New model of tertiary structure of plant 5S rRNA is confirmed by digestions with α-sarcin*

Miroslawa Z. Barciszewska[1], Siegfried Lorenz[2], Andrzej Joachimiak[1], Norbert Ulbrich[2], Volker A. Erdmann[2] and Jan Barciszewski[1]

[1]*Institute of Bioorganic Chemistry of the Polish Academy of Sciences, Noskowskiego 12, 61704 Poznan, Poland* and [2]*Institut für Biochemie, Freie Universität Berlin, Otto-Hahn-Bau, Thielallee 63, D-1000 Berlin 33, FRG*

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The cytotoxin α-sarcin was employed to test the model of secondary and tertiary structures of plant 5S rRNAs, which we recently proposed [(1990) Int. J. Biol. Macromol. (in press)]. α-Sarcin is a novel ribonuclease that hydrolyzes phosphodiester bonds adjacent to purines in nucleic acids [2–4]. The digestion pattern obtained for lupin and wheat germ 5S rRNAs strongly suggests the existence of tertiary interactions between residues C34, C35, C36, A37 and G85, G86, G87, U88 as previously proposed. The results on the secondary structure of plant 5S rRNA are in line with a previously proposed model [5]

Plant 5S rRNA; Tertiary structure model; α-Sarcin

1. INTRODUCTION

The ribosomal 5S RNA is an essential component of the large subunit of ribosomes occurring in almost all organisms [6]. The simple method of purification, along with fast techniques of determination of the nucleotide sequence make the 5S rRNA a very good molecule for biochemical, structural and evolutionary analysis [5,7]. The most important results of these studies have been primarily obtained through sequence comparisons and lead to generally accepted models of secondary structure for the 5S rRNAs of Eubacteria, Archabacteria and Eukaryotes [5,7]. Furthermore single- and double-stranded portions of the various 5S rRNAs structure have been identified by a number of enzymatic and chemical methods (for example, see [4–6, 8–17]).

We are interested in the structure and function of higher plant 5S rRNAs. With this goal in mind we have determined the primary structures of 9 different plant 5S rRNAs. Generally the secondary structure of plant 5S rRNAs seems to be very similar to that proposed earlier for eukaryotic 5S rRNAs [5,7]. However, detailed analysis of digestion patterns of wheat germ and lupin seed 5S rRNAs with S_1, T_1, mung bean and V_1 nucleases along with analysis of stable fragments of the 5S rRNAs, lead us to propose a new arrangement of structure for these molecules [1]. We would like to stress that tertiary interactions between parallel single-stranded portions of loops C and D are important points of this proposal. The aim of this work is to prove this model. As the probe for this study we have chosen α-sarcin, a novel ribonuclease, which cuts RNA chains at purine residues and preferentially in well accessible single stranded regions [2–4]. The enzyme seems to be quite sensitive to structural environment and can be used to study secondary and tertiary structures. The results presented in this communication strongly support our model of the three-dimensional structure of plant 5S rRNAs. The model also confirms previous findings concerning specificity of the enzyme and in addition proves that α-sarcin acts as a T_1 RNase-like enzyme. Precise analysis of the digestion patterns clearly shows that only guanosine residues located on the surface of the three dimensional model are digested.

Correspondence address: V.A. Erdmann, Institut for Biochemie, Freie Universitat Berlin, Otto-Hahn-Bau, Thielallee 63, D-1000 Berlin 33, FRG

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2. MATERIALS AND METHODS

Ribosomal 5S RNAs were isolated and purified from lupin seeds (*Lupinus luteus*) and wheat germ (*Triticum aestivum*) as described previously [7]. Labelling at the 3′ end was done with [5′-^{32}P]pCp (spec. radioact. 3000 Ci/mmol) and T4 ligase [18,19]. [5′-^{32}P]-labelled 5S rRNA was obtained by the exchange reaction using [γ-^{32}P]ATP, ADP and T4 polynucleotide kinase as described previously [18,19]. [^{32}P]-labelled 5S rRNAs were purified by 10% polyacrylamide gel electrophoresis with 7 M urea, eluted from the gel and renaturated [9]. The cytotoxic ribonuclease α-sarcin was isolated as published [4].

Digestion of 5S rRNAs with α-sarcin

The [^{32}P]-labelled 5S rRNAs (30 000 cpm) and 2 μg of cold lupin or wheat germ 5S rRNA were incubated at 37°C for 5 min with 0.5–1.5 μg toxin in 10 μl of 30 mM Tris-HCl, pH 7.5, containing 1 mM DTT. The reaction was stopped by addition of an equal volume

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of sample buffer containing 7 M urea in TEB (50 mM Tris/borate, pH 8.3, 1 mM EDTA), 0.02% Bromophenol blue and 0.02% xylene cyanol. To locate the positions of the cleavage sites in the RNAs, random alkaline hydrolysis and limited T_1 RNase digestion were employed [20].

3. RESULTS

α-Sarcin has been used widely to study ribosomal RNAs [4,21–25] including 5S rRNAs [4,21]. However, the fungus originating nuclease has not been tested so far on RNAs isolated from higher plants. Recently we proposed a new secondary and tertiary model for plant 5S rRNAs [1]. Since α-sarcin is sensitive to structural arrangements of the substrate [2–4] we use it as a tool to probe the structure in solution of two higher plant 5S rRNAs isolated from wheat germ and lupin seeds. Fig. 1 shows the digestion pattern of lupin [^{32}P]-labelled 5S rRNA with increasing amounts of α-sarcin. In general, the endonuclease cuts the 5S rRNA at the 3′ end of guanosine residues but occasionally it may also cut phosphodiester bonds at the 3′ end of adenosines as it was shown at the highest concentration of enzyme used (Fig. 1, line 6). It is clear that single-stranded guanosines are preferentially hydrolyzed with respect to those in double-stranded region. In fact all single-stranded guanosine residues of the 5S rRNA model except G66, G72, G85, G86, G87 and G99 are cleaved by α-sarcin. In addition several guanosines formally in double stranded segments are also hydrolyzed by the enzyme. These cuts are located in potentially destacked regions of double helix; G17 and G64 are on both sides of the U63 bulge (Fig. 2). Similarly G30, G48 and G51

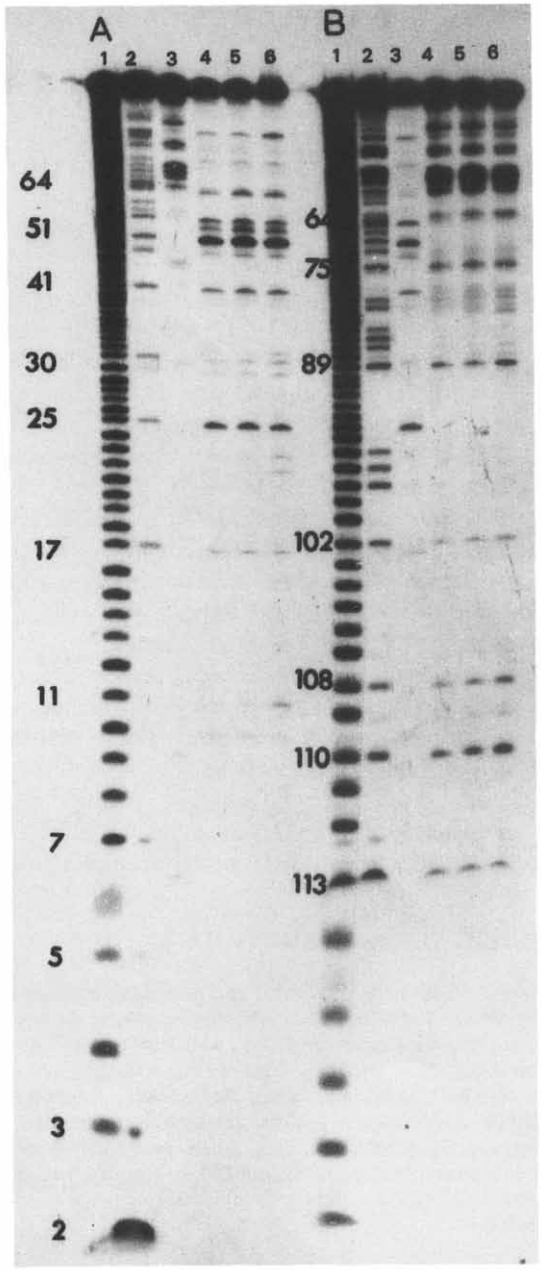

Fig. 1. Autoradiogram of 20% polyacrylamide gel electrophoresis of α-sarcin digestion of *Lupinus luteus* 5S rRNA labeled at 5′ end (A) and 3′ end with longer electrophoresis (B). Lanes: (1) alkaline ladder; (2) T_1 RNase digest; (3) control; (4) 0.5 μg sarcin, 5 min, 37°C; (5) as in (4) but with 1 μg sarcin; (6) as in 4 but with 1.5 μg sarcin.

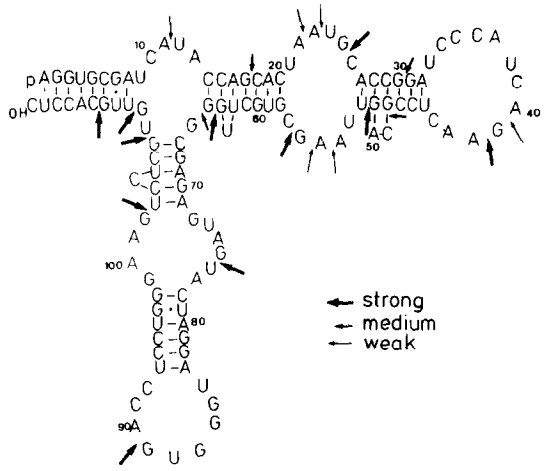

Fig. 2. Secondary structure model of lupin 5S rRNA (1) with α-sarcin cleavage sites as derived from the results of Fig. 1.

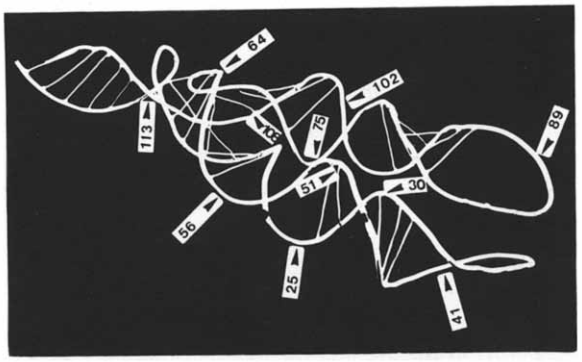

Fig. 3. Tertiary structure model of lupin 5S rRNA (1) showing guanylic acid residues hydrolysed by α-sarcin. Weak cleavage sites obtained for several adenosine residues are omitted.

are in proximity of C49, A50 bulge, while G113 is next to a G-U base pair stacked between two pyrimidine residues. At high α-sarcin concentration observed hydrolysis of adenosine phosphodiester bonds is restricted to single-stranded regions of the 5S rRNA molecule (Figs 1 and 2). In our analysis only the primary cuts in the RNA molecules were considered. Fig. 2 summarizes the results presented in Fig. 1 and the α-sarcin digestion sites are included in the secondary structure model of lupin 5S rRNA [1]. There are strong and medium cleavage sites specific for guanosines and weak for adenylic acid. The last ones were omitted from Fig. 3 where only G-specific hydrolysis are shown. It is clear from this figure that all cleavages of the 5S rRNA molecule occur on its surface, which should be easily reached by the α-sarcin. On the other hand all single-stranded guanosines which which are not hydrolyzed by the enzyme are hidden between β and λ domains of the three-dimensional model. The results shown in Figs 1–3 were obtained with lupin seed 5S rRNA. Similar studies performed with wheat germ 5S rRNA gave identical results (data not shown).

4. DISCUSSION

In order to test the new model of secondary and tertiary structure of plant 5S rRNAs, which was recently proposed [1], we have been looking for experimental tools, which should help us to address the question about tertiary interactions within these molecules. Based on limited digestions of plant 5S rRNAs with S_1 and V_1 nucleases we proposed tertiary interactions between parallel residues C34–G85, C35–G86, C36–G87 and A37–U88 [1]. Thus the cytotoxin α-sarcin, a purine and structure specific enzyme [2–4] seemed to be a very good choice for these extended studies. Here we report results of secondary and tertiary structure analysis of wheat germ (data not shown) and lupin seed 5S rRNAs in solution. To avoid misinterpretation of possible secondary cuts, analysis was done with RNA labeled at 3′ and 5′ ends. It is very interesting that nucleosides 85–88 are not digested at all even at the higher nuclease concentration. This result can be interpreted as the effect of strong tertiary interactions between residues 34–37 and 85–88. The digestion pattern obtained with α-sarcin is very similar to that of T_1 RNase [1]. As expected from the model, residues G42 and G89 occurring in single-stranded regions, were not hydrolyzed. These observations, together with other cleavage sites (Fig. 2) enabled us to conclude that α-sarcin hydrolyses primarily guanosine residues in single-stranded regions and as in the case of G51, G64 and G113, those present within destabilized portions of secondary structures. Our results concerning α-sarcin specificity, shown in Fig. 1 are in full agreement with previous studies [2–4]. Alpha sarcin is a G-specific nuclease, which may hydrolyse at high concentrations also single stranded adenosines.

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