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Sequence and secondary structure of the colicin fragment of Bacillus stearothermophilus 16S ribosomal RNA

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ABSTRACT

The sequence and the position of post-transcriptionally modified residues of the 3'-terminal end of *Bacillus stearothermophilus* 16S ribosomal RNA have been determined from the fragment that is cleaved off by bacteriocin treatment. The fragment contains 52 nucleotides, as compared to the 49 nucleotides of the corresponding fragment from *E. coli* ribosomes. The additional nucleotides are present in the sequence UCU very next to the 3'-terminus as was published earlier (1). The remainder of the sequence is identical to the one of *E. coli* except at six positions, due to three basepair alterations in a major hairpin stem. Measurements of the UV melting properties of the colicin fragment from *B. stearothermophilus* in comparison to the same fragment of *E. coli* show that the RNA from the thermophile has a more stable secondary structure.

INTRODUCTION

The 3'-terminal parts of the ribosomal RNA's of the small subunits from both prokaryotes and eukaryotes are, apart from the homology in primary structure, probably homologous in secondary structure (3-6). A nine basepair long hairpin with two dimethylated adenines in the loop is a universal feature of the 3'-end of the RNA's of small ribosomal subunits, while there is conservation of the sequence in single stranded regions. In *E. coli* this part of the 16S ribosomal RNA is strongly implicated in initiation of protein synthesis and in ribosomal subunit interaction (7,8). In the proposed models for the mechanism of action of the 3'-end of 16S rRNA it is suggested that intramolecular basepairing of this RNA is exchanged for intermolecular hybridisation with other RNA's (9,10).

In a recent study we showed that dimethylation of the adenines in the hairpin loop at the 3'-end of 16S RNA of *E. coli* destabilizes this hairpin and made the suggestion that methylation serves to facilitate intermolecular interactions at the expense of intramolecular basepairing (2). In order to extent these studies and also to determine the effect of nucleotide sequence

on the stability of the secondary structure we isolated the RNA fragment cleaved off from *Bacillus stearothermophilus* ribosomes by the bacteriocins cloacin DF_{13} or colicin E_3. Its nucleotide sequence was established and the thermal stability was determined by measuring the temperature dependent hyperchromicity.

MATERIALS AND METHODS

Materials. The bacteriocins colicin E_3 and cloacin DF_{13} were a generous gift of Dr. F.K. De Graaf. *Bacillus stearothermophilus* which was grown aerobically at 60°C was purchased from CAMR (England). The following were also commercial products: (γ-^{32}P)ATP (Amersham, U.K.), T_4-polynucleotide kinase (Boehringer-Mannheim Biochemicals) and T_4-RNA ligase (PL-Biochemicals).

Isolation of the 3'-terminal fragments. Isolation of the 3'-terminal fragments was as described before (11) except that in the case of *B. stearothermophilus* ribosomes were isolated after disrupting the cells by sonication and that the bacteriocin colicin E_3 was used.

Preparation of (5'-^{32}P)cytosine 3'5' diphosphate ((5'-^{32}P)pCp). The procedure of Stanley and Van Kammen (12) was essentially followed with the only exception that (γ-^{32}P)ATP from Amersham was used.

Enzymatic digestions. The fragments were labeled at their 5'-ends as described before (13). For the RNase T_1 digestion, the enzymatic conditions of Donis-Keller (14) were used.

Chemical sequencing. The fragments were labeled at their 3'-ends with T_4-RNA ligase and (5'-^{32}P)pCp under conditions similar to those of England and Uhlenbeck (15). The reactions of the chemical sequencing procedure were performed as described by Peattie (16).

Identification of modified nucleotides. The identification of modified nucleotides was as described before (17) except that 6 x 10 cm polyethyleneimine plates were used.

Melting experiments. The melting experiments were performed as described by Van Charldorp *et al.* (2).

RESULTS

1. Sequence of the Colicin Fragment of B. stearothermophilus

The bacteriocins colicin E_3 and cloacin DF_{13} cause a specific cleavage

near the 3'-end of 16S RNA in 30S ribosomal subunits of *E. coli* (18,19). In this study *Bacillus stearothermophilus* ribosomes and, as a control, *E. coli* ribosomes were treated with colicin E_3 or cloacin DF_{13} and the "colicin" fragments were isolated and purified (11).

Several approaches were taken to solve the nucleotide sequence and the positions of modification of the *Bacillus* fragment.

(*a*). The *E. coli* and *B. stearothermophilus* fragments were labeled with (^{32}P) at the 5'-end using (γ-^{32}P)ATP and polynucleotide kinase. Fig. 1A, lanes 1 and 2, show autoradiograms of polyacrylamide gels after electrophoresis of partial alkaline digests of these labeled fragments. The undigested fragment of *B. stearothermophilus* has a lower mobility than the fragment of *E. coli*. This is partly due to the fact that it contains 52 nucleotides (counted from ladders as in Fig. 1A, lane 2) as compared to the 49 of the *E. coli* fragment. Secondary structure may also contribute to differences in mobility as we showed previously (13). The ladder patterns of the alkaline digests (Fig. 1A, lanes 1 and 2) of the fragments from the two bacterial species are identical up till band no 15. This is indicative of identical sequence since a difference would show up in a slight change in mobility.

The 5'-labeled fragments were further used to locate the G residues. Partial RNase T_1 digests were electrophoresed and the autoradiograms are shown in Fig. 1A, lane 3 (*E. coli*) and lane 4 (*B. stearothermophilus*). Time of electrophoresis was such that the G at position 4 from the 5'-end in the *E. coli* fragment was at the bottom of the gel. All 13 G's from thereon present in the known sequence of the colicin fragment from *E. coli* can be seen in lane 3, except G23 which is methylated in the N^2 position (17). In the fragment of the thermophile (lane 4) only 6 G's can be found and those all correspond with positions of G residues in the *E. coli* fragment. In view of the homologies between the 3'-ends of RNA's of small ribosomal subunits of various sources (4-6, 20-26) this would be a very remarkable difference. Furthermore, there is a complete lack of G's in the *Bacillus* fragment in a region of the sequence where the *E. coli* fragment contains a hairpin stem (3,27), suggesting that the RNase T_1 treatment is insufficient to cleave at G residues in the *Bacillus* hairpin. Even extremely drastic conditions (7 M urea at 100°C, where the RNase T_1 is still active) does not result in additional bands with the *Bacillus* fragment. That this RNA indeed contains a very stable hairpin that is inaccessible to RNase T_1 is demonstrated in the experiment shown in lane 5 of Fig. 1A. Band 24 from the *Bacillus* fragment, presumably, in analogy with *E. coli*, containing the 5' part of the hairpin, was ex-

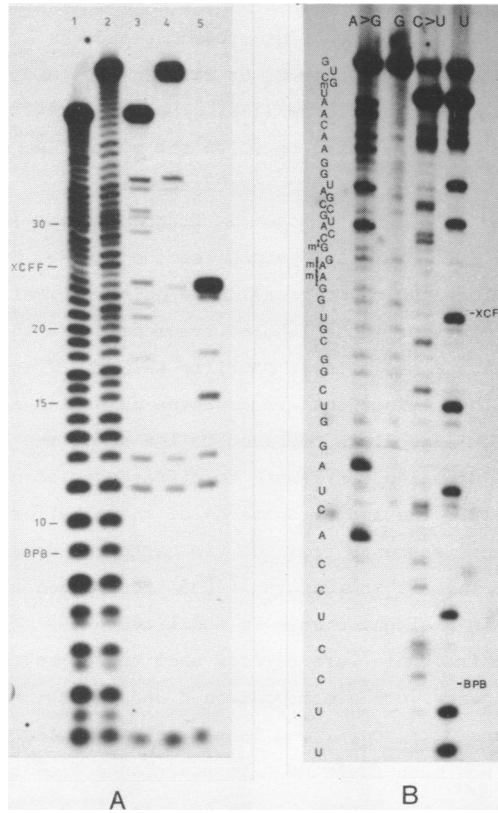

Figure 1.A. Autoradiogram of a sequencing gel using (5'-^{32}P) colicin fragments derived from ribosomes of *E. coli* and *B. stearothermophilus*.
Lane 1: partial alkaline digest of *E. coli* fragment. Lane 2: partial alkaline digest of *B. stearothermophilus* fragment. Lane 3: partial RNase T_1 digest of *E. coli* fragment. Lane 4: partial RNase T_1 digest of *B. stearothermophilus* fragment. Lane 5: partial RNase T_1 digest of the material from band number 24 in lane 2. The numbers of the bands correspond to the numbers of nucleotides from the 5'-end.
B. Cleavage pattern of the colicin fragment of *B. stearothermophilus* obtained by the direct chemical method of sequencing (16). For the position of modified nucleotides see Fig. 3. XCFF: xylene cyanol FF. BPB: bromophenol blue.

tracted from a gel containing a partial alkaline digest such as lane 2 in Fig. 1A. The isolated material was subjected to partial digestion by RNase T_1 and electrophoresed in lane 5 (Fig. 1A). The band just below the major band at position 24 in this lane is a contamination with band 23 from the original gel. This experiment clearly indicates that there are two additional G resi-

dues in the *Bacillus* fragment, one of which (at position 18) corresponds with *E. coli*.

(*b*). Both fragments were labeled at their 3'-end using (5'-^{32}P)pCp and RNA ligase (15) for application of the chemical sequence method (16). A difficulty was experienced here with the *Bacillus* fragment: it was apparently heterogeneous at the 3'-end, probably partly lacking the 3'-terminal A residue. In any event, the 52 nucleotide long fragment had to be purified from a gel after the labeling before a useful pattern could be obtained with this material. A result of the chemical sequence method with the *Bacillus* fragment is shown in Fig. 1B. In this specific experiment the A (except at positions 25 and 26), G (except at position 23; an ambiguous reactivity to dimethylsulphate and diethylpyrocarbonate), C and U residues between position 5 and 48 can readily be identified. Similar gels allowed the identification of the whole sequence (except the modified nucleotides, compare below).

(*c*). The colicin fragment of *E. coli* contains four modified bases (17): a methylated U at position 5, a N^2-monomethyl G at position 23 and two N^6,N^6-dimethyl A's at positions 25 and 26. To establish the position of modified residues in the *Bacillus* fragment, the previously described method (17) was also applied to the *Bacillus* fragment. The results are shown in Fig. 2. Modified nucleotides are found at positions 5 (Fig. 2A), 25 and 26 (Fig. 2B) and 23 (Fig. 2C). This strongly suggests, although it does not prove, that the *B. stearothermophilus* fragment contains the same modified nucleotides as the *E. coli* fragment.

Fig. 3 shows the sequence of the colicin fragment of *Bacillus stearothermophilus* drawn in a secondary structure model.

2. *Thermal Melting Characteristics of the Colicin Fragment*

Proton magnetic resonance studies (3), temperature dependent hyperchromicity measurements (2) and T-jump relaxation kinetics (27) have confirmed the presence of the central hairpin in the colicin (cloacin) fragment from *E. coli*. In addition, substantial basepairing of other nucleotides is obvious from these data.

Fig. 4 compares the UV melting curves of the colicin fragments from *E. coli* and *B. stearothermophilus* at 15 mM NaCl. As discussed previously (2), total hypochromicity and differentiation of the melting curve by computer show that the *E. coli* fragment contains at least three regions of secondary structures. The melting behaviour of the colicin fragment from the thermophile and its differential (not shown) is similar to that of *E. coli*, except that

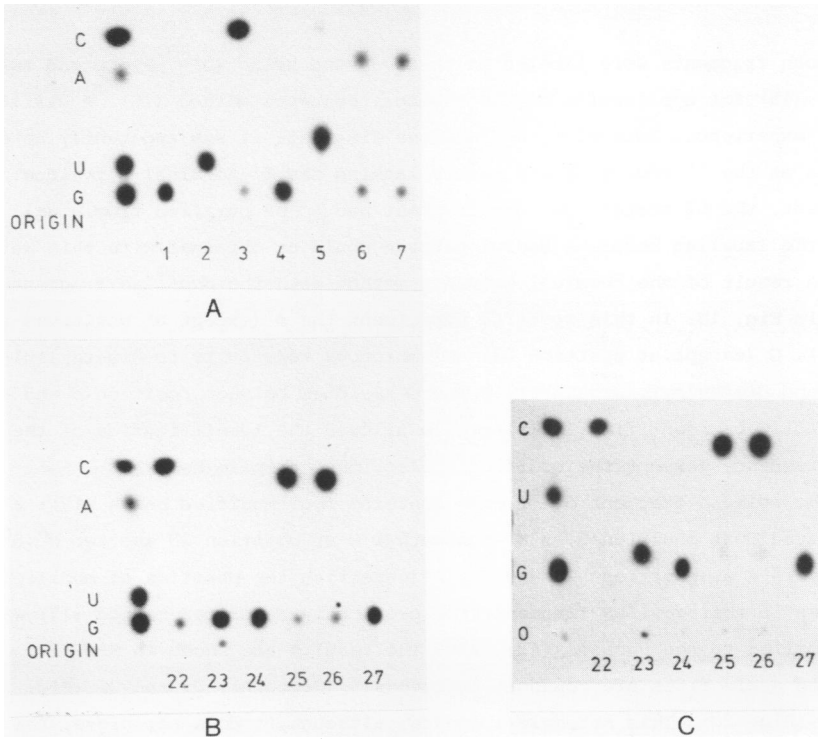

Figure 2. Nucleotide analysis of the colicin fragments of *B. stearothermophilus* on polyethyleneimine plates. The colicin fragment of *B. stearothermophilus* was labeled at its 5'-end with ATP and polynucleotide kinase, then subjected to partial formamide digestion. The digestion products were labeled with (γ-^{32}P)ATP and polynucleotide kinase and electrophoresed on a 20% acrylamide slab gel. The RNA from the separated bands was eluted and digested with RNase P_1 and fractionated by chromatography on polyethyleneimine plates. The plates were successively developed with H_2O, 0.5% formic acid and 0.15 M Li^+ formate. The numbering beneath the lanes refers to the position in the fragment (compare Fig. 3). (A) and (B). 6 x 10 cm polyethyleneimine plates. (C). 20 x 20 cm polyethyleneimine plate. Marker A nucleotide was absent.

the transitions are shifted considerably to higher temperature (Fig. 4). Data analysis by computer, not included in this paper, shows that the central hairpin of the *B. stearothermophilus* fragment has a T_m of 74°C at 15 mM NaCl, while the corresponding region in the colicin fragment melts with a T_m of 52°C at these conditions (2). A detailed analysis of UV melting curves of the *Bacillus* fragment will be presented somewhere else.

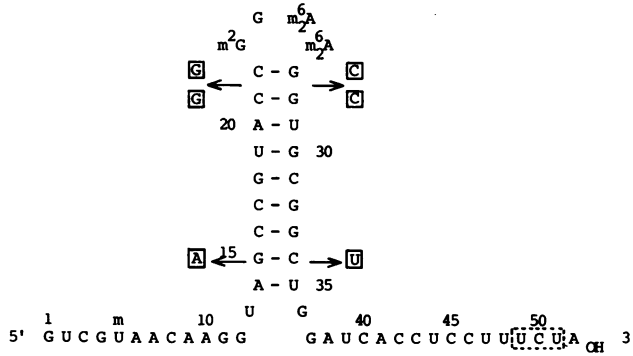

Figure 3. Sequence and secondary structure model of the colicin fragment of *B. stearothermophilus*. The boxed nucleotides are those found in the *E. coli* fragment. The nucleotides in the dashed box around position 50 are absent in *E. coli*. The exact nature of base modifications has not been established, but concurs with *E. coli*.

DISCUSSION

The 3'-ends of RNA of the small ribosomal subunits of both prokaryotes and eukaryotes are remarkably conserved (4-6, 20-26). For all the 3'-terminal parts of rRNA from the small subunits a hairpin can be drawn with (*i*) a helix of nine basepairs of which the A-U and U-G basepairs (third and fourth base-pair counting from the loop) are conserved in each helix, and (*ii*) a loop with the sequence $Gm_2^6Am_2^6A$, which is common to all species. In almost all the cases the loop consists of four nucleotides. In eukaryotic rRNA's this fourth nucleotide is an U, while in prokaryotic rRNA's this nucleotide is a modified

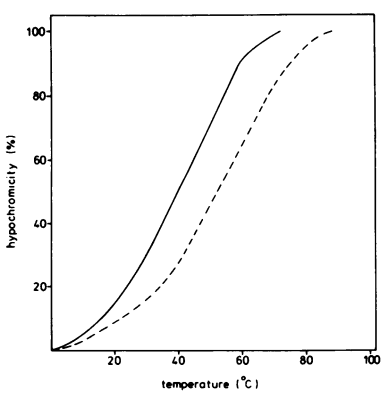

Figure 4. Corrected hyperchromic melting profiles at 260 nm of the 49 (———) and 52 (-----) nucleotide fragments derived from the 3'-ends of 16S rRNA of respectively *E. coli* and *B. stearothermophilus* at 15 mM NaCl.

G (17,28). Only in one case, namely the silkworm *Bombyx mori*, the loop was reported to consist of the sequence $Um^6_2Am^6_2A$. Considering the conservation of this part, it is unlikely that there is a G residue missing. Furthermore, the sequence 3' proximal to the helix appears to be GGAUCA, except for the mitochondrial rRNA's (24,25,29) and one chloroplast rRNA (30) where the homology ends with GGA. The conservation of the nucleotide sequence at the 5' side of the helix is more straightforward. Here we find the sequence 5' AAGUCGUAA (X)AAGGU. This oligonucleotide is preserved in all species which are sequenced untill now. The sequence of nucleotides at the 3'-end of *B. stearothermophilus*, shown in Fig. 3, is not very different from the corresponding 3'-termini of RNA's of small ribosomal subunits from other organisms. In comparison to *E. coli*, this thermophile contains an additional 3 nucleotides, as was already known (1) and 3 basepair alterations in the central stem region of the hairpin. Nevertheless, the optical melting experiments show that the secondary structure is far more stable than the corresponding one in *E. coli* (2). For one thing this illustrates that our understanding of how the sequence of basepairs in a RNA helix influences stability is very limited. The experimentally derived rules (31) are certainly not adequate to explain the difference in helix stability between *E. coli* and *B. stearothermophilus*. Probably additional rules, not yet established by *in vitro* experimenting, enable nature to vary the stability of RNA secondary structure by small changes in sequence or base modification.

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