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Short Communication

MIROSŁAWA Z. BARCISZEWSKA, JACEK KRAJEWSKI, IWONA GAWROŃSKA and JAN BARCISZEWSKI

RAPID SEPARATION OF TYROSINE-SPECIFIC tRNA FROM WHITE LUPIN*

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12, 61-704 Poznań, Poland

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During isolation of total ribonucleic acids from white lupin (*Lupinus albus*) and their subsequent separation by 10% polyacrylamide gel electrophoresis, a fast migrating RNA band is very well separated. The nucleotide sequence analysis of 76 nucleotide long sequence with many modified nucleosides was found to be identical with that of tyrosine specific tRNA of yellow lupin seeds (*Lupinus luteus*) and wheat germ (*Triticum aestivum*). Also this $tRNA^{Tyr}$ is identical with plant amber suppressor tRNA. The presented approach offers a very rapid method of purification of plant tRNA with UAG suppressor activity.

Presently about 40 primary structures of various plant tRNAs and different specificity are known [1]. These specific tRNAs in large quantities usually were purified on benzoylated diethylaminoethyl cellulose (BD-cel-

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lulose), DEAE Sephadex A-50, Sepharose 4B or by reversed phase column (r.p.c.) chromatography [2, 3].

The results presented demonstrate the possibility to avoid in some cases this long procedure for tRNA purification as exemplified by $tRNA^{Tyr}$ of white lupin.

MATERIALS AND METHODS

White lupin seeds were germinated for 10 days, RNA was extracted by phenol methods and separated on the 10% polyacrylamide gel in 7 M urea [4]. The gel was stained and tRNA band eluted as described earlier [5]. Finally tRNA was purified on hot sequencing gel (15% polyacrylamide gel, 7 M urea, 2000 V, 60°C). 5 μg of tRNA was dissolved in 10 μl of 80% formamide and heated for 5 min at 80°C. The tRNA hydrolyzate was dried and labeled with 100 μCi of [γ-^{32}P]ATP and T4 kinase. The oligonucleotides labeled at 5′-end were separated by two-dimensional gel electrophoresis as previously described [5], and the 5′-labeled nucleotides were liberated with P_1 nuclease and analysed on t.l.c. [5].

RESULTS AND DISCUSSION

As can be seen from Fig. 1 in denaturating conditions various species of RNA are separated as distinct bands, e.g. ribosomal 5S RNA, 5.8S RNA, small nuclear RNAs (e.g., U6 SnRNA) and tRNAs. Also one can notice a fast migrating band in the tRNA region of the gel (Fig. 1). The band was cut off and RNA eluted. For the sequence analysis, tRNA material was additionally purified by denaturing 15% polyacrylamide gel electrophoresis (7 M urea, 2000 V, 60°C). Determination of the primary tRNA structure was based on partial alkaline hydrolysis.

A short time (5 min) heating of RNA in 80% formamide (10 μl) revealed all fragments having a free 5′-hydroxyl group. The ^{32}P-labeled oligonucleotides were separated on polyacrylamide gel [5] and analysis of the 5′-nucleotide of each fragment allowed reconstruction of the entire tRNA molecule. The nucleotide sequence of *Lupinus albus* RNA of 76 residues long was found to be identical with that of tyrosine specific tRNA of yellow

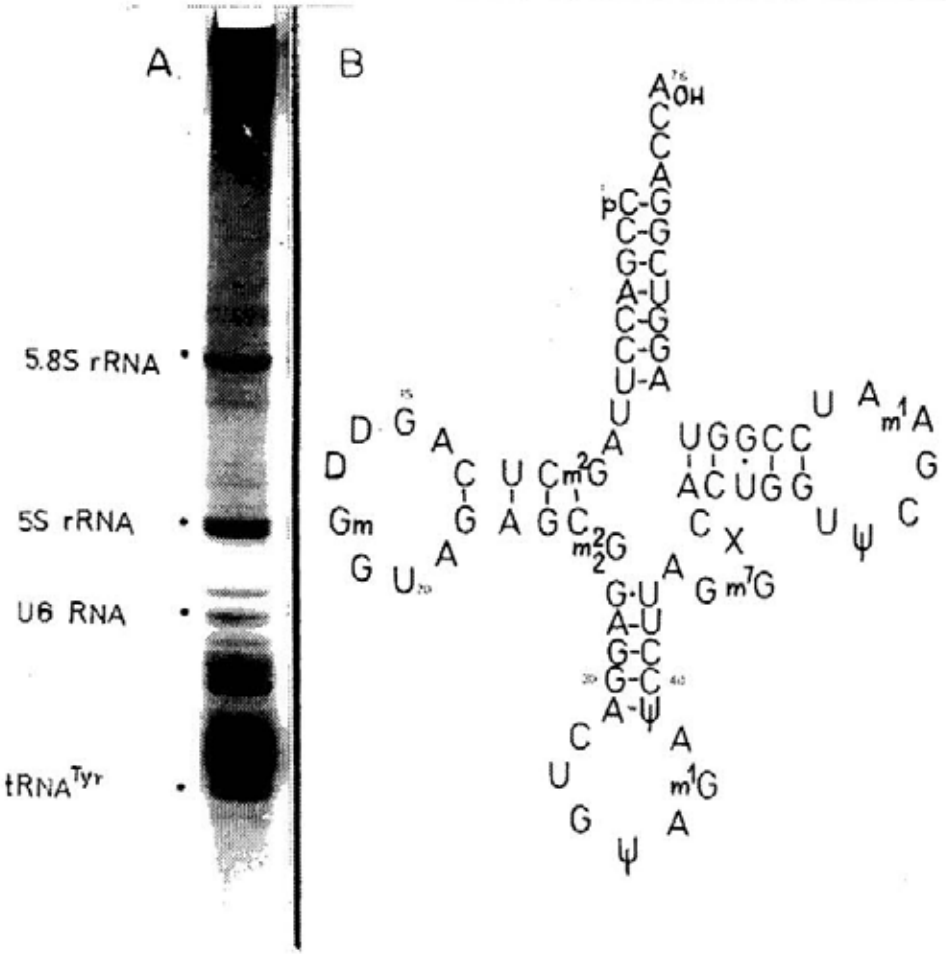

Fig. 1. A. Polyacrylamide gel electrophoresis of crude *Lupinus albus* RNAs; B, the cloverleaf model of secondary structure of white lupin $tRNA^{Tyr}$. The nucleoside X or acp^3U is 3-(3-amino-3-carboxypropyl) uridine

lupin seeds (*Lupinus luteus*) and wheat germ (*Triticum aestivum*) [1]. In all eukaryotic organisms two tyrosine tRNA isoacceptors are known to occur [6]. The difference between them results from a posttranscriptional modification of guanosine residue in the first position of the anticodon. The $tRNA^{Tyr}$ of *Lupin albus* analyzed here is identical to the plant lupin tRNA with GΨA anticodon [6]. Therefore the second $tRNA^{Tyr}$ isoacceptor of *L. albus* with nucleotide Q is migrating much slower. Since the band fast migrating on the 10% gel contains only one $tRNA^{Tyr}$ species, the presented

procedure offers an efficient method for purification of plant tRNA with UAG suppressor activity.

REFERENCES

1. Sprinzl, M., Hartmann, T., Weber, J., Blank, J. & Zeidler, R. (1989) *Nucleic Acids Res.*, **17**, r1.
2. Barciszewska, M., Joachimiak, A. & Barciszewski, J. (1989) *Phytochemistry*, **28**, 2039 - 2041.
3. Logermann, J., Shell, J. & Willmitzer, L. (1987) *Anal. Biochem.*, **163**, 16 - 20.
4. Barciszewski, J., Barciszewska, M., Suter, B. & Kubli, E. (1985) *Plant Sci.*, **40**, 193 - 196.
5. Barciszewska, M., Dirheimer, G. & Keith, G. (1983) *Biochem. Biophys. Res. Commun.*, **114**, 1161 - 1168.
6. Baier, H., Barciszewska, M. & Stickinger, H. (1984) *EMBO J.*, **3**, 1091 - 1096.