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A modified uridine in the anticodon of E. coli $tRNA_I^{Tyr}$ su_{oc}^+

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ABSTRACT:

The anticodon of an ochre-suppressing derivative of *E. coli* $tRNA_I^{Tyr}$, previously identified as UUA, can contain a modified uridine (U^+) in the first position. The novel modified nucleotide has been identified by two-dimensional thin layer chromatography following RNase T_2 digestion of anticodon-containing fragments. U_p^+ is found in less than stoichiometric molar yields in preparations of $tRNA_I^{Tyr}su_{oc}^+$. The electrophoretic mobility of U_p^+ is the same as Up at pH 3.5 and pH 7.5. U^+ probably does not contain sulfur since it cannot be labeled with ^{35}S *in vivo* incorporation experiments.

INTRODUCTION:

In several procaryotic tRNAs for which it may be anticipated from the corresponding codon that U should occupy the first anticodon position, it has been found that this position is taken by a modified U (1-7; see Table I, reference 8 for references to eucaryotic tRNAs). Modification of uridine may affect its hydrogen-bonding properties and, in particular, may alter the ability of U in the first anticodon position to "wobble" (3,4,8, 9). The anticodon of an ochre-suppressing derivative of *E. coli* $tRNA_I^{Tyr}$ has previously been shown to be UUA (10). However, since genetic experiments (S. Feinstein and S. Altman, manuscript in preparation) indicated that this ochre-suppressing tRNA does not recognize UAA and UAG codons equally well, the nature of the $tRNA_I^{Tyr}su_{oc}^+$ anticodon has been re-investigated. In this report we show that when the products of RNase T_2 enzymatic digestion of the anticodon fragment are analyzed in a chromatographic system different from that previously used (10), a novel modified uridine-containing nucleotide can be identified. Thus, *E. coli* $tRNA_I^{Tyr}su_{oc}^+$ is similar to many other procaryotic tRNAs with respect to the occurrence of a modified uridine in the first position of the anticodon (1-7).

METHODS

Bacterial and bacteriophage strains: *E. coli* CA 274 lac^-_{125}amber trp^-_{amber} and CA 293 lac^-_{659}ochre $trp^-_{amber}su_3^+$ were from the Cambridge strain collection.

BF 266 RC^{rel} (11) was received from P. Berg. The transducing phage ϕ80 $tRNA^{Tyr}$ su^{+}_{oc} (HA 221; (10)) and su^{+}_{3} A2 (12) carry respectively ochre and amber suppressing derivatives of $tRNA^{Tyr}$ as well as a second mutation making the expression of the suppressing phenotypes temperature sensitive.

Preparation of ^{32}P-labeled tRNAs: ^{32}P-labeled $tRNA^{Tyr}$ and its derivatives were prepared as described by Abelson et al (13). In some cases, the purification step involving stepwise elution from a benzoylated-DEAE cellulose column was omitted and the region in 10% polyacrylamide gels containing all species of $tRNA^{Tyr}$ was eluted and used directly.

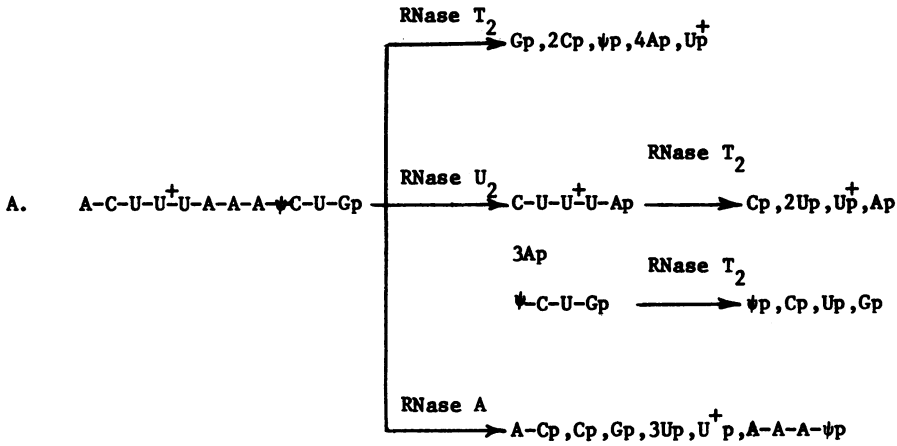
Preparation of anticodon-containing fragments: Two-dimensional fingerprints of RNase T_1 digestion of $tRNA^{Tyr}$ and its derivatives were prepared as described by Sanger and his colleagues (14,15), as summarized in reference 16. Fingerprints of radiochemically pure species were prepared after chromatography on benzoylated DEAE (BD) cellulose columns and polyacrylamide gel electrophoresis. Alternatively, fingerprints were made of mixtures of $tRNA^{Tyr}$ species, isolated directly from gels. In the latter case, the anticodon-containing fragment from the ochre-suppressing tRNA was well separated from other oligonucleotides because of its greater mobility in the first dimension of the chromatographic system (10). Methods for further digestion of these fragments by RNase U_2 (17), RNases A or T_2 or alkali have been described (14).

Thin layer chromatography: The products of the digestion of oligonucleotides by RNase T_2 were separated in the familiar two-dimensional thin layer chromatography system described by Nishimura (18). Thin layer chromatographic plates were autoradiographed after completion of the chromatography and nucleotides eluted by scraping the appropriate areas of the plates and then soaking the scraped cellulose in water. Electrophoresis on paper at pH 3.5 or on thin layer plates at pH 2.9 or 7.5 was carried out according to standard techniques (1,16).

^{35}S labeling experiments: Attempts to label U^{+} with ^{35}S were carried out by infecting cultures of E. coli BF 266 or E. coli CA 293 grown in the low sulfur medium described by Goodman et al (19). The tRNA extracted from these labeled cells was passed over BD cellulose columns and appropriate fractions then mixed with small amounts of ^{32}P-labeled $tRNA^{Tyr}$ to allow autoradiography following chromatography of the mixture on polyacrylamide gels. Sections of these gels containing P^{32} labelled material were eluted, fingerprints prepared and the anticodons subsequently analyzed for the presence of ^{35}S in U^{+}_{p} (see below). No positive results were obtained.

RESULTS:

The anticodon fragment obtained by RNase T_1 digestion of $tRNA_I^{Tyr}$ su_{oc}^+ should contain the following nucleotides: Up, Cp, Gp, Ap and ψp (Figure 1). The presence of these nucleotides can be demonstrated by RNase T_2 digestion



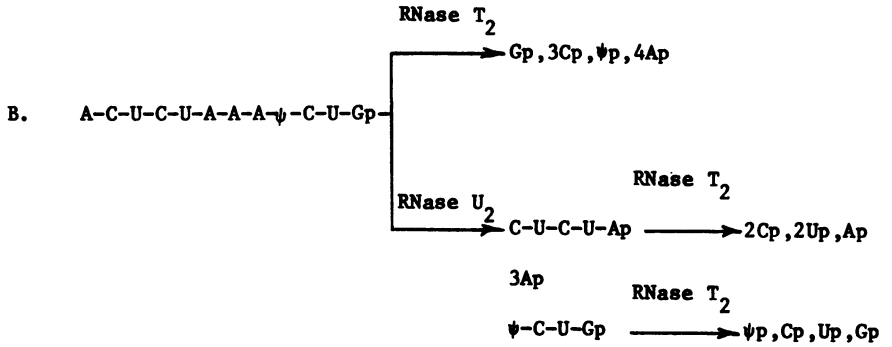


Figure 1: Scheme for the analysis of RNase T_1-produced fragments containing the anticodons of $tRNA_I^{Tyr}su_{oc}^+$ (A.), or $tRNA_I^{Tyr}su_3^+$ A2 (B.). Both tRNAs were isolated from cultures of BF 266 infected with the appropriate transducing phage (see Methods). The scheme illustrates the products expected after digestion of the oligonucleotides with various enzymes. The anticodon-containing fragments which were analyzed did not contain isopentenyl or 5-methyl-2-thioisopentenyl modifications of the A residue adjacent to the anticodon so this possible modification (20) is not indicated in the figure. Enzymatic reactions and subsequent separation of products were carried out as described in Methods.

followed by two-dimensional thin layer chromatography and autoradiography of the anticodon-containing fragment of ^{32}P-labeled $tRNA_1^{Tyr}$ su_{oc}^+ (Figure 2A). However, in addition to the expected nucleotides, there is ^{32}P-labeled material giving rise to a new spot in the autoradiogram (designated U_p^+ in Figure 2B). This new spot is not observed in a similar analysis of the anticodon-containing

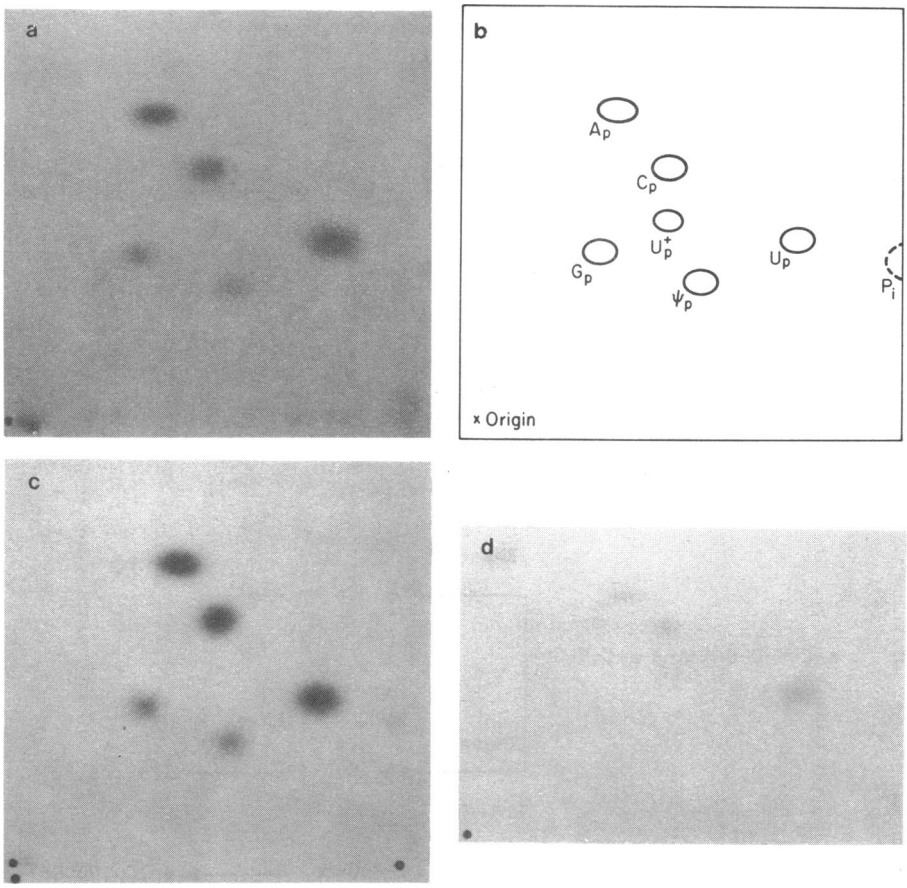


Figure 2: Autoradiograms of two-dimensional thin layer chromatography analysis of RNase T_2 digestion of oligonucleotides containing either the ochre-suppressing or amber-suppressing anticodon. A. RNase T_1-produced oligonucleotide containing the anticodon of $tRNA_1^{Tyr}su_{oc}^+$. B. A tracing of A. with identification of nucleotides. C. RNase T_1-produced oligonucleotide containing the anticodon of $tRNA_1^{Tyr}su_3^+$ A2. D. Analysis of the RNase U_2-produced fragment from the oligonucleotide analyzed in A (see Figure 1). A similar analysis of the analagous RNase U_2-produced fragment from the oligonucleotide analyzed in C. showed no U_p^+, nor did analyses of the fragment Ψ-C-U-Gp

fragment of $tRNA_I^{Tyr}$ su_3^+ A2 (Figure 2C). Furthermore, if the anticodon-containing fragment obtained by RNase T_1 digestion of the ochre-suppressing tRNA is digested with RNase U_2 and the resulting smaller anticodon-containing fragment digested with RNase T_2 (Figure 1) and subjected to an analysis similar to that described above, the presence of the novel nucleotide can again be demonstrated (Figure 2D). $\overset{+}{Up}$ is absent from a similar analysis of the RNase U_2-produced fragments of the amber-suppressing tRNA (data not shown). Quantitation of the molar yields of nucleotides isolated in experiments like those whose results are portrayed in Figure 2 show that the yield of $\overset{+}{Up}$ per mole of Gp is generally between 0.2 and 0.7 moles in the RNase T_1-produced fragment. The other nucleotides are found in the expected molar yields. The molar yield of Up was generally between 3.5 and 4.0. In some preparations made in E. coli CA 293 and CA 274 little or no $\overset{+}{Up}$ was found. Preparations made in E. coli BF 266 reliably gave $\overset{+}{Up}$ in the amounts indicated above. There is no correlation between the presence or absence of i^6A or ms^2i^6A and U^+ in the anticodon-containing fragments. (The data shown were assembled from analyses of fragments in which the A residue adjacent to the anticodon was unmodified.)

When $\overset{+}{Up}$ is eluted from thin layer plates and rechromatographed either by electrophoresis on paper or on thin layer plates at three different pH values, it chromatographs with or very close to Up (Table I) indicating that this

Table I

Electrophoretic mobility of $\overset{+}{Up}$ at different values of pH

pH	Buffer	Mobility (Relative to Up=1.0)	Number of Experiments
2.9	Ammonium formate	1.02	1
3.5	Pyridine-acetate	1.00	4
7.5	Potassium phosphate	1.00	1

Electrophoresis at pH 2.9 and 7.5 was carried out on Analtech Avicel 20 cm x 20 cm thin layer cellulose plates. pH 2.9 buffer: 0.05M ammonium formate. pH 7.5 buffer: 0.05 M potassium phosphate. Electrophoresis at pH 3.5 was carried out on Whatman 3MM paper in pyridine-acetate buffer as described in reference 16. No difference was observed in the mobility at pH 3.5 of $\overset{+}{Up}$ and Up whether or not the modified nucleotide had been treated with 0.3M KOH or H_2O at 37°C for 18 hrs. Controls for these latter experiments were samples of $\overset{+}{Up}$ which were eluted from the thin layer plates in glass-distilled water at 0°C, lyophilized and stored at -20°C until used.

nucleotide probably contains a modified uracil residue. Treatment of $\overset{+}{U}p$ with alkali prior to electrophoresis at pH 3.5 (see legend to Table I) does not alter its chromatographic properties. Previous analyses of the $tRNA_I^{Tyr}$ su_{oc}^+ anticodon were made using paper electrophoresis at pH 3.5 which explains how the novel uridine modification was overlooked.

Several attempts were made to label U^+ with ^{35}S but in no case were they successful (see Methods). While we may infer from this result that the modified nucleoside does not contain sulfur, the presence of sulfur cannot be rigorously excluded. The small amount of radioactivity recovered as ^{32}P-labelled nucleotide in any one preparation of $\overset{+}{U}p$ made further chemical analysis of its composition difficult.

When the U^+-containing anticodon fragment (obtained by RNase T_1 digestion) is treated with RNase A, no difference in the resulting products is seen compared to those produced in a similar analysis of the amber-suppressing derivative of $tRNA^{Tyr}$. Thus, the presence of U^+ did not inhibit the action of RNase A such that a product $\overset{+}{U}pUp$ would have been found in appreciable yield.

DISCUSSION:

When an RNase T_2 hydrolysate of the anticodon containing fragment of *E. coli* $tRNA_I^{Tyr}$ su_{oc}^+ is analyzed by two-dimensional thin layer chromatography, a novel, modified uridine-containing nucleotide can be detected. Since this ochre-suppressing tRNA was derived by a one step mutation of an amber-suppressing derivative (anticodon: CUA) of $tRNA_I^{Tyr}$ su_3^+ A2 and since no other U (aside from ψ) is found modified in the anticodon fragment, we may assume that the novel modified uridine is in the first position of the anticodon, i.e. $\overset{+}{U}UA$. In the electrophoresis system previously used (10) to analyze this anticodon, $\overset{+}{U}p$ comigrates with Up and is therefore undetectable. The molar yields of the modified nucleotide are variable and usually low, about 0.3 moles per mole of the ochre-suppressing tRNA. Preparations of $\overset{+}{U}p$ from an *E. coli* strain bearing the relaxed phenotype gave consistently higher yields than preparations from strains with the stringent phenotype. Thus, the low molar yields of $\overset{+}{U}p$ are probably not solely due to intrinsic lability of the modifying group.

The low level of modification may be a reflection of the overburdening of host cell modification enzymes by the amplification of the tRNA gene following infection with the transducing phage. On the other hand, the mutated $tRNA_I^{Tyr}$ may not be a substrate which can be used efficiently under any conditions by modification enzymes normally interacting with other tRNA substrates. This latter hypothesis is supported by the results of genetic

studies of the ochre-suppressing tRNA in which its suppressor properties have been compared when it is present in E. coli either as a single gene product or during infection with the transducing phage (S. Feinstein and S. Altman, manuscript in preparation). In vivo, in most cases tested $tRNA_I^{Tyr}$ su_{oc}^+ suppresses the amber codon, UAG, more efficiently than the ochre codon, UAA. Since the coding properties of the ochre-suppressing tRNA are unchanged in vivo whether single or multiple copies of the tRNA gene are present, we infer that the molar content of U^+ in the tRNA remains constant or approximately so.

With respect to the preferential recognition by $tRNA_I^{Tyr}$ su_{oc}^+ of UAG rather than UAA, the behavior of this tRNA is like that of $tRNA_2^{Glu}$ prepared from E. coli grown under sulfur-deficient conditions. E. coli $tRNA_2^{Glu}$ normally has a modified uridine (5-methylaminomethyl-2-thiouridine; mam^5-s^2-U) in the first anticodon position. Sulfur-deficient $tRNA_2^{Glu}$, in which the molar content of mam^5-s^2-U is drastically reduced, in vitro recognizes a codon ending in G more efficiently than one ending in A (8). Recent studies of E. coli $tRNA^{Lys}$ indicate that its coding properties may depend in a similar fashion upon the presence or absence of a thio-containing modification of uridine in the first anticodon position (21).

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