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The nucleotide sequence of 5S rRNA from an extreme thermophile, Thermus thermophilus HB8

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ABSTRACT

Using 3'- and 5'-end labelling sequencing techniques, the following primary structure for Thermus thermophilus HB8 5S RNA could be determined : pAA(U)CCCCCGUGCCCAUAGCGGCGUGGAACCACCCGUUCCCAUUCCG AACACGGAAGUGAAACGCGCCAGCGCCGAUGGUACUGGCGGACGACCGCUGGGAGAGUAGGUCGG UGCGGGGGA$_{OH}$. This sequence is most similar to Thermus aquaticus 5S RNA with which it shows 85% homology.

INTRODUCTION

It is accepted that 5S RNA is an essential component of large ribosomal particles from prokaryotic and eukaryotic organisms (1). In recent years, the primary sequences of 5S RNAs have been determined (2).

Thermus thermophilus HB8 is a bacterium which can grow at temperatures of up to 85°C where most biopolymers obtained from mesophilic organisms undergo thermal denaturation (3). Nevertheless, the thermostable ribosomes of this thermophile are capable of protein biosynthesis in the cell-free system from the mesophile E. coli (4). Furthermore, 5S RNA of T. thermophilus HB8 forms a specific complex with E-L5, E-L18 and E-L25, which are the ribosomal binding proteins of E. coli 5S RNA (5).

The primary sequence of 5S RNA from another extremely thermophilic bacterium, T. aquaticus, has already been reported (6). Evolutionary studies have suggested that T. aquaticus occupies a unique position in a phylogenic tree constructed by comparing the primary sequences of 5S RNAs (7,8).

In this paper, we report the nucleotide sequence of 5S RNA from T. thermophilus HB8. The sequence was determined by three kinds of gel sequencing methods using post-labelled material.

MATERIALS AND METHODS

T. thermophilus HB8 5S RNA was isolated and purified as previously described (3,9), labelled at the 3' and 5' ends as reported (10,11) and sequenced using the chemical degradation method of Peattie (12) and the enzymatic digestion method of Donis Keller (13). A rapid thin-layer readout technique was also used, essentially as described (14).

RESULTS AND DISCUSSION

(1) Sequence analysis of 3'-end labelled 5S RNA

RNase T_2-digestion of 3'-end labelled 5S RNA gave A[^{32}p] after TLC on a PEI-cellulose plate (15), thus identifying the 3'-terminal residue as A. The chemical sequencing method enabled us to analyze about 90 nucleotides starting from the 3'-end of the 5S RNA. The sequence obtained by the chemical method was confirmed by partial enzymatic digestion except for the nucleotides in positions 80 to 97. This region was found to be very resistant to RNases, suggesting the presence of a very tight secondary structure.

(2) Sequence analysis of 5'-end labelled 5S RNA

When nuclease P1-digests of 5'-end labelled 5S RNA were subjected to TLC on PEI-cellulose plates (15), only A was identified as the 5'-terminal residue. However, complete RNase T1 digestion of 5'-end labelled material gave two oligonucleotides differing in size by one nucleotide in electrophoresis on 20% polyacrylamide gel in 7M urea. Both of these oligonucleotides had the same 5'-terminal residue, namely A. This observation suggested a length heterogeneity in a region near the 5'-end of the RNA. Further purification of 5'-end labelled 5S RNA by 8% polyacrylamide gel electrophoresis (600 x 200 x 0.5mm) at 1.2 kV for 18 hours gave two bands. The ratio in radioactivity of the lower migrating RNA to the faster species was 2:1.

Both of these RNAs were sequenced by partial enzymatic digestion. Sequence analysis of the two species up to about 35 nucleotides from the 5'-end showed that these two species have the same sequence except for a U residue at position 3 (Figure 1) which is deleted in the faster migrating RNA. By partial enzymatic digestion of the slower migrating RNA, about 80 nucleotides were ana-

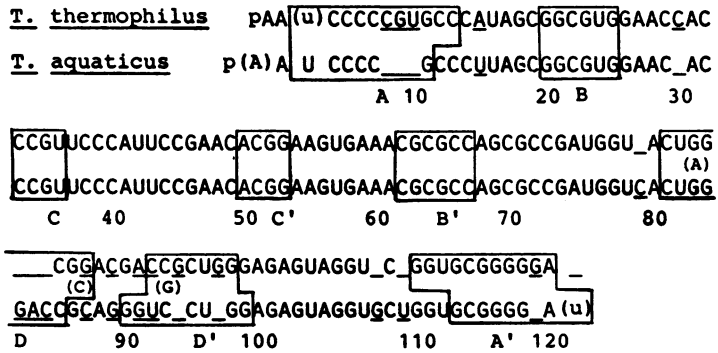

Figure 1. The nucleotide sequence of T. thermophilus HB8 5S RNA in comparison with that of T. aquaticus 5S RNA (6). The underlines show differences or gaps between these two sequences. The squared off regions are possibly involved in base pairing in the secondary structural model of Fox and Woese (18). Small letters mean heterogeneities observed in these 5S RNAs. Different nucleotides observed at positions 83, 86 and 93 by the TLC readout technique are indicated in brackets under the sequence of T. thermophilus HB8 5S RNA and are presumably misreadings due to the strong base-pairing suspected in this region.

lyzed starting from the 5'-end.

(3) Sequence analysis of 5S RNA by TLC readout sequencing method

Sequences derived by analyses from both the 3'-end and 5'-end of the 5S RNA molecule gave consistent overlapping in positions from 26 to 80. In order to confirm the overlapping sequence and get further structural information on the 5S RNA, a TLC readout sequencing technique was also employed. No modified nucleotides were encountered either in this technique or in the gel sequencing ladders described above.

The analysis of nucleotides in positions 25 to 110 by this method supported the results obtained with 3'- and 5'-end labelled materials except for positions 83, 86 and 93 (Figure 1).

The nucleotide sequence of T. thermophilus HB8 5S RNA is shown in Figure 1 in comparison to that of T. aquaticus 5S RNA (6). The homology of both primary sequences is approximately 85%. It is clear that the sequences of these two 5S RNAs are closer related to one another than to any other 5S RNA sequence so far determined (2). However, relatively low homology (85%) might suggest early

divergence of these two thermophile bacteria. The present sequencing study of T. thermophilus HB8 5S RNA further indicates that the genus Thermus is located at a unique position in a phylogenic tree among prokaryotic organisms (7).

When these two sequences are arranged in the secondary structure model of Fox and Woese (16), they are almost identical. The major difference in the secondary structure model is observed in stem A (16). With the sequence of T. thermophilus HB8, a longer stem A (11 nucleotide pairs including 8 G·C pairs) can be arranged than with that of T. aquaticus. This extended stem A may contribute to the increased thermostability of the 5S RNA (unpublished, Watanabe, K. et al.) from T. thermophilus, which is known to be able to grow at 85°C (3).

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