THE SEQUENCE OF THE 3′-OH END OF THE 16 S RNA OF *ESCHERICHIA COLI*

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1. Introduction

When 30 S ribosomes are treated with T1 RNase, the 16 S RNA is cleaved into several pieces. Among these pieces is a fragment from the 3′-OH end of the 16 S RNA which contains 26 nucleotides (band 3′) [1]. When colicin E3 reacts with 70 S ribosomes, either *in vivo* or *in vitro*, it releases a 50 nucleotide fragment from the 3′-OH end of the 16 S RNA [2]. This report deals with the sequence of these 2 bands. Our data are almost in complete agreement with the components found by Bowman et al. [2], in the colicin E3 released fragment but not with the sequence reported by Ehresmann et al. [3], for the terminal 38 nucleotides in the 3′-OH end of the 16 S RNA.

2. Experimental

The preparation of ^{32}P-labeled ribosomes and T1 RNase treatment of ribosomes was previously described [1]. Polyacrylamide gel electrophoresis of RNA fragments and retrieval of fragments from the gel were carried out according to Adams et al. [4]. All procedures for two-dimensional and one-dimensional electrophoretic separation of oligonucleotides have been described [4–6]. Colicin E3 was kindly supplied by Dr. Norton Zinder. Treatment of 70 S ribosomes with colicin was carried out according to Boone [7] using ^{32}P-labeled ribosomes. After colicin treatment, the 70 S ribosomes were treated with phenol. The RNA was precipitated with ethanol, washed with cold ethanol and dried. The RNA components were separated by electrophoresis on a 10% polyacrylamide flat gel. The fragment released by colicin E3 runs ahead of the 4 S (tRNA) band and was eluted according to Adams et al. [4].

3. Results

Previous studies had established the composition of band 3′ as: 3G, CG, UUG, $m_2^6Am_2^6ACCUG$ (oligonucleotide 71) [8] and AUC,C(UC,AC)UUCA_{OH} [1]. Fig. 1 shows a diagram of the radioautograph of the two-dimensional "fingerprint" pattern of band 3′ (after T1 RNase digestion) from 2 different cultures of *E. coli* MRE 600. Both patterns are identical.

A number of different enzyme treatments were carried out on band 3′. A very mild T1 RNase treatment removed two G residues from the 5′ end of the band [1]. Table 1 summarizes additional data, from which a sequence may be deduced. After partial T1 RNase digestion G,UUG and AUC,C(UC,AC)UUCA_{OH} are present in one fragment. A slightly larger fragment also contains CG. Since the product GGU is obtained from a pancreatic RNase digest of band 3′, the order of nucleotides adjacent to the 3′-OH fragment is CGGUUG. The position of $m_2^6Am_2^6ACCUG$ is established distal to CG because it is missing from product (b) in table 1 and it is found linked to CG after partial snake venom phosphodiesterase treatment.

The partial sequence of the 3′-OH oligonucleotide was previously published [1]. The data in table 1, in which the sequence GAU is found in the pancreatic RNase digest of band 3′, establishes AUCC as the 5′-end of this oligonucleotide. A partial snake venom phosphodiesterase hydrolysis gave a product containing A, 3C and 2U residues. The sequence compatible with these results is AUCCUC. The entire sequence of band 3′ is thus:
GG$m_2^6Am_2^6$ACCUGCGGUUGAUCCUCACUUCA_{OH}.

A colicin E3 fragment was prepared and divided in half and completely digested with T1 and pancreatic RNase. The diagram of the autoradiograph of the two-

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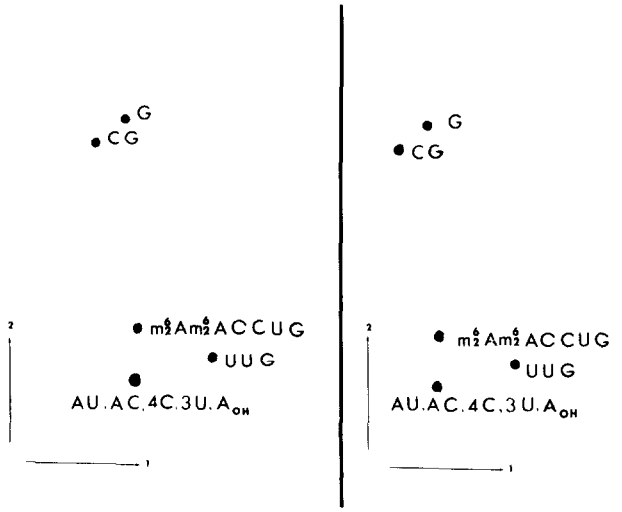

Fig. 1. Diagram of radiograph of the two-dimensional electrophoretic separation of the complete T1 RNase digests of band 3′ obtained from 2 different cultures of *E. coli* MRE 600. On the left: strain used by us in a previous study [1]; on the right: strain obtained from Dr. Robert Traut. 1 indicates cellulose acetate–pH 3.5 acetate buffer–7 M urea direction and 2 indicates the DEAE-cellulose–7% formic acid direction.

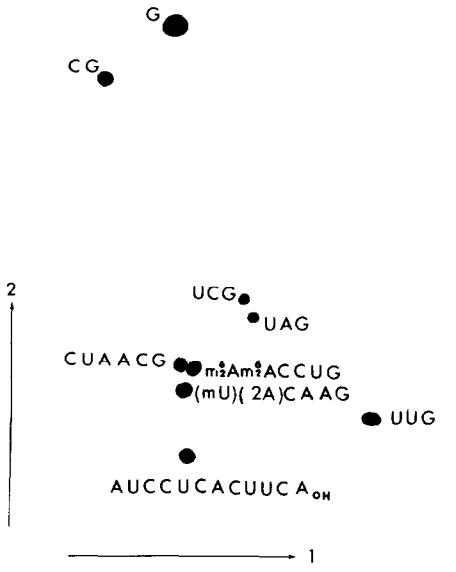

Fig. 2a. Diagram of radiograph of two-dimensional electrophoretic separation of complete T1 RNase digest of colicin E3 fragment. 1 indicates cellulose acetate–pH 3.5 acetate buffer–7 M urea direction and 2 indicates DEAE-cellulose-7% formic acid direction.

dimensional electrophoretic separation of the 2 different digests is shown in fig. 2. At the same time, another T1 RNase digest was carried out on an E3 fragment from another culture of *E. coli* MRE 600 obtained from Dr. Robert Traut. This "fingerprint" pattern is identical to the one shown in fig. 2a. Both E3 fragments contain all the components reported by Bowman et al. [2] except UG and AG. From the data in fig. 2 and table 2 and additional data discussed below, it is possible to deduce a sequence for the E3 fragment:

a) The presence of the oligonucleotide AGGGGGm$_2^6$Am$_2^6$AC establishes this sequence on the 5′ side of oligonucleotide 71.
b) The only source of an AG sequence in the E3 fragment is UAG which thus establishes the position of this trinucleotide.
c) From an independent experiment it was shown that CUAACG and UAG are linked together. RNA was obtained from T1 RNase treated 30 S ribosomes and separated by electrophoresis in polyacrylamide gels [1]. Some RNA material migrates much more rapidly than band 3′ in the gels. This material was retrieved from the gel and subjected to electro-

Table 1
Products obtained from various enzymatic treatments of band 3′.

T1 RNase	Partial T1 RNase*
G	(a) G
CG	UUG
UUG	AUC,C(UC,AC)UUCA$_{OH}$
m$_2^6$Am$_2^6$ACCUG	(b) CG
AUC,C(UC,AC)UUCA$_{OH}$	G
	UUG
Pancreatic RNase	AUC,C(UC,AC)UUCA$_{OH}$
U,C†	
AC	Snake venom phosphodiesterase*
GC	m$_2^6$Am$_2^6$ACCUG
GGU	CG
GAU	
m$_2^6$Am$_2^6$AC	

† The number of U and C residues was not estimated in this experiment.
* Partial digestion products were completely hydrolyzed with T1 RNase, followed by electrophoresis on DEAE-cellulose paper using 7% formic acid as solvent.

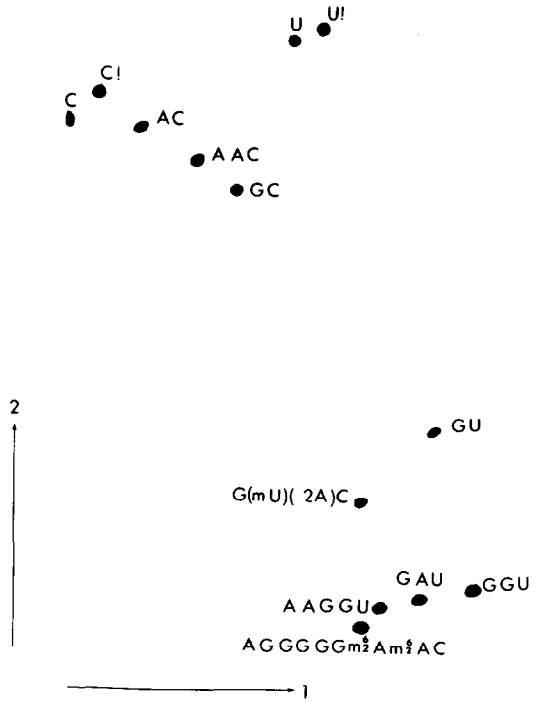

Fig. 2b. Diagram of radioautograph of two-dimensional electrophoretic separation of complete pancreatic RNase digest of colicin E3 fragment.

Table 2
Products of various enzymatic treatments of colicin E3 fragment.

T1 RNase	Pancreatic RNase
G (7 residues)	U,C†
CG	GGU*
UUG	GAU*
AUCCUCACUUCA$_{OH}$	GC*
UAG	GU*
UCG	AC**
C,UAAC,G	AGGGGGm$_2^6$Am$_2^6$AC**[2]
(mU; 2A)CAAG[3]	AAC**
	AAGGU**[2]
	G(mU[1],2A)C**

† U and C residues not estimated in this experiment.
* Complete T1 RNase digests yielded these compositions and sequences.
** Complete alkaline hydrolysis established the nucleotide composition of these oligonucleotides.
[1] "Fast" U residues on electrophoresis on Whatman #52 paper in pH 3.5 acetate buffer solvent system.
[2] T1 RNase digestion established the presence of an AG and an AAG sequence in these spots.
[3] This T1 RNase product, after complete pancreatic RNase digestion, yielded 2 radioactive spots after electrophoresis on DEAE-cellulose paper in the pH 3.5 acetate buffer solvent. The methylated U appears to be resistant to pancreatic RNase digestion.

phoresis on cellulose acetate in pH 3.5 acetate buffer containing 7 M urea and then chromatographed in the second direction on thin-layer DEAE-cellulose-cellulose plates, using "homomix C" [6] as solvent. One of the fragments yields, on complete T1 RNase hydrolysis, (C,UAAC)G and UAG. According to Fellner et al. [8], the sequence (C,UAAC)G is present only once in the 16 S RNA. This places CUAACG on the 5′-side of UAG.

d) The presence of G(mU,2A)C and AAGGU proves that the sequence at the 5′-end of the E3 fragment is G(mU,2A)CAAGGUCG since the UCG trinucleotide is the only remaining source of U following the AAGG sequence. The whole sequence is: G(mU,2A)CAAGGUCGCUAACGUAGGGGGm$_2^6$-Am$_2^6$ACCUGCGGUUGAUCCUCACUUCA$_{OH}$.

4. Discussion

The unique structural features of the 3′-OH end of the 16 S RNA are striking. Five methyl groups are found here [2], 4 of them on adjacent adenylic acid residues in oligonucleotide 71 [8]. The presence or absence of these 4 methyl groups determines kasugamycin sensitivity or resistance of 30 S ribosomes [9]. The resistant strain appears to lack a specific methylating enzyme which normally places all 4 methyl groups on the two adenylic residues in oligonucleotide 71 [10]. It is interesting to note, therefore, that the two A residues of oligonucleotide 71 are preceded by 6 purines including 5 guanine bases. This sequence may be part of the recognition site for the methylating enzyme.

It is not possible at this time to reconcile the different sequences reported by Ehresmann et al. [3] and by us. For example, Ehresmann et al. place CUAACG 12 nucleotides from the 3′-OH end of the 16 S RNA, while we place it 30 nucleotides from the end. Ehresmann et al. have not reported on the purity of their 3′-OH fragment, so it is not possible to judge whether this may account for the difficulties. The

possibility remains that there are strain differences, since there are minor differences between our results and those of Bowman et al. [2] on the composition of the E3 fragment.

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