Note

Natural Catalytic RNA with Ribonuclease P Activity from *Thermus thermophilus*†

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Ribonuclease P (RNase P) is an endonuclease responsible for the accurate 5'-processing of all tRNAs. This enzyme cleaves tRNA precursors to make mature 5' ends of tRNAs. All of these enzymes so far isolated are ribonuclease P proteins and the RNA component of eubacterial variants of the enzyme has catalytic activity *in vitro*. In contrast to the eubacterial RNA component, however, catalytic activities of RNA subunits alone from eukaryotic or archaeabacterial RNase P have not been demonstrated. To test whether the RNA subunit of RNase P from *Thermus thermophilus* has catalytic activity, we have attempted to isolate the RNA component from an extreme thermophile, *Thermus thermophilus* HB8. During the course of this study, the isolation of the structural gene of RNase P-RNA from *T. thermophilus* HB8 has been reported by Hartmann and Erdmann. They also reported that the *in vitro* transcript of the gene has catalytic activity *in vitro*. So far, however, the catalytic activity of the natural RNA component of the enzyme isolated from *T. thermophilus* has not been demonstrated.

Here we report that an RNA component can be obtained from *T. thermophilus* HB8 cells and such natural RNA preparation alone, without the protein subunit, has catalytic activity. *T. thermophilus* HB8 cells were grown at 80°C and harvested at the middle logarithmic phase as described. Cells (wet weight 15 g) were washed with water, frozen, ground with 2 times their weight of alumina, and extracted with 30 ml of PA200 buffer (50 mM Tris-HCl, pH 7.6, 200 mM NH₄Cl, 10 mM MgCl₂). Deoxyribonuclease (RNase-free, Promega) was added to a final concentration of 0.3 unit ml⁻¹. The suspension was centrifuged at 600 g for 20 min. The supernatant solution was centrifuged at 100,000 g for 1 h to remove the ribosomes. The resulting supernatant (S-100) was extracted with SDS (sodium dodecyl sulfate)–phenol. The RNA was precipitated with ethanol and the DNA was resuspended in TE buffer (10 mM Tris–HCl, pH 7.6, 1 mM EDTA). This RNA preparation still contained a small amount of protein. Protein in this preparation was measured by the Bradford method to be 0.06 mg/ml of total RNA. Approximately 2.4 mg of RNA was chromatographed on a Sephacryl S-300 column (1 cm × 45 cm) and fractions of 1 ml (total 30 fractions) were collected. Every fraction was tested for RNase P activity.

RNase P activity was assayed essentially as described. *Drosophila* initiator methionine tRNA precursor was used as the substrate. 32P-labeled substrate was prepared by *in vitro* transcription of plasmid pDfY using T7 RNA polymerase and [α-32P]UTP as described. For RNase P activity assay, the reaction mixture (10 μl) containing 50 mM Tris–HCl, pH 7.6, 0.1 M NH₄Cl, 80 mM MgCl₂, 5% polyethylene glycol, and 10,000 cpm of 32P-labeled tRNA precursor substrate, and the RNA sample was incubated at 55°C for 45 min. The samples were electrophoresed in 15% polyacrylamide/8 M urea gel with TBE (89 mM Tris–borate/2 mM EDTA buffer, pH 8.3). Labeled substrates and products were detected by autoradiography. In the Sephacryl S-300 column chromatography, RNase P activity was detected in fractions numbered 13 to 16, and tRNAs were eluted from fractions numbered 19 to 27 (data not shown). This indicated that an RNA fraction extracted from cells has catalytic activity. The reaction mixture using S' end 32P-labeled substrate was also electrophoresed with the sequence ladder of the same substrate. The cleavage site was identified by comparison of the mobility of the product with those of sequencing ladders of fragments prepared by partial alkaline digestion of the substrate as described. Figure 1 shows an autoradiogram of the reactions using 3'-end 32P-labeled substrate. Product bands are visible in lanes b, c, and d in Fig. 1. The mobilities of these product fragments did not correspond to those of any fragments produced by partial alkaline hydrolysis (Fig. 1, lanes a and e), which would contain 2'- or 3'-phosphate ends. This indicates that the fragments produced in the reaction (Fig. 1, lanes b, c, and d) had 3'-OH ends, as expected for cleavage catalyzed by RNase P activity. From this analysis, it has been confirmed that this active RNA cleaved at a specific

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Fig. 1. Cleavage of the 5'-End Labeled *Drosophila* Initiator Methionine tRNA Precursor by RNA Fractions from Sephacryl S-300 Column Chromatography.

Reactions were done as described in the text. Mixtures were analyzed in a 20% polyacrylamide/8 M urea gel. An autoradiogram of the gel is shown. Lanes a and e, partial alkaline digest of the substrate; b, c, and d, reaction mixtures using fraction numbers 14, 15, and 16 from Sephacryl S-300 column chromatography, respectively; f, substrate RNA only. The nucleotide sequence of residues 7 to +7, and the 5' leader and mature sequence regions of the tRNA precursor are shown on the left. The nucleotide sequence was identified as described. S', substrate.
Fig. 2. Electrophoretic Analysis of the Catalytic RNA from *T. thermophilus*.

(A): Catalytically active RNA fraction from Sephacryl S-300 column was electrophoresed in 6% polyacrylamide/8 M urea gel (lane a). Lane b, Synthetic MIRNA (E. coli RNase P-RNA, 428 nucleotides) as a size marker. The area assayed for RNase P activity (1–8) is shown on the left. (B): RNA fractions eluted from the area 1–8 (A) were assayed for RNase P activity as described in text. Samples were electrophoresed in 15% polyacrylamide/8 M urea gel. Autoradiogram of the gel is shown. Lanes 1–8, substrate RNA plus RNA fractions 1–8, respectively; lane 9, substrate RNA plus MIRNA as a positive control; lane 10, substrate RNA only. The positions of substrate and mature rRNA are indicated.

Site on a tRNA precursor to remove its 5'-leader sequence and that the termini produced by the cleavage had 5'-phosphoryl- and 3'-OH ends. We have therefore concluded that this RNA preparation has RNase P activity.

To measure the catalytically active RNA, this RNA was electrophoresed on a gel. This active RNA fraction from Sephacryl S-300 chromatography was precipitated with ethanol and a part of the precipitate was electrophoresed in 6% polyacrylamide/8 M urea gel. RNA was detected by ethidium bromide staining (Fig. 2A). An area of another unstained gel (corresponding to the area shown in Fig. 2A, 1–8) was cut into 8 pieces and those were numbered 1 to 8 from top to bottom. RNAs were eluted from these gel pieces by the method of Maxam and Gilbert and each RNA fraction was assayed for RNase P activity. The activity was detected mainly in fraction 4 (Fig. 2B). In Fig. 2B, lane 9, the reaction mixture with MIRNA (Escherichia coli RNase P-RNA) was electrophoresed. Synthesis of MIRNA and the reaction with MIRNA were done as described. Since this reaction is well-established, the pattern of Fig. 2B lane 9 is a good positive control. The position of the reaction product (mature tRNA) is indicated on the left of Fig. 2B. In Fig. 2B, lanes 3 and 4, the mature tRNA product could be obtained. This indicated that the size of active RNA from *T. thermophilus* HB8 cells was somewhat larger than the synthetic MIRNA (428 nucleotides) (Fig. 2A).

Using 32P-labeled *Drosophila* initiator methionine tRNA precursor as the substrate, this *T. thermophilus* RNA showed RNase P activity at the temperature between 55°C and 65°C, but no activity was observed at 37°C and 45°C. At 70°C, however, activity could not be detected, probably due to denaturation of *Drosophila* tRNA precursor or of this catalytic RNA itself. Although *T. thermophilus* is grown at 80°C, it is possible that higher order structures of this catalytic RNA are destroyed at 70°C in the absence of the protein subunit. Similarly, Hartmann and Erdmann reported that, using the *in vitro* transcript of *T. thermophilus* RNase P-RNA gene and an *E. coli* tRNA precursor substrate, the highest cleavage rates were obtained between 55 and 65°C. However, they also reported that this transcript still had 80% of the highest activity at 70°C. This difference of the data at 70°C from ours may be due to a difference in the tRNA precursor substrate used, or the difference between the properties of our natural preparation and their *in vitro* transcript. Identification of optimal temperature for the reaction may await the use of a thermophilic tRNA precursor.

In conclusion, we have obtained a natural RNA preparation having RNase P activity from *T. thermophilus* HB8. This RNA is catalytically active *in vitro* at 55°C or higher temperature. This is a naturally occurring thermophilic ribozyme.

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