PURIFICATION AND BASE COMPOSITION OF A CHLOROPLASTIC $tRNA^{Phe}$ FROM *PHASEOLUS VULGARIS*

P. GUILLEMAUT, R. MARTIN and J. H. WEIL

Institut de Biologie Moléculaire et Cellulaire, Université Louis Pasteur, 15, rue Descartes, 67000 Strasbourg, France

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1. Introduction

Chloroplast-specific tRNAs, different from their cytoplasmic counterparts, have been characterized in *Euglena,* [1–5], pea [6,7], bean [8–10], soybean [11], cotton [12,13], tobacco [14] and barley [15], but so far there is no information available on the structure of these chloroplastic tRNAs. We have purified a chloroplast-specific $tRNA^{Phe}$ from *Phaseolus vulgaris* and determined its base composition in order to compare it to that of prokaryotic and of plant cytoplasmic $tRNAs^{Phe}$.

2. Materials and methods

Bean leaf total tRNA was prepared as previously described [8]. The fractionation and purification of chloroplastic and cytoplastic $tRNAs^{Phe}$ was achieved by BD-cellulose chromatography [16–18]. The purity of bean chloroplastic $tRNA^{Phe}$ was checked by two-dimensional polyacrylamide gel electrophoresis [19]. Hydrolysis of the purified $tRNA^{Phe}$ and fractionation of the nucleosides by two-dimensional thin-layer chromatography was performed according to Rogg et al. [20]. To determine whether a Y base was present or absent, fluorescence spectra were recorded on a FICA 55 spectrofluorimeter under the conditions previously described [16].

3. Results

Upon BD-cellulose chromatography of bean leaf total tRNA [16] determination of phenylalanine accepting activity in the fractions reveals 3 peaks (fig.1). The first two peaks correspond to chloroplast-specific $tRNAs^{Phe}$, as shown by the following features: (i) these two peaks are only revealed using an *E. coli* enzyme or an enzyme from bean chloroplasts and it has already been shown that chloroplast-specific tRNAs can be aminoacylated by bacterial or chloroplastic enzymes, but not by the cytoplasmic enzyme [9,10,21,22]; (ii) peak 1 is predominant in preparations obtained from purified bean chloroplasts [22]; (iii) both peak 1 and peak 2 hybridize with chloroplast DNA, whereas peak 3 does not [23]. Peak 3 is the cytoplasmic $tRNA^{Phe}$ species.

Chloroplastic $tRNA^{Phe}$ peak 1 could be purified by two further chromatographic steps. In the first step the fractions corresponding to $tRNA^{Phe}_1$ from the previous column (fig.1) were chromatographed (without prior aminoacylation) on a BD-cellulose column, using a NaCl-ethanol gradient. Chloroplast $tRNA^{Phe}_1$ was eluted at 0.77 M NaCl, 9% ethanol (fig.2a). The fractions showing phenylalanine accepting activity (shown by the arrow on fig.2a) were pooled; at this stage this chloroplastic $tRNA^{Phe}_1$ can accept about 300 pmol/OD unit. This $tRNA^{Phe}_1$ was aminoacylated as previously described [8], re-extracted [18] and re-chromatographed on BD-cellulose using the same gradient as in the first step. In this second step, the chloroplastic [^{14}C]phenylalanyl-$tRNA^{Phe}_1$ is eluted at 0.85 M NaCl, 12% ethanol (fig.2b). The fractions containing more than 1650 pmol of phenylalanyl-tRNA/OD unit were collected, re-extracted and used for analysis.

In this way 1 mg of chloroplastic $tRNA^{Phe}_1$ was obtained from 250 mg of bean leaf total tRNA. It was shown to be pure and homogenous by two-

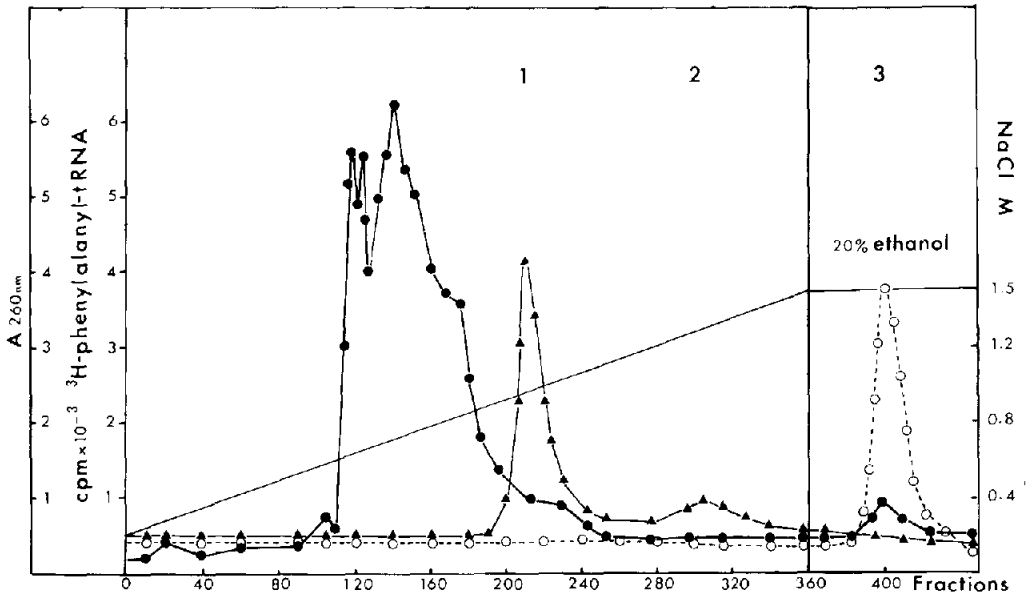
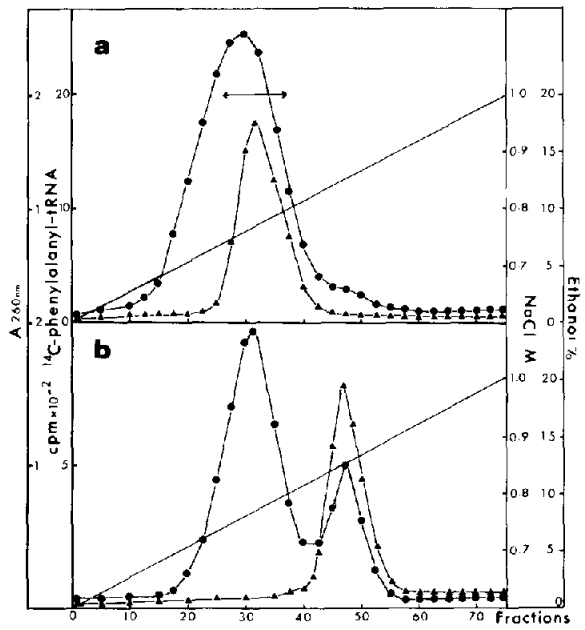


Fig.1. Fractionation of bean leaf total tRNA on BD-cellulose. Column size: 140 × 1.6 cm. 250 mg tRNA were loaded on the column. Elution with a 2 × 2 l gradient of NaCl 0.2 → 1.5 M in Tris-HCl 0.01 M pH 7.5, Mg^{++} 0.01 M, then by 1l of 20% ethanol in 1.5 M NaCl. Fractions of 11 ml were collected. (●——●) A_{260nm} (▲——▲) [^{3}H]phenylalanine accepting activity revealed using a chloroplast or *E. coli* enzyme (○——○) [^{3}H]phenylalanine accepting activity revealed using a cytoplasmic enzyme.



dimensional polyacrylamide gel electrophoresis [19].

After enzymatic hydrolysis of 5 OD units of this purified chloroplastic $tRNA^{Phe}$ the resulting nucleosides were fractionated by two-dimensional thin-layer chromatography as shown on fig.3. The nucleosides were characterized by their position on the chromatogram by comparison with the map of Rogg et al. [20];

Fig.2. Purification of chloroplastic $tRNA^{Phe}_1$ on BD-cellulose. (a) Fractions corresponding to peak 1 on fig.1 were pooled, precipitated with ethanol, re-extracted with phenol and loaded on a 40 × 1 cm column without prior aminoacylation. Elution with 2 × 150 ml gradient of NaCl 0.6 → 1.0 M and ethanol 0 → 20% in sodium acetate 0.01 M pH 4.5, Mg^{++} 0.01 M. Fractions of 4 ml were collected. (●——●) A_{260nm} (▲——▲) [^{14}C]phenylalanine accepting activity revealed using a *E. coli* enzyme. (b) Fractions corresponding to the peak (marked with an arrow) on fig.2a were pooled, precipitated, re-extracted, aminoacylated with [^{14}C]phenylalanine (12 000 cpm/nmole) using a *E. coli* enzyme, re-extracted and loaded on a 40 × 1 cm column. Elution with the same gradient as in fig.2a. Fractions of 4 ml were collected. (●——●) A_{260} nm. (▲——▲) Radioactivity due to [^{14}C]phenylalanyl-tRNA measured on a 25 μl aliquot.

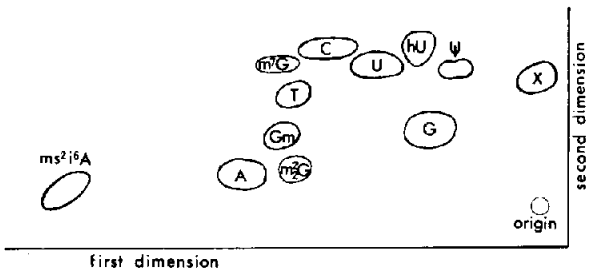


Fig.3. Thin-layer chromatography of the enzymatic hydrolysate of 5 OD units pure chloroplastic $tRNA^{Phe}_1$ on a 50 × 20 cm cellulose plate (DC-Alurolle Cellulose, Merck, Darmstadt). First dimension: *n*-butanol–isobutyric acid–25% ammonia–water (150 : 75 : 5 : 50, by vol). Second dimension: satured ammonium sulfate–0.1 M sodium acetate pH 6,–isopropyl alcohol (79 : 19 : 2, by vol). X = acp^3U = 3-(3-amino-3-carboxypropyl)uridine.

they were eluted with water and identified by their spectra in 0.1 N HCl and in 0.1 N KOH, which allowed a quantitative determination. Results are given in table I, where the nucleoside composition of *E. coli* and of wheat germ (cytoplasmic) $tRNAs^{Phe}$ have been listed for comparison.

To determine whether a Y-type base was present or not, fluorescence emission spectra (when excited at 310 nm) of the purified bean chloroplast $tRNA^{Phe}_1$, of bean cytoplasmic $tRNA^{Phe}$, of lupin (cytoplasmic) $tRNA^{Phe}$, of yeast (cytoplasmic) $tRNA^{Phe}$ and of *E. coli* total tRNA were compared. As shown on fig.4, the fluorescence characteristic of the Y bases present in yeast, bean and lupin cytoplasmic $tRNAs^{Phe}$ is absent in bean chloroplast $tRNA^{Phe}_1$. It should be noted that the fluorescence spectrum of bean cytoplasmic $tRNA^{Phe}$ is slightly different from that of yeast $tRNA^{Phe}$, but identical to that of lupin $tRNA^{Phe}$ which is known to contain a peroxy-Y base [26], as in the case of wheat-germ [27] and barley [15,28] $tRNAs^{Phe}$.

Bean chloroplast $tRNA^{Phe}_1$ does not contain s^4U, as shown by the absence of any absorbance peak at 336 nm [29].

Table 1
Nucleoside composition of chloroplastic $tRNA^{Phe}_1$ as compared to that of *E. coli* and of wheat germ (cytoplasmic)$tRNAs^{Phe}$.

	E. coli $tRNA^{Phe}$ [24]	Bean chloroplastic $tRNA^{Phe}_1$	Wheat germ (cytoplasmic) $tRNA^{Phe}$ [25]
A	14	17	16
U	8	10 or 11	7
C	21	19	19
G	23	20	20
ms^2i^6A	1	1	–
m^1A	–		1
T	1	1	1
ψ	3	2	3
hU	2	2	3
acp^3U	1	1	–
s^4U	1	–	–
Cm	–	–	1
m^2G	–	–	1
m^2_2G	–	1	1
m^7G	1	1	1
Gm	–	1 or 2	1
Y	–	–	1
	76	76 (?)	76

The results are expressed as moles of nucleosides/mole tRNA. In the case of chloroplast $tRNA^{Phe}_1$ the calculations were made with the assumption that this tRNA is also 76 nucleotides long and the values were rounded of to the closest unit.

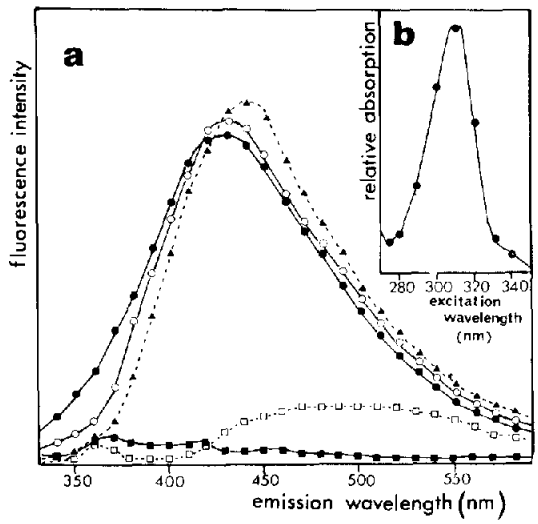


Fig.4. Fluorescence emission spectra (a) of bean cytoplasmic (●——●) and chloroplast (■——■) $tRNA^{Phe}_1$, and of yellow lupin (○——○), yeast (▲--▲) cytoplasmic $tRNAs^{Phe}$ and *E. coli* total tRNA (□--□). The excitation wavelength was 310 nm. Excitation spectrum (b) of bean cytoplasmic $tRNA^{Phe}$ (●——●) monitored at 430 nm. The different spectra were obtained at the following tRNA concentrations: Bean cytoplasmic $tRNA^{Phe}$ (500 pmol/OD): 0.8 OD unit/ml. Bean chloroplastic $tRNA^{Phe}_1$ (1750 pmol/OD): 3 OD units/ml. Yellow lupin (cytoplasmic) $tRNA^{Phe}$ (1500 pmol/OD): 0.26 OD unit/ml. Yeast (cytoplasmic $tRNA^{Phe}$ (1600 pmol/OD): 0.25 OD unit/ml. *E. coli* (unfractionated) tRNAs: 9.5 OD unit/ml.

4. Discussion

As far as the minor nucleosides are concerned, chloroplastic $tRNA^{Phe}_1$ resembles *E. coli* $tRNA^{Phe}$ in that it also contains one m^7G, one acp^3U (sometimes called X) and one ms^2i^6A. In *E. coli* $tRNA^{Phe}$, the first two are present in a m^7G-acp^3U-C sequence located in the extra-arm at position 47–49 [30]. The existence of a m^7G in this tRNA is consistent with the presence of a m^7G tRNA methylase found in bean chloroplasts [31]. All $tRNAs^{Phe}$ sequenced so far have a G-A-A (or a Gm-A-A) anticodon and either a Y or ms^2i^6A adjacent (on the 3′ end) of the anticodon. As chloroplastic $tRNA^{Phe}_1$ has no Y, it is likely that the ms^2i^6A detected is located next to the anticodon.

But chloroplastic $tRNA^{Phe}_1$ differs from *E. coli* $tRNA^{Phe}$ in that it contains no s^4U (present in *E. coli* $tRNA^{Phe}$) and does contain one m^2_2G and Gm (absent in *E. coli* $tRNA^{Phe}$).

On the other hand, chloroplastic $tRNA^{Phe}_1$ resembles wheat germ (cytoplasmic) $tRNA^{Phe}$ which also contains one m^7G, one m^2_2G and one Gm, but differs in that it has no Y, no m^1A and no m^2G.

Chloroplastic $tRNA^{Phe}_1$ appears close to *E. coli* $tRNA^{Phe}$ since the hypermodified nucleosides, namely acp^3U and ms^2i^6A are similar. This result is consistent with those showing that quite often heterologous aminoacylation reactions can take place between chloroplastic and prokaryotic tRNAs and aminoacyl-tRNA synthetases [9,10,15,21,22,32,33].

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