Studies on human tRNA I. The rapid, large scale isolation and partial fractionation of placenta and liver tRNA

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ABSTRACT

A procedure for the large scale isolation of mammalian tRNA has been applied to the isolation of several grams of human liver, human placenta, rabbit liver and rat liver tRNA. This procedure entails an initial grinding of the tissue in phenol-sodium acetate at acidic pH, followed by DEAE cellulose chromatography. Procedures are also described for analysis of the purified tRNA on the basis of size, using controlled pore glass bead columns. In addition, the acceptor activity of isolated tRNAs has been determined using both the heterologous and homologous synthetases. The chromatographic profile of individual isoaccepting species using BD cellulose chromatography is shown and the 3' terminal nucleoside content was also determined.

The methods described now make it feasible for large scale studies of mammalian tRNA enabling us to better understand the relationships between the structure of mammalian tRNA and its many diversified functions.

INTRODUCTION

In recent years the study of tRNA has progressed from the isolation, characterization and sequence analysis of tRNA from microorganisms, such as *E. coli* and yeast, to subsequent studies on eukaryote tRNA. In the future, nucleic acid sequence analysis will be used extensively as a tool in the study of many specific biochemical phenomena.

Three examples where nucleic acid sequence analysis has already been important in obtaining the chemical nature of a biological phenomenon include the role of specific modified nucleotides in tRNA, studies on the functions of tRNA in regulation and control, and the exact nature of the differences in tRNA found during cellular development. In order to carry out these studies, procedures are required for the large scale

isolation of tRNA. Several such procedures have been described using rat liver¹⁻⁴, rabbit liver⁵⁻⁶, beef liver⁷, calf liver⁸, and human placenta⁹⁻¹⁰. In addition a recent review on this subject has been published by Weinstein & Fink¹¹.

We attempted to isolate gram quantities of tRNA from human liver and placenta, but in our hands yields from the above mentioned procedures were quite low and had a low acceptor activity. All of these procedures involved an initial homogenization at either neutral or basic pH. Recently however, Anandaraj and Cherayil¹² described a procedure for the isolation of chick embryo tRNA at acidic pH. We have modified this procedure and have applied it to the isolation of gram quantities of tRNA from human liver and placenta. In addition, we have used this procedure to obtain tRNA from rabbit and rat liver in yields comparable to those reported for other methods. The major advantage of this procedure is that 6 to 10 kg of tissue may be processed in less than 8 hours to yield 1 to 3 grams of a tRNA preparation which has a purity of 18 to 19 A₂₆₀/mg, depending on the tissue used, and is essentially free of high molecular weight contaminants. Subsequent fractionation of the tRNA on BD cellulose column not only is indicative of the stability of the isolated crude tRNA, but also will allow us to obtain large quantities of pure human tRNA for the purpose of nucleic acid sequence analysis.

MATERIALS AND METHODS

Chemicals and Reagents: Human liver and placenta were obtained frozen from local area hospitals and coroner's office. Rat and rabbit liver were 24 hour fasted Type I from Pel-Freez, Rogers, Arkansas. All tissues were stored at -20°C until used. Bentonite was obtained from Baroid Division, National Lead Co., ATP dipotassium salt from Nutritional Biochemicals, dithiothreitol (DDT) from Calbiochem, and 2-mercaptoethanol from Eastman. Amino acids, ¹⁴C label amino acids, enzyme grade ammonium sulfate and BD-cellulose were purchased from Schwarz-Mann. Chloroform, Methanol, isopropanol and anhydrous ether were the highest purity available from Fisher

Scientific. Phenol (90%) was a product of Dow Chemical Co. Tris (hydroxymethyl) amino methane was Trizma base purchased from Sigma, Sephadex G-25 was obtained from Pharmacia Fine Chemicals, DEAE cellulose (0.91 meg/g) from Schleicher and Schuell, and controlled pore glass (CPG-10) from Electro-Nucleonics, Inc.

All other chemicals were of highest purity available and all solutions were prepared with glass distilled, deionized water.

Measurement of amino acid acceptor activity: The standard assay mixture contained in a total volume of 1.0 ml:100 mM Tris-HCl pH 7.6, 5mM $MgCl_2$, 50mM KCl, 0.5mM EDTA, 2.5mM ATP, 0.01mM ^{14}C amino acid, 1-5 A_{260} units of tRNA, and a saturating amount of crude synthetase. After incubation for 30 minutes at 37°, the reaction was terminated with 2 ml of ice cold 10% TCA. The precipitate then was collected on a glass fiber filter, washed with 2% TCA, and dried as previously described¹³. When crude *E. coli* synthetase was used for determination of methionine initiator tRNA, the standard assay mixture was identical to mix A, described previously¹³ except that the concentration of ^{14}C methionine was 0.005 mM.

Radioactivity retained on the filter disc was measured in a Nuclear Chicago refrigerated Isocap 300 in 10 ml of Omnifluor-toluene (New England Nuclear) at a counting efficiency of greater than 85%.

All experiments were done at least in duplicate with blanks being parallel tubes incubated without tRNA. In all cases the amino acid acceptor activity of a given tRNA preparation was linearly dependent on added tRNA.

Acceptor activities are expressed as pmoles of amino acid incorporated per A_{260} units of tRNA as measured in distilled water. All absorbance measurements were taken on a Gilford 240 spectrophotometer. One absorbance unit, A_{260} (A_{280}), is defined as the amount of material which, in a volume of 1 ml and with a path length of 1 cm, gives an absorbance of 1.000 at 260nm (280nm).

RESULTS AND DISCUSSION

Preparation of tRNA: The following isolation procedure is carried out at room temperature (unless otherwise noted) using glass distilled water throughout.

One Kg of liver, or 3 placenta with umbilical cord removed, were partially thawed, chopped into pieces approximately 8 cm³, rinsed with distilled water and placed in a one gallon Waring blender. 1400 ml of liquid phenol (90%) and 1400 ml of 0.14 M sodium acetate (pH 4.5) were added and the tissue was homogenized for 0.2 min at low speed, 0.2 min. at medium speed, and 2.6 min. at high speed.

After the 3 min. grinding, the mixture was filtered through cheesecloth and centrifuged for 15 min. at full speed in either an ambient International Model UV Centrifuge equipped with a 4 liter swinging bucket head or a refrigerated International PR-6000 equipped with a 6 liter swinging bucket head.

The upper aqueous phase was poured off and combined with 0.5 volumes of phenol (i.e. 1 liter of phenol for each 2 liters of aqueous phase), stirred for 15 minutes and then allowed to stand at room temperature, while the remaining 9 Kg of tissue (27 placenta) was processed in 1 Kg (3 placenta) batches.

A phase separation occurred during the settling period allowing the bulk of the lower phenol layer to be siphoned off and discarded. The remaining upper phase and interphase were then centrifuged as before. The lower and interphase then were siphoned off and discarded. (These two phases could be re-extracted with an additional 500 ml of 0.14 M sodium acetate, pH 4.5, to increase the yield 2-5%.)

Next, the upper phases from 10 kg of tissue (30 placenta) were pooled, extracted a third time with an additional 500 ml phenol per 2 liters of upper aqueous phase, stirred 15 minutes, and centrifuged as before. The lower phase and interphase were siphoned off and discarded. (These two phases could be re-extracted with 500 ml of 0.14 M sodium acetate, pH 4.5, to give a 2-5% increase in yield.)

Then, the phenol extracted aqueous phase was stirred in 3 liter batches with approximately 75 grams of washed DEAE

cellulose. The washed DEAE cellulose was prepared as previously described¹³ and equilibrated with 0.14 M sodium acetate pH 4.5. This slurry was poured into a 2 liter glass Buchner funnel, and allowed to drain. Two additional batches of phenol extracted upper phase were then stirred with DEAE cellulose and added to the same Buchner funnel. Any remaining phenol extracted aqueous upper phase was added directly to the DEAE cellulose already in the Buchner funnel. During the building and elution of the DEAE cellulose column in the Buchner funnel, a gentle suction obtained from a water aspirator was applied to reduce the time required to pack and elute the DEAE cellulose.

Once packed, the DEAE cellulose was initially washed with 12 liters of 0.14 M sodium acetate pH 4.5 followed by 16 liters of 0.14 M sodium acetate pH 4.5 containing 0.3 M NaCl. The time required for the 2 liter washes was reduced to approximately 1 hour by using suction obtained from a water aspirator. The A_{260} of each 4 liters of effluent was measured, and after 16 liters of the 0.3 M NaCl wash had passed through the DEAE cellulose, the A_{260} approached 0.05. (In some experiments up to 24 liters of 0.3 M NaCl wash was required to reduce the A_{260} of the effluent to less than 0.1 A_{260} .) The tRNA was then eluted with 4 liters of 0.14 M sodium acetate pH 4.5 containing 1 M NaCl, and immediately precipitated with 2.5 volumes of ice cold 95% ethanol.

After standing overnight at -20°C the precipitate was collected by centrifugation, washed with ethanol, washed with ether, and dried over phosphorous pentoxide and potassium hydroxide in vacuo.

In some preparations, especially those from human liver, if the tRNA was eluted from the DEAE cellulose before the A_{260} dropped below 0.1, the dried product had an A_{260} of less than 15 units per mg. In these cases the material was further purified by either an additional phenol extraction or absorption to DEAE cellulose. For the phenol extraction, the material was dissolved in 0.14 M sodium acetate, pH 4.5, containing 0.1% Benonite and 0.001 M sodium thiosulfate to a concentration of

approximately 40A₂₆₀/ml. It was then extracted 2 times with 1 liter of liquid phenol, precipitated with 2 volumes of ethanol, washed two times with ether and dried as before. When DEAE cellulose was used to further purify the tRNA, the procedure was identical to the initial DEAE cellulose procedure described earlier in this paper.

The yields for various preparations of tRNA ranged from 0.8 gram to 2.5 grams per 10 kg tissue depending on the tissue used and the care taken in recovering the aqueous upper phase during the phenol extraction steps. The average yield and purity of the tRNA prepared from various tissues is given in Table I and an outline of this procedure is shown in Figure 1. The isolation conditions were varied in an attempt to determine the optimum conditions for maximum yield and specific activity of tRNAs from both human liver and placenta. These variations in specific conditions and results obtained are listed below.

- (1) If the grinding mixture contained 0.01 M Tris HCl pH 7.6 and 0.3 M NaCl the yield of tRNA was reduced by 50% due to the inability of the interphase to be distinguished from the upper phase during the phenol extraction steps.
- (2) Precipitation of the upper aqueous phase from the final phenol extraction prior to the DEAE step caused a 50% decrease in yield. This loss was due to the difficulty in solubilizing the precipitate prior to its absorption to DEAE.
- (3) A preliminary isopropanol fractionation was attempted on the upper phase prior to absorption to DEAE. In this experiment 0.30 volume of isopropanol was added, with stirring, to the upper phase (similar to that described by Robison and Zimmerman⁷), stirred 1 hour at room temperature and centrifuged. The supernatant was collected, an additional 0.80 volume of isopropanol was added, and the precipitate was collected by centrifugation after standing 2 hours at -20°C. The yield from this procedure was reduced approximately 50% over that of tRNA obtained by direct absorption to DEAE but could be increased by several reextractions of the 0.30 volume isopropanol precipitate.

FIGURE I - PREPARATION OF HUMAN tRNA

1 kg tissue (or 3 placenta)

+ 1400 ml phenol

+ 1400 ml 0.14 M sodium acetate pH 4.5

Homogenize 3 min., filter through cheesecloth,
centrifuge 15 min.

Lower and interphase discarded Upper phase

Add 1 liter phenol per
2 liters upper phase

Stir, centrifuge 15 min.

Lower and interphase discarded Upper phase

Add 500 ml phenol per
2 liters upper phase

Stir, centrifuge 15 min.

Lower and interphase discarded

Pool upper phases from
10 such preparations
and absorb to 225
grams DEAE-cellulose

Elute DEAE cellulose with:

- (1) 12 liter 0.14 M sodium acetate pH 4.5 - discard effluent
- (2) 16 liter 0.14 M sodium acetate pH 4.5 containing 0.3M NaCl
- discard effluent
- (3) 4 liter 0.14 M sodium acetate pH 4.5 containing 1.0 m NaCl
 - Precipitate effluent with 2.5 volumes of 95% ethanol
 - Collect precipitate by centrifugation
 - Wash precipitate once with 1 liter of 95% ethanol followed
by two washes of 1 liter each of anhydrous ether
 - Air dry, followed by vacuum drying over phosphorus
pentoxide - potassium hydroxide.

TABLE I

Source	Quantity of Tissue	Yield(gm)	A ₂₆₀ /mg
Human Placenta	22 placenta (10 kg)	0.94	18.24
Human Liver	10 kg	2.05	18.63
Rabbit Liver	6 kg	2.25	19.20
Rat Liver	6 kg	2.45	19.11

(4) Extraction of the upper aqueous phase with ether or chloroform before absorption to DEAE or inclusion of 0.1% Bentonite, 0.1% SDS, or 0.001 M sodium thiosulfate in the grinding buffer caused no significant effect on the final yield of tRNA based either on acceptor activity or A_{260}/mg .

(5) Extraction of the upper aqueous phase with chloroform: methanol, (2:1,v:v) caused a complete loss of acceptor activity of the tRNA, a phenomenon also observed at pH 7.6 using Tris buffer during the isolation of beef liver tRNA (B. Dudock-personal communication).

(6) Thawing of either placenta or liver to room temperature and then grinding in phenol/acetate also caused a marked decrease in the yield of tRNA and therefore these tissues were only partially thawed and chopped into small pieces immediately before homogenization.

(7) Since the yield obtained from human liver and placenta was lower than that reported for either rat or rabbit liver¹⁻⁶, we attempted the isolation of tRNA from these two additional mammalian tissues. This data is summarized in Table I and indicates that this procedure produces yields for rat and rabbit liver tRNA comparable to those reported by others¹⁻⁶.

The reduced yield in tRNA from human placenta and liver could be attributed to several possibilities. First, placenta and liver might contain less tRNA per gram of net weight of tissue due to the large amount of connective tissue in placenta or fat and fibrous tissue in human liver. Second, although we have received the excellent cooperation from several area hospitals and coroners' offices in obtaining large quantities of frozen placenta and frozen human liver, there is always the question of degradation of the sample prior to freezing. On the other hand, the rat and rabbit liver were obtained from Pelfreeze, and frozen within minutes after sacrifice. As was noted earlier, the yield of tRNA was greatly reduced if the placenta or liver was thawed to room temperature before homogenization.

(8) The efficacy of employing an incubation of the preparation of tRNA at pH 8.2 in 1 M Tris buffer at 37° for 1 hour as a final step in the purification procedure was investigated.

Our results indicate that stripping causes no increase in acceptor activity and therefore was not included in the isolation procedure.

Isolation of mammalian synthetase: The procedures used for isolation of crude rabbit liver, human liver, and placenta synthetase are essentially those described by several groups¹⁻⁹. All procedures were carried out at 4° (unless otherwise noted), using glass distilled water throughout.

Tissue (100 grams of rabbit liver, 200 grams human liver, or two placenta) was thawed, cut into thin slices and washed with two 500 ml portions of a grinding mix (0.05M Tris-HCl pH 7.6, 0.01M MgCl₂, 0.25M Sucrose, 15% Glycerol, 0.01M 2-mercaptoethanol, 0.1M KCl, 0.5mM EDTA) to remove the excess blood. The washed tissue was then homogenized for 1 minute in a 1 quart Waring blender at full speed in 250 ml of grinding mix and centrifuged for 30 minutes at 10,000 Xg in a Lourds refrigerated centrifuge to remove particulate matter and cell debris. After filtering through glass wool to remove lipid, the crude extract was either (A) precipitated with ammonium sulfate or (B) subjected to DEAE cellulose chromatography.

Method A: 40 grams of solid ammonium sulfate was added per 100 ml of supernatant, and stirred for 1 hour at 4°. The precipitate was then collected by centrifugation at 10,000 Xg for 30 minutes and stored frozen. Prior to use, a small amount of this precipitate was dissolved in column buffer A (0.02M Tris-HCl pH 7.6, 0.01M MgCl₂, 0.01M 2-mercaptoethanol, 15% glycerol) and desalted on a 2 x 50 cm Sephadex G-25 column. The enzymes prepared in this manner are stable in the form of the ammonium sulfate pellet or G-25 effluent for at least 3 months at -20°C. If the G-25 effluent is adjusted to 50% glycerol and stored at -20°, a rapid loss in acceptor activity occurs within 2-3 weeks.

Method B: The crude extract, after passage through glass wool, was adjusted to 0.2 M KCl and applied to a 600 ml DEAE cellulose column (4.5 x 40 cm), which had been built and washed in column buffer A, containing 0.2 M KCl. (The DEAE cellulose used in this step had been washed as previously described¹³).

This column was eluted with column buffer A at a flow rate of 2-3 ml/min. The A_{280} absorbing material was pooled, precipitated with solid ammonium sulfate, collected by centrifugation and stored as described in Method A. Prior to use in assays, the protein was desalted as described in Method A. The enzyme prepared using Method B was similar in its stability but higher in specific activity when compared to that obtained using Method A. Because of recent reports that the DEAE cellulose step causes a diminution in specific synthetase activities¹⁴, we have isolated crude synthetase using both procedures.

Analysis of tRNA Preparations on CPG-10: When this study began, it soon became apparent that we needed a method of analysis of the size distribution of the A_{260} absorbing material during various stages of the isolation procedures. Original analysis using Sephadex G-100 proved to be too slow (6-8 hours), and in addition highly impure material caused a marked decrease in flow rate. We therefore attempted to use a controlled pore glass bead column for our analysis.

Controlled pore glass beads (CPG-10-170 or CPG-10-240) were soaked in sulfuric acid-dichromate cleaning solution for 2 hours, thoroughly rinsed with distilled water, and finally equilibrated with 0.1M cacodylate-KOH buffer pH 7.0. After the glass bead slurry had been thoroughly de-gassed, it was packed into a 5 mm x 50 cm stainless steel column fitted with Swagelok fittings top and bottom. A wad of glass wool in the lower fitting was used as a bed support. The column was packed using a Mil-Roy-D-pump at a flow rate of 2 ml/min. and washed with 100 ml of 0.1 M cacodylate-KOH (pH 7.0) buffer which had been de-gassed by heating at 50°C while under a partial vacuum. The A_{260} of the column effluent was monitored using a Gilford Spectrophotometer, equipped with an IEC Model 3631 flow cell and recorded on a Sargent Welch Model SR chart recorder. Figure 2 shows (solid line) a typical elution profile using CPG-10-240 for *E. coli* tRNA (obtained from Biogen Inc.) and tryptophane. The broken line represents an overlay of the profiles for human liver, human placenta, rabbit liver, and

Figure 2: Comparison of the elution profiles of isolated human tRNA, and a mixture of commercial *E. coli* tRNA and tryptophane on controlled pore glass beads (CPG-10-240).

Figure 3: Comparison of the elution profiles of isolated human tRNA, and a mixture of commercial *E. coli* tRNA and tryptophane on controlled pore glass beads (CPG-10-170).

rat liver tRNA's isolated using the pH 4.5 method described herein, since all four tRNA's gave identical elution profiles.

As can be seen, this method of analysis not only is highly reproducible, rapid (10 to 20 minutes for complete analysis) and sensitive to small changes in molecular size, but also it can be used with microliter quantities of sample.

Figure 3 shows similar elution profiles using CPG-10-170 for the same samples analyzed using CPG-10-240. It should be noted that as expected the 240A beads give better resolution of the larger molecular weight species while the 170A beads give better resolution of tRNA, and smaller molecular weight species.

This rapid, sensitive method of analysis clearly indicates that the size of the A_{260} absorbing material, isolated by the pH 4.5 procedure from several tissues, corresponds to tRNA, with a slight contamination with higher molecular weight RNA.

Acceptor activity of isolated tRNA: The average amino acid acceptor activity for several tRNA preparations of human liver, placenta, and rabbit liver are shown in Table II. It can be seen that the tRNAs prepared using the procedure discussed herein contain the full complement of individual tRNAs. We have also found that there is very little variation in the acceptor activity from one preparation to another, if all the tissues are only partially thawed before homogenization, and all three phenol extractions are done prior to DEAE cellulose chromatography. The difficulties found in using other procedures¹⁻⁶ stem from a degradation of tRNA which occurs while dissolving crude tRNA, after ethanol precipitation, prior to further purification.

In addition, of the three different sources of synthetase, rabbit liver gave the most active preparation, placenta gave a preparation with moderate activity, while human liver synthetase had very low activity. Although several different human livers were used to isolate synthetase, to date, we have been unable to obtain an active preparation of human liver synthetase for alanine, methionine, and leucine from frozen liver. Perhaps the difficulty in obtaining a highly active preparation

TABLE II - AMINO ACID ACCEPTOR ACTIVITY

<u>Amino Acid</u>	<u>Placenta tRNA</u>	<u>Acceptance (pmoles/mg)</u>	
		<u>Human Liver tRNA</u>	
	<u>Placenta Synthetase</u>	<u>Rabbit Liver Synthetase</u>	<u>Human Liver Synthetase*</u>
Ala	280	376	—
Arg	394	2	---
Asn	362	10	---
Asp	74	145	---
Cys	224	127	---
Glu	78	2	---
Gln	32	22	---
Gly	475	67	80
His	404	106	80
Ile	83	137	---
Met	105	176	—
Lys	568	242	240
Leu	178	361	—
Phe	220	220	240
Ser	506	529	360
Val	299	392	340
Tyr	134	194	80
Trp	301	112	200
Pro	246	86	100
Thr	420	239	185

* — inactive --- not done

of human liver synthetase stems from our inability in obtaining fresh, unfrozen liver.

Also, in the case of placenta synthetase we have found that two placenta (about 1 Kg of tissue) had to be processed in the procedure discussed earlier, to yield a synthetase activity comparable to that obtained from 100 grams of fresh, frozen rabbit liver.

Therefore, although the human tRNAs contain the full complement of isoaccepting tRNA species, the acceptor activities observed are strongly dependent on the activity of the individual synthetase preparation.

We have also observed that the tRNAs from these three sources (human liver, placenta, and rabbit liver) are capable of being aminoacylated by several synthetases from any of these sources. These results support the hypothesis that mammalian tRNAs have a similar primary structure (and/or synthetase recognition site), as has been demonstrated from mammalian tRNA^{phe} 15,16 and tRNA^{met} 17,18 from several different organs and species.

Terminal nucleoside content of the isolated tRNA: Both human liver and human placenta tRNA isolated by the above mentioned procedure and commercial E. coli tRNA were analyzed for terminal nucleoside content and compared.

The procedure used entailed hydrolysis of the crude tRNA with RNase T₂ as described¹⁹, followed by chromatography on RPC-5 with ammonium carbonate buffer²⁰ to separate nucleosides from nucleotides.

The ribonucleoside peak was lyophilized to dryness and analyzed on Aminex-A 25²¹ using formic acid (pH 2.35). The results showed that the mammalian tRNA and E. coli tRNA contained both adenosine and cytosine as terminal nucleosides in a ratio of 2:1.

Although we have not tested our crude synthetase preparations for tRNA ribonucleotidyl transferase (CCA adding enzyme), low levels of activity for this enzyme could account for the slightly reduced acceptor activity of the mammalian

tRNA preparations, a point we are presently investigating.

Chromatographic profile of human liver and human placenta tRNA on a preparative BD cellulose column: The isolated crude tRNA from human liver and placenta was partially fractionated using a 1 liter benzoylated DEAE (BD) cellulose column (3.6 x 100 cm) as shown in Figures 4 and 5. The BD cellulose obtained, from Schwarz-Mann, was washed with 2M NaCl containing 20% ethanol, followed by several liters of distilled water, equilibrated with BD buffer (0.01M Tris-HCl pH 7.6 0.01M $MgCl_2$, 0.001M $Na_2S_2O_3$) containing 0.4M NaCl and thoroughly degassed. The column was built and packed using a Mil-Roy-D-pump at a flow rate of 10 ml/min. and washed with several liters of BD buffer containing 0.4M NaCl. Human liver tRNA (4.5 grams) was dissolved in 500 ml of BD buffer containing 0.4M NaCl and pumped onto the column. After washing with 200 ml of BD buffer containing 0.4M NaCl, a 6 liter linear gradient was generated (3 liters of BD buffer containing 0.4 M NaCl to 3 liters of BD buffer containing 0.6 M NaCl). At tube #490, a 4 liter linear gradient was generated (2 liters of BD buffer containing 0.6 M NaCl to 2 liters of BD buffer containing 2.0 M NaCl), and at tube #740 a 2 liter linear gradient was generated (1 liter of BD buffer containing 2.0 M NaCl to 1 liter of BD buffer containing 2.0 M NaCl 20% ethanol). The flow rate was maintained at 15 ml/1.25 min./ tube using the Mil-Roy-D-pump. Human placenta tRNA (3.5 grams) was fractionated as shown in Figure 5 with conditions identical to those for human liver tRNA except that the total volume of the first gradient was 4 liters rather than 6 liters and the second and third gradients were begun at tubes 295 and 540 respectively.

Column effluent was monitored manually at A_{260} using a Gilford spectrophotometer. Five ml portions from every 10th tube were precipitated with ethanol, collected by centrifugation and dried. After dissolving in 1 ml of distilled water, aliquots from this concentrated assay set were used in the standard assay procedure described earlier in this paper to determine the elution profiles for various isoaccepting tRNAs.

Fluorescence was measured at room temperature in an Aminco-Bowman spectrofluorometer with a mercury-xenon arc lamp as described by Yoshikami, et al.²².

For assays, fluorescence was measured at 440 nm upon excitation at 310 nm. The fluorescence emission spectrum (uncorrected) is shown in Figure 6 for the peak tubes of fluorescence from human liver tRNA chromatographed on the BD cellulose column shown in Figure 4.

A comparison of Figures 4 and 5 shows a clear separation of several tRNA's and their isoaccepting species. An analysis of the elution profiles obtained for the ten specific tRNAs tested indicates some striking similarities and differences. Both human liver and placenta contain at least one species of tRNA^{val} which elutes early during the first gradient. The tRNA^{ala} which also elutes early is partially fractionated into at least three isoaccepting species. Other similarities between human and placenta tRNAs include at least three isoaccepting species of tRNA^{ser} and two isoaccepting species of tRNA^{lys} while tRNA^{met} was also fractionated into several isoaccepting species. In addition, the initiator tRNA (tRNA^{met}_i), which was aminoacylated with methionine using a crude *E. coli* synthetase preparation, eluted earlier than the tRNA^{met}_m which was not an acceptable substrate for the crude *E. coli* synthetase.

For the tRNA^{phe}, we observed two peaks. The first is a minor species which elutes just prior to the major peak and is probably the tRNA^{phe} without the fluorescent nucleotide. The major peak has a tendency to tail and therefore was eluted as two peaks with the latter coming out during the ethanol gradient, a phenomenon we have observed with *E. coli* tRNA^{phe}.

Differences in the elution profile for the tRNAs specific for tyrosine, glycine and tryptophane were also observed. As can be seen, tRNA^{tyr} from human placenta contains at least four species, while the tRNA^{tyr} from human liver probably only contains the first two species. The tRNA^{gly} from human placenta contains one major isoaccepting species which elutes early in the first salt gradient, while the tRNA^{gly} from human liver

Figure 4: Chromatography of human liver tRNA on BD-cellulose. Assays were carried out using 10 μ l of concentrated column effluent, and a saturating amount of either human liver aminoacyl tRNA synthetase or rabbit liver aminoacyl tRNA synthetase, both prepared using Method B.

Figure 5: Chromatography of human placenta tRNA on BD-cellulose. Assays were carried out using 50 μ l column effluent directly, and a saturating amount of either human placenta aminoacyl tRNA synthetase or rabbit liver aminoacyl tRNA synthetase, both prepared using Method B.

Figure 6: Fluorescence spectra of tubes 720, 870 and 950 from human placenta tRNA chromatographed on BD-cellulose (Figure 5).

contains at least three isoaccepting species. There is little similarity between the isoaccepting species of tRNA^{tyr} in human liver and human placenta, as the liver tRNA^{tyr} consists of at least two isoaccepting species which elute during the first salt gradient, and placenta tRNA^{tyr} consists of at least one major species which elutes during the second salt gradient.

After the chromatographic profiles were determined both runs were assayed for fluorescence. No fluorescence was observed until at least half of the high salt gradient had been eluted. In human liver tRNA we observed three distinct fluorescence peaks. Spectral analysis of the peak tubes of fluorescence (Figure 6) indicates that the first two peaks (at tube 720 and 870) give identical fluorescence spectra and also correspond to the observed peak of acceptor activity for phenylalanine. The species responsible for the late eluting fluorescence peak is presently under investigation but is unknown at this time.

Human placenta tRNA also contained three peaks of fluorescence activity. However, it is the last two peaks which correspond to tRNA^{phe} in human placenta as judged by fluorescence spectra (Figure not shown) and amino acid acceptor activity for phenylalanine. The species responsible for the early eluting peak of fluorescence activity in placenta is presently under investigation but is unknown at this time.

CONCLUSION

We have described a procedure for the rapid isolation of gram quantities of mammalian tRNA and have tested this procedure using human liver, human placenta, rat liver, and rabbit liver. The yields of tRNA from these sources are comparable to those from other procedures, but the time for isolation is greatly reduced.

Analysis of the isolated tRNA on the basis of size, acceptor activity and chromatographic profile show that the material is highly purified, active in the aminoacylation reaction, and contains the full complement of individual tRNA species.

Presently we are purifying several individual tRNAs for the purpose of sequence analysis and other studies, in an attempt to learn more about the nature of human tRNA and the differences in the tRNA isolated from distinct human organs.

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REFERENCES

1. Brunngraber, E. F. (1962) Biochem. Biophys. Res. Comm. 8, 1-3.
2. Delihias, N. and Staehelin, M. (1966) Biochim. Biophys. Acta 119, 385-391.
3. Rogg, H., Wehrli, W., and Staehelin, M. (1969) Biochim. Biophys. Acta 195, 13-15.
4. Fink, L. M., Lanks, K. W., Goto, T., and Weinstein, I. B. (1971) Biochemistry 10, 1873-1878.
5. Igarashi, S. J. and Dufresne, M. J. (1972) Analytical Biochem. 47, 102-108.
6. Petrissant, G., Boissard, M., and Puissant, C. (1971) Biochimie 53, 1105-1109.
7. Robison, B. and Zimmerman, T. P. (1970) Analytical Biochem. 37, 11-20.
8. Pearson, R. L., Hancher, C. W., Weiss, J. F., Holladay, D. W., and Kelmers, A. D. (1973) Biochim. Biophys. Acta 294, 236-249.

9. Abadom, P. N., and Elson, D. (1970) *Biochim. Biophys. Acta* 199, 528-531.
10. Matthaei, J. H. and Schoech, G. K. (1967) *Biochem. Biophys. Res. Comm.* 27, 638-643.
11. Weinstein, I. B. and Fink, L. M. (1971) in *Methods in Cancer Research*, Vol. VI, pp 311-343. Academic Press, Inc. New York.
12. Anandaraj, M. P. J. S., and Cherayil, J. D. (1974) *Analytical Biochem.* 58, 190-194.
13. Roe, B., Sirover, M., and Dudock, B. (1973) *Biochemistry* 12, 4146-4154.
14. Mushinski, J. F. (1974) *Nature* 248, 332-334.
15. Keith, G., Picaud, F., Weissenbach, J. Ebel, J. P. Petrissant, G., and Dirheimer, G. (1973) *FEBS Letters* 31, 345-347.
16. Keith, G. (Personal Communication).
17. Simsek, M., Raj Bhandary, U. L., Boissard, M., and Petrissant, G. (1974) *Nature* 247, 518-520.
18. Piper, P. W., and Clark, B. F. C. (1974) *Nature* 274, 516-518.
19. Harada, F., Kimura, F. and Nishimura, S. (1971) *Biochemistry* 10, 3269-3277.
20. Roe, B., Marcu, K., and Dudock, B. (1973) *Biochim. Biophys. Acta* 319, 25-36.
21. Singhal, R. P., and Cohn, W. E. (1973) *Biochemistry* 12, 1532-1537.
22. Yoshikami, D., Katz, G., Keller, E. B., and Dudock, B. S. (1968) *Biochim. Biophys. Acta* 166, 714-717.