COMPARATIVE STUDY OF THE TOTAL tRNAs OF THE SEEDS OF THE COTTON PLANT AND THE SEEDS OF Ginkgo biloba

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For the purposes of a comparative study of the tRNAs from higher plants, we have isolated and fractionated the total tRNAs from the seeds of the cotton plant and the seeds of Ginkgo biloba Z. (ginkgo, family Ginkgoaceae). The comparison of the chromatographic behaviors of the tRNAs from the two materials was made on the methionine and phenylalanine tRNAs. In the process of fractionating the total tRNAs of the cotton plant on a column of BD-cellulose using the homologous aminoacyl-tRNA-synthetases (ARSases) in the analysis of the tRNA activity, three peaks of methionine-acceptor activity that were eluted in the 0.4-0.7 M NaCl range were detected. Acylation of the fractions with [^{14}C]methionine with the aid of the ARSases from E. coli showed only one peak of methionine-acceptor activity, which apparently corresponds to initiator tRNA^{Met}.

When the tRNAs from ginkgo seeds were separated under the same conditions, again three peaks of methionine activity were revealed, but only the first was acylated with the participation of the E. coli ARSases.

The fractionation of the tRNAs from cotton seeds showed no less than four poorly resolved peaks of phenylalanine-acceptor activity. The elution profile of the tRNAs from ginkgo seeds showed only two peaks accepting phenylalanine.

The fractions accepting phenylalanine were eluted at high salt concentrations, in the range of 1.1-1.8 M NaCl, in the separation of the tRNAs both of the cotton plant and of ginkgo. This is evidence in favor of the assumption that the tRNA^{Phe} from both materials contains strongly hydrophobic groupings similar to the nucleosides Y and peroxy-Y found in the tRNA^{Phe} of a series of plant and animal materials [1-4].

The results that we have obtained are similar to those given in the literature. The existence of three isoacceptor forms of tRNA^{Phe} has been reported in the fractionation of the total rRNA from cotton-plant shoots [5]. Two isoacceptor forms of cytoplasmatic tRNA^{Phe},s and one form of tRNA^{Met} have been found in an investigation of the tRNA from cotton seeds [6].

The additional peaks that we isolated may be true isoacceptors or conformers, productors of incomplete post-transcription modification, or products of the splitting out of the terminal adenosine from the 3'- end of the tRNA.

EXPERIMENTAL

UV absorption was measured on a Spektromom-204 spectrophotometer (Hungary). Radioactivities were counted in a LS-100C liquid scintillation counter (Beckman, U.S.A.) with a counting efficiency for ^{14}C of 65%.

The following reagents were used: [^{14}C]phenylalanine, 315 mCi/mmole, UVVVR, Czechoslovakia; [^{14}C]-methionine, 10 mCi/mmole, Poland. Toluene or xylene of "scintillation" grade. The BD-cellulose was synthesized by the method of Gillam and Tener [7], and DEAE-cellulose from the firm Reanal (Hungary) and yeast RNA from Olaine were used, the other reagents being of kh.ch. ["chemically pure"] or ch.d.a. ["pure for analysis"] grade.

Isolation of the tRNAs. The total tRNAs from cotton seeds were isolated by a known method [8]. To isolate the total tRNAs from ginkgo seeds, the seeds were freed from hulls, ground in a mortar in previously cooled acetone, and filtered off with suction on a Buchner funnel. Then the seeds were again covered with cold acetone and ground, and the acetone was sucked off. The powder of the seeds was dried in the air and was passed through a sieve (0.25 mm). The tRNAs were isolated as described for cotton seeds [8] using 0.14 M NaCl as the aqueous phase. The volume of the aqueous phase was three parts to one part of seed powder. The yield of total tRNA was 50-70 mg per 1 kg of dry seed powder.

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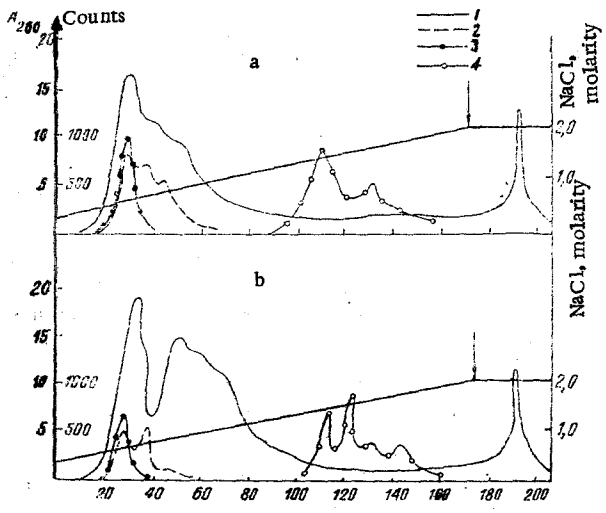

Fig. 1. Fractionation of ginkgo tRNA (a) and of cotton tRNA (b) on a column of BD-cellulose: 1) optical density at 260 nm; 2) acceptor activity for methionine with the homologous ARSases; 3) acceptor activity for methionine with E. coli ARSases; 4) acceptor activity for phenylalanine. The arrows show the beginning of elution with 2 M NaCl in 20% ethanol.

A crude preparation of the ARSases from cotton seeds was obtained by a method that we have described previously [9]. In analyzing the activity of the tRNAs of ginkgo seeds, we used the preparation of ARSases from cotton seeds. The total ARSases of E. coli were isolated by the method of Kelmers et al. using the nuclease-free strain MRE-600.

Fractionation of the tRNAs. A column with dimensions of 1.2 × 40 cm was filled with BD-cellulose equilibrated with 0.25 M NaCl, 10 mM $MgCl_2$, and about 3000 optical units of tRNAs were deposited on it. Elution was carried out with a gradient of 0.25-2.0 M NaCl, 10 mM $MgCl_2$, and then with 2.0 M NaCl in 20% ethanol. The total volume of gradient was 1.7 liters, and the rate of elution 0.8 ml/min.

To analyze the activity of the fractions from the column of BD-cellulose, we used the following method, which enables the influence of high concentrations of NaCl on the degree of aminoacylation to be excluded. Aliquots of the fractions to be analyzed were taken, and a solution of yeast RNA was added to a final concentration of 5-6 OU/ml. Then 2.5 volumes of ethanol was added and the mixture was left in the cold for 2-3 h. After centrifuging, the supernatant was decanted off and the precipitate was dissolved in buffer for aminoacylation. The further analysis was carried out as described previously [9].

SUMMARY

1. Three methionine tRNAs have been found both in the total tRNAs of cotton seeds and in those of Ginkgo biloba. In both cases only the first was acylated by methionine with the aid of E. coli ARSases.

2. Four phenylalanine tRNAs have been found in the total tRNAs of cotton seeds, and two in the tRNAs of ginkgo seeds.

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