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# tRNA fluorescent labeling at 3′ end inducing an aminoacyl-tRNA-like behavior

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A fluorescent tRNA derivative labeled at 3′-*O* position of the ultimate adenosine residue by reaction, under mild conditions, of tRNA with isatoic anhydride [3,1-benzoxazine-2,4(1*H*)-dione] was obtained. The labeling selectivity was determined by several criteria: digestion with RNase, followed by HPLC of the digest, produces only one labeled nucleoside, identified as 3′-*O*-anthraniloyladenosine; the ratio of the absorbance at 260 nm to 332 nm also suggests a 1:1 molar ratio between the nucleic acid and the fluorophore, finally, the incapacity of the labeled tRNA to be charged by the specific aminoacyltransferase further demonstrates the engagement of the 3′-*O* position.

Although the 3′-*O*-anthraniloyl-labeled tRNA does not seem to be functionally active, as far as the aminoacyl charging activity is concerned, surprisingly we found that it is able to form the ternary complex with elongation factor Tu (EF-Tu) and GTP with an affinity consistently higher than uncharged tRNA. From fluorescence anisotropy measurements the ternary complex dissociation constant was estimated as 73 nM for *Escherichia coli* and 140 nM for yeast anthraniloyl-tRNA$^{Phe}$. These results may be interpreted in terms of the particular structure of the anthraniloyl group that makes the labeled tRNA similar to an aminoacyl-tRNA.

The reason why the codon–anticodon interaction between aminoacyl-tRNA and mRNA on ribosomes requires the formation of an EF-Tu · GTP · aatRNA complex is still arguable. Several methods have been used to study the ternary complex formation. Among these, the most incisive in determining the complex topology are the fluorescence methods. However, for this technique to be successfully employed requires chemical modifications of tRNA that leave it substantially unmodified regarding the interaction with EF-Tu and ribosome. The majority of the chemical modification reactions performed involve the 4-thiouridine base at position 8 (Adkins et al., 1983; Janiak et al., 1990), the replacement of wybutine or dihydrouracil with ethidium (Wintermayer and Zachau, 1979) or proflavine (Robbins and Hardesty, 1983) and modification of the 3′ end (Abrahams et al., 1990; Nagamatsu, 1989).

The 3′ terminus of tRNA can be directly modified, via periodate oxidation of the ribose moiety, to introduce a fluorescent label. Unfortunately this technique inactivates tRNA in the aminoacylation reaction so that it cannot be recognized by EF-Tu (Churchrich, 1963; Yang and Söll, 1973). Alternatively very selective modification of the CCA end can be achieved by enzymatic methods; in this way the penultimate cytidine residue can be substituted by 2-thiocytidine, which can be selectively alkylated by iodoacetamide derivatives (Sprinzl et al., 1977). However, the alkylation reaction must occur after aminoacylation of tRNA, otherwise the aminoacylation activity is lost (Ott et al., 1989).

In this paper we describe a new fluorescent tRNA derivative, obtained by reaction of the nucleic acid with isatoic anhydride, which introduces the anthraniloyl group at the 3′-*O* position of the ultimate adenosine. This tRNA derivative is obviously no longer chargeable by the specific amino acid. However, we found that it forms the ternary complex with EF-Tu and GTP, thus behaving as an aminoacyl-tRNA.

## MATERIALS AND METHODS

### Materials

tRNA$^{Phe}$ from yeast and tRNA$^{Phe}$ from *Escherichia coli*, phosphodiesterase from *Crotalus durissus*, guanyl nucleotides, phosphoenolpyruvate and pyruvate kinase were purchased from Boehringer Mannheim. Ribonuclease A and T$_1$ were obtained from Sigma. Isatoic anhydride [3,1-benzoxazine-2,4(1*H*)-dione] was from Molecular Probes; Aurodox was a gift of Dr G. Chinali. Other reagents were of analytical grade.

EF-Tu was purified as a complex with GDP from *E. coli* A19 strain at mid-log phase, as described by Arai et al. (1972). The complex concentration was determined by the absorbance at 280 nm using an absorption coefficient of 41600 $M^{-1}$ $cm^{-1}$ (Abrahamson et al., 1985).

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*Abbreviations*: anl-Ado, 3′-anthraniloyladenosine; anl-tRNA, 3′-anthraniloyl-tRNA; EF-Tu, elongation factor Tu.

*Enzymes*: Pyruvate kinase (EC 2.7.1.40); phosphodiesterase (EC 3.1.4.1); ribonuclease A (EC 3.1.27.5); ribonuclease T$_1$ (EC 3.1.27.1); aminoacyl-tRNA synthetases (EC 6.1.1); phenylalanine-tRNA synthetase (EC 6.1.1.20).

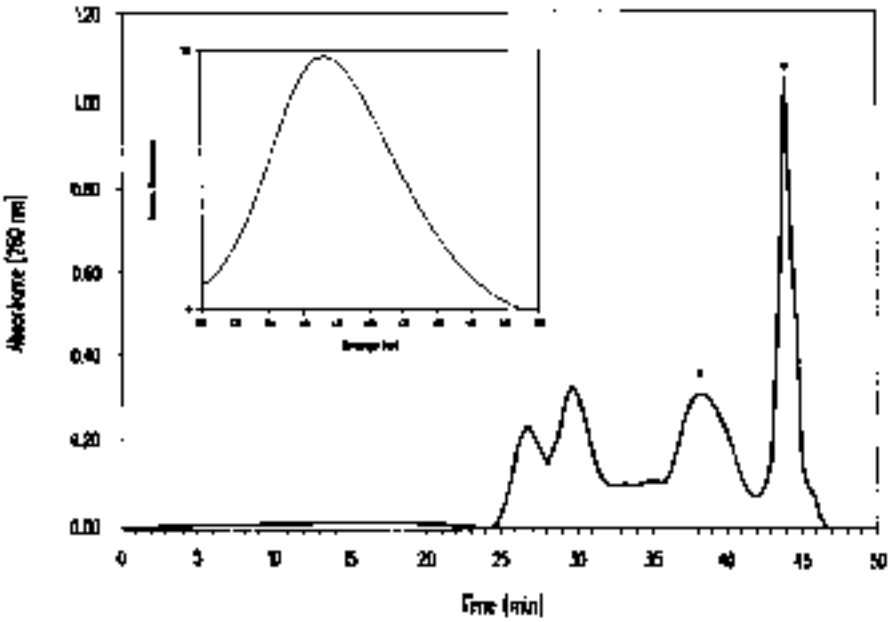


**Fig. 1. Chromatography of *E. coli* anthraniloyl tRNA$^{Phe}$ on phenyl-Superose column.** The column was developed as described in Materials and Methods. The peaks marked with the asterisks were fluorescent. The inset shows the fluorescence emission spectrum upon excitation at 330 nm of the last peak.

## EF-Tu · GTP complex preparation

EF-Tu·GDP (15 μM) was incubated at 35°C for 20 min with 10 μM GDP, 30 μM phospho*enol*pyruvate and 5 U/ml of pyruvate kinase in a buffer containing 50 mM Tris/HCl pH 7.4, 30 mM ammonium chloride, 10 mM magnesium acetate and 5 mM dithiothreitol. The GDP→GTP conversion was estimated by HPLC, as described below.

## Labeling of tRNA$^{Phe}$

tRNA$^{Phe}$ (from *E. coli* or from yeast) was dissolved in water at a concentration of 80 μM, the pH was adjusted to 8.8 with NaOH and a solution of isatoic anhydride in acetonitrile (3 mg/ml) was added, with continuous stirring, to final concentration of 1600 μM. The pH was maintained at 8.8 by titration with 0.1 M NaOH and the reaction was allowed to proceed for 3 h at room temperature. The anthraniloyl-tRNA$^{Phe}$ adduct was purified from the reaction mixture by ethanol precipitation. This procedure was repeated three times. Then, the adduct was resuspended in 100 mM potassium acetate pH 5 and purified by gel-filtration on Sephadex G-25. Two fluorescent peaks were eluted from the column. The first contained the labeled tRNA$^{Phe}$, the second, eluted at a higher volume, contained anthranilic acid. Finally, the fluorescently labeled tRNA$^{Phe}$ was purified from the unreacted tRNA$^{Phe}$ by hydrophobic interaction chromatography on a phenyl-Superose prepacked column (HR 5/5 (Pharmacia), using an FPLC apparatus (Pharmacia). The fractions containing the labeled tRNA$^{Phe}$ obtained from the Sephadex G-25 column were pooled, precipitated with ethanol, resuspended in 50 mM sodium phosphate pH 7 containing 1.7 M ammonium sulfate and loaded onto the phenyl-Superose column. The column was run at room temperature with a flow rate of 0.5 ml/min and developed with a stepwise gradient obtained by mixing solution A (50 mM sodium phosphate pH 7, 1.7 M ammonium sulfate) and solution B (50 mM sodium phosphate pH 7) in the following manner: 0% B (2 min), a linear gradient from 0% B to 50% B (30 min), 50% B (5 min), 100% B (10 min).

## 3′-*O*-Anthraniloyl-adenosine preparation

The anthraniloyl-adenosine (anl-Ado) was prepared according to Hiratsuka (1983). It was purified further by HPLC on a reverse-phase $C_{18}$ column by isocratic elution with the solvent methanol/50 mM ammonium formate pH 4 (1:1, by vol.) at flow rate of 1 ml/min, the purified anl-Ado being used as standard.

## Nucleotide separation

The separation of nucleoside monophosphates was achieved by HPLC using an anion exchange column (4.6 × 250 mm) of Ultrasil AX (Beckman). The column was eluted with 20 mM potassium phosphate pH 4 (solvent A) for 20 min, then with a linear gradient of 0–80% 1 M potassium phosphate pH 5 (solvent B) for 15 min. The elution was monitored simultaneously for absorbance and fluorescence by placing in series an ultraviolet detector set at 254 nm and a fluorescence detector with an excitation wavelength set at 330 nm and emission at 430 nm.

The GDP→GTP analysis was performed on the same column by isocratic elution at 60% of solvent B and a flow rate of 1 ml/min, monitoring the absorbance at 254 nm.

## Aminoacylation of *E. coli* tRNA$^{Phe}$

tRNA$^{Phe}$ (10–50 pmol) was incubated at 37°C for 15 min in 100 μl buffer containing 50 mM Tris/HCl pH 7.5, 10 mM magnesium acetate, 80 mM ammonium chloride, 2 mM dithiothreitol, 2 mM ATP, 5 mM phospho*enol*pyruvate, 2 U pyruvate kinase, 2 μM [$^{14}$C]phenylalanine (513 Ci/mol) and 5 μl aminoacyl synthetase solution purified from *E. coli* A19 strain according to Wagner and Sprinzl (1979). The reaction was stopped by adding 2 ml cold 5% trichloroacetic acid solution and the mixtures were left for 10 min in an ice bath and filtered on glass-fiber filters. The filters were rinsed three times with 2 ml 5% trichloroacetic acid solution and, after drying, $^{14}$C measured in a liquid scintillator.

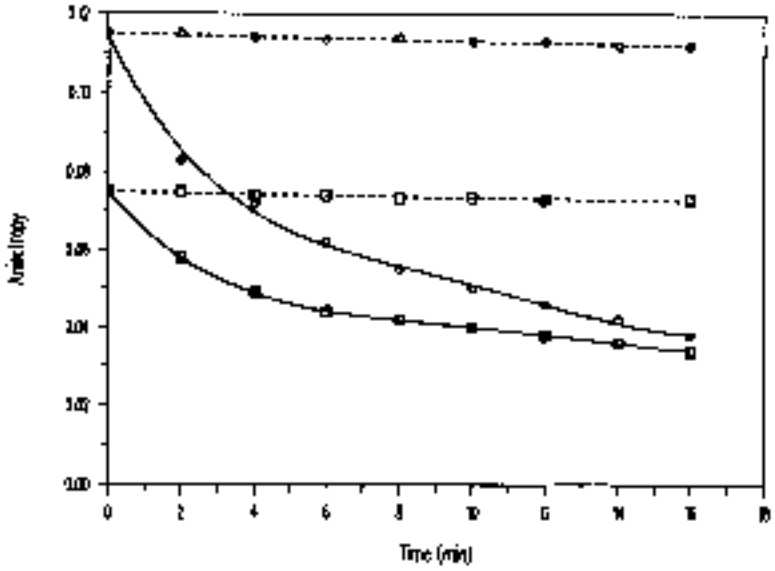


**Fig. 2. Time course of nuclease digestion of *E. coli* and yeast anthraniloyl tRNA$^{Phe}$.** The fluorescence anisotropy was measured at 420 nm upon excitation at 330 nm. The incubation mixture contained in 1 ml final volume 20 mM Tris/HCl pH 7.6, 100 mM KCl, 10 mM magnesium acetate, 1 mM dithiothreitol, 50 nM *E. coli* anti-tRNA (□) or yeast anti-tRNA (○) and 1 μg RNase A ( ) or 1 μg phosphodiesterase (– – –).

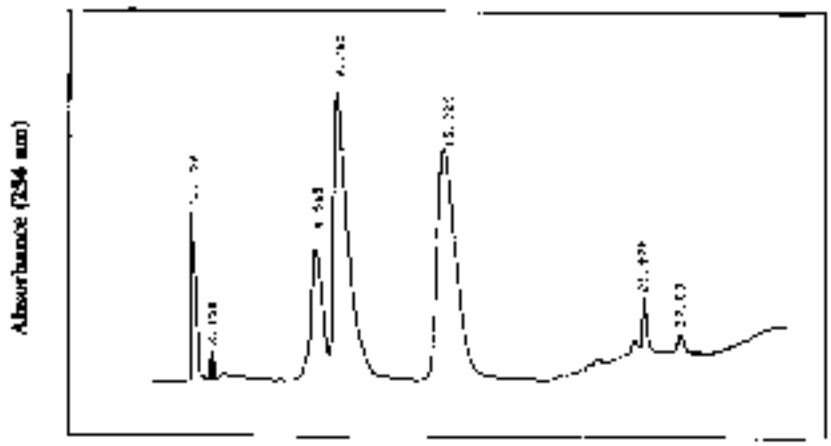


**Fig. 3. HPLC analysis of the RNase digest of *E. coli* anthraniloyl tRNA$^{Phe}$.** Anti-tRNA (0.2 mg) was digested with 5 μg RNase A for 5 h at 37°C, then the pH was lowered to 4.5 and 20 units RNase $T_1$ was added. The incubation was protracted overnight at 37°C. The nucleotides were analyzed by HPLC as described in Materials and Methods. The blackened peak is the only fluorescent peak detected and has the same retention time as anti-Ado. The other peaks were CMP and UMP (retention time = 8.565 min), AMP (9.765 min), GMP (15.328 min).

## Fluorescence anisotropy measurements

Fluorescence anisotropy measurements were made on a Perkin-Elmer MPF 66 spectrofluorometer interfaced to a Perkin-Elmer 7700 calculator. The band pass was 10 nm on both excitation (330 nm) and emission (420 nm). The absorbance of the samples never exceeded 0.01 at the excitation wavelength. The anisotropy ($r$) is defined as:

$$r = I_{vv} - GI_{vh}/I_{vv} + 2GI_{vh}$$

where the $G$ factor ($= I_{hv}/I_{hh}$) corrects for unequal transmission of horizontally and vertically polarized light.

The ternary complex formation was obtained in 1 ml buffer containing 50 mM Tris/HCl pH 7.4, 30 mM ammonium chloride, 10 mM magnesium acetate, 5 mM dithiothreitol, 45 nM 3'-anthraniloyl-tRNA$^{Phe}$ (anti-tRNA$^{Phe}$) (from *E. coli* or yeast) and varying amounts of EF-Tu · GTP complex. The solution was thermostatted at 25°C. The fluorescence intensity measurements were corrected for blanks made up as above except for the fluorescent anti-tRNA. Equilibrium constants were determined by measuring fluorescence anisotropy 10 min after each addition of a known amount of EF-Tu · GTP. The measured fluorescence anisotropy is, in fact, linearly correlated to the fraction of free and EF-Tu · GTP bound anti-tRNA according to the equation:

$$r = f_f r_f - f_b r_b$$

where $r_f$ and $r_b$ are the anisotropies of the free and bound anti-tRNA and $f_f$ and $f_b$ the fraction of the free and bound anti-tRNA in the experimental conditions of the measurement (Lakowicz, 1983).

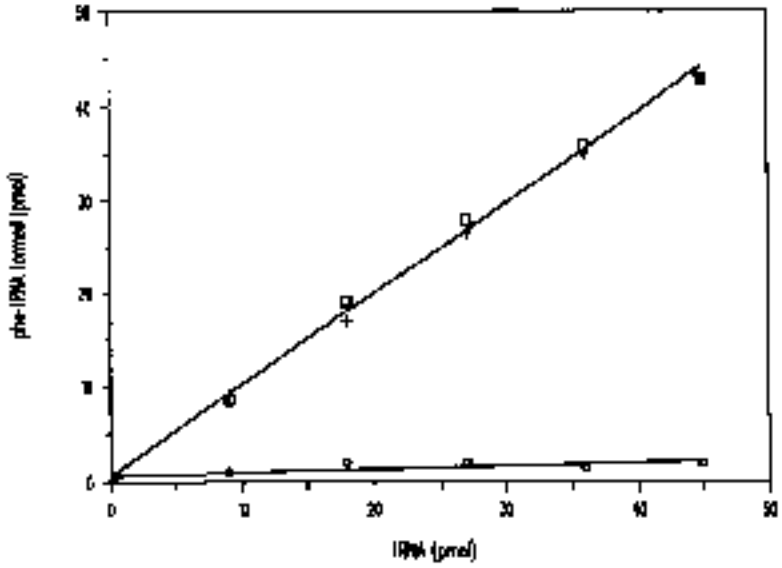


**Fig. 4. Aminoacylation of *E. coli* anthraniloyl tRNA$^{Phe}$ with phenylalanine-tRNA synthetase.** Increasing amounts of anl-tRNA$^{Phe}$ were incubated as described in Materials and Methods with the specific aminoacylsynthetase (○); tRNA$^{Phe}$ was used as control in the presence (□) and absence (+) of 45 pmol anr anl-tRNA$^{Phe}$.

**Table 1. Fluorescence anisotropy of anl-tRNA$^{Phe}$ in various conditions.** The solution of anl-tRNA was added with the listed components at the indicated concentration and allowed to stand 30 min at 25°C before the anisotropy measurements.

| Addition | Concn | Anisotropy of 45 nM anl-tRNA$^{Phe}$ | |
|---|---|---|---|
| | | yeast | *E. coli* |
| | nM | | |
| None | | 0.115 | 0.075 |
| EF-Tu · GTP | 70 | 0.131 | 0.096 |
| EF-Tu · GTP | 200 | 0.146 | 0.111 |
| EF-Tu · GTP | 3400 | 0.170 | 0.126 |
| EF-Tu · GTP | 3400 | 0.117 | 0.076 |
| + Phe-tRNA$^{Phe}$ | 50 | | |
| EF-Tu · GTP | 3400 | 0.165 | 0.122 |
| + tRNA$^{Phe}$ | 400 | | |
| EF-Tu · GTP | 3 000 | 0.168 | 0.120 |
| + Aurodox* | 12 000 | | |
| EF-Tu · GTP | 3 000 | 0.107 | 0.070 |
| + Aurodox* | 12 000 | | |

* Measured immediately after Aurodox addition.
* Measured after 3-h incubation at 37°C.

## RESULTS

The elution profile of derivatized *E. coli* tRNA$^{Phe}$ from the phenyl-Superose column, developed as reported in Materials and Methods, is shown in Fig. 1. Only two fluorescent peaks were eluted from the column, the last one, which was the most abundant, was eluted at the end of the gradient (at zero concentration of ammonium sulfate) and was utilized for successive experiments. The other fluorescent peak is probably non-chargeable-tRNA that was derivatized. In fact, only the second of the two non-fluorescent peaks eluted was found to be aminoacylable by the specific aminoacyl synthetase.

The absorption spectrum of the last fluorescent peak shows two maxima centered at 260 nm and 332 nm. The ratio $A_{260}/A_{332}$ was found to be 110. This indicates an incorporation of about 1 mol labelmed tRNA$^{Phe}$, assuming an absorption coefficient for the anthraniloyl residue of 4600 $M^{-1}$ $cm^{-1}$ at 332 nm (Hiratsuka, 1983) and for tRNA of $5 \times 10^5$ $M^{-1}$ $cm^{-1}$ (Scott and Schulman, 1979). A similar elution pattern was observed starting from derivatized yeast tRNA$^{Phe}$ and, in this case also, $A_{260}/A_{332}$ of the last peak was about 110.

The label specificity was demonstrated by subjecting the modified tRNAs to RNase A digestion. The reaction was followed by measuring the fluorescence anisotropy with the time. In fact, the modified tRNAs show a fluorescence anisotropy consistently higher than the free fluorophore (0.075 for *E. coli*; 0.115 for yeast). In the presence of RNase (Fig. 2), the anisotropy decreased to 0.03, which corresponds to the anisotropy of anl-Ado that has been synthesized as described by Hiratsuka (1983). Instead, the labeled tRNAs, when subjected to phosphodiesterase hydrolysis, do not modify their anisotropy during an incubation time of 20 min (Fig. 2), thus indicating a considerable resistance to this enzyme. Moreover, the incubation of both anl-tRNAs in 0.1 M NaOH for 1 h at 25°C resulted in the complete hydrolysis of the fluorophore which was separated by gel filtration on Sephadex G-25 from tRNA and demonstrated to be anthranylic acid by TLC.

In order to identify the labeling site(s) the modified tRNAs, from *E. coli* and from yeast, were separately subjected to exhaustive digestion with RNase A and RNase $T_2$. The products were analyzed by anion-exchange HPLC and the chromatogram compared to that of anl-Ado used as a standard. The elution profile was simultaneously monitored for absorption at 254 nm and for fluorescence intensity. Fig. 3 reports the results for *E. coli* anl-tRNA digestion. Only one fluorescent peak, showing the same retention time as that of anl-Ado, was detected after the front peaks. For a further identification of the fluorescent peak, the fractions were pooled, concentrated and analyzed by TLC on two different media, i.e. silica gel and cellulose plates, as described by Hiratsuka (1983). In both cases, the same $R_f$ of anl-Ado, utilized as a standard, was found for the unique fluorescent spot visualized on the two plates.

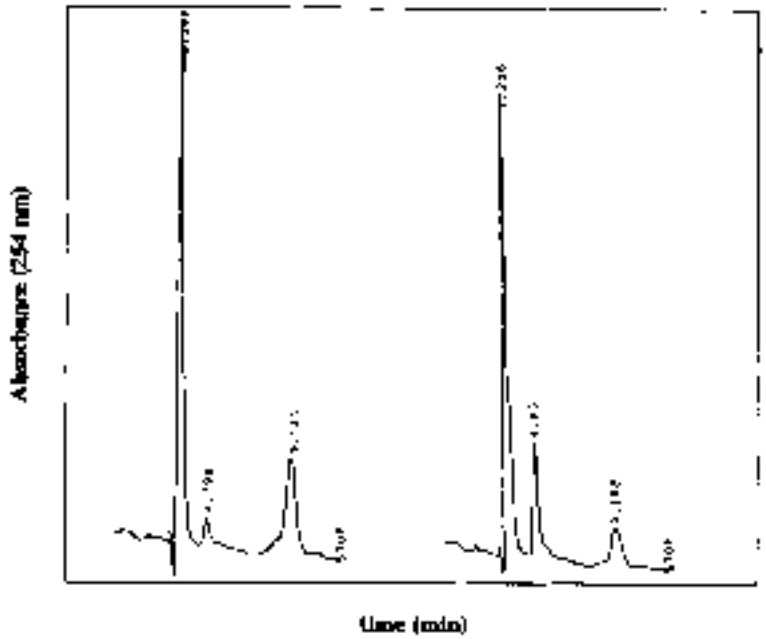


**Fig. 5. HPLC analysis of GDP and GTP.** The analysis was performed as described in Materials and Methods. The retention time of GDP was 4.8 min and that of GTP was 9.1 min. The EF-Tu · GTP · anti-tRNA complex was incubated with 12 mM Aurodox at 37°C. Aliquots were taken at time 0 (left) and after a 3-h incubation (right) and analyzed.

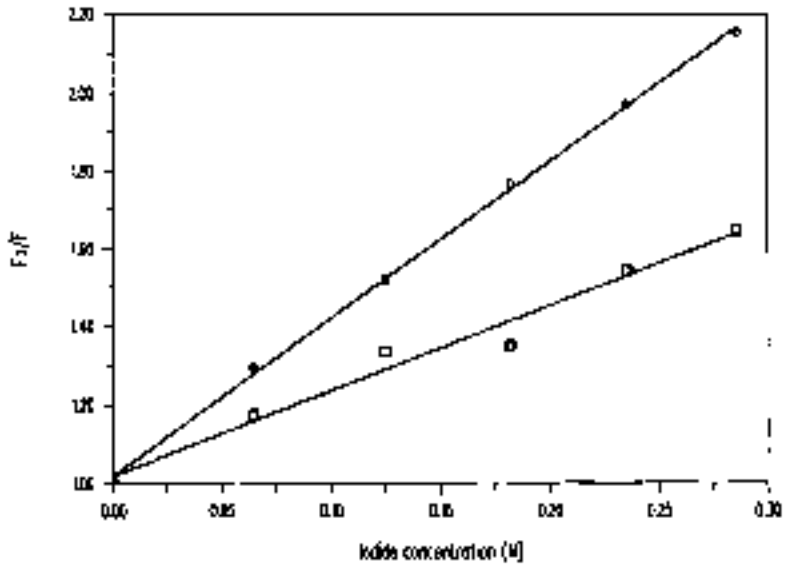


**Fig. 6. Fluorescence emission quenching of anti-tRNA$^{Phe}$ by Iodide Ions.** The fluorescence emission at 420 nm was plotted against iodide concentration according to Stern-Volmer equation. The experiments were performed in the presence of EF-Tu · GTP complex (○) or in the presence of EF-Tu · GDP complex (□).

*E. coli* anti-tRNA$^{Phe}$ was assayed, as described in Materials and Methods, for the capacity to accept the $^{14}$C-labeled phenylalanine in the aminoacylation assay with homologous synthetase. Fig. 4 clearly shows that anti-tRNA$^{Phe}$ has no ability to charge the phenylalanine, compared to the unlabeled tRNA$^{Phe}$ in the same conditions. However, we found that unlabeled tRNA$^{Phe}$ is normally aminoacylated in the presence of anti-tRNA (Fig. 4).

To examine the behavior of anti-tRNA with EF-Tu, anti-tRNA from *E. coli* and from yeast were assayed in the same conditions where aminoacyl-tRNAs form the ternary complex with EF-Tu and GTP. The addition of EF-Tu · GTP to a solution of anti-tRNA$^{Phe}$ causes an increase of fluorescence anisotropy. To evaluate the affinity constant of EF-Tu · GTP · anti-tRNA, the concentration dependence of fluorescence anisotropy increasing the EF-Tu · GTP concentration was measured. After the addition of an excess of EF-Tu · GTP the fluorescence anisotropy reached the maximum value of 0.126 for *E. coli* and 0.170 for yeast (Table 1). These values were utilized in the calculation of $K_d$ as the anisotropies of the two anti-tRNAs bound in the ternary complex. From analysis of the data reported in Table 1, values of $K_d$ = 7.3 nM for *E. coli* and $K_d$ = 140 nM for yeast were calculated.

It is worth noting that when assaying anti-tRNA in the presence of the complex EF-Tu · GDP in the same concen-

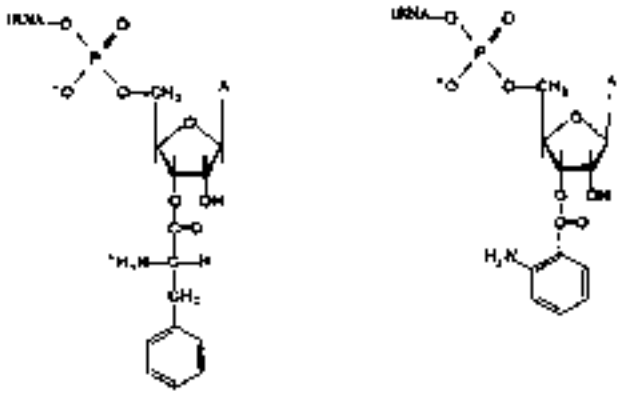

**Fig. 7. Chemical structure of anti-tRNA.** The chemical structure of the adduct formed between the ribose of the ultimate adenosine residue of tRNA and the isatoic anhydride (right) is compared to that of phenylalanyl adenosine terminus of Phe-tRNA$^{Phe}$ (left).

tration range of the previous experiment, no increase of the fluorescence anisotropy was observed. This excludes the formation of appreciable amount of ternary complex among EF-Tu, GDP and anti-tRNA in these experimental conditions.

More evidence that these results can be ascribed to the formation of the ternary complex among anti-tRNA$^{Phe}$, EF-Tu and GTP arise when titrating the ternary complex with Phe-tRNA$^{Phe}$. In fact, after addition of increasing amounts of Phe-tRNA$^{Phe}$ to the solution containing the complex, a decrease of the fluorescence anisotropy occurred that indicates a displacement of fluorescent anti-tRNA$^{Phe}$ by Phe-tRNA$^{Phe}$ in the ternary complex (Table 1). Conversely, the addition of about ninefold excess of uncharged tRNA$^{Phe}$ does not affect the fluorescence anisotropy (Table 1). We also examined the effect of addition of Aurodox (*N*-methylkirromycin) to the ternary complex solution of anti-tRNA with EF-Tu · GTP. A time-dependent decrease of fluorescence anisotropy with a concomitant hydrolysis of GTP to GDP was observed (Table 1 and Fig. 5). This is evident for either ternary complex formation or the engagement of the 3′-*O* position of the ultimate adenosine of tRNA by an anthraniloyl residue. It is known, in fact, that kirromycin and Aurodox stimulates, in the presence of aa-tRNA or of aminoacylated fragments of tRNA, the GTPase activity of EF-Tu (Parlato et al., 1981; Bhuta and Chladek, 1980).

In order to gain an insight into the environment of the anthraniloyl moiety when anti-tRNA$^{Phe}$ is involved in the ternary complex, fluorescence quenching experiments on solutions of anti-tRNA$^{Phe}$ alone and in the presence of EF-Tu and GTP were performed. We found that the anthraniloyl fluorophore behaves differently when anti-tRNA$^{Phe}$ is free or involved in the ternary complex. Fig. 6 reports, in fact, fluorescence quenching experiments with iodide ions in conditions which either prevented or promoted ternary complex formation. As one can see, the Stern-Volmer constant increases when the ternary complex is formed, thus indicating a more efficient quenching of the fluorophore when the complex is formed than in the free anti-tRNA$^{Phe}$.

## DISCUSSION

The labeling procedure described in this paper represents a simple and general method to introduce at the 3′ terminus of tRNAs an anthraniloyl residue with good characteristics for fluorescence studies. It is demonstrated, in fact, that isatoic anhydride could specifically label tRNAs at the 3′ terminus by a single-step reaction. This finding is not surprising since it is known that the reactivity of the 3′ terminus is higher than other tRNA zones, this region being single-stranded and consequently well exposed to chemical reagent attack (Bhanot et al., 1977).

The single labeling site is demonstrated by several independent tests: the $A_{260}/A_{280}$ ratio; the finding of a single fluorescent nucleoside, after RNase A and RNase T$_1$ exhaustive digestion, identified as 3′-anthraniloyl-adenosine; the resistance of the modified tRNA to phosphodiesterase digestion which can be explained by assuming that anti-tRNA is not recognized by the enzyme since the 3′ terminus is modified by the anthraniloyl group. Actually, in the case of *E. coli* tRNA$^{Phe}$, which possesses in position 47 the rare base 3-(3-amino-3-carboxypropyl)uridine (acp$^3$U) bearing a free amino group, the possibility of labeling at this point by isatoic anhydride cannot be ruled out. However, we did not observe this event in our reaction conditions. In fact, mild alkali hydrolysis easily cleaves the esteric anthraniloyl-3′-*O*-adenosine link (Fallbrard et al., 1967; Guillory and Jeng, 1977), whereas the amide bond would be much more stable. Moreover, it was reported (Schiller and Schechter, 1977) that the acp$^3$U base of *E. coli* tRNA$^{Phe}$ can be labeled by the *N*-hydroxysuccinimide ester of *N*-methyl-anthranylic acid or of 1-dimethylaminonaphthalene-5-sulfonyl-glycine. These modified tRNAs were only poorly aminoacylated and were found to be strong competitive inhibitors of phenylalanine-tRNA synthetase. Thus it was inferred (Schiller and Schechter, 1977) that the integrity of the polar amino acid group of acp$^3$U is required for the biologically active conformation of this tRNA. Therefore, our finding that the tRNA$^{Phe}$ derivatized with isatoic anhydride does not inhibit phenylalanine-tRNA synthetase would further exclude the labeling of acp$^3$U base. On the other hand, the observation that isatoic anhydride can selectively label an alcoholic group in the presence of amino groups has been reported (Moorman and Abeles, 1982) for its reaction with α-chymotrypsin. These authors found that only one mole anthraniloyl residue was incorporated/mole protein at a serine residue.

For the purification of anti-tRNA$^{Phe}$ from other possible by-products and from unreacted tRNA$^{Phe}$ a new procedure was developed. It relies on a hydrophobic interaction chromatography on phenyl-Superose. It is well known that higher hydrophobicity of many fluorescently labeled tRNAs can be advantageously employed for their purification from the unreacted tRNAs. On this basis, the use of the benzoylated DEAE-cellulose (Yang and Soll, 1973; Gillam et al., 1967) and of RPC-5 chromatography (Johnson et al., 1982; Wintermayer and Zachau, 1979) has been reported. The chromatographic technique described in this paper is equally as effective as those reported but is faster.

It is worth noting that an anthraniloyl-modified tRNA$^{Phe}$ has been described (Yang and Soll, 1973). However, in this case, the labeling group was attached by a pyrophosphate linkage at the 5′ end of the tRNA and could still be aminoacylated at the 3′ terminus by the homologous enzyme. Instead, as was expected in our case, the anti-tRNA$^{Phe}$ is no longer chargeable. This fact would exclude the possibility of employing the 3′-anti-tRNAs in studies of its interaction with other macromolecules requiring the aminoacylation of the 3′ terminus. Surprisingly, we found that the 3′anti tRNA$^{Phe}$ interacts with EF-Tu · GTP forming the ternary complex. The most simple explanation of this behavior is that the particular structure of the anthraniloyl moiety resembles that of an ami-

noacyl residue, particularly the phenylalanine (Fig. 7). However it should be mentioned that the protonated α-amino group seems to be essential for the recognition of the aminoacyl-tRNA by EF-Tu · GTP (Miller et al., 1973; Miller and Weissbach, 1977; Pingoud and Urbanke, 1980; Thompson et al., 1985). Instead, the amino group of the anthraniloyl moiety, being aromatic, should be unprotonated in experimental conditions at physiological pH. This could explain the lower affinity of anl-tRNA for EF-Tu GTP than phe-tRNA$^{Phe}$.

The ability of anl-tRNA$^{Phe}$ to behave as an aminoacylated tRNA makes it potentially useful for structural studies by fluorescence spectroscopy of its interactions, especially those involving the 3′ terminus, with many macromolecules, including ribosomes, in the course of protein biosynthesis. In this respect, the fluorescence quenching experiment on the ternary complex anl-tRNA$^{Phe}$ · EF-Tu · GTP is an example. We found, in fact, a more efficient iodide quenching on the anl-tRNA$^{Phe}$ when it is engaged in the ternary complex than when it is free in solution. It was previously reported that an oligonucleotide complementary to the CCA end of tRNA binds less efficiently to the free aminoacyl tRNA than when it is engaged in the ternary complex (Kruse et al., 1980). This observation led the authors to infer that the CCA end of tRNA is not covered by EF-Tu in the ternary complex. The same conclusion was reached by other authors (Ott et al., 1989) from the observation that the introduction of a bulky fluorescent group on the penultimate thiocytidine residue of a modified tRNA does not impair ternary complex formation. Our findings from fluorescence quenching experiments indicate a better exposure in the ternary complex of the reporter anthraniloyl moiety, and thereof of the tRNA 3′ terminus. This observation not only strengthens the hypothesis that the 3′ end of tRNA is not covered by EF-Tu but suggests that the interaction between EF-Tu and tRNA induces a conformational change at the 3′ end, leading to its better solvent exposure.

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