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The nucleotide sequence of asparagine tRNA from Escherichia coli

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ABSTRACT

The nucleotide sequence of *Escherichia coli* asparagine tRNA was determined to be pU-C-C-U-C-U-G-s^4U-A-G-U-U-C-A-G-D-C-G-G-D-A-G-A-A-C-G-G-C-G-G-A-C-U-Q-U-U-t^6A-A-ψ-C-C-G-U-A-U-m^7G-U-C-A-C-U-G-G-T-ψ-C-G-A-G-U-C-C-A-G-U-C-A-G-A-G-G-A-G-C-C-A_{OH}. Its D-stem and D-loop have almost the same sequence as *Escherichia coli* asparatate tRNA.

INTRODUCTION

Asparagine tRNA in *E. coli* is one of the few acceptor RNAs which contain the hypermodified nucleoside Q in the anticodon (1). This family of tRNAs can be easily purified by chromatography on dihydroxyboryl-substituted cellulose (2). Since the knowledge of the primary structure of $tRNA^{Asn}$ was important for our studies on the precursors to the Q-base containing tRNAs in *E. coli* (3), we undertook to determine its nucleotide sequence.

MATERIALS AND METHODS

General. Pancreatic RNase, snake venom phosphodiesterase and *E. coli* alkaline phosphatase (BAPF) were obtained from Worthington Biochemical Corp. T1 and T2 RNase (Sanko) were purchased from Calbiochem. U2 RNase was a gift of Dr. H. Okazaki of the Sankyo Co., Tokyo. Silkworm nuclease was a gift of Dr. J. Mukai. PEI (4) plates (Polygram Cel 300 PEI) were a product of Macherey-Nagel. RP-14, RP-54, and NST-54 X-ray films were from Eastman Kodak; Whatman DE81 DEAE-cellulose paper and Whatman #1 chromatography paper from Fisher Scientific. Cellogel was a product of Colab Inc.

Preparation of tRNA. For the early sequence work unlabelled tRNA from *E. coli* B was purified by DEAE-Sephadex chromatography and reversed phase chromatography as described (1). Uniformly [^{32}P]-labelled tRNA was purified from *E. coli* K12 strain A49 (5) as described (6). From this tRNA the mixture of Q-base containing tRNAs was isolated as described earlier (2).

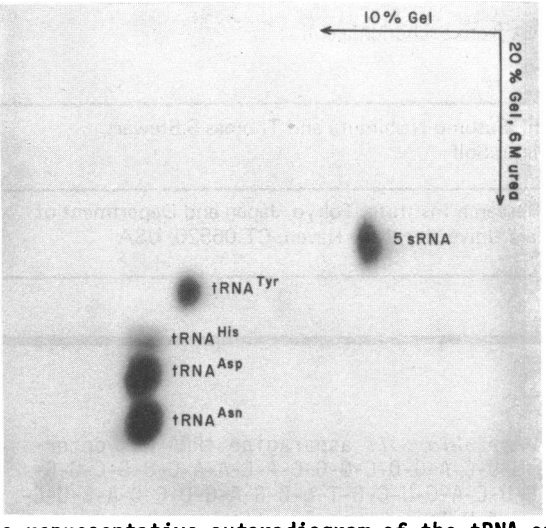

Figure 1
Autoradiogram of [^{32}P]-tRNA species separated by 2-dimensional polyacrylamide gel electrophoresis.

The individual tRNA species were then separated by standard two-dimensional polyacrylamide electrophoresis (7) using a 10% gel in the first-dimension followed by a 20% gel in 6M urea for the second dimension. Figure 1 shows a representative autoradiogram of the tRNA species after separation by two-dimensional gel electrophoresis.

Sequencing Techniques. Standard procedures (8) were used for enzyme degradation and fingerprinting of [^{32}P]tRNA and also the identification of the oligonucleotides resulting from enzymatic digestion. Reaction conditions for polynucleotide phosphorylase and silkworm nuclease have been described (9,10). Larger oligonucleotide fragments derived by chemical cleavage at m^{7}G (11) or D (12) were separated by polyacrylamide gel electrophoresis on 15% gels containing 6M urea (13).

Characterization of Modified Nucleotides. All oligonucleotide fragments were digested exhaustively with T2 RNase. The hydrolysate was subjected to thin layer chromatography (on cellulose) in the following two systems: solvent I, isobutyric acid - 0.5 M ammonium hydroxide (5:3, v/v); solvent II, t-butanol-concentrated hydrochloric acid-water (70:15:15). The fragments were detected by autoradiography (for [^{32}P]-labelled oligonucleotides) or by UV-absorbance (cold oligonucleotides). In the latter case they were eluted and their spectra determined.

RESULTS

Since both unlabelled and [^{32}P]-labelled tRNA^{Asn} was used for sequence analysis we have combined the description of the results. Only in some cases will it be indicated which source of tRNA was used.

Pancreatic RNase Digestion Products. The [^{32}P]-labelled tRNA^{Asn} was digested with pancreatic RNase and the fragments separated by electro-

TABLE I

Pancreatic RNase End Products

Fragment Number	Sequence	Molar Yields	
		Measured	From final sequence
p1	C-	7.2	13
p2+2a*	Q-U-	0.9	1
p3	m^7G-U-	0.7	1
p4	U- + ψ-	3.7	7
p5	A-C-	1.2	1
p6	A-U-	1.1	1
p7	G-U-	2.4	2
p8	A-G-D-	1.0	1
p9	A-G-U-	2.5	2
p10+10a†	t^6A-A-ψ-	0.7	1
p11	G-G-D-	0.9	1
p12	G-G-T-	1.0	1
p13	G-A-G-U-	1.2	1
p14	G-G-C-	0.9	1
p15	G-G-A-C-	1.1	1
p16	A-G-A-A-C-	1.3	1
p17	A-G-A-G-G-A-G-C-	0.9	1
p18	pU-	0.9	1

* Upon further analysis both fragments, p2 and p2a, gave the sequence Q-U-. Possibly there is a difference in the state of modification of Q.

† Fragment p10a was shown to be U-t^6A-A-ψ-. Possibly the adjacent modified nucleotide slows down the rate of RNase A reaction.

TABLE II

Analysis of Pancreatic RNase End Products

Fragment Number	T2 RNase Products					T1 RNase Products	Conclusion
	A	C	G	U	Others		
p1		+					C-
p2				1	Q-(1.0)	Q-U-	Q-U-
p3				1	m^7G-(0.7)	m^7G-U-	m^7G-U-
p4				+[a]	ψ		U- + ψ-
p5	1.1	1				A-C-	A-C-
p6	1.2			1		A-U-	A-U-
p7			0.9	1		G-(0.9),U-(1)	G-U-
p8	1.1		0.9		D-(1)	A-G-(1.0),D-(1)	A-G-D-
p9	1.0		0.9	1		A-G-(0.9),U-(1)	A-G-U-
p10	1.1				t^6A-(0.9),ψ-(1)	t^6A-A-ψ-	t^6A-A-ψ-*
p11			1.8		D-(1)	G-(1.8),D-(1)	G-G-D-
p12			1.8		T-(1)	G-(1.8),T-(1)	G-G-T-
p13	1.1		1.9	1		AG-(1.1),G-(0.9),U-(1)	G-A-G-U-*
p14		1	1.7			G-(1.7),C-(1)	G-G-C-
p15	0.9	1	1.8			AC-(1),G-(1.8)	G-G-A-C-
p16	0.9	1	2.8			AG-(1.4),A-A-C-(1)	A-G-A-A-C-
p17	2.9	1	3.7			AG-(3.1),G-(1.2),C-(1)	A-G-A-G-G-A-G-C-*
p18					pU-		pU-

*Sequence determined from data in Table V.

[a]This material identified as a mixture of U- and ψ- by tlc.

phoresis and homochromatography on PEI plates. Figure 2 shows the resulting fingerprint. Eighteen fragments were found. The experimentally deter-

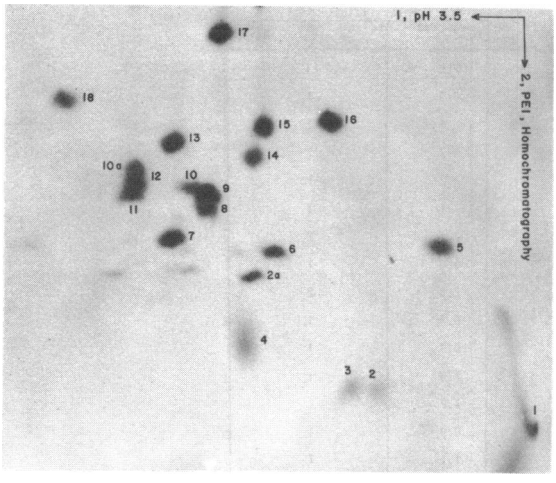

Figure 2.
Fingerprint of complete RNase A digest of asparagine tRNA.

mined molar yields for each fragment are shown in Table I. Analysis of the pancreatic RNase end products and their deduced sequences are shown in Table II. With the exception of fragments p10, p13, and p17, all oligonucleotides could be elucidated by the combined results of T2 and T1 RNase digestions. For these three fragments additional enzymatic analyses are described in Table V.

Sequence of Fragment p10. The results in Table V show that t^6A is at the 5'-end of the oligonucleotide; thus its sequence is t^6A-A-ψ-.

Sequence of Fragment p13. Since G was found to be the 5'-terminal residue (see Table V) the sequence of this tetranucleotide is G-A-G-U-.

Sequence of Fragment p17. Silkworm nuclease digests yielded A-G-A- as the 5'-end and -A-G-C- as the 3'-end of this oligonucleotide (Table V). Combined with the results of the T1 RNase digest the sequence was deduced as A-G-A-G-G-A-G-C-.

T1 RNase Digestion Products. Digestion of *E. coli* $tRNA^{Asn}$ with T1 RNase gave the standard two-dimensional fingerprint shown in Figure 3. Fifteen

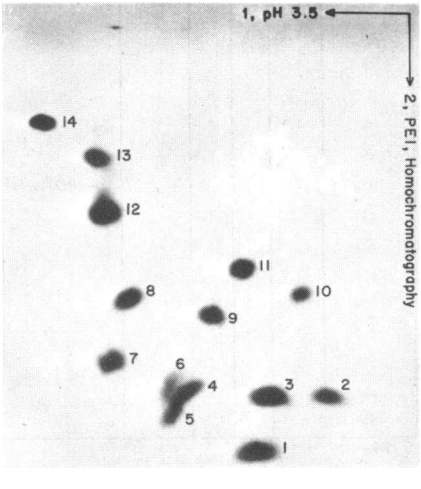

Figure 3.
Fingerprint of complete T1 RNase digest of asparagine tRNA. Fragment 15 is outside of the area of this autoradiogram.

TABLE III

T1 RNase End Products

Fragment Number	Sequence	Molar Yields	
		Measured	From final sequence
t1	G-	5.4	5
t2	C-G-	1.3	1
t3	A-G-	3.4	3
t4	D-C-G-	1.2	1
t5	D-A-G-	0.9	1
t6	U-A-G-	0.7	1
t7	T-ψ-C-G-	1.2	1
t8	U-U-C-A-G-	1.1	1
t9	U-C-A-G-	1.1	1
t10	A-A-C-G-	0.9	1
t11	U-C-C-A-G-	1.1	1
t12	U-A-U-m^7G-U-C-A-C-U-G-	1.0	1
t13	A-C-U-Q-U-U-t^6A-A-ψ-C-C-G-	0.8	1
t14	pU-C-C-U-C-U-G-	0.9	1
t15	C-C-A_{OH}	0.8	1

fragments were found. The experimentally determined molar yields of each fragment are shown in Table III. Analysis of the T1 RNase end products

TABLE IV

Analysis of T1 RNase End Products

Fragment Number	T_2 RNase Products					Pancreatic RNase Products	Conclusions
	A	C	G	U	Others		
t1			+				G-
t2		1.1	1			G-(1),C-(0.9)	C-G-
t3	1.0		1			AG-	A-G-
t4		1.0	1		D-(0.9)	G-(1),C-(1.1),D-(0.9)	D-C-G-*
t5	1.1		1		D-(1.1)	A-G-(1),D-(1.1)	D-A-G-
t6	1.1		1	1.0		A-G-(1),U-(1.1)	U-A-G-
t7		0.9	1		T-(1.0), ψ-(1.1)	G-,C-,T-,ψ-	T-ψ-C-G*
t8	1.0	1.0	1	2.0		A-G-(1),C-(1.0),U-(1.3)	U-U-C-A-G-*
t9	1.0	1.0	1	1.0		A-G-(1),C-(0.4),U-(0.7)	U-C-A-G-*
t10	2.4	0.9	1			A-A-C-(0.7),G-(1)	A-A-C-G-
t11	1.1	2.2	1			A-G-(1.1),C-(1.0),U-(0.2)	U-C-C-A-G-*
t12	1.9	2.2	1	3.7	m^7G-(1.0)	A-U-(0.9),A-C-(1.0),m^7G-U-,C-(2.7)	U-A-U-m^7G-U-C-A-C-U-G-*
t13	+	+	+	+	Q-,t^6A-,ψ-	t^6A-A-ψ-, A-C-, Q-U-, G-, C-, U-	A-C-U-Q-U-U-t^6A-A-ψ-C-C-G-†
t14		2.9	1	2.0	pU-(0.9)	pU-(0.7),G-(1),G-(2.4),U-(0.9)	pU-C-C-U-C-U-G-*
t15		+					

*Sequence determined from data in Table V.

†Sequence determined earlier (1).

TABLE V

Further Analysis of some T1 or Pancreatic RNase End Products

Treatment	Fragment Number	Products
Phosphatase followed by venom phosphodiesterase (cold oligonucleotide)	p10	$t^6A(1)$, -A(1.0), -ψ(1.0)
	p13	G(1), -A(1.3), -G(0.7), -U(0.7)
	t4	D(0.8), -C(0.8), -G(1)
	t9	U(0.7), -C(0.8), -A(0.7), -G(1)
Phosphatase followed by PNPase (cold oligonucleotide)	t8	U-U-C
	t11	U-C-C
Venom phosphodiesterase	t15	-C(1.0), -A(0.9)
Silkworm nuclease (^{32}P-labelled oligonucleotides)	t14	pU-C, pU-C-C, pU-C-C-U (5'-end) -U-G- (3'-end)
	p17	A-G-A (5'-end), -A-G-C- (3'-end)
U2 RNase (cold oligonucleotides)	t12	U-A-, (m^7G-,C-,2U-)A-, (C-,U-)G-
m^7G cleavage (cold oligonucleotide)	t12	U-A-U-, -U-C-A-C-U-G-

and their sequences are shown in Table IV. Only six of the fifteen fragments could be unambiguously sequenced by the combined results of pancreatic and T2 RNase digestions. Further analyses of the remaining T1 RNase end products by U2 RNase, snake venom phosphodiesterase, polynucleotide phosphorylase, and silkworm nuclease are shown in Table V.

Sequence of Fragment t4. Since D is at its 5'-terminus (Table V), the sequence is D-C-G-.

Sequence of Fragment t7. The sequence T-ψ-C-G- for this oligonucleotide was established by partial digestion with spleen phosphodiesterase (data not shown).

Sequence of Fragment t8. Treatment with polynucleotide phosphorylase yielded U-U-C- as the 5'-terminal sequence; thus fragment t8 is U-U-C-A-G-.

Sequence of Fragment t9. Since U is the 5'-terminal base (Table V), the oligonucleotide sequence is U-C-A-G-.

Sequence of Fragment t11. Treatment with polynucleotide phosphorylase gave U-C-C as the 5'-terminal sequence (Table V); thus fragment t11 is U-C-C-A-G-.

Sequence of Fragment t12. The combined results of m^7G cleavage and U2 RNase digestion of this oligonucleotide (Table V) establish its sequence as U-A-m^7G-C-U-U-A-C-U-G-.

Sequence of Fragment t13. The sequence of this oligonucleotide has been determined earlier (1).

Sequence of Fragment t14. Silkworm nuclease digestion gave pU-C-C-U- as 5'-terminus and U-G- as the 3'-end of this oligonucleotide (Table V); thus its sequence is pU-C-C-U-C-U-G-.

Sequence of Fragment t15. The combined results of T2 RNase and venom phosphodiesterase digestion establish the sequence as C-C-A_{OH}.

Large Overlapping Fragments and Determination of the Total Sequence. Large oligonucleotide fragments of the $tRNA^{Asn}$ were obtained by chemical cleavage at m^7G, chemical cleavage at D, and by partial digestion with T1

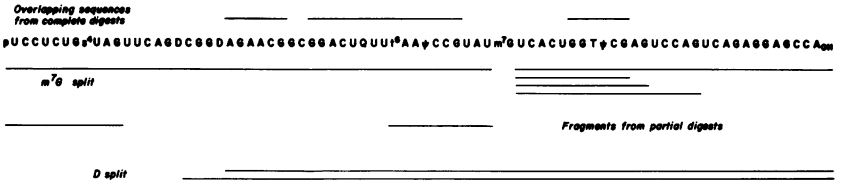

Figure 4. Summary of overlapping fragments.

RNase. The products were fractionated by two-dimensional polyacrylamide gel electrophoresis. The bands from the second gel were eluted and one half was digested completely with T1 RNase and the other half with pancreatic RNase. The resulting products were fractionated by homochromatography and identified by base analysis and enzymatic digestion. The results are summarized in Figure 4. The total nucleotide sequence of *E. coli* $tRNA^{Asn}$ is shown in Figure 5 in the familiar cloverleaf form.

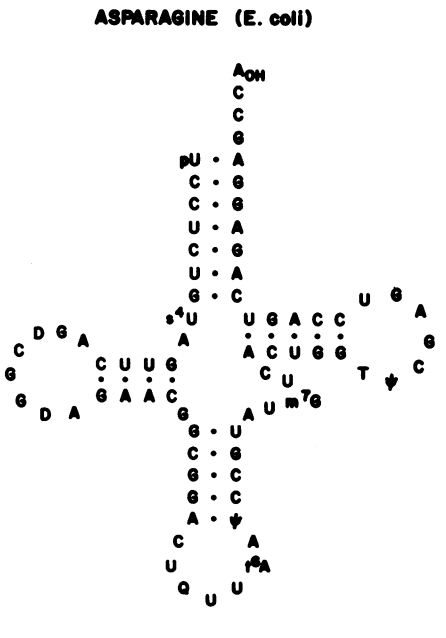

Figure 5.
Cloverleaf model of *E. coli* $tRNA^{Asn}$.

Characterization of Modified Nucleotides. The modified nucleotides s^4Up, Dp, ψp, Qp, m^7Gp, and Tp, were found in the tRNA. s^4Up was not found in any of the radioactive preparations, and even in some of the cold preparations of tRNA. However, in one T1 RNase digestion of unlabelled tRNA s^4U-A-G- was isolated. Also undegraded tRNA had a higher UV absorbance at 330 nm. Therefore we believe that 4-thiouridine is present in $tRNA^{Asn}$ at position 8.

DISCUSSION

The nucleotide sequence of *E. coli* $tRNA^{Asn}$ does not show any unusual features. It is interesting to note that its D-stem and D-loop have almost the same sequence as *E. coli* $tRNA^{Asp}$.

ACKNOWLEDGEMENTS

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