THE 5'-TERMINI OF THE OLIGONUCLEOTIDES FROM RIBONUCLEASE T_1 DIGESTED E. COLI RIBOSOMES*

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SUMMARY

Oligonucleotides remaining in the 70s Escherichia coli ribosomal particles after varying degrees of digestion with ribonuclease T_1 were phosphorylated with polynucleotide kinase in the presence of γ-labeled ^{32}P-ATP. The resulting radioactively labeled RNA molecules were further digested with pancreatic ribonuclease and analyzed by a two-dimensional finger-printing technique. The numbers of labeled oligonucleotides were proportional to the duration of T_1 digestion; most of these oligonucleotides yielded *pAp and/or *pCp as their 5'- end groups upon alkaline hydrolysis.

INTRODUCTION

Controlled enzymatic digestion of ribosomes has been used as an approach in studying the overall conformation of these particles. Such studies on ribosomes of Escherichia coli have indicated that certain portions of the RNA chains are accessible to these enzymes and thus may be located on the surface of the particle (1-4). A defined number of oligonucleotides are produced in most cases (5-9). It might be informative to characterize these oligonucleotide fragments. Recently, Santer and Santer (3) reported on the isolation and partial characterization of three RNA fragments from RNase T_1 digested 30s E. coli ribosomes. We also reported that the 3'-terminal ends of the original rRNA chains are among these oligonucleotide fragments which lie within the protected regions of the 70s ribosomal particles (1).

In this paper we report on the characterization of the 5'-termini of the oligonucleotides isolated from E. coli 70s ribosomes which have been digested for varying extents with RNase T_1. Our data indicate that the majority of these newly generated 5'-end groups consist of *pAp and/or *pCp.

EXPERIMENTAL PROCEDURE

The isolation of E. coli 70s ribosome, digestion of ribosome with a water-insoluble derivative of RNase T_1 and the isolation of RNA were carried out as previously described (1). Polynucleotide kinase was prepared from cultures of T_4-infected E. coli as described by Richardson (12) and the RNA was phosphorylated with γ-^{32}P-ATP under conditions described by Hanggi et al. (13). Incubation was carried out at 37° for 30 min. The reaction mixture was then passed through a small column of phosphocellulose (1 x 10 cm) prewashed with 0.01 M sodium phosphate buffer (pH 7.5). The nucleotide material was eluted from the column and lyophilized.

The lyophilized nucleotide material was dissolved in 0.1 ml distilled water. Pancreatic ribonuclease was added in an enzyme/RNA ratio of 1 to 100. The pH of the reaction mixture was maintained at 7.2 for 18 hr at 38°. The enzymatic digest was then subjected to electrophoresis on Whatman 3MM paper in 1% formic acid, pH 2.7, followed by chromatography in t-butanol:0.02 M NH_4-formate, pH 3.8 (1:1 by volume) (14). Radioactive oligonucleotides, detected by autoradiography, were then quantitatively determined by scintillation counting after elution with 0.05 M NH_4OH.

To characterize the nature of the 5'-termini, ^{32}P-labeled oligonucleotides were hydrolyzed in 0.3 N KOH for 18 hr at 37° and subjected to electrophoresis on Whatman 3MM paper in 0.05 M ammonium formate at pH 3.5 for 4 hr at 30 v/cm with standard nucleoside diphosphates.

RESULTS AND DISCUSSION

We previously showed that one could digest E. coli 70s ribosomal particles with RNase T_1 and obtain particles which retained a portion of original biological activities. In order to characterize the 5'-termini of these ribosomal RNA fragments produced as a result of such enzymatic treatment, these fragments were used as substrates of the polynucleotide kinase. RNA preparations from ribosomes which retained 85% (Sample I) and 70% (Sample II) of their biological activities after RNase treatment were

phosphorylated to the extent of 2.7 x 10^5 and 4.3 x 10^5 dpm/ODU at 260 mμ, respectively. Thus, the numbers of phosphorylatable termini in the RNA were inversely proportional to their biological activities remaining after the nuclease treatment. Further incubation did not result in any significant additional phosphorylation. A comparison of the electrophoretic patterns on polyacrylamide gel (2.5%) of the original RNA preparation and the kinase reaction product showed no detectable difference indicating that no extensive endonucleolytic degradation occurred during the kinase incubation. Hence, the only available sites for phosphorylation by the polynucleotide kinase were those 5'-termini of the RNA fragments generated by the degradation of ribosomal particles by RNase T_1.

To characterize the 5'-phosphorylated RNA, they were digested with pancreatic RNase to completion and fractionated by a two-dimensional mapping technique. Figure 1 shows tracings of the autoradiograms of the complete pancreatic RNase digestion of RNA samples I and II. The distribution of radioactivity among these oligonucleotides is presented in Table I. The numbers of phosphorylated oligonucleotides, an index of the number of accessible points in the RNA chain, are directly proportional to the duration of ribonuclease treatment of the ribosomal particles. However, in both samples there were only a limited number of oligonucleotides that could be phosphorylated. This finding confirms our previous observation (1) and is in agreement with reports from other laboratories (3,4,15) that there are relatively few susceptible sites in the ribosomal RNA chain when they are complexed with ribosomal proteins. It is interesting to note that in both samples the majority of the radioactivity resides in oligonucleotides which are rich in adenylic acid content as judged by their mapping position as compared to published data (16,17).

To identify the 5'-terminus of the phosphorylated oligonucleotides, the radioactive spots on the chromatograms were cut out, soaked in dilute NH_4OH to recover the radioactive oligonucleotides and

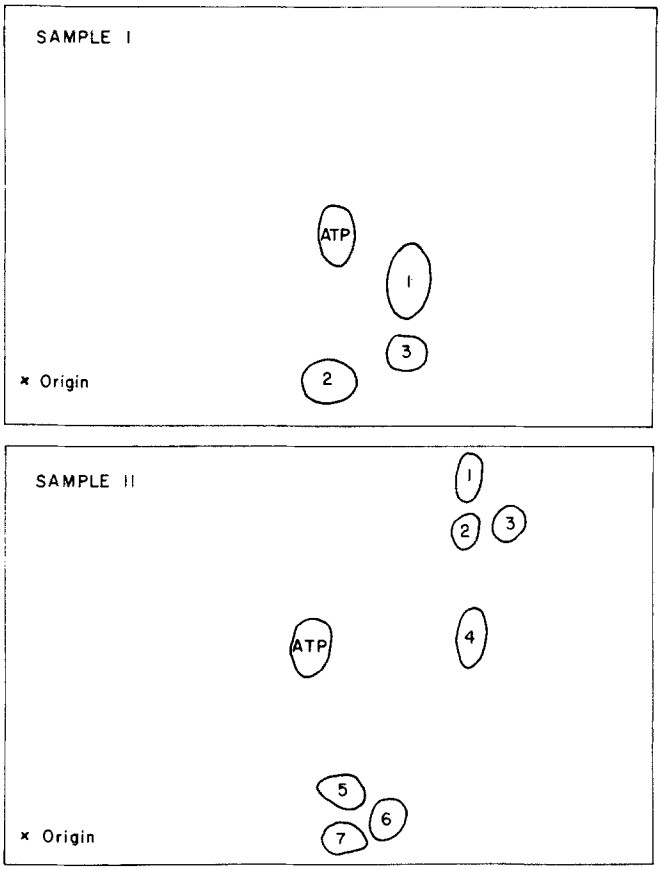

Figure 1. Diagram of a two-dimensional fractionation of a pancreatic ribonuclease digest of a kinase phosphorylated RNA product. First dimension, electrophoresis (bottom to top) in 1% formic acid, pH 2.7, for 17 hours and 6 volts per cm; second dimension, paper chromatography (left to right), descending, with t-butyl alcohol-0.02 M ammonium formate, pH 3.8 (1:1, by volume). Running time was 22 hours at room temperature. (a) RNA sample I from ribosomes with 85% biological activities remaining (1). (b) RNA sample II from ribosomes with 70% biological activities remaining.

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hydrolyzed in KOH. The resulting components were fractionated by electrophoresis at pH 3.5. The distribution of the radioactivity between the various nucleoside diphosphates in each of the radioactive spots is summarized in Table I; however, because cytidine diphosphate and adenosine

TABLE I

Distribution of ^{32}P-Radioactivity

In Pancreatic RNase Digest of RNA Fragments (%)		In 5'-Termini of RNA Fragments (%)		
		$^{*}pAp + {}^{*}pCp$	$^{*}pGp$	$^{*}pUp$
Sample I				
Spot #				
1	35.5	74	4	22
2	29.3	2	-	98
3	35.2	73	4	23
Sample II				
Spot #				
1	3.2	--	-	100
2	0.6	--	-	100
3	3.0	28	-	72
4	6.8	65	7	28
5	26.6	86	5	9
6	38.3	60	-	40
7	21.5	65	3	32

diphosphate move together even at this pH, no further attempts were made to determine the distribution of radioactivity between the two. Our results showed that a few oligonucleotides (viz. Spot #2 in Sample I and spots #1 and #2 in Sample II) yielded $^{*}pUp$ as their sole 5'-terminus. On the other hand, $^{*}pAp$ and/or $^{*}pCp$ were the predominant end-groups in other oligonucleotides from both RNA samples, although there appeared to be some heterogeneity indicated by the presence of other nucleoside diphosphates. We also observed the presence of $^{*}pGp$ though in a very low percentage. We do not think its presence is entirely due to endonuclease contamination of

the kinase preparations, judging from the electrophoretic pattern on polyacrylamide gel of the kinase reaction product. It is possible that these termini were the result of a small degree of non-specific nuclease degradation of the original ribosomal RNA chains during isolation of the ribosomal particles.

In conclusion, our results indicate that relatively few RNA fragments were produced even after considerable treatment of the 70s ribosomal particles in the presence of 10^{-2} M Mg^{++} with RNase T_1. Such findings support the idea that only a limited number of sites in the rRNA chains is accessible to nuclease action (3,15,18). In addition, our results indicate that the majority of these newly generated 5'-end groups are *pAp and/or *pCp.

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