THE PRESENCE OF N-[9-(β-D-RIBOFURANOSYL)PURIN-6-YLCARBAMOYL]THREONINE IN SERINE, METHIONINE AND LYSINE TRANSFER RNA's FROM ESCHERICHIA COLI

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Received October 20, 1969

Summary: The isolation and characterization of N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine from $tRNA_3^{Ser}$, $tRNA_1^{Met}$ and $tRNA^{Lys}$ of E. coli is described. The specific role of this minor component of tRNA in the recognition of codons starting with A is discussed.

Chheda et al. (1) isolated a threonine-containing adenine derivative from acid hydrolyzates of unfractionated tRNA's of various origins and its structure was determined as N-(purin-6-ylcarbamoyl)threonine by Schweizer et al. (2). This communication reports that E. coli $tRNA_3^{Ser}$, $tRNA_1^{Met}$ and $tRNA^{Lys}$, all of which recognize codons starting with A, contains this minor adenosine derivative, N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine (referred to as N hereafter). This strongly suggests that this minor component has a specific role in the recognition of codons stating with A.

Materials and Methods: E. coli $tRNA^{fMet}$, $tRNA_1^{Met}$ and $tRNA_2^{Met}$ were prepared as described previously (3). E. coli $tRNA_1^{Ser}$[for codons of the UC series (4)] $tRNA_2^{Ser}$[for codons of the UC series (4)], $tRNA_3^{Ser}$[for codons, AGU and AGC (4)], $tRNA^{Phe}$, $tRNA^{His}$, $tRNA^{Asp}$ and $tRNA^{Leu}$ were described previously (5). E. coli $tRNA^{Lys}$ was obtained by the combined use of DEAE-Sephadex A-50 (6), benzoylated DEAE-cellulose (7) and reversed-phase column chromatographies (8). The $tRNA^{Lys}$ preparations accept more than 0.8 mole of lysine and less than 0.05 mole of other amino acids per tRNA molecule (calculated assuming that 1 A_{260} unit corresponds to 1.66 mμmole of tRNA). A synthetic sample of N (9) was kindly provided by Dr. G. B. Chheda.

Paper and thin layer electrophoresis were carried out on Whatman 3MM paper and on Avicel SF cellulose, respectively. Thin layer chromatography was carried out by the ascending technique on Avicel SF cellulose (11 x 11 cm).

To test for the presence of N in a given tRNA sample, 30 A_{260} units of dialyzed tRNA were hydrolyzed with ribonuclease (RNase) T_2, and the digest was chromatographed two-dimensionally on paper using the system used for detection of minor components of Seno et al. (3).

N was isolated as follows. About 800 A_{260} units of a given tRNA prepara-

tion were extensively hydrolyzed with RNase T_1 and chromatographed on a column of DEAE-Sephadex A-25 with an NaCl gradient in the presence of 7 M urea at pH 7.5. The oligonucleotide fraction containing N was hydrolyzed with RNase T_2 (12 units of enzyme per 100 A_{260} units of oligonucleotide) for 18 hours at 37°. Then the reaction mixture was neutralized and treated with E. coli alkaline phosphatase. In this way major components are converted to nucleosides, whereas almost all the N is obtained as 2',3'-cyclic phosphate. The hydrolyzate was subjected to paper electrophoresis for 40 minutes at 20 volts/cm with 0.05 M triethylammonium bicarbonate buffer (pH 7.5). The band traveling to the anode with a mobility slightly less than that of adenosine 2',(3')-phosphate was eluted with water. To convert the 2',3'-cyclic phosphate of N to the nucleoside, the eluate was hydrolyzed with 10 times as much RNase T_2 as in the previous digestion for 18 hours at 37° and then digested with E. coli alkaline phosphatase. The hydrolyzate was again subjected to paper electrophoresis under the same conditions as described above. The band of N, which moved with a mobility between those of adenosine 2',(3')-phosphate and adenosine, was eluted with water. Finally N was absorbed on a column of Dowex-1 x 2 (0.2 x 15 cm, Cl type), washed with 10 ml of water and eluted with 0.01 M HCl. The hydrochloric acid was removed by repeated evaporation under reduced pressure.

Results: Using RNase T_2 digestion, the 2',3'-cyclic phosphate of N was detected in $tRNA_3^{Ser}$, $tRNA_1^{Met}$ and $tRNA^{Lys}$ at a position between cytidine 3'-phosphate and pseudouridine 3'-phosphate on the two-dimensional paper chromatogram. However, it was not detected in $tRNA^{fMet}$, $tRNA_2^{Met}$, $tRNA_1^{Ser}$, $tRNA_2^{Ser}$, $tRNA^{Phe}$, $tRNA^{His}$, $tRNA^{Asp}$ or $tRNA^{Leu}$. Nearly 1 mole of the 2',3'-cyclic phosphate of N per tRNA molecule was detected in $tRNA_1^{Met}$ and $tRNA^{Lys}$. More than 0.7 mole of 2',3'-cyclic phosphate of N was always detected in $tRNA_3^{Ser}$, but the yield varied in different preparations.

Fig. 1 show a comparison of the ultraviolet (UV) absorption spectra of the nucleosides obtained from $tRNA_3^{Ser}$, $tRNA_1^{Met}$ and $tRNA^{Lys}$ with those of a synthetic sample of N. The four spectra were identical in all respects. An extra negative charge of N was demonstrated under neutral conditions by thin layer electrophoresis. As Table I shows, all three nucleoside samples traveled to the anode with the same mobility as that of the synthetic nucleoside. The identity of the nucleoside was further confirmed by thin layer chromatography with seven different solvent systems, as shown in Table II. In all solvent systems, the Rf values of the nucleoside samples were exactly the same as those of the synthetic material. An hydrolysis with 0.01 M NaOH at 100° for 3 hours, the nucleoside gave a product with UV-absorption and a product reacting with ninhydrin. The former was identified as adenosine by its UV absorption

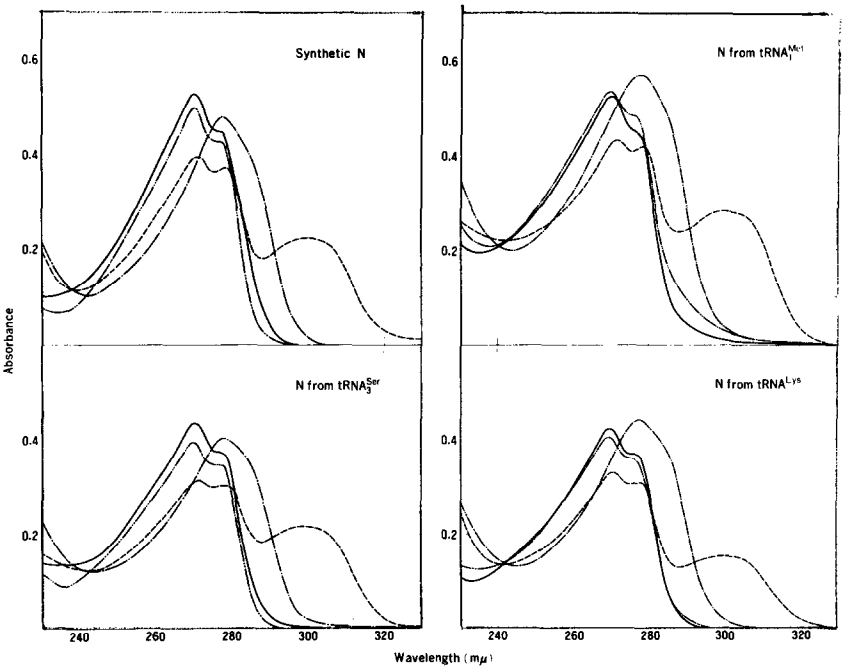

Fig. 1: UV spectra of a synthetic sample of N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine and the nucleosides obtained from tRNA$_3^{Ser}$, tRNA$_1^{Met}$ and tRNA^{Lys}. -·-·-·-, pH 0.2; ————, pH 2.8 and pH 7.6; —··—··—, pH 11.3; --------, pH 12.9.

Table I: Electrophoretic Mobilities

Compound	Migration from Origin (cm)
Adenosine 2',(3')-phosphate	+ 4.0
Adenosine	0
N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine	+ 2.0
Nucleoside from tRNA$_3^{Ser}$	+ 2.0
Nucleoside from tRNA$_1^{Met}$	+ 2.0
Nucleoside from tRNA^{Lys}	+ 2.0

Electrophoresis was carried out on Avicel SF cellulose at 30 volts/cm for 20 minutes in 0.05 M triethylammonium bicarbonate buffer (pH 7.5).

Table II: Thin Layer Chromatography

Compound	Rf Values						
	A	B	C	D	E	F	G
Adenosine	0.52	0.29	0.50	0.72	0.51	0.51	0.64
N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine	0.50	0.04	0.43	0.60	0.40	0.58	0.67
Nucleoside from $tRNA_3^{Ser}$	0.50	0.04	0.43	0.60	0.40	0.58	0.67
Nucleoside from $tRNA_1^{Met}$	0.50	0.04	0.43	0.60	0.40	0.58	0.67
Nucleoside from $tRNA^{Lys}$	0.50	0.04	0.43	0.60	0.40	0.58	0.67

Solvent Systems

A: 2-Propanol-1 % aqueous ammonium sulfate (2:1, v/v)
B: 1-Butanol-water-concd. ammonium hydroxide (86:14:5, v/v/v)
C: 2-Propanol-water-concd. ammonium hydroxide (7:2:1, v/v/v)
D: Isobutyric acid-water-concd. ammonium hydroxide (66:33:1, v/v/v)
E: 1-Propanol-water-concd. ammonium hydroxide (55:35:10, v/v/v)
F: Ethanol-1 M ammonium acetate (7:3, v/v)
G: 1-Butanol-water-acetic acid (4:2:1, v/v/v)

spectra and by thin layer chromatography and the latter as threonine by thin layer chromatography. The amount of threonine was determined by ion exchange chromatography using a JEOL JLC-5AH automatic amino acid analyzer. The molar ratios of threonine to adenosine were 0.75, 0.65 and 0.70 with nucleoside samples from $tRNA_3^{Ser}$, $tRNA_1^{Met}$ and $tRNA^{Lys}$, respectively.

Discussion: It is well known that the position adjacent to the 3'-end of the anticodon is occupied by a modified purine nucleoside (10). We have previously shown that all *E. coli* tRNA's which recognize codons starting with U contain 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine [(5), and S. Nishimura, Y. Yamada and H. Ishikura, unpublished data]. These results indicate that this adenosine derivative located in the position next to the 3'-end of the anticodon has a specific role in the function of tRNA to recognize a codon sequence starting with U. A similar observation was made by Armstrong *et al.* who reported that cytokinins were found only in fractions enriched with yeast tRNA species corresponding to codons beginning with U (11). Now the question arises of wether there is a similar type of correlation between the structure of a minor component and codon recognition in other classes of tRNA. As described

in this paper, all three E. coli tRNA's in which we have detected N recognize codons starting with A. The sequence of the oligonucleotide containing N obtained from an RNase T_1 digest of $tRNA_3^{Ser}$ was determined to be CpUpNpApGp (H. Ishikura, Y. Yamada and S. Nishimura, unpublished data). This oligonucleotide is a possible candidate for the presumed anticodon sequence (GCU). It is reasonable to conclude that N is located at the position adjacent to the anticodon in $tRNA_3^{Ser}$. Our preliminary data showed that in $tRNA_1^{Met}$, N occupies the position occupied by A* in the sequence proposed for $tRNA^{Met}$ (12). Although the exact location of N in $tRNA^{Lys}$ is not yet known, it is most probably also next to the anticodon in $tRNA^{Lys}$. Thus our finding strongly suggests that N located to the 3'-end of the anticodon of tRNA has a specific role in the recognition of codons starting with A. In support of this, it should be mentioned that Takemura et al. (13) isolated the same compound from yeast $tRNA^{Ile}$ and demonstrated that this minor component is located next to the 3'-end of the anticodon. The absence of N in E. coli $tRNA_1^{Ser}$, $tRNA_2^{Ser}$, $tRNA^{Phe}$, $tRNA^{His}$, $tRNA^{Asp}$ and $tRNA^{Leu}$ also supports this hypothesis. The absence of N in $tRNA^{fMet}$ might be related with its function as initiator tRNA (12).

It should be mentioned that a different adenosine derivative with spectra similar to those of N was detected in $tRNA_2^{Met}$ in our laboratory. It behaved differently from N on two-dimensional paper chromatography. Another derivative with spectra similar to those of N was also isolated from an oligonucleotide fraction obtained by RNase T_1 digestion of $tRNA^{Lys}$. The yield was comparable to that of N and it gave a spot at the same position as the 2',3'-cyclic phosphate of N on chromatography after RNase T_2 digestion. The elution profile of the RNase T_1 digest showed that the $tRNA^{Lys}$ preparations used contained two rather than a single $tRNA^{Lys}$ species. It is concluded that one of the $tRNA^{Lys}$, contains N while the other contains the similar but different derivative. It is possible that N is replaced by similar derivatives in some tRNA's which correspond to codons starting with A.

Acknowledgment: We are indebted to the Laboratories of Kaken Chemicals for large scale isolation of crude E. coli tRNA. We wish to thank Drs. R. H. Hall

and S. Takemura for showing us their manuscripts prior to publication and for their valuable discussion. Thanks are also due to Dr. G. B. Chheda for a sample of synthetic N. This work was supported in part by a grant from the Ministry of Education.

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