FLUORESCENCE OF TRYPTOPHANYL-tRNA^{Trp} FROM *E.coli*; AN INTERACTION BETWEEN THE INDOLE AND tRNA AND ITS DEPENDENCE ON tRNA CONFORMATION

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1. Introduction

While investigating potential fluorescent probes of conformation in tRNA we have measured the fluorescence of tryptophan esterified to tRNA^{Trp} from *E. coli*. We find that this fluorescence is highly quenched by comparison with tryptophan in free solution. A similar observation has recently been reported, using tRNA^{Trp} from rabbit liver [1]. In pursuing the basis of this quenching, we have isolated the terminal tryptophanyl adenosine, and report here that the quenching of tryptophan fluorescence in this compound explains partly the high quenching in tryptophanyl-tRNA^{Trp} (Trp-tRNA^{Trp}) but that other interactions must also be involved. Thus, the two conformational forms of Trp-tRNA^{Trp} [2], active and inactive with respect to enzymatic discharging, differ significantly in fluorescent yield. This shows that tryptophan interacts differently with the two forms.

2. Materials and methods

Tryptophanyl-tRNA^{Trp} was isolated on benzoylated DEAE-cellulose (Schwartz/Mann) from *E. coli* B tRNA (General Biochemicals) essentially by the method of Joseph and Muench [3], but omitting the final chromatography on hydroxylapatite. The specific activity of the product was 1.5 nmoles tryptophan/A_{260} unit. Active and inactive conformational forms were obtained by incubation in 0.1 M sodium acetate buffer, pH 4.1 and pH 5.6, respectively, the latter containing 4 mM EDTA Na_2, for 20 min at 37°. After cooling to 0°, the Trp-tRNA^{Trp} was precipitated with 2.5 vol cold ethanol and re-dissolved in 50 mM Tris-acetate buffer, pH 7.2, 50 mM K-acetate, 20 mM Mg-acetate, or 20 mM sodium-acetate buffer, pH 5, 5 mM $MgCl_2$. These incubation conditions and those of the enzymatic discharge assay were as described by Gartland and Sueoka [2]. Tryptophanyl-tRNA-synthetase for charging crude tRNA^{Trp} was isolated from *E. coli* B, according to Joseph and Muench [3], but omitting the chromatography on CG-50.

Tryptophanyl-adenosine was isolated from unfractionated *E. coli* B tRNA (2000 A_{260} units) charged with [^{14}C]tryptophan (20 pmoles/A_{260} unit; 2.8 μCi/μmole). tRNA was recovered from the charging mixture by phenol extraction and ethanol precipitation [3], and nucleotides, phenol, and ethanol were removed by passing the tRNA through a column (2 × 10 cm) of Sephadex G-25 in 10 mM sodium-acetate buffer, pH 5. The effluent fractions containing tRNA were pooled (total vol 6 ml) and digested with 2 mg ribonuclease (Worthington RAF) for 10 min at 25°. The digest was fractionated on DEAE-Sephadex A-25 (28 × 2.5 cm) in the same buffer. [^{14}C]Tryptophanyl-adenosine was excluded and emerged in the void volume, free from all other products of digestion and of any free tryptophan. The overall recovery of the ester was 62%, and solutions of the ester were transferred from H_2O to D_2O by ion-exclusion from DEAE-Sephadex A-25 on a small column, pre-equilibrated with D_2O. Preparations of Trp-tRNA^{Trp} and tryptophanyl-adenosine were discharged after adjustment of the pH to 8.8 by addition of 2 M NaOH to Tris buffer, or 2 M Tris to acetate buffer. Incubation

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Table 1
Fluorescence of tryptophanyl-tRNA^{Trp} and tryptophanyl-adenosine.

Substance	pH	Fluorescence as % free Trp	Fluorescence in D_2O / Fluorescence in H_2O
	5.0 or		
Tryptophan	7.2	100	1.59
Trp-adenosine	5.0	23	0.96
Trp-ethyl ester	4.5	17*	
Trp-tRNA^{Trp} (active)	5.0	3.5 ± 0.2	
Trp-tRNA^{Trp} (inact.)	5.0	4.9 ± 0.2	
Trp-tRNA^{Trp} (active)	7.2	6.9 ± 0.2	
Trp-tRNA^{Trp} (inact.)	7.2	8.4 ± 0.2	

* Value from Weinryb and Steiner [4].

Fluorescent intensities were measured in 50 mM Tris-acetate buffer, pH 7.2, 50 mM K-acetate, 20 mM Mg-acetate, or 20 mM sodium acetate buffer, pH 5.0, 5 mM $MgCl_2$. After ester hydrolysis at pH 8.8, the pH was re-adjusted to the initial value and the fluorescence of the released tryptophan measured. The fluorescence of each tryptophanyl compound is expressed as a percentage of that of the tryptophan released by hydrolysis.

was for 2 hr at 37°. Discharging the Trp-tRNA^{Trp} followed first order kinetics with a period for half exchange of 15 min. Solutions were re-adjusted to their initial pH before measurement by fluorescence of the released tryptophan. The increase in buffer concentration had no effect on the quantum yield of free tryptophan.

Fluorescent spectra were recorded on a FICA 55 absolute differential spectrofluorimeter equipped with a Hewlett–Packard XY recorder. All measurements were made at 3°.

Labelled tryptophan and D_2O were obtained from C.E.A., Saclay, France.

3. Results and discussion

Fluorescence measurements on Trp-tRNA^{Trp} and Trp-adenosine are presented in table 1. The fluorescent yield from Trp-adenosine is close to that quoted

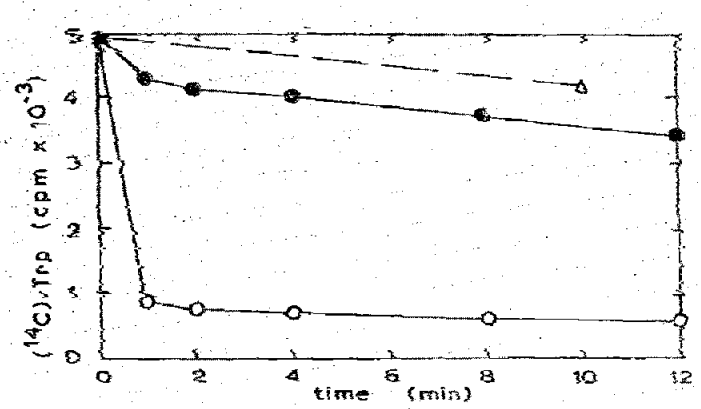

Fig. 1. Enzymatic discharge of active and inactive Trp-tRNA^{Trp}. Active and inactive forms of Trp-tRNA^{Trp} were made by incubation at pH 4.1 or 5.6 in sodium-acetate buffer, as described under Methods. After incubation for 2 hr at 5° in 50 mM Tris-acetate buffer, pH 7.2, 50 mM K-acetate, 20 mM Mg-acetate, or 20 mM sodium-acetate buffer, pH 5, 5 mM $MgCl_2$, aliquots containing 20 pmoles [^{14}C]Trp-tRNA (29 mCi/mmole) were incubated with tryptophanyl-tRNA-synthetase in 0.1 M Tris-acetate buffer, pH 7.2, 5 mM $MgCl_2$, 4 mM AMP, and 4 mM pyrophosphate. The abscissa shows the [^{14}C]tryptophan remaining esterified to the active form (●—●—●), the inactive form (○—○—○), and to either form with no enzyme.

for tryptophan-ethyl ester and it seems likely that the two compounds are quenched by a similar mechanism though the nature of the mechanism is not yet clear (for a discussion of tryptophan-ethyl ester quenching see [4]). The data indicate, however, that the mechanism is not sensitive to substitution of solvent hydrogen by deuterium, in contrast to tryptophan [5], though it should be noted that no attempt was made to distinguish a deuterium effect from one due to differences in dissolved oxygen.

The greater fluorescence of Trp-tRNA^{Trp} at pH 7.2 than at pH 5.0 is consistent with partial deprotonation of the tryptophanyl amino group associated with the decrease in pK of this group upon ester formation. Different conformational forms of Trp-tRNA^{Trp} were obtained as described by Gartland and Sueoka [3] and their stability under the conditions of fluorescent measurement were confirmed by enzymatic discharge assay (fig. 1). At both pH 5 and pH 7.2 the fluorescence of the active form was significantly lower than that of the inactive form. The striking decrease in the fluorescence of Trp-tRNA^{Trp} compared to Trp-adenosine shows that there must be

a direct interaction between the indole ring and the tRNA. Furthermore, this interaction is modified when the tRNA assumes the inactive conformation. In the absence of much direct evidence one can only attempt to predict the probable nature of the indole–tRNA interaction. One possible mechanism for the quenching observed would involve proton transfer from the indole NH group in the excited state [6] to a site on the tRNA mediated possibly by a hydrogen bond present in the ground state complex. Our data on the effect of heavy water on the fluorescence of Trp-tRNA^{Trp} was not sufficiently accurate to allow any conclusion which would bear on this mechanism. A further possible type of interaction is that observed in complexes between tryptamine or 5-hydroxy-tryptamine and DNA and poly A where there is stacking of the indole ring and nucleotide bases and strong quenching of the indole fluorescence [7]. Such a preferred conformation is also seen between the p-metoxyphenylalanyl side chain and base moeities in crystalline puromycin [8]. The interaction here is intramolecular and the structure is an "open" conformation, with aromatic ring and base on opposite sides of the peptide bond. It is thought that the "open" conformation will predominate also in solution.

Similar considerations are likely to apply to tryptophanyl-adenosine, so that the intramolecular stacked configuration is unlikely, and this is supported by our fluorescence data. Molecular orbital calculations also favour an "open" structure, though they suggest that aminoacyl-adenosine moeities may have somewhat more conformational freedom than puromycin [9]. A stacking interaction in Trp-tRNA^{Trp} would favour models with the 3′-terminus rather close to one loop, or a sugar-phosphate backbone conformation in the terminal region which brings the amino acid into proximity with other bases in the acceptor stem. There is experimental evidence in favour of an interaction between the 3′-terminal base and other parts of the tRNA molecule [10].

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