A Simple Method for Isolating Highly Purified Yeast Phenylalanine Transfer Ribonucleic Acid*

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ABSTRACT: An extensive fractionation of yeast transfer ribonucleic acids (tRNAs) may be obtained by elution from a column of benzoylated DEAE-cellulose with a gradient of NaCl. This salt at a concentration of 1 M elutes all amino acid acceptor activities with the exception of that for phenylalanine. The latter activity may be eluted by addition of a small proportion of ethanol to the eluent. By this means a 17-fold purified preparation of phenylalanine tRNA was obtained from commercial yeast soluble ribonucleic acid in one rapid and simple step. Further chromatography on a column of silicic acid coated with benzoylated DEAE-cellulose gave a final preparation which was 25-fold purified in phenylalanine tRNA. This material was capable of accepting 1.83 nmoles of phenylalanine/A_{260} unit. The purity of the preparation was estimated as 97.5 ± 4%.

Yeast sRNA[1] may be extensively fractionated after adsorption on to a column of benzoylated DEAE-cellulose (BD-cellulose[2]) by elution with a gradient of increasing concentration of sodium chloride (Tener *et al.*, 1966; Gillam *et al.*, 1967). Phenylalanine tRNA ($tRNA^{Phe}$) is unique in that it is not eluted by NaCl alone at concentrations up to 1 M. Inclusion of a small proportion of ethanol or 2-methoxyethanol in 1 M NaCl in the eluent results in removal of $tRNA^{Phe}$ from the column. Only small traces of acceptor activity for other amino acids are present in the eluate (Gillam *et al.*, 1967).

As described in the present communication, utilization of the above property of $tRNA^{Phe}$ enabled a 17-fold purification to be obtained in a single step. A final purification of 25-fold was obtained after further chromatography on a column of silicic acid coated with BD-cellulose (BDCS). It is considered that this technique has advantages over other methods of isolating $tRNA^{Phe}$ (*e.g.*, countercurrent distribution (Hoskinson and Khorana, 1965) and reverse-phase chromatography (Kelmers, 1966)) in terms of simplicity and rapidity.

Experimental Procedures

Materials

A commercial preparation of sRNA from brewer's yeast was used as the starting material. This was obtained from Boehringer & Soehne, Mannheim, Germany.

Fully benzoylated DEAE-cellulose was prepared as described by Gillam *et al.* (1967). Uniformly labeled L-[^{14}C]phenylalanine (393 mCi/mmole), uniformly labeled L-[^{14}C]tyrosine (352 mCi/mmole), L-[3-^{14}C]tryptophan (22.0 mCi/mmole), and a mixture of 15 uniformly labeled L-[^{14}C]amino acids (NEC-445; 1.5 mCi/mg) were obtained from New England Nuclear Corp., Boston, Mass.

The enzyme preparation used in most experiments was a mixture of aminoacyl-tRNA synthetases prepared from baker's yeast by method B of Gillam *et al.* (1967) except that the 105,000*g* supernatant was applied directly to a column of Sephadex G-25. The column was eluted with 10 mM potassium phosphate buffer (pH 7.5) containing 1 mM 2-mercaptoethanol, 0.1 mM EDTA (sodium salt), and 40% v/v glycerol and the high molecular weight fraction was collected. In one experiment (the test for other acceptor activities in the purified $tRNA^{Phe}$) the enzyme used was prepared by method A of Gillam *et al.* (1967) and was eluted from Sephadex G-25 with the same medium as above.

Methods

Coating of Silicic Acid with BD-cellulose. The reagents were first dried as follows. Silicic acid (Mallinckrodt, analytical grade, 100 mesh) was heated at 125° for 18 hr and BD-cellulose was heated at 75° for 24 hr in a vacuum oven at 100 mm. Methylene chloride was passed through a dry-packed column of oven-dried alumina.

BD-cellulose (17 g) was dissolved in methylene chloride (1100 ml) and then silicic acid (470 g) was added. After thorough mixing, the organic solvent was removed by distillation at reduced pressure and the residue was left under vacuum overnight. The dried material was pulverized and was passed through a 40 mesh sieve. It was then suspended in 20% v/v ethanol and was poured into a column. Washing with 10% v/v ethanol–1.8 M

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[1] The term sRNA is used to denote the complete mixture of low molecular weight ribonucleic acids used as the starting material for the purification.

[2] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: BD-cellulose, benzoylated diethylaminoethylcellulose; BDCS, silicic acid coated with BD-cellulose; TCA, trichloroacetic acid.

NaCl was carried out until no further ultraviolet-absorbing material was removed.

Estimation of Amino Acid Acceptor Activity of Crude and Purified tRNA^{Phe} Preparations. Mixtures (total volume 0.5 ml) contained the following components at the concentrations stated: 0.12 M Tris-Cl buffer (pH 7.5), 15 mM $MgCl_2$, 30 mM KCl, 4 mM EDTA (sodium salt), 3 mM ATP (sodium salt), 13 mM 2-mercaptoethanol, 8 μM each of 18 unlabeled L-amino acids, 32 μM unlabeled L-phenylalanine, 0.5 or 1.0 μCi/ml of L-[^{14}C]phenylalanine, 0.8 mg of protein/ml of aminoacyl-tRNA synthetases, and sRNA (5–10 A_{260} units/ml) or purified tRNA^{Phe}. The quantity of RNA in the mixture was decreased as the extent of purification increased so that the concentration of tRNA^{Phe} present remained approximately constant. When it was desired to estimate acceptor activity for another amino acid, the above assay system was modified to have 8 μmoles of phenylalanine and 32 μmoles of [^{14}C]amino acid (0.5 or 1.0 μCi/ml). Control mixtures to which no RNA was added were also prepared.

After incubation for 15 min at room temperature (approximately 23°), samples (50 μl) were withdrawn on to filter paper disks (Whatman No. 3MM, 2.4-cm diameter) which were immediately placed in an ice-cold solution of TCA (10% w/v). The filters were then passed through the following series of ice-cold solutions, being allowed to stand for 10 min in each solution: 5% w/v TCA, 5% w/v TCA, 95% v/v ethanol, and diethyl ether. (The volume of each solution was at least 10 ml/filter.) The filters were then dried at room temperature before being placed in vials with 5 ml of toluene scintillation fluid (0.3% w/v 2,5-diphenyloxazole and 0.03% w/v 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene in toluene). Radioactivity was determined in a Nuclear-Chicago Unilux scintillation counter. The counting efficiency was 52%. The counts were corrected for the values obtained for the controls to which no RNA had been added.

Estimation of Phenylalanine Acceptor Activity of RNA in Column Fractions. Acceptor activity in fractions from columns was estimated using a slight modification of the method described by Cherayil and Bock (1965) and Cherayil *et al.* (1968). A mixture of the components required for charging tRNA, having the composition given above, was applied to the dried paper disks containing RNA from column fractions. After incubation at room temperature for 30 min the disks were placed in ice-cold 10% w/v TCA. Washing and measurement of radioactivity bound to the disks were carried out as described above. Disks which had not been loaded with RNA were put through the entire procedure to serve as blanks. These gave about 300 cpm/disk.

Control experiments showed that the counts per minute obtained by this method for a given amount of tRNA were reproducible but were only about 70% of the counts per minute obtained when the same amount of tRNA was assayed by incubation in solution in test tubes.

Concentrating RNA Solutions. Usually tRNA solutions were concentrated by ultrafiltration (Blatt *et al.*, 1965) in a Diaflo, Model 50 or Model 400, ultrafiltration cell (Amicon Corporation, Cambridge, Mass.) using a UM-1 membrane. Sometimes it was considered preferable to concentrate by adsorption of the RNA on small columns of DEAE-cellulose (chloride form). The RNA solution was diluted to a salt concentration of 0.15 M or lower before passing through the column. The RNA was removed from the column by elution with 1.5 M NaCl.

When tRNA was to be precipitated, the solution, containing at least 5 A_{260} units[3]/ml, was mixed with sodium chloride solution to give a final concentration of 1–1.5 M and then two volumes of cold ethanol (95% v/v) was added. The mixture was left at −20° for at least 1 hr before the precipitated RNA was removed by centrifugation.

Estimation of Total Phosphorus in the Purified tRNA. Total phosphorus was estimated in small samples (0.1–0.3 A_{260} unit) of the purified tRNA^{Phe} by the method described by Ames (1966).

Test for Ability of the Purified tRNA^{Phe} to Accept Amino Acids Other Than Phenylalanine. A mixture (total volume 0.45 ml) was prepared containing the following components: 0.22 M sodium cacodylate buffer (pH 7.6), 18 mM $MgCl_2$, 36 mM KCl, 4.5 mM EDTA (sodium salt), 3.3 mM ATP (sodium salt), 14 mM 2-mercaptoethanol, 9 μM L-phenylalanine (unlabeled), 5 μCi of [^{14}C]amino acids mixture, 0.44 μCi of L-[^{14}C]tryptophan, 0.2 μCi of L-[^{14}C]tyrosine, 1.8 mg of protein containing aminoacyl-tRNA synthetases (prepared by method A; Gillam *et al.*, 1967), and 1.03 A_{260} units of purified tRNA^{Phe}. A control mixture was prepared having the same composition except for the omission of tRNA^{Phe} and unlabeled phenylalanine. Subsequent operations were performed with the two mixtures in parallel.

Incubation was carried out at room temperature (25°) for 20 min and then to each mixture was added 0.1 ml of 5 M NaCl, 1.2 ml of cold 95% v/v ethanol, and 0.02 ml of unlabeled sRNA solution (36 mg/ml). The mixtures were left at −20° overnight and then the precipitates were sedimented by centrifugation. These were then washed four times with cold 67% v/v ethanol containing 0.5 M NaCl by resuspension and centrifugation. The final pellets were each dissolved in 0.5 ml of 1.5 M NH_4OH and the solutions were incubated at 37° for 25 min to strip amino acids from the tRNA. The solutions were then mixed with 1 ml of cold 95% v/v ethanol. Some material was precipitated and this was removed by low-speed centrifugation. The turbid supernatants were then evaporated to dryness in a stream of air at 37°. The residues were extracted with 1 ml of 67% v/v ethanol at 37° for 10 min and then the clear liquids were decanted. Samples of these liquids counted in dioxan-based aqueous scintillation fluid showed that most of the radioactivity which had been present in the NH_4OH digest had been recovered. The amino acid solutions were evaporated to dryness, the residues were each dissolved

[3] An A_{260} unit of RNA is defined as that quantity which, when dissolved in 1 ml of 50 mM Tris-Cl buffer (pH 7.5) containing 20 mM $MgCl_2$, gives a solution having an absorbance of 1 at a wavelength of 260 nm.

TABLE I: Concentration Factors and Percentage Recoveries of Phenylalanine Acceptor Activity at Various Stages during the Purification of $tRNA^{Phe}$.

Stage of Purification	Total No. of A_{260} Units	% Recov of Total Initial Phenylalanine Acceptor Act.	Concn Factor
Starting material (yeast sRNA)	4350	100	1
Eluted from BD-cellulose with 9.5% v/v ethanol (combined fractions of highest specific activity)	179	70	17
Applied to BDCS	158	62	17
Final purified $tRNA^{Phe}$	68	39	25

in 30 μl of water, and 20-μl samples were spotted on to Whatman No. 1 chromatography paper. Unlabeled amino acid markers were also applied to the paper and a descending chromatogram was run for 16 hr using the solvent system 1-butanol–acetic acid–water (60:15:25, v/v). Strips (width 3.5 cm) corresponding to each radioactive spot were cut from the paper and were analyzed for radioactivity using a Nuclear-Chicago, Actigraph chromatogram strip scanner.

Results

Isolation of Crude $tRNA^{Phe}$ Using a Column of BD-cellulose. A column (2.5 × 25 cm) of BD-cellulose was equilibrated with standard buffer (10 mM $MgCl_2$ and 50 mM sodium acetate, pH 5) containing 0.3 M NaCl. A sample of yeast sRNA (250 mg, 4350 A_{260} units), dissolved in 40 ml of the same solution, was then applied to the column. All operations were at room temperature and the solutions used for eluting were all made up in the above standard buffer. After loading with sRNA, 115 ml of 0.3 M NaCl was passed through the column. The eluate had no absorbance at 260 nm.

The column was then eluted with a linear gradient (total volume 2 l.) of NaCl from 0.3 to 0.9 M, followed by a solution of 1.0 M NaCl (0.5 l.). The eluate which was collected as a single fraction contained 3460 A_{260} units. The final A_{260} of the liquid emerging from the column was 0.11. The RNA present in this eluate was subsequently concentrated and assayed for phenylalanine acceptor activity. It contained only 4.8% of the total activity of the starting material. Elution was continued, using 1.0 M NaCl containing 9.5% v/v ethanol, fractions (18.5 ml) now being collected. Figure 1 shows the absorbances and phenylalanine acceptor activities of these fractions. The ratio of activity to absorbance was clearly not constant across the peak, indicating the presence of a considerable amount of absorbing material that was not $tRNA^{Phe}$. The fractions of highest specific activity (8–10) were pooled. These contained 4.1% of the total absorbance and 70% of the total phenylalanine acceptor activity of the starting material (see Table I). Total recovery of absorbance from the column was 87%.

After concentrating by ultrafiltration and removal of a sample for estimation of acceptor activity, the remainder of the $tRNA^{Phe}$ (158 A_{260} units) was purified further by chromatography on BDCS.

Properties of BDCS. Preliminary experiments showed that in the presence of 10 mM Mg^{2+}, yeast sRNA could be adsorbed on to BDCS and could be eluted by NaCl at slightly lower concentrations than those required for elution from BD-cellulose. The over-all pattern of absorbance in the eluate obtained with a gradient of NaCl was qualitatively similar to that observed with BD-cellulose (Gillam *et al.*, 1967). Increasing the Mg^{2+} concen-

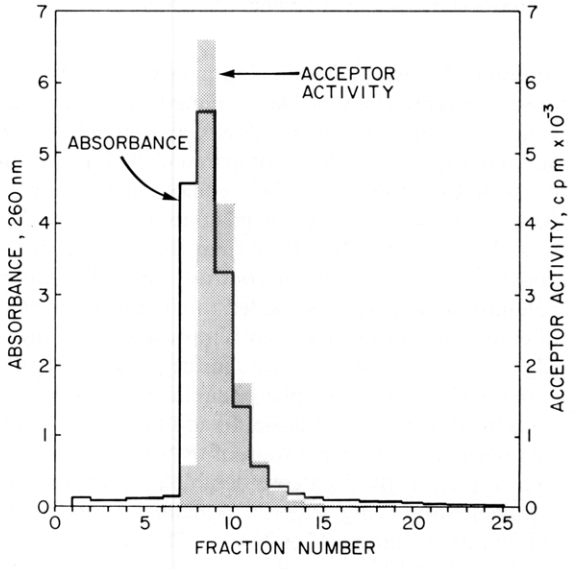

FIGURE 1: Absorbance and phenylalanine acceptor activity of fractions eluted from a column of BD-cellulose with 9.5% v/v ethanol in 1.0 M NaCl, 10 mM $MgCl_2$, and 50 mM sodium acetate (pH 5). The column (2.5 × 25 cm) had previously been loaded with 250 mg of sRNA and eluted with a gradient of NaCl as described in the text. The volume of each fraction was 18.5 ml and the flow rate was 90 ml/hr. Acceptor activity was estimated in duplicate 0.1-ml samples of fractions 6 and 11–18. Samples of fractions 7–9 were diluted tenfold and of fraction 10, threefold with water. Estimation of acceptor activity was performed using duplicate 0.1-ml quantities of the diluted samples of these fractions. Phenylalanine acceptor activities are expressed in terms of each fraction (undiluted).

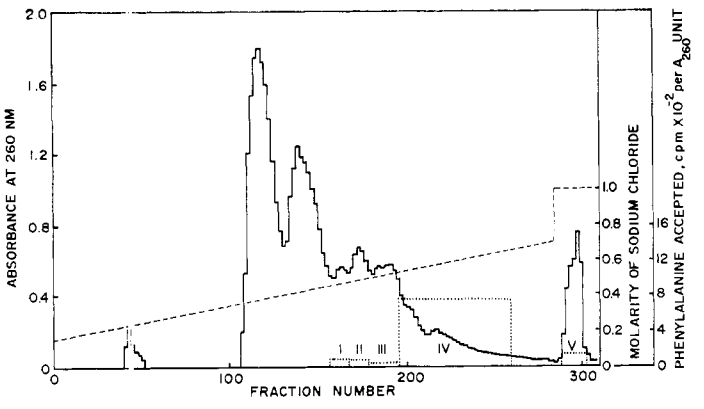

FIGURE 2: Fractionation of 100 mg of yeast sRNA (Calbiochem) on a column (3.2 × 105 cm) of BDCS. The sample was applied in 20 ml of 0.15 M NaCl and 0.05 M $MgCl_2$ and eluted with the indicated (broken line) gradient of sodium chloride (a total of 4 l.) containing 0.05 M $MgCl_2$. Fractions were 14 ml/10 min. At the end of the gradient more absorbing material was eluted with 1 M NaCl–0.05 M $MgCl_2$ containing 10% (v/v) 2-methoxyethanol. Fractions I–V were concentrated, the sRNA of each fraction was precipitated with ethanol. Aliquots of 5–10 A_{260} units were assayed for phenylalanine acceptor activity as described in Experimental Procedures, except a larger amount of unlabeled L-phenylalanine (80 μM) and larger amounts of each of 18 unlabeled L-amino acids (10 μM) were used. After incubation at 22° for 60 min, the reaction was stopped by the addition of 0.2 ml of 5 M NaCl and 1.4 ml of ethanol at 0°. Complete precipitation was assured by addition of 2 mg of carrier sRNA to each tube. The precipitates were washed three times with cold 67% v/v ethanol containing 0.5 M NaCl. They were then dissolved in water and aliquots were counted in Bray's (1960) scintillation fluid (dotted line).

tration to 50 mM resulted in elution of the sRNA at still lower concentrations of NaCl (Figure 2). Under these conditions, the bulk of the sRNA was eluted between about 0.3 and 0.5 M NaCl[4] compared with about 0.5 and 0.8 M NaCl for sRNA on BD-cellulose in 10 mM Mg^{2+} (Gillam *et al.*, 1967). Phenylalanine acceptor activity was eluted from BDCS later than the bulk of sRNA, above 0.5 M NaCl, but in contrast with BD-cellulose columns no alcohol was needed to elute it. Its position of elution from BDCS was not simply due to the higher Mg^{2+} concentration since even in the presence of 50 or 100 mM Mg^{2+} very little phenylalanine acceptor activity was eluted from BD-cellulose. In addition, it was found that when a crude preparation of $tRNA^{Phe}$ was applied to a column of BDCS a considerable proportion of the ultraviolet-absorbing material remained bound to the column after elution of $tRNA^{Phe}$. This material could be eluted with 10% ethanol and was found to have little phenylalanine acceptor activity. These observations pointed to a convenient method of further purifying the crude $tRNA^{Phe}$.

Chromatography of $tRNA^{Phe}$ on a Column of BDCS. Chromatography was at room temperature (approximately 23°) and all solutions contained 50 mM $MgCl_2$ and 50 mM sodium acetate (pH 5) with NaCl concentrations as stated. A column (1.9 × 64 cm) of BDCS was equilibrated with 0.4 M NaCl. The crude $tRNA^{Phe}$ (158 A_{260} units), dissolved in 0.4 M NaCl (3.6 ml), was applied to the column and was washed in with two portions (2 ml) of 0.4 M NaCl. Elution was carried out with a linear gradient made from 1 l. each of 0.4 and 0.7 M NaCl. Fractions were collected for 30 min each. The flow rate (17 ml/hr) was low since in this experiment the eluent was fed to the column by gravity only. Flow rates of up to 60 ml/hr had been used previously, without apparent adverse effect on the resolution. After collection of 130 fractions (final NaCl concentration 0.57 M), the absorbance of the eluate was less than 0.01 and elution with the gradient was stopped. The column was then eluted with 0.9 M NaCl (250 ml), followed by 1.0 M NaCl containing 9.5% v/v ethanol. During elution with the latter, fractions of 17 ml each were collected.

Figure 3 shows absorbances, phenylalanine acceptor activities, and specific activities (ratio of acceptor activity to absorbance) for the fractions from the column. $tRNA^{Phe}$ was eluted as a peak whose maximum coincided with that of an absorbance peak at 0.45 M NaCl. The specific activity across the peak was fairly constant. Little absorbing material was eluted by 0.9 M NaCl but 13% of the absorbance loaded on the column was present as a peak in the ethanol region where there was very little phenylalanine acceptor activity. The total recovery of absorbance from the column was 88%.

Fractions 30–63, for which the specific activity was approximately constant, were combined and the tRNA was concentrated using a small column of DEAE-cellulose. The final solution of $tRNA^{Phe}$ in 1.0 ml of water contained 68.4 A_{260} units (Table I). Its phenylalanine acceptor activity per A_{260} unit was 25 times that of the starting material (*i.e.*, the sRNA applied to the BD-cellulose column).

Estimation of the Purity of the Final $tRNA^{Phe}$ Preparation. The maximal acceptance of phenylalanine was determined in mixtures containing approximately 0.2 or 0.4 A_{260} unit per ml of $tRNA^{Phe}$ by measuring the TCA-insoluble radioactivity after incubation for 15 min at room temperature. Other components of the mixtures were as given above. The concentration of enzyme was sufficient to give maximal charging within 5–10 min and there was no significant fall in TCA-insoluble radioactivity up to 30 min. The efficiency of the scintillation counter was determined using samples (0.025 μCi) of five different [^{14}C]amino acids (New England Nuclear Corp.), dried on to paper disks, and placed in vials with 5 ml of toluene scintillation fluid.

The values obtained for the three parameters involved in calculating the purity of the $tRNA^{Phe}$ were as follows (expressed as mean ± approximate standard error of the mean): uptake of radioactivity, 51,000 ± 560 cpm per A_{260} unit; counting efficiency, 52.3 ± 0.7%; total phosphorus content 143 ± 1.5 ng-atoms per A_{260} unit. These values were equivalent to a phenylalanine acceptance of 1830 ± 50 pmoles per A_{260} unit and to a phosphorus content of 78 ± 3 g-atoms per mole of phenylalanine accepted.

The structure of yeast $tRNA^{Phe}$ has been determined and the molecule contains 76 nucleotides (RajBhandary

[4] In considering elution of tRNA species using gradients of sodium chloride, the concentrations of sodium chloride referred to are those present at the top of the column.

et al., 1967). Thus, material of 100% purity should accept 1 mole of phenylalanine for every 76 moles of phosphorus. The purity of the present preparation may therefore be estimated to be 97.5 ± 4%.

Tests for the Presence of Other Acceptor Activities in the Purified tRNA^{Phe}. The procedure described in Methods was carried out in which tRNA^{Phe} was incubated with a mixture of [^{14}C]amino acids under conditions suitable for charging. [^{14}C]Amino acids absent were methionine, cysteine, asparagine, and glutamine. The amino acids which combined with the tRNA were then isolated and separated by paper chromatography. The average specific activity of the [^{14}C]amino acids in the incubation mixture was approximately three times that of [^{14}C]phenylalanine.

Appreciable radioactivity was found in the final solution applied to the chromatogram obtained from the control mixture which had not contained tRNA^{Phe}. When the chromatogram was scanned it was found that this radioactivity was present as peaks in the region of basic amino acids, in valine, in leucine, and in isoleucine. Radioactivity was slightly above background in a number of other amino acids. The chromatogram obtained from the mixture containing tRNA^{Phe} showed a single large peak of radioactivity corresponding to phenylalanine. The remainder of the radioactivity trace was superimposable on that from the control mixture with the possible exception of the isoleucine region. The peaks noted above which were present on both chromatographs were small compared with the large phenylalanine peak. It was estimated that on the chromatogram obtained from the mixture containing tRNA^{Phe} the presence of radioactivity in another amino acid of the order of 3% of that in phenylalanine would have shown clearly as a difference between the two chromatograms. With the relative specific activities used, charging of another tRNA species present as a 1% impurity in the tRNA^{Phe} would have given this amount of radioactivity.

As a further check on purity, the tRNA^{Phe} was assayed directly for uptake of [^{14}C]isoleucine and [^{14}C]tyrosine. tRNA^{Tyr} was considered the most likely contaminating species for reasons discussed later. The amounts of isoleucine and tyrosine accepted per A_{260} unit were only 0.1 and 0.35%, respectively, of the amount of phenylalanine accepted (1.5 and 6.3 compared with 1830 pmoles/A_{260} unit). It seems likely that the isoleucine impurity suspected from the chromatogram was due to a slight irregularity in the leading edge of the adjacent phenylalanine spot.

Discussion

The results presented above demonstrate the isolation of a highly purified sample of yeast tRNA^{Phe} in two simple chromatographic steps. The first step depends upon the fact that tRNA^{Phe} binds more strongly to BD-cellulose than do other species of tRNA. The latter may be eluted by NaCl, while ethanol or 2-methoxyethanol is required for elution of tRNA^{Phe}. In general tRNAs bind more strongly to BD-cellulose at lower Mg^{2+} concentrations (*cf.* Gillam *et al.*, 1967) and the inclusion of 10 mM

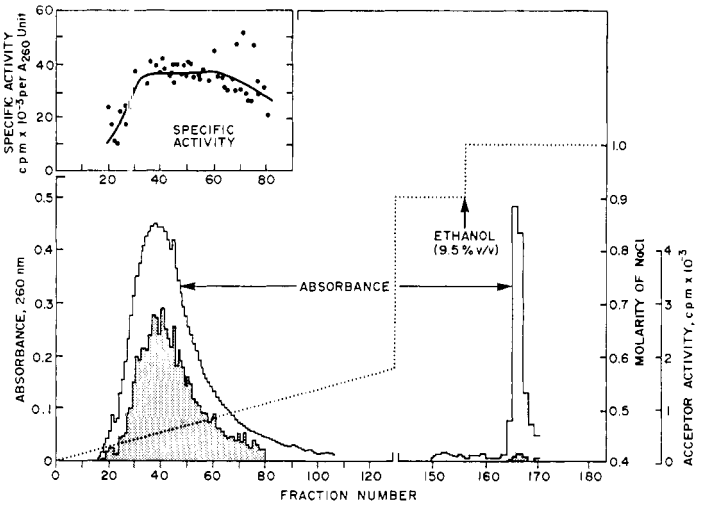

FIGURE 3: Absorbance, phenylalanine acceptor activity, and specific activity of fractions eluted from a column of BDCS (dimensions 1.9 × 64 cm) with a gradient of NaCl, followed by 0.9 M NaCl, and then by 1.0 M NaCl containing 9.5% v/v ethanol. All solutions also contained 50 mM $MgCl_2$ and 50 mM sodium acetate (pH 5). The broken line shows the NaCl concentration at the top of the column. The column had been loaded with 158 A_{260} units of crude tRNA^{Phe}. The volume of each fraction of the aqueous eluate was 8.5 ml and of the ethanolic eluate 17 ml. The flow rate was 17 ml/hr. Estimation of acceptor activity (shaded area) was carried out using a 0.15-ml sample of each fraction. The specific activity was calculated for each fraction and is expressed as counts per minute per A_{260} unit.

$MgCl_2$ in the eluents was necessary to ensure elution of other tRNA species with NaCl. In 5 mM Mg^{2+}, a considerable portion of tRNA^{Tyr} is found in the ethanol fraction, which also contains tRNA^{Ser} (unpublished observations).

A considerable amount of ultraviolet-absorbing material other than tRNA^{Phe} is eluted from BD-cellulose by ethanol (9.5% v/v). The nature of this material, which does not accept amino acids (Gillam *et al.*, 1967), is unknown. The possibility of separating tRNA^{Phe} from this material by eluting with an ethanol gradient (0–12% v/v in 1.0 M NaCl) was investigated. No better separation was obtained, however, than when elution was performed simply with 9.5% v/v ethanol in 1.0 M NaCl (Figure 1), the inactive material being eluted before, together with, and after the phenylalanine acceptor activity (unpublished observations). The inactive material may be partially separated from tRNA^{Phe} by gel filtration on Sephadex G-100, when it appears as a shoulder which is eluted in advance of the tRNA^{Phe} peak (unpublished observations). Thus it seems likely that at least some of this material is of higher molecular weight than tRNA^{Phe}.

In the second purification step, tRNA^{Phe} was eluted from a BDCS column by NaCl at a concentration of approximately 0.45 M. Most of the inactive material remained bound to the column until ethanol was included in the eluent. BDCS apparently binds tRNAs less strongly than does BD-cellulose in that lower NaCl concentrations are required for elution. This property suggests that BDCS may prove useful in fractionating nu-

cleic acids which bind too strongly to BD-cellulose for elution with conventional salt gradients, *e.g.*, rRNA and mRNA (Gillam *et al.*, 1967; Sedat *et al.*, 1967). It should be noted that the capacity of BDCS is much less than that of BD-cellulose.

The purity of the final preparation of $tRNA^{Phe}$ was estimated as 97.5 ± 4%. As noted above, the tRNA species which binds to BD-cellulose most strongly, next to $tRNA^{Phe}$, is $tRNA^{Tyr}$, which is partly eluted in the ethanol fraction if the Mg^{2+} concentration of the eluent is lowered from 10 to 5 mM. The most likely contaminant of the $tRNA^{Phe}$ might therefore be expected to be $tRNA^{Tyr}$. The tyrosine acceptor activity of the final preparation was only 0.35% of its phenylalanine acceptor activity. The results of the chromatographic separations of amino acids taken up by the $tRNA^{Phe}$ failed to detect any other acceptor activities. In this experiment the presence of 1% of a contaminating tRNA species should have been detectable.

No definite answer is available to the question of why $tRNA^{Phe}$ binds to BD-cellulose much more strongly than all other tRNA species. However it has been observed that the attachment of an aromatic group to a tRNA molecule produces a similar effect. Thus, tyrosyl-tRNA and tryptophanyl-tRNA (Maxwell *et al.*, 1968) and several *N*-phenoxyacetylated aminoacyl-tRNAs (Tener *et al.*, 1966) require ethanol for elution from BD-cellulose. The primary structure of yeast $tRNA^{Phe}$ has been determined (RajBhandary *et al.*, 1967) with the exception of one unidentified nucleoside, denoted Y. Since there are no obvious peculiarities of $tRNA^{Phe}$ when compared with other tRNA species of known sequence, it has been suggested tentatively that Y may contain a lipophilic group which could account for the unique behavior of $tRNA^{Phe}$ on BD-cellulose (Gillam *et al.*, 1967). This must be regarded as purely speculative until the nature of Y has been determined.

It is not yet known whether the same method can be used for isolation of $tRNA^{Phe}$ from other organisms. The method does not appear to be directly applicable to *Escherichia coli*. When sRNA from *E. coli* B was chromatographed on BD-cellulose at least 90% of the $tRNA^{Phe}$ was eluted by 1 M NaCl in the absence of ethanol. Material subsequently eluted from the column with 9.5% v/v ethanol was found to be enriched in tRNAs specific for tyrosine, alanine, and serine. Smaller amounts of other tRNAs were also present in this material (unpublished observations). It would be of considerable interest to examine the chromatographic behavior of $tRNA^{Phe}$ from a range of different organisms on BD-cellulose.

In another communication the isolation of yeast tyrosine and tryptophan tRNAs is described (Maxwell *et al.*, 1968). The method used depends upon the increased affinity for BD-cellulose conferred on a tRNA molecule when it becomes charged with an aromatic amino acid. This method should also be applicable to the isolation of phenylalanyl-tRNAs which are less strongly bound to BD-cellulose than is yeast $tRNA^{Phe}$.

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