DEGRADATION OF A SPECIFIC 5 S RNA – 23 S RNA – PROTEIN COMPLEX BY PANCREATIC RIBONUCLEASE

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Received 23 May 1972

1. Introduction

The formation of a specific complex containing 23 S RNA, 5 S RNA and only a few of the *E. coli* 50 S subunit proteins has been previously described [1, 2]. The high degree of stability of this complex suggested that its degradation by a ribonuclease might lead to the identification of the portion of the 5 S RNA molecule which directly interacts with the other components.

The present communication describes the isolation and nucleotide sequence of 5 S RNA fragments obtained after pancreatic RNAase digestion of the 23 S RNA – 5 S RNA – protein complex.

2. Experimental

The preparation of ^{32}P-5 S RNA, of 23 S RNA and of fraction I proteins from the 2 M LiCl-split of 50 S ribosomal subunits, as well as the conditions for the formation of the ^{32}P-5 S RNA–23 S RNA–protein complex have been described previously [1]. ^{32}P-5 S RNA (1.5 μg), 23 S RNA (40 μg) and 1 molar equivalent of fraction I proteins were mixed in a total volume of 0.1 ml of reconstitution buffer [3] (10 mM Tris-HCl, pH 7.6; 300 mM KCl, 6 mM 2-mercaptoethanol, 20 mM $MgCl_2$), incubated for 15 min at 30° and quickly chilled to 0°. All manipulations on the complex up to the polyacrylamide gel electrophoresis of the degradation products, were performed in the reconstitution buffer in order to maintain the proper ionic environment for specific protein – RNA interactions.

The overall rate of pancreatic RNAase degradation was followed by adding appropriate amounts of the enzyme to 0.1 ml of complex solution, incubating at 25° for 30 min and precipitating with 5 ml of cold 5% trichloracetic acid (TCA). The samples were left to precipitate for 3 min and filtered on Millipore filters. Two control experiments were performed. The first omitted the fraction I proteins from the initial incubation mixture, and the second omitted proteins prior to digestion and then added the proper amount of fraction I proteins prior to the addition of the TCA solution. The dried filters were then counted in a Packard Tri-carb spectrometer.

In the experiments in which RNAase degradation was followed by polyacrylamide gel electrophoresis, 1 μg of pancreatic RNAase was used per 0.1 ml of complex solution. After incubation at 25°, the samples were chilled and applied to 10% cyanogum (BDH) gels prepared in 40 mM Tris-HCl, pH 7.4 in a model EC 470 (E.C. Apparatus Co., Philadelphia, Pa, USA). Electrophoresis was performed for 4 hr at 150 V and 100 mA. The temperature of the cooling circulating bath was 2°. After autoradiography, the ^{32}P-containing bands were extracted in the presence of phenol, precipitated with ethanol and fingerprinted according to conventional techniques [4].

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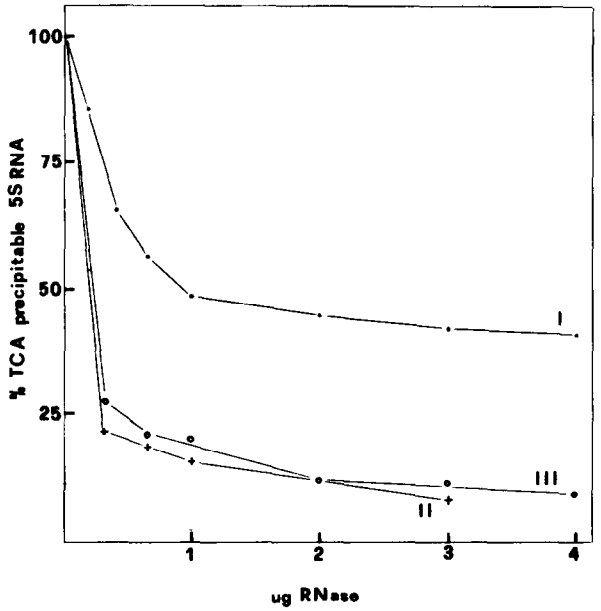

Fig. 1. Degradation of ^{32}P-5 S RNA in a specific 23 S RNA–protein-containing complex as a function of pancreatic RNAase concentration.
I: Complex degradation.
II: Control experiment in the absence of fraction I proteins. Fraction I proteins were added after degradation and before precipitation.
III: Control experiment in the absence of fraction I proteins.

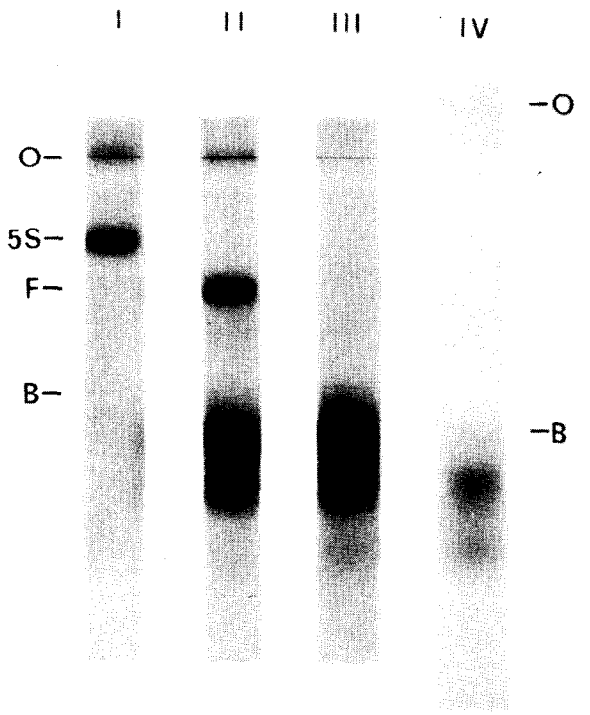

Fig. 2. Polyacrylamide gel electrophoresis fractionation of pancreatic RNAase digest of ^{32}P-5 S RNA-containing complexes. I: 5 S RNA non-degraded. II: Digest of complex containing 5 S RNA, 23 S RNA and fraction I proteins. III: Digest of 5 S RNA and 23 S RNA in the absence of fraction I proteins. IV: Digest of 5 S RNA, 23 S RNA and fraction I proteins prepared from the 30 S ribosomal subunit.
O is the origin; B is the position of the bromphenol-blue marker; F is the position of the protein specific fragment.

3. Results and conclusion

The effect of the fraction I proteins from the 50 S ribosomal subunit on the degradation of complexed 5 S RNA by pancreatic RNAase is apparent from the results of the experiment in which a ^{32}P-5 S RNA-containing complex was exposed to increasing RNAase concentrations and the digest precipitated with trichloracetic acid (TCA). As illustrated in fig. 1, after digestion of the complex with up to 4 μg of enzyme for 30 min at 25°, 40% of the ^{32}P-5 S RNA radioactivity remained TCA-precipitable. When the 50 S subunit proteins were omitted from the incubation mixture, less than 8% was still precipitable under the same conditions. This preliminary observation helped in the determination of suitable conditions for further experiments.

Polyacrylamide gel electrophoresis was performed immediately after enzymatic degradation of various ^{32}P-5 S RNA-containing incubation mixtures. In all cases where the 50 S subunit fraction I proteins were present, autoradiographic analysis of the gels revealed one or two well defined bands of radioactivity which migrated between 5 S RNA and normal degradation products. These were not observed when the 50 S subunit proteins were omitted or replaced by a similar fraction of 30 S subunit proteins (fig. 2).

Immunological analysis of the radioactive bands, performed by R. Garrett and G. Stöffler (manuscript in preparation), has demonstrated the presence of those proteins previously identified as being specifc for the 5 S RNA binding in 50 S subunits [2]. The fragments of ^{32}P-5 S RNA which are responsible for the bands observed after pancreatic RNAase digestion

Table 1

Molar yields of oligonucleotides in a T_1 RNAase complete digest of the 5 S RNA fragments.

Oligonucleotide	Molar yields		
	Theoretical in 5 S RNA	Theoretical in sequence 69–110	Found in fragments
1 G	10.70	7.00	7.30
2 CG	5.00	1.00	0.91
3 AG	2.00	2.00	2.73
4 UG	4.00	2.00	2.08
5 CCG	3.50	1.00	0.83
6 AAG	1.00	0	– *
7 CCAG	0.67	0	0.10
8 AAACG	1.00	0	–
9 CCUG	0.83	0	0.12
10 UAG	4.00	2.00	1.34
11 AUG	1.00	1.00	0.71
12 AACUG	1.00	0	0.09
13 AACUCAG	1.00	0	–
14 ACCCCAUG	1.00	0	–
15 UCCCACCUG	1.00	0	–
16 UCUCCCCAUG	0.83	0.83	0.02
18 pUG	1.00	0	0.04
19 CAU_{OH}	1.00	0	–
20 CAG	0.50	0	–
31 UCUG	0.17	0	0.06
32 UCUCCUCAUG	0.17	0.17	–
34 CCAUG	0.33	0	–
– U	0	0	0.97
– C	0	0	0.42
– AAC	0	1.00	0.78
– CCCCAUG	0	0	0.73
– UCCCCAUG	0	0	0.25

* – indicates that the corresponding oligonucleotide was not detected in the digest.

Table 2

Molar yields of oligonucleotides in a pancreatic RNAase complete digest of the 5 S RNA fragments.

Oligonucleotide	Molar yields		
	Theoretical in 5 S RNA	Theoretical in sequence 69–110	Found in fragments
1 C	17.33	5.83	5.20
2 U	5.17	1.17	0.74
3 AC	1.00	0	– *
4 GC	7.16	2.00	2.74
5 AU	2.33	1.00	1.37
6 GAC	1.00	0	–
7 AGC	2.00	0	–
8 GAAC	1.00	0	–
9 GAAAC	1.00	0	–
10 GGC	2.00	0	0.29
11 AGGC	0.67	0	0.15
12 GU	2.67	1.00	1.24
13 GAU	1.00	1.00	0.94
14 AGU	1.50	1.00	1.18
15 GGU	2.00	1.00	0.99
16 AGGGAAC	1.00	1.00	0.29
17 AGAAGU	1.00	0	–
18 GAGAGU	1.00	1.00	0.94
19 GGGGU	1.00	1.00	0.26
20 pU	1.00	0	0.20
21 AU_{OH}	1.00	0	0.11

* – indicates that the oligonucleotide was not detected in the digest.

of the complex, remain bound to the specific proteins during gel electrophoresis. They must therefore contain part or all of the specific nucleotide sequences which directly interact with these proteins during the assembly of the 50 S subunit.

The radioactive material in the slowest moving band was recovered from the gels and fingerprinted after complete digestion with either T_1 or pancreatic RNAase. The results are given in tables 1 and 2. All the T_1 oligonucleotides, which can originate from sequence 69–110 of 5 S RNA were present in the T_1 RNAase digest. With a few exceptions, the yields are as expected. Oligonucleotide 16 (UCUCCCCAUG) was found in extremely low yield, but three degradation products, U, UCCCCAUG and CCCCAUG, which would not be produced if the 69–110 sequence were intact, were found instead. Similarly, the yield of oligonucleotide 10 (UAG) is low, but, correlatively, that of oligonucleotide 3 (AG) is higher than would be expected from the intact sequence.

In addition, several oligonucleotides, which cannot be derived from sequence 69–110, were found with average molarities of 0.1. Upon examination of the primary structure of 5 S RNA, it can be seen that they all derive from either sequence 111–120 or sequence 1–11 (fig. 3). Particularly significant in this respect is the presence, in the T_1 RNAase fragment digest, of oligonucleotide 18 (pUG), which corresponds to the 5′-end of the molecule.

These data clearly show that the 5 S RNA fragments which remain bound to proteins after extensive

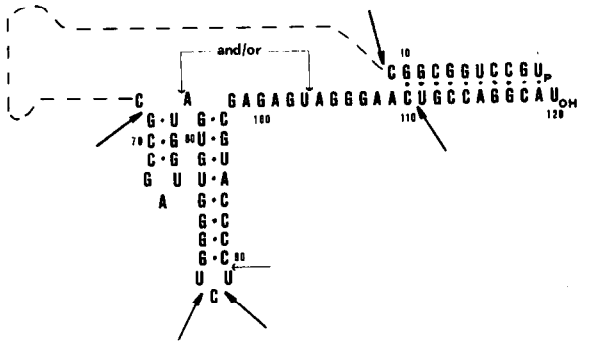

Fig. 3. A model of the 5 S RNA nucleotide sequences from which the protein specific identified fragments are derived. The large arrows indicate the major nucleotide positions subject to nuclease attack. The smaller arrows indicate positions less frequently digested. See text for further explanation.

pancreatic RNAase digestion of the specific 5 S RNA–50 S protein complex are derived from either sequence 69–120 or sequence 1–11. Fingerprint analysis of the fragments after complete pancreatic RNAase digestion entirely supports this conclusion (table 2). No oligonucleotide which is specific for sequence 12–68 has ever been found in complete digests of the fragments.

The following pattern of degradation of protein-bound 5 S RNA by pancreatic RNAase is suggested by these results (see fig. 3, heavy arrows). Two chain cleavages, located at C_{11} and C_{68}, are responsible for the severing of the protein-interacting sequences from the rest of the molecule. Two other very accessible internucleotide bonds belong to residues U_{87} and C_{88}. Hydrolysis at U_{87} and C_{88} occurs in about 98% of the molecules. These splits are responsible for the low yield of oligonucleotide 16 and the presence of U and UCCCCAUG in the T_1 RNAase digest of the fragments. The bond located at C_{110} is also highly accessible, since the low yields of the oligonucleotides belonging to sequence 111–120 indicate that it is broken in 80–90% of the molecules. Only 10–20% of the fragments contain this sequence and sequence 1–11. The bond at U_{89}, the hydrolysis of which is responsible for the presence of CCCCAUG in the T_1 RNAase digest, appears to be split to an extent of 70–75%. The respective yields of oligonucleotides 3 (AG) and 10 (UAG) (table 1) clearly indicate that bonds U_{77} and/or U_{103} also are accessible to some extent. However, a definite choice between these two positions cannot be made on the basis of the present data.

It is clear that the observed electrophoretic mobility of the uninterrupted nucleotide sequences, which can be deduced from these results, must be dependent on their interaction with proteins. As a matter of fact, when the radioactive products recovered after electrophoresis are run on a second polyacrylamide gel in the presence of the detergent Sarkosyl, they separate into 2 or 3 discrete bands with higher mobilities. The secondary structure of the original 5 S RNA molecule is also probably important in determining the behaviour of the fragments. In particular it is clear that the presence of the oligonucleotides, which belong to sequence 1–11, is due to the pairing of this sequence with sequence 110–120. A schematic interpretation of the results, as well as the pairing scheme that they suggest in the relevant portions of the 5 S RNA molecule, are presented in fig. 3.

The present results show that the nucleotide residues, which are the most important for the recognition of 5 S RNA by specific ribosomal proteins, belong to sequence 69–110. Several nucleotide residues in this sequence, such as U_{87}, C_{88} and U_{89}, are nevertheless very accessible to pancreatic RNAase in the complex. It is unlikely that they directly participate in protein–5 S RNA interactions. Such considerations can help to further delineate the essential nucleotide stretches. Although about 10% of the analyzed fragments also contained sequences 110–120 and 1–11, it is unlikely that these sequences have a strong interaction with the proteins. Their occurrence in the fragments may rather be explained by partial protection in the complex of the bond joining C_{110} and U_{111} and to the pairing of both ends of the 5 S RNA molecule into a stem-like double helix.

Acknowledgements

This work was supported in part by grants from the C.E.A., the D.G.R.S.T. and the Fondation pour la Recherche Médicale Française. P.N. Gray is a post doctoral fellow of the Damon Runyon Fund for Cancer Research, DRF-628; G. Bellemare is a post doctoral fellow of M.R.C. of Canada.

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