The Isolation of Yeast Tyrosine and Tryptophan Transfer Ribonucleic Acids*

I. H. Maxwell,† E. Wimmer,‡ and G. M. Tener§

ABSTRACT: Uncharged yeast (*Saccharomyces cerevisiae*) transfer ribonucleic acids (tRNAs) may be entirely eluted from a column of benzoylated DEAE-cellulose (BD-cellulose) by 1 M NaCl, with the sole exception of phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$). Tyrosine ribonucleic acid ($tRNA^{Tyr}$) and tryptophan ribonucleic acid ($tRNA^{Trp}$) when charged with their respective amino acids bind strongly to BD-cellulose. Tyrosyl-$tRNA^{Tyr}$ is not eluted by NaCl alone at concentrations up to 1 M but may be eluted with 1 M NaCl containing a small proportion of ethanol. Tryptophanyl-$tRNA^{Trp}$, although partly eluted by NaCl at concentrations greater than 0.8 M, requires the presence of ethanol for complete elution. These properties of the aminoacyl-tRNAs were utilized to obtain highly purified preparations of $tRNA^{Tyr}$ and $tRNA^{Trp}$ in three simple chromatographic steps, each step using a column of BD-cellulose. First, $tRNA^{Phe}$ was removed together with some nontransfer ribonucleic acid material. Secondly, the remaining mixture of tRNAs was charged with either tyrosine or tryptophan and the charged species were isolated. Finally, the charged tRNA was stripped of amino acid and rechromatographed to obtain further purification. The final preparations accepted 1.86 and 1.93 nmoles of tyrosine or tryptophan, respectively, per A_{260} unit, corresponding to purification factors of approximately 32 and 28. It was estimated that both preparations were more than 94% pure.

All species of yeast tRNA when uncharged, with the exception of $tRNA^{Phe}$, may be eluted from BD-cellulose[1] by solutions containing 10 mM $MgCl_2$ and NaCl at appropriate concentrations (Gillam *et al.*, 1967). A fraction of sRNA[2] containing the $tRNA^{Phe}$ and some unidentified material has a high affinity for BD-cellulose and is not removed without the addition of ethanol to the eluting system. It has been called the "ethanol fraction." When $tRNA^{Tyr}$ and $tRNA^{Trp}$ are charged with their respective aromatic amino acids, their affinity for BD-cellulose is markedly increased and they are eluted with the ethanol fraction. These observations form the basis of the purification method reported here.

The method is briefly the following. The sRNA is first freed of the ethanol fraction by elution from BD-cellulose and the mixture of tRNAs is charged with either tyrosine or tryptophan. The charged tRNA is separated from the uncharged tRNAs by repeating the elution from BD-cellulose. The ethanol fraction from this second column contains the aminoacyl-tRNA. This is then stripped of its amino acid and purified further by chromatography on BD-cellulose. The final preparations obtained by this method are 32- and 28-fold purified in $tRNA^{Tyr}$ and $tRNA^{Trp}$, respectively.

Purification of yeast $tRNA^{Tyr}$ by countercurrent distribution has been described (Apgar *et al.*, 1962; Holley *et al.*, 1963; Madison *et al.*, 1966). Preparation of highly purified $tRNA^{Trp}$ has not previously been described.

Materials and Methods

Mixtures of aminoacyl-tRNA synthetases were prepared from baker's yeast as described (Wimmer *et al.*, 1968). Enzymes used for assay of acceptor activity of purified tRNAs were prepared by method B of Gillam *et al.* (1967). Those used for charging sRNA and in the test for other acceptor activities in the purified tRNAs were prepared by method A.

Other materials and methods were as described (Wimmer *et al.*, 1968)

Measurement of Radioactivity in Fractions Obtained from Columns. Samples (1.0 ml) of the fractions were each mixed with 1 ml of cold 10% w/v TCA and with carrier RNA (3.7 A_{260} units[3] of sRNA in 0.05 ml of water). The mixtures were left at 0° for 10–30 min and were then filtered on glass fiber filters (Reeve Angel, 934

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

[2] The term sRNA is used for the crude mixture of lower molecular weight polynucleotides from which tRNAs may be purified.

[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 of absorbance 1 at a wavelength of 260 nm.

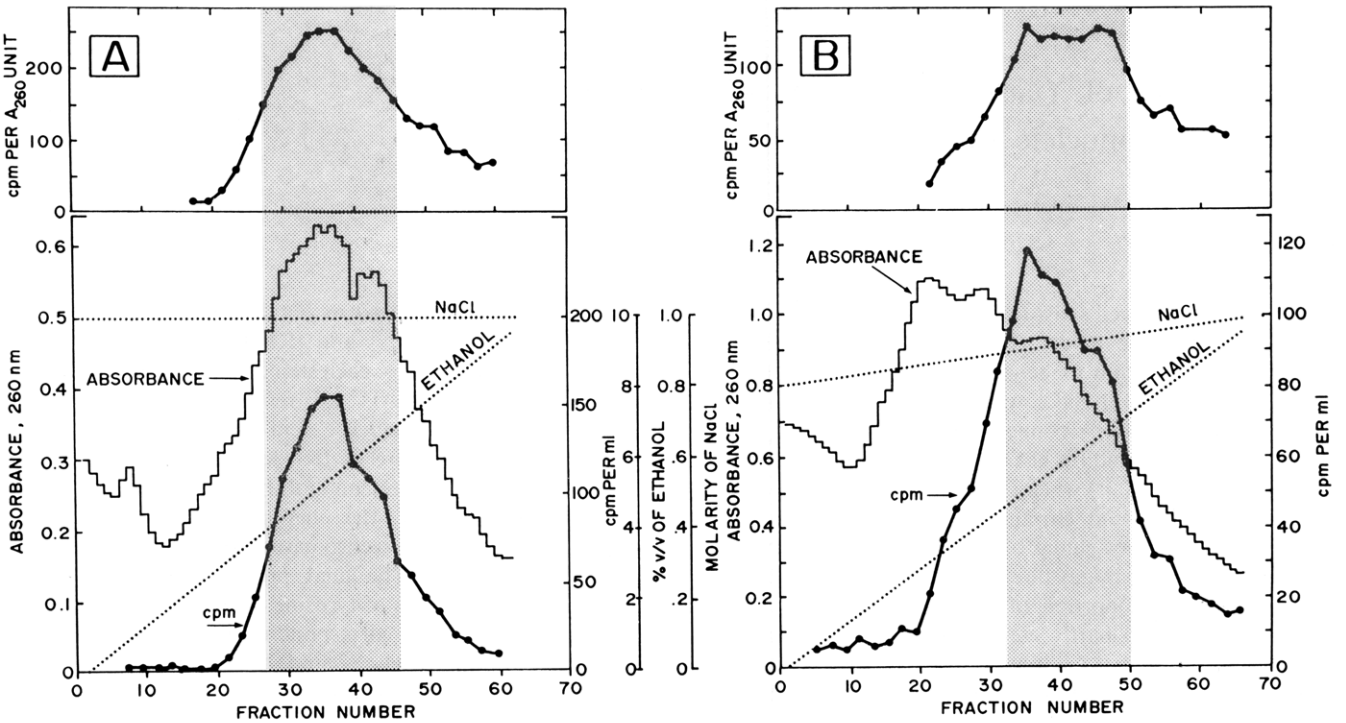

FIGURE 1: Absorbance and radioactivity of fractions eluted from columns of BD-cellulose by gradients of ethanol (0–9.5%, v/v). The columns (2.5 × 28 cm) had previously been loaded with sRNA charged with (A) [^{14}C]tyrosine or (B) [^{14}C]tryptophan, and had then been eluted with aqueous salt solutions as described in the text. All operations were at 2°. In A the solutions used to make the ethanol gradient (total volume 1 l.) contained 1.0 M NaCl. In B a gradient of NaCl (0.8–1.0 M) was superimposed on the ethanol gradient (total volume 1 l.). All solutions contained standard buffer (10 mM $MgCl_2$ and 50 mM sodium acetate, pH 5). The volume of each fraction was 15 ml and the flow rates were approximately 2 ml/min. Radioactivity was measured in alternate fractions and is expressed as counts per minute per milliliter.

AH, 2.4-cm diameter). Each filter was washed with three portions (2 ml) of cold 5% w/v TCA and with one portion (2 ml) of 2% v/v acetic acid. The filters were then placed in vials and were dried at 70° for 1 hr. After addition of 5 ml of scintillation fluid radioactivity was determined in a scintillation counter.

Removal of Esterified Amino Acid from tRNA. Esterified tyrosine or tryptophan was stripped from tRNA by incubation at 37° for 60 min in 1.8 M Tris-acetate (pH 8) (Sarin and Zamecnik, 1964). Negligible TCA-insoluble radioactivity remained after incubation.

Estimation of Amino Acid Acceptor Activities of tRNA Preparations. Estimations of tyrosine or tryptophan acceptor activities were performed as described for phenylalanine (Wimmer *et al.*, 1968) except that unlabeled and [^{14}C]phenylalanine were replaced by either unlabeled L-tyrosine (29 μM) plus uniformly labeled L-[^{14}C]tyrosine (1.0 μCi/ml; 352 mCi/mmole) or L-[3-^{14}C]-tryptophan (1.0 μCi/ml; 22.0 mCi/mmole). For estimation of other acceptor activities appropriate amounts of unlabeled and [^{14}C]amino acid were added to give a final specific activity of 20 mCi/mmole.

Experimental Procedures and Results Section

Removal of the Ethanol Fraction from sRNA. sRNA (1.0 g) (from brewer's yeast) was dissolved in 13 ml of 0.4 M NaCl, 10 mM $MgCl_2$, and 50 mM sodium acetate (pH 5). Operations were performed at room temperature and all solutions mentioned below containing NaCl also contained 10 mM $MgCl_2$ and 50 mM sodium acetate (pH 5.0).

The solution was loaded on a column of BD-cellulose (2.5 × 29 cm) equilibrated with 0.4 M NaCl. Elution was with a linear gradient of NaCl (0.4–1.0 M; total volume 2 l.), followed by 1.0 M NaCl (1 l.). The RNA in the combined eluate (15,500 A_{260} units, equivalent to 94% of the absorbance loaded on the column), after concentrating by ultrafiltration, was precipitated with ethanol. The RNA pellets obtained by centrifugation were drained and most of the ethanol was removed by evaporation *in vacuo* for 10 min. The RNA was then dissolved in water (total volume 30 ml; 458 A_{260} units/ml).

Charging of sRNA with Tyrosine or Tryptophan. The mixtures used for charging (total volume 100 ml) contained the following components: 0.10 M sodium cacodylate buffer (pH 7.6), 20 mM $MgCl_2$, 20 mM KCl, 1 mM EDTA (sodium salt), 0.5 or 2.0 mM ATP (sodium salt), 10 mM 2-mercaptoethanol, 0.5 mM L-tyrosine or L-tryptophan (unlabeled), 10 μCi of L-[^{14}C]tyrosine or L-[^{14}C]tryptophan, 5000 A_{260} units of sRNA from which the ethanol fraction had been removed, and 25 ml of enzyme solution (containing approximately 10 mg of protein/ml). ATP concentrations of 0.5 and 2.0 mM were found to be optimal for charging with tyrosine and tryptophan, respectively. It was found that the efficiency of charging decreased as the sRNA concentration was increased. Charging was therefore carried out at a com-

TABLE I: Percentage Recoveries of Absorbance and Acceptor Activities at Various Stages in the Purification of $tRNA^{Tyr}$ and $tRNA^{Trp}$.

Stage of Purification	$tRNA^{Tyr}$			$tRNA^{Trp}$		
	A_{260} Units	% Initial A_{260}	% Initial Acceptor Act.	A_{260} Units	% Initial A_{260}	% Initial Acceptor Act.
sRNA (ethanol fraction removed)	5000	100	100	5000	100	100
Charged tRNA applied to first column	4830	97	*a*	4860	97	*a*
Total aqueous eluate	4150	83	14	3760	75	18
Ethanol fraction (selected fractions only)	176	3.5	*a*	224	4.5	*a*
Stripped tRNAs applied to second column	107	2.1	*a*	154	3.1	*a*
Final purified tRNAs	27.4	0.55	16.8	34.9	0.70	18.7

[a] Values not determined.

paratively low concentration of sRNA (50 A_{260} units/ml).

The mixtures were incubated at 25° for 20–25 minutes and then 50 ml of 1 M sodium acetate (pH 5) was added to each. They were then extracted with an equal volume of water-saturated phenol for 5 min at room temperature. RNA was precipitated from the aqueous phases by addition of 20 ml of 5 M NaCl and 160 ml of cold ethanol. The RNA was redissolved in sodium acetate buffer (pH 5) and reprecipitated with ethanol twice. The final pellets were dissolved in 0.4 M NaCl buffer.

Isolation of Charged tRNAs. A sample of charged sRNA was loaded at 2° on a column of BD-cellulose previously equilibrated with standard buffer containing 0.4 M NaCl. Elution was performed at 2°. For material charged with tyrosine a linear gradient (total volume 1 l.) of NaCl from 0.4 to 0.9 M was used followed by a solution of 1.0 M NaCl (0.5 l.) and then by a gradient of ethanol (0–9.5% in 1 M NaCl (see Figure 1A). For tryptophenyl-tRNA the salt gradient (total volume 2 l.) was from 0.4 to 0.8 M NaCl and this was followed by a gradient of NaCl from 0.8 to 1 M which also changed from 0 to 9.5% alcohol (see Figure 1B). Table I shows the absorbance and acceptor activities present in the eluates. The fractions of highest specific radioactivity were pooled as indicated by the shaded areas in Figure 1 and the RNA was concentrated by ultrafiltration and then precipitated with ethanol.

Rechromatography of Stripped tRNAs. After incubation in Tris buffer (pH 8) to remove the esterified amino acid the RNA was again precipitated with ethanol. The precipitates were dissolved in standard buffer containing 0.4 M NaCl and were loaded on fresh columns of BD-cellulose previously equilibrated with this solution. The columns were eluted with NaCl as described in Figure 2, which shows the absorbances and tyrosine or tryptophan acceptor activities of fractions collected.

In each case a peak of absorbance coincided with the peak of acceptor activity, maximal absorbance occurring in fractions eluted[4] at 1.1 M NaCl for $tRNA^{Tyr}$ and 0.58 M NaCl for $tRNA^{Trp}$. (The apparent higher salt concentrations needed to elute these fractions was the result of using a relatively large column and steep salt gradient.) The specific acceptor activities (Figure 2) across the peak were fairly constant for about 17 fractions for $tRNA^{Tyr}$ and about 10 fractions for $tRNA^{Trp}$. These fractions from each column were combined and the tRNAs were concentrated by adsorption on to small columns of DEAE-cellulose, followed by elution with 1.5 M NaCl containing 10 mM $MgCl_2$. The final solutions, each in 1.0 ml of water, contained 27.4 A_{260} units of $tRNA^{Tyr}$ and 34.9 A_{260} units of $tRNA^{Trp}$. These preparations showed, respectively, 32 and 28 times the acceptor activities per A_{260} unit for tyrosine and tryptophan of untreated sRNA (see Table I).

Purity of Final Preparations. The values obtained for the parameters involved in calculating the purity of the tRNAs (expressed as mean plus and minus standard error) are given in Table II. The structure of yeast $tRNA^{Tyr}$ has been determined (Madison *et al.*, 1966). The molecule contains 78 nucleotides. The calculated purity of the present preparation, based on this value, was 98 ± 4%. The number of nucleotides per molecule of $tRNA^{Trp}$ has not been determined. Taking the arbitrary value 77 (the mean of the values for $tRNA^{Phe}$ and $tRNA^{Tyr}$) the calculated purity of the present preparation of $tRNA^{Trp}$ was 100 ± 5%.

Tests for the Presence of Other Acceptor Activities in the Purified tRNAs. Samples of the purified tRNAs were incubated with a mixture of [^{14}C]amino acids under conditions appropriate for charging. Esterified

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

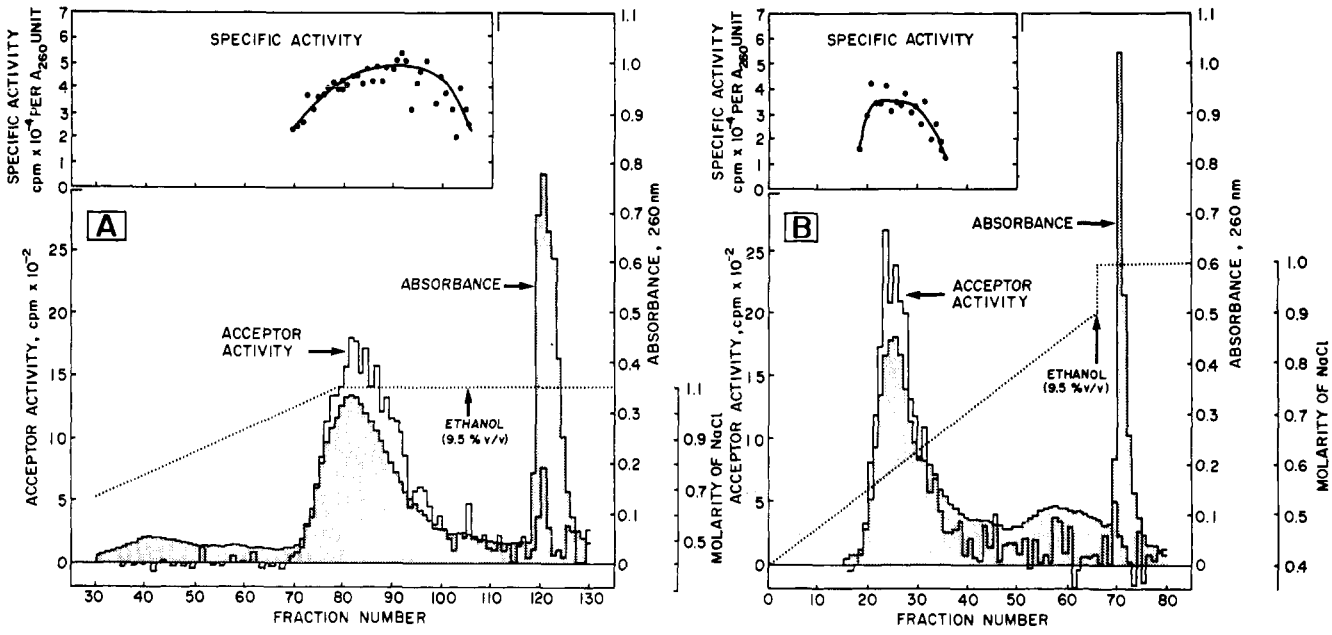

FIGURE 2: Absorbance and tyrosine or tryptophan acceptor activities of fractions eluted from columns of BD-cellulose loaded with partially purified $tRNA^{Tyr}$ or $tRNA^{Trp}$. (A) The column (2.5 × 28 cm) was loaded with 107 A_{260} units of stripped $tRNA^{Tyr}$. Elution was carried out at room temperature (22°) with a linear gradient of NaCl from 0.4 to 1.1 M (total volume 1 l.) followed by 1.1 M NaCl (0.3 l.) and then by 1.1 M NaCl containing 9.5% v/v ethanol. The volume of each fraction was approximately 13 ml and the flow rate approximately 1.8 ml/min. (B) The column (1.6 × 30 cm) was loaded with 154 A_{260} units of stripped $tRNA^{Trp}$. Elution was carried out at 2° with a linear gradient of NaCl from 0.4 to 0.9 M (total volume 1 l.) followed by 1.0 M NaCl containing 9.5% v/v ethanol. The volume of each fraction was 15 ml and the flow rate approximately 1.4 ml/min. All solutions used in A and B contained standard buffer (10 mM $MgCl_2$ and 50 mM sodium acetate, pH 5.0). Acceptor activity was estimated using 0.15-ml samples of the fractions. Specific activities are expressed as the measured counts per minute divided by the absorbance of the same fraction.

TABLE II: Calculation of the Purity of the tRNA Preparations.

	Uptake of Radioactivity (cpm/A_{260} unit)	Counting Efficiency (%)	Amino Acid Acceptance (pmoles of Tyr or Trp per A_{260} unit)	Total P Content (ng-atoms/A_{260} units)	g-atoms of P/mole of Amino Acid	Calcd Purity (%)
$tRNA^{Tyr}$	53,800 ± 800	52.3 ± 0.7	1,860 ± 54	147 ± 1.5	79 ± 3	98 ± 4[a]
$tRNA^{Trp}$	44,000 ± 1,000	52.3 ± 0.7	1,930 ± 71	148 ± 1.5	77 ± 4	100 ± 5[b]

[a] Calculated using the published value of 78 nucleotide residues/molecule of yeast $tRNA^{Tyr}$ (Madison *et al.*, 1966).
[b] Calculated using an assumed value of 77 nucleotide residues/molecule of $tRNA^{Trp}$.

amino acids were identified as described (Wimmer *et al.*, 1968). The chromatograms showed large peaks of radioactivity in the regions of tyrosine or tryptophan, as expected. There were no other major peaks but in some regions the radioactivity was slightly above that of the control. These regions corresponded to phenylalanine, tryptophan, and the basic amino acids for $tRNA^{Tyr}$ and to tyrosine, valine, and phenylalanine for $tRNA^{Trp}$. It was difficult to obtain an accurate estimate of the proportions of these possible contaminating species from the chromatograms. The tRNA preparations were therefore assayed directly for acceptor activity for these amino acids and also for other amino acids whose tRNAs chromatograph in the same region on BD-cellulose. The latter are serine and glutamine for $tRNA^{Tyr}$ and leucine, isolucine, threonine, proline, and methionine for $tRNA^{Trp}$ (Gillam *et al.*, 1967).

The results are shown in Table III. The major contaminants in $tRNA^{Tyr}$ were $tRNA^{Phe}$ and $tRNA^{Ser}$, present to the extent of 1.7 and 1.4%. The contaminant of $tRNA^{Trp}$ present in highest amount (0.5%) was $tRNA^{Ile}$.

Discussion

A number of factors influence the binding of tRNA to BD-cellulose. The concentration of magnesium

ion in the eluting solution is particularly important. On chromatography of crude tRNA on BD-cellulose to remove $tRNA^{Phe}$ (the ethanol fraction), the bulk of the tRNA was eluted with 1 M NaCl containing 10 mM $MgCl_2$ and then $tRNA^{Phe}$ was eluted with a solution containing the same concentration of salts in 10% ethanol. The resulting $tRNA^{Phe}$ fraction was essentially free of $tRNA^{Tyr}$. However, when 5 mM magnesium was used in the eluent a considerable portion of the $tRNA^{Tyr}$ was found in this fraction. Likewise some $tRNA^{Ser}$ was also present in this fraction when 5 mM $MgCl_2$ or EDTA was used in place of the 10 mM $MgCl_2$ (Wimmer *et al.*, 1968).

It is apparent that the addition of tyrosine or tryptophan to their respective tRNAs markedly increases the interaction between the tRNAs and BD-cellulose. This enhanced binding must be a consequence of changing the lipophilic nature of the tRNA by introducing the aromatic amino acid since analogous experiments with $tRNA^{Ala}$ indicated that the charged and uncharged species were eluted at approximately the same salt concentration (Gillam *et al.*, 1967).

The binding of tyrosyl-$tRNA^{Tyr}$ was stronger than that of tryptophanyl-$tRNA^{Trp}$. The former required the addition of ethanol to the 1 M NaCl eluting system before it was released whereas tryptophanyl-$tRNA^{Trp}$ was partially eluted from BD-cellulose with concentrated salt solutions (0.85–1.0 M NaCl). However, its complete removal required the addition of ethanol. To overcome this distribution of product between two fractions a gradient of salt to 0.8 M was used to elute most uncharged tRNAs and then the whole of the $tRNA^{Trp}$ fraction was eluted in the system containing ethanol.

Removal of the tyrosine and tryptophan regenerated the tRNAs which could be eluted from BD-cellulose with salt gradients alone.

This method of shifting the elution position of the given tRNA leads to products essentially free of contaminants. The trace amounts of other acceptor activities found in the purified material are probably a consequence of the small amount of tRNA which is nonspecifically adsorbed to BD-cellulose. From the determinations of the ratios of total phosphorus content to maximal amino acid acceptance and from the low percentages of contaminating acceptor activities it can be concluded that the final preparations of $tRNA^{Tyr}$ and $tRNA^{Trp}$ were at least 94% pure. The over-all yields (17 and 19%, respectively) were rather low. But since at both stages of purification by column chromatography (Figures 1 and 2) only the fractions of highest specific activity were combined, a considerable portion of the total acceptor activity was discarded at each stage. The second column should separate the tRNAs from most of the contaminating materials carried over from the first column. It might therefore be possible to combine a larger number of fractions from the first column to obtain a higher final yield of purified tRNA.

The method used for the above purifications apparently depends upon the interaction of the aromatic amino acids with benzoyl groups on the BD-cellulose. It should therefore be possible to isolate $tRNA^{Tyr}$, $tRNA^{Trp}$, or $tRNA^{Phe}$ from the sRNA of any organsim by this method providing that the uncharged tRNA can be eluted by a salt. The previously described method (Wimmer *et al.*, 1968) for the isolation of $tRNA^{Phe}$ was shown to be applicable only where this species is bound strongly to the exchanger, as with sRNA from yeast but not from *Escherichia coli*.

TABLE III: Acceptor Activities of the Purified tRNAs for Various Amino Acids.

tRNA	Amino Acid	pmoles/ A_{260} Unit	Acceptor Act. as Percentage of That for Tyr or Trp
$tRNA^{Tyr}$	Phenylalanine	32.3	1.7
	Serine	25.9	1.4
	Tryptophan	10.1	0.5
	Lysine	5.5	0.3
	Arginine	5.1	0.3
	Glutamine	0	0
			Sum 4.2
$tRNA^{Trp}$	Isoleucine	10.1	0.5
	Valine	4.6	0.2
	Methionine	3.7	0.2
	Tyrosine	3.6	0.2
	Threonine	3.4	0.2
	Proline	2.9	0.2
	Phenylalanine	1.3	0.07
	Leucine	1.0	0.05
			Sum 1.6

Gillam *et al.* (1967) have reported the elution of two partially resolved peaks of acceptor activity from BD-cellulose for both tyrosine and tryptophan. Two fractions of yeast $tRNA^{Tyr}$ separable by countercurrent distribution have been shown to differ only in the presence or absence of the terminal adenosine residue (Madison *et al.*, 1966). According to these workers there is a possibility that another species of yeast $tRNA^{Tyr}$ may exist but that it must be present in very small amount. It seems likely that the partially resolved peaks of tyrosine acceptor activity observed by Gillam *et al.* (1967) represented $tRNA^{Tyr}$ with and without the terminal adenosine. This would account for the failure to observe two peaks in Figure 2 above, since all the $tRNA^{Tyr}$ applied to the column had previously been charged with tyrosine and must therefore have contained terminal adenosine. A similar situation may exist for $tRNA^{Trp}$.

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Polynucleotides Containing 2′-*O*-Methyladenosine. I. Synthesis by Polynucleotide Phosphorylase*

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ABSTRACT: 2′-*O*-Methyladenosine 5′-phosphate, which was chemically synthesized from adenosine, was converted into 2′-*O*-methyladenosine 5′-diphosphate with rabbit muscle myokinase. This nucleoside diphosphate was slowly polymerized by polynucleotide phosphorylase to form poly-2′-*O*-methyladenylic acid. The rate of incorporation of this modified substrate was only $^1/_{100}$ that obtained with adenosine 5′-diphosphate. Addition of oligonucleotide primer stimulated the incorporation of 2′-*O*-methyladenosine 5′-diphosphate in both crude and primer-dependent polynucleotide phosphorylase preparations. The apparent K_m for 2′-*O*-methyladenosine 5′-diphosphate in the presence of oligonucleotide primer was determined to be 1.8×10^{-2} M. Studies on the size of the product revealed that poly-2′-*O*-methyladenylic acid synthesized in the absence of primer sedimented at approximately 13–15 S while polymer formed in the presence of primer sedimented at 5–6 S. 2′-*O*-Methyladenosine 5′-diphosphate was polymerized by polynucleotide phosphorylase in the presence of uridine 5′-diphosphate. The product of this reaction was shown to be a heteropolymer resulting from mixed incorporation of both substrates by its susceptibility to both alkali and ribonuclease.

The occurrence of 2′-*O*-methylribose in RNA was first reported by Smith and Dunn (1959). It has since been found in RNA obtained from many sources (Biswas and Myers, 1960; Hall, 1964; Singh and Lane, 1964; Correll, 1965) including purified phenylalanyl-tRNA (RajBhandary *et al.*, 1967). Recent studies on rRNA isolated from HeLa cells indicate that the major site of methylation in this RNA molecule is the 2′-hydroxyl group of ribose (Wagner *et al.*, 1967).

In addition to conferring resistance to nucleases which hydrolyze polynucleotides through the formation of 2′,3′ cyclic intermediates, 2′-*O*-methyl groups have been shown to influence the susceptibility of RNA components toward other enzymes. Snake venom phosphodiesterase attacks oligonucleotides containing 2′-*O*-methyl groups with difficulty (Gray and Lane, 1967), and 2′-*O*-methylribonucleoside 5′-phosphates are resistant to snake venom 5′-nucleotidase (Honjo *et al.*, 1964). Recently, Norton and Roth (1967) have reported the isolation of a ribonuclease from *Anacystis nidulans* which is specific for phosphodiester linkages containing a 2′-*O*-methyl group.

The participation of 2′-hydroxyl groups in internal hydrogen bonding between adjacent nucleotides in RNA has been postulated to be a significant component of the forces favoring ordered structure within polynucleotide chains (Riley *et al.*, 1966; Sato *et al.*, 1966; Ts'o *et al.*, 1966). Therefore, substitution at the 2′ position with an *O*-methyl group may influence both the physical and biological properties of an RNA molecule.

In considering possible approaches to the synthesis of RNA molecules containing large amounts of 2′-*O*-methyl groups, polynucleotide phosphorylase-catalyzed polymerization seemed rather unlikely in light of previous work on the substrate specificity of this enzyme. Although the enzyme has been shown to utilize nucleoside diphosphates which are extensively modified in the base moiety, alterations of the ribose diphosphate moiety were believed to be unacceptable (Grunberg-Manago, 1963). Thus, inversion of the 2′-hydroxyl of ribose to form the arabinose derivative produced an inactive substrate (Michelson *et al.*, 1962; Lucas-Lenard and Cohen, 1966). Conversion of either the 2′- or 5′-hydroxyl of the nucleoside diphosphate into the corresponding deoxy analog likewise resulted in molecules lacking substrate activity for polynucleotide phosphorylase (Grunberg-Manago, 1963; Yengoyan and Rammler, 1966).

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