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Fractionation of the Nonpolar Transfer Ribonucleic Acids from Rat Liver, Yeast, and *Escherichia coli* by Partition Chromatography*

Walter Wehrli and Matthys Staehelin

ABSTRACT: tRNAs from rat liver, yeast, and *Escherichia coli* have been fractionated by partition chromatography. Acceptor activity profiles have been established for all 20 amino acids in the case of rat liver and for 18 amino acids in the case of yeast. The nonpolar tRNAs of all three organisms have been further purified by partition and reversed-phase chromatography. It has been found that all three organisms have nonpolar tRNAs specific for phenylalanine, leucine, serine, and tryptophan. In addition, tyrosine and cysteine tRNA are nonpolar in *E. coli* and yeast, whereas methionine, lysine, and arginine tRNA are nonpolar in yeast and rat liver.

The tRNAs specific for all other amino acids are more polar. The relation between tRNA polarity and the genetic code as well as the influence of N^6-(Δ^2-isopentenyl)adenosine are discussed.

In recent years, many methods of separating and isolating the various tRNAs from *Escherichia coli* and yeast have been published and quite a number of nucleotide sequences of purified tRNAs from these organisms are known at present. However, only a few attempts have been made to study mammalian tRNAs (see also Nishimura and Weinstein, 1969, for further literature). This may be due to the lack of methods of preparing tRNAs from mammalian tissues in sufficiently large amounts for sequence studies. In order to elucidate the chemical structure of mammalian tRNAs (Staehelin *et al.*, 1968), we have developed a simple and rapid method for the large-scale preparation of mammalian tRNA from liver (Rogg *et al.*, 1969). In this communication we describe the fractionation of gram quantities of crude liver tRNA and especially of the least polar phenylalanine, leucine, serine, tryptophan, methionine, and lysine tRNAs. The tRNAs of *E. coli* and yeast were fractionated in an analogous

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way and their content of nonpolar species was compared to that of rat liver tRNA.

The method of fractionation employed was a modification of the partition chromatography system developed by Muench and Berg (1966a) for *E. coli*. Further purification was achieved by reversed-phase chromatography as described by Weiss and Kelmers (1967).

Materials

Sephadex G-75 was purchased from Pharmacia Fine Chemicals; 2-ethoxyethanol and 2-butoxyethanol were distilled and the fractions containing less than 0.2 and 0.4 A_{260} units per ml, respectively, were used. Triethylamine was obtained from Fluka (Switzerland), and cetyltrimethylammonium bromide from Serva (Germany). [^{14}C]Amino acids were supplied by the Radiochemical Centre, Amersham (England), and adjusted to a specific activity of 5 μCi/μmole by dilution with the corresponding L-[^{12}C]amino acids obtained from Calbiochem. *E. coli* B tRNA was also purchased from Calbiochem. Brewer's yeast tRNA was prepared according to the method of Monier *et al.* (1960) and liver tRNA was isolated as described by Rogg *et al.* (1969) from 10-kg batches of rat liver.

Methods

Preparation of Aminoacyl-tRNA Synthetases. FROM RAT LIVER. The pH 5 enzymes of rat liver were prepared as described by Schweet (1962). The mixture of the various synthetases was not purified further, because the enzymes were stable for several months when kept in liquid nitrogen or in 50% glycerol at −20°. In addition, the preparation was essentially free of nucleases and tRNA. This enzyme preparation was used to charge both rat liver and yeast tRNAs.

FROM *E. coli*. The synthetases were prepared as described by Muench and Berg (1966b), except that the chromatography on hydroxylapatite was omitted.

Assay of Amino Acid Acceptor Activity of tRNA. Aminoacyl acceptor activity was measured under the following conditions: 30 μmoles of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer (Good *et al.*, 1966) at pH 7.0 for Ala, Arg, Asp, Cys, Gln, Pro, and Tyr, pH 7.5 for Asn, Gly, His, Leu, Trp, and Val, and pH 8.0 for Ile, Lys, Met, and Thr; 60 μmoles of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 8.0, for Phe and Ser; 30 μmoles of KPO_4, pH 6.5, for Glu; 4 μmoles of $MgCl_2$; 0.1 μmole of EDTA; 2 μmoles of ATP; 10 mμmoles of ^{14}C-labeled amino acid (5 μCi/μmole); 0.5 μmole of dithiothreitol (not added for Leu, Lys, Met, Phe, Ser, and Trp); 100 mμmoles of each of the 19 unlabeled amino acids; 0.2–2.0 A_{260} units of tRNA; 0.1–0.2 mg of rat liver pH 5 enzyme for liver and yeast tRNA; 0.02–0.1 mg of *E. coli* tRNA synthetases for *E. coli* tRNA. The total assay volume was 0.25 ml. Incubation was carried out at 37° for 20 min; tRNA was precipitated with 0.5 ml of 2 M HCl and collected on a glass filter disk (Whatman GF/C, 2.4 cm diameter), washed with dilute HCl, dried, and counted on a Packard Tri-Carb liquid scintillation counter. A scintillation solution of 6 g of Bu-PBD (Scintillator CIBA) in 1 l. of toluene was used.

Partition Columns. The biphasic partition mixture for the preparation of the columns and for chromatography was prepared as described by Muench and Berg (1966a). A total of 6 volumes of 1.25 M potassium phosphate (pH 6.88)

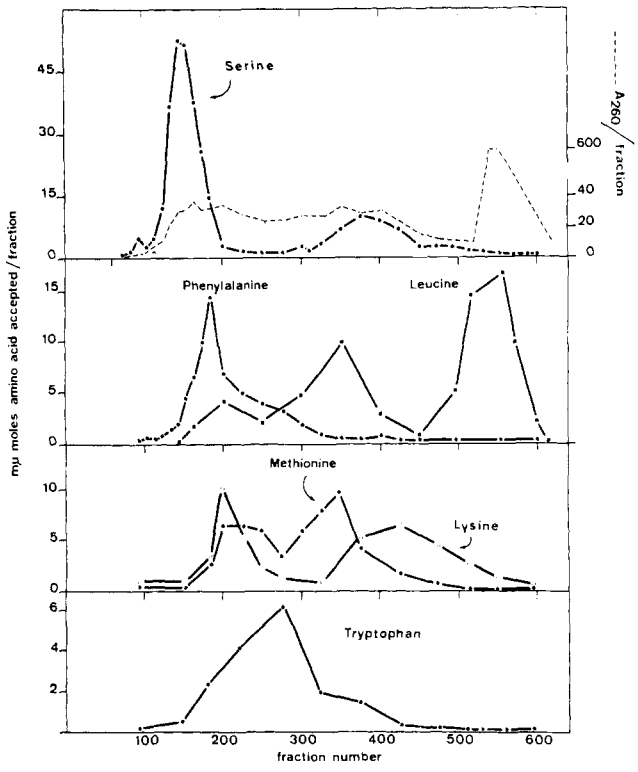

FIGURE 1: Fractionation of 36,000 A_{260} units of crude rat liver tRNA on a partition column (12 × 60 cm) at 20°. The tRNA was dissolved in 10 ml of H_2O and 40 ml of organic phase was added. This mixture was applied to the column. The column was eluted as described in Methods using 13 l. of organic phase for the first 12,000 A_{260} units and 2.5 l. of polar phase for the remaining material. The fraction size was 25 ml. The recovery of desalted tRNA was 80%. The figure indicates only the amino acid acceptance of the most lipophilic species. For a complete elution pattern see Figure 11.

(equimolar in KH_2PO_4 and K_2HPO_4), 2 volumes of 2-ethoxyethanol, 1 volume of 2-butoxyethanol, 0.01 volume of triethylamine, and 0.001 volume of 2-mercaptoethanol was mixed and equilibrated at 22°. Since large amounts of the lower water phase were required for the preparation of the columns, its composition was determined by gas chromatography and phosphate analysis (Lowry and Lopez, 1946). The lower phase was accordingly prepared by mixing 92 volumes of 2.42 M potassium phosphate, pH 6.88 (equimolar to KH_2PO_4 and K_2HPO_4), 7.5 volumes of 2-ethoxyethanol, 0.8 volume of 2-butoxyethanol, 0.01 volume of triethylamine, and 0.001 volume of 2-mercaptoethanol. Three column sizes were used, 12 × 60, 7 × 40, and 2.5 × 90 cm. All the columns were equipped with a cooling jacket to regulate the temperature. To prepare the largest column, 500 g of Sephadex G-75 were suspended in 20 l. of lower phase for 48 hr with occasional stirring. The column was then packed with this suspension and washed with upper phase, as described by Muench and Berg (1966a). The flow rate without a pump was about 25 ml/hr, and in order to avoid shrinkage of the column, the hydrostatic pressure was reduced by attaching a long thin polyethylene tube to the outflow of the column in such a way that its end was only 10–30 cm below the top layer of the eluent. The 7 × 40 and 2.5 × 90 cm columns were similarly prepared, 120 and 30 g of Sephadex G-75 being suspended in 5 and 2.5 l. of lower phase, respectively. The flow rates were adjusted to about 20 and 10 ml per hr, respectively. tRNA was dissolved in a solution of 1 volume

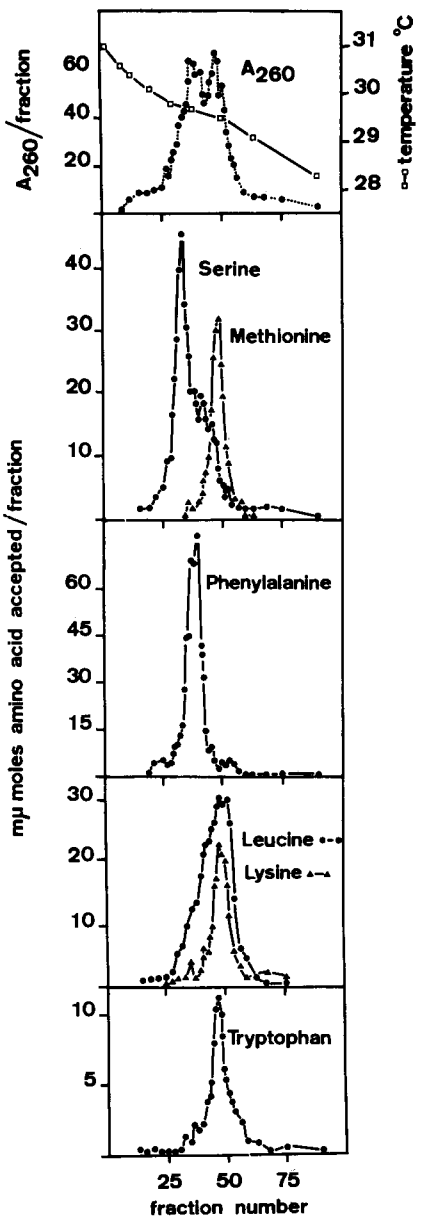

FIGURE 2: Fractionation of 2376 A_{260} units from fractions 170–250 (Figure 1) on a partition column (2.5 × 90 cm) with a temperature gradient from 31 to 28.3°, as indicated. The tRNA was dissolved in 1.0 ml of H_2O and 7 ml of organic phase was added. This mixture was applied to the column. Elution as described in Methods. Fraction size 25 ml. Recovery of desalted tRNA was 75%.

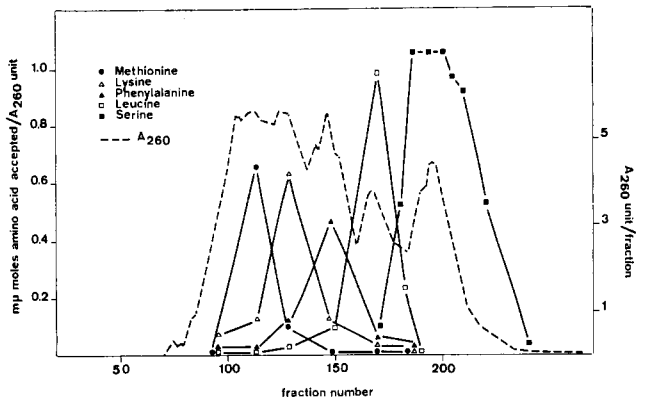

FIGURE 3: Fractionation of rat liver tRNA by reversed-phase chromatography. Rat liver tRNA (540 A_{260} units), prepurified by partition chromatography and similar in composition to fractions 170–190 in Figure 1, was dissolved in 5 ml of initial elution buffer and applied on a reversed-phase column (1 × 250 cm) at 25° (system 2 of Weiss and Kelmers, 1967). The gradient used for elution was 0.2–1.0 M NaCl (3000 ml) in 0.01 M sodium acetate (pH 4.5) at a flow rate of 10 ml/4.5 min. Fraction size 10 ml. The following fractions were pooled: 80–106, 107–117, 118–135, 136–159, 160–176, 177–184, 185–190, 191–196, 197–201, 202–207, 208–215, 216–230, and 231–250. Isolation of the pooled tRNA fractions as described in the accompanying paper (Müller *et al.*, 1971).

of water and 4–10 volumes of upper phase to a maximum concentration of 40 mg/ml. Up to 4 g of tRNA (80,000 A_{260} units measured in 0.1 M NaCl) was applied to the largest column, up to 1 g (20,000 A_{260} units) to the medium one, and up to 200 mg (4000 A_{260} units) to the small one. The two large columns were used to separate crude tRNA, whereas on the 2.5 × 90 cm column prepurified tRNA was repartitioned.

The columns were eluted with upper phase collecting fractions of 25 ml. When the desired tRNAs were separated, the remaining material was washed out with lower phase until the concentrations were less than 0.2 A_{260} unit/ml. The columns were then again washed with upper phase to remove excess lower phase. The two large columns could be reused at least 6–10 times, whereas the flow rate of the small column became too slow after the second or third run.

The two large columns were run at a constant temperature of 20°; the temperature of the small column was reduced slowly from 31 to 28° during elution, as described in Results.

Reversed-Phase Columns. The columns were prepared and run as described by Weiss and Kelmers (1967) (reversed-phase chromatography system 2) at pH 4.5 in the absence of $MgCl_2$. To obtain a good separation not more than 30 mg (600 A_{260} units) was applied to a 1 × 250 cm column.

Isolation of tRNA. The flotation method described by Mirzabekov *et al.* (1964) was used. Fractions (25 ml) were extracted twice with 20 ml each of diethyl ether containing 0.01% 2-mercaptoethanol. Then 10 ml of H_2O was added to the water phase, followed by 1.0 ml of 0.1 M cetyltrimethylammonium bromide. When the fraction contained more than 25 A_{260} units of tRNA/ml, more cetyl salt had to be added. After mixing, 20 ml of ether–mercaptoethanol was added, and after rigorous shaking the mixture was incubated for 30 min at 37°. The tRNA appeared as a precipitate at the interphases. If the lower phase was not completely clear, it had to be extracted again with cetyltrimethylammonium bromide and ether. Both lower and upper phases were discarded, and the tRNA was dissolved in 10 ml of 1 M NaCl and precipitated with 20 ml of ethanol. The tRNA was collected by centrifugation and finally dissolved in water. The tRNA solutions were stable when kept frozen at −20°. Up to 25 fractions from the large column were pooled for the extraction of the tRNA. The recovery of tRNA chromatographed and isolated as described was usually more than 80% of the starting material.

Results

Preliminary Fractionation Experiments. First we tried to separate yeast tRNA according to the method of Muench and Berg (1966a). The most lipophilic tRNAs, *i.e.*, tyrosine, phenylalanine, and serine tRNA, passed through the column with little retardation and the separation was consequently unsatisfactory. The ratio of the stationary polar phase

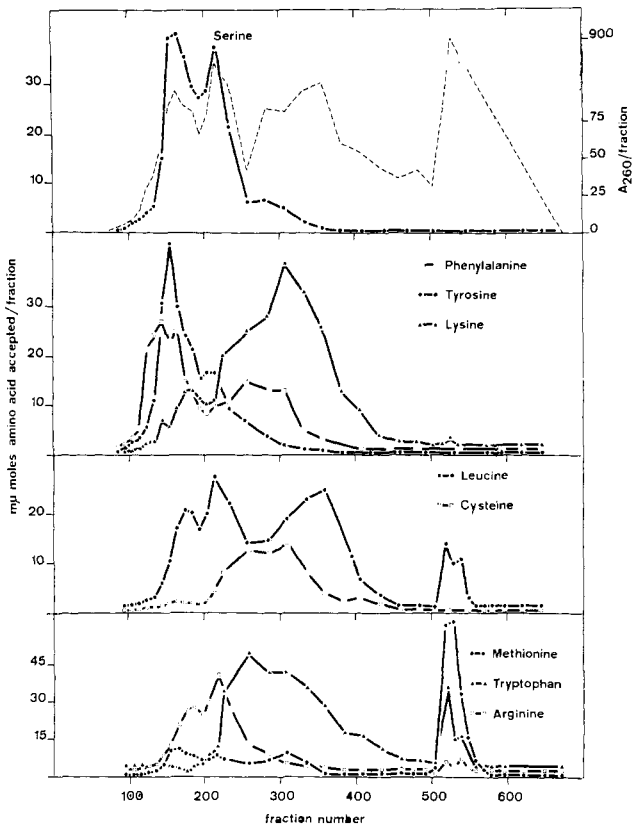

FIGURE 4: Fractionation of 70,000 A_{260} units of crude yeast tRNA on a partition column (12 × 60 cm) at 20°. The tRNA was applied as in Figure 1 and the column was eluted using 13 l. of organic phase for the first 28,000 A_{260} units and 4 l. of polar phase for the remaining material. Fraction size 25 ml. The recovery of desalted tRNA was 84%. The figure indicates only the amino acid acceptance of the most lipophilic species. For a complete elution pattern, see Figure 10. The amino acid acceptor activity was determined with rat liver pH 5 enzyme.

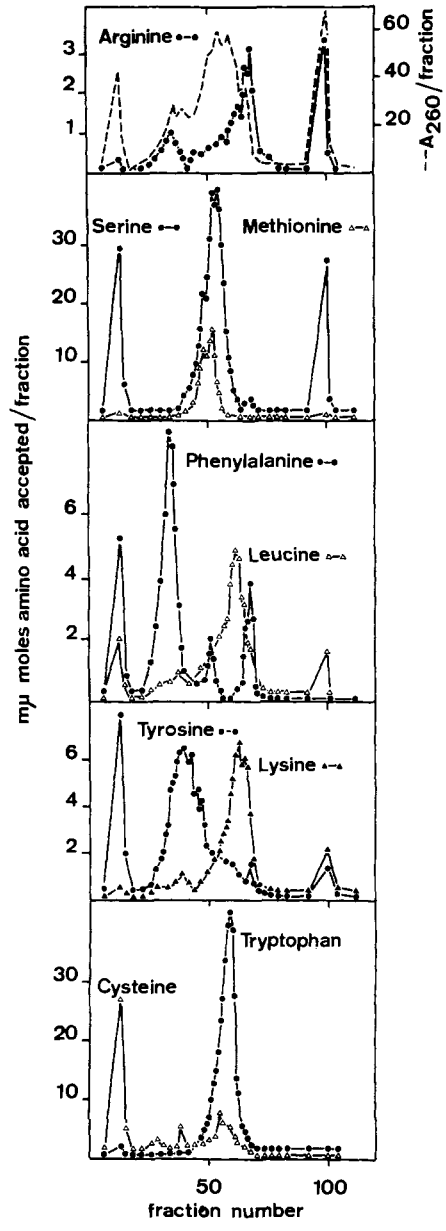

FIGURE 5: Fractionation of 2180 A_{260} units from fractions 151–220 (Figure 4) on a partition column (2.5 × 90 cm) with a linear temperature gradient from 31° at fraction 1 to 28.3° at fraction 94. Fraction size 25 ml. Application of the tRNA and elution of the column as in Figure 2. The recovery of desalted tRNA was 87%. Determination of amino acid acceptor activity as in Figure 4.

to the mobile organic phase was therefore increased by using Sephadex gel with a higher inner volume, V_i. Of the various gels tested Sephadex G-75 proved to be the most suitable. To obtain a good flow rate the column length and the hydrostatic pressure were reduced, as described in Methods.

In another series of experiments the influence of the column temperature on the elution pattern of the various tRNAs was tested. It was found that above 30° all tRNAs remained in the stationary phase on the column and that when a temperature gradient was employed increasing amounts of tRNA were eluted with a good separation of the lipophilic tRNAs.

Considering these characteristics of the partition system, we decided to separate tRNAs in two steps, using first a short column with a large diameter (12 × 60 or 7 × 40 cm) at a constant temperature of 20° and then applying selected fractions for further purification to a 2.5 × 90 cm column with a temperature gradient running from about 31 to 28°. As a third purification method reversed-phase chromatography was used.

The elution profiles obtained with these methods for tRNAs of liver, yeast, and *E. coli* are described below.

Fractionation of Rat Liver tRNA. Crude rat liver tRNA was chromatographed on a large partition column. Figure 1 shows the elution profiles of the most lipophilic tRNAs with acceptor activities for serine, phenylalanine, leucine, lysine, methionine, and tryptophan. For all amino acids, except for tryptophan, two or three tRNA peaks were found. Apart from the fact that we obtained three leucine-accepting tRNA species, this is in keeping with the findings of Nishimura and Weinstein (1969). The first fractions contained serine tRNAs in a purity of up to 70%, measured in terms of their serine acceptor activity.[1] Figure 11 shows the peaks obtained for the tRNAs of all 20 amino acids. In order to further purify the various tRNAs, partition chromatography with a temperature gradient was used.

Figure 2 indicates the elution profiles of the most lipophilic

[1] A serine tRNA shown by sequence analysis to contain less than 10% impurities was used to determine the amino acid acceptor activity of a pure tRNA.

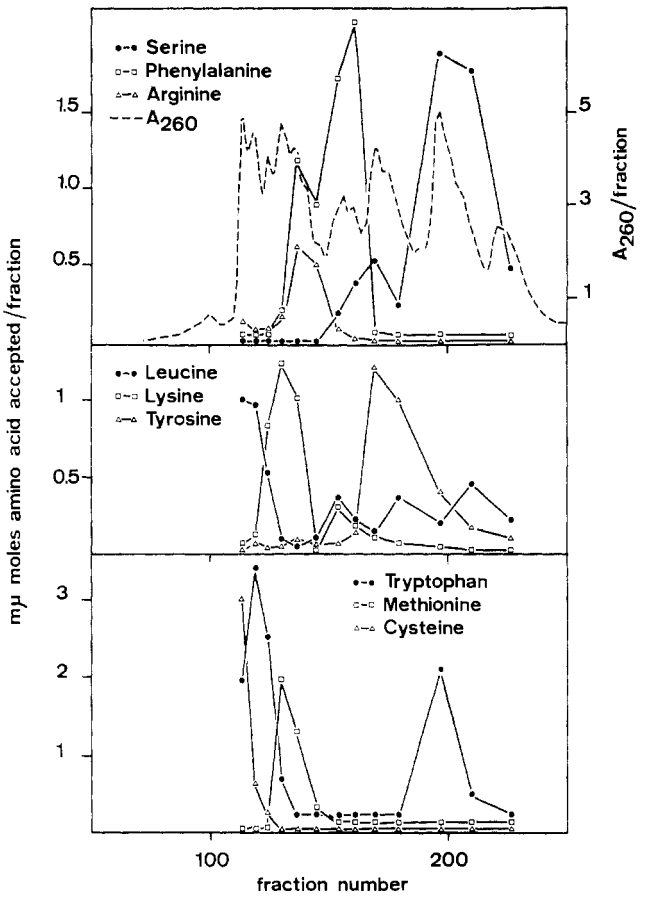

FIGURE 6: Fractionation of 460 A_{260} units from fraction 151–220 (Figure 4) on a reversed-phase column (1 × 250 cm). Conditions as in Figure 3. The recovery of desalted tRNA was 89%. Determinations of amino acid acceptor activity as in Figure 4.

tRNAs specific for serine, phenylalanine, leucine, lysine, methionine, and tryptophan. Serine and phenylalanine tRNA were about 70–80% pure, judging from their amino acid acceptor activity. However, serine tRNA consisted of several chemically different species, which could be separated by reversed-phase chromatography, as described in the accompanying paper (Müller *et al.*, 1971). Leucine, lysine, methionine, and tryptophan tRNA were eluted in sharp, but not well-separated, peaks.

We therefore examined the chromatographic behavior of these lipophilic tRNAs on reversed-phase chromatography, using a fraction of tRNA which was prepurified by partition chromatography and showed acceptor activity for the five amino acids serine, phenylalanine, leucine, lysine, and methionine.

Figure 3 shows the elution pattern obtained. All five tRNA species were clearly separated. Furthermore, the order of elution did not correspond to the partition column, since leucine tRNA appeared between serine and phenylalanine tRNA. Tryptophan tRNA was not included in this experiment. From similar studies in which a larger fraction of total tRNA was used, however, it was known that it would appear between methionine and lysine tRNA.

Hence, to separate the five lipophilic tRNAs specific for serine, phenylalanine, leucine, lysine, and methionine in high yields, it was most convenient to isolate first the phenylalanine and serine tRNA by partition chromatography and then separate the other three on a reversed-phase column.

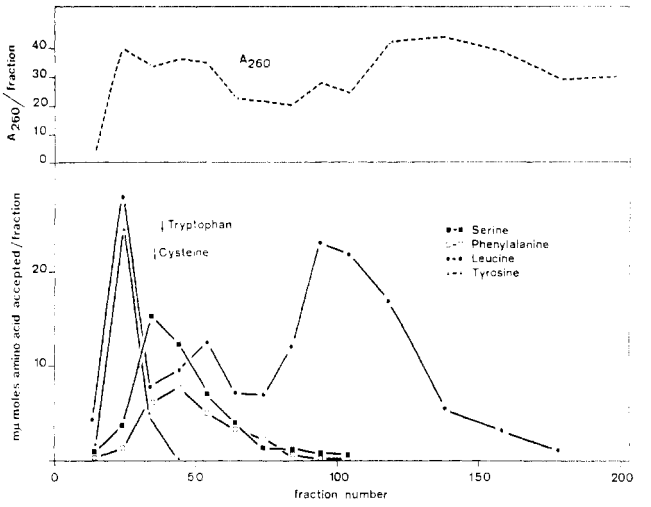

FIGURE 7: Fractionation of 10,370 A_{260} units of crude *E. coli* tRNA on a partition column (7 × 40 cm) at 20°. The tRNA was dissolved in 2 ml of H_2O and 13 ml of organic phase was added. The column was eluted using 5.2 l. of organic phase for the first 6400 A_{260} units and 0.75 l. for the remaining material. Fraction size 25 ml. The recovery of desalted tRNA was 86%. The figure indicates only the amino acid acceptance of the most lipophilic species. For a complete elution pattern, see Figure 9.

Tryptophan tRNA did not interfere, because it could be removed by the first large partition column. However, it was rather difficult to isolate this species in a pure form with good recovery.

In order to compare the elution patterns of tRNAs from rat liver with that of the better known organisms yeast and *E. coli*, tRNA samples from these two organisms were chromatographed in an analogous way. Crude yeast tRNA was chromatographed on a large partition column, as described for rat liver tRNA. Figure 4 shows the elution profile of the yeast tRNAs specific for serine, phenylalanine, tyrosine, lysine, leucine, cysteine, methionine, tryptophan, and arginine. It is evident that yeast tRNA contained a greater number of lipophilic tRNAs, cysteine and especially tyrosine tRNA being much less polar than in rat liver. In Figure 10 the peaks obtained for the tRNAs of 18 amino acids are summarized. No activity for glutamic and aspartic acid was obtained with the aminoacyl-tRNA synthetases isolated from rat liver.

The lipophilic tRNAs were further purified on a small partition column with a temperature gradient and by reversed-phase chromatography. Figures 5 and 6 show the elution profiles from these columns for the tRNAs specific for the same nine amino acids as in Figure 4.

Although yeast tRNA contained many more lipophilic species and several minor tRNA peaks occurred besides the main tRNA species for each amino acid, all major species could be separated by the combination of partition and reversed-phase chromatography. To compare the partition system used here with that developed by Muench and Berg (1966a) crude *E. coli* tRNA was partitioned on a large partition column in the same way as rat liver tRNA. Figure 7 shows the elution pattern of the tRNAs specific for serine, phenylalanine, leucine, tyrosine, tryptophan, and cysteine. The nonpolar tRNAs were further purified on a small partition column with a temperature gradient (Figure 8). Leucine tRNA is separated into three peaks. Thus, together with the more polar peak separated in the first chromatography (Figure 7), four leucine tRNA species were isolated. The

TABLE I: Amino Acids with at Least One Lipophilic tRNA.[a]

Amino Acid	Origin of tRNA: *E. coli*	Yeast	Rat liver	First Base of Codon
Phenylalanine	+	+	+	U
Leucine	+	+	+	U(C)
Serine	+	+	+	U(A)
Tyrosine	+	+	−	U
Cysteine	+	+	−	U
Tryptophan	+	+	+	U
Methionine	−	+	+	A
Lysine	−	+	+	A
Arginine	−	+	±	A(C)

[a] A tRNA is termed lipophilic when it is eluted on a partition column within the first 20% of the total tRNA.

first leucine tRNA peak and the main serine tRNA peak appear to be pure; with tyrosine, tryptophan, phenylalanine, and cysteine tRNA some overlapping occurs, but further separation should easily be achieved by reversed-phase chromatography (see, *e.g.*, Nishimura *et al.*, 1967, Weiss and Kelmers, 1967, and Weiss *et al.*, 1968).

Discussion

Muench and Berg (1966a) developed a separation procedure for *E. coli* tRNA using partition chromatography. The great advantage of this chromatography system is that, in contrast to most other commonly used systems, the nonpolar tRNAs are eluted at the beginning of the chromatogram. The system described by Muench and Berg (1966a) was modified in such a way that it was possible to separate several grams of rat liver, yeast, and *E. coli* tRNA and to purify the lipophilic species.

The recovery of the tRNAs isolated by these methods is usually more than 80% for both A_{260} units and acceptance activity for the various amino acids. All steps were performed under such conditions as to leave the sensitive rare bases undegraded. This has been tested in the case of serine tRNA from which a virtually quantitative yield of N^6-acetylcytidine was obtained.

As has been found with tRNAs of all the organisms studied so far, the separation methods used in our studies yield a large number of multiple components for most of the amino acids. However, without determining the chemical sequences of the various species, no definitive conclusions as to the nature of these components can be drawn; in particular it cannot be decided whether they are homogenous and occur naturally or whether they are mixtures of several species or artefacts due to aggregation or partial degradation. We therefore set out to isolate and characterize the various serine tRNAs from rat liver by sequence determination (Staehelin *et al.*, 1968; Müller *et al.*, 1971).

Some interesting conclusions can be drawn from the separation patterns by comparing the polarity of the various tRNAs from *E. coli*, yeast, and rat liver.

In Table I, the amino acids having the most lipophilic tRNAs from these organisms are listed. Four amino acids, serine, phenylalanine, leucine, and tryptophan, have a

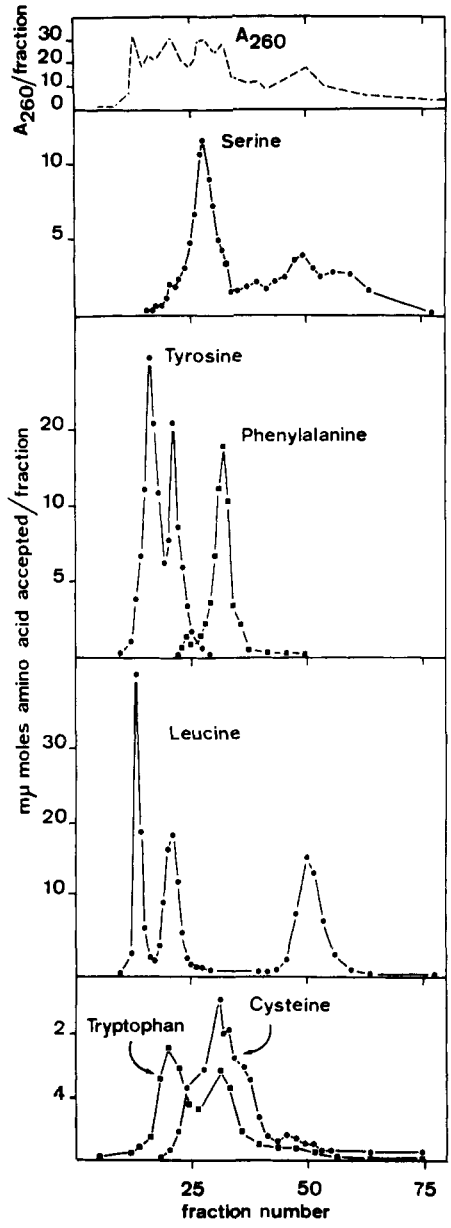

FIGURE 8: Fractionation of 1245 A_{260} units from fractions 9–68 (Figure 7) on a partition column (2.5 × 90 cm) with a linear temperature gradient from 30° at fraction 1 to 27.9° at fraction 76. Fraction size 25 ml. Application of tRNA and elution of the column as in Figure 2. The recovery of desalted tRNA was 74%.

lipophilic tRNA in all three organisms, whereas eleven, namely, proline, histidine, glutamic acid, glutamine, aspartic acid, asparagine, isoleucine, threonine, valine, alanine, and glycine, always have relatively polar tRNAs. Lysine and methionine tRNA are lipophilic only in yeast and rat liver, whereas tyrosine and cysteine tRNA are lipophilic only in yeast and *E. coli*. Arginine tRNA is a borderline case and tends to be more polar in *E. coli* than in yeast and rat liver.

Thus it is apparent that in general the polarity of various tRNAs remains constant throughout the evolution and might therefore be directly connected with their function.

Indeed, if one compares the codon triplets of the various amino acids, it is very striking that in *E. coli* all amino acids having uridine as first base also have a corresponding lipo-

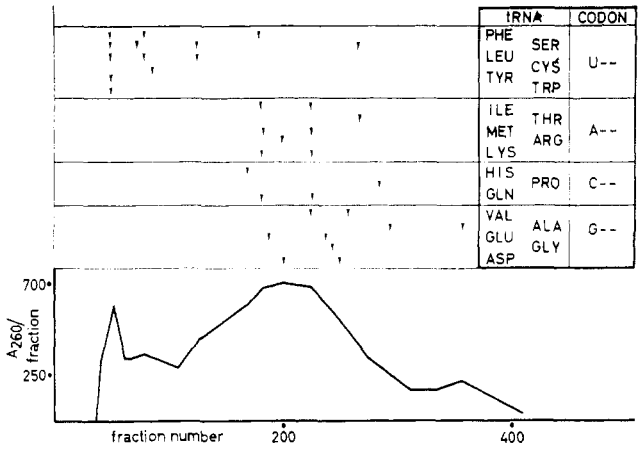

FIGURE 9: Order of elution of *E. coli* tRNAs on partition chromatography. This composite diagram is based on data from Muench and Berg (1966a) and Muench and Safille (1968) as well as from our own experiments. tRNA specific for asparagine is eluted at the same place as tRNA specific for aspartic acid. To elute the more polar tRNAs, Muench and Berg (1966a) used a triethylamine gradient.

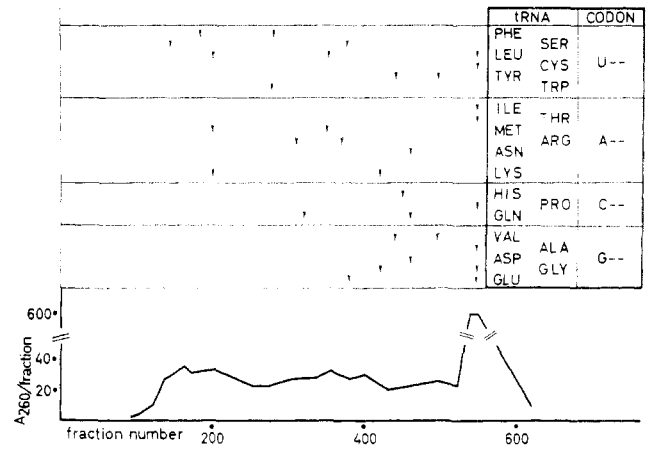

FIGURE 11: Order of elution of liver tRNAs on partition chromatography. Summary of the experiment described in Figure 1. The more polar tRNAs (fractions 520–610) were eluted as indicated in Figure 10.

philic tRNA (Figure 9). The same is true of yeast tRNA, but in addition, methionine, lysine, and arginine, all of which have adenine as first base and a purine base at the 5′ end of the codon, also have lipophilic tRNAs (Figure 10). Rat liver tRNA is very similar to yeast tRNA; however; tyrosine and cysteine tRNA are rather polar species (Figure 11). Serine is an interesting case, since four codons have U and two A as first letter. The coding experiments described in the following paper (Müller *et al.*, 1971) showed that the most lipophilic rat liver serine tRNAs respond to the codons with U as first base, whereas the tRNA responding to AGU and AGC is considerably more polar. An analogous result can be deduced for *E. coli* serine tRNA from the work reported by Ishikura and Nishimura (1968). Leucine also has six codons, and again the tRNA responding to the codons with U as first base is the most lipophilic one (Weisblum *et al.*, 1965).

These results all suggest a direct relation between polarity and coding properties.

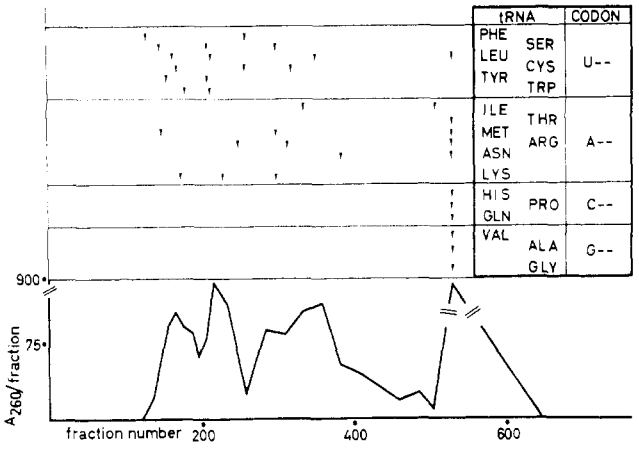

FIGURE 10: Order of elution of yeast tRNAs on partition chromatography. Summary of the experiment described in Figure 4. For the tRNAs of glutamic and aspartic acid no amino acid acceptor activity was found in the assay used. To elute the more polar tRNAs (fractions 500–620) lower water phase was used as indicated in Methods.

Recently, various authors have described experiments concerned with the relation between the isopentenyladenosine content and the coding properties of tRNAs (Armstrong *et al.*, 1969a,b; Peterkofsky and Jesensky, 1969; Nishimura *et al.*, 1969). These studies are all consistent with the hypothesis that N^6-(Δ^2-isopentenyl)adenosine and its derivatives are only found in those tRNA species responding to codons beginning with U. Since the very lipophilic isopentenyl derivatives always occur in the unpaired region of the anticodon, where the bases are known to be exposed, it is conceivable that they render a whole tRNA molecule lipophilic.

Thus, the fact that nonpolar tRNAs respond to codons having U as first base seems to be related to the presence of a lipophilic base in the anticodon.

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Isolation and Characterization of Serine Transfer Ribonucleic Acids from Rat Liver*

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ABSTRACT: Partition chromatography yields two peaks of rat liver serine tRNA. One of them is 70–80% pure, as judged from its serine acceptor activity. However, coding experiments and sequence studies show that this material is still heterogeneous. By additional partition chromatography and reversed-phase chromatography, four chemically different serine tRNAs could be purified and characterized. In coding experiments species I responds to UCU, UCC, and UCA, species IIa to UCG, and species III to AGU and AGC. Species IIb apparently responds only to UCU and UCA.

Serine is one of the three amino acids coded for by six different triplets. Whereas the six codons of the other two amino acids, arginine and leucine, have at least one common base, in the case of serine no single base is common to all six codons. By carrying out ribosomal binding experiments with trinucleotides Caskey *et al.* (1968) have obtained evidence suggesting that there are three species of guinea pig liver serine tRNA. One species was bound to ribosomes in the presence of UCU, UCC, and UCA, the second in the presence of UCG, and the third in the presence of AGU and AGC. However, there is chemical and genetic evidence indicating the existence of a great many tRNAs with identical coding properties but different primary sequences (*e.g.*, Zachau *et al.*, 1966, and Goodman *et al.*, 1968). On the other hand, only one seryl-tRNA synthetase has been found in rat liver (Rouge, 1969). It therefore seemed of interest to isolate the various species of serine tRNA present in rat liver in order to study their structural differences. This paper describes the separation of several species of rat liver serine tRNA as well as their coding responses to different trinucleotides.

Methods and Materials

tRNA was prepared as described by Rogg *et al.* (1969) from the livers of Sprague–Dawley rats of both sexes. Partition chromatography was carried out according to Wehrli and Staehelin's modification (1971) of the method devised by Muench and Berg (1966). Several grams of rat liver tRNA were fractionated on columns (12 × 60 cm) at 20°. Fractions from these large columns were rechromatographed on columns measuring 2.5 × 90 cm; in this instance a temperature gradient was used. The tRNA was recovered from pooled fractions and the serine acceptor activity determined as described by Wehrli and Staehelin (1971).

Reversed-phase chromatography was carried out according to Weiss and Kelmers (1967) (system 2) at pH 4.5 in the absence of $MgCl_2$, and Weiss *et al.* (1968) (system 4). When tRNA was to be aminoacylated before rechromatography, the fractions from the partition chromatography were desalted on small (1 × 1 cm) DEAE-cellulose columns, which were washed with 0.1 M potassium acetate (pH 5) and eluted with 1 M potassium acetate. The tRNA was precipitated with 2 volumes of ethanol and aminoacylated as described by Wehrli and Staehelin (1971), 1.0 ml of the assay mixture being used for the aminoacylation of 100 A_{260} units of tRNA. The specific activity of labeled serine varied as indicated in the figures. The aminoacylation mixture was extracted with phenol, and the tRNA was precipitated with 2 volumes of ethanol and dissolved in 5–10 ml of the starting elution buffer. tRNA fractions that were not aminoacylated were dialyzed overnight at 4° against the starting elution buffer. For analytical purposes, aliquots of each fraction were precipitated with HCl, with 0.25 mg of carrier tRNA added to facilitate precipitation, and counted as described previously (Wehrli and Staehelin, 1971). Fractions to be recovered were diluted 10-fold with water and absorbed onto small (1 × 1 cm) DEAE-cellulose columns. These were washed with an equal volume of 0.2 M potassium acetate (pH 5.0) and eluted with 3 ml of 1 M potassium acetate (pH 5.0). tRNA was precipitated with 2 volumes of ethanol.

Methylated albumin kieselguhr columns (3.0 × 40 cm) were prepared in three layers as described by Mandell and

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