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Sequence of E.coli $tRNA^{Glu1}$ by automated sequential degradation

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ABSTRACT

A minor $tRNA^{Glu}1$ constituent of a preparation of E. coli B $tRNA^{Glu}$ (Oak Ridge) has a guanosine residue at position 66 rather than an adenosine as in the $tRNA^{Glu}2$ described by Ohashi et al. (3). Automated sequential degradation was used to sequence this region.

INTRODUCTION

Preparations of E. coli $tRNA^{Glu}$ contain at least two species (1, 2). A sequence for the major component has been published (3), but quantitative sequence data (4, 5) indicate some uncertainty in the sequence near the connection of the acceptor stem and the T-ψ-C-G stem (Fig. 1). Ohashi et al. (3) report some difficulty in the isolation and analysis of RNase T_1 and pancreatic RNase A oligonucleotides from this region, and Singhal (5) and Chang (4) show low recoveries of these components in column nucleotide assays. In a pancreatic digest of the same lot of E. coli $tRNA^{Glu}$, all the oligonucleotides were present in molar ratios (90–105%) except for two oligonucleotides (5). The regions in question are rich in guanosine residues, often a source of difficulty in enzymic degradation. The stoichiometry of oligonucleotides (5) suggests that the sequence of the minor component is almost identical to the major component.

Since the sequential degradation process can quantitatively degrade oligo(G) sequences, we have used our automated sequential degradation apparatus (7) to study the sequence in this region.

Materials: We used $tRNA^{Glu}$ prepared at the Oak Ridge National Laboratory (2). This material is composed of two $tRNA^{Glu}$ components (1), which comprise 78% of the preparation. The remainder is not $tRNA^{Glu}$. About 25% of the $tRNA^{Glu}$

fraction appears in a minor chromatographic position (B. Z. Egan, personal communication), and the remainder as a single peak on RPC-5 chromatography. This distribution in this does not necessarily reflect the distribution in the intact E. coli.

Reagents of the highest purity available were used. $NaIO_4$ was purchased from Matheson Coleman Bell, and lysine monohydrochloride was purchased from A & M Bulk Biochemicals of Los Angeles, California.

Methods: The elimination of the terminal residue from $tRNA^{Glu}$ was carried out in 0.5 M lysine chloride at pH 6.5 after periodate oxidation (6). The terminal phosphate was removed by passage over immobilized E. coli alkaline phosphatase (7). The released bases were assayed by chromatography on Biogel P-2 at pH 5.2. Although the instrument design is different, the principles of operation have been described (7a).

The above steps were repeated automatically in our sequenator as described in the Results.

RESULTS

Two runs were made on the $tRNA^{Glu}$ preparation (Table I). On the basis of the distribution of tRNA's in the sample, the quantity of base released would be 56% from $tRNA^{Glu}$ (major), about 19% from the minor $tRNA^{Glu}$, and the remainder would be derived from the contaminating tRNA's. The results through 16 cycles are consistent with these estimates. Beyond cycle 16 there is a partial cessation of sequential degradation. This reduced the yield in each succeeding step, but the base released still corresponded quantitatively to that in $tRNA^{Glu}_2$.

The minor $tRNA^{Glu}$ component has a sequence at position 66 different from that of the major sequence (Table I). At position 66, 56% of the end groups are adenine [as predicted from the major component (3)] and the remainder is guanine. Thus the minor component must have G at this position. Since there are four G residues preceding No. 66, there is some probability that asynchrony is responsible for the amount of G. If this were the case, the bases obtained from the succeeding position (66) would reflect that asynchrony. However, we find 95% of the amount of uracil expected in cycle 12 (position 65) and either no uracil or less than 9% uracil in step 13 (position 64). These results indicate the degradation is synchronous and the tRNA is responsible for the unusual base distribution at position 66.

TABLE I. Analysis of E. coli $tRNA^{Glu}$ by sequential degradation[a]

Sequence position	Cycle number	Sequence	Find[b] Run 1 (%)	Find[b] Run 2 (%)	Recovery (bases)[c] Run 1 (%)	Recovery (bases)[c] Run 2 (%)
76	1	A	100	100	100	100
75	2	C	89	95	96	110
74	3	C	89	98	92	96
73	4	G	81	100	75	-
72	5	C	80	98	84	62
71	6	A	76	74	70	64
70	7	G	84	75	62	65
69	8	G	89	85	58	66
68	9	G	77	82	57	56
67	10	G	83	83	47	48
66	11	A,G	55A, 38G	55A, 45G	38	48
65	12	U	65	70	43	44
64	13	C	64 (9U)	86[d]	42	48
63	14	C	81		33	
62	15	C	81		33	
61	16	C	75		30	
60	17	U	48		34	
59	18	A	60 (25U)		25	
58	19	A	75		18	
57	20	G	47 (37A)		24	
56	21	C	47		24	
55-46	22-29	29 nmoles recovered after 29 cycles (37% of original RNA).				

[a] E. coli K12 MO ORNL Lot No. 15-291: 56% $tRNA^{Glu}_1$, 19% $tRNA^{Glu}_2$, 22% tRNA (not glutamyl tRNA) (B. Z. Egan, unpublished).

[b] The entire composition is not presented. The individual values indicate the fraction present as that base in that analysis.

[c] The values refer to the fraction of the initial number of end groups available for reaction.

[d] No uracil was released in this cycle.

An unexpected finding was that the amount of base released by the sequenator fell to zero as it approached the pseudouridine at position 55. This does not appear to be a loss of sensitivity due to asynchrony, since we have carried the process through 29 cycles and all the T and ψ are still present in the truncated $tRNA^{Glu}$ (Table II). We have tested the enzyme activity by sequentially degrading mixed tRNA through four cycles and find that all the end groups are dephosphorylated, so that reduced phosphatase activity is not the cause of the cessation of sequencing. The end groups indicate that there was considerable asynchrony in the late stages. We have observed the same effect with E. coli $tRNA^{fMet}$ (unpublished observation) and $tRNA^{Phe}$ (8). Since the end groups are intact and present as nucleosides, it appears that the periodate oxidation step has become a major rate-limiting step.

TABLE II. Analyses of residual $tRNA^{Glu}$ after 29 cycles[a]

Composition	Moles/mole tRNA						
	ψ	U	T	m^2A	G	C	A
Find	2.1	8.1	1.1	1[b]	16	21	10
Theory (21 cycles)	2	7	1	1[b]	16	19	8
End group (OH^-)		0.18	0.03		0.18	0.24	0.14
End group (P'ase, then OH^-)		0.18	0.03		0.18	0.24	0.14
Yield: 64%[b]							

[a] E. coli K12 MO, Lot 15-291 (ORNL).

[b] The final product contained 1.1 nmole of m^2A/A_{260} and was used as the reference for molar ratios. The initial tRNA contained 1.55 nmole of end group/A_{260} and 1.25 nmole Glu/A_{260}.

The truncated $tRNA^{Glu}$ remaining has been hydrolyzed with RNase T_1 to confirm the cessation of sequential degradation in the T-ψ-C loop (Table III). Only a small fraction (~10%) of the T-ψ-C-G oligonucleotide was attacked by sequential degradation so its appearance in the RNase T_1 digest was expected. The oligonucleotides derived from positions 58-76 are totally absent. This indicates that sequential degradation had virtually ceased prior to cycle 19. The yield of oligonucleotides C_3-A-G_p and A-C-A-C_2-G_p is unusually low.

There is no pGp, due to phosphatase action, and the RNase T_1 oligonucleotide from the anticodon region has been transformed to several products due to the reaction

of 5-methylaminomethyl-2-thiouridine with the periodate/amine reagent.

TABLE III. Oligonucleotide recovery from RNase T_1 hydrolysates of intact and partially sequenced $tRNA^{Glu}$ [a]

Oligonucleotide	Ratio[b]
C-C-A	0
Gp	1.26
C-Gp	1.0
pGp	0
A-Gp	0.90
A-C-Gp[c]	0 (0.15)
C_3-A-Gp	0.78
T-ψ-C-Gp	1.24
A-C-A-C_2-Gp	0.65
U-C-ψ-A-Gp	0.96
U-A_2-C-A-Gp	1.10
A_2-U-C_4-U-A-Gp	0
C_3-U-mnm^{2}sU-U-C-m^{2}A-C-Gp [d]	—

[a] End product of 29 cycles. See Table I.

[b] Since this preparation is 78% $tRNA^{Glu}$, the expected ratio of oligonucleotides present in all the tRNA's is 1.24. Ratio = moles oligoN from total tRNA/moles $tRNA^{Glu}$ oligoN.

[c] Since A-C-G is an ubiquitous trinucleotide, this small quantity is probably derived from the contaminating tRNA.

[d] The mnm^{2}sU (5-methylaminomethyl-2-thiouridine) is attacked by the periodate amine reagent giving rise to several products that chromatograph differently from the intact oligonucleotide. It was not quantitated except that there was no oligonucleotide in the original position.

DISCUSSION

Sequential degradation of a mixture of both the major and minor components of E. coli $tRNA^{Glu}$ shows they differ. The minor component has a G instead of an A at position 66. This is the first time such an "exchange" has been found at the end of a base-paired stem. The inability to detect this sequence difference by standard enzyme fragmentation, chromatographic resolution, and intellectual reconstitution arises largely from the difficulty in separating oligonucleotides containing a large proportion of guanine (3, 4, 5) as well as the relatively small proportion of the minor components. Since the periodate/amine treatment is quantitative even with sequences of oligo(G) (6, 8), the sequence difference was readily characterized by this procedure. A further point to be made is that 100% homogeneity of the RNA is not required for sequence analysis by sequential degradation. However, it is advantageous to have a

synchronous starting material. This is essentially the opposite of the requirements for sequencing by selective fragmentation, where high purity is required but full sequence synchrony is not.

Two observations lead us to suggest that the sequence of the minor component differs from that of the major by the presence of G at position 66 (Fig. 1). This preparation has been shown to have stoichiometric amounts of each oligonucleotide (RNase A hydrolysis) except for those in position 50-54 and 66-72. Our RNase T_1 hydrolysates show that the region 45-57 is present in stoichiometric quantity but the region 66-72 differs qualitatively in the presence of a G instead of an A.

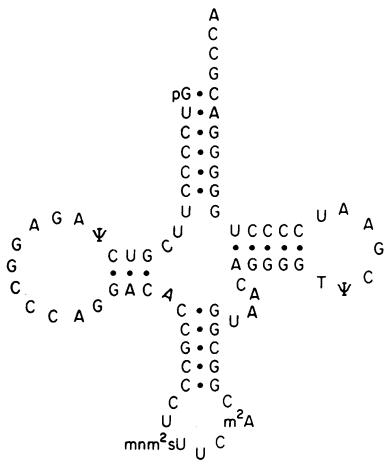

Figure 1. Sequence of $tRNA^{Glu_1}$. The S* is 5-methylaminomethyl-2-thiouridine. Position numbering begins with pG and goes to 76 at the terminal adenosine. Cycle numbering begins at adenosine and goes toward the pG.

The cessation of sequential degradation near position 58 (T-ψ-C-G loop) was unexpected, yet the same result was observed during manual sequencing (8). After we went through 29 cycles (9 more than required to remove the G from the T-ψ-C-G sequence), the termini are still nucleosides (Table II); and it appears that the periodate oxidation has become rate-limiting. This can be rationalized by one of two mechanisms: the conformation of the residual tRNA prevents the approach of the periodate anion or there is an alkali-labile group attached to the terminal diol. We are presently investigating the properties of the limiting step(s).

It is interesting that the oligonucleotides recovered in lower yield from the truncated tRNA are all in or near loop I. Whether this has any relationship to the proposed 3 dimensional structure of tRNA (10) is unknown.

The presence of G at position 66 poses a novel structural question. This residue is at the junction of the acceptor and T-ψ-C-G stems. The NMR studies of Shulman et al. (9) have shown that of the pure tRNA's studied only $tRNA^{Glu_2}$ has properties proving that the two stems (ending in A·U and U·A) are stacked upon each other in the same fashion as the stems are stacked in crystals of yeast $tRNA^{Phe}$ (10, 11). The minor $tRNA^{Glu_1}$, on the other hand, would have the structure U·G and A·U. The intrinsic strength of the terminal G·U base pair is negligible compared to the normal Watson-Crick type of base-pair (12), which would presumably weaken the interaction. To understand the nature of the forces holding the two stems together, it would be of value to know if the stacked-stem structure exists in $tRNA^{Glu_1}$ as well. If the structure is not as stable as $tRNA^{Glu_2}$, there is a potential for this to be a regulatory site in protein synthesis.

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