Comparative Studies on Mammalian and Yeast Phenylalanine Transfer Ribonucleic Acids

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ABSTRACT: Rat liver tRNA^{Phe} displays a high affinity for benzoylated DEAE-cellulose, thereby providing a simple method for its purification. This property is due to the presence of a fluorescent and hydrophobic nucleotide, "Y_R," similar to the nucleotide "Y" present in tRNA^{Phe} obtained from yeast.

Phenylalanine tRNA from adult rat liver, fetal rat liver, rabbit reticulocytes, Novikoff hepatoma, rat liver nuclei, and rat liver mitochondria exhibited similar chromatographic behavior suggesting that this nucleotide (or closely related substances) is widely distributed in mammalian systems. The fluorescent nucleotide from rat liver tRNA^{Phe} appears to reside in the anticodon region and its fluorescence was environment sensitive in much the same manner as with yeast tRNA^{Phe}. However, fluorescence spectra of the purified tRNAs and thin-layer chromatography, absorption spectra and fluorescence excitation, and emission spectra of the purified fluorescent bases from yeast and rat liver tRNA^{Phe} suggest that these compounds have similar but not identical structures.

Despite abundant studies in microorganisms, relatively little is known about the structure of mammalian tRNAs. Staehelin *et al.* (1968) have determined the primary structure of a serine tRNA of rat liver. An extensive profile of the isoaccepting species of rat liver tRNA, and their patterns of codon recognition, has been elucidated (Nishimura and Weinstein, 1969). Purification and characterization of additional mammalian tRNAs are of interest for several reasons. (1) There is extensive aminoacylation of yeast and mammalian tRNAs by the heterologous synthetases (Doctor and Mudd, 1963); comparison of the structures of mammalian and yeast tRNAs may indicate which regions of the tRNA are recognition sites for the synthetase. (2) The structure and metabolism of certain unusual nucleotides in mammalian tRNA may provide important clues to the role of tRNA in cellular regulation and differentiation. (3) The role of minor nucleotides in the recognition of mRNA and ribosome binding may be elucidated (Thiebe and Zachau, 1968; Ghosh and Ghosh, 1970). For a review of these subjects, see Weinstein and Fink (1970).

In the present study, we describe the purification of a highly hydrophobic and fluorescent residue, Y_R,[1] present in $tRNA^{Phe}_{liver}$, and compare this material to the corresponding base obtained from phenylalanine tRNA of yeast.

Materials

Benzoylated DEAE-cellulose (20–50 mesh) was purchased from Schwarz BioResearch. Silicic acid coated with BD-cellulose was prepared as described by Wimmer *et al.* (1968). Cellulose plates (type MN 300, Brinkman) were used for thin-layer chromatography. Uniformly labeled [^{14}C]phenylalanine (specific activity either 50 (Stanstar) or 500 mCi/mmole) and [^{14}C]serine (specific activity 120 mCi/mmole) were obtained from Schwarz BioResearch. Phenol was analytical reagent grade (Mallinckrodt); 8-hydroxyquinoline was from Fisher Scientific; sodium lauryl sulfate was purchased from Sigma Co. Bentonite (Fisher) was washed by the method of Fraenkel-Conrat *et al.* (1961). Baker's yeast tRNA was purchased from Schwarz BioResearch or Plenum Scientific. Yeast tRNA^{Phe} isolated from this material had a purity of greater than 90%. A preparation of nuclear 4–7S RNA was kindly supplied by Dr. Harris Busch (Hodnett and Busch, 1968) and samples of rat liver mitochondrial tRNA and mitochondiral aminoacyl-tRNA synthetase were prepared by Dr. Clayton Buck (Buck and Nass, 1968). Ribonuclease T_1 was obtained from Sankyo.

Methods

Spectra absorption measurements were determined on a Zeiss spectrophotometer (Model PMQ II) or a Cary recording spectrophotometer (Model 14). Fluorescence measurements were made on an Hitachi Perkin-Elmer spectrophotofluorimeter, Model MPF-2A, empolying a xenon lamp and 1-cm light path. One A_{260} unit is that amount of material which has an absorbance of 1 when dissolved in 1 ml of water and measured with a 1-cm light path. The theoretical acceptance capacity of a pure tRNA was assumed to 1.67 mmoles of amino acid/A_{260} unit of tRNA (Hoskinson and Khorana, 1965).

Preparation of tRNA. The following modification of the method of Delihas and Staehelin (1966) was used for the isolation of rat liver tRNA, and tRNAs from other tissues. Rats were decapitated and their livers removed and quickly frozen in liquid nitrogen. Batches of 100 g were homogenized

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[1] Abbreviations used are: $tRNA^{Phe}_{liver}$, phenylalanine tRNA from rat liver; $tRNA^{Phe}_{yeast}$, phenylalanine tRNA from yeast; BD-cellulose, benzoylated DEAE-cellulose; SBD-cellulose, silicic acid coated with BD-cellulose; $tRNA^{Phe}_{liver}$ (HCl) or $tRNA^{Phe}_{yeast}$ (HCl), phenylalanine tRNA treated at pH 2.9 for 2.5 hr at 37° and eluted from BD-cellulose columns with 1 M NaCl; Y_R^+, for fluorescent, chloroform-soluble product of mild acid hydrolysis of $tRNA^{Phe}_{liver}$; Y^+, the major fluorescent, chloroform-soluble product of mild acid hydrolysis of $tRNA^{Phe}_{yeast}$; Y^{2+}, a minor, fluorescent, chloroform-soluble product of mild acid hydrolysis of $tRNA^{Phe}_{yeast}$; Y, the fluorescent nucleoside adjacent to the 3′-OH terminus of the anticodon region of $tRNA^{Phe}_{yeast}$; Y_R, the fluorescent nucleoside in the anticodon loop of $tRNA^{Phe}_{liver}$.

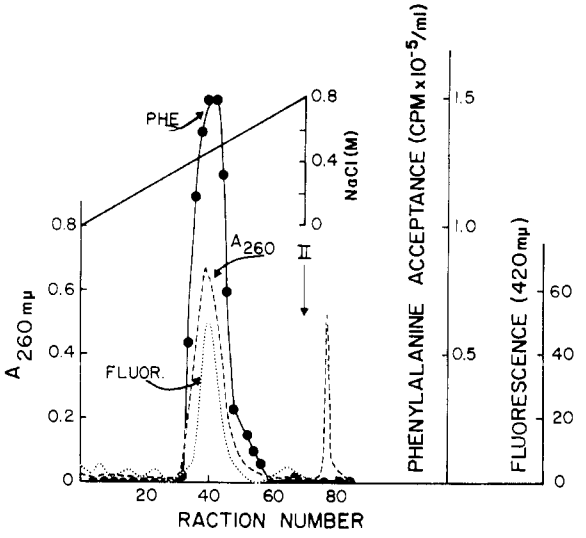

FIGURE 1: Chromatography of the partially purified rat liver phenylalanine tRNA from BD-cellulose (see text) on SBD-cellulose. The column (1 × 120 cm) was equilibrated with 0.05 M sodium acetate (pH 5.0) and 0.05 M $MgCl_2$ at room temperature, and 120 A_{260} units of tRNA in 3 ml of starting buffer was applied. The tRNA was eluted with a linear gradient of 600 ml of 0.05 M sodium acetate (pH 5.0) and 0.05 M $MgCl_2$ in the mixing chamber and 600 ml of 0.8 M NaCl, 0.05 M sodium acetate (pH 5.0), and 0.05 M $MgCl_2$ in the reservoir. The remaining tRNA was removed with 1 M NaCl, 0.05 M sodium acetate (pH 5.0), 0.05 M $MgCl_2$, and 10% ethanol (v/v). The fractions were assayed for [^{14}C]phenylalanine acceptance and for fluorescence at 420 mμ when excited at 310 mμ (see text).

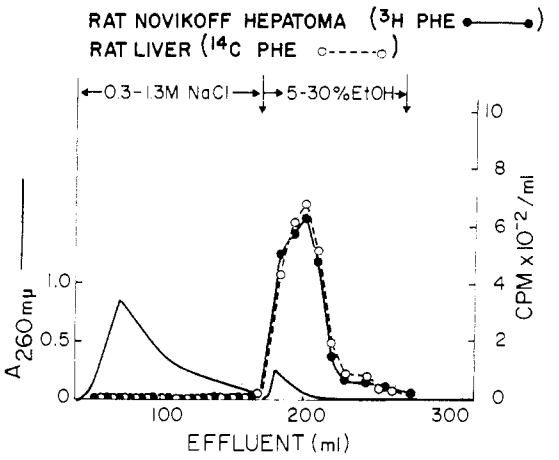

FIGURE 2: Cochromatography of rat Novikoff hepatoma tRNA, which had been previously acylated with [^{3}H]phenylalanine, and rabbit liver tRNA, which had been acylated with [^{14}C]phenylalanine, on a BD-cellulose column. The column (0.5 × 12 cm) was equilibrated with 0.3 M NaCl, 0.05 sodium acetate (pH 5.0), and 0.01 M $MgCl_2$. The tRNA was eluted with a linear gradient of 75 ml of 0.3 M NaCl, 0.05 M sodium acetate (pH 5.0), and 0.01 M $MgCl_2$ in the mixing chamber and 75 ml of 1.3 M NaCl, 0.05 M sodium acetate (pH 5.0), and 0.01 M $MgCl_2$ in the reservoir. The remaining tRNA was eluted with a linear gradient of 50 ml of 1.3 M NaCl, 0.05 M sodium acetate (pH 5.0), 0.01 M $MgCl_2$, and 5% v/v ethanol in the mixing chamber and 1.3 M NaCl, 0.05 M sodium acetate (pH 5.0), 0.01 M $MgCl_2$, and 30% v/v ethanol in the reservoir. The flow rate was 1 ml/min and the fraction size was 8 ml. The optical density at 260 mμ for each fraction was measured. Aliquots (1 ml) of the fractions were counted directly in Bray's solution to determine [^{3}H]- and [^{14}C]phenylalanine distribution.

with 200 ml of 0.01 M Tris-HCl (pH 7.0), 0.2% bentonite, 0.3 M sucrose, and 200 ml of water-saturated phenol containing 0.1% 8-hydroxyquinoline, in a Waring blender at high speed for 2 min. The homogenate was stirred vigorously at 20° for 1 hr and then centrifuged at 13,000*g* for 25 min at 4°. The aqueous layer was reextracted three times with water-saturated phenol containing 0.1% sodium lauryl sulfate and 0.1% 8-hydroxyquinoline. Two volumes of 95% ethanol and 0.1 volume of 2 M sodium acetate (pH 5.0) were added to the aqueous layer and the mixture placed at −20° for 4 hr or longer. The precipitate was collected by centrifugation at 13,000*g* for 30 min at 4°, suspended in 40 ml of 1 M NaCl, and stirred gently at 4° for 8 hr, and the insoluble RNA (largely rRNA) removed by centrifugation at 13,000*g* at 4° for 30 min. The 1 M NaCl-soluble RNA was precipitated with two volumes of 95% ethanol at −20°; the precipitate was collected at 13,000*g* for 30 min and resuspended in distilled water containing 0.01 M $MgCl_2$. Analysis of this material by sucrose gradient centrifugation and polyacrylamide electrophoresis revealed predominantly 4S RNA (tRNA), a small amount of 5S RNA, and less than 5% contamination with rRNA. The yield for rat liver was between 0.5 and 0.8 mg of tRNA per g wet weight of liver.

The above preparation of tRNA contained variable amounts of polysacharides and low molecular weight contaminants which were removed during the subsequent fractionation on BD-cellulose.

Assay of Amino Acid Acceptor Activity. A crude preparation of rat liver aminoacyl-tRNA synthetase was obtained as previously described (Nishimura and Weinstein, 1969). The assay systems for column fractions, the processing of samples, and radioactivity measurements were also as previously described (Nishimura and Weinstein, 1969).

Column Chromatography. Columns of BD-cellulose and SBD-cellulose were prepared and run as previously described (Gillam *et al.*, 1967; Wimmer *et al.*, 1968; Fink *et al.*, 1968). Oligonucleotides from a ribonuclease T_1 digest were separated in 7 M urea on a DEAE-cellulose column essentially as described by Reeves *et al.* (1968).

Results

Purification of Rat Liver Phenylalanine tRNA. When uncharged rat liver tRNA was applied to a BD-cellulose column, approximately 95% of the material eluted with a 0.4–1.5 M gradient of NaCl. As originally reported with yeast $tRNA^{Phe}$ (Gillam *et al.*, 1968), $tRNA^{Phe}_{liver}$ was tighlty bound to the column and eluted only when the column was washed with ethanol (Fink *et al.*, 1968). The BD-cellulose procedure resulted in an approximately 17-fold enrichment for $tRNA^{Phe}$. Figure 1 indicates the results obtained when the phenylalanine tRNA obtained from the latter procedure was applied to an SBD-cellulose column and eluted with a gradient of 0–0.8 M NaCl. A sharp optical density peak was coincident with phenylalanine acceptance activity and was paralleled by fluorescence which had an emission maximum at 430 mμ when excited at 310 mμ. This fluorescence is attributed to the presence in $tRNA^{Phe}_{liver}$ of a residue, Y_R, which is similar to the Y residue originally identified by RajBhandary *et al.* (1968) in $tRNA^{Phe}_{yeast}$. The properties of Y_R are described in greater detail below. The fractions at the peak had an acceptance capacity for [^{14}C]phenylalanine of approximately 1400 mmoles/A_{260}, corresponding to a purity of 84%.

In the course of purification of $tRNA^{Phe}$ from either rat liver or yeast, we have observed a peak of serine tRNA which elutes slightly earlier than the $tRNA^{Phe}$, in the ethanol

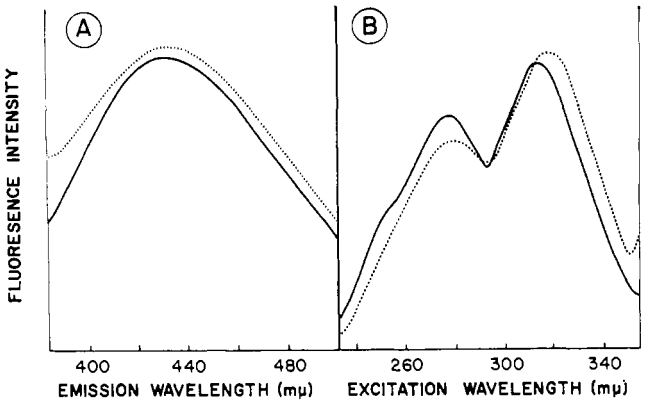

FIGURE 3: Uncorrected fluorescence emission (A) and excitation (B) spectra of $tRNA^{Phe}$ yeast (--------) and $tRNA^{Phe}$ liver (———) in 0.01 M potassium phosphate (pH 7.0) and 0.01 M $MgCl_2$.

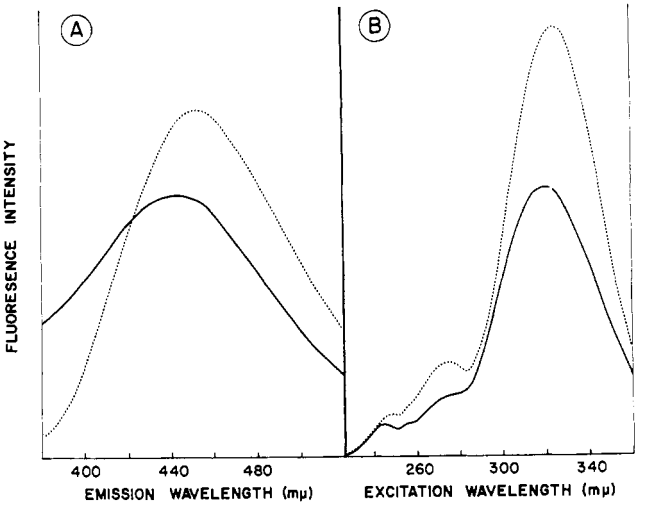

FIGURE 4: Uncorrected fluorescence emission (A) and excitation (B) spectra of Y^+ (--------) and Y_R^+ (———) in 0.01 M potassium phosphate (pH 7.0) and 0.01 M $MgCl_2$.

eluate of the BD-cellulose column. A similar result has been described with $tRNA_{yeast}$ (Gillman *et al.* 1967) and with chicken liver tRNA (Maenpaa and Bernfield, 1969). This serine tRNA was well separated from $tRNA^{Phe}$ by the SBDC procedure, since the former material remained bound to this column during the 0–0.8 M NaCl gradient.

Other Mammalian Phenylalanine tRNAs. Figure 2 indicates that $tRNA^{Phe}$ of the Novikoff hepatoma, when charged with liver synthetase, also eluted in the ethanol region of BD-cellulose. Similar results were obtained with fetal rat liver tRNA, rabbit reticulocyte tRNA, and a low molecular weight fraction of rat liver nuclear RNA (see materials). When rat liver mitochondrial tRNA was acylated with [^{14}C]phenylalanine, employing mitochondrial synthetase, this material also cochromatographed with rat liver cytoplasmic phenylalanyl-tRNA.

Although the above studies were done with tRNAs precharged with phenylalanine, this in itself would not cause them to elute in the ethanol region of BD-cellulose since precharged *E. coli* tRNA (Roy and Söll, 1968), which does not contain the Y residue (Uziel and Gassen, 1969; Barrell and Sanger, 1969), and precharged $tRNA^{Phe}_{liver}$, from which the Y_R residue has been excised, elute in the NaCl region of this column (see below). The high affinity for BD-cellulose of the above described phenylalanine tRNAs, as well as data presented below, suggest that the phenylalanine tRNAs from a variety of mammalian sources contain a residue similar to Y_R.

Comparison of the Fluorescent Residue in Rat Liver and Yeast Phenylalanine tRNA. Yeast $tRNA^{Phe}$ contains an unidentified nucleotide (Y) which is adjacent to the anticodon and fluoresces (RajBhandary *et al.* 1968). It seemed likely that $tRNA^{Phe}_{liver}$, because of its high affinity for BD-cellulose and its fluorescence, might contain a similar residue. Figure 3 gives the fluorescence spectra of purified rat liver $tRNA^{Phe}_{liver}$. It can be seen that when examined in 0.01 M potassium phosphate (pH 7.0) and 0.01 M $MgCl_2$ the material displayed excitation maxima at 275 and 313 mμ and a shoulder at 750 mμ. The emission maximum, when excited at any of the three former wavelengths, was 430 mμ. Yeast $tRNA^{Phe}$ studied earlier under identical conditions displayed excitation maxima at 320 and 280 mμ with a shoulder at 254 mμ; the emission maximum was at 435 mμ. Beardsley *et al.* (1970) have reported that in the presence of 0.1 M Mg^{2+} the intensity of fluorescence at 430 mμ of $tRNA^{Phe}_{yeast}$ increases approximately 2.5-fold. We have found a similar effect when $tRNA^{Phe}_{liver}$ was studied in the presence and absence of Mg^{2+}.

We next employed the mild acid hydrolysis and chloroform extraction procedure of Thiebe and Zachau (1968) to excise the fluorescent residues of both tRNAs. The tRNAs were then rechromatographed on BD-cellulose where they now eluted at 0.7–0.8 M NaCl, rather than in the ethanol region, and retained essentially 100% of their total phenylalanine acceptance capacities. Similar results have previously been reported for $tRNA^{Phe}_{yeast}$ (Thiebe and Zachau, 1968). With both materials the intensity of the fluorescence at 420 mμ of the $tRNA^{Phe}_{HCl}$ was approximately 5% of the $tRNA^{Phe}$ prior to mild acid hydrolysis and was qualitatively different.

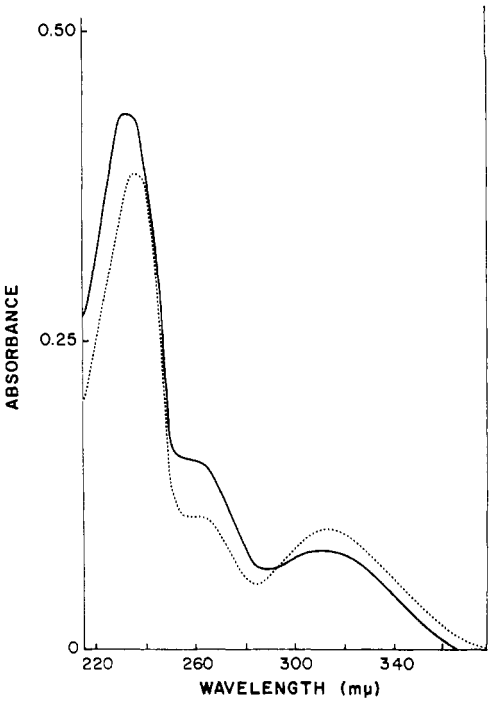

FIGURE 5: Absorption spectra of Y^+ (--------) and Y_R^+ (———) in 0.01 M potassium phosphate (pH 7.0) and 0.01 M $MgCl_2$.

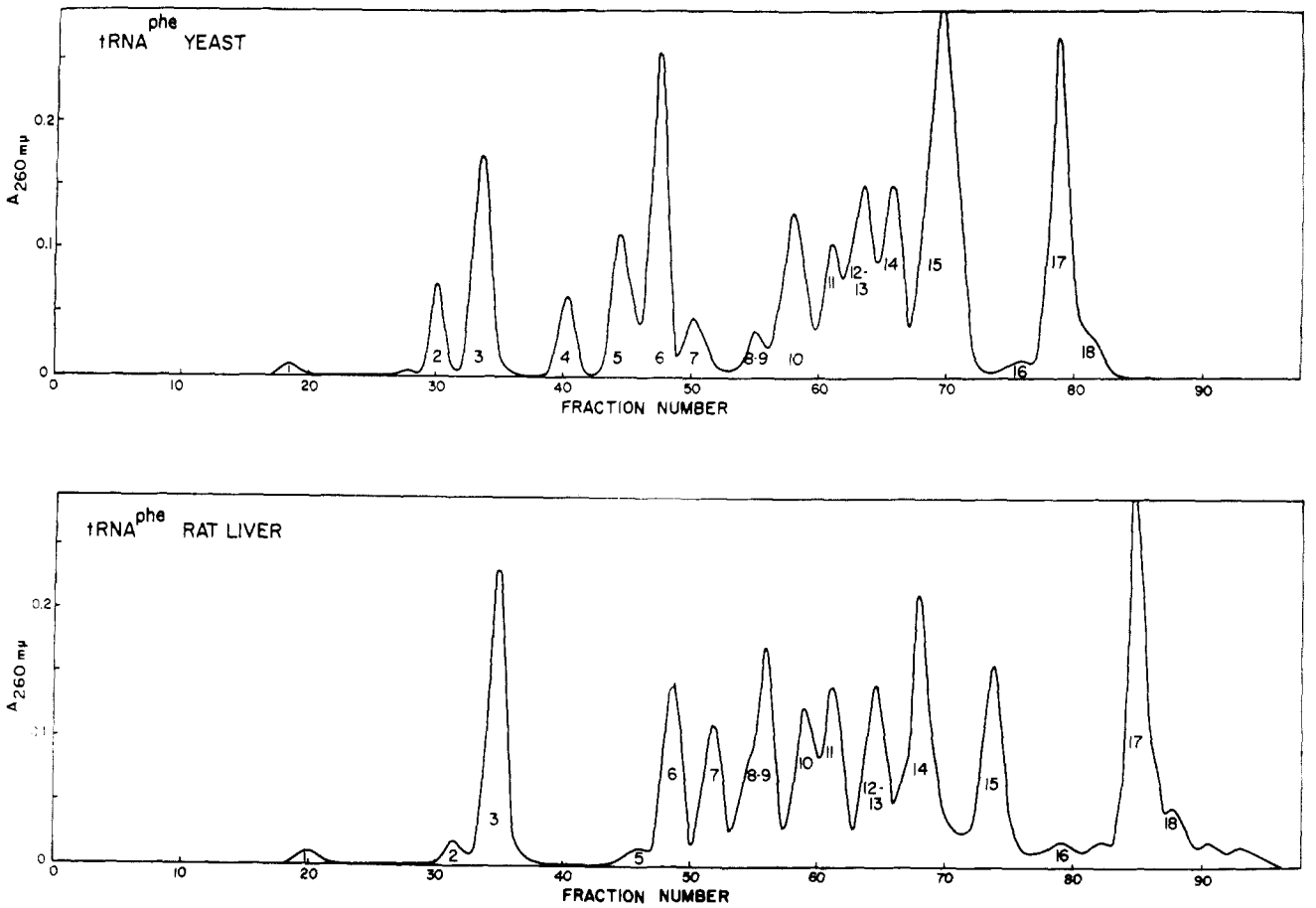

FIGURE 6: Fractionation of T_1 RNase digests of purified $tRNA^{Phe}_{liver}$ (a) and $tRNA^{Phe}_{yeast}$ (b) by chromatography on DEAE-cellulose. $tRNA^{Phe}$ (5 A_{260} units) in 0.4 ml of 0.02 M Tris-HCl (pH 7.5) was digested with T_1 ribonuclease (6.5 units) for 16 hr at 37°. This solution was then adjusted with 8 M urea and 2 M Tris-HCl (pH 8.0) to the molarity of the column starting buffer, and placed on a 0.5 × 105 cm DEAE-cellulose (Cl^-) column. A linear gradient elution was carried out using 300 ml of 7 M urea in 0.02 M Tris-HCl (pH 7.5) and 0.3 M NaCl in the reservoir and 300 ml of 7 M urea in 0.02 M Tris-HCl (pH 7.5) in the mixing chamber. The flow rate was 12.4 ml/hr. The effluent was monitored in a Beckman DU spectrophotometer with a Gilford recorder. All peaks were also monitored for fluorescence at 420 mμ when excited at 310 mμ.

The $tRNA^{Phe}_{HCl}$ displayed shoulders in the excitation and emission spectra which were not well resolved from the Raman scatter peaks. Weak fluorescence with an excitation maximum of approximately 300 mμ was observed. It is apparent that the decreased affinity for BD-cellulose, following mild acid hydrolysis and chloroform extraction, correlates with a loss of intense fluorescence at 430 mμ. The residual weak fluorescence is similar to that of free 7-methylguanosine, a known constituent of $tRNA^{Phe}_{yeast}$ (RajBhandary *et al.*, 1968). In contrast to its enhancing effect on the fluorescence of the original materials, 0.01 M Mg^{2+} did not affect the intensity of this residual fluorescence.

The fluorescence spectrum of the Y^+ residue from $tRNA^{Phe}_{yeast}$ (Thiebe and Zachau, 1968) was compared to that of the material obtained from $tRNA^{Phe}_{liver}$, henceforth referred to as $Y_R{}^+$. The Y^+ and $Y_R{}^+$ were extracted with chloroform following acid hydrolysis and chromatographed on thin-layer cellulose plates MN 300 at 21° in ethyl acetate–1-propanol–water (4:1:2, v/v) (Thiebe and Zachau, 1968) the upper phase of the system was used). The Y^+ had an R_F of 0.81 (a minor component from yeast, Y^{2+}, had R_F 0.49), while $Y_R{}^+$ had an R_F of 0.52. The fluorescent spots were eluted and their spectra were studied. Figure 4 indicates that Y^+ had excitation maxima at 324 and 248 mμ with a shoulder at 276 mμ and an emission maximum of 453 mμ. $Y_R{}^+$ had excitation maxima at 245, 276, and 320 mμ, and an emission maximum at 442 mμ.

Comparison of Absorption Spectra. The absorption spectra of Y^+ and $Y_R{}^+$ are given in Figure 5. Y^+ had absorption maxima at 237, 265, and 321 mμ. The spectrum of $Y_R{}^+$ was similar to but not identical with that of Y^+. With $Y_R{}^+$ the absorption in the 310–320-m and 265-mμ regions, relative to that in the 235-mμ region, was less intense than in the case of Y^+.

Comparison of Ribonuclease T_1 Digests of Rat Liver and Yeast $tRNA^{Phe}$. Purified $tRNA^{Phe}_{liver}$ was digested with ribonuclease T_1; the resulting oligonucleotides were separated in 7M urea on a DEAE-cellulose column. The profile was compared to that obtained with a T_1 digest of purified $tRNA^{Phe}_{yeast}$ analyzed under identical conditions. The profile of the latter material was similar to that obtained by RajBhandary *et al.* (1968). A comparison of the oligonucleotide profiles obtained with $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{liver}$ revealed several similarities, as well as differences (Figure 6). The peaks from the $tRNA^{Phe}_{liver}$ were numbered so as to give the best correspondence with those of the $tRNA^{Phe}_{yeast}$. Although we have not done a complete analysis of all oligonucleotides, we have determined the compositions of a few of the simpler fragments. Our data on the liver material indicate that, as with yeast (RajBhandary *et al.* 1968), peak I was G-cyclic-p, peak 3 was

G_p, and peak 6 was ApGp. Peak 4 of yeast was missing in the liver profile. Since it derives from the CCA stem region of yeast, this suggests that the nucleotide sequence in the CCA stem of $tRNA^{Phe}_{liver}$ differs from that of yeast. Several other peaks in the T_1 profile of liver differed appreciably when compared to the yeast profile, suggesting additional sequence differences in the two tRNAs. With both yeast and liver phenylalanine tRNAs, peaks 13, 17, and 18 were fluorescent. We attribute the fluorescence of peak 13 to the presence of 7-methylguanosine, a known constituent in the corresponding fragment of $tRNA^{Phe}_{yeast}$ (RajBhandary *et al.* 1968). Consistent with this is the fact that, like free 7-methylguanosine, this peak had a fluorescence emission maximum of 350 mμ when excited at 290 mμ. The fluorescence of peak 17 and 18, in both the yeast and liver profile, can be attributed to the Y residue. With the yeast peak 17 is a dodecanucleotide representing the entire anticodon region (RajBhandary *et al.*, 1968). Peak 17 of liver eluted somewhat later than that of yeast, suggesting that the anticodon region of the liver material is similar to but probably not identical with that of yeast. Peak 18 appears to be very similar to peak 17 (RajBhandary *et al.*, 1968) and occurs in both yeast and rat liver $tRNA^{Phe}$.

Discussion

The modified nucleotide, "Y," has been described as being present in yeast, wheat germ, rat, and beef liver $tRNA^{Phe}$ (RajBhandary *et al.*, 1968; Dudock *et al.*, 1968; Yoshikami *et al.*, 1968; Fink *et al.*, 1968; Zimmerman *et al.*, 1970). This study demonstrates that the $tRNA^{Phe}$ from fetal rat liver, rabbit reticulocytes, Novikoff hepatoma rat liver nuclei, and rat liver mitochondria as well as that from adult rat liver exhibited similar chromatographic behavior with respect to their elution from BD-cellulose which suggests that they contain nucleosides similar to or identical with the Y_R residue. A larger number of phenylalanine tRNAs from different sources need to be tested for their hydrophobicity and fluorescence properties before it can be stated that this is a characteristic of all eukaryotic organisms.

Because of the reports of chromatographic differences between cytoplasmic and mitochondiral tRNAs of eukaryotes (Buck and Nass, 1968, 1969; Brown and Novelli, 1968; Suyama and Eyer, 1967; Fournier and Simpson, 1968; Epler, 1969), and the suggestion that the protein synthesizing mechanism of mitochondria is more similar to bacterial systems than to the cytoplasm of the eukaryote (Raven, 1970) the tRNA from liver mitochondria was studied for the presence of Y by its elution profile on BD-cellulose. The results suggest that the $tRNA^{Phe}$ from mitochondria must also contain a base with hydrophobic properties similar to the present in cytoplasmic $tRNA^{Phe}$.

Since methylation of tRNAs has been shown to occur mainly in the cytoplasm of mammalian cells (Muramatsu and Fujisawa, 1968; Burdon *et al.*, 1967) it can be inferred that the nucleus contains precursor tRNAs which are undermethylated and may lack their complete complement of other unusual bases. The nuclear tRNA from rat liver, which was prepared from washed nuclei, was acylated with phenylalanine and cochromatographed with acylated cytoplasmic tRNA. Both cytoplasmic and nuclear $tRNA^{Phe}$ are eluted from BD-cellulose only with ethanol. Since all of the acylated $tRNA^{Phe}$ contained Y, this moiety must have been synthesized on the tRNA before conversion to an acylatable molecule occurred. Therefore, there may still be a nonacylatable Y-deficient $tRNA^{Phe}$ precursor which we cannot as yet identify. These studies do not imply that the Y base is identical from all sources studied.

In fact, the differences in R_F of the Y_R^+ and of Y^+ and Y^{2+} of yeast suggest that there may be extensive diversity among the Y residues, though they have similar fluorescence and absorption properties and confer similar hydrophobicity to $tRNA^{Phe}$. The differences between the excitation spectra of the excised Y^+ or Y_R^+ and the intact tRNA when the emission is measured at 430 mμ are of two types. The blue shift is consistent with that expected if the $\pi^*-\pi$ transition responsible for fluoresence were occurring in a more hydrophobic environment in the intact tRNA. As suggested by Eisinger *et al.* (1970), the Y residue may be stacked with adjacent bases or it may interact with unpaired bases elsewhere in the molecule. The data on fluoresence depolarization of Y obtained by Beardsley and Cantor (1970) and by Thiebe and Zachau (1971) also suggest that the rotation of Y may be hindered by interaction with some part of the molecule.

In addition to the blue shift, the excitation spectrum of Y in the intact tRNAs has a more intense absorption band in the region of 280 mμ than expected. This is not due to a difference in spectrum between the base and the nucleoside since Thiebe and Zachau (1971) have shown these spectra to be quite similar with regard to the intensities of the various bands. At this time, none of the remaining possibilities have been eliminated, *i.e.*, there may be energy transfer to Y from an adjacent nucleotide which absorbs near 280 mμ or some very peculiar characteristic of the local environment may enhance the efficiency of fluorescence dependent on excitation at 280 mμ relative to that at 310 mμ.

The comparison of the T_1 ribonuclease digestion products from $tRNA^{Phe}_{yeast}$ and $tRNA^{Phe}_{liver}$ establishes that the Y residue occurs in comparable fragments, even though the elution profiles of the products of T_1 ribonuclease digestion on DEAE-cellulose are not identical. Analysis of the fragments reveals fluorescence in peak 13 similar to the fluorescence of 7-methylguanosine. When the $tRNA^{Phe}$ is exposed to mild acid hydrolysis there is residual fluorescence, again demonstrating that there are other fluorescent bases in $tRNA^{Phe}$. This residual fluorescence is not sensitive to the magnesium concentration in the environments as is the fluorescence from the Y nucleotide in $tRNA^{Phe}$; possibly this is because the residual fluorescence emanates from a region that is in an extremely stable conformation.

Added in Proof

Recently the structure of Y base in $tRNA^{Phe}$ from yeast was established (Nakanishi *et al.*, 1970).

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

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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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