# ISOLATION OF THE TOTAL tRNA FROM COTTON SEEDS

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A number of methods of isolating tRNA from the cotton plant have been described in the literature [1, 2], but they are unsuitable for obtaining tRNA in large amounts. To isolate tRNA from cotton seeds we have tested various methods: I — the phenol method using the following compositions of the aqueous extractant: 1) water; 2) 0.14 M NaCl; 3) sodium acetate buffer, pH 4.5, in the presence of sodium dodecyl sulfate; and II — a nonphenolic method of extraction with a hot solution of dodecyl sulfate (DDS), which gives good results in the isolation of tRNA from microorganisms [3]. The tRNA isolated was characterized spectrophotometrically, and also from its amino-acceptor activity for [$^{14}$C]phenylalanine.

From a comparison of the yields, spectrophotometric characteristics, and results of aminoacylation, it follows that all the phenolic methods are superior to the nonphenolic method in relation to the amount and quality of the total tRNA studied (Table 1). This can be seen particularly clearly from a comparison of the amino-acceptor activity of the tRNAs obtained.

It is likely that disruption or some other irreversible changes take place in the structure of the tRNA in its isolation by hot detergent. The suggestion that the tRNA is denatured in the extraction process was not confirmed, since after incubation of the tRNA preparation at 65°C in the presnece of magnesium ions its acceptor activity did not increase.

We tested the method of hot extraction on bakers' yeast and pea seeds. In the case of the yeast, the preparation did not differ appreciably in its characteristics from the tRNA obtained from the same material by the phenol method, and the amino-acceptor activity for [$^{14}$C]phenylalanine even rose somewhat: phenolic extraction — 26.6 pmole/o.u; hot extraction — 28 pmole/o.u.

In the case of the tRNA from peas, isolated by the nonphenolic method, the acceptor activity was, as in the case of the cotton plant, very low — 2.2 pmole/o.u. It may be concluded that this convenient method without the use of phenol is unsuitable for higher plants, unlike microorganisms.

On the basis of what has been said above, for the preparative isolation of tRNA we used the extraction of a powder of the seeds with a mixture of phenol and 0.14 M NaCl. In the isolation of tRNA from kilogram amounts of the initial raw material, considerable difficulties were caused by the necessity for centrifuging large volumes of extracts. Consequently, in this case we used the separation of the aqueous phenol into layers described by Monier et al. [4]. For additional purification we used a SS-40 flow-through supercentrifuge after treatment of the aqueous phase with chloroform. The chloroform treatment facilitates the centrifuging in the supercentrifuge, since it eliminates phenol from the aqueous phase and thereby leads to an increase in the difference in densities of the supernatant and the protein precipitate. Furthermore, in the process of treatment with chloroform part of the protein collects at the boundary of separation between the chloroform and the aqueous phase and is eliminated, as a result of which the aqueous layer is additionally clarified.

When the SS-40 centrifuge was used, two phenol treatments were usually sufficient for satisfactory deproteination.

Treatment of the ethanolic precipitate after extraction with 2 M NaCl was used to eliminate highly polymeric RNAs. For the further purification of the preparation from protein and polysaccharide impurities and from pigments we used the sorption of the RNA in bulk from 0.1 M NaCl solution on to DEAE-cellulose followed by desorption with 2 M NaCl [5]. The treatment of the RNA in the bulk permits the time of purification to be shortened severalfold in comparison with the usual purification on a column of DEAE-cellulose.

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TABLE 1. Comparison of the Total tRNAs Isolated by Various Methods from Cotton Seeds

| Index | Phenolic extraction, composition of the aqueous phase | | | Hot extraction |
|---|---|---|---|---|
| | acetate buffer | 0.14 M NaCl | water | |
| Yield per 100 g, mg | 13 | 22,5 | 17,3 | 20 |
| pmole o.u. | 16,5 | 21,6 | 18.2 | 4.1 |
| $\frac{E_{260}}{E_{230}}$ | 2,0 | 2,0 | 1,95 | 1,90 |
| $\frac{E_{260}}{E_{280}}$ | 1,96 | 1,96 | 1,96 | 1,98 |

The yield of total tRNA after the final stage of purification was 200-300 mg per 1 kg of powdered seeds.

According to gel electrophoresis in 7% polyacrylamide gel, the preparation of total tRNA contained about 13% of 5S RNA and about 30% of high-molecular-weight RNAs.

## EXPERIMENTAL

UV absorption was measured on a Spektromom-204 spectrophotometer (Hungary). In the calculations it was assumed that the absorption of a solution of tRNA with a concentration of 1 mg/ml at 260 nm ($A_{260}$) was 24.

Radioactivities were counted in an LS-100C liquid scintillation counter (Beckman, USA) with a counting efficiency in respect to $^{14}C$ of 65%. The acceptor activity was expressed in picomoles of $^{14}C$-labeled amino acid added to one optical unit of tRNA.

The following reagents were used: DEAE-cellulose (Olaine), os.ch. ["particularly pure"] of "scintillation" toluene or xylene, and [$^{14}C$]phenylalanine 315 mCi/mmole UVVVR, Czechoslovakia. The other reagents were of kh.ch. ["chemically pure"] or ch.d.a. ["pure for analysis"] grade.

Isolation of tRNA by the Phenol Method. Comminuted cotton seeds were defatted by repeated treatment with acetone and were passed through a 0.25-mm sieve. The dry powder was mixed with 8-10 parts of an aqueous phase (water, 0.14 M NaCl, or sodium acetate buffer, pH 4.5) and with 4 parts of water-saturated phenol. Cotton seeds contain a large amount of reserve proteins and therefore to improve separation of the mixtures into layers we used large volumes of extractants. The mixtures were homogenized in a Waring-type homogenizer for 3-5 min and were shaken for 1-1.5 h. Then the mixture was left to settle at 4°C for 48 h. The upper phase was taken off, 0.5 volume of chloroform was added to it, and the mixture was shaken and was left at 4°C for 1 h. The residual phenolic phase was treated with 4 parts of water, 0.14 M NaCl, or acid buffer, shaken for 1.5 h, and left to settle overnight. The aqueous phase after the chloroform treatment was centrifuged in a SS-40 flow-through centrifuge, and then half a volume of phenol was added and the mixture was left overnight for settling. The aqueous layer was separated off and it was again treated with chloroform and centrifuged on a SS-40 supercentrifuge.

The aqueous phase obtained after the second treatment of the seeds was separated from the phenolic layer and was subjected to the same treatment as the aqueous phase after the first extraction. The combined aqueous phases were treated with 2.5 volumes of ethanol. The precipitate that deposited was collected by centrifuging and was suspended in a small volume of 2 M NaCl. The suspension was shaken for 15-20 min and was centrifuged in the cold at 6000 rpm. Then 2.5 volumes of ethanol was added to the supernatant and the precipitate was dissolved in 0.1 M NaCl.

Water-swollen DEAE-cellulose was added in a proportion of 1 ml per 100-150 o.u. The suspension was shaken at room temperature for 15 min and was centrifuged at 3000 rpm for 10 min. The supernatant was taken off, and the cellulose was eluted with 0.1 M NaCl once or

twice more, and the tRNA was then eluted with 2 M NaCl. The tRNA was precipitated from the solution with ethanol, and the precipitate was washed with ethanol and ether, and dried.

Isolation of tRNA by Extraction with a Hot Solution of Sodium Dodecyl Sulfate. The isolation of the tRNA was carried out by the method of Crestfield et al. [3] with slight modifications. The composition of the buffer for extraction was 0.05 M Tris-HCl, pH 7.5, 0.14 M NaCl, 1-3% DDS. The ratio of the extractant to the powdered seeds was 10:1. After incubation of the mixture at 95°C, the solution was cooled, and 2.5 volumes of ethanol was added. The further working up of the ethanolic precipitate was the same as in phenolic extraction.

Determination of the Activity of the tRNA. The isolation of the aminoacyl-tRNA synthetase and the analysis were performed as described previously [6]. The optimum conditions for the acylation of the total tRNA with [$^{14}$C]phenylalanine were: pH 7.8; $MgCl_2$ — 2.5 μmole; ATP — 0.75 μmole; time — 35 min.

The gel electrophoresis of the total tRNA preparation was carried out by Loening's method [7]. After staining, the bands corresponding to the various RNAs were cut out, comminuted, and incubated in 0.3 N KOH for 18 h. The absorption was measured on a Spektromom-204 spectrophotometer.

## SUMMARY

1. A comparative study has been made of various methods of isolating the tRNA from cotton seeds. It has been found that the best results are given by extraction of cotton seeds with a mixture of 80% phenol and 0.14 M NaCl.

2. The conditions have been worked out for the preparative isolation of the total tRNA.

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