Terminal heterogeneity and corrections of the nucleotide sequence of 5S rRNA from an extreme thermophile, *Thermus thermophilus* HB8

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ABSTRACT

The nucleotide sequence of 5S rRNA from an extreme thermophile *Thermus thermophilus* HB8 has been re-examined and determined as (pA)A¹⁰U¹⁰CCCCG²⁰UG²⁰CCCA³⁰UAG³⁰CGG⁴⁰CGUG⁴⁰GAAC⁵⁰CCG⁵⁰U⁵⁰UCCCA⁵⁰U⁵⁰CCGAACA⁵⁰CGGAAGUGAAACGCGCCAGCCGCGCAUGGUACUGGGCGGGCGACCGCCUGGGAGAGUAGGUCG⁶⁰GUGCGGGGA(U⁷⁰_{OH})¹²⁰. The present study could resolve previously ambiguous residues and find two additional residues (G₈₃, C₉₇), G at position 89 and two terminal heterogeneities which are exactly the same as reported with *Thermus aquaticus* 5S rRNA.

INTRODUCTION

It has been shown that 5S rRNAs from extreme thermophiles have more rigid structure than those from mesophilic organisms (1, unpublished results by Watanabe, K. et al.). Therefore, these 5S rRNAs may be suitable for some physicochemical studies of 5S rRNA. In fact, the crystallization of the 5S rRNA from *Thermus thermophilus* HB8 has been reported and the preliminary X-ray crystallographic studies are encouraging (2).

Recently, we have reported the nucleotide sequence of 5S rRNA from *T. thermophilus* HB8 (3). However, we have encountered some ambiguous residues in positions 83-97, probably due to the strong secondary structure involved in this region (3). Independently, our colleagues Komiya et al. have done the sequence study of the same RNA and resolved the previous ambiguous sequence by using high-temperature sequence gels. Therefore, *T. thermophilus* HB8 5S rRNA samples were exchanged between both laboratories and checked again for their nucleotide sequence.

The present paper reports the terminal heterogeneities and

the complete nucleotide sequence of T. thermophilus HB8 5S rRNA.

MATERIALS AND METHODS

5S rRNA samples were isolated and purified from T. thermophilus HB8 as described previously by Kumagai and Erdmann (3) and by Komiya et al. (4,5). The gel sequencing analyses with the 3'- and 5'-end labeled RNAs were carried out as described (3,4), and the two-dimensional homochromatography was done as reported (6). The unlabeled RNA was completely digested with RNases A and T₁ and the digests were analyzed by conventional column chromatographic procedures and UV-spectrophotometry (7).

RESULTS AND DISCUSSION

Figure 1 shows analysis of T. thermophilus HB8 5S rRNA samples from both groups on a stained 10% polyacrylamide gel in the presence of urea at 4°C.

Clearly, two species are detectable (upper band a and lower one b) in both samples although the relative amounts of these species seem to differ between both samples. In the previous studies using rapid gel sequencing methods, we had ignored the species a. However, since the 5S rRNA sample from one of our groups contained relatively large amounts of this species, in the present study, we have checked the nucleotide sequence of species a as well as species b.

The corrected nucleotide sequence of the 5S rRNA from T. thermophilus HB8 is shown in the abstract. The present chemical sequence analysis with the 3'-labeled 5S rRNA using a high temperature gel (20% polyacrylamide-8M urea gel at 2.5-3.5kV) could resolve the previously ambiguous residues at positions 84, 87 and 94 (present numbering) (3) and add additional residues G 83 and C 97 resulting in the formation of one additional G-C pair in this region. Since in some experiments the band resulted from scission at G 89 was very faint, G 89 might have been misread as A. These results obtained from gel sequencing studies were fully supported by cataloguing the oligonucleotides obtained by complete digestions with RNases A and T₁. The species b shown in Fig. 1 was further separated into two species by using electrophoresis on a long 8% polyacrylamide gel (as previously re-

Figure 1: Analysis of 5S rRNA samples on 10% polyacrylamide gel (20x20x0.15cm) electrophoresis in the presence of 7M urea. Electrophoresis was done at 400V and 4°C for 15 hours. The buffer used was 90mM Tris-borate, pH 8.3, 4mM EDTA. After electrophoresis the gel was stained using 0.1% toluidine blue - 1% acetic acid solution. Lane 1: 5S rRNA sample prepared by Komiya and Takemura. Lane 2: 5S rRNA sample prepared by Kumagai and Erdmann.

Figure 2: Temperature dependence of electrophoretic mobilities of two different 5S rRNA species on 10% polyacrylamide gel (40x20x0.05cm) in the presence of 7M urea. The buffer used was 50mM Tris-borate, pH 8.3, 2mM EDTA. Electrophoresis was done at room temperature until xylene cyanol dye migrated to 20cm. After electrophoresis, the RNA bands were detected by autoradiography. Lane A: Electrophoresis, 250V, 3mA for 21 hours. Lane B: Electrophoresis, 1.2kV, 25mA for 3 hours. Lanes a and b show 5'-end labeled 5S rRNA species a and b from Figure 1 respectively.

ported (3)). When the sequences near 5'-ends of both species were checked again by two-dimensional homochromatography with 5'-labeled materials, the results with the slower migrating species were consistent with the 5'-terminal sequence of major species reported before. However, the faster migrating species (minor species) was found to lack only one A at 5'-end which was supported by cataloguing the oligonucleotides but was inconsistent with the previous assignment obtained by the Donis-Keller's method.

On the other hand, the sequence studies with species a revealed that this species contains one additional U at the 3'-end. It turned out that this species should therefore have one additional A-U base pair in the helix formed between 5'- and 3'-end regions. Figure 2 shows analyses of 5'-end labeled species a and b on a 10% polyacrylamide gel electrophoresis in the presence of urea at different temperature. As shown in Fig. 2-A at low temperature species a migrated much slower than species b. Under those conditions, species b could not be separated into the two species differing by the one nucleotide at the 5'-end. At high temperature (>50°C in Fig. 2-B), species a migrated significantly closer to species b. Therefore, one additional A-U base pair formed between the 5'- and 3'-end regions of species a seems to influence the conformational stability of this molecule. Finally, it should be noted that the present studies also revealed that the 5S rRNA of T. thermophilus HB8 contains at least two terminal heterogeneities, one at 5'-end and the other at 3'-end, which are exactly the same as reported with T. aquaticus 5S rRNA (8), although some parts of its sequence differ in an unusual manner from that of T. thermophilus HB 8 (3).

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