A SIMPLE PROCEDURE FOR THE PURIFICATION OF YEAST PHENYLALANINE TRANSFER RNA

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Received June 28, 1968

For the past several years we have been attempting to study the secondary and tertiary structure of tRNAs[+] by means of chemical probes. In the course of this work it became necessary to obtain a highly purified species of tRNA. For two reasons we chose to purify yeast Phenylalanine tRNA. The nucleotide sequence of this molecule has been completely worked out (RajBhandary and Chang [1968]). Secondly, a simple and highly effective partial purification of this species has been obtained by Gillam et al. (1967) on benzoylated DEAE cellulose. In this communication we report the use of BD-cellulose to complete the purification of $tRNA^{Phe}$.

Experimental

BD-cellulose was prepared, washed and packed into columns according to the methods described by Gillam et al. (1967). Unfractionated yeast soluble RNA was obtained from Schwarz Bioresearch, Orangeburg, N.Y. In some cases this crude RNA was submitted to ammonium sulfate fractionation (Lindahl and Fresco [1967]) before further use. [^{14}C]-Phenylalanine (U.L.) was obtained from New England Nuclear Corp. Amino acid acceptor assays were carried out essentially according to Scott (1968) except that glass

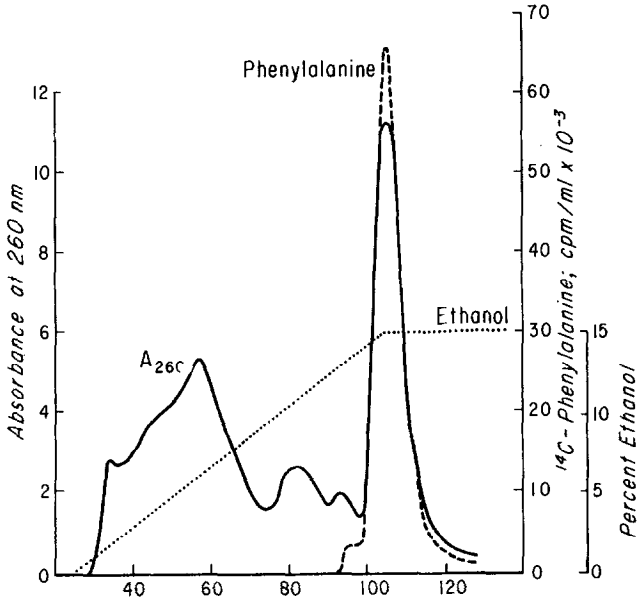
[+] Abbreviations used in this paper are the same as those used by RajBhandary, Stuart and Chang (1968) with the following additions: BD-cellulose, benzoylated DEAE cellulose; pmole, 10^{-12} mole.

fiber filters (Whatman GF/C) were used. The crude aminoacyl-tRNA synthetase was prepared by the method of Madison, Everett and Kung (1967). ^{14}C was counted in a liquid scintillation counter at an efficiency of 75%.

As a first step in the purification procedure about 70,000 A_{260} units of commercial tRNA were fractionated on a 4X90 cm BD-cellulose column according to the method of Gillam et al. (1967). In agreement with these authors, we found that $tRNA^{Phe}$ remained on the column during elution with a salt gradient but finally emerged as a sharp peak when the column was washed with 1M NaCl-.01M Mg SO_4 in 10% ethanol.

The partially purified $tRNA^{Phe}$ from the large BD-cellulose column was recovered by ethanol precipitation and redissolved in a small volume of water. Portions of this material were aminoacylated with [^{14}C]-Phenylalanine (1.6 mc/mmole) after a series of small scale experiments had been run to determine the conditions for optimum charging. [^{14}C]-Phenylalanyl tRNA was freed of protein by phenol extraction (followed by ether extraction to remove phenol) or by adsorption to and elution from DEAE-cellulose at pH 4.5 and 4°C. After alcohol precipitation the recovered Phenylalanyl tRNA was dissolved in a small volume of starting buffer (0.80M NaCl- 0.01M Na Acetate, pH 4.5-0.02M Mg SO_4) and applied to a 1.2x84 cm column of BD-cellulose at room temperature. After washing with 150 ml of starting buffer the column was eluted with a linear gradient comprised of 200 ml starting buffer and 200 ml limit buffer (1.0M NaCl- 0.01M Na Acetate, pH 4.5- 0.02M Mg SO_4 in 15% ethanol). Finally the column was eluted with limit buffer until the A_{260} of the eluate fell to < 0.5.

The flow rate was about 55 ml/hr; collection of 5 ml fractions was begun when elution with the linear gradient was commenced. The elution profile of a typical chromatographic purification is shown in the Figure. Appropriate fractions were combined, made 1.0 M in Tris-Cl^-, pH 8.0, incubated at 37°C for 30 minutes to deacylate the tRNA, and desalted on Sephadex G-25. The $tRNA^{Phe}$ was recovered by lyophilization.



Chromatography of Phenylalanyl t RNA on BD-cellulose

T_1 RNAse digestion and fractionation of the T_1 oligonucleotides from $tRNA^{Phe}$ on a DEAE - cellulose - urea column was performed essentially as outlined by RajBhandary, Stuart and Chang (1968) except that the eluting system contained .02M M Tris-borate, pH 8.0 instead of .02M Tris-Cl, pH 7.5.

Results and Discussion

In different experiments the Phenylalanine acceptor activity of tRNA recovered from the first BD-cellulose column ranged from 250-500 pmoles/A_{260} unit. Material of higher purity was obtained when the commercial RNA was fractionated with ammonium sulfate prior to chromatography.

The Phenylalanine content per A_{260} unit in the final peak to emerge from the second BD-cellulose column was constant across the peak. It varied from 1600-2000 pmoles/A_{260} unit in different experiments. Assuming an extinction coefficient of 21 A_{260} units per milligram, this corresponds to a purity of 80-100%. This value for the extinction coefficient is the value

cited by Lindahl and Fresco (1967) for unfractionated tRNA in 0.15M KCl-0.01M cacodylate - 0.005M $Mg\ Cl_2$ - 0.0005M EDTA, pH 7.0. To obtain our A_{260} values we diluted an aliquot of each column fraction appropriately with water.

A preliminary study on the oligonucleotide ratios of an RNAse T_1 digest of our $tRNA^{Phe}$ confirms that this material is of high purity. Thus we find that the dodecanucleotide Ap2'OMeCpUp2'OMeGpApApYpψp5-MeCpUpGp corresponding to peak 17 of RajBhandary, Stuart and Chang (1968) accounts for 13.9% of the total A_{260} units recovered from the column. This compares with a value of 14.0% calculated from the data in Table I of RajBhandary, Stuart and Chang (1968).

A summary of our most recent and most successful purification is given in the Table. The yields are not always as high as those shown in this Table; heavy losses sometimes occur during the work-up of the amino-acylation mixture. It appears probable (G. M. Tener, personal communication)

Table Summary of Purification of $tRNA^{Phe}$

After purification step	total RNA (A_{260}units)	specific activity (pmoles/A_{260}unit)	total activity (μmoles)	percent yield
Ammonium sulfate	77,000	36	2.78	-
First BD-cellulose column	6,050	445	2.69	97
Second BD-cellulose column	515	1990	1.02	73*

* The yield shown here has been corrected for the fact that only one-half of the material isolated from the first BD-cellulose column was aminoacylated and chromatographed on the second BD-cellulose column.

that the phenol extraction or DEAE-cellulose steps used to free the aminoacyl-tRNA of protein prior to the second BD-cellulose column could be omitted without deleterious effects. This should greatly decrease the losses which may occur during the work-up of the aminoacylation mixture.

Acknowledgements

It is a pleasure to acknowledge the capable technical assistance of Joann Van Dolah.

This work was supported by grants from the National Science Foundation and the National Institutes of Health.

References

1. Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M., Biochemistry 6, 3043 (1967).
2. Lindahl, T., and Fresco, J. R., Methods in Enzymology 12A, 601 (1967).
3. Madison, J. T., Everett, G. A., and Kung, H., J. Biol. Chem. 242, 1318 (1967).
4. RajBhandary, U. L., and Chang, S. H., J. Biol. Chem. 243, 598 (1968).
5. RajBhandary, U. L., Stuart, A., and Chang, S. H., J. Biol. Chem. 243, 584 (1968).
6. Scott, J., Methods in Enzymology (1968), In press.