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# PURIFICATION AND CHARACTERIZATION OF ISOACCEPTING SPECIES OF PHENYLALANYL-TRANSFER RIBONUCLEIC ACID FROM RAT LIVER

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## SUMMARY

Four isoacceptor species of phenylalanyl-tRNA, from rat-liver total tRNA, have been purified to homogeneity using sequential chromatography on BD-cellulose and reversed-phase-5 (RPC-5) columns. Re-chromatography of individual species (I–IV) on RPC-5 columns revealed chromatographic homogeneity (single peak, same site and height of elution) and biological homogeneity since none of the species accepted amino acids other than phenylalanine. Thermal denaturation profiles and circular dichroism spectral patterns suggested that these species exist in two major conformations.

## INTRODUCTION

Alterations in the tRNAs of systems undergoing changes in the metabolic processes and differentiation have been studied by many workers[1]. Differences between tRNAs of neoplastic and normal tissues have also been reported[2–3]. One of the well-studied systems has been the tRNAs of normal rat liver and minimal deviation hepatoma (5123D)[4–6], where the differences in the chromatographic profiles of various amino acid-accepting tRNAs, including that of phenylalanyl-tRNA, have been reported[5–6]. As a continuation of these studies, we have isolated, purified to homogeneity and characterized four isoaccepting species of phenylalanyl-tRNA from rat liver.

## MATERIALS AND METHODS

### *Transfer RNA*

tRNAs were isolated as described by Taylor *et al.*[2] with slight modifications. Rat liver (Buffalo rat, Microbiological Associate) was homogenized in Tris buffer (0.01 *M*, pH 7.4) containing 0.1% bentonite, sucrose (1.0 *M*) and phenol–water (73:27) and deproteinized with phenol–chloroform (1:1). tRNAs were separated from other RNA species by differential salt precipitation with 1.0 *M* sodium chloride and trace contaminants were further eliminated by subjecting the tRNAs to 0.2 to 2.0 *M* KCl gradient through a DEAE-cellulose column. The resulting tRNAs were com-

pletely deacylated by incubating in 0.01 *M* Tris–HCl buffer (pH 8.5) containing 0.001 *M* magnesium chloride at 37° for 2 h.

*Synthetases*

Aminoacyl-tRNA synthetases were prepared from rat liver as described by Taylor *et al.*[2]. The preparation was freed of endogenous tRNA, RNase and other low-molecular-weight impurities by passage through a Sephadex G-50 column and stored at −20° without any loss in aminoacylating activity.

*Aminoacylation*

The tRNAs were acylated *in vitro* with [$^3$H]phenylalanine (5.02 Ci/mmole; New England Nuclear Corp., Boston, Mass., U.S.A.) as described by Taylor *et al.*[2]. The charged tRNAs obtained after three precipitations with 95% ethanol at −20° were resuspended in 0.05 *M* sodium acetate buffer (pH 4.5) containing 0.3 *M* sodium chloride and 0.01 *M* magnesium chloride and then purified by BD-cellulose chromatography.

*BD-cellulose chromatography*

The preparation of BD-cellulose and subsequent chromatography were performed as described by Gillam *et al.*[7]. Unacylated tRNAs were eluted with a 1-l linear gradient of 0.3 to 1.0 *M* sodium chloride in 0.05 *M* sodium acetate buffer (pH 4.5) containing 0.01 *M* magnesium chloride. The acylated tRNA species, still bound to the column after the linear gradient elution, were eluted either with 250 ml of 1.0 *M* sodium chloride containing 15% ethanol or with a 400-ml co-linear gradient of 1.0 to 2.0 *M* sodium chloride and 0 to 30% (v/v) ethanol in 0.05 *M* sodium acetate buffer (pH 4.5) containing 0.01 *M* magnesium chloride. The ethanol fractions rich in [$^3$H]-phenylalanyl-tRNAs were concentrated by Amicon ultrafiltration and further purified by reversed-phase-5 (RPC-5) chromatography.

*RPC-5 chromatography*

The preparation of RPC-5 columns and subsequent chromatography were performed as described by Pearson *et al.*[8]. RPC-5 column prepared by Method C of Pearson *et al.*[8] and that obtained commercially (Miles Labs., Elkhart, Ind., U.S.A.) showed identical elution profiles of phenylalanyl-tRNA. The acylated tRNAs were eluted with a 100-ml linear gradient of 0.5 to 0.9 *M* sodium chloride in 0.01 *M* sodium acetate buffer (pH 4.5) containing 0.001 *M* β-mercaptoethanol and 0.01 *M* magnesium chloride at 37°. The positions of the peaks of [$^3$H]phenylalanyl-tRNA were identified by monitoring radioactivity and the peaks were pooled separately. Each peak was re-chromatographed on RPC-5 to eliminate cross contaminations. After this, individual peaks were pooled, concentrated and deacylated as described earlier. The acceptor activities of the various peaks for other amino acids than phenylalanine were also examined. The tRNAs thus isolated were used for further studies.

*Recovery monitoring*

Throughout the purification of these four isoaccepting species of phenylalanyl-tRNA, percent recovery was monitored by measuring the radioactivity. Radioactivity was measured either as 10% trichloroacetic acid-precipitable counts on Whatman

No. 3 filter discs after hydrolyzing with 0.1 *M* sodium hydroxide and solubilizing with Nuclear-Chicago Solvent (NCS), or in Triton X-100 as suggested by Kelmers and Heatherly[9]. The samples were counted in a Beckman Model LS-150 scintillation spectrometer.

*Thermal denaturation studies*

The isoacceptor species thus isolated from the RPC-5 column were resuspended, dialyzed and the thermal denaturation profiles were recorded as described by Patnaik and Taylor[10] using a Gilford Model 2000 spectrophotometer with an ultrathermostat attachment coupled to a thermosensor unit. Optical density was measured both at 260 and 280 nm.

*Circular dichroism studies*

Circular dichroism spectra were recorded in a Jasco ORD-UV-5 spectrophotometer with an ultrasensitive SS-10 circular dichroism modification, using 1-cm path length. The data are presented as mean residue ellipticity as described by Willick and Kay[11]. The ellipticity values reported for each species were the average of analysis of at least three samples. Absorption spectra of the samples were recorded on a Cary 14 spectrophotometer.

## RESULTS AND DISCUSSION

*Purification of phenylalanyl-tRNA*

Phenylalanyl-tRNA has been eluted as a single peak from BD-cellulose in 15% ethanol–1.0 *M* sodium chloride (figure not shown). However, with a co-linear gradient of both ethanol (0 to 30%) and sodium chloride (1.0 to 2.0 *M*) at least two additional minor peaks were resolved from BD-cellulose column (Fig. 1). While less than 1% of the total radioactivity was eluted with the initial salt gradient (0.3 to 1.0 *M*), most of the radioactivity (recovery in counts per minute as 94%) appeared in the ethanol fraction. This peak showed serine acceptor activity as a major contaminant with ar-

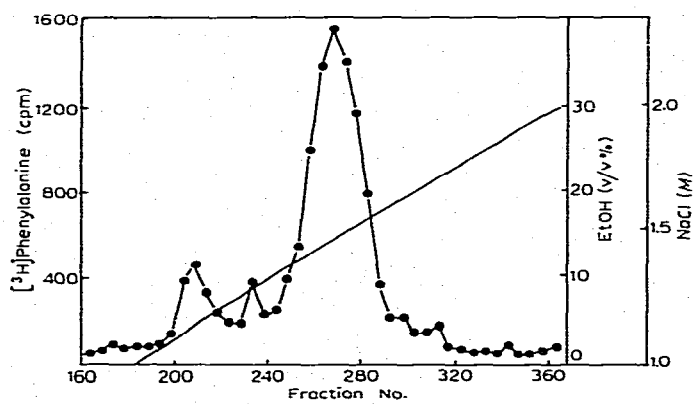


Fig. 1. BD-cellulose elution profile of phenylalanyl-tRNA with a co-linear gradient of ethanol (0–30%) and NaCl (1.0–2.0 *M*) in sodium acetate buffer (pH 4.5) containing 0.01 *M* $MgCl_2$. The column was previously eluted with a linear NaCl gradient (0.3–1.0 *M*) in the above buffer

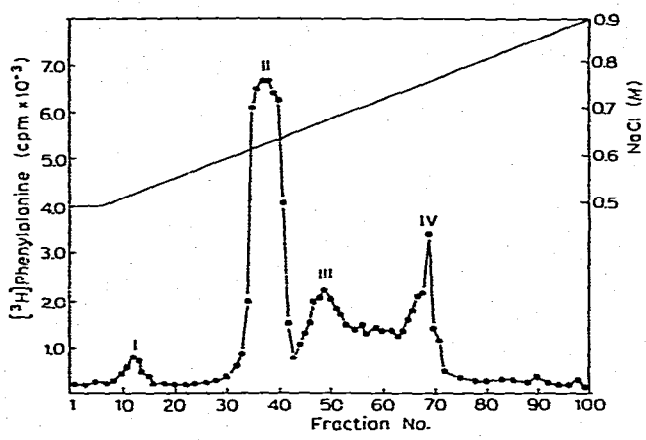


Fig. 2. RPC-5 elution profile of phenylalanyl-tRNA, previously fractionated through the BD-cellulose column. RPC-5 column was eluted with a linear NaCl gradient (0.5–0.9 *M*) in sodium acetate buffer (pH 4.5) containing 0.001 *M* β-mercaptoethanol and 0.01 *M* $MgCl_2$, at 37 .

ginine, tyrosine, aspartic acid and glutamic acid acceptors as trace contaminants. This is in agreement with our earlier observations[12].

*Resolution of isoacceptors of phenylalanyl-tRNA*

The phenylalanyl-tRNA was further purified by fractionation on a RPC-5 column. The elution profile is shown in Fig. 2. With a linear gradient of 0.5 to 0.9 *M* sodium chloride, four trichloroacetic acid-precipitable radioactive peaks were distinctly resolved. It should be emphasized that this profile was very reproducible, that two of these peaks (peaks I and III) were always comparably minor ones and that peaks I and II did not exhibit any serine acceptor activities whereas peak IV exhibited greatly reduced serine acceptor activity. Furthermore, the minor contaminants observed after BD-cellulose fractionation were almost undetectable in all the peaks except for tyrosine, which was observed in peak III. However, re-chromatography of the isolated peaks from RPC-5 column individually showed no further cross contamination. Furthermore, purity of the species I and II and that of III and IV was 100% as judged from phenylalanine acceptance alone. Thus all the four (I–IV) species of phenylalanyl-tRNA, obtained after re-chromatography on RPC-5 columns, exhibited chromatographic homogeneity (single peak, same site and height of elution) and biological homogeneity since no one accepted amino acids other than phenylalanine. Although three isoaccepting species of phenylalanyl-tRNA from mouse liver have been reported earlier[13], this is the first occasion to detect and purify to chromatographic and biological homogeneity four species of phenylalanyl-tRNA from rat liver since with an RPC-2 column only one species could be isolated[5,6].

*Thermal denaturation studies*

The physical state of the four species was examined by thermal denaturation in 1 × SSC (0.15 *M* sodium chloride–0.015 *M* sodium citrate, pH 7.0) buffer con-

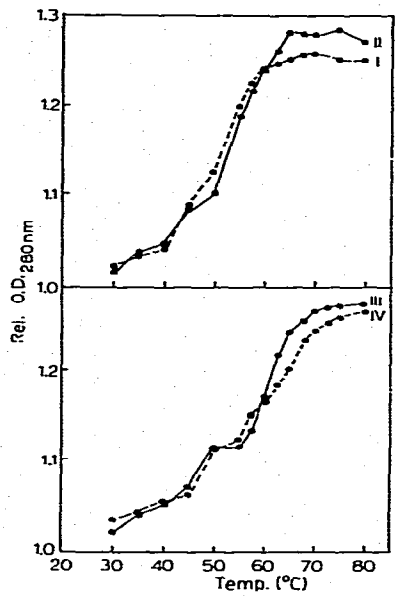


Fig. 3. Thermal denaturation profiles of isoaccepting species of phenylalanyl-tRNA in 1 × SSC buffer containing 0.002 *M* $MgCl_2$. The species numbers refer to those reported in Fig. 2.

taining 2 m*M* magnesium chloride (Fig. 3). Hyperchromicity, due to thermal denaturation, varied between 26 and 29%. After denaturation in 0.01 × SSC without any added magnesium chloride, hyperchromicities of species I and II were reduced to 7–9%, whereas species III and IV maintained 13–15% hyperchromicity. This shows that whereas species I and II lost major portions of their structure, species III and IV retained about 50% of their structure under the condition of denaturation (Table I). It was further observed that species III and IV exhibited a bimodal denaturation, the first mode appearing between 30 and 54° and the second mode having major portion of the hyperchromicity appeared between 55 and 70°. This is indicative of the absence

TABLE I

SOME CHARACTERISTICS OF THE FOUR ISOACCEPTING SPECIES OF PHENYLALANYL-tRNA FROM RAT LIVER

| *Species* of tRNA* | *Circular dichroism positive maxima* | | | | *Thermal denaturation hyperchromicity* | |
|---|---|---|---|---|---|---|
| | *Native* | | *Denatured*** | | *Native* | *Denatured*** |
| | *λ (nm)* | *[θ] × 10⁻⁴* | *λ (nm)* | *[θ] × 10⁻⁴* | | |
| I | 264.6 | 1.892 | 266.4 | 1.304 | 0.2693 | 0.0735 |
| II | 264.2 | 1.943 | 267.0 | 1.459 | 0.2879 | 0.0933 |
| III | 261.8 | 2.068 | 265.2 | 1.638 | 0.2789 | 0.1386 |
| IV | 262.2 | 2.079 | 264.8 | 1.589 | 0.2743 | 0.1562 |

* Species numbers refer to those specified in Fig. 2.
** Suspended and dialyzed in 0.01 × SSC without any added $MgCl_2$ ($<10^{-6}$ *M* $MgCl_2$).

of simultaneous melting of the entire molecule. Similar types of melting profiles have been observed earlier by others[14]. These observations were quite reproducible.

*Circular dichroism spectral measurements*

The circular dichroism spectra of the four isoaccepting species in their native state are presented in Fig. 4. Under the conditions of measurement, the position of the positive maxima of the various species varied between 261 and 265 nm. The band positions in species I and II were 264.6 and 264.2 nm, respectively, and in the case of species III and IV, these were 261.8 and 262.2, respectively. The magnitude of the posi-

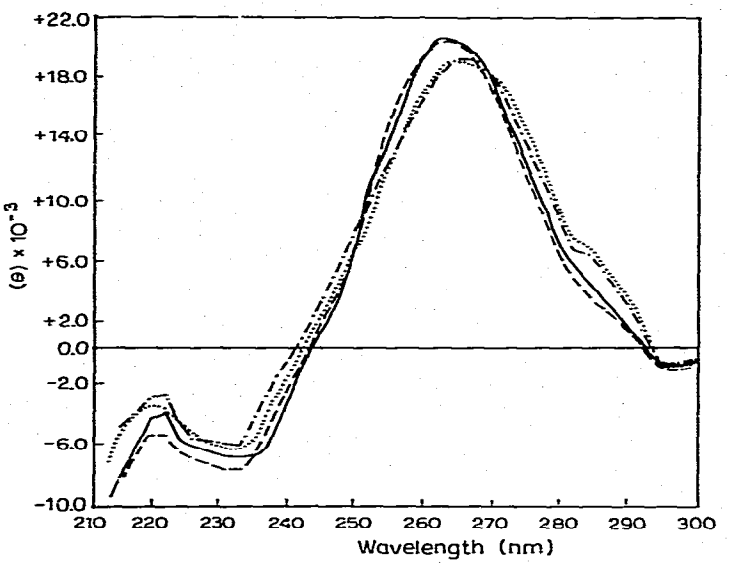


Fig. 4. Circular dichroism spectra of isoaccepting species of phenylalanyl-tRNA in 0.01 *M* potassium phosphate buffer (pH 7.0) containing 0.01 *M* $MgCl_2$. · · · · ·, species I; — · —, species II; – – –, species III; ———, species IV. Species numbers refer to those reported in Fig. 2.

tive maxima was higher in species III and IV than in species I and II. The negative minima at 295 nm could be detected in all of the four species. Similar circular dichroism spectra of phenylalanyl-tRNA from other sources have been reported[15,16]. Of great interest was the appearance of a yet unreported shoulder in the positive maxima around 282 nm. This shoulder was more pronounced in species I and II (Fig. 4). Although the origin of this shoulder with respect to the structural characteristics of these species is at present not known, it is hoped to reveal important distinguishing information about the conformational status of the species.

In all the species, denaturation of the ordered structure was accompanied by a red shift in the wavelength of the positive maxima with subsequent decrease in the magnitude of this band. The isoacceptor species were denatured by dialyzing in 0.01 × SSC for 24 h with several changes of the buffer. In species I and II this band was shifted to about 267 nm, whereas in species III and IV the shift was only up to about 265 nm. In all the species, the negative minima around 295 nm were absent. Under the conditions of denaturation, alteration of the circular dichroism spectra

around 260 nm and disappearance of the negative minima around 295 nm are consequences of destruction of the secondary as well as the tertiary structure[15–17]. However, as the circular dichroism spectra of the denatured species I and II were virtually different from those of species III and IV, these have perhaps not been transformed into a common denatured conformation under our experimental conditions.

## CONCLUSION

These observations along with the thermal denaturation profiles suggest that the four homogeneous isoaccepting species of phenylalanyl-tRNA present in normal rat liver perhaps exist in two major conformations, the significance of which is not clear at this stage. Further studies are being undertaken to investigate differences existing between each of these species.

## ACKNOWLEDGEMENT

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