HIGH-MOLECULAR-WEIGHT rRNAs OF COTTON SEEDS

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The electrophoretic mobilities of the 25S and 18S rRNAs of cotton seeds in polyacrylamide gel have been studied. A pyrimidyl-RNase hydrolysate of the high-molecular-weight rRNAs was separated into isopleths containing up to decanucleotides. The mono-, di-, and trinucleotide isopleths were separated, respectively, into CP and Up; ApCp, GpCp, ApUp, and GpUp; ApApCp, GpApCp, ApGpCp, ApApUp, GpGpCp, ApGpUp + GpApUp, and GpGpUp on a KhZh 1305 microcolumn liquid chromatograph.

Ribosomal RNAs of seeds of the cotton plant *Gossypium hirsutum* have been isolated from the ribosomes by phenol-detergent deproteination. The ribosomes were isolated from the postmitochondrial supernatant of a homogenate of defatted cottonseed flour [1–4]. The high-molecular-weight rRNAs (hm rRNAs) were separated from the low-molecular-weight ones. The UV spectra of the preparations of hm rRNAs obtained corresponded to the characteristic UV spectra of RNA preparations freed from protein and polysaccharide impurities. The spectral characteristics OU_{260}:OU_{230} and OU_{260}:OU_{280} amounted to 2.4-2.6 and 2.1-2.2, respectively.

The dependence of the electrophoretic mobilities of the 25S and 18S rRNAs of cotton seeds on the concentration of acrylamide in the polyacrylamide gel (PAG) and the dependence of the resolution of the RNA zones on the time of electrophoresis were studied by gel electrophoresis [5, 6]. The results obtained agree well with those given in the literature [5–8] for hm rRNAs isolated from pea shoots, radishes, and maize. Figure 1 shows patterns of the electrophoretic separation of the hm rRNAs of cotton seeds in PAG containing 2.8% of acrylamide, which has proved to be effective for studying the hm rRNAs of the seeds of the cotton plant. The 2.8% PAG possesses a rigidity that is convenient for manipulation and gives good electrophoretic resolution of rRNA zones even when up to OU_{260} of hm rRNA is deposited on one PAG tube (6 × 90 mm). In the case of electrophoresis in buffer B (see the Experimental part), it is possible to deposit up to 3 OU_{260} of hm rRNA.

Electrophoresis showed that the bulk of the hm rRNAs was made up of the 25S and 18S rRNAs while there was a very small amount of RNAs with an electrophoretic mobility similar to that of the 23S and 16S rRNAs (the origin of which has not yet been established), and trace amounts of mRNAs and of 5S rRNAs + tRNAs. This shows the undegraded state of the 25S and 18S rRNA isolated from cotton seeds [8, 9].

The high-molecular-weight rRNAs of cotton seeds were hydrolyzed with Reanal 4X crystallized pancreatic RNase [10]. The pyrimidyl-RNase hydrolysate of the hm rRNAs was separated into isopleths by microcolumn liquid chromatography on a KhZh 1305 instrument [11, 12]. The chromatogram showed that the enzymatic hydrolysate was separated up to the isopleth containing decanucleotides (Fig. 2a).

The chromatographic separation of the mononucleotides into Cp and Up was carried out by methods previously described [13, 14] (Fig. 2b). The di- and trinucleotides were separated on a column (1 × 45 mm) of DEAE-cellulose by the method of V. V. Vlasov and D. G. Knorre [12, 15] (Fig. 3). In agreement with the elution sequence, the dinucleotides were separated into ApCp, GpCp, ApUp, and GpUp, and the trinucleotides into ApApCp, GpApCp, ApGpCp, ApApUp, GpGpCp, ApGpUp + GpApUp, and GpGpUp. The oligonucleotides were identified from their charges, UV spectra, and nucleotide compositions [11, 16, 17].

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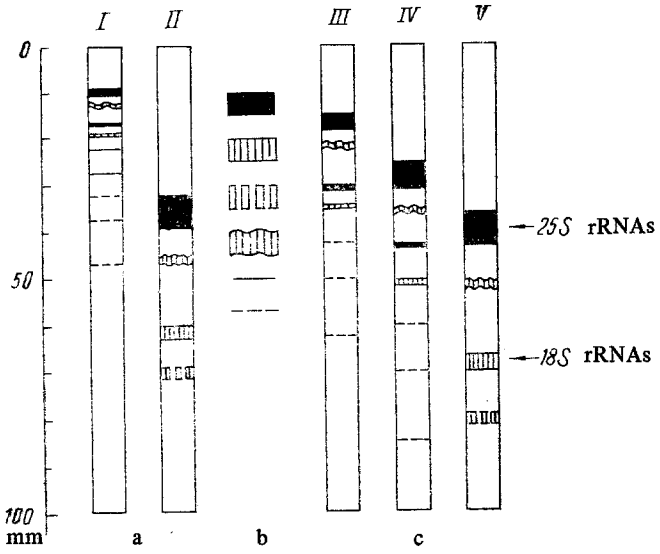

Fig. 1. Electrophoresis in 2.8% PAG of the hm rRNAs of cotton seeds in buffers A (a) and B (c) (b shows the degree of staining of the RNA zones; the symbols from top to bottom are arranged in order of weakening coloration): I) time of electrophoresis 60 min; II) 300 min; III) 140 min; IV) 190 min; V) 340 min.

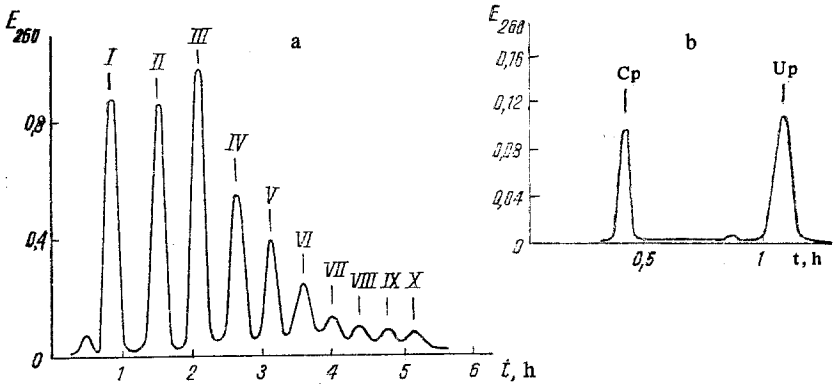

Fig. 2. Microcolumn chromatographic separation into isopleths of a pyrimidyl-RNase hydrolysate of the hm rRNAs of cotton seeds on a KhZh 1305 instrument (a), and the microcolumn chromatography of the mononucleotides (b): I) mononucleotides; II) dinucleotides; III) trinucleotides, and so on.

EXPERIMENTAL

Isolation of the High-Molecular-Weight (hm) rRNAs. The cytoplasmic ribosomes were isolated from the postmitochondrial supernatant of a homogenate of defatted cottonseed flour by Mg^{2+}-ethanolic precipitation without an increase in the concentration of KCl to 0.5 M [1-4]. The total rRNAs isolated from the ribosomes by phenol-detergent (sodium dodecyl sulfate – SDS) deproteination (twice) were precipitated from an aqueous phase with three volumes of 96% ethanol cooled to –20°C. The precipitate was collected by centrifugation and was washed with ethanol. The ethanol-washed precipitate was dissolved in 0.15 M CH_3COONa buffer, pH 6, with 0.5% of SDS, and from this solution the total rRNAs were again precipitated with three volumes of ethanol cooled to –20°C. The precipitate was collected by centrifugation and was suspended in 3 M CH_3COONa, pH 6, solution, and the suspension was left in the refrigerator at –20° for 12 h. The precipitate of hm rRNAs that had deposited was

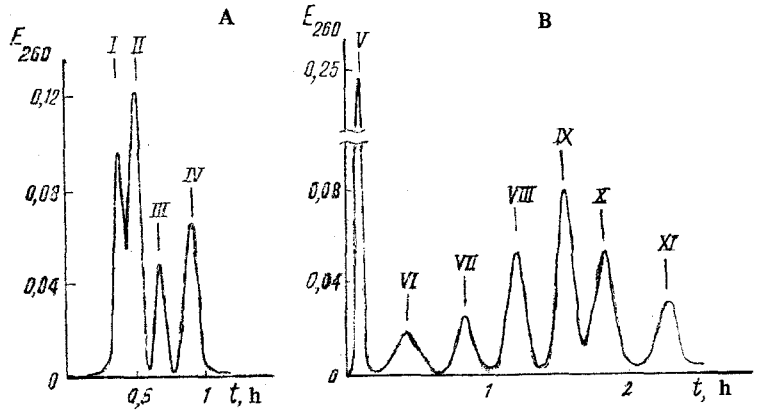

Fig. 3. Microcolumn chromatograph separation of the dinucleotides (A) and trinucleotides (B) on the KhZh 1305 instrument: I) ApCp; II) GpCp; III) ApUp; IV) GpUp; V) ApApCp; VI) GpApCp; VII) ApGpCp; VIII) ApApUp; IX) GpGpCp; X) AgGpUp + GpApUp; XI) GpGpUp.

collected by centrifugation and was again suspended in 3 M CH_3COONa solution, pH 6. The suspension was left in the refrigerator. This extraction of the low-molecular-weight rRNAs was performed three times. Then the precipitate of hm rRNAs was dissolved in 0.15 M CH_3COONa, pH 6, with 0.5% of SDS, three volumes of ethanol were added to the solution, and the mixture was left in the refrigerator for storage at −20°C [6]. The yield of the preparation of hm rRNAs amounted to 0.12–0.13% of the dry weight of the defatted cottonseed flour.

Electrophoresis in PAG. Acrylamide was recrystallized from chloroform. Bisacrylamide was recrystallized from acetone. The gels were prepared by U. E. Loening's method [5] in glass tubes (6 × 100 mm). The precipitate of hm rRNAs was collected by centrifugation from the storage solution and was washed twice with 75% ethanol containing 0.5% of SDS and dried in vacuum. Then it was dissolved in the electrophoresis buffer to give a concentration of 100 OU_{260} ml, and sucrose was added to a concentration of 10%. The electrophoreses were performed in two buffer solutions — A and B [5, 6]. Buffer A consisted of 0.036 M Tris, 0.03 M NaH_2PO_4, and 0.001 M Na_2EDTA, pH 7.8, and buffer B of 0.04 Tris, 0.02 M CH_3COONa, and 0.002 M Na EDTA, pH 7.8 (glacial CH_3COOH was added at room temperature). Pre-electrophoresis was performed at a current strength of 5 mA per tube of gel for 30 min, and then 20 μl of the prepared solution of hm rRNAs was added to each of the gels. Electrophoresis was performed at a current strength of 5 mA per tube of gel for the necessary time. The gels were extracted from the tubes after they had been separated from the walls of the tubes by spraying with water. The RNA zones in the gels were stained by Peacock and Dingman's method [18].

The microcolumn liquid chromatography of the pyrimidyl-RNase hydrolysate of the hm rRNAs was performed on a KhZh 1305 instrument. A 7 M solution of urea was freed from ionic impurities on columns containing cation- and anion-exchange resins. The pyrimidyl-RNase hydrolysis was carried out in a buffer solution containing 0.01 M Tris-CH_3COOH, 0.2 M CH_3COONa, pH 7.5, using an enzyme–substrate ratio of 1:200 (v/v) at 37°C for 40 h [10]. After the end of incubation, the hydrolysate was diluted with a 20-fold volume of 7 M urea solution. A 1 × 45 mm column containing DEAE-cellulose was equilibrated with a buffer solution of 0.02 M Tris-HCl in 7 M urea, pH 7.55. On the column was deposited 0.1 OU_{260} of the pyrimidyl-RNase hydrolysate of the hm rRNAs. The chromatographic separation of the isopleths was carried out with a gradient concentration of NaCl of from 0 to 0.3 M in a buffer solution of 0.02 M Tris-HCl in 7 M urea, pH 7.55 [1, 12, 19].

The microcolumn chromatographic separation of the mono- di-, and trinucleotides was performed by methods described previously [12–15]. Each oligonucleotide peak was collected separately and was desalted on a column (1 × 20 mm) containing DEAE-cellulose in the bicarbonate form. The desalted oligonucleotides were hydrolyzed in 0.3 N KOH at 37°C for 18 h into the 3'(2')-ribomononucleotides. The nucleotide compositions of the oligonucleotides were determined by the chromatographic separation of the alkaline hydrolysate corresponding to them into individual 3'(2')-ribomononucleotides as described in the literature [13, 14].

SUMMARY

The electrophoretic mobilities of the 25S and 18S rRNAs of cotton seeds in PAG have been studied.

The microcolumn liquid chromatography of a pyrimidyl-RNase hydrolysate of the hm rRNAs of cotton seeds on a KhZh 1305 instrument has been performed.

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