# BINDING OF UGA TO WILD TYPE AND SUPPRESSOR TRYPTOPHAN tRNA FROM *E. COLI**

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Received 2 December 1974

## 1. Introduction

Suppression of nonsense triplets normally occurs by a mutation in the anticodon of a tRNA [1]. A nucleotide exchange gives rise to a new anticodon sequence which is complementary to the nonsense triplet. This mutation therefore enables the tRNA to read a nonsense codon and incorporate an amino acid into the polypeptide chain. One exception to this mechanism has been described by Hirsh [2] who observed that $tRNA^{Trp}$ from *E. coli* CAJ 64, a suppressor-tRNA which responds to the triplet UGA, possesses an unaltered –CCA sequence in the anticodon. The mutation-induced base exchange that renders this tRNA suppressor positive was found to occur at position 24 of the polynucleotide chain, a part of the dihydro-uridine-stem. In $tRNA^{Trp}$ from CAJ 64 ($su^{+}_{UGA}$) a guanosine residue which occurs in wild type tryptophan tRNA in this position, is substituted by an adenosine.

The mechanism by which the base substitution in the dihydro-U-stem of $tRNA^{Trp}$ affects the codon recognition is still unknown. In an attempt to elucidate this mechanism, ribosome-free binding [3] of UGA to both $tRNA^{Trp}$ from *E. coli* CAJ 64 ($su^{+}_{UGA}$) and from *E. coli* CA 265 ($su^{-}_{UGA}$) was measured. The results are presented in this paper.

## 2. Materials and methods

$^{14}C$ Tryptophan (52 mCi/mmole), [$^{3}H$] ADP (15 Ci/mmole) and [$^{3}H$] CTP (22 Ci/mmole) were obtained from The Radiochemical Centre, Amersham, Great Britain. UpG and ADP were from Papierwerke Waldhof-Aschaffenburg, Mannheim, Germany. Unfractionated *E. coli* B tRNA was purchased from Schwarz/Mann, Orangeburg, N.Y., USA. Snake venom phosphodiesterase was a product of Sigma, St. Louis, Mo., USA, polynucleotide phosphorylase was obtained from Boehringer Mannheim, Germany. $tRNA^{Trp}$ from CA 265 ($su^{-}_{UGA}$) was a gift from Dr. B.F.C. Clark. A culture of *E. coli* CAJ 64 was kindly supplied by Dr. J.D. Smith.

### 2.1. *Preparation of $tRNA^{Trp}$ from E. coli CAJ 64 ($su^{+}_{UGA}$)*

*E. coli* CAJ 64 was grown in a full medium at 37°C to the late logarithmic phase and collected by centrifugation in a continuous flow rotor. From 500 g of wet cell paste the crude tRNA was isolated as described by Avital and Elson [4]. The tRNA was subjected to reverse-phase chromatography 3 at pH 7 [5]. The fractions containing tryptophan-acceptor activity were further purified by chromatography on benzoylated DEAE-cellulose with $MgCl_2$-containing elution buffers and subsequently on the same ion exchanger with an EDTA-containing elution medium [6]. The material collected from the pooled fractions accepted 1150 pmoles tryptophan per $A_{260}$ unit.

### 2.2. *Synthesis of $^{3}H$-labeled UpGpA*

[$^{3}H$] ADP (300 $\mu$Ci) was dried and the residue dissolved in 100 $\mu$l of a mixture containing 430 $\mu$g cold ADP, 500 $\mu$g UpG, 25 $\mu$g polynucleotide phos-

* A brief account of this work has been presented at the 9th International Congress of Biochemistry, Stockholm 1973.



*North-Holland Publishing Company – Amsterdam*

phorylase, 200 mM Tris–HCl, pH 9.5, 10 mM $MgCl_2$ and 120 mM NaCl. After an overnight incubation at 34°C the product was chromatographed and recovered as described before [3].

2.3. *Removal of the –CCA terminus from tRNA*

The phosphodiesterase digestion method of Philipps [7] was followed without the Sephadex G-100 step. The effect of various incubation periods ranging from 30–120 min was examined whereby 60 min were found to give an optimal effect. Reconstitution was determined by incubating for 60 min at 37°C 0.1–0.3 $A_{260}$ units of phosphodiesterase-treated tRNA in 100 μl of a solution containing, in addition to buffer, salts and a SH-reagent (42 mM HEPES, pH 7, 26 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 10 mM dithioerythritol), 4.5 nmoles [$^3$H]CTP (sp. act. 2.2 Ci/mmole), 0.1 μmole ATP and an *E. coli* 100 000 *g* supernatant (280 μg protein) from which tRNAs had been removed by passage through a DEAE-cullulose column and which was then extensively dialyzed. After addition of ice-cold 10% trichloroacetic acid the mixture was filtered on a SARTORIUS SM 11306 nitrocellulose filter and washed four times with the trichloroacetic acid solution. After drying the filters were placed in scintillation fluid and counted.

The procedure for the estimation of amino acid acceptor activities and the experimental details of the micro-dialysis measurements have been previously described [3,8]. The binding constants were calculated using the formulae described in an earlier article [3]. The mean of duplicate measurements are listed in table 1. The molar extinction coefficient of 31800 for UGA was calculated according to Cantor and Tinoco [9].

Table 1
Binding constants of UGA–$tRNA^{Trp}$ complexes.

| | Native | Phosphodiesterase treated |
|---|---|---|
| $tRNA^{Trp}$ from $su^-$-strain | 497 ± 3 $M^{-1}$ | 275 ± 22 $M^{-1}$ |
| $tRNA^{Trp}$ from $su^+$-strain | 408 ± 88 $M^{-1}$ | 470 ± 55 $M^{-1}$ |

## 3. Results and discussion

The mutation-induced replacement of a guanosine by an adenosine in the dihydro-U-stem of a wild type $tRNA^{Trp}$ makes this tRNA suppressor positive for the triplet UGA [2]. In addition to this newly acquired codon response the suppressor-tRNA still fulfils its normal function as a UGG-coded tryptophan-tRNA. The UGA-recognition requires the formation of an A–C base-pair in the wobble position which is forbidden by the rules outlined in Crick's wobble hypothesis [10]. Since the mutation does not occur in the anticodon but at a rather distant part of the tRNA molecule, a novel and as yet unknown mechanism must be causing the different codon response. Two explanations seem plausible and would account for the effect:

1) An altered stereostructure of the antocodon, created by the base exchange in the dihydro-U-stem, may enable the $tRNA^{Trp}$ to pair with the triplet UGA. Evidence supporting the suggestion of a change of the tertiary structure by the suppressor mutation is provided by the observation of a different behaviour of wild type and suppressor-$tRNA^{Trp}$ towards 'denaturating' ionic conditions [2]. Thus the possibility of a stereochemically changed anticodon appears to be realistic.

2) Alternatively, as a result of the suppressor mutation, a change in the geometry of the $tRNA^{Trp}$-binding to the ribosomal A-site may be envisaged. This different ribosomal binding could lead to a different stereochemistry of the codon–anticodon interaction, thereby allowing the formation of a normally forbidden A–C wobble pair.

If the first of the two possible mechanisms was involved in this type of suppression, a difference in the ribosome-free binding of UGA to either wild type or suppressor-tRNA should be observed. The wild type tRNA is expected to have little or no affinity for UGA, while the suppressor-tRNA should be able to form complexes with an association constant comparable to other ribosome-free codon–anticodon associations. If the second mechanism is correct, both wild type and suppressor-tRNA should bind UGA with the same strength in the absence of ribosomes.

In order to test these two possibilities, one measured ribosome-free binding of the $^3$H-labeled trinucleotide UGA to both $tRNA^{Trp}$ from *E. coli* CA

265 ($su^{-}_{UGA}$) and to $tRNA^{Trp}$ from *E. coli* CAJ 64 ($su^{+}_{UGA}$) in the equilibrium dialysis system described earlier [3,8]. As shown in table 1, both tryptophan tRNAs are able to bind UGA with nearly the same binding constant. In order to show that the binding site for UGA is the anticodon and not the 3′-terminal –CCA sequence, both tryptophan tRNAs were exposed to snake venom phosphodiesterase for one hour at 37°C. Using unfractionated *E. coli* tRNA as a model compound, maximal reconstitution of the –CCA terminus was observed if diesterase treatment laster for 1 hr. Longer incubation resulted in a reduced reconstitution of the tRNA for still unknown reasons. Endonucleolytic contaminations of the diesterase may either have caused too many chain internal scissions or the exonuclease may have proceeded beyond the –CCA terminus. In both cases tRNA would no longer be expected to serve as substrate for the reconstitution enzyme. Unexpectedly, a difference in diesterase susceptibility between the wild type and suppressor-$tRNA^{Trp}$ was discovered when the –CCA terminus was reconstituted. If expressed per mole of tRNA, 0.7 moles CTP were incorporated by suppressor-tRNA and 0.31 moles by wild type tRNA, while an identically diesterase-treated sample of unfractionated *E. coli* tRNA accepted 0.40 moles CTP. This different susceptibility which has not been described so far, seems to be additional evidence for an altered tertiary structure caused by the base exchange in position 24 of the polynucleotide chain. Both diesterase-treated tryptophan tRNAs still bind the trinucleotide UGA as measured by equilibrium dialysis. The binding constants calculated from these experiments were in the same range as the values obtained with native tRNAs (table 1). Thus it is inferred that wild type and suppressor-$tRNA^{Trp}$ are capable of attaching the triplet UGA to their anticodon with the same affinity.

The first of the two possible mechanisms outlined above which would be based on an altered stereostructure of the anticodon is, therefore, incompatible with this experimental finding. Consequently the ability of $tRNA^{Trp}$ from the $su^{+}$-strain to read UGA must be caused by its different and mutation-induced attachment to the ribosome as compared to wild type tRNA. Although both suppressor and wild type $tRNA^{Trp}$ have the ability to interact with UGA, only the suppressor-tRNA can read ribosome-bound UGA on account of its altered ribosome binding capacity. Normal, restrictive tRNA binding to ribosomes possibly limits the codon–anticodon interaction of wild type tRNA to the degree permitted by the wobble hypothesis.

## Acknowledgements

The expert technical assistance of Mrs. Barbara Stumper and Mr. Peter Wegerer's help with the preparation of the suppressor-tRNA are gratefully acknowledged. I also wish to extend my gratitude to Dr. B.F.C. Clark for a gift of $tRNA^{Trp}$, to Dr. J.D. Smith for supplying me with the *E. coli* strains and for fruitful discussions, and to Dr. R.D. Nolan for critically reading and correcting the manuscript.

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