A FRAGMENT OF 23S RNA CONTAINING A NUCLEOTIDE SEQUENCE COMPLEMENTARY TO A REGION OF 5S RNA

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1. Introduction

Investigations of the functional role of ribosomal RNA utilizing the guanine-specific reagent Kethoxal [1] have led us to the problem of determining the sites of reaction within the ribosome. Diagonal paper electrophoresis methods have provided a convenient system for the isolation and sequence determination of oligonucleotides surrounding the sites of Kethoxal attack on the 5S RNA [2], 16S RNA [3], and 23S RNA [4]. Limitations in the ability of paper electrophoresis methods to resolve large oligonucleotides have prompted us to use polyacrylamide gel electrophoresis. Use of this approach has allowed us to resolve a Kethoxal-modified fragment of 23S RNA containing a base sequence complementary to a 12 nucleotide-long region of 5S RNA.

2. Materials and methods

^{32}P-labeled 50S ribosomal subunits were prepared from *E. coli* (strain MRE 600) as previously described [3]. Subunits (100–600 μg, 0.5-4 $\times$ 10^9 cpm) were reacted with Kethoxal for two hr at 37°C in 0.5 ml of a solution containing 3 mg/ml Kethoxal (Nutritional Biochemicals), 10 mM $MgCl_2$, 0.1 M potassium cacodylate, pH 7.0. Ribosomal subunits were precipitated with ethanol and extracted with phenol as previously described [3]. RNA was digested with T1 RNAse (Sankyo) at an enzyme: substrate ratio of 1:20 in a solution containing 0.025 M sodium borate, 0.001 M EDTA, 0.01 M Tris–HCl, pH 7.4, for 30 min at 37°C. The digest was applied to a 15/20 percent composite polyacrylamide slab gel (0.15 $\times$ 10 $\times$ 15 cm) made as previously described [5], and run in 8 M urea, 0.02 M boric acid, 0.001 M EDTA, 0.01 M Tris–HCl, pH 8.3, for 14 hr at 10 V/cm at 6°C. RNA fragments were located by autoradiography, eluted by three successive extractions of the macerated gel slice by vortexing with 0.3 ml portions of 0.2 M NaOAc, pH 5.0, and recovered by precipitation with 2 vol of ethanol. Further purification was achieved by homochromatography on DEAE-cellulose thin-layer plates (Brinkmann) using homomix b [6].

Before complete digestion of the RNA fragment with T1 nuclease, Kethoxal was removed by incubation for 30 minutes at 37°C in 0.1 ml triethylamine carbonate (pH 10). After lyophilization, digestion was carried out with 0.01 ml T1 nuclease (0.1 mg/ml, in 0.001 M EDTA, 0.01 M Tris–HCl, pH 7.4) for 30 min at 37°C and fingerprinted by standard methods [6]. RNA sequence methods were as described by Sanger and his collaborators [6], except for use of U2 and silkworm nucleases, which was described elsewhere [3]. The products of combined digestion with silkworm nuclease and alkaline phosphatase were separated by electrophoresis on cellulose acetate (pH 3.5) followed by electrophoresis on DEAE paper at pH 3.5. Products of RNAse A digestion, with or without prior blocking by 1-cyclohexyl-3-(2-morpholinyl)-4-ethylcarbodiimide metho-*p*-toluene sulfonate (CMCT) were further analyzed by alkaline hydrolysis. Products of U2 nuclease or combined silkworm nuclease-alkaline phosphatase digestion were analyzed further by alkaline hydrolysis as well as by complete digestion with snake venom phosphodiesterase. Nucleotide compositions of some of the silkworm-phosphatase products were established with the help of electrophoretic mobility values.

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3. Results

Guanine residues modified by Kethoxal are resistant to Tl nuclease. Thus, digestion of Kethoxal-modified RNA will give rise to fragments longer than those observed in digests of unmodified RNA. One such fragment can be readily identified by its slower mobility on polyacrylamide gel electrophoresis of Tl digests of Kethoxal-modified 23S RNA (fig.1). Analysis by two-dimensional paper electrophoresis after removal of Kethoxal and complete T1 digestion reveals two oligonucleotides (fig.2). Their sequences were determined from the data shown in tables 1 and 2.

The order of oligonucleotides 1 and 2 was determined by two independent techniques. Prior to secondary cleavage with Tl, the complete fragment was treated with alkaline phosphatase to remove its 3′ terminal phosphate. After Tl digestion, only the guanine residue arising from the internal position bears a 3′-phosphate. Only oligonucleotide 1 is dephosphorylated by this treatment, placing it at the 3′ position. Digestion of the complete fragment with RNAse A yields, in addition to the products listed in table 1, the overlapping oligonucleotide GAC, confirming the result obtained by the phosphatase treatment. The complete sequence of the fragment and the site of attachment of Kethoxal are shown in fig.3.

4. Discussion

From the specificity of Kethoxal for unpaired guanines, it can be concluded that the internal guanine residue in the 23S RNA fragment (fig.3) is in an exposed, single-stranded region within the intact 50S subunit.

A striking feature of the nucleotide sequence of this fragment is its extensive complementarity to 5S RNA. Fig.4 shows the complementarity of residues 72–83 of 5S RNA [7] with a 12-nucleotide sequence within the 23S RNA fragment. Although there is no direct evidence that base pairing between these two sequences occurs in the ribosome, such a model is consistent with a number of observations. 1) The internal guanine of the 23S RNA fragment is not involved in the proposed base-pairing, consistent with its reactivity toward Kethoxal. 2) None of the 6 guanines in residues 72–83 of 5S RNA are accessible

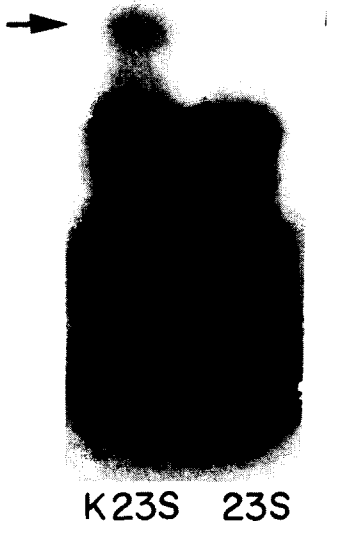

Fig.1. Gel electrophoresis of ribonuclease Tl digests of ^{32}P-labeled 23S RNA from Kethoxal-treated (K23S) and control (23S) 50S ribosomes. The Tl-resistant Kethoxal containing fragment is indicated by the arrow.

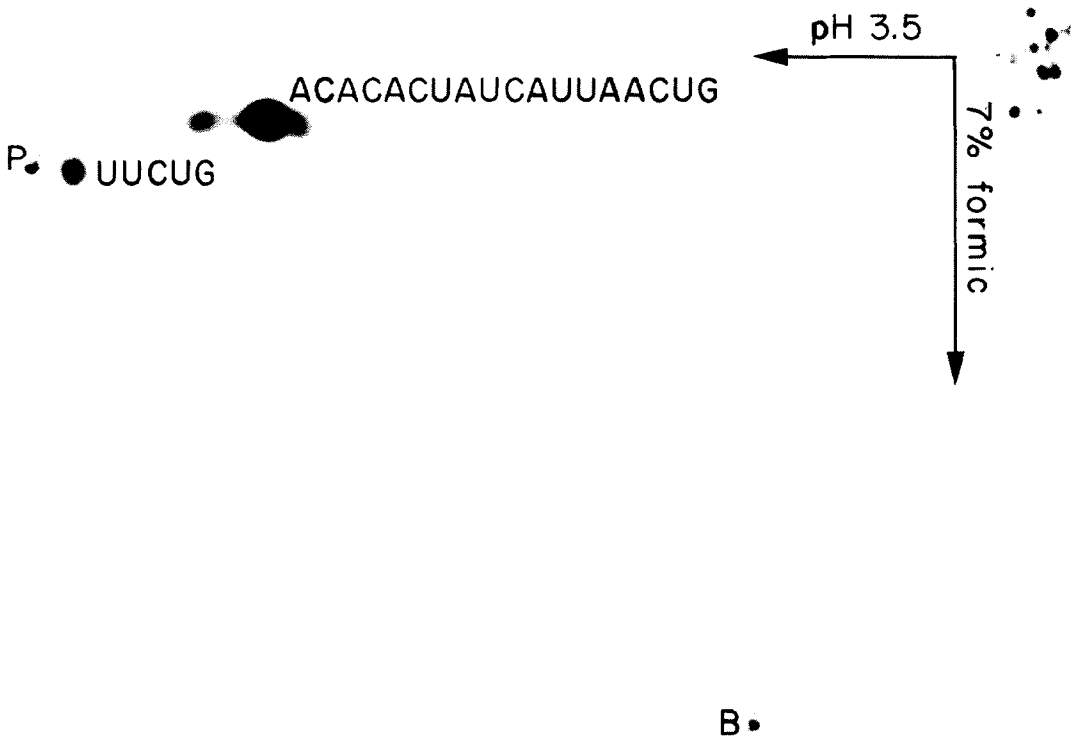

Fig. 2. Two-dimensional paper electrophoresis of a ribonuclease T1 digest of the 23S RNA fragment isolated by gel electrophoresis (fig. 1) after removal of Kethoxal. The positions of the blue (xylene cyanol FF) and pink (acid fuchsin) dye markers are indicated by B and P, respectively.

Table 1
Digestion products of T1 oligonucleotides

Oligo-nucleotide	RNAse A	U2		CMCT	Alkaline hydrolysis	snake venom phosphodiesterase
1	$AAC_{1.0}$	$UUA_{1.1}$		$\dot{U}\dot{G}$		
	$AU_{2.1}$	$CUG_{1.0}$		$(\dot{U}_2A)C$		
	$AC_{2.9}$	CUA	2.0	$(\dot{U}_2A_3)C$		
	$G_{0.8}$	UCA		$\underline{\underline{AC}}$		
	$C_{1.3}$	$CA_{1.8}$				
	$U_{3.1}$	$A_{2.5}$				
2	$G_{1.0}$			$\dot{U}\dot{U}C$	$\underline{U}$	$\underline{U}$
	$C_{1.0}$			$\dot{U}\dot{G}$	C	C
	$U_{3.1}$				G	pGp

Table 2
Sequence derivation of T1 oligonucleotides

Oligonucleotide 1:

A C A
A C A
A C A C A
A C A C U
C A C U
A C U
U A U C A U — Silkworm nuclease
A U C
A U U
A U U A
A U U A A C
A A C U G
A C U G

A C A C A C U A U C A U U A A C U G — CMCT, U2

Oligonucleotide 2:

U U C U G — CMCT

K
UUCUGACACACUAUCAUUAACUG

Fig. 3. The nucleotide sequence of the 23S RNA fragment, showing the site of attachment of Kethoxal (indicated by the letter K).

to Kethoxal in the intact ribosome [2]. 3) Studies on the binding of 5S RNA to 23S RNA, mediated by proteins L18 and L25, have shown the region comprising nucleotides 70–103 of 5S RNA to be important for this interaction [8,9]. 4) Sequence analysis of 5S RNA from a variety of procaryotic organisms has shown that the region around residues 72–83 is highly conserved ([7,10–13]; this observation was pointed out to us by Dr C. R. Woese).

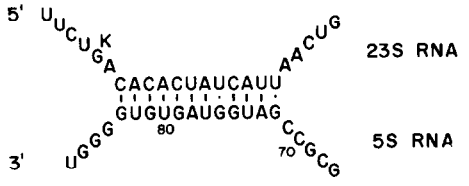

Fig. 4. Complementarity between the 23S RNA fragment and residues 72–83 of 5S RNA.

Thus, in addition to the 5S RNA-protein interactions and 23S RNA-protein interactions hitherto reported [14,15], the model presented here predicts a specific interaction between 5S RNA and 23S RNA.

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