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**ISOLATION OF LARGE OLIGONUCLEOTIDE FRAGMENTS FROM THE ALANINE RNA**

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The discovery that very brief treatment of the alanine RNA with RNase T1 cleaves the RNA specifically into two large fragments suggested that this enzyme might give additional specific cleavages if digestion conditions were a little more vigorous. The isolation of several large oligonucleotides resulting from such specific cleavages is the subject of this communication.

Yeast alanine RNA was digested with 225 units of RNase T1 (Sankyo, Ltd., Tokyo) per mg of RNA in 0.2 N pH 7.5 Tris buffer for 1 hr at 0°C; and, after removal of the RNase T1 by phenol extraction, the digest was chromatographed on DEAE-cellulose in 7 M urea. The chromatographic pattern is shown in Figure 1. For comparison, a pattern obtained after complete digestion of the alanine RNA with RNase T1 and chromatography under identical conditions is shown in Figure 2.

Comparison of Figures 1 and 2 indicates that many small fragments present in Figure 2 are absent in the limited digest shown in Figure 1. In particular, peaks 1, 2, 5, 9, 10, 12, 13, and 15 are missing or greatly reduced in Figure 1. A number of large fragments, present in peaks 16–22, are found instead. Analyses of four of these large fragments are summarized below.

Complete digestion of peak 16 with RNase T1, followed by chromatography, gave peaks 9 and 12, as is shown in Figure 3. Peak 9 is UpCpCpApCpC, the amino

![Figure 1](image-url)
acid-acceptor end of the alanine RNA molecule. (The 3'-terminal pA is missing from most of the transfer RNA's isolated from commercial baker's yeast.) Peak 12 is ApCpUpCpGp. Since peak 9 is the end of the RNA molecule, ApCpUpCpGp must be attached to this; and the sequence of the amino acid-acceptor end of the alanine RNA is therefore -ApCpUpCpGpUpCpApCpA.

Analyses of peak 18 suggested that the peak was heterogeneous. After further study, it was found that peak 18 contained three large oligonucleotides which could be separated by rechromatography at 55°C (Fig. 4). Subsequent digestion of each of these three large fragments, 18a, 18b, and 18c, with RNase T1, followed by chromatography of the digests, gave the results shown in Figures 5, 6, and 7.

As shown in Figure 5, fragment 18a gave peaks 1 and 15. These peaks are, respectively, CpN²-DiMeGp (with a terminal 2',3'-cyclic phosphate) and Cp(Cp,Cp,Up)Cp-UpUpIp (with a terminal 2',3'-cyclic phosphate in peak 15a, and as the 3'-phosphate in peak 15b). The amounts of the dinucleotide and octanucleotide were equivalent. Since there is only one Ip in the alanine RNA, and it is found in the sequence IpGpCp in a pancreatic RNase digest of the RNA, the CpDiMeGp cannot be placed to the right of the Ip. Therefore, the dinucleotide must be attached to the left of the octanucleotide; and the sequence of 18a is CpDiMeGpCp(Cp,Cp,Up)CpUpUpIp.

Fragment 18b gave peaks 3, 5, and 10 (Fig. 6). These peaks are Gp, ApGp, and Cpl-MelpIpGp, respectively. They were obtained in molar ratios of 2:2:1. It is known that the two ApGp's are present in the alanine RNA in the octanucleotide...

Fragment 18c gave peaks 2, 3, 4, and pGp (pGp is part of peak 9 in Fig. 2). Peak 2 is Upl-MeGp (with a 2',3'-cyclic phosphate), peak 3 is Gp, and peak 4 is a mixture of CpGp and UpGp. The molar ratios of the different fragments were: pGp, 1; Gp, 3; CpGp, 1; UpGp, 1; and UpMeGp, 1. The presence of pGp establishes that 18c is the 5'-phosphate end of the alanine RNA molecule. Since it is known from the analysis of the pancreatic RNase digest of the RNA that pGp is present in the sequence pGpGpGpCp, the positions of two of the three Gp's and the CpGp are known; and the terminal five nucleotides must be pGpGpGpGpCpG.- The positions of the UpGp, UpMeGp, and one Gp are established by the following information. Since it is known that MeGp appears in a pancreatic RNase digest in the sequence MeGpGpGpCp, the UpGp cannot be placed to the right of the MeGp without an additional CpGp. Therefore, the arrangement must be pGpGpGpGpCpGpUpGp-UpMeGp. If the remaining Gp is placed at either hyphen in this structure, there will be a GpGpUp sequence preceded by a pyrimidine nucleotide, and GpGpUp should appear in a pancreatic RNase digest of the RNA. Since GpGpUp is not found, the final Gp must be placed to the right of the MeGp. The sequence of 18c is therefore pGpGpGpGpUpGpUpGpGpApGp. This sequence has been confirmed by the isolation of MeGpGp from a pancreatic RNase digest of fragment 18c.

As indicated above, fragments 18c and 16 are known to be the two ends of the alanine RNA molecule. The analyses reported in the accompanying paper indicate that fragment 18a comes from the left half of the molecule, and fragment 18b comes from the right half. Therefore, the four fragments described here are present in the alanine RNA molecule in the order 18c-18a-18b-16.

Further work will be required to determine the structures of larger fragments found in peaks 19-22, and to establish the basis for the selectivity of enzymatic attack by RNase T1 under conditions of limited digestion.


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