UNUSUAL CCA-STEM STRUCTURE OF *E. COLI* B $tRNA_1^{His}$

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1. Introduction

E. coli $tRNA^{His}$ contains two rare minor nucleosides, i.e. 2-methyladenosine [1] and an unidentified nucleoside, "Q", [2] which was previously found in *E. coli* $tRNA^{Tyr}$. A preliminary sequencial study of *E. coli* $tRNA_1^{His}$ suggested that Q seems to be located in the first position of the anticodon and 2-methyladenosine in the position next to the 3′-hydroxyl end of the anticodon [2]. In order to confirm this, we extended the sequencial analysis of this tRNA with the final aim of obtaining a total primary sequence. The present communication deals with oligonucleotide sequences derived from RNase T_1 and pancreatic RNase digestion of *E. coli* $tRNA_1^{His}$. From these results, it was concluded that *E. coli* $tRNA^{His}$ has an unusual CCA-stem structure having only 3 unpaired nucleotide residues at the 3′-hydroxyl end.

2. Materials and methods

2.1. *Isolation of E. coli B tRNA*$_1^{His}$

Purified *E. coli* $tRNA_1^{His}$ was obtained by successive application of DEAE-Sephadex A-50 column chromatography at pH 7.5 and reverse phase partition column chromatography at pH 4.3 [3]. Histidine tRNA was separated into 2 fractions by the reverse phase chromatography. A major species, designated as $tRNA_1^{His}$, was eluted first from the column and was further purified by benzoylated DEAE-cellulose column chromatography [3], followed by DEAE-Sephadex A-50 column chromatography at pH 4.0 [4]. The purity of the $tRNA^{His}$ thus obtained was estimated to be more than 90% as judged from its amino acid acceptor ability and the chromatographic profiles of its RNase T_1 and pancreatic RNase digests [2].

* *Abbreviations:* s^4U, 4-thiouridine; D, dihydrouridine; m^7G, 7-methylguanosine; m^2A, 2-methyladenosine; Q, unknown nucleoside located in the first position of the anticodon of *E. coli* $tRNA^{Tyr}$; Y, pyrimidine nucleoside; O.D. unit, an amount of material which has an absorbance of 1.0 at 260 mμ when dissolved in 1 ml of water and measured with a 1 cm light path.

3. Results and discussion

The products of complete digestion of 150 O.D. units (see footnote*) of *E. coli* $tRNA_1^{His}$ with RNase T_1 or pancreatic RNase were first separated by DEAE-Sephadex A-25 column chromatography at pH 7.5 [2, 5]. The fractions in each peak thus obtained were desalted, and further fractionated either by DEAE-Sephadex A-25 column chromatography at pH 2.7 or Dowex 1 column chromatography, when it was found to be necessary [5]. The nucleotide sequence of each oligonucleotide has been determined by conventional methods as already described [2, 5]. Tables 1 and 2 show the identity and quantities of materials in the peaks obtained by RNase T_1 and pancreatic RNase digestion. The results of the two analyses are in excellent agreement, and overlapping sequences obtained by the 2 methods are as follows: Y-G-G-G-T- -C-Gp, Y-G-m^2 A- - -C-C-A-Gp, Y-G-A-A-U-C-C-C-A-U-U-A-Gp and Y-G-G-A-U-U-Q-U-Gp. *E. coli* $tRNA_1^{His}$ contained 1 mole each of 4-thiouridine, ribothymidine, 7-methyl-

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Table 1
Analysis of product obtained by degradation of *E. coli* $tRNA_1^{His}$ with RNase T_1.

Peak	Nucleotide sequence	Molar ratio	
		observed	theoretical
1	Gp	6.2	6.0[b]
2	U-Gp	2.1	2.0
3	A-Gp	1.1	1.0
4	D-D-Gp	1.1	1.0
5	D-A-Gp	1.1	1.0
6	pGp	1.0	1.0
7	T-ψ-C-Gp	1.0	1.0
8[a]	A-U-U-Q-U-Gp	0.9	1.0
9-1	C-C-A-C-C-C-C-A	0.9	1.0
9-2-1	C-U-C-A-Gp	1.0	1.0
9-2-2	C-C-C-U-Gp	1.0	1.0
9-3	U-U-m^7G-U-C-Gp	0.7	1.0
10[a]	m^2A-ψ-ψ-C-C-A-Gp	0.9	1.0
11	C-U-A-s^4U-A-Gp	0.7	1.0
12	A-A-U-C-C-C-A-U-U-A-Gp	0.8	1.0

[a] The procedure for sequencing the oligonucleotides corresponding to peaks 8 and 10, which consist of the anticodon region, has been already described [2].

[b] The amounts of Gp were estimated from the sequence of oligonucleotides derived from the pancreatic RNase digest.

Table 2
Analysis of products obtained by degradation of *E. coli* $tRNA_1^{His}$ with pancreatic RNase.

Peak	Nucleotide sequence	Molar ratio	
		observed	theoretical
1	A	1.0	1.0
2-1	Cp	14.5	14.0[a]
2-2	Dp	0.9	1.0
2-3	ψp	2.0	2.0
2-4	Up	6.5	6.0[a]
2-5	Q-Up	1.1	1.0
2-6	m^7G-Up	0.8	1.0
3-1	A-Cp	1.1	1.0
3-2	A-Up	1.1	1.0
4	G-Up	1.2	1.0
5	G-m^2A-ψp	0.8	1.0
6-1	A-G-Cp	2.0	2.0
6-2	A-s^4Up	0.9	1.0
6-3	G-G-Cp	0.9	1.0
6-4	A-G-Dp	0.8	1.0
6-5	A-G-Up	1.0	1.0
6-6	G-G-Dp	0.9	1.0
7-1	G-A-A-Up	1.1	1.0
7-2	G-G-A-Up	1.0	1.0
7-3	G-G-G-Tp	0.9	1.0
8-1	A-G-A-G-Cp	1.1	1.0
8-2	pG-G-Up	1.0	1.0

[a] The amounts of Cp and Up were estimated from the sequence of oligonucleotides derived from the RNase T_1 digest.

guanosine, 2-methyladenosine and Q together with 3 moles of pseudouridine and dihydrouridine, as minor constituents. It should be noted that C-C-A-C-C-C-C-A was isolated from the RNase T_1 digest as an oligonucleotide derived from the 3′-hydroxyl end, and pG-G-Up was isolated from the pancreatic RNase digest as an oligonucleotide derived from the 5′-hydroxyl end, Since identification of the sequence of peak 9-1 as C-C-A-C-C-C-C-A is most important for the conclusion emphasized in this communication, the procedure for this analysis will be described in more detail as follows. On two-dimensional thin layer chromatography of an RNase T_2 digest of peak 9-1 with system 1 [2], A, Ap and Cp were obtained in the ratio of 1.00:1.02:5.60. Digestion of the oligonucleotide peak 9-1 with RNase U_2 and subsequent separation of the products by two-dimensional thin layer chromatography with system 2 [2] gave 2 clear spots, 9-1-1 and 9-1-2. Hydrolysis of the oligonucleotide 9-1-1 with RNase T_2 gave Ap and Cp in the ratio of 1.00:2.20, showing that the sequence of this oligonucleotide is C-C-Ap. The RNase T_2 digest of the oligonucleotide 9-1-2 contained A and Cp in the ratio of 1.00:3.98, indicating that the sequence is C-C-C-C-A. This was further confirmed by the complete digestion with snake venom phosphodiesterase. pA, pC and C were obtained in the ratio of 1.00:2.91:1.18. It should be also noted that oligonucleotide peak 9-1 was eluted in a pentanucleotide fraction from a column of DEAE-Sephadex A-25 at neutral pH. It was previously observed that C-C-A was eluted faster than Gp from the same column [6, 7] and C-A-A-C-C-A was eluted in the position

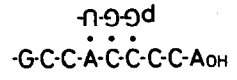

Fig. 1. Probable secondary structure of terminal region of *E. coli* B $tRNA_1^{His}$.

between trinucleotide and tetranucleotide fractions [8]. Thus, elution position of the oligonucleotide peak 9-1 indicated that the chain length of the oligonucleotide must be not more than 8 residues.

The expected secondary structure of the terminal region of *E. coli* $tRNA_1^{His}$ was obtained as shown in fig. 1. The fourth nucleotide from the 3′-hydroxyl end of *E. coli* $tRNA_1^{His}$ was occupied by C instead of A or G which is generally present in most tRNA's (see review by Zachau [9]). It is very probable that pG-G-U- from the 5′-hydroxyl end forms base-pairing with an -A-C-C- sequence starting from the fourth residue at the 3′-hydroxyl end, since there is no alternative way to make maximal base pairing between the 2 sequences. Singer and Smith [10, 11] recently determined the nucleotide sequence of $tRNA^{His}$ from *Salmonella typhimurium* which contain the *E. coli* K12-derived episome F′14, carrying a $tRNA^{His}$ structural gene. Their results on oligonucleotide sequences derived from RNase T_1 or pancreatic RNase digestion were completely identical with those reported here, except for uncertainty as to whether the oligonucleotide derived from the 3′-hydroxyl is C-C-A-C-C-C-C-A or C-C-A-C-C-C-C-A. The cloverleaf structure given by either sequence has the C-C-A stem consisting of 8 base-pairs. Based on their results, it is most likely that *E. coli* B $tRNA_1^{His}$ contains the same length of the C-C-A stem as other *E. coli* tRNA, but with one extra base-pair. Therefore it is reasonable to conclude that the presence of 4-unpaired nucleotide residues from the 3′-terminal of tRNA is not absolutely essential for amino-acylation of the tRNA and subsequent transfer of amino acid to protein in the course of protein synthesis.

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