (1994) 76, 1183–1191
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tRNA-guanine transglycosylase from *Escherichia coli*: Recognition of dimeric, unmodified tRNA^{Tyr}

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(Received 5 May 1994; accepted 20 September 1994)

Summary — In order to probe the interaction between tRNA and the tRNA hypermodifying enzyme, tRNA-guanine transglycosylase (TGT) from *Escherichia coli*, we have undertaken the generation of *E coli* tRNA^{Tyr} and analogues. During efforts to adapt currently available *in vitro* transcription techniques we encountered difficulties attributable to dimerization of the tRNA products. *E coli* tRNA^{Tyr} has previously been characterized for its ability to form a dimer in solutions of suitable salt concentrations at appropriate temperatures (Yang SK, Söll DG, Crothers DM (1972) *Biochemistry* 11, 2311–2320; Rordorff BF, Kearns DR (1976) *Biochemistry* 15, 3320–3330). We have applied similar techniques to our unmodified analogue of *E coli* tRNA^{Tyr} and produced both monomeric and dimeric forms of *E coli* tRNA^{Tyr}. In this report we find that the dimer does serve as a substrate for modification by TGT. While both the conformers are equal in terms of V_{max} (within experimental error) a 2.5-fold increase in K_M occurs when going from monomer to dimer. This suggests that TGT preferentially binds the monomer but once either conformer is bound will catalyze the modification reaction equally well. We have also compared the results for the two conformers to our previous data of an RNA minihelix corresponding to the anticodon arm of *E coli* tRNA^{Tyr}. Here we find that our earlier conclusion, that the recognition elements for TGT are localized within the anticodon arm of cognate tRNAs, is supported.

queuine / transglycosylase / tRNA / modified nucleotide / band shift assay

Introduction

Molecular recognition is a critically important process which is necessary for enzymes to distinguish substrate molecules from structurally similar molecules. The tRNAs and the various enzymes with which they interact provide particularly good examples of this phenomenon. In these cases recognition, and more importantly discrimination between cognate and non-cognate tRNAs, can be quite difficult due to the large degree of conserved sequence [1] and the common tertiary structure [2] in tRNAs.

One enzyme which encounters this situation is tRNA-guanine transglycosylase (TGT, EC 2.4.2.29) [3]. In *Escherichia coli*, this enzyme catalyzes the replacement of the anticodon guanine (guanine 34) in tRNAs Tyr, Asp, Asn, and His with 7-aminomethyl-7-deazaguanine, preQ$_1$. Ultimately, preQ$_1$ is converted to the hypermodified base queuine. In order to investigate the nature of this modification we undertook the synthesis of an unmodified analogue of *E coli* tRNA^{Tyr} (fig 1) *via* the *in vitro* transcription method suggested by Milligan and Uhlenbeck [4]. As a consequence of conditions during transcription and purification, two distinct tRNA conformers were obtained. These conformers appeared to correspond to monomeric and dimeric tRNA.

Dimerization has been demonstrated in heterogeneous RNA mixtures [5, 6] and characterized for specific tRNAs including: yeast tRNA^{Ser} [7], tRNA^{Gly} [8] and tRNA^{Ala} [9], and the most relevant to this work, *E coli* tRNA^{Tyr} [10]. While various groups have studied the effects of aggregate formation in several enzyme systems the biological significance of such interactions has not yet been fully established.

Loehr and Keller [9] found that alanyl-tRNA synthetase was able to correctly charge dimers of yeast tRNA^{Ala} with alanine and proposed a structure for a possible tRNA^{Ala} dimer conformation. Investigations with yeast tRNA^{Ser} produced analogous results in that

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Abbreviations: HEPES, hydroxyethylpiperazine-ethylsulfonate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; NTP, ribonucleotide triphosphate; ECY2M, *E coli* tRNA^{Tyr} monomeric form; ECY2D, *E coli* tRNA^{Tyr} dimeric form; TGT, tRNA-guanine transglycosylase.

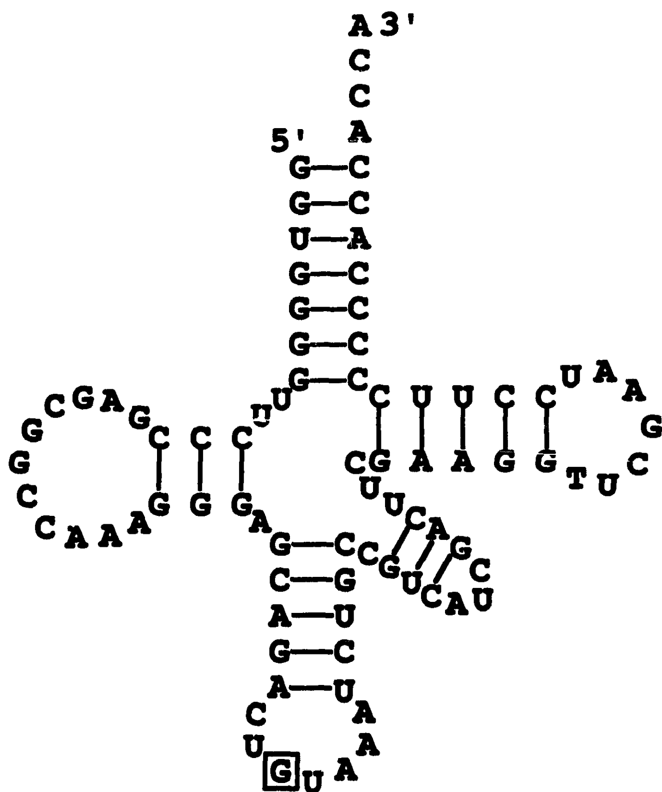


Fig 1. The primary sequence and secondary structure of *E coli* tRNA^{Tyr}. Guanosine-34 is highlighted.

aggregation of the tRNA species did not seem to inhibit the aminoacylation by the cognate aminoacyl-tRNA synthetase [7]. The researchers surmised, in both instances, that the motifs related to recognition were maintained in the aggregate forms. On the contrary, Yang *et al* [10] found that, once formed, a dimer of *E coli* tRNA^{Tyr} was no longer capable of aminoacylation by tyrosyl-tRNA synthetase. In addition, when the dimer was heated in an appropriate buffer system the monomer tRNA could be reformed and aminoacylation proceeded. Finally, a structure for the tRNA^{Tyr} dimer was proposed.

Subsequently, a proton NMR study of the base pairing in the *E coli* tRNA^{Tyr} dimer and monomer led to a solution structure model for the dimer being proposed (fig 2) [11]. In this structure an extended, hybrid double helix is formed from the acceptor stems and TΨC arms of each individual tRNA molecule while the anticodon arms of each monomer remain intact.

In previous work [12] we established that an RNA minihelix analogous to the anticodon arm of *E coli* tRNA^{Tyr} acts as a substrate for modification by TGT. Since the proposed dimer maintains the structure of the anticodon arms we endeavored to investigate the nature of the interaction between TGT and an unmodified dimer of *E coli* tRNA^{Tyr}.

Materials and methods

Reagents

Chemical reagents were purchased from Sigma, Boehringer Mannheim, and Gibco BRL. Nucleoside triphosphates and Rnasin were from Boehringer Mannheim. *Bst* NI restriction endonuclease was from Stratagene. 8-[^{14}C] Guanine was from Moravek Biochemicals. TGT was isolated from an overexpressing clone as described previously [13]. T7 RNA polymerase was isolated from *E coli* BL21/pAR1219 following the procedure of Grodberg and Dunn [14].

Preparation and purification of the tRNA transcript

Unmodified *E coli* tRNA^{Tyr} was produced *via* the transcription of double-stranded DNA template in an *in vitro* system as previously described [12].

Formation of ECY2 conformers

Monomerization and dimerization is based on the work of Yang *et al* [10]. The monomer of *E coli* tRNA^{Tyr} (ECY2M) was formed in 5 mM HEPES (pH 7.5) and 400 μM $MgCl_2$ with the concentration of tRNA varying from 20 to 40 μM. Monomerization proceeds by incubating the tRNA at 68°C for 1 h and then cooling quickly to 4°C and maintaining at 4°C for 1 h. The dimer (ECY2D) is formed in a buffer containing 5 mM HEPES (pH 7.5) and 0.5 to 1 M NaCl with the concentration of tRNA in an optimal range of 50 to 100 μM. Dimerization occurs when the tRNA is incubated at 50°C for 4 h and then cooled quickly to 4°C and maintained for 1 h.

Gel filtration chromatography

The chromatography was carried out on a Pharmacia FPLC system using a Superose 12 column (HR 10/30, Pharmacia). The samples were injected onto the column, and eluted isocratically in 10 mM HEPES (pH 7.5), 1 mM EDTA, and 250 mM NaCl. The peak fractions were collected and subjected to polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis

All electrophoretic analyses were performed on a PhastSystem (Pharmacia) following PhastSystem protocols. Native protein gels were run by applying the samples and standards directly on an 8 to 25% gradient gels (Pharmacia PhastGel) and were electrophoresed using PhastGel native buffer strips (880 mM L-alanine, 250 mM Tris (pH 8.8), 2% agarose). For native tRNA gels, the samples were directly applied to 20% homogeneous gels (Pharmacia PhastGel) and electrophoresed using native buffer strips. In the case of tRNA denaturing gels, high density gels (Pharmacia PhastGel) were prepared by saturating with TBE/urea buffer (8.9 mM Tris base, 8.9 mM boric acid, 0.2 mM EDTA, 7 M urea (pH 8.0) and the samples were prepared by mixing the tRNA 1:1 with RNA loading buffer (10 M urea, 5 mM Tris pH 8.0), 5 mM boric acid, 1 mM EDTA, 0.01% xylene cyanole, 0.01% bromophenol blue) and

heating at 70°C for 10 min. The samples were then applied to the gel and electrophoresed using TBE buffer strips (8.9 mM Tris base, 8.9 mM boric acid, 0.2 mM EDTA, 2% agarose). For the gel shown in figure 6, *ca* 5 µg of protein (4 µl of incubation mixture) was loaded in each lane. TGT (15 µM) was incubated for 15 min at 37°C in 100 mM HEPES (pH 7.5), alone (lane 2) and + 10 µM tRNA ECY2 (lane 4). Both of the samples were divided into two equals volumes and ribonuclease A (50 µg/ml final concentration) was added to one sample of each (lanes 3, 5). All four samples were incubated for an additional 15 min at 37°C. Upon completion of electrophoresis, protein gels were stained with Coomassie blue and tRNA gels were stained with ethidium bromide and visualized on a UV transilluminator.

Kinetic assays

The kinetic parameter determinations were performed in a buffer consisting of 100 mM HEPES at pH 7.5,10 mM $MgCl_2$, and 100 mM DTT. The concentration of [8-^{14}C]-guanine was maintained at 10 µM while the concentration of the tRNA conformers was varied from 0.5 µM to 20 µM. The general procedure included incubating a 400 µl mixture of buffer, substrate, and 12.6 µg/ml TGT at 37°C. At 2 min intervals 75 µl aliquots were removed from the reaction mixture. The aliquots were quenched in 5% TCA and filtered onto glass fiber filters (Whatman, GF/C). The filters were then washed three times with 5% TCA and once with ethanol, dried, and quantitated *via*

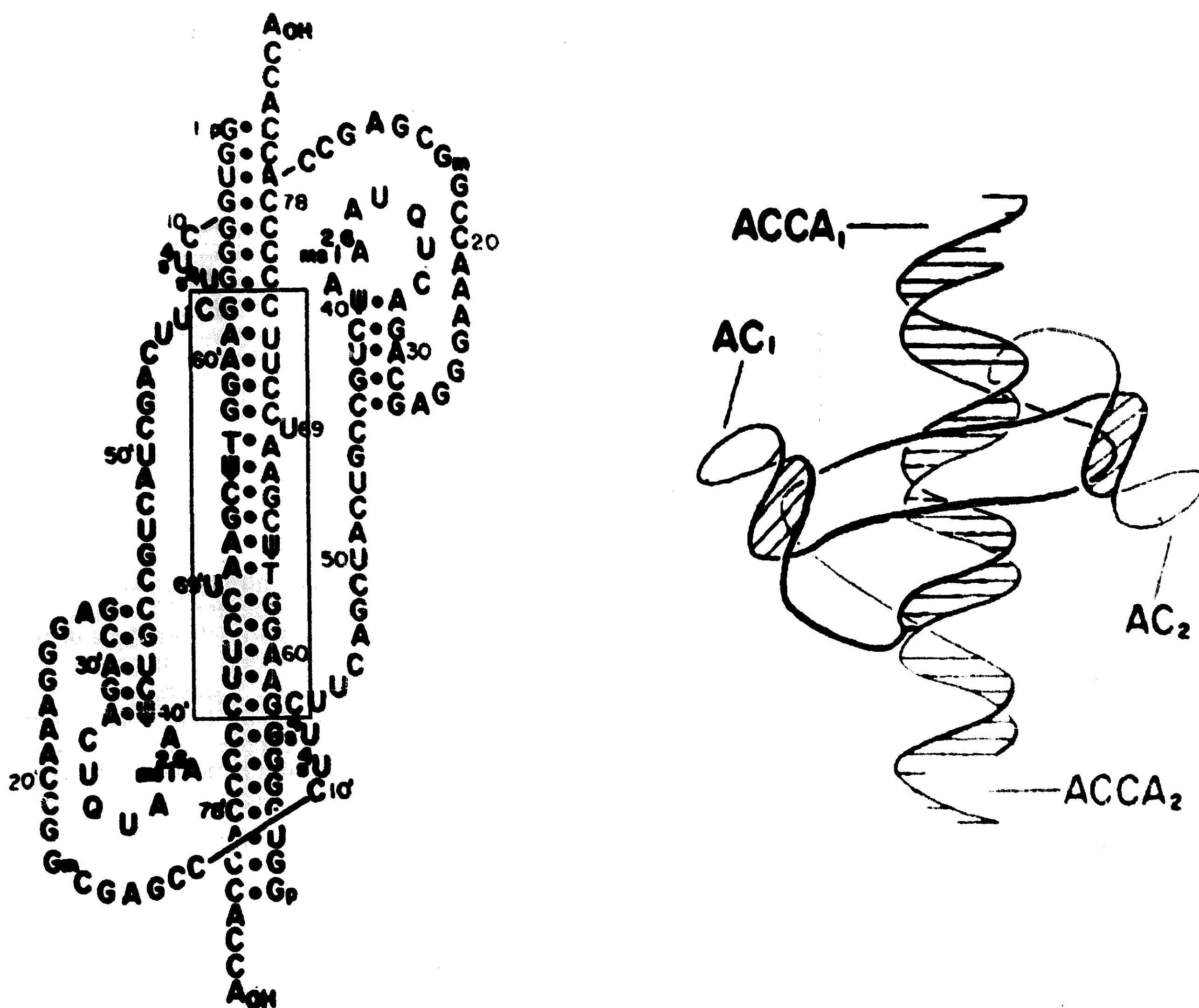


Fig 2. Secondary (left) and tertiary (right) structure models of the dimer of *E coli* tRNA^{Tyr} proposed by Rordorf and Kearns [11]. The intermolecular base pairs in the secondary structure model are enclosed in a box (adapted with permission from Rordorff and Kearns, *Biochemistry* 15, 3320–3330. Copyright (1976) American Chemical Society).

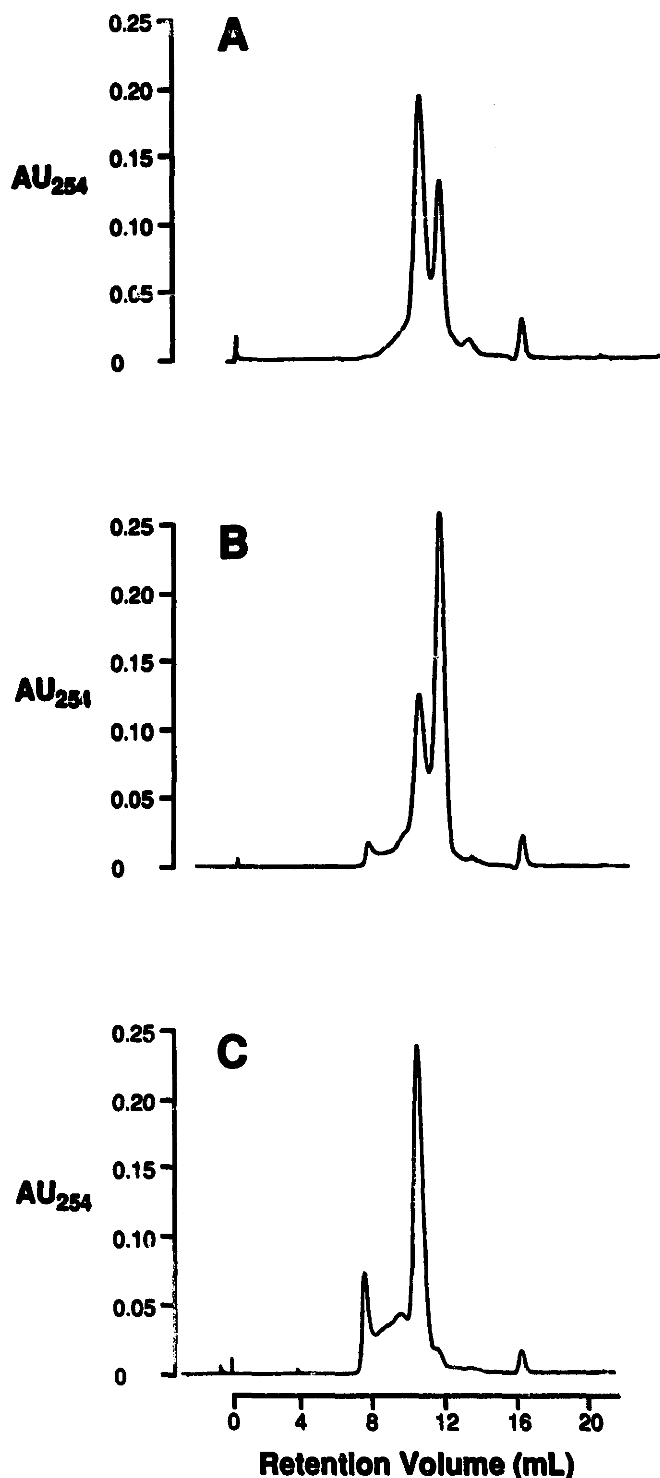


Fig 3. Superose 12 gel filtration separation of ECY2M and ECY2D. A. Untreated *E coli* tRNA^{Tyr}. B. Transcript after heating at 68°C for 1 h in 400 μM $MgCl_2$. C. Transcript after heating at 50°C for 4 h in 1 M NaCl as described in *Materials and methods*.

liquid scintillation counting. Initial velocities were determined by linear regression and from these data Michaelis-Menten parameters were determined by non-linear regression.

Results

Formation and characterization of E coli tRNA^{Tyr} conformers

We have modified published procedures so that both dimeric and monomeric *E coli* tRNA^{Tyr} can be produced in a buffer system compatible with our TGT assay. Gel filtration chromatography was used to characterize the conformers. Figure 3 shows the results of the separation: (A), the untreated *E coli* tRNA^{Tyr} transcription product resuspended in gel filtration buffer; (B), sample from A heated at 68°C in the presence of 400 μM $MgCl_2$; and (C) sample from A heated at 50°C in the presence of 1 M NaCl. The incubation at 68°C forces the tRNA to assume the monomer conformation, while lower temperature and high salt concentration generate the dimer. At identical points in both chromatograms, prior to the major peak, a small peak elutes. This peak is presumably due to some contaminating, high molecular mass species generated during the prolonged exposure to high salt and elevated temperatures.

The conformers were also separated *via* polyacrylamide gel electrophoresis (fig 4). Under native conditions (fig 4A) two bands are clearly distinguishable that correspond to ECY2M and ECY2D (lane 1). Lanes 2 and 3 represent the monomerization procedure, lane 2 is the sample prior to gel filtration and lane 3 is the predominant peak from figure 3B which agrees with ECY2M. The dimerization product is represented by lanes 4 and 5. Here lane 4 is the dimerized sample before gel filtration and lane 5 is the major peak, ECY2D, from figure 3C.

Figure 4B shows the results from denaturing polyacrylamide gel electrophoresis. In lane 1 is the untreated *E coli* tRNA^{Tyr} transcript, a mixture of ECY2M and ECY2D and in lane 3 is a sample of ECY2D. After being exposed to 7 M urea both samples denature and migrate to positions equivalent to an ECY2M sample which has been similarly treated (lane 2).

tRNA·TGT interaction

The interaction of ECY2M and ECY2D with TGT in the absence of guanine was investigated by native PAGE (fig 5). Lane 2 shows the protein alone migrating to an apparent molecular mass of 127 kDa. This represents the protein in its native trimeric state. The enzyme incubated in the presence of ECY2M appears in lane 3. In this instance the protein dissociates into two bands, 69 kDa, the ECY2M·TGT (monomer) complex, and 150 kDa, the ECY2M·TGT

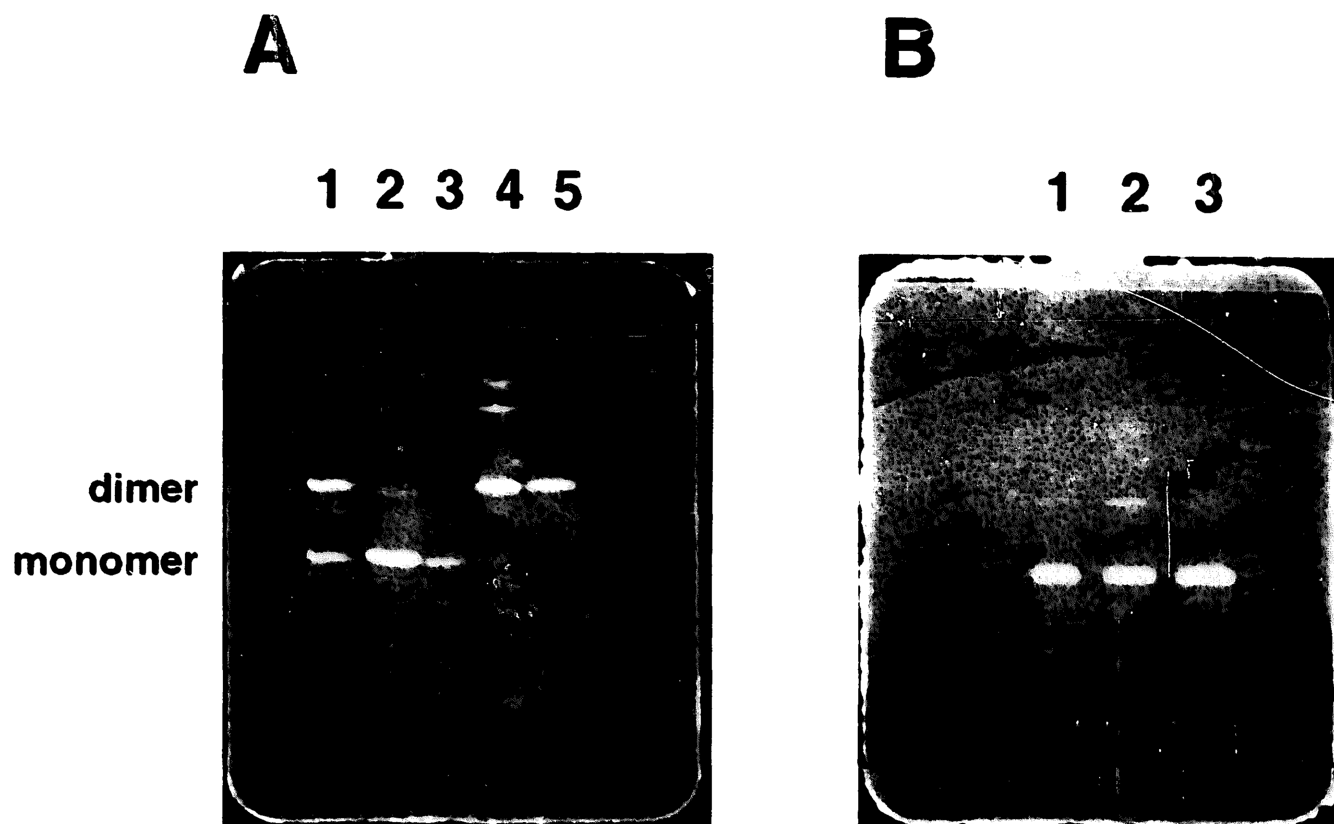


Fig 4. Native-PAGE of ECY2 and ECY2D and denaturing-PAGE of ECY2 and ECY2D. **A.** Native-PAGE of ECY2 and ECY2D. Lane 1, untreated *E coli* tRNA^{Tyr} (prior to separation in figure 3A); lane 2, ECY2 (prior to separation in figure 3B); lane 3, major peak from figure 3B; lane 4, ECY2D (prior to separation in figure 3C); lane 5, major peak from figure 3C. **B.** Denaturing-PAGE of ECY2 and ECY2D. Lane 1, untreated *E coli* tRNA^{Tyr} (prior to separation in figure 3A); lane 2, ECY2 (prior to separation in figure 3B); lane 3, ECY2D (prior to separation in figure 3C).

(trimer) complex. In lane 4 the enzyme is incubated with ECY2D and again two bands appear. Here the bands correspond to 96 kDa, the ECY2D•TGT (monomer) complex, and 180 kDa, the ECY2D•TGT (trimer) complex.

In order to further investigate the band shift phenomenon, we incubated ECY2M•TGT complex with ribonuclease. In figure 6 the TGT trimer and ECY2M•TGT complex bands appear (lanes 3, 4). When the ECY2M•TGT complex sample is treated with ribonuclease three bands appear corresponding to the TGT trimer, a band that migrates at a slightly higher M_r and a fainter band at a lower M_r than the ECY2M•TGT complex (lane 5). The lower band may be due to a TGT complex with partially digested ECY2M. The band at the slightly higher M_r than the TGT trimer may be due to a TGT trimer complex with partially digested ECY2M.

In vitro kinetics

The kinetic studies were performed in the presence of 10 μM 8-[^{14}C] guanine. This was selected due to pre-

vious experiments which established a guanine K_M = 0.98 μM [12]. Plots of initial velocity *versus* RNA concentration (fig 7) demonstrate that Michaelis-Menten kinetics are followed for each conformer and

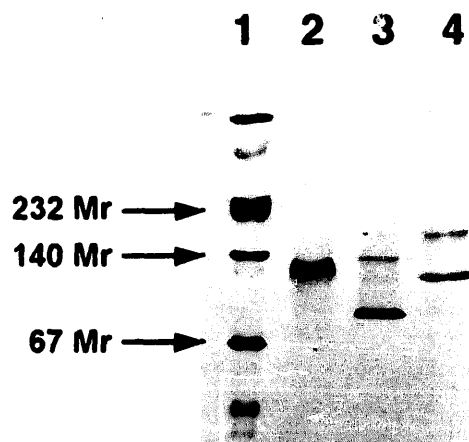


Fig 5. Native-PAGE of ECY2M and ECY2D complexes with TGT. Lane 1, M_r standards; lane 2, 5 μM TGT without substrates; lane 3, 5 μM TGT + 15 μM ECY2; lane 4, 5 μM TGT + 15 μM ECY2D. The enzyme was incubated at 37°C for 10 min in 100 mM HEPES, pH 7.5 with the indicated concentration of substrate. The gel was then run at a constant temperature of 5°C.

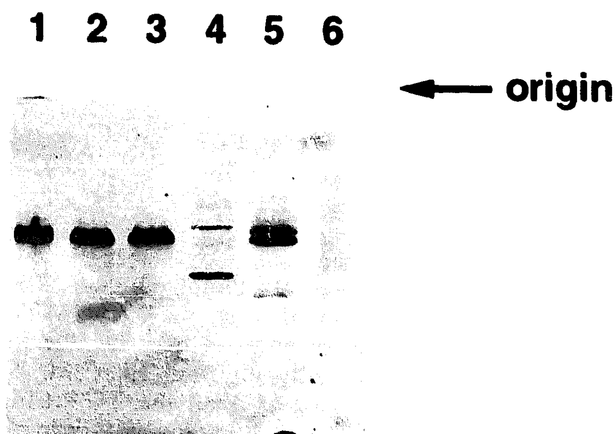
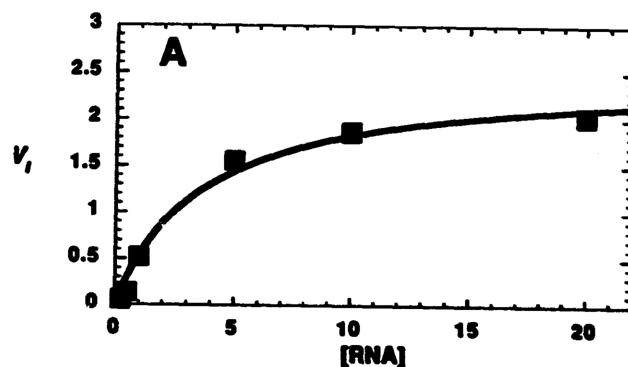
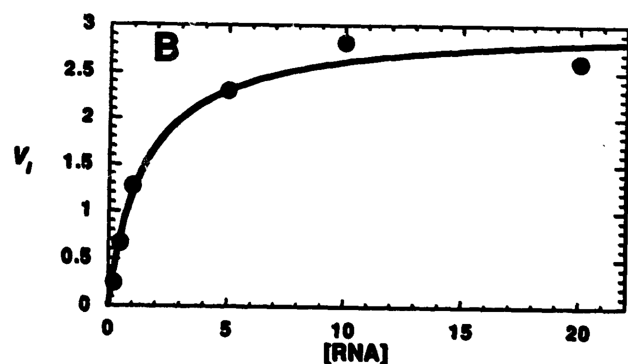


Fig 6. Native-PAGE of TGT and TGT·tRNA complex. Approximately equal amounts of TGT protein were applied to each lane. Lane 1, TGT standard; lane 2, TGT control; lane 3, TGT + ribonuclease; lane 4, TGT + ECY2M; lane 5, TGT + ECY2M + ribonuclease; lane 6, ribonuclease.





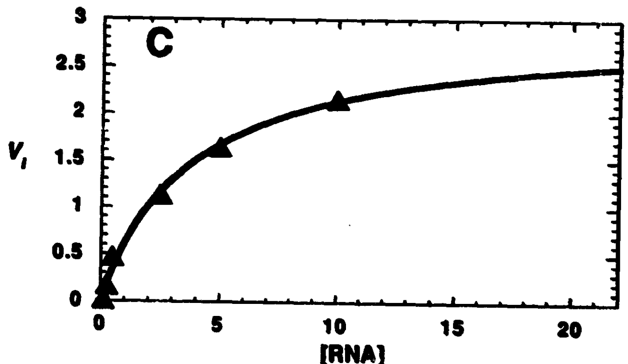


Fig 7. Michaelis-Menten plots of untreated *E coli* $tRNA^{Tyr}$, ECY2M, and ECY2D. **A.** Untreated *E coli* $tRNA^{Tyr}$. **B.** ECY2M. **C.** ECY2D. Solid curves are calculated fits of the data by non-linear regression. V_i units are μM s^{-1} mg^{-1} and (RNA) units are in μM.

a mixture of both conformers. Non-linear regression analysis was used to fit the initial velocity data and are displayed as the solid curves in figure 7. The kinetic parameters (K_M, V_{max}, and V_{max}/K_M) for ECY2, ECY2D, and a mixture of the two from the above fits are shown in table I along with the standard errors of the fits.

Discussion

Using the *in vitro* generation system suggested by Milligan and Uhlenbeck [4], we successfully implemented a system for the *in vitro* generation and purification of *E coli* $tRNA^{Tyr}$ analogues to investigate the molecular processes involved in substrate recognition by *E coli* TGT. One problem related to the system was the dimerization of the transcripts during the purification process. Dimerization of tRNA is not rare albeit most reports of the phenomenon are associated with the isolation and recovery of *in vivo* generated tRNA and predate *in vitro* generated tRNA studies. Furthermore, the biological significance of tRNA dimerization remains unresolved. To further our investigation into the elements required for recognition and modification of tRNA and to expand the knowledge base regarding the biological significance of tRNA aggregation we embarked on studies to produce both a monomeric and dimeric *E coli* $tRNA^{Tyr}$ (ECY2M and ECY2D respectively) in a buffer system conductive to characterization of the interaction of each species with TGT.

As demonstrated in figures 3 and 4 we have successfully generated $tRNA^{Tyr}$ monomer (ECY2M) and dimer (ECY2D) in the appropriate buffer system. The gel filtration and native-PAGE experiments show that the transcription and purification process provides two major products which display migration patterns consistent with the monomer and dimer conformations (fig 3A, fig 4, lane 1). Upon heating at appropriate temperatures in the presence of different cations the transcript conformation can be driven into either the monomeric (fig 3B, fig 4A, lanes 2 and 3) or dimeric (fig 3C, fig 4A, lanes 4 and 5) conformations. An additional peak, presumably due to higher order aggregates of tRNA, appears in the treated samples. The fact that the peak is larger in the ECY2D sample than in the ECY2M sample suggests that the formation of higher order aggregates is dependent upon the length of time the nucleic acid is exposed to elevated temperatures, the concentration at which the nucleic acid is treated, and/or the valency of the cation in the treatment buffer. The higher order aggregates also appear in the native-PAGE with a more intense band in the ECY2D lane (fig 4A, lane 4). The most compelling evidence for dimer and monomer formation is provided by the denaturing-PAGE. After the tRNA is denatured by exposure to 7 M urea at 70°C for 10 min (see *Materials and methods*) each of the three samples exhibits a single band which migrates to a point corresponding to monomeric ECY2.

We have previously reported that *E coli* TGT purified from our overexpressing clone appears to be oligomeric [13]. Native-PAGE of TGT prepared from an optimized protocol shows only a single band corresponding to an apparent trimeric form of TGT which has been confirmed by chemical cross-linking and denaturing PAGE (Garcia and Chong, unpublished). When incubated in the presence of saturating amounts of tRNA, the TGT band shifts to a position consistent with the formation of a tRNA•TGT monomer complex (fig 6, lane 4). Ethidium bromide staining of the native-PAGE indicates that tRNA is present in the TGT•tRNA complex band (data not shown). This trimer to monomer dissociation appears to be reversible as after treatment with ribonuclease, the TGT•tRNA complex migrates predominantly as the TGT trimer (fig 6, lane 5). The exact significance of the TGT trimer to monomer dissociation upon binding tRNA is unknown.

It has been suggested that tRNA aggregation could act as a mechanism for the control of tRNA function. For instance dimerization of *E coli* $tRNA^{Tyr}$ has been found to lead to the loss of the aminoacylation activity by tyrosyl-tRNA synthetase [10]. Studies to elucidate which recognition elements found in *B stearothermophilus* $tRNA^{Tyr}$ allow for aminoacylation by tyrosyl-

Table I. Kinetic parameters for TGT substrates. Standard errors are shown in parentheses. The concentrations of the untreated ECY2 transcript were calculated assuming the presence of monomer only. The values for ECY2-A1 have been recalculated from the data in Curnow *et al* [12] to account for the difference in the amount of enzyme used and to make the units consistent. The V_{max}/K_M ratio was calculated relative to ECY2M.

Conformer	K_m (μM)	V_{max} ($\mu M \cdot s^{-1} \cdot mg^{-1}$)	V_{max}/K_m ($s^{-1} \cdot mg^{-1}$)	V_{max}/K_m ratio
Untreated ECY2 transcript	3.7 (0.9)	2.5 (0.19)	0.68 (0.02)	1:2.8
Monomer (ECY2M)	1.6 (0.3)	3.0 (0.17)	1.95 (0.02)	1:1
Dimer (ECY2M)	3.8 (0.7)	2.9 (0.23)	0.77 (0.02)	1:2.5
Stem-loop (ECY2-A1)	9.6 (0.7)	0.39 (0.01)	0.04 (0.01)	1:50

tRNA synthetase have shown that adenine-73 acts as a discriminator base for tyrosyl-tRNA synthetase recognition but only in conjunction with the overall structure of *B stearothermophilus* $tRNA^{Tyr}$ [15]. If a similar gross structural requirement exists for the *E coli* tyrosyl-tRNA synthetase, then formation of the dimer and concomitant loss of native structure could result in loss of aminoacylation activity.

Other tRNA species, such as yeast $tRNA^{Ser}$ and yeast $tRNA^{Ala}$, retain the ability to be aminoacylated by their cognate aminoacyl-tRNA synthetase upon dimer formation [7, 9]. Loehr and Keller [9] demonstrated that, once formed, dimers of yeast $tRNA^{Ala}$ were correctly charged with alanine by alanyl-tRNA synthetase. They went on to propose a structure for the $tRNA^{Ala}$ dimer conformation. In this structure two G3–U70 discriminator base pairs and two acceptor stems are formed as hybrids of the two individual tRNA molecules. In independent work, alanyl-tRNA synthetase has displayed aminoacylation activity with RNA duplexes comprising the CCA acceptor, the G3–U70 discriminator base pair, and as few as four base pairs of *E coli* $tRNA^{Ala}$ acceptor stem [16], thus demonstrating that the only structural requirement for aminoacylation is the acceptor stem itself. These investigations illustrate that when key structural recognition elements are maintained in grossly varied structures enzyme recognition and catalysis can be preserved.

We have previously shown that ECY2-A1, a minihelix analogue of *E coli* $tRNA^{Tyr}$, corresponding to the anticodon stem and loop, had a K_M of 9.6 μM and a V_{max} of 0.39 $\mu M{\cdot}s^{-1}{\cdot}mg^{-1}$ with TGT, indicating that up to 80% of the tRNA sequence can be removed resulting in only a 20-fold decrease in substrate specificity [12]. This 20-fold difference could be attributed to other specific and/or non-specific interactions between TGT and the full length tRNA which do not occur with the minihelix. Considering the satisfactory activity of TGT with ECY2-A1 and the published structure of dimerized $tRNA^{Tyr}$, with the key TGT structural recognition element intact, *ie* the anticodon stem and loop motifs, we felt that ECY2D was very likely to be a substrate for TGT modification.

In table I we present the results of the kinetic parameter determinations. We find that in cases where the full length tRNA is involved the V_{max} is equal within experimental error. However, both the dimer and the untreated *E coli* $tRNA^{Tyr}$ have K_M's which are elevated approximately 2.5-fold over the K_M for the monomer resulting in a V_{max}/K_M which is decreased 2.5-fold accordingly. This suggests that the enzyme preferentially binds the monomer over dimer, but once bound either conformation undergoes catalysis equivalently.

In our earlier study we found that ECY2-A1 had a 20-fold lower specificity, reflected in V_{max}/K_M, than full-length, unmodified *E coli* $tRNA^{Tyr}$. Here we find a 50-fold difference in specificity between ECY2M and ECY2-A1. Evidently our previous preparation of unmodified $tRNA^{Tyr}$ contained a significant proportion of the dimeric conformer, as can be seen for the untreated ECY2 transcript in figure 4A and table I. The present observation that the untreated tRNA has kinetic parameters almost identical to those for ECY2D is almost certainly due to the fact that the concentration of the untreated tRNA was calculated assuming that it was entirely in the monomeric form. This would result in an erroneously high K_M and low V_{max}/K_M, in this case coincidently identical to those for ECY2D. The fact that most of the 50-fold difference between ECY2M and ECY2-A1 is recovered in the dimer, which shares no structural similarities to ECY2M (except for the anticodon arms) suggests that most of the additional binding is of a non-specific nature. It should be noted that ECY2-A1, the microhelix, may also form a dimeric structure This could explain some of the observed difference in activity *versus* the full length tRNA. We have performed thermal denaturation/renaturation experiments upon ECY2-A1 at relatively low concentrations (*eg* 0.5 μM) and find that the product is indistinguishable from the untreated material (data not shown). We found no evidence for dimerization of the microhelix. Further studies of the microhelix and microhelix analogues are in progress. This report supports our earlier conclusion which postulated that most, if not all of the positive elements for recognition are localized in the anticodon arm.

Gu and Santi have suggested that the tRNA (m^5U54)-methyltransferase might recognize and methylate other RNA species due to the fact that only the T-arm loop and stem structure is necessary for methylation [17]. These same workers have subsequently found that this enyzme does indeed methylate 16S rRNA *in vitro* [18]. Our results regarding the tRNA structural elements required for TGT recognition and catalysis suggest that TGT may very well exchange $preQ_1$ into other RNA species, perhaps leading to queuine incorporation. Further studies are needed to address this possibility.

While the results presented here obtain for the *E coli* enzyme, they may not hold true for eukaryotic TGTs. Grosjean and coworkers have shown that the entire tRNA molecule appears to be necessary for queuine modification in *Xenopus* oocytes [19]. This finding, in addition to previous reports that prokaryotic TGTs incorporate a queuine precursor ($preQ_1$) and do not recognize queuine whereas the eukaryotic TGTs do recognize queuine, highlights the significant differences between prokaryotic and eukaryotic TGTs with respect to substrate recognition.

Acknowledgments

This work was supported by National Institutes of Health Grants GM45968 and GM07767 and the University of Michigan, College of Pharmacy.

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