THE PRESENCE OF *N*-[9-(c-D-RIBOFURANOSYL)PURIN-6-YLCARBAMOYL] THREONINE IN ISOLEUCINE, THREONINE AND ASPARAGINE tRNAs FROM *ESCHERICHIA COLI*

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1. Introduction

We have previously reported that *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl] threonine (tA) was isolated from *E. coli* $tRNA_3^{Ser}$, $tRNA_1^{Met}$ and $tRNA^{Lys}$ [1]. We have proposed the hypothesis that most tRNAs which recognize codons starting from A always contain tA [1]. In order to confirm this hypothesis, purified *E. coli* $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$ were isolated to check whether or not these tRNAs actually contain tA. In fact, as described in this communication, it was found that *E. coli* $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$ contain tA. Characterization of tA in these tRNAs has been accomplished by comparing ultraviolet absorption spectra, and electrophoretic and chromatographic mobilities of tA isolated from each tRNA with those of the authentic sample, in addition to the identification of threonine as the amino acid component.

2. Materials and methods

2.1. *Isolation of E. coli $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$*

Unfractionated *E. coli* tRNA used for the preparation of the amino acid specific tRNAs was prepared from cells of *E. coli* harvested at the late log phase. *E. coli* $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$ were obtained by combinations of DEAE-Sephadex A-50 column chromatography [2] with other column chromatographic procedures such as reverse phase partition chromatography [3], benzoylated DEAE-cellulose chromatography [4], DEAE-Sephadex A-50 column chromatography at pH 4.0 [5] and/or QAE-Sephadex A-50 column chromatography at pH 9.5. Details of these purification procedures will be published elsewhere. The purities of the preparations of $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$ were estimated to be more than 80% from their amino acid acceptor abilities and from the chromatographic profiles of their digests with RNase T_1 and bovine pancreatic RNase.

2.2. *Isolation of tA from E. coli $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$*

Approx. 100 OD units each of $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$ were extensively hydrolyzed by incubating with 25 units of RNase T_2 for 18 hr at 37° as described previously [6]. The hydrolysate was fractionated by two-dimensional paper chromatography using Whatman 3 MM paper (30 × 30 cm) [7]. The spot located between Cp and Ψp was cut out, and eluted with water. The eluate which contained tA > p was again incubated with 8 units of RNase T_2 for 18 hr at 37° to open the cyclic phosphate moiety, and further treated with *E. coli* alkaline phosphomonoesterase to obtain the nucleoside, tA.

Abbreviations: tA: *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl] threonine; N_1, N_2 and N_3: unknown nucleosides which were later characterized as tA; OD unit: the amount of material which has an absorbance of 1.0 at 260 nm when dissolved in 1 ml of water and measured with a 1-cm light path.

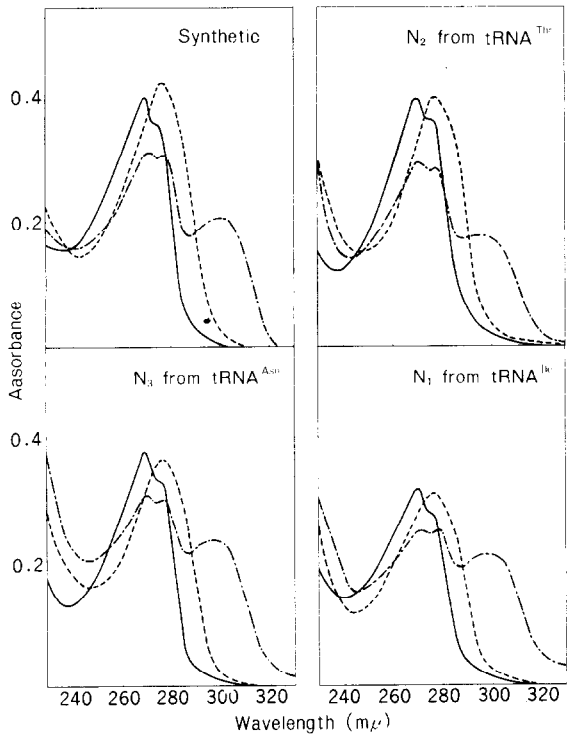

Fig. 1. UV absorption spectra of N_1, N_2 and N_3 and authentic tA. (———): pH 7.5; (- - - - -): 1 N HCl; (— — — —): 0.1 N NaOH.

Purified tA was finally obtained by paper electrophoresis at 20 V/cm with 0.05 M triethylammonium bicarbonate buffer, pH 7.5, for 50 min, since only tA moved to the anode, whereas other nucleoside contaminants remained at the origin. Yield of tA: 0.05 μmole from $tRNA^{Thr}$; 0.03 μmole from $tRNA^{Ile}$; 0.08 μmole from $tRNA^{Asn}$.

2.3. *Thin layer electrophoresis and chromatography*

Thin layer electrophoresis was carried out using a glass plate coated with Avicel SF cellulose, at 20 V/cm for 15 min with 0.05 M triethylammonium bicarbonate buffer, pH 7.5. Thin layer chromatography was also carried out using the same plate. Solvent systems used were: A) ethanol–1 M ammonium acetate (7/3, v/v); B) 1-propanol–water–conc. ammonium hydroxide (55/35/10, v/v/v); C) 2-propanol–conc. hydrochloric acid–water (70/15/15, v/v/v). It should be also mentioned that designations of solvent systems described in the previous communication [1] should be read as F for E, G for F, E for G, respectively.

2.4. *Amino acid analysis for detection of threonine in tA*

Approx. 0.05 μmole each of tA from $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$ was heated for 3 hr at 100° in 0.2 ml of 0.1 M NaOH to liberate the amino acid as described by Chheda [8]. The neutralized alkaline hydrolyzate was analyzed for detection of amino acids by using an automatic amino acid analyzer, JEOL JLC-5 AH.

3. Results

For detection of minor components in *E. coli* $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$, 2 OD units each tRNA was extensively hydrolyzed by RNase T_2, and the resulting nucleotide mixture was analyzed by two-dimensional thin layer chromatography as described previously [7]. A minor nucleoside 3′-phosphate which is designated as N > p hereafter, and which was later characterized as tA > p, was found in a position between Cp and Ψp [9, 10]. Fig. 1. shows the UV absorption spectra of N_1 from *E. coli* $tRNA^{Ile}$, N_2 from $tRNA^{Thr}$ and N_3 from $tRNA^{Asn}$ isolated on a preparative scale as described in Materials and methods. Their spectra are all identical with that of an authentic sample of *N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]-threonine (tA) at three different pH values. Table 1 shows the R_f values of N_1, N_2, N_3 and authentic tA on thin layer chromatography and their electrophoretic mobilities. The nucleoside preparations, N_1, N_2 and N_3, behaved identically with the authentic tA in all systems, indicating that N_1, N_2 and N_3 is actually tA. Further proof that N_1, N_2 and N_3 are identical with tA was obtained by the analysis of alkaline hydrolysates of these nucleosides. As described by Chheda [8], nucleosides formed by the alkaline treatment was identified as adenosine in all cases. In addition, threonine was detected as a major amino acid component by automatic amino acid analysis. The molar ratio of threonine to the original nucleoside was found to be 0.52, 0.52 and 0.44 for N_1, N_2 and N_3, respectively. No other amino acid was detected except glycine from $tRNA^{Ile}$, $tRNA^{Thr}$

Table 1
Relative chromatographic mobilities and electrophoretic mobilities of N_1, N_2, N_3, tA and related compounds.

Compound	Thin layer chromatography R_f in solvent system A	B	C	Electrophoresis Migration from origin (cm)
Adenosine 2',(3')-phosphate				3.1
Adenosine	0.51	0.64	0.21	0
tA	0.36	0.67	0.30	1.5
Nucleoside N_1 from $tRNA^{Ile}$	0.36	0.67	0.30	1.5
Nucleoside N_2 from $tRNA^{Thr}$	0.36	0.67	0.30	1.5
Nucleoside N_3 from $tRNA^{Asn}$	0.36	0.67	0.30	1.5

and $tRNA^{Asn}$, alanine from $tRNA^{Thr}$, and serine from $tRNA^{Asn}$, each approx. one tenth in amount as compared with threonine. At present it is not certain whether or not such amino acids detected in small quantities are actual components of the minor nucleosides.

4. Discussion

Yield of tA varied depending upon *E. coli* tRNAs used as a source. It is partly due to the presence of other minor nucleosides with structures which seem closely similar to that of tA in these *E. coli* tRNAs. Recently, a new minor nucleoside was isolated from *E. coli* $tRNA^{Thr}$ in our laboratory, and its structure was determined as *N*-[9-(β-D-ribofuranosyl)purin-6-yl-*N*-methylcarbamoyl] threonine [11].

Results reported in this communication clearly show that tA was present in *E. coli* $tRNA^{Ile}$, $tRNA^{Thr}$ and $tRNA^{Asn}$. Since we have already shown that $tRNA_1^{Met}$, $tRNA_3^{Ser}$ and $tRNA^{Lys}$ from *E. coli* contain tA [1], it has been now proved that 6 out of 8 tRNAs which recognize codons starting from A contain tA. Preliminary sequential study on *E. coli* $tRNA^{Asn}$ indicated that tA was probably located at the position next to the 3'-hydroxyl end of the anticodon [10]. Yarus and Barrell recently reported that an unknown minor component was located adjacent to the anticodon of *E. coli* $tRNA^{Ile}$, and suggested that this minor nucleoside is probably tA [12]. Sequential study on *E. coli* $tRNA^{Met}$ [13] and $tRNA_3^{Ser}$ [1] suggested that tA is also located in the position adjacent to the anticodons of these tRNAs. It is therefore very likely that tA is located in the same position in $tRNA^{Lys}$ and $tRNA^{Thr}$. A survey of the distribution of tA in individual *E. coli* tRNAs available in our laboratory so far indicated that it is absent in any other *E. coli* tRNAs which recognize codons starting either from U, C or G. In fact, it was shown that *E. coli* tRNAs which recognize codons starting from U always contain 2-methylthio-N^6-isopentenyladenosine, presumably in the position adjacent to the anticodons [14–17]. In addition, N^6-methyladenosine in *E. coli* $tRNA_1^{Val}$ [7], 2-methyladenosine in *E. coli* $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{His}$ and $tRNA^{Arg}$ (for CG series) [9], 1-methylguanosine in *E. coli* $tRNA_1^{Leu}$ and $tRNA_2^{Leu}$ ([18, 19], K. Ishii, Y. Yamada, S. Nishimura and H. Ishikura, unpublished results), and adenosine in *E. coli* $tRNA^{Gly}$ [20] and $tRNA_2^{Val}$ [21] were found in the positions next to the anticodons. Thus, except for the case of *E. coli* $tRNA^{fMet}$, which contains adenosine instead of tA in that position [13], there is strict regularity between the presence of tA and codon recognition of tRNA. Namely, *E. coli* tRNAs which recognize codon starting from A always contain tA or its related compound adjacent to anticodon. The exact explanation for the presence of tA with such regularity is still unknown. However, it is reasonable to speculate that tA may facilitate precise formation of A–U base pairing between the first letter of the codon and the third position of the anticodon by stabilizing the three-dimensional structure of anticodon loop. Theoretical and experimental studies on the role of tA as well as other

minor nucleosides next to the anticodon still remain to be carried out.

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