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Biochemistry

Isomeric aminoacyl-tRNAs are both bound by elongation factor Tu

[CTP(ATP):tRNA nucleotidyltransferase/aminoacyl-tRNA deacylation/protein biosynthesis/positional specificity]

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ABSTRACT Recent suggestions that elongation factor Tu (EF-Tu) is specific for 2′-*O*-aminoacyl-tRNA, as compared with the 3′-isomer, prompted us to assay [^{3}H]aminoacyl-tRNAs from *Escherichia coli* terminating in 2′- or 3′-deoxyadenosine for binding to EF-Tu to determine the possible positional specificity of the factor. Binding of modified aminaoacyl-tRNAs to EF-Tu·GTP was measured both as a function of the ability of EF-Tu·GTP to diminish the rate of chemical deacylation of [^{3}H]aminoacyl-tRNAs and by gel filtration of the individual ternary complexes. Fifteen different tRNA isoacceptors were tested by the deacylation procedure, including three (tRNA^{Asp}, tRNA^{Cys}, and tRNA^{Tyr}) for which isomeric modified aminoacyl-tRNAs were available. All of the modified aminoacyl-tRNAs were protected from deacylation, although generally to a lesser extent than the corresponding unmodified species.

Six modified tRNA isoacceptors (including tRNA^{Trp} and tRNA^{Tyr}, for which both modified aminoacyl-tRNAs were accessible by enzymatic aminoacylation) were used in gel filtration experiments to permit direct measurement of the individual aminoacyl-tRNA-EF-Tu·GTP complexes. These experiments were also done in the presence of equimolar amounts of the corresponding unmodified [^{14}C]aminoacyl-tRNAs, and the relative affinities for a limiting amount of EF-Tu·GTP were measured. The results were completely consistent with those obtained by the deacylation procedure and indicated that EF-Tu can bind to both positional isomers of aminoacyl-tRNA with no obvious preference for either.

Although aminoacyl-tRNA is thought to consist of a mixture of 2′- and 3′-*O*-aminoacyl species which equilibrate rapidly, the specificity generally associated with enzymic processes makes it not unlikely that single isomers of tRNA may be utilized exclusively for certain of the partial reactions of protein biosynthesis. The rapid equilibration of the two isomers of aminoacyl-tRNAs has precluded a determination of positional specificities involved in such transformations, but data of this type are now accessible by the use of certain modified tRNAs, e.g., those terminating in 2′- and 3′-deoxyadenosine (1–6). Results obtained from the study of such modified tRNAs have suggested, e.g., that tRNAs are aminoacylated exclusively on a single (2′ or 3′) OH group (7–9). The use of isomeric phenylalanyl-tRNAs has also permitted the study of positional specificities involved in the binding of (*N*-acetyl)phenylalanyl-tRNA to the ribosomal A- and P-sites and in participation of phenylalanyl-tRNA as an acceptor in the peptidyltransferase reaction (2, 4, 5).

Elongation factor Tu (EF-Tu) forms a ternary complex with GTP and aminoacyl-tRNA and is essential for proper binding of the latter to the ribosomal A-site (10). The binary complex (EF-Tu·GTP) does not bind deacylated or *N*-acylated aminoacyl-tRNAs (11, 12) to a significant extent; that ternary complex formation involves the aminoacyl moiety of tRNA is further suggested by the greatly reduced rate of chemical hydrolysis of bound aminoacyl-tRNA (13, 14). This report deals with the binding of aminoacylated *Escherichia coli* tRNAs terminating in 2′- or 3′-deoxyadenosine to EF-Tu from *E. coli*, as measured both by the ability of the factor to retard chemical deacylation of the modified aminoacyl-tRNAs and by gel filtration studies. The results, which are not consistent with conclusions reached recently on the basis of fragment reaction studies (15, 16) and experiments utilizing positionally defined but nonisomeric tRNAs (17, 18), are presented for 16 different tRNA isoacceptors, including four for which both aminoacyl-tRNA isomers were accessible enzymatically.

Abbreviations: tRNA-C-C$_{OH}$, tRNA missing the 3′-terminal adenosine moiety; DBAE-cellulose, *N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethyl-cellulose; nucleoside Q, 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine; Na^{+}-Mes, sodium 2-(*N*-morpholino)ethanesulfonate; NH_4^{+}-Pipes, ammonium piperazine-*N*,*N*′-bis-(2-ethanesulfonate); EF-Tu, elongation factor Tu; 1 A_{260} unit, that amount of material that gives an absorbance of 1 when dissolved in 1 ml of solvent when the light path is 1 cm.

MATERIALS AND METHODS

[^{3}H]Arginine, aspartate, glutamate, glycine, isoleucine, phenylalanine, threonine, tryptophan, valine, cordycepin, 2′-dATP, and GDP were purchased from New England Nuclear; [^{3}H] alanine, leucine, and serine were from ICN. [^{3}H]Lysine and asparagine were from Schwarz/Mann and [^{3}H]cystine from Amersham/Searle. Phospho*enol*pyruvate and pyruvate kinase were obtained from Sigma Chemicals, as were CTP, ATP, GTP, GDP, 2′-dATP, and cordycepin [which was converted to 3′-dATP chemically (19, 20)]. Aminoethyl-cellulose (Sigma) was converted to (acetylated) DBAE-cellulose (*N*-[*N*′-(*m*-dihydroxyborylphenyl)succinamyl]aminoethyl-cellulose) by known methods (ref. 21 and references therein). DEAE-cellulose was from Whatman, glass fiber disks from Schleicher and Schuell, and nitrocellulose filters (45 μm) from Millipore. Unfractionated *E. coli* tRNA and partially fractionated *E. coli* aminoacyl-tRNA synthetase were prepared as described (9); venom phosphodiesterase was from Boehringer Mannheim. The EF-Tu was isolated from *E. coli* by a published method (22); the factor was shown to have a molecular weight of approximately 42,000 and to be free of nuclease activities.

Preparation of Modified *E. coli* tRNAs. *E. coli* tRNA species **2a** and **3a** (Fig. 1) were prepared as described (2, 9). *E. coli* tRNA (2000 A_{260} units) was digested with 200 μg of venom endonuclease for 30 min. (One A_{260} unit is that amount of material that gives an absorbance of 1 when dissolved in 1 ml of solvent when the light path is 1 cm.) The recovered tRNA was applied to a DBAE-cellulose column (1.4 × 28 cm) that had been equilibrated with 50 mM morpholine·HCl, pH 8.7, containing 1 M NaCl and 0.1 M $MgCl_2$. The column was washed with 400 ml of the same buffer at 25° to remove those venom-treated tRNAs containing no more than one *cis*-diol moiety; tRNAs containing 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl-7-deazaguanosine (nucleoside Q) were

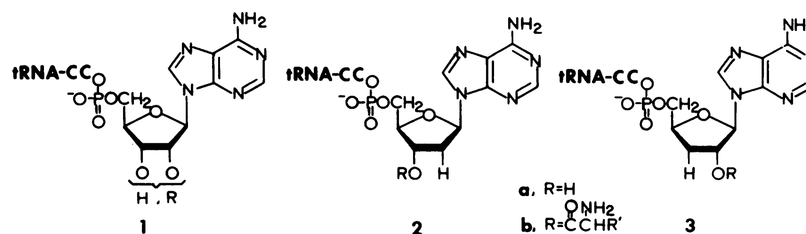

FIG. 1. Isomeric aminoacyl-tRNAs.

recovered by elution with 50 mM Na^+-Mes [sodium 2-(*N*-morpholino)ethanesulfonate], pH 5.5, containing 1 M NaCl.

The venom-treated tRNAs lacking and containing nucleoside Q (23) were reconstituted separately with CTP and yeast CTP(ATP):tRNA nucleotidyltransferase to afford the respective abbreviated tRNAs. Portions of the two tRNA-C-C_{OH} samples were further treated with 2′- or 3-′dATP and CTP(ATP):tRNA nucleotidyltransferase to afford tRNA species **2a** and **3a**, respectively. The modified tRNAs containing nucleoside Q were purified by chromatography on DEAE-cellulose and then on acetylated DBAE-cellulose, elution with 50 mM morpholine·HCl buffer, pH 8.7, containing 1.0 M NaCl and 0.1 M $MgCl_2$. Unreacted tRNA-C-C_{OH} was recovered by elution with 50 mM Na^+-Mes, pH 5.5, containing 1.0 M NaCl. The modified tRNA species (**2a** and **3a**) derived from tRNAs not containing nucleoside Q were purified on DEAE-cellulose and then applied to DBAE-cellulose columns that had been preequilibrated at 4° with 50 mM morpholine·HCl buffer, pH 8.7, containing 1.0 M NaCl, 0.1 M $MgCl_2$, and 20% dimethylsulfoxide. Elution with the same buffer afforded purified tRNA species **2a** and **3a**, and unreacted tRNA-C-C_{OH} was recovered by washing at 4° with 50 mM Na^+-Mes (pH 5.5) containing 1 M NaCl.

Deacylation of Aminoacyl-tRNAs. The aminoacylation mixture consisted of 360 μl (total volume) of 13 mM NH_4^+-Pipes buffer [ammonium piperazine-*N,N′*-bis-(2-ethanesulfonate)], pH 7.0, containing 0.13 M KCl, 20 mM $MgCl_2$, 0.7 mM EDTA, 1.5–8 μCi of ^3H-labeled amino acid at 2–10 μM concentration, 8 μM of each of 18 other unlabeled amino acids (except cysteine) to prevent misacylations (24), 2 mM ATP, and 0.8–1.6 A_{260} units of unfractionated tRNA species **1a**, **2a**, or **3a**. The reaction was initiated by the addition of 30 μl of *E. coli* aminoacyl-tRNA synthetase solution and maintained at room temperature for 30 (tRNA species **1a**) or 60 (**2a** and **3a**) min. The aminoacylation mixture was extracted with phenol and the aqueous layer was washed with ether. A separate solution (150 μl) containing 0.13 M Tris·HCl, pH 8.6, 20 mM $MgCl_2$, 1.7 mM dithiothreitol, 1.7 mM GTP, 20 mM phospho*enol*pyruvate, 20 units of pyruvate kinase, and an appropriate amount (typically 60–400 pmol) of EF-Tu·GDP was incubated at 37° for 60 min.

A 150-μl aliquot of the aminoacyl-tRNA solution was added to the EF-Tu·GTP solution; a second 150-μl aliquot of the tRNA solution was added to a 150-μl preincubated solution of 0.13 M Tris·HCl, pH 8.6, containing 20 mM $MgCl_2$ and 1.7 mM dithiothreitol. (Controls were also run omitting only EF-Tu·GDP or only GTP and phospho*enol*pyruvate. These gave the same results obtained without the entire EF-Tu·GTP-generating system.) Each combined solution was incubated at 37°, and 45-μl aliquots were removed after 0, 15, 30, 60, and 90 min and added to glass fiber disks that had been soaked with 0.05 M cetyltrimethylammonium bromide in 1% HOAc. The dried disks were washed (1% HOAc) and then used to determine radioactivity. In some assays, aliquots were removed over a period of 240 min.

RESULTS

Modified tRNAs were prepared by treatment of unfractionated *E. coli* tRNAs with venom exonuclease until the tRNA was without significant acceptor activity for phenylalanine, aspartic acid, or asparagine. The venom-treated tRNAs were then applied to a DBAE-cellulose column that had been equilibrated with 50 mM morpholine-HCl, pH 8.7, containing 1 M NaCl and 0.1 M $MgCl_2$. The column was washed with the same buffer solution at room temperature, thus effecting elution of all tRNAs not containing nucleoside Q. The remaining four species ($tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{His}$, and $tRNA^{Tyr}$) containing nucleoside Q, which has a *cis*-diol moiety, were washed from the column with 50 mM Na^+-Mes buffer, pH 5.5, containing 1 M NaCl. The two types of tRNAs (i.e., with and without nucleoside Q) were then treated separately with CTP and CTP(ATP):tRNA nucleotidyltransferase to afford the respective abbreviated tRNAs (tRNA-C-C_{OH}), and portions of each were further treated with 2′- and 3′-deoxyadenosine 5′-triphosphate and yeast CTP(ATP):tRNA nucleotidyltransferase to afford tRNA species **2a** and **3a** derived from the two types of tRNA. Final purification of the tRNAs was effected by chromatography on (acetylated) DBAE-cellulose; since the conditions required to separate tRNA species lacking *cis*-diols from those containing one such group (e.g., $tRNA^{Phe}$ species **2a** from $tRNA^{Phe}$-C-C_{OH}) are different from those used to separate species containing one and two *cis*-diol groups (e.g., $tRNA^{His}$ species **2a** from tRNA-^{His}-C-C_{OH}), the initial fractionation of the venom-treated tRNAs on DBAE-cellulose facilitated purification of the modified tRNAs.

Experiments utilizing several unmodified [^3H]aminoacyl-tRNAs (**1b**) revealed that a limited amount of EF-Tu·GTP was quite efficient at preventing deacylation of certain tRNAs, but less effective for others. For example, deacylation of [^3H]arginyl-tRNA (in the presence of other unlabeled aminoacyl-tRNAs) was much slower in the presence of limited amounts of EF-Tu·GTP (Fig. 2), but the same EF-Tu·GTP preparation was relatively ineffective at protecting from deacylation histidyl-, isoleucyl-, or threonyl-tRNAs (present in the same mixture of unfractionated tRNA species as arginyl-tRNA). Therefore, it was necessary to determine for each tRNA isoacceptor the extent to which EF-Tu·GTP would prevent deacylation so that the amount utilized could be adjusted accordingly. That the diminution in the rate of deacylation of any single aminoacyl-tRNA was due to the presence of EF-Tu·GTP and proportional to the amount of the factor available may be judged from Fig. 3, which shows that increasing amounts of the factor afforded increased protection from deacylation of arginyl-tRNA; in the absence of added GTP, EF-Tu·GDP did not prevent the deacylation. Similar results were obtained with phenylalanyl-tRNA. Also consistent with the belief that this assay system measured the binding of EF-Tu·GTP to aminoacyl-tRNA was the observation that methionyl-$tRNA_f^{Met}$, which is known not to bind to EF-Tu (25), was not protected from deacylation by the factor.

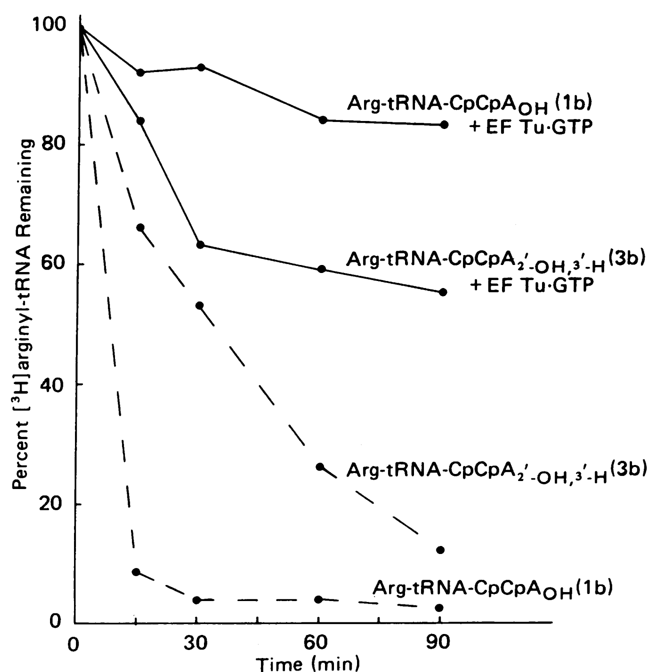

FIG. 2. Deacylation of [^{3}H]arginyl-tRNAs **1b** and **3b** in the presence (●—●) and absence (● - - - ●) of EF-Tu·GTP. Experimental details are given in *Materials and Methods;* arginyl-tRNA **1b** (8.7 pmol) was incubated in the presence of 1.3 molar equivalents of EF-Tu·GTP, species **3b** (10.5 pmol) in the presence of 1.1 equivalents. Deacylation of **1b** and **3b** in the absence of EF-Tu·GTP was found to be first order with respect to aminoacyl-tRNA, as expected. Since the ternary complex is in rapid equilibrium with EF-Tu·GTP and aminoacyl-tRNA (12, 26), and since deacylation from the ternary complex is much slower than from free aminoacyl-tRNA, in the presence of EF-Tu·GTP the rate of deacylation of a single aminoacyl-tRNA can be specified as $-$ d[aminoacyl-tRNA]$_T$/(dt) = k_1[aminoacyl-tRNA]$_T$ $-$ (k_1[aminoacyl-tRNA]$_F$[EF-Tu·GTP]$_F$/K_s) where [aminoacyl-tRNA]$_T$ is the total concentration of aminoacyl-tRNA, [aminoacyl-tRNA]$_F$ is the concentration of aminoacyl-tRNA not bound to EF-Tu·GTP, K_s is the dissociation constant of the ternary complex, and k_1 is the rate constant for chemical deacylation. In the present case the incubation mixtures included several unlabeled aminoacyl-tRNAs, each of which would also compete for the limited amount of EF-Tu·GTP with a unique rate constant, thus greatly complicating kinetic analysis.

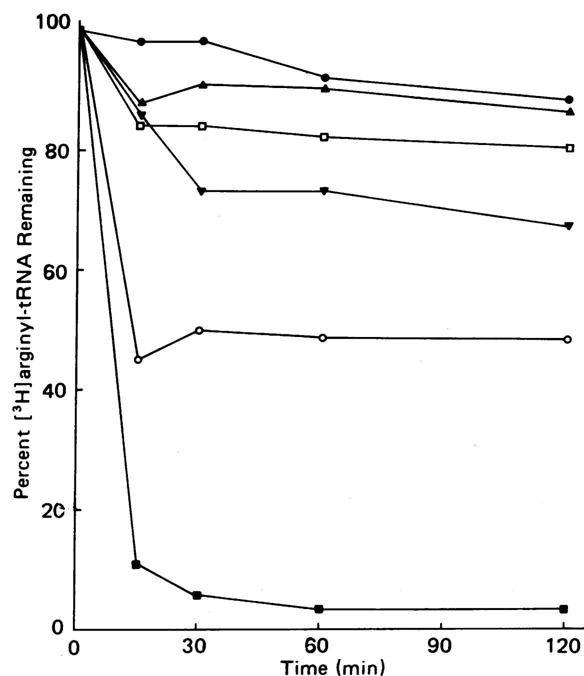

FIG. 3. Deacylation of [^{3}H]arginyl-tRNA in the absence of EF-Tu·GTP (■) and in the presence of 0.47 (○), 0.93 (▼), 1.4 (□), 1.9 (▲), and 2.3 (●) equivalents of EF-Tu·GTP. EF-Tu·GDP afforded no protection from deacylation. See *Materials and Methods* for experimental details.

The measurement of EF-Tu·GTP interaction with each modified tRNA involved the determination of four deacylation curves. Two were measured using a single [^{3}H]aminoacyl-tRNA (**1b**; in the presence of other unlabeled aminoacyl-tRNAs), both in the absence of EF-Tu·GTP, and in the presence of sufficient factor to afford reasonable (but not complete) protection from deacylation. Additional deacylations were carried out utilizing the corresponding tRNA species **2b** or **3b**, or both in the cases of asparaginyl-, cysteinyl-, and tyrosyl-tRNAs. One of these curves was obtained in the absence of EF-Tu·GTP, while the other was measured utilizing approximately the same ratio of EF-Tu·GTP and [^{3}H]aminoacyl-tRNA species **2b** or **3b** as was used for species **1b**.

The deacylation curves obtained for species **1b** were indicative of the extent of protection from deacylation afforded each unmodified aminoacyl-tRNA under the specific reaction conditions chosen. The curve obtained for species **1b** in the presence of EF-Tu·GTP could be compared directly with that obtained for the corresponding tRNA species **2b** or **3b** to indicate the relative extent of protection of the latter. However, interpretation of the results was complicated by the fact that aminoacyl-tRNAs of type **2b** and **3b** undergo chemical hydrolysis less quickly than species of type **1b** (3). Therefore, the deacylation of **2b** or **3b** was also determined in the absence of EF-Tu·GTP for comparative purposes.

Deacylation of unmodified [^{3}H]arginyl-tRNA was 50% complete after 7–8 min in the absence of EF-Tu·GTP, but only 17% deacylation occurred after 90 min in the presence of the factor (Fig. 2). For arginyl-tRNA species **3b**, deacylation was 50% complete after 34 min, but in the presence of EF-Tu·GTP, deacylation proceeded to the extent of only 40% in 90 min. Thus, arginyl-tRNA species **3b** was protected from chemical deacylation by EF-Tu·GTP, although not to the same extent as the corresponding unmodified tRNA. All of the other modified aminoacyl-tRNAs tested, including both isomers of asparaginyl-, tyrosyl-, and cysteinyl-tRNAs, were also protected from deacylation by EF-Tu·GTP when assayed in the presence of an amount of the factor that gave reasonable protection of the corresponding unmodified tRNAs (Table 1). For example, leucyl-tRNA species **3b** underwent 50% deacylation in >120 min in the presence of the factor but after only 26 min in the absence of EF-Tu·GTP. Generally, the extent of protection of individual tRNA species **2b** or **3b** by EF-Tu·GTP seemed to be somewhat less than that obtained with the corresponding unmodified aminoacyl-tRNA, although variations in the ratio of EF-Tu·GTP and aminoacyl-tRNA utilized, as well as inherent differences in the rates of chemical deacylation of **2b** and **3b** as compared with **1b** and in the distribution of unlabeled aminoacyl-tRNAs associated with each modified and unmodified [^{3}H]aminoacyl-tRNA isoacceptor, made it difficult to assess the relative extents of protection on a quantitative basis using this assay.

A more direct measure of the relative affinities of the 2′- and 3′-*O*-aminoacyl isomers of tRNA for EF-Tu·GTP was obtained by gel filtration experiments. Gel filtration of tyrosyl-tRNA species **2b** and **3b**, for example, indicated that both would form ternary complexes with EF-Tu·GTP. The relative affinities of

Table 1. Deacylation of modified tRNAs[a]

	50% Deacylation time (min)			
	tRNA species 2b		tRNA species 3b	
Amino acid	+EF-Tu	−EF-Tu	+EF-Tu	−EF-Tu
Alanine	38.5	18		
Arginine			90[b]	28[b]
Asparagine	45	21	30	18
Cysteine	120[c]	75[c]	94	62
Glutamic acid			90[c]	23[c]
Glycine	52.5	25		
Histidine	105	54.5		
Isoleucine			105[d]	75[d]
Leucine			>120[e]	26
Lysine	88.5	49.5		
Phenylalanine			120	80
Serine	113.5[f]	17[f]		
Threonine	50	40		
Tyrosine	107[d]	59[d]	109	54.5
Valine			103[g]	60[g]

[a] Experimental procedure given in *Materials and Methods*.
[b] Time required to effect 45% deacylation (50% deacylation not reached in presence of EF-Tu).
[c] Time required to effect 20% deacylation.
[d] Time required to effect 40% deacylation.
[e] Less than 10% deacylation of the modified tRNA had occurred after 120 min.
[f] Time required to effect 30% deacylation.
[g] Time required to effect 35% deacylation.

the factor for the two species, as compared to unmodified tyrosyl-tRNA, was measured by carrying out the same experiment with an equimolar mixture of [^{14}C]tyrosyl-tRNA species **2b** or **3b** and [^{3}H]tyrosyl-tRNA species **1b** in the presence of a limiting amount of EF-Tu·GTP. The complex formed in the presence of **1b** and **2b** contained 49% of tRNA species **2b** and 51% of the unmodified tRNA, while that formed in the presence of tyrosyl-tRNA's **1b** and **3b** contained 34% of the modified tRNA. When the experiment was repeated with tryptophanyl-tRNA species **1b–3b**, the complex containing **1b** and **2b** consisted of 38% of the modified tRNA, while that containing **1b** and **3b** had 29% of the modified species (Fig. 4). These experiments were repeated for single isomers of modified alanyl-, arginyl-, lysyl-, and phenylalanyl-tRNAs. In each case, the modified tRNA was able to participate in the formation of a ternary complex. In agreement with the results obtained by the deacylation assay, the modified tRNAs were generally slightly less effective in binding to EF-Tu·GTP than the corresponding unmodified species; no specificity for a single positional isomer was apparent from the gel filtration studies.

DISCUSSION

Chinali *et al.* (4) studied the EF-T-directed ribosomal binding of yeast phenylalanyl-tRNA^{Phe} species **1b** and **3b** to *E. coli* ribosomes in the presence of 10 mM $MgCl_2$. Incubation of the tRNAs (50 pmol) with approximately 45 pmol of ribosomes resulted in binding of 19.0 pmol of species **1b** and 16.8 pmol of species **3b** after 5 min at 30°. The same authors later reported (17) that the 3′-*N*-phenylalanyl-tRNA^{Phe} analog, terminating in the nucleoside 3′-amino-3′-deoxyadenosine (5), underwent EF-T-directed ribosomal binding weakly (18), if at all (17), and the same results have recently been reported for a heterologous eukaryotic system that utilized rabbit reticulocyte ribosomes (5). On the basis of these observations, it was suggested (17, 18)

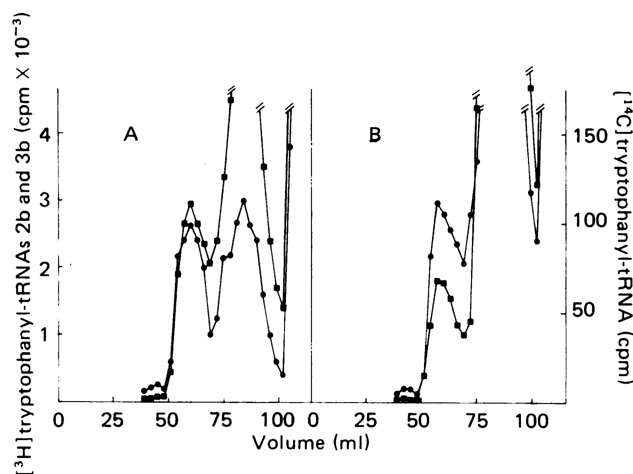

FIG. 4. Gel filtration on Sephadex G-100 of (A) tryptophanyl-tRNA **2b** (■) and (B) tryptophanyl-tRNA **3b** (■) in the presence of approximately equimolar amounts of unmodified tryptophanyl-tRNA (**1b**) (●) and limiting amounts of EF-Tu·GTP. As demonstrated previously (12, 27, 28), the ternary complex eluted at 58–60 ml, followed by unbound tryptophanyl-tRNA (V_e = 84 ml). The elution volume of aminoacyl-tRNA was also verified in the absence of the other components of this system, as were the values for aminoacyl-tRNA synthetase (V_e = 44 ml) and EF-Tu·GDP (V_e = 75 ml). For the experiment depicted in panel B, the aminoacylations were carried out in 220 µl of 0.09 M NH_4^+-Pipes buffer, pH 7.0, containing 0.09 M KCl, 13.5 mM $MgCl_2$, 0.45 mM EDTA, 1.8 mM ATP, and 0.5 A_{260} unit of tRNA^{Trp} species **1a** plus 7.5 µM L-[^{14}C]tryptophan, 57 Ci/mol, or 0.55 A_{260} unit of tRNA^{Trp} species **3a** plus 7.5 µM L-[^{3}H]tryptophan, 5.5 Ci/mmol. The reactions were initiated by the addition of 20 µl of a preparation of partially fractionated aminoacyl-tRNA synthetases; the mixture was maintained at room temperature for 30 min and then placed in an ice bath. Solutions containing EF-Tu·GTP were prepared in 300 µl of 50 mM Tris·HCl at pH 7.4, containing 10 mM $MgCl_2$, 50 mM NH_4Cl, 5 mM dithiothreitol, 3.5 mM GTP, 40 mM phospho*enol*pyruvate, 10 units of pyruvate kinase, and 110 pmol of EF-Tu·GDP. The reaction mixture was maintained at 37° for 15 min, chilled for 15 min, and then mixed with the tryptophanyl-tRNA solution. After 10 min in an ice bath, the combined solution was treated with 0.5 ml of cold 50 mM Tris·HCl at pH 6.5, containing 10 mM $Mg(OAc)_2$, 0.1 M NH_4OAc, 1 mM EDTA, and 10 mM 2-mercaptoethanol, and applied to a 1.3 × 105 cm column of Sephadex G-100 that had been equilibrated with the same buffer at 3°. Washing of the column (3°, 24 ml/hr, 1.5-ml fractions) gave the elution profile shown in panel B. Analysis of the graph revealed that 57.6 pmol of tryptophanyl-tRNAs **1b** and **2b** had been subjected to gel filtration (total, 115.2 pmol). The quantitative results of the experiment are discussed in the *text*. Controls were done in the absence of EF-Tu·GDP to demonstrate that the aminoacyl-tRNA would not form complexes with tryptophanyl-tRNA synthetase under the experimental conditions, nor with EF-Tu·GDP.

that "the 2′-aminoacyl-tRNAs are the substrates required for the elongation factor Tu" (18). Ringer and Chládek have also suggested that EF-Tu is specific for 2′-*O*-aminoacyl-tRNA on the basis of their observation that both cytidyl-1′(3′ → 5′)-2′,3′-*O*-phenylalanyladenosine and cytidylyl-(3′ → 5′)-2′-*O*-phenylalanyl-3′-deoxyadenosine, but not cytidylyl-(3′ → 5′)-2′-deoxy-3′-*O*-phenylalanyladenosine, were able to effect dissociation of EF-Tu·GTP when the aminoacylated dinucleoside monophosphates were utilized at concentrations of 10^{-6}–10^{-4} M (15, 16). The only direct comparison of isomeric aminoacyl-tRNAs in an EF-Tu-dependent system was reported by Hecht *et al.* (2). They found that *E. coli* phenylalanyl-tRNA species **1b–3b** were all inhibitory to the EF-Tu-dependent binding of unmodified [^{3}H]phenylalanyl-tRNA to *E. coli* ribosomes, which suggested strongly that all three were bound by EF-Tu.

The observation that EF-Tu·GTP binds specifically to aminoacyl-tRNAs (10–12), and thereby protects the aminoacyl moiety from chemical hydrolysis (13, 14), suggested that protection from deacylation could be utilized as a measure of aminoacyl-tRNA-EF-Tu·GTP interaction. Preliminary experiments indicated that not all unmodified aminoacyl-tRNAs were protected from deacylation to the same extent, but that all except $tRNA_f^{Met}$ could be protected somewhat by using an appropriate amount of EF-Tu·GTP. The experiments utilizing modified aminoacyl-tRNA analogs **2b** and **3b** were subsequently carried out for each modified isoacceptor with the same relative amounts of EF-Tu·GTP and aminoacyl-tRNA species (**2b** or **3b**) as was observed to afford reasonable protection of the unmodified tRNA (**1b**) from which it was derived. As illustrated for arginyl-tRNA species **1b** and **3b** (Fig. 2), EF-Tu·GTP did prevent deacylation of both species, but the protection of **3b** was much less pronounced. This may be attributed both to a weaker interaction between EF-Tu·GTP and **3b** and to the inherently less facile chemical hydrolysis of **2b** and **3b** as compared with **1b**. EF-Tu was found to have a similar effect on the rate of chemical deacylation of tyrosyl-tRNA species **1b–3b**. As in the case of arginyl-tRNA, the unmodified species (**1b**) deacylated more quickly than the modified species (**2b** and **3b**) in the absence of the factor, but less quickly when EF-Tu·GTP was present. Both of the modified species were protected from deacylation, although the relative extent of protection was difficult to determine from this assay. Fifteen other modified aminoacyl-tRNAs, including both isomeric species (**2b** and **3b**) derived from $tRNA^{Asn}$ and $tRNA^{Cys}$, were also tested for interaction with EF-Tu·GTP (Table 1). Under conditions that afforded protection of tRNA species **1b**, each of the corresponding modified species was also protected from hydrolysis.

To verify the results obtained using the chemical deacylation assay and provide a more direct method for determining the relative strengths of binding of tRNA species **1b–3b** by EF-Tu·GTP, the possible formation of aminoacyl-tRNA–EF-Tu·GTP ternary complexes was also studied by gel filtration. Six isoacceptor activities were studied, including two ($tRNA^{Trp}$ and $tRNA^{Tyr}$) for which species **2b** and **3b** were both accessible. All of the modified aminoacyl-tRNAs formed ternary complexes with EF-Tu·GTP. In the presence of the corresponding unmodified tRNAs and a limited amount of EF-Tu·GTP, each of the eight modified species was bound to the factor, and there was no obvious preference for 2′- or 3′-*O*-aminoacylated tRNAs.

Note Added in Proof. Recent results by Sprinzl and Cramer (personal communication) have indicated that *E. coli* tyrosyl-tRNAs 2 and 3 are both bound by EF-Tu · GTP, but that neither isomer of the phenylalanyl-tRNAs terminating in 2′- or 3′-aminoadenosine was bound.

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