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Primary structure of $tRNA^{Arg}_{II}$ of E. coli B

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ABSTRACT.

$tRNA^{Arg}_{II}$ of E. coli has 77 nucleotides. There are eight minor nucleotides including inosine and 2-methyladenosine. Except for a few differences, the structure of $tRNA^{Arg}_{II}$ is very similar to the structure of $tRNA^{Arg}_{I}$ reported by Murao et al.[3]. The major difference is in the size of dihydrouridine loop. $tRNA^{Arg}_{II}$ does not contain 2-thiocytosine. The unidentified nucleoside X seems to be a different modification other than nucleoside N reported to be present in $tRNA^{Arg}_{I}$.

INTRODUCTION.

During studies on the arginyl tRNA synthetase of E. coli, $tRNA^{Arg}$ was isolated and purified. To study the interaction of the cognate enzyme with the tRNA and the oligonucleotides derived from it, it was necessary to carry out structural studies on the nucleic acid. Arginine specific transfer RNA separates into five isoacceptors on a reverse phase chromatographic system[1,2]. Two of these species are known to interact with the codons CGA, CGU and CGC[2]. In this work, ^{32}P labelled $tRNA^{Arg}$ was purified on BD-cellulose and RPC-5 columns. The isoacceptor emerging as a major peak from the RPC-5 column was selected for analysis. It recognizes the codons $CG\begin{matrix}U\\A\\C\end{matrix}$ and contains inosine at the anticodon. While this work was in progress, a structure for $tRNA^{Arg}_{I}$ from E. coli was published by Murao et al.[3]. This $tRNA^{Arg}$ also contains inosine at the anticodon. The structure of the $tRNA^{Arg}_{II}$ isoacceptor isolated from the RPC-5 column is similar but not identical to the structure of $tRNA^{Arg}_{I}$. This report describes the structure of $tRNA^{Arg}_{II}$ and the differences compared to the sequence of $tRNA^{Arg}_{I}$.

EXPERIMENTAL.

Wild type E. coli B was grown in low phosphate medium containing ^{32}P[4]. Transfer RNA from these cells was isolated following the procedure of Zubay[5]. Arginine specific transfer RNA was separated on a BD-cellulose column

(0.5 x 5 cm), following the procedure of Gillam et al.[6]. Separation of the isoacceptor was achieved on a RPC-5 column[7]. The specific acceptance for amino acid was determined by using tritiated L-arginine of very high specific activity (10 mc/μmole). The amino acid acceptance of $tRNA^{Arg}$ at this stage was between 1.1 and 1.3 nmoles per A_{260}. Fingerprinting and the structural analysis of this material were carried out according to the procedures of Brownlee and Sanger[8]. The nature of the modified nucleosides was established from their mobilities on thin layer cellulose plates using the solvent systems described by Nishimura[9].

RESULTS.

Figure 1 shows the two dimensional fingerprint of $tRNA^{Arg}_{II}$ after ribonuclease T_1 digestion and Figure 2 is a fingerprint obtained after digestion with ribonuclease A. Quantitative analysis of the oligonucleotides indicates less than 5% contamination with any other nucleic acid. There are 77 nucleotides in the structure. Eight of them are minor bases. The modified nucleosides present in the structure of $tRNA^{Arg}_{II}$ include 4-thiouridine, ribothymidine, inosine, dihydrouridine, 2-methyladenosine, 7-methylguanosine, pseuduridine and an unidentified nucleoside N.

Table 1 shows the calculated molar yield of the oligonucleotides obtained from the analysis of the fingerprints.

To determine the primary structure of the tRNA, partial T_1 and pancreatic ribonuclease digestion products were isolated by a combination of electrophoresis and thinlayer homochromatography[8]. The partial products were further characterized by complete T_1 or ribonuclease A digestion.

Figure 3 summarizes the sequence overlaps of partial digests.

The nucleotide sequence of $tRNA^{Arg}_{II}$ as deduced from these analyses is shown in Figure 4.

DISCUSSION.

There are six codons for the amino acid L-arginine. At least five isoacceptors have been separated on the reverse phase chromatographic system[1,2]. Two of the five species of the isoacceptors have been reported to interact with the codons $CG\begin{matrix}A\\C\end{matrix}$[1,2]. Ribosome binding studies of the ^{32}P labelled $tRNA^{Arg}$ agree with this observation (Chakraburtty, K. unpublished results.)

The primary structure of E. coli $tRNA^{Arg}_{I}$ reported by Murao et al.[3] is similar but not identical to the structure reported in this paper. The major difference between the two structures is the size of the dihydrouridine

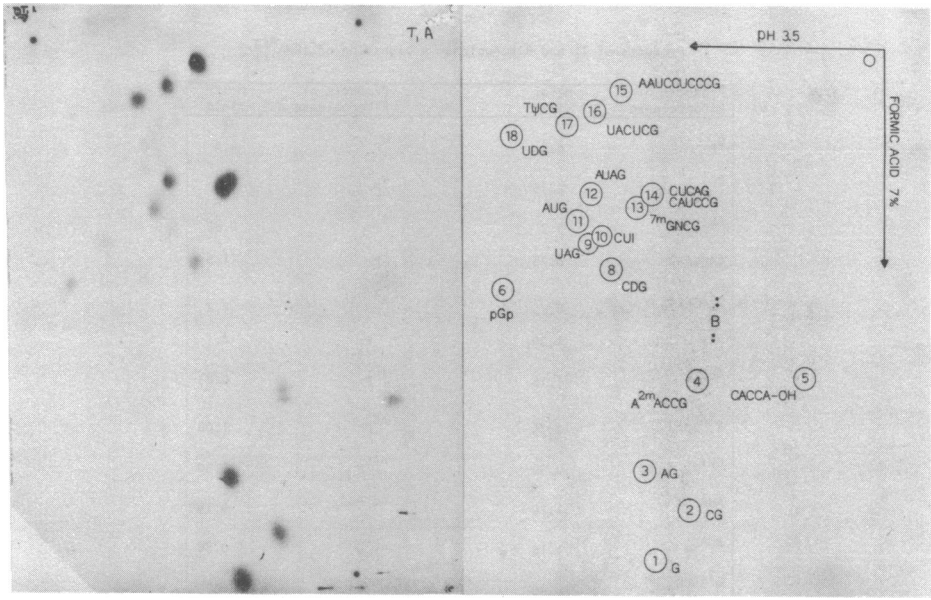

Figure 1. A two dimensional fractionation of a ribonuclease T_1 digest of $tRNA^{Arg}$. The diagram shows the sequences of nucleotides. B is the blue marker.

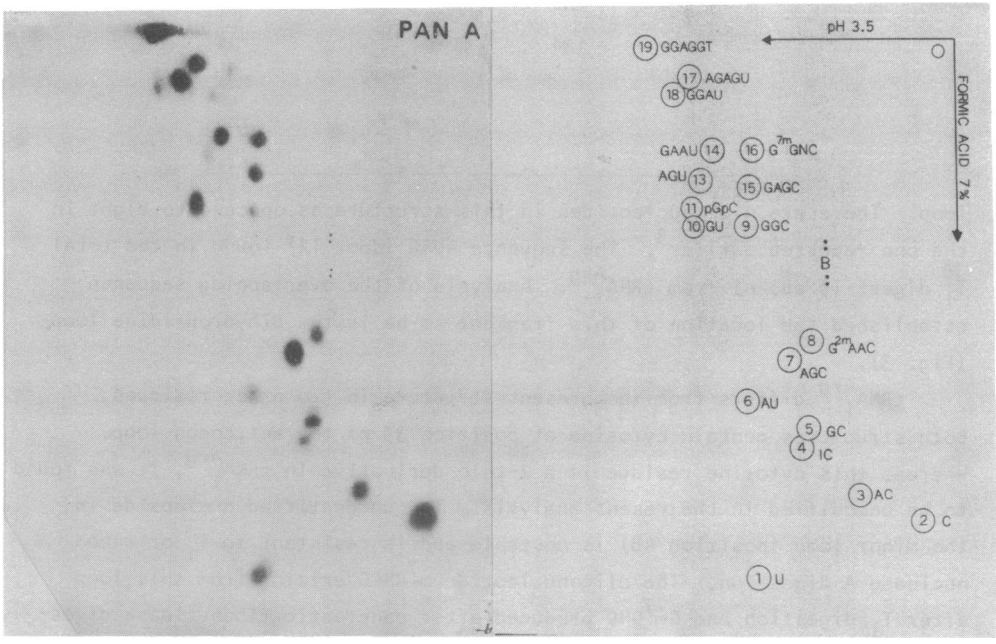

Figure 2. A two dimensional fractionation of a pancreatic ribonuclease digest of $tRNA^{Arg}$. The diagram shows the sequences of the oligonucleotides. B is the blue marker.

TABLE 1

Products of T_1 and Ribonuclease A Digestion of $tRNA^{Arg}_{11}$

Products of T_1 Ribonuclease	Molar Yield	Products of Ribonuclease A	Molar Yield
G_p	6.3	U_p	4.4
CG_p	2.35	Ψ_p	0.90
AG_p	3.3	D_p	0.85
CACCA-OH	0.75	C_p	11.5
m^2AACCG_p	0.75	AC_p	2.2
p^GP	1.00	GC_p	2.1
CDG_p	1.15	IC_p	0.83
s^4UAG_p	0.75	AU_p	1.00
CUI_p	0.50	AGC_p	1.90
AUG_p	1.10	Gm^2AAC_p	0.75
$AUAG_p$	1.10	Gs^4U	0.86
m^7GNCG_p	0.90	$p^{GC}P$	0.75
$CUCAG_p$	1.25	GGC_p	0.80
$CAUCCG_p$	1.00	$GAGC_p$	0.90
$AAUCCUCCCG_p$	1.10	Gm^7GNC_p	0.86
$UACUCG_p$	1.00	$GAAU_p$	1.20
$T\Psi CG_p$	1.15	$AGAGU_p$	1.20
		$GGAU_p$	2.20
		$GGAGGT_p$	1.20

loop. There are nine nucleotides in this structure as opposed to eight in the one reported earlier[3]. The sequence AUAG (spot 12) found in the total T_1 digest is absent from $tRNA^{Arg}_1$. Analysis of the overlapping sequence established the location of this fragment to be in the dihydrouridine loop (Fig. 3).

$tRNA^{Arg}_1$ differs from the present structure in two other residues. Both structures contain cytosine at position 33 of the anticodon loop. Whereas this cytosine residue is a 2-thio derivative in $tRNA^{Arg}_1$, it was found to be unmodified in the present analysis. The unidentified nucleoside in the minor loop (position 48) is unstable and is resistant to T_1 or ribonuclease A digestion. The oligonucleotide m^7GNCG arising from this loop after T_1 digestion and Gm^7GNC produced after pancreatic ribonuclease digestion both have very different mobilities in the two dimensional fingerprints compared to that from $tRNA^{Arg}_1$[10]. Nucleoside X, which is reported to occur in several species of E. coli tRNA, has recently been identified by Ohashi

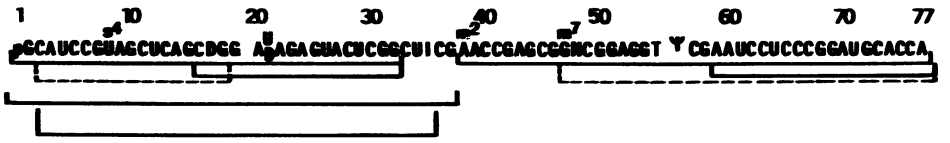

Figure 3. Overlapping sequence of ^{32}P $tRNA^{Arg}$ as deduced from the partial T_1 digestion products.

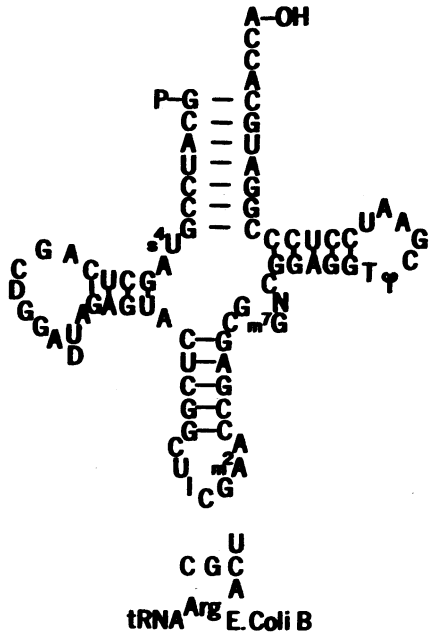

Figure 4. Cloverleaf structure of $tRNA^{Arg}_{11}$.

et al.[11] and subsequently by Friedman et al.[12] as 3-(3-amino-3-carboxypropyl) uridine. Reaction of this nucleoside with phenoxyacetoxysuccinimide shifts the elution of tRNA containing this nucleoside on BD-cellulose[13]. The purification procedure for this tRNA involved sham acylation to remove any reactive transfer RNA. Therefore, it is likely that the modified nucleoside in this structure is different from the recently identified nucleoside X.

Inosine is present in both $tRNA^{Arg}$ structures. Using an antibody against inosine, Inouye et al. have established the occurrence of this minor nucleoside in E. coli $tRNA^{Arg}$[14]. In the present analysis, the modification of adenosine to inosine at this wobble position was observed to be incomplete. The ratio of adenosine to inosine in this position varies from preparation to preparation; the maximum yield obtained was a little over half

a mole of inosine per mole of $tRNA^{Arg}$. When unmodified, the T_1 fragment CUACG became inseparable from the oligonucleotide CUCAG present in another part of this structure. This caused ambiguity in the sequence reported earlier[15].

It is not clear whether the differences between the two E. coli $tRNA^{Arg}$ are technical or biological. In a recent personal communication Dr. S. Nishimura indicated the possibility of an error in the analysis of the dihydrouridine loop of $tRNA_I^{Arg}$ reported by Murao et al.[3], who omitted one guanosine residue (G18). It remains to be determined whether the major arginine-accepting components in the two laboratories differ because of a difference in the organism, a difference in the growth conditions or the isolation of different isoacceptor species. Since the earlier reported structure is referred to as $tRNA_I^{Arg}$, I refer to this structure as $tRNA_{II}^{Arg}$.

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