Two distinct conformations of rat liver ribosomal 5S RNA

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ABSTRACT

Three different conformers of rat liver 5S ribosomal RNA were investigated by partial nuclease cleavage technique using S₁ nuclease and cobra venom endoribonuclease (CVE) as conformational probes. Urea-treated and renatured 5S RNA co-migrate on non-denaturing gels, but exhibit distinct differences in their nuclease cleavage patterns. The most prominent differences in S₁ nuclease and CVE accessibility of these conformers are located in region 30-50 and around nucleotides 70 and 90. The third form of 5S RNA with higher electrophoretic mobility was generated by EDTA treatment. The cleavage patterns of this 5S RNA conformer are similar to that characteristic for the renatured 5S RNA. The results demonstrate the difference in secondary structure and possibly different tertiary base-pairing interactions of 5S RNA conformers.

INTRODUCTION

Despite numerous efforts, the final version of the secondary and tertiary structure model of 5S RNA is still absent (for recent review see [1]). For the prokaryotes, 5S RNA secondary structure model, proposed by Fox and Woese [2] on the basis of evolutionally conserved bases is now widely used, but does not fit for all the chemical modification and physical measurement data [3-5]. The issue is even more complicated since 5S RNA may exist in different conformational states [6,7]. Conformers of *E. coli* 5S RNA were found because of their different electrophoretic and chromatographic mobilities [6,8]. They can also be distinguished by their different chemical reactivity, oligonucleotide binding, nuclease accessibility, physical behaviour and in affinity for ribosomal proteins [9]. A thermodynamic study by Richards et al. [10] showed that two forms of *E. coli* 5S RNA exist in solution at equilibrium. It is not yet under-

stood how these differences are manifested on the level of the 5S RNA base-pairing scheme. Even less is known about eukaryotic 5S RNA. Conformational heterogeneity of rat liver 5S RNA was shown by us earlier [11]. Recently the extent of base stacking and basepairing of yeast 5S RNA in the presence and absence of Mg^{++} ions were monitored by UV, CD and 360 MHz 1H NMR spectroscopy [12]. Here we extend these observations using two controversially acting secondary structure-specific nuclease, single-strand specific S_1 nuclease and double-strand specific cobra venom endoribonuclease (CVE) in combination with rapid sequencing [13,14] for the investigation of three different conformers of the rat liver 5S RNA.

MATERIALS AND METHODS

Materials

Alkaline phosphatase, S_1 nuclease and T4 RNA ligase were the products of SKTB BAV (Novosibirsk, USSR). T_1 ribonuclease was purchased from Sankyo Co. Ltd. Polynucleotide kinase was isolated according to [15] by Dr. M.Ustav. Cobra *Naja naja oxiana* venom endoribonuclease (CVE) was kindly provided by Dr. S.K.Vasilenko. γ - ^{32}P ATP with specific activity 1000 Ci/mmol was from V/O Isotop, USSR. 5'- ^{32}P -pCp was synthesized as described by England et al. [16] and used without further purification. Rat liver 5S RNA was isolated as described earlier [17].

Preparation of terminally labelled 5S RNA conformers

5S RNA was dephosphorylated as described in [18]. 100-300 pmol of dephosphorylated 5S RNA was labelled with 100-500 pmol of γ - ^{32}P ATP and 0.5-1 μ l of polynucleotide kinase in 10-15 μ l 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM dithiothreitol and 10 mM $MgCl_2$ for 30 min. at 37°C. 5S RNA was labelled at their 3'-termini with ^{32}P -pCp and T4 RNA ligase, essentially as described earlier [14]. Labelled 5S RNA was mixed with 7 M urea-containing sample buffer, incubated for 3 min at 60°C, rapidly cooled, purified by polyacrylamide gel electrophoresis and eluted from the gel [19]. After repeated ethanol precipitation in the presence of cold homologous carrier, 5S RNA was dissolved in 5 mM Tris-HCl, pH 7.5, containing 50 mM KCl and 1.5 mM

MgCl₂ (TKM buffer) at the final concentration of 10 A₂₆₀ units/ml. 5S RNA treated in this way is further designated as conformer B. For renaturation, 5S RNA solution in TKM was heated for 15-20 min at 65°C and cooled to room temperature during 3-4 hr (designated as conformer A). EDTA-treated 5S RNA was generated in the same manner but in the presence of 5 mM EDTA-Na in TKM buffer and is designated below as conformer C. The 5S RNA conformers were analyzed on the 10% polyacrylamide gel. Electrophoresis was carried out in a continuous buffer system, containing 100 mM Tris-borate, pH 8.3, 2 mM EDTA at room temperature with 100 V for 4-5 hr. The positions of radioactive material were detected by autoradiography.

Structural analysis

For digestion of 5S RNA with S₁ nuclease 1/10 volume of 300 mM NaOAc, pH 4.6, 1 M NaCl, 10 mM ZnCl₂, 10 mM MgCl₂ and 50% glycerol was added to the solution of 5S RNA in TKM buffer and the reaction was performed with 10-15 units of the enzyme for 20 min at 5°C. Partial cleavage with 1-5 µg of CVE was carried out in TKM buffer, containing additionally 100 mM Tris-HCl, pH 7.5, 0.2 M NaCl and 20 mM MgCl₂ for 20 min at 5°C. Hydrolysis with T₁ RNase was performed in the same buffer with 0.5 units of the enzyme for 5 min at room temperature. "Ladder" was generated with 0.1 M NaOH. About 5 µg of ³²P-5S RNA was used in the reaction volume 10-15 µl. The reactions were stopped by precipitating RNA with approx 5 volumes of cold ethanol. The pellet of hydrolysis products was dissolved in the sample buffer [19], heated for 1 min at 60°C and loaded onto the thin 20% sequencing gel [20].

RESULTS

Three preparations of 5S RNA

Electrophoresis of three differently prepared rat liver 5S RNA samples in non-denaturing conditions is shown in Fig. 1. The characteristic mobilities of these 5S RNA preparations do not change if the samples are incubated for 30 min at 0°C in S₁ nuclease and CVE digestion buffers. As it is evident from Fig. 1, conformer B behaves identically with conformer A. On the other hand, the EDTA-treatment generates a novel conformer of 5S RNA

Fig.1. Electrophoresis of rat liver 5S RNA conformers in a 10% non-denaturing polyacrylamide gel.
1 - conformer B; 2 - conformer A;
3 - conformer C. For details see Materials and Methods.

with higher electrophoretic mobility (Fig.1). This observation is consistent with relevant observations on *E.coli* 5S RNA [6,8]. Contrary to our results, urea-treated and renatured forms of *E.coli* 5S RNA differ significantly in electrophoretic mobilities [7]. Thus, three different preparations of 5S RNA give, by the electrophoretic criterion, two distinct conformers of this molecule. It might be worthwhile to add that at denaturing conditions, in urea-containing polyacrylamide gel, all three preparations of 5S RNA migrate identically (see samples on Fig.2).

Enzymatic tests

The patterns of partial cleavage of the three 5S RNA preparations with S_1 nuclease and CVE are presented in Fig. 2. The first and most striking result of these experiments is that no striking differences between the 5S RNA conformers A and C can be found (compare Fig. 2 slots 5, 6, 8 and 9). At the same time these two samples of 5S RNA exhibit clear difference in their electrophoretic mobilities (Fig. 1, slots 2 and 3). On the contrary, two co-migrating preparations of 5S RNA: conformers A and B, clearly differ in their partial S_1 nuclease and CVE cleavage patterns (Fig. 2, compare slots 4, 5, 7 and 8). These results are summarized in Fig. 3a and b. For S_1 nuclease target sites, common for both these forms of 5S RNA, are located at the following positions: loop b: 21, 22, 23; loop c: 37, 38, 39, 40;

Fig.2. Separation of partial cleavage products of 5'- 32 p-5S RNA from rat liver in a sequencing gel [19].

1 - control, conformer C; 2 - T₁ nuclease; 3 - "ladder"; 4 - conformer B cleaved with S₁ nuclease; 5 - conformer A, S₁; 6 - conformer C, S₁; 7 - conformer B, CVE; 8 - conformer A, CVE; 9 - conformer C, CVE. For other details see Materials and Methods.

loop d: 74, 75 and 100-101. The most prominent differences in S₁ nuclease cuts are: i) region from 37 to 49 is largely accessible in conformer B; there is an obvious "slippage" of the S₁ sensitive area in loop c and helix III towards 3'-direction in the case of 5S RNA conformer B, compared with conformer A; ii) the regions 25-30 and 87-90 of the 5S RNA conformer B are completely protected against S₁ nuclease attack while accessible in the renatured molecule. For CVE, the following differences are most apparent: i) covalent cuts after U₅₃, U₇₂ and U₇₆, well manifested in the conformer A, disappear in conformer B (Fig.3a,

Fig.3. Comparison of the cleavage sites of S₁ nuclease (▼) and CVE (▽) on the model of eukaryotic 5S RNA described by Nishikawa and Takemura [24].

A - conformer A; B - conformer B. Possible intramolecular interactions are indicated with dots (antiparallel interactions) and rings (parallel interactions) respectively.

b); ii) the region around U₆₉ becomes more accessible to CVE in the 5S RNA conformer B. We found similar differences with 3'-³²P-labelled 5S RNA preparations.

DISCUSSION

The situation we face here differs considerably from that of *E. coli* 5S RNA, described earlier. The urea-treated *E. coli* 5S RNA exhibits an altered accessibility toward various chemical and enzymatic probes and also migrates faster than the renatured one [7,8,21-23]. About 8 base-pairs should be melted out and re-

formed in a different way for the conformational transition of *E.coli* 5S RNA [10]. Here, however, we found that the 5S RNA conformers A and B exhibit identical electrophoretic mobilities, but exhibit distinct differences in their secondary structures. The fact that two apparently different conformers of an RNA may migrate together in a non-denaturing polyacrylamide gel should be seriously considered in similar studies elsewhere, since it shows that seemingly homogeneous RNA preparations may well be heterogeneous if more sensitive techniques are used. On the other hand, we found that rat liver 5S RNA conformers A and C, despite their different electrophoretic mobilities, do not differ in their nuclease accessibilities as significantly as conformers A and B. However, we cannot explain the obvious difference in the electrophoretic mobility of the conformers A and C with the differences in their S₁ nuclease and CVE cleavage patterns (Fig.2). It possibly implies that at least at these particular experimental conditions 5S RNA conformers A and C may differ in some more subtle way, changing its overall charge and/or shape, but leaving the principal base-pairing scheme of the molecule unaltered.

What intramolecular events actually take place during the conformational transition between rat liver 5S RNA A and B conformers? Above (see Materials and Methods) and in ref. [14] we, while interpreting our enzymatic cleavage results, used the model of 5S RNA by Nishikawa and Takemura [24]. Recently, a parallel tertiary base pairing interaction between the universal invariant GUA sequence at position 75-77 and the complementary CPuU sequence of eukaryotic and prokaryotic 5S RNA at positions 36-38 and 38-40, respectively, was proposed [25,26]. Alternative schemes with antiparallel tertiary base-pairing interactions have been suggested for *E.coli* 5S RNA [3,29-31]. Similar antiparallel tertiary interactions between nucleotides G41-C39 and U73-G75 could be constructed also for the rat liver 5S RNA. The digestion results of the 5S RNA conformer A with CVE do not exclude the existence of tertiary interactions between these regions of the rat liver 5S RNA molecule but do not distinguish between both kinds of interactions. This enzyme which has been shown to be sensitive to secondary and tertiary interactions [27,28],

cleaves strongly positions U72 and U76. Contradictorily, S_1 nuclease cleaves positions 35-41 in conformer A which should be protected partially both by the suggested parallel and anti-parallel tertiary interactions. Possibly these tertiary interactions are not stable under the conditions of S_1 nuclease digestion and therefore not detectable with this test. For the *E.coli* 5S RNA conformer B base pairing has been proposed between the sequences G33-A39 and U82-C88 [32], which are consistent with enzyme digestion studies [21]. Our enzyme digestion data agree with a structure of rat liver 5S RNA conformer B, where nucleotides C30-C36 and G87-G93 are base paired (the corresponding nucleotides are marked by dots in Fig.3b). In case of this tertiary interaction base pairs between nucleotides U84-G86 and G93-C91, and between C29-A32 and G48-U45, respectively, of the Nishikawa-Takemura model disappear. The resistance of the regions 27-36 and 87-90 of the 5S RNA conformer B and the high sensitivity of region 37-49 to S_1 nuclease cleavage are consistent with the proposed tertiary interaction.

Even though there is at present no strong indication whether the different conformational states of 5S RNA are functionally relevant (but see refs. [32-34], their occurrence and detailed characterization is still important since 5S RNA is a popular model compound to study RNA structure as well as RNA-protein in interactions.

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