NUCLEOTIDE SEQUENCES OF PANCREATIC RIBONUCLEASE DIGESTION PRODUCTS FROM THE 16S RIBOSOMAL RNA OF *E. COLI*

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1. Introduction

We have recently reported the nucleotide sequences of the products arising from T_1 ribonuclease digestion of the 16 S ribosomal RNA from *E. coli* [1]. This catalogue of sequences is proving very useful in our current efforts to determine the primary structures of some larger sections of this molecule, obtained by partial enzymic hydrolysis. We anticipated that a similar study of the products released by pancreatic ribonuclease hydrolysis of the 16 S RNA would also be helpful in this way. In this paper we list the sequences of many of the larger products arising from the action of this enzyme upon the 16 S RNA.

2. Methods

^{32}P-Labelled 16 S RNA was prepared from *E. coli* (MRE 600) as previously described [2].

Pancreatic ribonuclease digestion of the RNA was carried out using the following conditions: an enzyme-substrate ratio of 1:25, with an RNA concentration of 5–10 mg/ml; incubation for 45 at 37°, in the presence of 0.01 M tris (pH 7.4) and 1 mM EDTA. These conditions were not entirely satisfactory, since some 'over-splitting' of larger oligonucleotides occurred (see Results and discussion section), precluding any valid quantitative studies of the relative amounts of different products released. However, if milder conditions were employed, large amounts of oligonucleotides containing 3'-terminal cyclic phosphates arose. This gave rise to double spots of products during subsequent fractionation.

The fingerprinting and sequence analysis of the enzymic digestion products was carried out according to Sanger et al. [3]. Sequences of some products could be determined by hydrolysis with alkali and T_1 ribonuclease. In the sequence analysis of many products it was also necessary to use the technique of partial digestion with spleen phosphodiesterase. A solution of purified spleen phosphodiesterase was kindly supplied to us by Dr. G.Bernardi, having an activity of 20 U/ml. We found that a satisfactory range of digestion products could usually be obtained by digesting an oligonucleotide for 30 or 60 min at 37°C, with 0.01 ml of the enzyme (at a concentration of 0.004 U/ml) in the presence of 0.01 M ammonium acetate (pH 6.0) and 2 mM EDTA. The products were fractionated by electrophoresis on DEAE-paper in 7% formic acid. The nucleotide sequence could be elucidated from the compositions and M values [see 3] of these digestion products.

3. Results and discussion

A fingerprint of pancreatic ribonuclease digest of the 16 S RNA is shown in fig. 1-a. The sequences of certain prominent oligonucleotides are marked directly upon the corresponding plan (fig. 1-b). The remainder are listed in table 1. An extended electrophoresis was carried out in the second dimension of the fingerprint

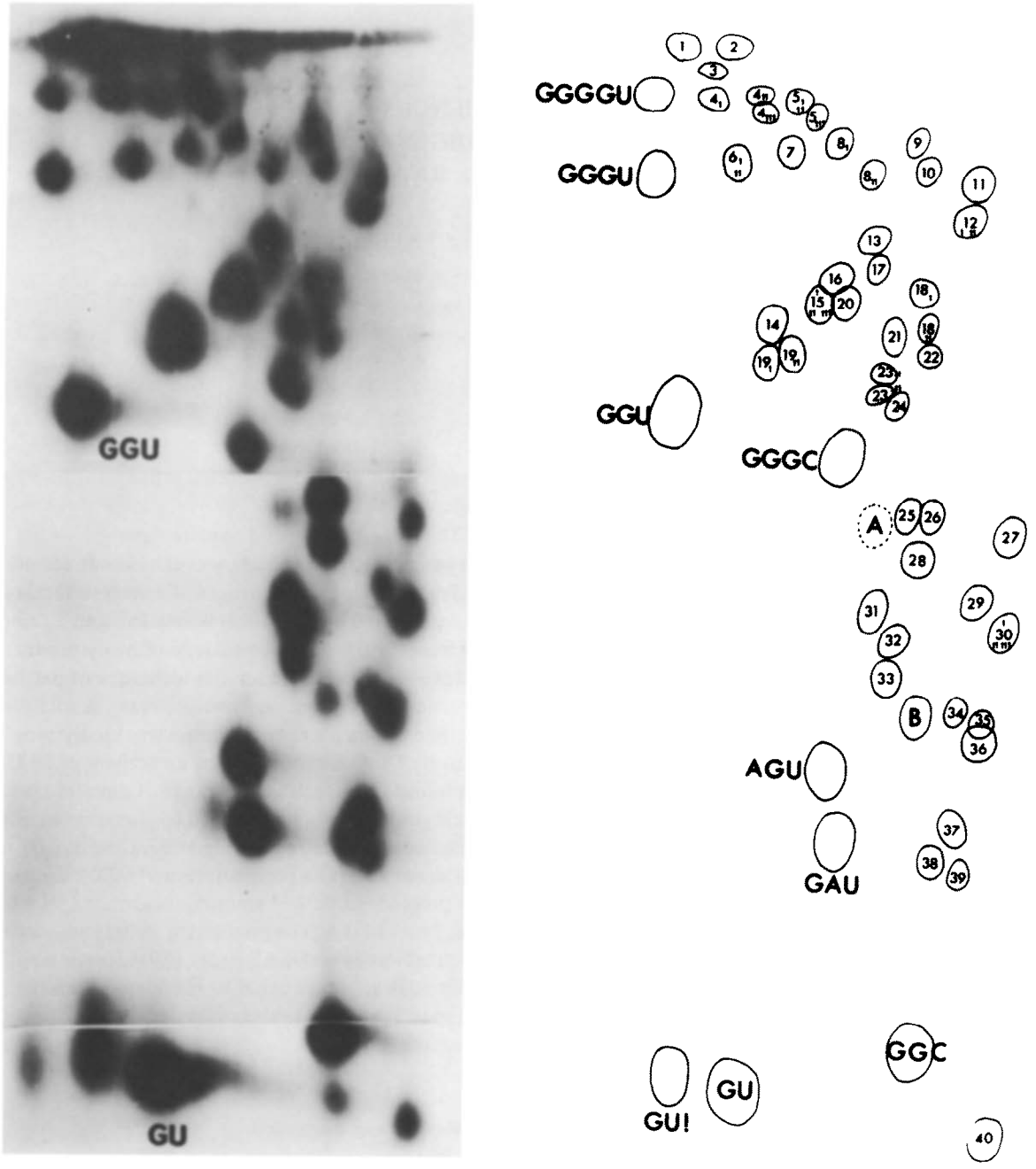

Fig. 1. (a) A fingerprint of a pancreatic ribonuclease digest of the 16 S ribosomal RNA. Fractionation was carried out by electrophoresis on a strip of cellulose acetate at pH 3.5 (first dimension- right to left) and on DEAE-paper in 7% formic acid (top to bottom). (b) The corresponding plan.

Table 1
The nucleotide sequences of the products released by digestion of the 16 S RNA with pancreatic ribonuclease.

	Sequence	Spot	Sequence
1	$[(A\text{-}G)_2G_6]$Up	19_i	G-A-G-Up
2	$(A\text{-}G,A\text{-}A\text{-}G,G_5)$Up	19_{ii}	G-G-A-Up
3	$(A\text{-}G,G_4)$Up	20	A-A-G-G-Up
4_i	G-G-A-G-G-Up	21	G-G-G-A-A-A-Cp
4_{ii}	G-G-G-G-A-A-Up	22	$(A\text{-}A\text{-}G,G_2)$Cp
4_{iii}	G-G-G-G-G-Cp	23_i	G-G-A-G-Cp
5_i	$(A\text{-}G,G_3)$A-A-Up	23_{ii}	G-A-G-G-Cp
5_{ii}	$[(A\text{-}G)_2G_2]$A-Up	24	A-G-G-G-Cp
5_{iii}	$(A\text{-}G,G_4)$Cp	25	A-G-A-A-Up
6_i	G-G-A-G-Up	26	A-A-A-G-Up
6_{ii}	G-G-G-A-Up	27	G-A-A-G-A-A-Cp
7	G-G-A-A-G-Up	28	G-A-A-A-Up
8_i	G-G-A-A-A-G-Up	29	G-G-A-A-A-Cp
8_{ii}	$(A\text{-}G,G_3)$A-A-Cp	30_i	(A-A-G,A-G)Cp
9	G-G-A-A-G-A-A-G-Cp	30_{ii}	(A-A-A-G,G)Cp
10	A-A-A-A-G-A-A-G-Up	30_{iii}	G-A-A-G-A-Cp
11	(A-A-A-G,A-A-G,A-G)Cp	31	A-G-A-Up
12_i	(A_5G_3)Cp	32	A-A-G-Up
12_{ii}	(A_5G_3)Cp	33	G-A-A-Up
13	A-G-A-G-A-Up	34	G-G-A-A-Cp
14	A-G-G-Up	35	(G_2A_2)Cp
15_i	G-G-A-A-Up	36	(G_2A_2)Cp
15_{ii}	G-A-G-A-Up	37	A-G-G-Cp
15_{iii}	A-G-G-A-Up	38	G-G-A-Cp
16	G-A-A-G-Up	39	G-A-G-Cp
17	G-A-G-A-A-Up	40	A-A-A-Up
18_i	G-G-G-A-A-A-Cp	A	pA-A-A-Up
18_{ii}	$[(A\text{-}G)_2G]$Cp	B	G-A(A,mU)Cp
41_i	A-A-A-G-Cp	47	G-A-Cp
41_{ii}	A-A-G-A-Cp	48	A-A-A-A-Cp
42	A-G-A-Cp	49	A-A-A-Cp
43	A-A-Up	50	A-Up
44_i	G-A-A-Cp	51_i	G-Cp
44_{ii}	G-m^6_2A-m^6_2A-Cp	51_{ii}	G-m^4Cm-Cp
45	A-A-G-Cp	52	A-A-Cp
46	A-G-Cp	53	m^7G-Cp
		54	A-Cp

1) The m^2Gp (3 moles) and m^5Cp (2 moles) residues which are present within the 16 S RNA [1, 2] have not been separately identified, and must be listed here as the corresponding non-modified nucleotides.

2) The sequence A-A-A-A-Gp is present in T_1 ribonuclease digests of the 16 S RNA [1], arising from the sequence G-A-A-A-A-Gp. The latter sequence has not been found in the pancreatic RNAase digest. We are unclear as to why this is. Our unpublished results on partial enzymic hydrolysis products confirm that the sequence A-A-A-A-G-A-A-G-Up arises from C-A-A-A-A-G-A-A-G-Up as a legitimate pancreatic RNAase product. The corresponding sequence C-A-A-A-A-Gp is encountered in T_1 ribonuclease digests [1].

in order to obtain an adequate fractionation of most of the larger oligonucleotides. Therefore, 17 of the smaller, most rapidly migrating oligonucleotides, together with Up and Cp, are absent from this fingerprint but are listed separately in table 1. We have determined the complete nucleotide sequences of 65 of the 81 various products. Two principal difficulties were encountered in determining the remaining nucleotide sequences. The fraction of the larger oligonucleotides achieved with this fingerprinting system was less effective for the pancreatic ribonuclease products than for the T_1 ribonuclease products [1], because of the narrower range of nucleotide compositions of the former. In a number of cases, isomeric oligonucleotides were not separated, and could not be subjected to satisfactory sequence analysis. Furthermore, in a few instances, substantial amounts of internal splitting of large oligonucleotides occurred during partial digestion with spleen phosphodiesterase, preventing useful sequence analysis.

All the oligonucleotides shown in fig. 1 contained single pyrimidine residues. However, a certain amount of Gp and A-Gp were also released under the conditions of digestion employed, indicating that some secondary splitting adjacent to purine nucleotides was also occurring. We carried out quantitative studies of the relative amounts of the products arising in enzymic digests. If some of the larger sequences were considered as present only once within the 16 S RNA, many of the small common oligonucleotides were present in amounts greater than suggested from studies of T_1 ribonuclease digests, which appear to be rather accurate quantitatively from other criteria [1]. We therefore believe that, because of secondary splitting, our quantitative data obtained with these digests are not valid, and they are not presented here. For the same reason, we cannot rule out that certain spots present may have arisen from other larger oligonucleotides by secondary splitting. However, we think it unlikely that any of the large oligonucleotides would be missing entirely. In many cases, any possible relationship of this type between the different spots can be excluded by consideration of the sequences.

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