MODIFICATION OF THE GUANINE IN THE INVARIANT SEQUENCE $^{5'}CCG_{44}AAC^{3'}$ OF THE *ESCHERICHIA COLI* 5 S RNA IN SOLUTION BY KETHOXAL

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1. Introduction

The 5 S rRNA of all prokaryotic organisms contain the invariant sequence $^{5'}CCGAAC^{3'}$ [1]. Part of this sequence is complementary to the $^{5'}GT\psi CG^{3'}$ sequence invariant in most prokaryotic tRNAs [2]. Thus this invariant 5 S RNA sequence has been hypothesized to form a ribosomal binding site for tRNAs via base pairing of these invariant, complementary regions [3,4]. This hypothesis has been refined to a binding role in the ribosomal A site [5]. Much circumstantial evidence has been gathered to support such a scheme [6–9]. However, the guanine within the invariant sequence is not modifiable by the guanine specific reagent kethoxal, which blocks RNase T_1 cleavage, when the 5 S RNA is within the 70 S ribosome of *Escherichia coli* [11]. Neither is this guanine is also inaccessible to kethoxal within the *Bacillus subtilis* or *Bacillus licheniformis* [12]. This guanine is also innaccessible to kethoxal within the *E. coli* 50 S ribosomal subunit [13].

We have shown [14] that when the 5 S RNA is released from the ribosome of *E. coli* in moderate concentrations of Tris–HCl by kethoxal, this guanine within the invariant sequence is available for modification. This is in apparent contradiction with [15] which showed that free 5 S RNA reacted with kethoxal in a phosphate buffer, was not available for modification at this position. Therefore, we have studied the availability of guanines in a renatured *E. coli* 5 S RNA in solution to kethoxal in a 75 mM Tris–HCl buffer. We report that the guanine in the invariant sequence is indeed modifiable, confirming that in a buffer containing moderate concentrations of Tris, the favored conformation of the *E. coli* 5 S RNA contains the guanine in the invariant sequence in a highly exposed position. This is to be expected from the accepted secondary structure models of the 5 S RNA [16,17].

2. Methods

5 S [^{32}P]RNA was extracted and purified from *E. coli* MRE 600 70 S ribosomes as in [11]. For renaturation, the 5 S RNA was heated to 60°C in 5 mM Tris–acetate (pH 7.8), 10 mM $Mg(OAc)_2$ and slowly cooled (1°C/min) to room temperature [18]. After renaturation, the 5 S RNA was dialyzed into the reaction buffer: 75 mM Tris–HCl (pH 7.5); 50 mM NH_4Cl, 10 mM $Mg(OAc)_2$. Reaction was under limiting conditions, with 75 μmol/ml kethoxal for 60 min at 37°C. β-ethoxy-α-ketobutylaldehyde (kethoxal) was the kind gift of Dr D. Stringfellow of The Upjohn Company.

After reaction, kethoxal was removed by ethanol precipitation of the 5 S RNA. Oligonucleotides resulting from RNase T_1 digestion were separated by the homochromatography procedure [19] as modified [14]. Kethoxylated fragments were analyzed after deblocking with triethylammonium carbonate (pH 10) by digesting these oligonucleotides to completion with a mixture of RNase T_1 and pancreatic RNase.

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The resulting fragments were separated on MN300 cellulose plates by ionophoresis at pH 3.5 and 1 kV. Assignment of the oligonucleotides was done by reference to its mobility to dye markers and comigration with known marker oligonucleotides.

3. Results

The homochromatography fingerprint pattern of oligonucleotides arising from *E. coli* 5 S [^{32}P]RNA that was reacted with kethoxal and digested with RNase T_1 after renaturation, contains 4 oligonucleotides not found in the fingerprint patterns from unreacted 5 S [^{32}P]RNA (fig.1). These fragments are identical to those found in kethoxal-reacted 5 S RNA that is released from the ribosome [14] and have been labelled 18, 19, 20 and 21, in like manner. Fragments 18 and 20 are the results of kethoxylation events at positions 13 and 41 of the 5 S RNA. Fragment 19 is the result of kethoxylation at position 44 which is within the $^{5'}CCG_{44}AAC^{3'}$ invariant sequence. Fragment 21 is the result of kethoxylation at both position 41 and 44 in the same molecule.

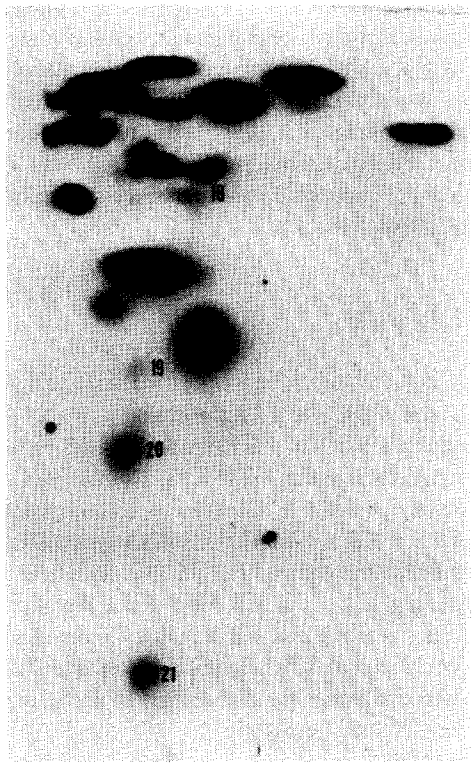

Fig.1. Separation by electrophoresis-homochromatography of oligonucleotides from a ribonuclease T_1 digestion of kethoxal-reacted *E. coli* 5 S RNA. The direction of electrophoresis in the first dimension was from left to right. Homochromatography was from bottom to top.

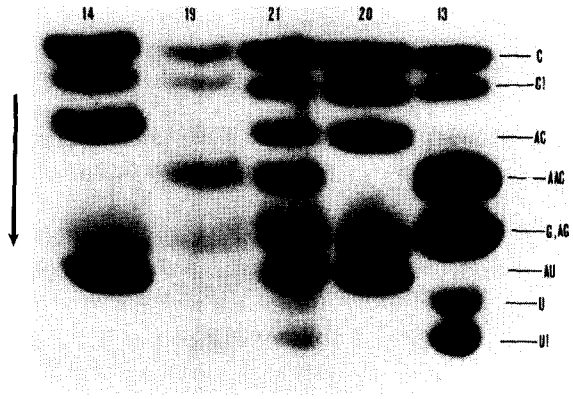

Fig.2. Electrophoretic separation of oligonucleotides derived by RNase T_1 + pancreatic RNase digestion after deblocking possible kethoxylated sites by incubation in triethylammonium carbonate (pH 10) overnight. Fragments 13 and 14 are unique fragments derived from the *E. coli* 5 S RNA sequence containing the marker fragments C, AC, AAC, (G, AG), AU and U. G and AG are not separable in this system. Cyclic nucleotides arise from absence of a 0.1 N HCl treatment prior to separation.

Figure 2 shows the RNAse (T_1+panc.) pattern of fragments 19, 20 and 21, after removal of kethoxal and allows us to unequivocally give a nucleotide assignment to these fragments. Notice that fragment 21 contains both an AC and an AAC within its sequence. This could only arise from kethoxylation at both positions 41 and 44 as all AC sequences within the 5 S RNA of *E. coli* are on the 5′ side of G_{41} and all AAC sequences are on the 3′ side of G_{44}. The patterns for fragments 19 and 21 are consistent with their assignments and both these guanines have been shown to be reactive [11,13–15].

The molar yield of fragment 21 corresponds to the predicted molar yield based on the probability of obtaining the double reaction; i.e., the product of

Table 1
Kethoxylated fragments that appear during a limited reaction

Fragment no.	Assigned nucleotide sequence	Molar yield of kethoxylated guanines
18	$CCG^{K}_{13}UAG$[a]	–
19	$CCG^{K}_{44}AACUCAG$	0.290[b]
20	$ACCCCAUG^{K}_{41}CCG$	0.588[b]
21	$ACCCCAUG^{K}_{41}CCG^{K}_{44}AACUCAG$	0.183

[a] Assignment based on the known migration of the fragment arising from a reaction of G_{13} [14]

[b] The molar yields for kethoxylated guanines that give rise to fragments 19 and 20 are the total yields for the respective guanines reacted and include the molar yield for fragment 21

the molar yields of the single reactions (table 1). This further strengthens the oligonucleotide assignments. Since kethoxylation at either position does not seem to affect the probability of kethoxylation at the other position, neither event greatly changes the conformation of the molecule in this region.

4. Discussion

The prokaryotic 5 S rRNA contains an invariant sequence $^{5'}CCGAAC^{3'}$ which is complementary to the $^{5'}GT\psi CG^{3'}$ arm of the tRNA. Although this sequence has been implicated in the binding of the tRNA to the A site [5,6] and is predicted to be part of a large, conserved, 13 nucleotide long, single stranded loop [16,17], the guanine within this invariant sequence is not accessible to the guanine specific reagent, kethoxal within the 70 S ribosome of both Gram-positive and Gram-negative bacteria [11,12]. Neither is it accessible within the 50 S ribosomal subunit of *E. coli* [13]. We have shown [14], this guanine to be accessible to kethoxal when the 5 S RNA is released from the *E. coli* 70 S ribosome in moderate concentrations of Tris–HCl [14]. We now show that renatured 5 S RNA reacted in the same buffer, also contains this guanine in a highly exposed configuration.

If this invariant sequence is exposed in solution, but not within the ribosome, it must be shielded within the ribosome by RNA–RNA interactions, RNA–protein interactions, or an inramolecular rearrangement of the molecule itself. We feel that an RNA–protein interaction is the most likely, and that there is enough evidence to indicate that protein L5 plays a decisive role. Protein L5 binds to the region from nucleotide 18–57 in *E. coli* [20]. Thus within the ribosome, L5 would shield the invariant sequence $^{5'}C_{42}CGAAC^{3'}$ of the 5 S RNA. It has been proposed that conformational changes during protein synthesis expose this oligonucleotide sequence [11]. We now predict that this conformational change involves an alteration of the interaction between protein L5 and the 5 S RNA exposing the invariant sequence and making it available for possible interactions with the tRNA. This conformational change need not be extensive since the guanine at position 41 is accessible to kethoxal within the 70 S ribosome [11,12]. This position is immediately to the 5′ side of the invariant sequence $^{5'}C_{42}CGAAC^{3'}$.

These results are in apparent contradiction with the report [15] that reaction of the *E. coli* 5 S RNA with kethoxal and glyoxal in a phosphate buffer, resulted in the modification of positions 13 and 41. We confirm the reactivity of these sites but also find reactivity at position 44. This may be due to the differences in the buffers used for the reaction or it may be due to the different methods used for oligonucleotide separation. Two dimensional ionophoresis was used in the separations of [15]. This gives good resolution of smaller oligonucleotides but larger fragments remain near the origin. We used homochromatography for the second dimension, which gives enhanced separation of larger oligonucleotides as can be seen in fig.1.

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