Purification of Human Placenta Phenylalanine, Valine, Methionine, Glycine, and Serine Transfer Ribonucleic Acids†

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ABSTRACT: By using column chromatography on varied media, the purification of several individual tRNAs from human placenta has been achieved. The crude human placenta tRNA was isolated using phenol extraction at pH 4.5 followed by DEAE-cellulose chromatography (B. Roe (1975), *Nucleic Acids Res. 2,* 21–42) and initially fractionated on BD-cellulose at neutral pH. Subsequent chromatography of the partially purified tRNA using high-speed, high-pressure liquid chromatography on RPC-5 and Aminex A-28 coupled with chromatography on BD-cellulose at acidic pH and on DEAE-Sephadex A-50 significantly shortened isolation time for milligram quantities of several pure tRNA species. Those tRNAs from human placenta obtained in a purity greater than 1.2 nmol/A_{260} unit are $tRNA^{Phe}$, $tRNA^{Met}_i$, $tRNA^{Val}_{1a}$, $tRNA^{Val}_{1b}$, and $tRNA^{Gly}_1$, while those obtained at purity of at least 0.8 nmol/A_{260} unit are $tRNA^{Ser}_2$ and $tRNA^{Ser}_3$. In addition, the use of Aminex A-28 as a chromatographic system for the isolation of tRNA is discussed.

In recent years studies on the relationship between the structure of tRNA and its diversified functions have expanded into mammalian systems. To date, however, these studies have been confined to non-human mammalian tissues.

The difficulties encountered in the large-scale preparation of total human tRNA have now been overcome by using the rapid isolation procedure recently developed in our laboratory (Roe, 1975). Using this method we have isolated several grams of human liver and human placenta tRNA in a short time period.

We now wish to report the fractionation of human placenta tRNA and demonstrate for the first time the preparation of milligram quantities of several highly purified tRNA species. The purification of these tRNAs has been facilitated by the use of high-pressure, high-speed liquid chromatography on reverse phase chromatography number 5 (RPC-5) (Pearson et al., 1973; Roe et al., 1973) and a new chromatographic system, Aminex A-28 (Singhal, 1974).

Fractionation on DEAE-Sephadex A-50 (Nishimura, 1971) and BD-cellulose (Roe, 1975; Gillam et al., 1967) were also used as additional chromatographic steps. These techniques are similar to those reported by others for fractionation of either bacterial or other mammalian tRNA (Gillam et al., 1967; Nishimura, 1971; Pearson et al., 1973) except that now we have made extensive use of chromatography on Aminex A-28 (Singhal, 1974). By using these rapid isolation techniques, coupled with the newly developed nucleic acid sequence analysis procedures of Randerath (Randerath et al., 1972, 1974), RajBhandary (Simsek et al., 1973), Söll (Szeto and Söll, 1974), and Keith and Gilham (Keith and Gilham, 1974) we have recently completed the determination of the nucleotide sequence of two human placental tRNAs (Roe et al., 1975; Gillum et al., 1975).

These are necessary first steps in studies dealing with the changes in human tRNA during normal and abnormal cellular development, the role of modified nucleotides in human tRNA, and the function of specific human tRNAs in regulation and control. In such studies, the determination of a complete nucleotide sequence will not be an end in itself, but will lead to a larger series of experiments to determine the exact nature of the diverse functions of specified human tRNA.

Materials and Methods

Human placenta tRNA was isolated using phenol extraction at pH 4.5 in 0.14 *M* sodium acetate buffer, followed by DEAE-cellulose chromatography. Crude rabbit liver aminoacyl-tRNA synthetase was prepared by passage of a 30000*g* supernatant of the homogenized tissue through DEAE-cellulose followed by ammonium sulfate precipitation and Sephadex G-25 chromatography. Both methods are described elsewhere in detail (Roe, 1975).

The standard aminoacylation reactions were performed at pH 7.6 in Tris-HCl buffer using a saturating amount of crude aminoacyl-tRNA synthetase as previously described (Roe, 1975). Placenta $tRNA^{Met}_i$ was distinguished from $tRNA^{Met}_m$ by its ability to be aminoacylated with methionine using crude *Escherichia coli* aminoacyl-tRNA synthetase (Piper and Clark, 1974).

Radioactive amino acids were purchased from Schwarz/Mann (Stan-STAR), and unless otherwise stated diluted to 10 Ci/mol with nonradioactive amino acid. All other reagents were of the highest purity available. Radioactivity was determined in Omnifluor–toluene using a Nuclear Chicago, refrigerated Isocap 300 liquid scintillation counter. All solutions were prepared with deionized, distilled water and were thoroughly degassed before use. Fluorescence was measured as previously described (Roe, 1975).

BD-cellulose chromatography (column 1 of Figure 1) of 5-g batches of human placenta tRNA was reported earlier (Roe, 1975). Those fractions from several BD-cellulose col-

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[1] Abbreviations used are: A_{260} unit, the quantity of material contained in 1 ml of solution which has an absorbance of 1 at 260 nm when measured in a 1-cm light-path cell; FI, relative fluorescence intensity.

umn runs which were enriched in the specific tRNAs of interest were pooled, precipitated with two volumes of 95% ethanol, and stored overnight at −20°. The precipitated tRNA was collected by centrifugation, dried in vacuo over phosphorus pentoxide and solid potassium hydroxide, and stored at −20°.

Individual tRNAs were further purified by using RPC-5 at neutral and then acidic pH (Roe et al., 1973), BD-cellulose at acidic pH (Gillam et al., 1967), and DEAE-Sephadex A-50 at neutral pH (Nishimura, 1971). In addition, chromatography on Aminex A-28 (Singhal, 1974) in the final phase of purification was utilized for several of the human tRNAs.

Chromatography on RPC-5 was carried out using a 1-l. stainless steel column at a flow rate of 15 ml per 1.5 min per fraction. Plaskon was coated with Adogen 464 as described by Pearson et al. (1971) with modifications by Roe et al. (1973). The building of this RPC-5 column has been described previously (Roe et al., 1973). In this and subsequent column chromatographic steps, the tRNA sample was dissolved in the appropriate buffer containing 0.1 *M* sodium chloride to a concentration of 4–5 mg/ml and loaded directly onto the column. In most RPC-5 column chromatography at pH 7.6 (column 2, Figure 1) the tRNA was eluted with a 3-l. concave gradient consisting of 2 l. of 0.01 *M* Tris-HCl (pH 7.6), 0.01 *M* magnesium chloride, and 0.001 *M* sodium thiosulfate (buffer A) containing 0.4 *M* sodium chloride as the low salt buffer and 1 l. of buffer A containing 1.2 *M* sodium chloride as the high salt buffer. A constant flow rate was maintained by using a Mil-Roy D pump. After the A_{260} for alternate fractions had been determined, aliquots of every fifth tube were then used in the standard aminoacylation reaction to determine the elution profile for individual isoaccepting tRNA species.

Chromatography on RPC-5 at pH 4.5 (column 3 of Figure 1) was performed on the 1-l. column described above. After loading, the tRNA was eluted with a 3-l. concave gradient consisting of 2 l. of 0.01 *M* sodium acetate (pH 4.5), 0.01 *M* magnesium chloride, and 0.001 *M* sodium thiosulfate (buffer B) containing 0.4 *M* sodium chloride as the low salt buffer and 1 l. of buffer B containing 1.2 *M* sodium chloride as the high salt buffer. The flow rate was maintained at 15 ml per 1.5 min per fraction by using a Mil-Roy D pump. Absorbance and assay measurements were carried out as described above.

For chromatography on BD-cellulose at pH 4.5 (column 4 of Figure 1) a 70-ml column (1 cm × 90 cm) was built in a glass tube as described (Gillam et al., 1967). After loading, the tRNA was eluted with a 1-l. linear gradient, consisting of 500 ml of 0.4 *M* sodium chloride in buffer B as the low salt and 500 ml of 0.8 *M* sodium chloride in buffer B as the high salt. The flow rate was maintained at 4.5 ml per 6 min per fraction by a Milton Roy minipump. Upon completion of the gradient the column was washed with buffer B containing 1.0 *M* sodium chloride and 20% ethanol to elute tightly bound tRNA.

The DEAE-Sephadex A-50 (column 5 of Figure 1) column was built in a glass tube (1.4 cm × 100 cm) as described by Nishimura (Nishimura, 1971). After dissolving in buffer A containing 0.35 *M* sodium chloride and loading onto the column, the tRNA was eluted with a 1 l. linear gradient consisting of 500 ml of buffer A containing 0.35 *M* sodium chloride as the low salt and 500 ml of 0.52 *M* sodium chloride as the high salt. The flow rate was 5 ml per 24 min per fraction.

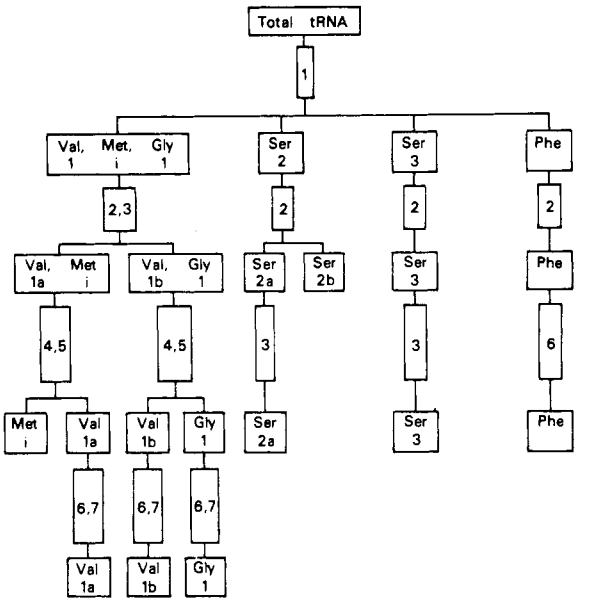

FIGURE 1: Isolation scheme for the purification of seven human placenta tRNAs. Details are given in Materials and Methods.

Recently, Singhal (Singhal, 1974) has reported a procedure for using Aminex A-28 for analysis of nucleotides, for tRNA fragments, and for the fractionation of tRNA. The reported superiority of this column packing over RPC-5 prompted us to use it as an additional column in our isolation scheme. A column containing Aminex A-28 (Bio-Rad) was built in a 0.5-in. o.d. stainless steel tube (1 cm i.d. × 30 cm) with Swagelock fittings top and bottom. The lower fitting contained a sintered stainless steel filter element with a 5–10-μ pore size (Swagelock No. SS-810-6-1-SR-17 Akron Valve and Fitting Co., Akron, Ohio). The bed support must be of some less porous material other than the glass wool packing used in RPC-5 (Roe et al., 1973) since at the running pressure (500–2000 psi), the Aminex A-28 was extruded through the glass wool bed support. Pretreatment of Aminex A-28 and equilibration in the acetate form were as described by Singhal and Cohn for Aminex A-25 (Singhal and Cohn, 1973). The flow rate was maintained at 2 ml per min per fraction using a Mil-Roy D pump (column 6 of Figure 1). After loading, the tRNA was eluted with a 175-ml concave gradient consisting of 100 ml of buffer B containing 0.3 *M* sodium chloride as the low salt buffer and 75 ml of buffer B containing 0.8 *M* sodium chloride as the high salt buffer. Upon completion of the gradient, the column was washed with buffer B containing 1.2 *M* sodium chloride and reequilibrated with at least five column volumes of buffer B containing 0.1 *M* sodium chloride. We found that by reducing the salt concentration of the loading buffer to 0.1 *M* sodium chloride, it was possible to load up to 1 mg of tRNA/ml of column volume. We have also observed that the selection of gradient for Aminex A-28 chromatography was as sensitive to sample size as was RPC-5 (Roe et al., 1973) and that regeneration of the column required more extensive washing with low salt buffer than did RPC-5. In some cases the Aminex A-28 column was run at neutral pH (column 7 of Figure 1) using buffer A in place of buffer B with all other conditions identical with that just described.

Any changes in the procedure for individual columns which differ from those mentioned above are included in the figure legends.

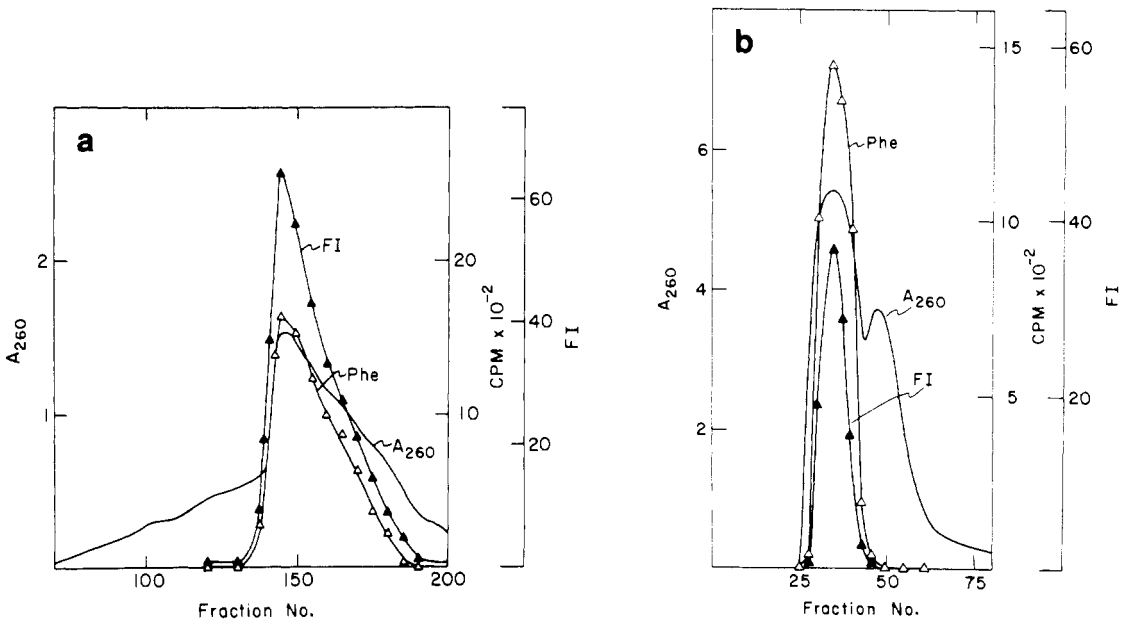

FIGURE 2: (a) Chromatography of 61 mg of partially purified $tRNA^{Phe}$ on RPC-5 at neutral pH. Column conditions were as described in Materials and Methods except that the low salt buffer A contained 0.5 *M* sodium chloride. Assays were carried out using 50 μl of column effluent in the standard assay. Fluorescence was measured at 440 nm upon excitation at 310 nm. (b) Chromatography of 20 mg of purified $tRNA^{Phe}$ on Aminex A-28. Column conditions were as described in Materials and Methods except that the high salt buffer B contained 1.2 *M* sodium chloride. Assays were carried out using 10 μl of column effluent in the standard assay. Fluorescence was measured at 440 nm upon excitation at 310 nm.

Results and Discussion

The scheme for the purification of seven human placenta tRNAs is shown in Figure 1 and briefly described below.

BD-cellulose was specifically chosen as the initial column in these studies aimed at isolation of specific isoaccepting tRNAs from human placenta because of its large capacity and relatively good separation of several tRNAs (Roe, 1975; Gillam et al., 1967). Several specific fractions of tRNA from two BD-cellulose column runs (5 g of crude tRNA fractionated on each run) were pooled, precipitated, and dried as described in Materials and Methods.

The choice of RPC-5 as the second column used for the isolation of the individual tRNAs was based not only on its relatively high capacity, but also on the short time period required to achieve a severalfold purification. Figures 2a, 3a, and 5a are typical chromatographic profiles obtained for various pooled fractions from the initial BD-cellulose columns when chromatographed on RPC-5 at pH 7.6.

Figure 2a and b show the elution profiles for placenta $tRNA^{Phe}$ under neutral conditions on RPC-5 and acidic pH on Aminex A-28, respectively. It can be seen that during chromatography on these two columns, both the amino acid acceptor activity for phenylalanine and the fluorescence in $tRNA^{Phe}$ were greater than 1.2 nmol/A_{260} unit across the peak region.

Having purified $tRNA^{Phe}$ we then attempted to purify other placenta tRNAs using a similar series of column chromatographic techniques. For the following several reasons, the purification of $tRNA^{Val}_1$, $tRNA^{Met}_i$, and $tRNA^{Gly}_1$ was attempted next. First, these three tRNAs which elute together on the initial BD-cellulose column runs could be pooled as one fraction and then processed further. Second, since the nucleotide sequence of $tRNA^{Met}_i$ from several mammalian tissues has been determined (Simsek et al., 1973; Piper and Clark, 1974), it would be of interest to compare these known sequences to that of the human placenta initiator tRNA. Third, since $tRNA^{Val}_1$ from mouse myeloma cells (Piper and Clark, 1974) and $tRNA^{Gly}_1$ from wheat embryo (Marcu et al., 1973) have been found to lack the nucleoside ribothymidine, a comparative study of the nucleotide sequences of these two tRNAs with the corresponding human tRNAs would also be of interest.

The scheme for the isolation of these three tRNAs is shown in Figure 1. The pooled fractions of tRNA enriched in $tRNA^{Val}_1$, $tRNA^{Met}_i$, and $tRNA^{Gly}_1$ from BD-cellulose were chromatographed on RPC-5 at pH 7.6 (Figure 3a). Under these conditions the $tRNA^{Val}_1$ was separated into two distinct species, $tRNA^{Val}_{1a}$ and $tRNA^{Val}_{1b}$. The $tRNA^{Val}_{1a}$ eluted early in the salt gradient with the $tRNA^{Met}_i$, while the $tRNA^{Val}_{1b}$ eluted later with the $tRNA^{Gly}_1$. Subsequent chromatography of both fractions on RPC-5 at acidic pH caused only a slight increase in the purity and failed to separate the tRNAs of interest (figure not shown). Further chromatography of the fraction enriched in $tRNA^{Val}_{1a}$ and $tRNA^{Met}_i$ yielded only partial separation of these tRNAs (figure not shown) while chromatography on DEAE-Sephadex A-50 gave a clear separation of these two tRNAs (Figure 3b). At this stage of purification the $tRNA^{Val}_{1a}$ was 0.5 nmol/A_{260} unit at its peak, and the $tRNA^{Met}_i$ was 1.3 nmol/A_{260} unit. Further chromatography of $tRNA^{Val}_{1a}$ on Aminex A-28 at pH 4.5 yielded this tRNA at a purity of 0.8 nmol/A_{260} unit (figure not shown), while additional chromatography on Aminex A-28 at neutral pH (Figure 3c) yielded a preparation that had a final purity of 1.2 nmol/A_{260} unit over the peak region.

When the tRNAs enriched in $tRNA^{Val}_{1b}$ and $tRNA^{Gly}_1$ (Figure 3a) were further purified using BD-cellulose at acidic pH followed by DEAE-Sephadex A-50 at neutral pH, a separation of these two tRNAs was observed (figures not shown). Upon further fractionation on Aminex A-28 at acidic, then neutral pH, the $tRNA^{Val}_{1b}$ was further resolved into two species, each with a final purity of 1.2 nmol/A_{260} unit (Figure 4a), while the $tRNA^{Gly}_1$ obtained had a purity of 1.2 nmol/A_{260} unit (Figure 4b).

The initial BD-cellulose column chromatography also

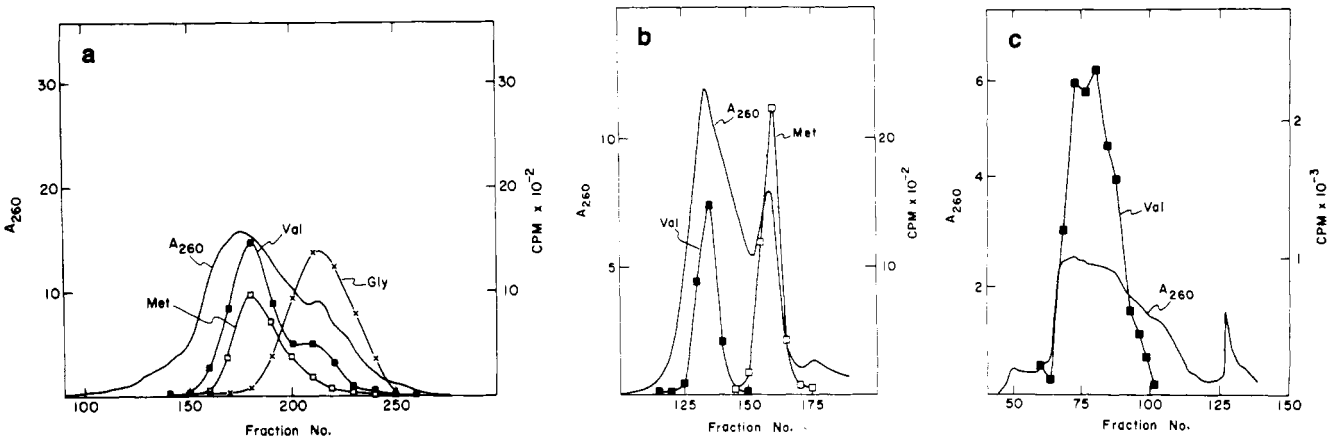

FIGURE 3: (a) Chromatography of 778 mg of partially purified human placenta tRNA enriched in $tRNA^{Val}_1$, $tRNA^{Met}_i$, and $tRNA^{Gly}_1$ on RPC-5. Column conditions were identical with those described in Materials and Methods for RPC-5 at neutral pH, except that the low salt buffer A contained 0.3 *M* sodium chloride. Assays were carried out using 25 μl of column effluent in the standard assay mixture. (b) Chromatography of 100 mg of tRNA enriched in $tRNA^{Val}_{1a}$ and $tRNA^{Met}_i$ on DEAE-Sephadex A-50 at neutral pH. Column conditions were as described in Materials and Methods and 25 μl of column effluent was used in the standard assay. (c) Chromatography of 8 mg of purified $tRNA^{Val}_{1a}$ on Aminex A-28 at neutral pH. Column conditions were as described in Materials and Methods except that buffer A was used throughout. Assays were carried out using 10 μl of column effluent in the standard assay with [^{14}C]valine of a specific activity of 50 Ci/mol.

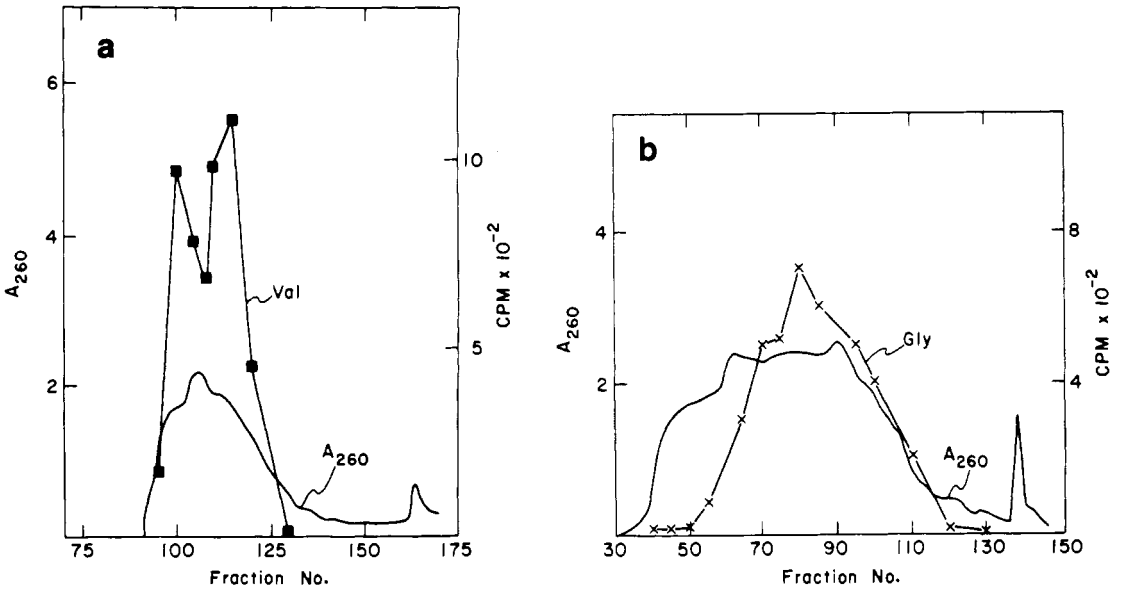

FIGURE 4: (a) Chromatography of 5 mg of purified $tRNA^{Val}_{1b}$ on Aminex A-28. Column conditions were as described in Materials and Methods except that buffer A was used throughout. Assays were carried out using 10 μl of column effluent in the standard assay with [^{14}C]valine of a specific activity of 50 Ci/mol. (b) Chromatography of 12 mg of purified $tRNA^{Gly}_1$ on Aminex A-28. Column conditions were as described in Materials and Methods except that buffer A was used throughout. Assays were carried out using 5 μl of column effluent in the standard assay.

gave a severalfold purification of many other tRNAs of interest. Since in their early studies Staehelin and co-workers (Staehelin et al., 1968) had determined the complete nucleotide sequence of rat liver $tRNA^{Ser}$, we felt that an isolation of the human $tRNA^{Ser}$ would be important. These tRNAs were initially fractionated into three major species on BD-cellulose (Roe, 1975). The third species, $tRNA^{Ser}_3$, which eluted after $tRNA^{Phe}$ in the ethanol gradient, was at least tenfold purified over crude tRNA. Chromatography of the partially purified $tRNA^{Ser}_3$ on RPC-5 at neutral pH yielded a twofold purification (figure not shown). Subsequent chromatography on RPC-5 under acidic conditions brought the final purity of $tRNA^{Ser}_3$ to 1.0 nmol/A_{260} unit (Figure 5a).

Upon fractionation using RPC-5 at neutral pH, the $tRNA^{Ser}_2$ was separated into two distinct species, $tRNA^{Ser}_{2a}$ and $tRNA^{Ser}_{2b}$ (figure not shown). Further chromatography of the $tRNA^{Ser}_{2a}$, using RPC-5 at acidic pH (Figure 5b), brought the final purity of this tRNA to 0.8 nmol/A_{260} unit.

The resolving power of Aminex A-28 is further demonstrated in Figure 6. For this column run, a portion of the pooled tRNA from the initial BD-cellulose chromatography was purified through RPC-5 at neutral, then acidic pH, and then chromatographed on Aminex A-28 at acidic pH. The $tRNA^{Asn}$, $tRNA^{Ile}$, $tRNA^{Lys}_1$, and $tRNA^{His}$ were all eluted as sharp peaks in different fractions under these conditions. All four tRNAs had a purity of approximately 0.4–0.5 nmol/A_{260} units at their respective peaks.

Conclusions

Table I gives the yield and purity of those human placenta tRNAs we have purified to date. By processing a total of 10 g of crude human placenta tRNA, we have obtained rea-

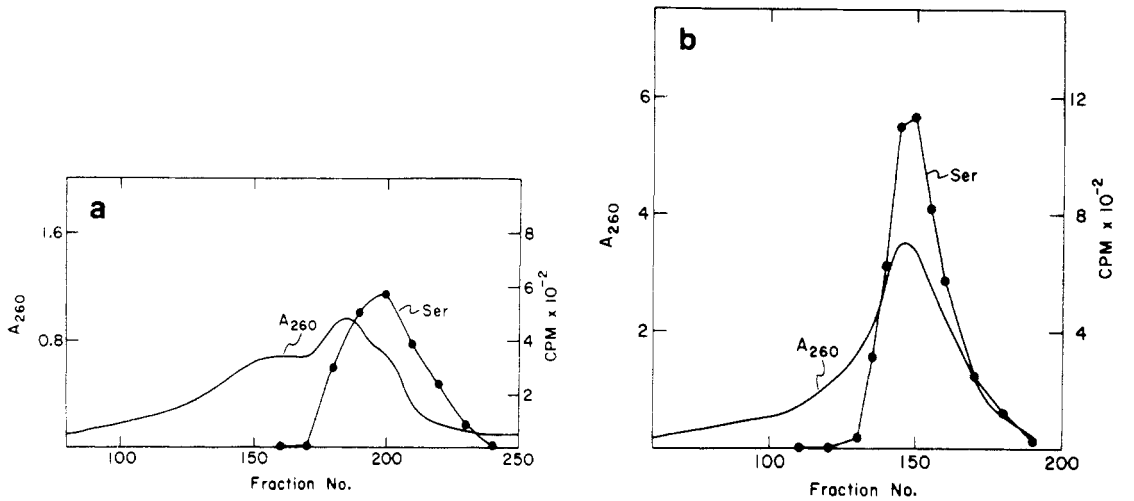

FIGURE 5: (a) Chromatography of 50 mg of tRNA enriched in $tRNA^{Ser}_3$ on RPC-5 at acidic pH. Column conditions were as described in Materials and Methods except that the low salt buffer B contained 0.5 *M* sodium chloride. Assays were carried out using 50 μl of column effluent in the standard assay. (b) Chromatography of 125 mg of purified $tRNA^{Ser}_{2a}$ on RPC-5 at acidic pH. Column conditions were identical with those described above. Assays were carried out using 25 μl of column effluent in the standard assay mixture.

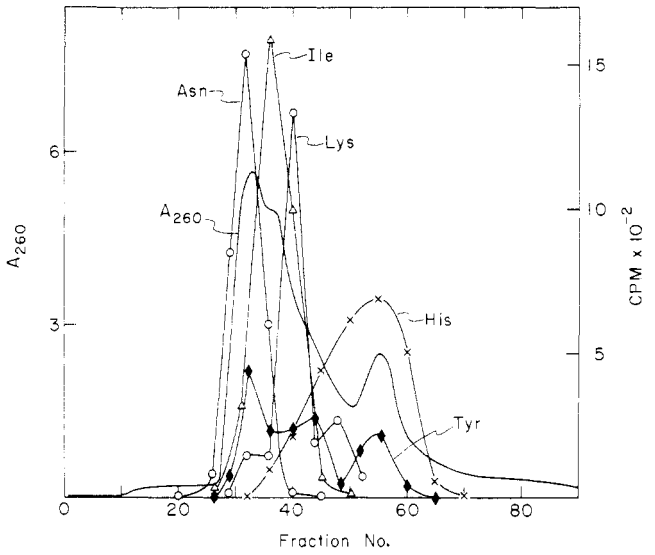

FIGURE 6: Chromatography of 10 mg of partially purified $tRNA^{Asn}$, $tRNA^{Ile}$, $tRNA^{Lys}_1$, and $tRNA^{His}$ on Aminex A-28 at acidic pH. Column conditions were as described in Materials and Methods. Assays were carried out using 50 μl of column effluent in the standard assay mixture.

Table I: Highly Purified Human Placenta tRNAs.

	Purity (nmol/A_{260})	Yield (mg)
$tRNA^{Met}_i$	1.3	20
$tRNA^{Phe}$	1.3	20
$tRNA^{Val}_{1a}$	1.2	10
$tRNA^{Val}_{1b}$	1.2	10
$tRNA^{Gly}_1$	1.2	20
$tRNA^{Ser}_2$	0.8	10
$tRNA^{Ser}_3$	1.0	10

sonably large quantities (10–20 mg) of several highly purified (0.8–1.3 nmol/A_{260} unit) individual tRNA species. Although these tRNAs have not been shown to be chromatographically pure, we have recently completed several studies which lead us to believe that they are greater than 90% pure. The complete nucleotide sequence of human placenta $tRNA^{Phe}$ and $tRNA^{Met}_i$ (whose isolation has been described herein) has now been determined and will be reported elsewhere in detail. The complete nucleotide sequence of human placenta $tRNA^{Phe}$ is identical with that of calf liver and rabbit liver $tRNA^{Phe}$ (Keith et al., 1974) while $tRNA^{Met}_i$ is identical with that of rabbit liver, mouse myeloma, and salmon $tRNA^{Met}_i$ (Piper and Clark, 1974; Simsek et al., 1973). These nucleotide sequence studies were completed in collaboration with Dr. K. Randerath at Baylor College of Medicine and Dr. U. L. RajBhandary at Massachusetts Institute of Technology, respectively. Throughout these determinations little, if any, contamination with additional tRNA fragments was observed. In addition, hexagonal bipyramid crystals of this human placenta $tRNA^{Phe}$ have not been obtained (N. Woo and A. Rich, personal communication). These studies just mentioned, in addition to the high degree of aminoacylation, confirm that the individual tRNAs which we have obtained were very highly purified.

In the isolation scheme discussed herein, the use of a small Aminex A-28 column as the final step was chosen because of its excellent resolving power over other chromatographic media. In addition, since the total running time for each column was less than 3 hr, we could easily complete the isolation in a relatively short time period. We have found that these columns gave quite reproducible results and showed no loss in either capacity or resolution with repeated use.

One difficulty we have encountered with the isolation of pure human tRNAs is that they seem to require more extensive and varied chromatography than had previously been observed for *E. coli* tRNA (Roe et al., 1973; Roe and Dudock, 1972). While a specific *E. coli* tRNA of a purity of 1.2 nmol/A_{260} unit could be obtained in two or three chromatographic steps, we have found that as many as seven different column runs were required to achieve such a purity for human tRNA. This phenomenon has also been observed in the preparation of pure species of calf liver tRNA (Pearson et al., 1973). At present one can only postulate that because of different structural features such as unique modified nucleotides or incomplete modification, the mammalian tRNA may exist in many different conforma-

tions causing elution as broad rather than narrow peaks. It could also be possible that through point mutations or other alterations of the DNA template for mammalian tRNA, we are observing microheterogeneity. At present these are only speculations.

In the experiments described in this communication, we have demonstrated the isolation of several human tRNAs in relatively high yield and purity. It remains for us now to study these highly purified tRNAs in greater detail. Presently, we are pursuing the nucleic acid sequence analysis of other human tRNAs whose isolation has been reported here. Since we now have sufficient quantities of human tRNA, we also will study the modified nucleotides in individual human tRNAs, and attempt to elucidate the structures of any unique mammalian modified nucleotides. Further studies to isolate and compare the primary structure of human tRNA from normal and cancerous human liver with those of placenta are now in progress. These studies will lead to a better understanding of the exact nature of the alterations which occur in tRNA during normal and abnormal human cellular development.

Acknowledgments

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References

Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M. (1967), *Biochemistry 6,* 3043–3056.

Gillum, A., Roe, B. A., Anandaraj, M. P. J. S., and RajBhandary, U. L. (1975), *Cell* (in press).

Keith, G., Ebel, J. P., and Dirheimer, G. (1974), *FEBS Lett. 48,* 50–52.

Keith, G., and Gilham, P. T. (1974), *Biochemistry 13,* 3601–3605.

Marcu, K., Mignery, R. Reszelbach, R., Roe, B., Sirover, M., and Dudock, B. (1973), *Biochem. Biophys. Res. Commun. 55,* 477–483.

Nishimura, S. (1971), in Procedures in Nucleic Acid Research, Vol. 2, Cantoni, G. L., and Davis, D. R., Ed., New York, N.Y., Harper and Row, p 542–564.

Pearson, R. L., Hancher, C. W., Weiss, J. F., Holladay, D. W., and Kelmers, A. D. (1973), *Biochim. Biophys. Acta 294,* 239–249.

Piper, P. W., and Clark, B. F. C. (1974), *Eur. J. Biochem. 45,* 589–600.

Randerath, E., Yu, C. T., and Randerath, K. (1972), *Anal. Biochem. 48,* 172–198.

Randerath, K., Randerath, E., Chia, L. S. Y., Gupta, R. C., and Sivarajan, M. (1974), *Nucleic Acids Res. 1,* 1121–1141.

Roe, B. (1975), *Nucleic Acids Res. 2,* 21–42.

Roe, B. A., Anandaraj, M. P. J. S., Chia, L. S. Y., Randerath, E., Gupta, R. C., and Randerath, K., (1975), *Biochem. Biophys. Res. Commun.* (in press).

Roe, B., and Dudock, B. (1972), *Biochem. Biophys. Res. Commun., 49,* 399–406.

Roe, B., Marcu, K., and Dudock, B. (1973), *Biochim. Biophys. Acta 319,* 25–36.

Simsek, M., Ziegenmeyer, J., Heckman, J., and RajBhandary, U. L. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 1041–1045.

Singhal, R. P. (1974), *Eur. J. Biochem. 43,* 245–252.

Singhal, R. P., and Cohn, W. E. (1973), *Biochemistry 12,* 1532–1537.

Staehelin, M., Rogg, H., Baguley, B. C., Ginsberg, T., and Wehrli, M. (1968), *Nature (London) 219,* 1363–1365.

Szeto, K. S., and Soll, D. (1974), *Nucleic Acids Res. 1,* 1733–1738.