Plant Physiol. (1979) 63, 79–86
0032-0089/79/63/0079/08/$00.50/0

Purification of Leucine tRNA Isoaccepting Species from Soybean Cotyledons

I. BENZOYLATED DIETHYLAMINO CELLULOSE FRACTIONATION, N-HYDROXYSUCCINIMIDE MODIFICATION, AND CHARACTERIZATION OF PRODUCT[1]

Received for publication May 1, 1978 and in revised form August 15, 1978

BRUCE R. LESTER[2] AND JOE H. CHERRY[3]
Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Transfer RNA from soybean (*Glycine max*) cotyledons was purified to homogeneity followed by the purification of the family of leucine tRNA via benzoylated diethylaminoethyl cellulose (BDC) chromatography. Nonacylated total purified tRNA was salicylhydroxamate (SHAM) modified by the phenoxyacetyl method and fractionated into three peaks on a BDC column. The first peak containing bulk tRNA with no hydrophobic character amounted to 78% of the added tRNA. The second peak containing 19% of the added tRNA and represents the tRNA with intrinsic hydrophobic properties. The third peak containing 3% of the tRNA represents the SHAM modified tRNA and nonspecifically modified tRNA. Transfer RNA peaks I and II were pooled and subsequently stoichiometrically acylated in two batches, one containing [^{14}C]leucine while the other contained unlabeled leucine. The acylated tRNA was loaded on and step-eluted from a BDC column. The purified acylated-tRNA was phenoxyacetyl modified and following ethanol precipitation was fractionated on a BDC column. A double peak eluted from the column in the ethanol gradient contained 5.3% of the starting optical density and 85.3% of the starting counts per minute. Characterization of this leucine tRNA showed typical ultraviolet spectra properties and appeared to be homogeneous on a G-100 Sephadex column. The minimum purity of the tRNA was 32 to 35%. Finally, the acylated tRNA was chromatographed on an RPC-2 column giving six leucine isoaccepting tRNAs. The data indicate that leucine tRNA was highly purified without losing the integrity of the family of isoacceptors.

Work from this laboratory has demonstrated that cytokinins, benzyladenine and zeatin, affected the relative levels of charging activity of three isoaccepting species of leucine tRNA (1). Following exogenous cytokinin (N^6-benzyladenine) treatment of soybean cotyledons, two leucine tRNAs were greatly increased and another leucine tRNA was decreased (2). This result raised the possibility that qualitative and quantitative alterations in leucine tRNA isoaccepting species following cytokinin treatment may regulate protein synthesis. The changes may be at least partially responsible for the mechanism of action of cytokinins in soybean tissue. To study this possibility further it was imperative to purify total leucine tRNA and to purify the various isoaccepting species of leucine tRNA present in soybean.

[1] Journal Paper No. 6632 of the Purdue Agricultural Experiment Station.

[2] Present address: Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

[3] To whom correspondence should be addressed.

MATERIALS AND METHODS

Plant Material. Soybean seeds were imbibed overnight in water without aeration, and planted in moist Vermiculite in shallow pans. The pans were kept in a dark germinator maintained at 30 C and at 100% RH, and watered once a day during their 4-day tenure in the germinator. Cotyledons were harvested routinely at 4 days after imbibition, although 5-day cotyledons were occasionally used. Cotyledons from etiolated soybean seedlings were used as a source of both tRNA and leucine tRNA synthetase.

tRNA. tRNA was prepared from total RNA extracted by the phenol technique of Kanabus and Cherry (5) with certain modifications necessitated by a *scaling up* of the procedure (Lester, Locy, Rychter, and Cherry, unpublished). The total phenol extracted RNA was precipitated by making it 2.0 M with respect to K-acetate. This solution was allowed to stir for 3 hr at 4 C after which it was centrifuged at 10,000*g* for 15 min. The soluble RNA was precipitated from the supernatant liquid by the addition of 2 volumes of cold 95% ethanol. This solution was allowed to stir for 3 hr at 4 C after which it was centrifuged at 10,000*g* for 15 min. The soluble RNA was precipitated from the supernatant liquid by the addition of 2 volumes of cold 95% ethanol and the solution was stored overnight at −20 C.

The precipitate was collected by centrifugation and dissolved in 100 ml buffer containing 0.01 M Na-acetate (pH 4.5), 0.01 M $MgCl_2$, and 3 mM NaN_3 for each 1,000*g* starting material. The resulting suspension was cleared by centrifugation at 10,000*g* for 10 min and the supernatant was added to a DEAE-cellulose column bedded in the same buffer and containing 2.0 ml of bed volume for each 100 g of starting material.

After simple application the column was washed with the Na-acetate until the OD_{260} dropped below 0.02. The soluble tRNA was eluted from the column with 1.0 M NaCl in the Na-acetate. The resulting 1.0 M NaCl eluate was immediately chromatographed on a column of G-100 Sephadex (2.5 × 90 cm) equilibrated with 0.01 M Na-acetate, 10 mM $MgCl_2$, and 3 mM NaN_3 at room temperature. Routinely, the tRNA peak was identified by inspection of the OD_{260} profile. The tRNA fraction from the Sephadex G-100 column was pooled, and concentrated by applying the pooled fraction to a DEAE-cellulose column. The column contained 1 ml of bed volume for each 200 OD_{260} of tRNA. This DEAE-cellulose column was previously equilibrated with 10 mM Na-acetate (pH 4.5), 10 mM $MgCl_2$, 3 mM NaN_3, and 0.4 M NaCl. The tRNA was eluted from the column using 1.0 M NaCl in the same buffer. The tRNA fractions were pooled (recovery was 93–99%) and dialyzed overnight against 100 volumes of distilled H_2O. The dialyzed tRNA solution was diluted to 100 or 200 OD_{260} units/ml for subsequent use.

Leucine tRNA Synthetase. Extraction, purification, and frac-

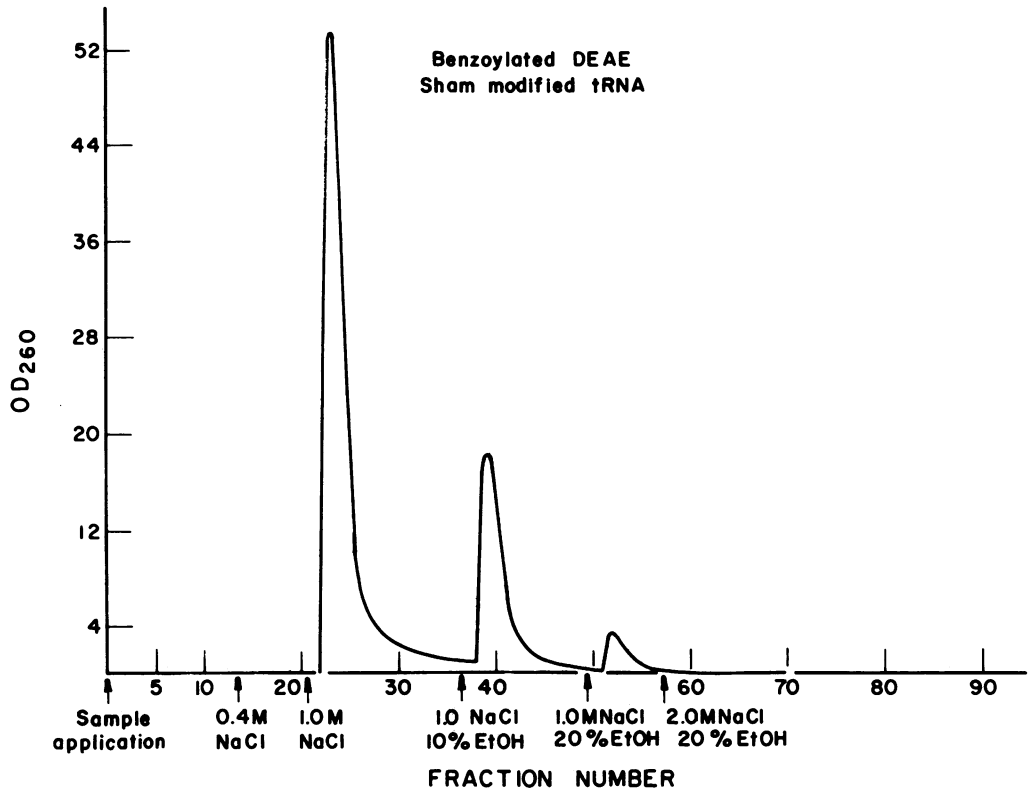

FIG. 1. BDC chromatography of SHAM-modified total tRNA. A tRNA sample containing 2,500 OD_{260} units was modified as outlined under "Materials and Methods" by the pheoxyacetyl ester of N-hydroxysuccinimide except that the tRNA was not previously acylated with leucine. The sample was in 10 mM Na-acetate (pH 4.5), 10 mM $MgCl_2$, and 3 mM NaN_3 as was the column.

tionation of the enzyme were performed at 4 C. Freshly harvested cotyledon tissue was chilled on ice and ground in a Sorval Omni-Mixer for six 1-min bursts of alternating speed (100% and 40% line voltage). The enzyme extraction buffer consisted of 25 mM K-phosphate (pH 7.8), 10 mM 2-mercaptoethanol, 10 μM phenylmethylsulfonylfluoride and 1 μM leucine. Typically, two 400-g lots of cotyledons were ground with 1,200 ml of enzyme extraction buffer plus 40 g of insoluble PVP (Polyclar AT) each. The homogenate was strained through four layers of cheesecloth and to the resulting homogenate enough solid ammonium sulfate was added to bring it to 35% saturation (209 g/l). An aliquot of this solution was removed before ammonium sulfate addition and assayed for protein and enzyme activity to determine the amount of enzyme in the crude homogenate. After 45 min this solution was centrifuged at 13,000*g* for 25 min. The clear supernatant was collected through a Miracloth filter to remove the floating lipid layer. To this filtered supernatant was added enough solid ammonium sulfate to bring it to 45% saturation (65 g/l). The 35 to 45% fraction was collected by centrifugation at 13,000*g* for 30 min. The resulting pellet was washed with 45% ammonium sulfate in enzyme extraction buffer. The 35 to 45% fraction was used either immediately or stored at −84 C for future use. The precipitate was dissolved in buffer A (25 mM K-phosphate [pH 7.8], 0.5 mM DTT, 10 μM phenylmethylsulfonylfluoride, 1 μM leucine, and 10% glycerol). This solution was added to a DEAE-cellulose column (2.6 × 60 cm) bedded in buffer A and washed with 400 ml of the same buffer. The enzyme was step eluted with 0.1 M K-phosphate (pH 7.8) in buffer A and concentrated by Ficoll. The resulting solution was added to a G-150 Sephadex column (2.5 × 90 cm) and eluted with buffer A at pH 6.5. The enzyme activity was pooled and dialyzed against buffer A (pH 7.8) in 50% glycerol and stored at −84 C until used.

tRNA Aminoacylation Assay. The reaction was carried out at 30 C. Under all conditions the reaction mixture contained (final concentrations) 10 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 0.5 mM ATP, and 0.2 g/l soluble PVP. The reaction size, amount of tRNA, and amount of amino acid varied with the particular assay purpose. Reactions were performed prior to phenoxyacetylesterification of acylated leucine tRNA for benzoylated DEAE-chromatography to determine the optimum conditions for obtaining maximum acylation in the least amount of time, and to determine the maximum extent of acylation for a particular tRNA preparation. These conditions were not designed to obtain typical initial velocity reaction kinetics but were designed to obtain stoichiometric acylation, *i.e.* high enzyme concentrations in relation to substrate. In addition to the standard components mentioned earlier, sufficient enzyme was added to obtain completion of the charging reaction in 10 to 15 min (routinely this ranged from 0.2 ml to 0.5 ml/ml reaction volume). For each ml of reaction volume 0.05 of radioactive L-[^{14}C]leucine having a specific radioactivity of 312 mCi/mmol (0.05 mCi/ml) was used. The usual reaction mixture was 0.2 ml and 0.02-ml aliquots were withdrawn over a 40-min period and radioactivity was determined as described above.

Column Chromatography. RPC-2 support medium was prepared according to Weiss and Kelmers (8). RPC-5 was purchased from Miles Laboratories. DEAE-Cellulose was purchased from Reeve Angel Corporation as Whatman DE23 and processed as per their recommendations. Sephadex G-50, G-100, and G-150 were purchased from Pharmacia Fine Chemicals. L-[3,5-^{3}H]Leucine at 46.0 Ci/mmol, L-[^{14}C]leucine at 312 mCi/mmol, and BDC[4] were purchased from Schwarz/Mann, and used according to methods of Gillam *et al.* (3) and Gillam and Tener (4). Ficoll and hydroxylapatite were purchased from Sigma. Aliquot 336 was purchased from General Mills and Freon 214 from E. I. du Pont

[4] Abbreviations: BDC: benzoylated diethylaminoethyl cellulose; SHAM: salicylhydroxamate; B-DEAE: benzoylated diethylaminoethyl.

de Nemours and Co. Chromosorb W-HP was purchased from Johns-Manville. BDC was stored in 2.0 M NaCl and 20% ethanol. The BDC column was formed using 2.0 ml column volume for each 100 OD_{260} units of tRNA to be added. The column was extensively washed with 0.01 M Na-acetate (pH 4.5) and 0.4 M NaCl until the OD_{260} dropped below 0.025. Due to swelling of the BDC in ethanol and the concomitant shrinkage with removal of ethanol, the column was reformed and again washed with Na-acetate buffer with 0.4 M NaCl to prevent channeling.

RESULTS

After the tRNA had been purified to homogeneity from the other nucleic acid components of the cell (as determined by

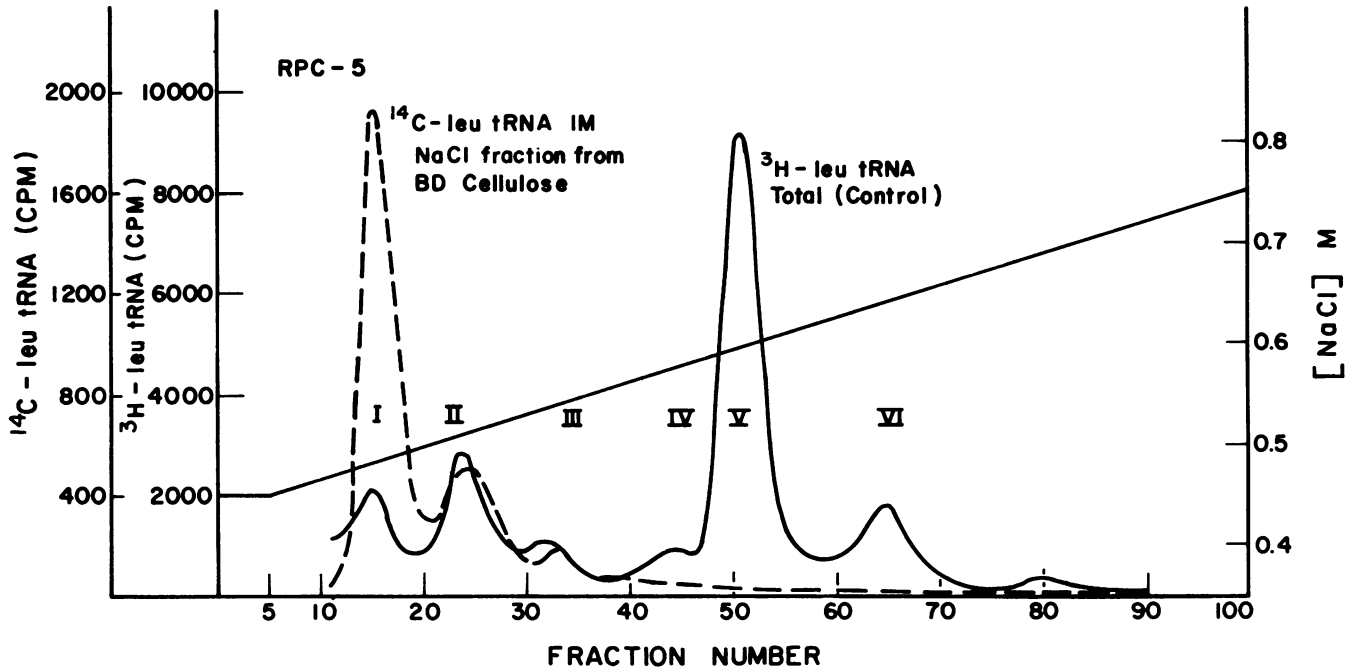

FIG. 2. Co-chromatography of [^{14}C]leucine tRNA (----) previously eluted from a BDC column (Fig. 1) in 1 M NaCl against [^{3}H]leucine total tRNA (——) on a RPC-5 column. An aliquot of 10 OD_{260} units of the first peak from BDC eluted with 1.0 M NaCl (Fig. 1) was acylated with [^{14}C]leucine. In addition, 40 OD_{260} units of total tRNA was acylated with [^{3}H]leucine. After processing both samples over DEAE-cellulose the samples were combined and co-chromatographed.

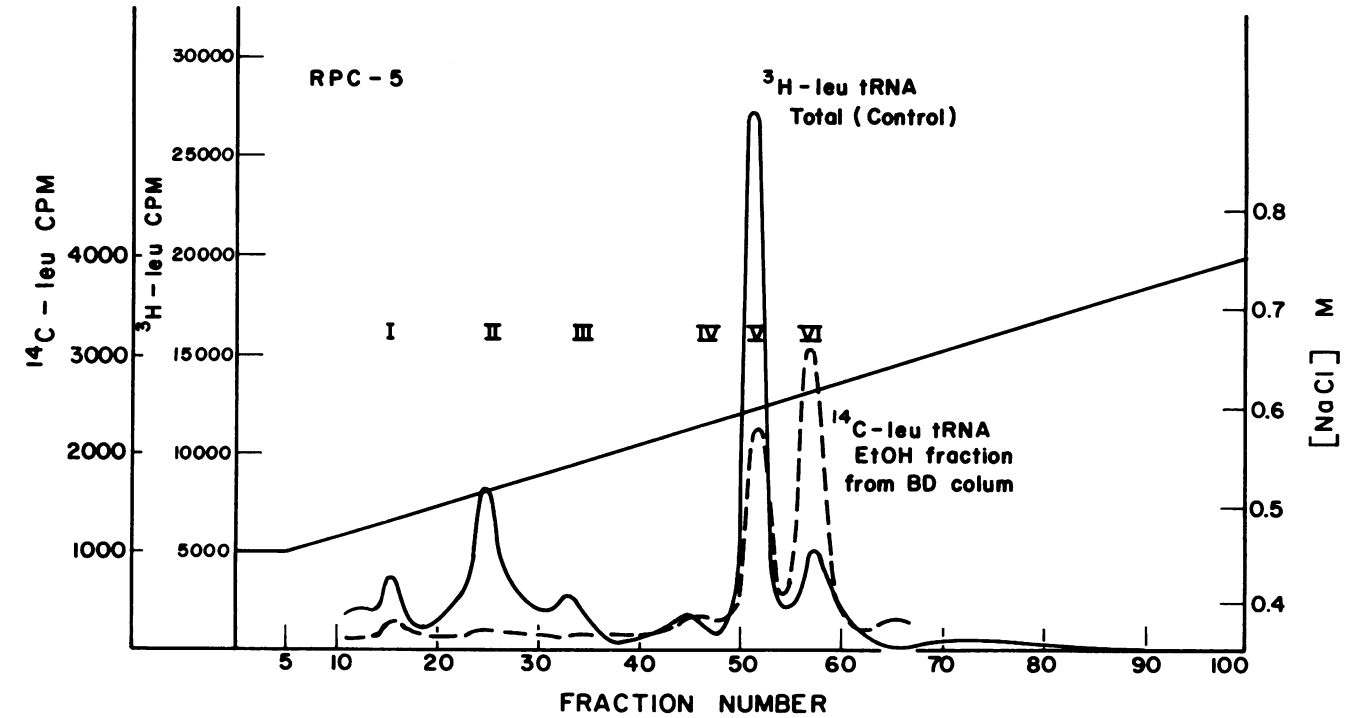

FIG. 3. Co-chromatography of [^{14}C]leucine tRNA (----) previously eluted from a BDC column (Fig. 1) in 10% ethanol against [^{3}H]leucine total tRNA (——) on a RPC-5 column. An aliquot of 10 OD_{260} units of the second peak from BD-cellulose eluted with 1.0 M NaCl and 10% ethanol (Fig. 1) was acylated with [^{14}C]leucine. In addition, 40 OD_{260} units of total tRNA acylated with [^{3}H]leucine. After processing both samples over DEAE-cellulose the samples were combined and co-chromatographed.

acrylamide gel electrophoresis), the purification of the leucine isoaccepting species was begun. The *modus operandi* was to purify the family of leucine tRNA via benzoylated DEAE and subsequently to characterize the product to insure maintenance of the leucine tRNA integrity.

BDC separates on the basis of hydrophobic interaction between the tRNA and the benzene groups attached to the cellulose support media. The usual method for purification involves charging total tRNA with the desired amino acid, and modifying the exposed amino group by phenoxyacetyl esterification, thus imparting high aromatic character to the desired family of tRNAs. These modified tRNAs will then bind preferentially to the column. Prior to modification those tRNAs which bind to the BDC due to their intrinsic hydrophobic character must be eliminated. These include phenylalanine tRNA, with the odd Y base, and any tRNAs which might become nonspecifically modified in the phenoxyacetyl esterification step.

Benzoylated DEAE Profile. The procedure for separating the nonessential tRNAs involved SHAM modification of the tRNA. The tRNA was carried through the modification procedure (see below, under "Modification and Fractionation of Leucine tRNA") except that it was not charged with leucine, therefore, any modification which might have taken place was nonspecific. The resulting profile (Fig. 1) shows three peaks. The first peak, eluted with 1.0 M NaCl in 10 mM Na-acetate (pH 4.5), 10 mM $MgCl_2$ and 3 mM NaN_3, contains 77% of the added tRNA (2,500 OD_{260} units) and represents the bulk of the tRNA which had no hydrophobic character either intrinsic or through SHAM modification. The second peak contains 19.3% of the original tRNA and represents the tRNA with intrinsic hydrophobicity such as phenylalanine

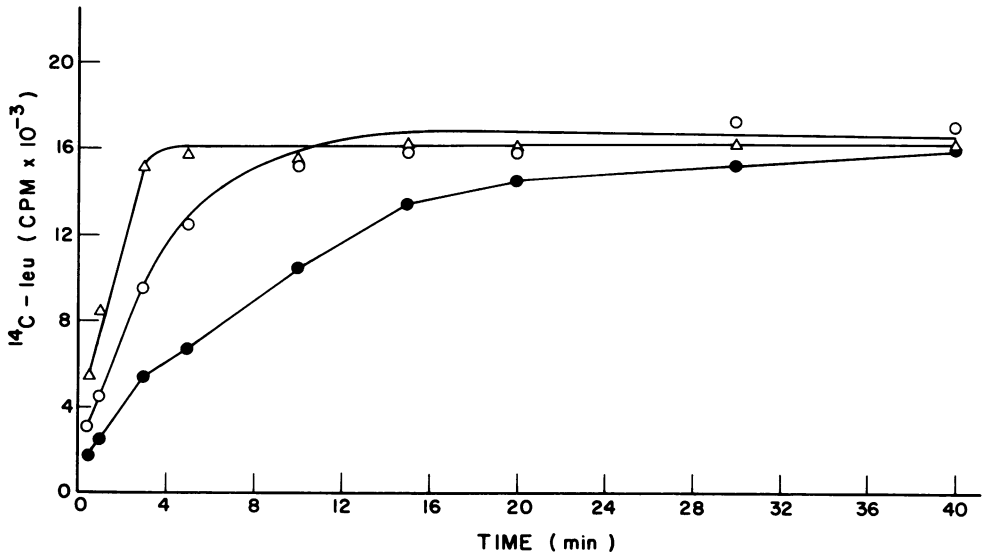

FIG. 4. Stoichiometric acylation of tRNA at different enzyme levels to determine optimum enzyme concentration. Various enzyme concentrations were used to determine maximum acylation plateau level and time to achieve this level. The reaction conditions are those outlined under "Materials and Methods" under stoichiometric acylation. The 0.2-ml reaction mixture contained 8 OD_{260} units of tRNA and 0.01 ml (●), 0.02 ml (○), and 0.04 ml (△) of leucine tRNA synthetases (4.14 mg protein/ml) per reaction mixture.

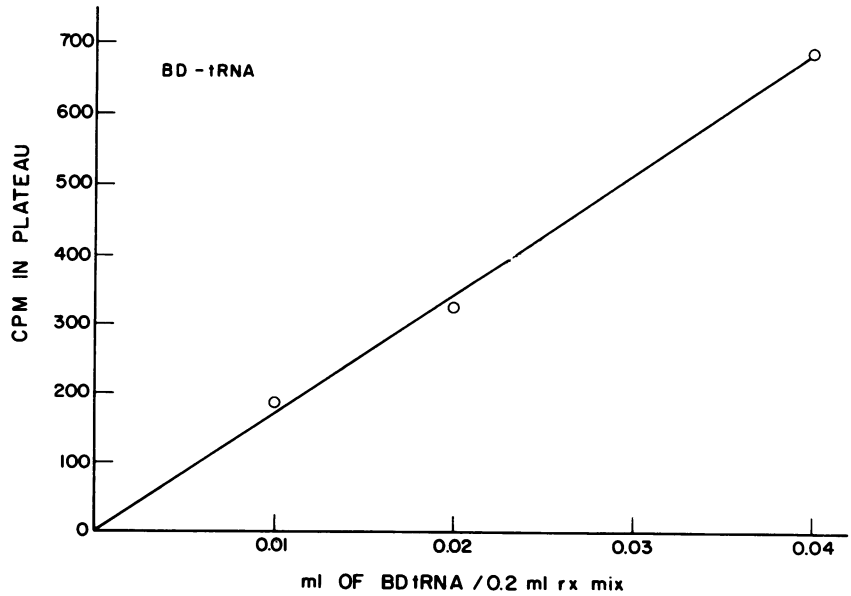

FIG. 5. Plateau acylation cpm *versus* concentration of tRNA. Secondary plot of maximum acylation plateau achievable for each concentration of tRNA. Various tRNA concentrations were acylated as outlined under "stoichiometric acylation." The 0.2-ml reaction mixture contained 0.04 ml of leucine tRNA synthetase (4.14 mg protein/ml) and 4.0, 8.0, and 12.0 OD_{260} units of tRNA per reaction mixture.

tRNA and as mentioned earlier probably cytokinin containing tRNA (9) which also imparts hydrophobicity to tRNA. Finally, the third peak contains 3.7% of the original tRNA and probably represents the nonspecific modification of tRNA by the phenoxyacetyl ester. The first and second peaks were pooled and used in the ultimate acylation and modification step thus excluding the nonspecifically modified tRNA.

Salt and Ethanol Fractions; Charging Specification. Samples of tRNA obtained from 1.0 M NaCl and 1.0 M NaCl plus 10% ethanol fractions were aminoacylated with a total leucine tRNA synthetase enzyme and chromatographed on RPC-5. Figure 2 shows that tRNA from the 1.0 M NaCl fraction represents leucine isoacceptors I, II, III, and possibly IV, indicating that they probably do not contain the cytokinin moiety in their structure. Figure 3 shows that tRNA from the first ethanol fraction includes leucine isoacceptor tRNAs V and VI and possibly IV, indicating that they do possess some intrinsic hydrophobicity which probably represents the presence of isopentenyl adenosine in the structure of the tRNAs. These results represent only presumptive evidence; however, they tend to substantiate subsequent findings (6).

Stoichiometric Acylation. To ensure maximum purification of leucine tRNA in the BDC step it was necessary to achieve essentially complete acylation of the leucine tRNA in a stoichiometric procedure, *i.e.* to completion and in 10 to 15 min to minimize base-catalyzed hydrolysis. Under these conditions the concentration of enzyme had to be greater than the concentration of substrate and the limiting component had to be tRNA so that the reaction did not stop due to depletion of another component. Figure 4 shows completion of charging under various enzyme concentrations to determine which enzyme concentration was optimum with respect to time and stoichiometry. Figure 5 shows the results of an experiment to insure that tRNA is the limiting component in the reaction. As can be seen the plateau acylation values are linear with respect to tRNA concentration showing that stoichiometric acylation is taking place. The calculated values of the percentage of leucine tRNA in the total tRNA preparation are quite close to theoretical values and values in the literature (*i.e.* 2.4–4%).

The tRNA (2,500 OD units) was acylated in two batches using the enzyme and tRNA concentration calculated above. The first reaction mixture contained the bulk of the tRNA (2,000 OD units) with unlabeled leucine and the second reaction mixture was only 25% as large as the first but contained labeled [^{14}C]leucine. The reaction mixtures contained the same components as described above for stoichiometric acylation and in the same concentrations except scaled up proportionately. After the reaction was complete (10 min) the reaction mixtures were combined and diluted with Na-acetate such that the final concentration was 10 mM Na-acetate (pH 4.5) and 10 mM $MgCl_2$, 3 mM NaN_3, and 0.3 M NaCl. This solution was added to a 13-ml DEAE-column and washed with Na-acetate buffer in 0.4 M NaCl until the OD_{260} dropped below 0.025. The bound and acylated tRNA was eluted with 1.0 M NaCl in Na-acetate buffer and precipitated in 2 volumes of cold ethanol overnight. Ninety per cent of the OD_{260} units and cpm were recovered. This sample was subsequently modified as per the phenoxyacetyl ester modification outlined below.

Modification and Fractionation of Leucine tRNA. After tRNA had been stoichiometrically acylated with leucine and precipitated

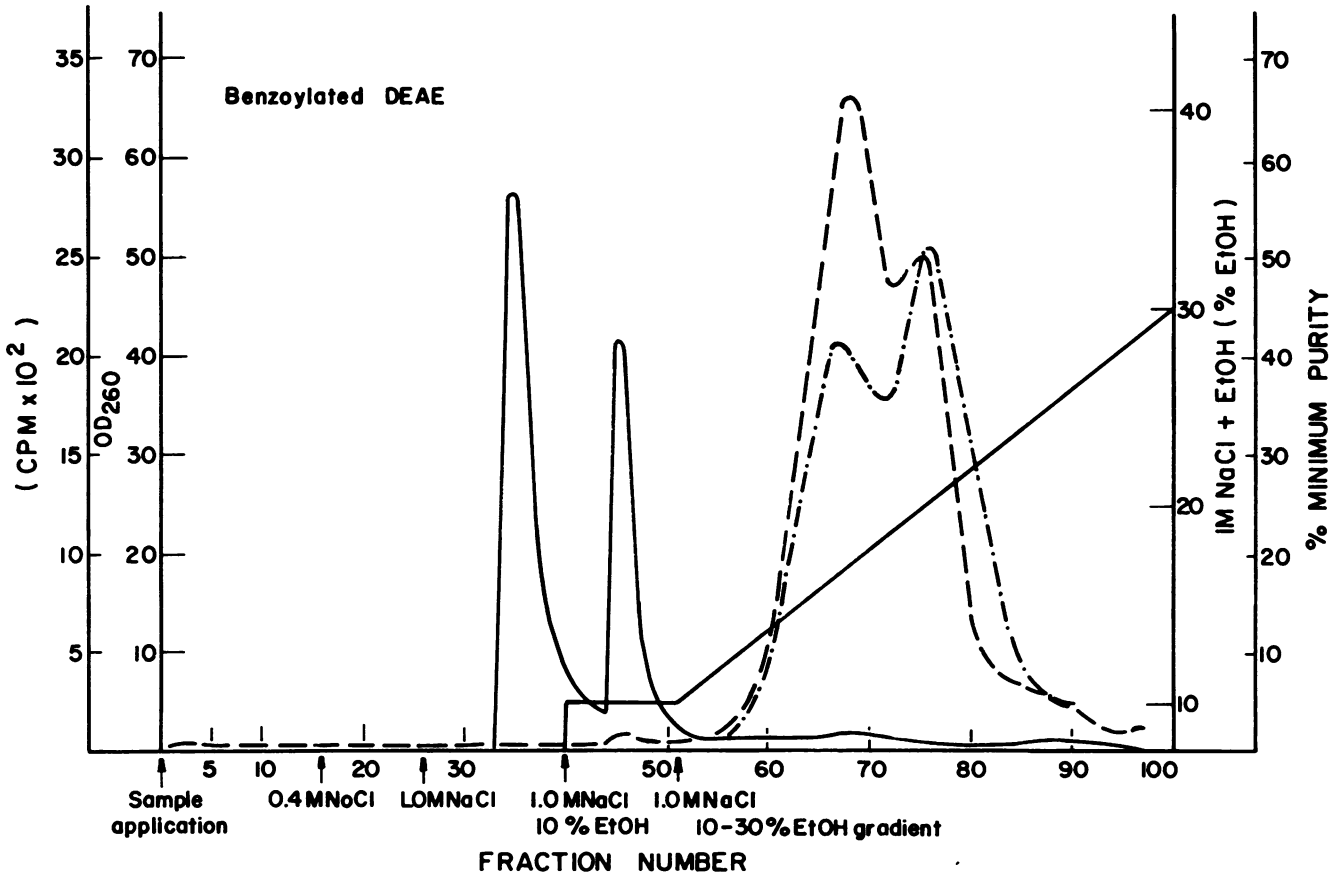

FIG. 6. BDC chromatography of leucine acylated tRNA, modified with the phenoxyacetyl ester of N-hydroxysuccinimide. The tRNA (2,500 OD_{260} units) was acylated as outlined under "Materials and Methods" under stoichiometric acylation using the optimum enzyme and tRNA concentrations determined in stoichiometric acylation under "Results" (Figs. 4 and 5) and modified as described in "Results" under "Modification and Fractionation of Leucine tRNA" and added to the BDC column. It was eluted as shown in the graph. The OD_{260} (——), [^{14}C]leucine-acylated and modified tRNA (---) and per cent minimum purity (·····) were determined in each 4.0-ml fraction. The column (30.0 ml) was eluted at 40 ml/hr.

in ethanol overnight, it was suspended the next day in 5.0 ml of 0.5 M triethanolamine buffer (pH 8.1) to which, after determination of total OD_{260} and cpm, was added 3.0 ml of 1,4-dioxane containing 50 mg/ml of phenoxyacetyl ester of N-hydroxysuccinimide (100 M excess of the ester to the acylated tRNA). This solution was left to incubate in an ice bath at 4 C for 15 min after which the reaction was terminated and the modified tRNA precipitated by the addition of 20 ml of 1,4-dioxane. The precipitate was collected by centrifugation and resuspended in 10 ml of 0.1 M Na-acetate (pH 4.5) with 10 mM $MgCl_2$. The solution was precipitated with 3 volumes of cold ethanol and left in the freezer overnight or until fractionation on BDC. The ethanol pellet was taken up in 50 ml of Na-acetate (pH 4.5) and loaded into a preequilibrated BDC column. The tRNA solution contained 94% of the starting OD_{260} units and cpm. After the tRNA was added, the column was washed with 0.4 M NaCl in Na-acetate and the tRNA fractions were eluted in stepwise sequence (Fig. 6) with 1.0 M NaCl, 1.0 M NaCl plus 10% ethanol and a linear gradient of 10 to 30% ethanol plus 1.0 M NaCl, all solutions were in Na-acetate (pH 4.5). Figure 6 shows that the acylated and modified leucine tRNA was separated from both the salt fraction tRNA and the 10% ethanol fractions. The double peak in the ethanol gradient probably represents the noncytokinin and cytokinin containing leucine tRNA, respectively. Ninety-five per cent of the tRNA and cpm added to the BDC column were recovered. Fractions 61 through 80 were pooled, and dialyzed against 4,000 ml of distilled H_2O and lyophilized to dryness. This fraction contained 5.3% of the starting OD and 85.3% of the starting cpm.

CHARACTERIZATION OF THE BDC PRODUCT

The leucine tRNA fraction from BDC was precipitated by the addition of 2 volumes of cold 95% ethanol overnight. The precipitate was recovered the next day by centrifugation. The resulting pellet was suspended in 10.0 ml of 1.0 M Tris-HCL (pH 9.0) and incubated for 30 min at 37 C. Incubation under these conditions caused base-catalyzed hydrolysis resulting in the discharging of the phenoxyacetyl ester modified leucine from leucine tRNA. The tRNA was then precipitated from solution by the addition of 2 volumes of cold 95% ethanol and stored overnight. The precipitate was collected by centrifugation, and the pellet was suspended in 0.01 M Na-acetate (pH 4.5), 10 mM $MgCl_2$, and 3 mM NaN_3. The unacylated leucine tRNA fraction from BDC was then characterized as to its optical properties, size and charging integrity, including minimum purity calculations from stoichiometric acylation and charging specificity, *i.e.* number of isoaccepting species on RPC-2.

UV Spectra. The UV spectra of the purified tRNA were determined to ensure that no contribution to total *A* was due to amino acids or protein binding to the BDC column or the purified tRNA. The spectra observed from 220 nm to 310 nm were a typical nucleic acid absorption pattern. The 280/260 ratio was 0.4818 indicating 100% nucleic acid.

Size and Homogeneity Tests. The purified tRNA was loaded onto a G-100 Sephadex column and a symmetrical peak was eluted (Fig. 7) with no shoulders or competing peaks. The single symmetrical peak eluted at the position for 4S molecules.

Stoichiometric Charging. The tRNA from BDC was next acylated under stoichiometric conditions. Figure 8 shows the plateau values achieved with different concentrations of BDC-tRNA and demonstrates the linear dependence of plateau acylation upon BDC-tRNA concentration. From these data it was calculated that 32 to 35% of the BDC-tRNA was acylatable by leucine tRNA synthetase. This is a minimum purity calculation which most probably depends upon the integrity of the CCA terminus of the tRNA molecule since the BDC-tRNA has been shown not to include cleavage products or portions of smaller broken tRNAs (Fig. 7).

Isoacceptor Integrity of Leucine tRNA. The BDC-tRNA was acylated with tRNA synthetase and chromatographed on an RPC-2 column to determine if there was a preferential loss of any of the six leucine isoaccepting tRNAs. Figure 9 demonstrates that the integrity of the leucine tRNA family of isoacceptors is maintained through the acylation, modification and subsequent fractionation of leucine tRNA on BDC.

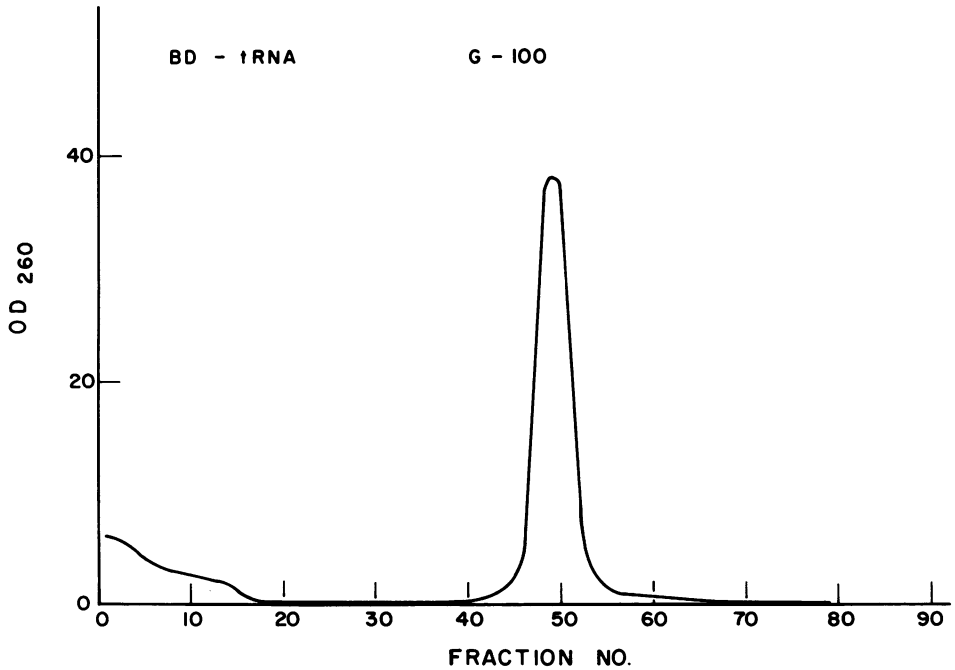

FIG. 7. Sephadex G-100 elution pattern of BDC leucine tRNA fraction. The discharged and concentrated BDC leucine tRNA fraction was added to a Sephadex G-100 column (2.5 × 90 cm) bedded in 10 mM Na-acetate (pH 4.5), 10 mM $MgCl_2$, and 3 mM NaN_3 and eluted in 2.0-ml fractions at the rate of 20 ml/hr in the same buffer.

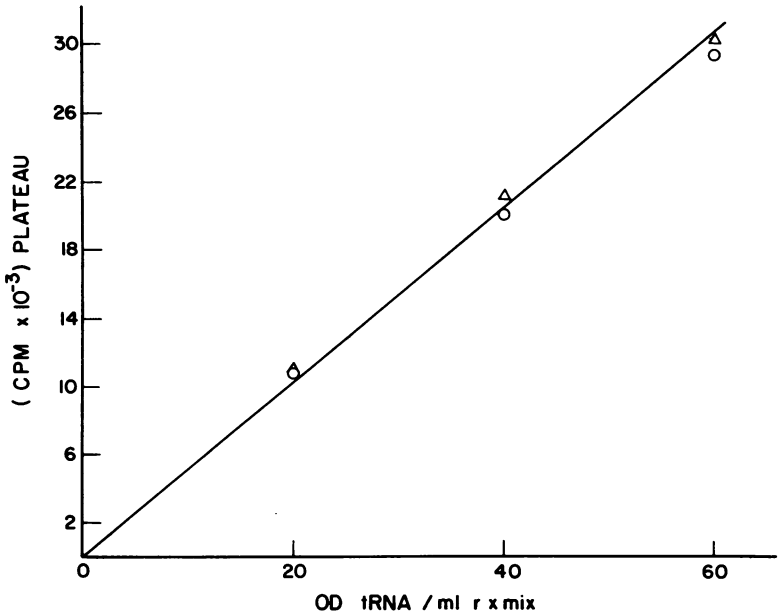

FIG. 8. Maximum plateau acylation cpm *versus* concentration of BDC leucine tRNA (0.6 OD_{260}/ml). Secondary plot of maximum acylation plateau achievable for each concentration of BDC leucine tRNA. Various BDC leucine tRNA concentrations were acylated as outlined under "Stoichiometric Acylation." The 0.2-ml reaction mixture contained 0.04 ml of leucine tRNA synthetase (4.14 mg protein/ml) and 0.01, 0.02, and 0.04 ml of 1.10 dilution of BDC leucine tRNA (6.0 OD_{260}/ml) per reaction mixture.

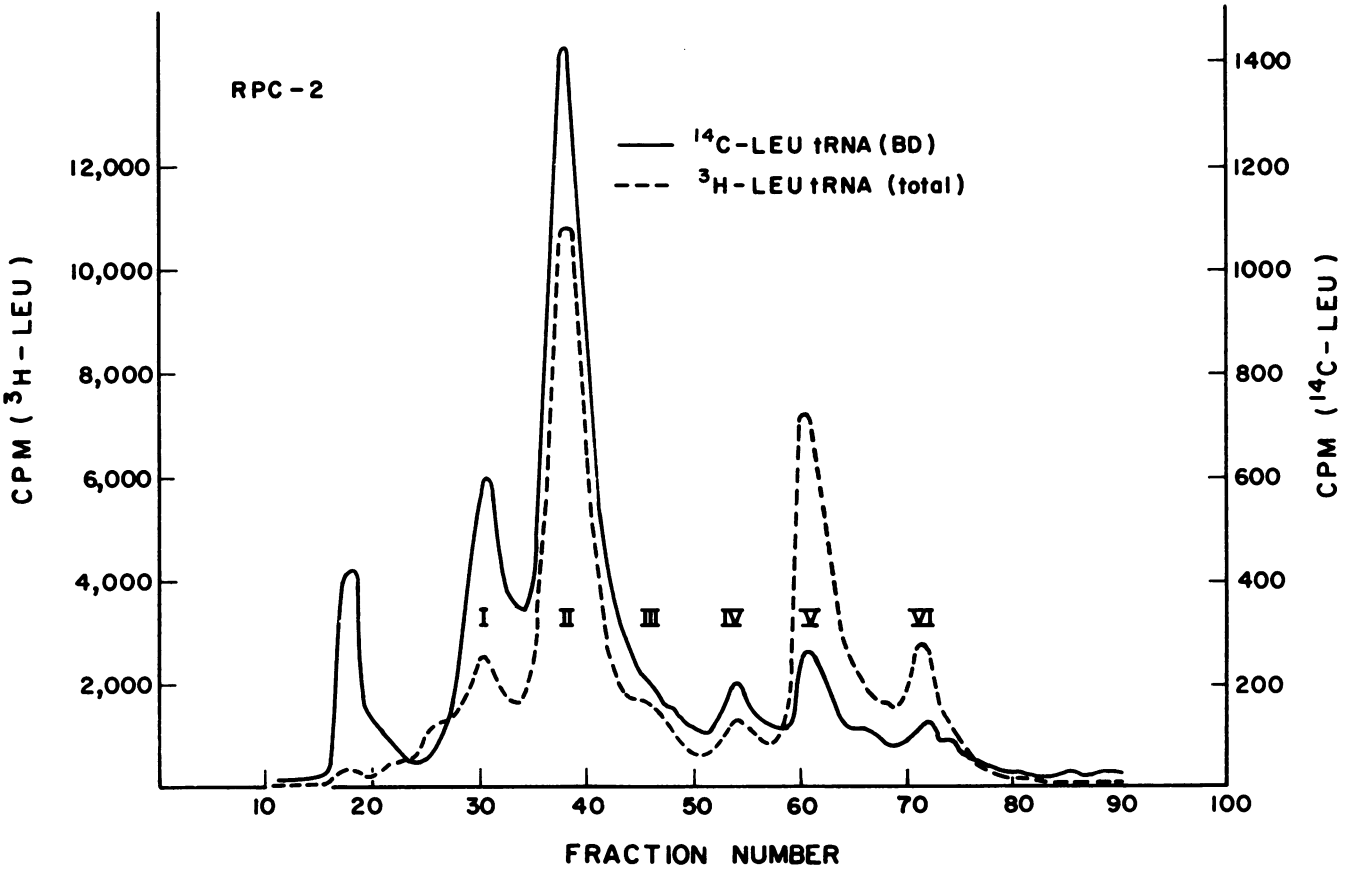

FIG. 9. RPC-2 co-chromatography of BDC leucine tRNA acylated with [^{14}C]leucine (——) and total tRNA acylated with [^{3}H]leucine (---). An aliquot of 2.0 OD_{260} units from BDC leucine tRNA was acylated with [^{14}C]leucine in the manner outlined under "Materials and Methods" for RPC acylation as was 25 OD_{260} units of total tRNA acylated with [^{3}H]leucine. After processing both samples over DEAE-cellulose the samples were combined and co-chromatographed.

DISCUSSION

One objective of this study was to develop a method for the purification of the isoaccepting species of leucine tRNA from soybean cotyledons in order to examine the possibility that they were involved in hormonally mediated translational regulation. We decided to utilize the phenoxyacetyl ester modification of leucine tRNA followed by separation on BDC columns as the method of purification. This fractionation resulted in leucine tRNA which was 35 to 45% pure. The partially purified leucine tRNA family was then discharged and rechromatographed on RPC-2 to separate further the isoaccepting species of leucine from each other and extraneous tRNA carried along in the BDC step (6). The calculated purity of the leucine tRNA from BDC was a minimum purity and tests were performed to insure that extensive damage or alteration had not taken place during purification. The data demonstrate that the material from BDC was nucleic acid and not protein or contaminating amino acids (UV spectra), that its integrity *via-a-vis* size and homogeneity was maintained (Fig. 7), that its integrity with respect to acylation competency was maintained (Fig. 8), and that its substituent components were all present (Fig. 9).

The results summarized here with respect to specificities of salt and ethanol fractions from BDC show that leucine isoaccepting species V, VI, and possibly IV contain a high degree of intrinsic hydrophobicity due to their elution in the ethanol fraction from BDC. Vold *et al.* (7) have shown that elution in the ethanol fractions of uncharged isoaccepting tRNA species from BDC columns, excluding phenylalanine tRNA with the highly hydrophobic Y base, is probably due to the presence of cytokinin or cytokinin-like compounds in the structure of the tRNA.

LITERATURE CITED

1. ANDERSON MB, JH CHERRY 1969 Differences in leucyl-tRNA's and synthetase in soybean seedlings. Proc Nat Acad Sci USA 62: 202–209
2. CHERRY JH, MB ANDERSON 1972 Induced changes in transfer RNA species. DJ Carr, ed, Plant Growth Substances. Springer-Verlag, Heidelberg, pp 181–189
3. GILLAM I, S MILLWARD, D BLEW, M VON TIGERSTROM, E WIMMER, GM TENER 1967 The separation of soluble ribonucleic acids on benzoylated diethylaminoethyl-cellulose. Biochemistry 6: 3043–3056
4. GILLAM IC, GM TENER 1971 The use of BD-cellulose in separating tRNAs. Methods Enzymol. 20: 55–70
5. KANABUS J, JH CHERRY 1971 Isolation of an organ-specific leucyl-tRNA synthetase from soybean seedlings. Proc Nat Acad Sci USA 68: 873–876
6. LESTER BR, RO MORRIS, JH CHERRY 1979 Purification of leucine tRNA isoaccepting species from soybean cotyledons. II. RPC-2 purification, ribosome binding, and cytokinin content. Plant Physiol 63: 87–92
7. VOLD BS, GM CLINTON, J SPIZIZEN 1970 An effect of temperature on the *B. subtilis* tRNA's which respond to codons beginning with U and A correlation with cytokinin activity. Biochim Biophys Acta 209: 396–404
8. WEISS JR, AD KELMERS 1967 A new chromatographic system for increased resolution of tRNA. Biochemistry 6: 2507–2513
9. WIMMER E, IH MAXWELL, GM TENER 1968 A simple method for isolating highly purified yeast phenylalaine transfer ribonucleic acid. Biochemistry 7: 2623–2628