CONSERVATION OF THE STRUCTURES OF PLANT tRNAs AND AMINOACYL-tRNA SYNTHETASES

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Received 2 April 1979

1. Introduction

Since the determination of the first nucleotide sequence of a transfer ribonucleic acid ($tRNA^{Ala}$) in 1965 [1], almost 200 primary structures of other tRNAs have been determined [2,3] Among them tRNAs specific for phenylalanine and methionine are of most interest The former for their easy purification by benzoylated DEAE-cellulose column chromatography [4] and the latter in respect to their special functions in protein biosynthesis [5]. Both tRNAs were isolated from different sources [6] and some similarities appeared. At the same time many aminoacyl-tRNA synthetases from different organisms were purified and some generalisations concerning their structures have been formulated [7,8].

Among large number of organisms from which tRNAs and synthetases were characterized, higher plant material was little studied, one reason being difficulty in preparation of sufficient quantities of these macromolecules in the pure state [9,10] As far as tRNA is concerned, phenylalanine specific tRNA was sequenced from wheat germ [11], pea [12], yellow lupin [13] and barley [14] Methionine initiator tRNA structure was determined for wheat germ [15] and tentatively, yellow lupine seeds [16]

Aminoacyl-tRNA synthetases (AARS) charging cognate tRNA with phenylalanine (PheRS), methionine (MetRS), arginine (ArgRS) and leucine (LeuRS) from wheat germ [17–19] and yellow lupin seeds [20–22] were characterized.

The purpose of this work is to compare and analyse the similarities and differences in the structures of higher plant tRNAs and synthetases based on literature information and new results obtained in our laboratory. We will aim to show that within the limits presently available of experimental data, plant tRNAs and aminoacyl-tRNA synthetases structures are largely conserved

2 Materials and methods

Phenylalanine specific tRNA from yellow lupin seeds was purified and sequenced as in [13] Methionine-initiator tRNA of lupin was characterized and compared to wheat germ initiator tRNA as in [16] Methionine-tRNA ligase from lupin was isolated as in [20] Phenylalanyl- and arginyl-tRNA synthetases from lupin seeds have been characterized [21] The enzymes were $\geqslant 90\%$ pure as judged by polyacrylamide gel electrophoresis

Aminoacylation reaction was carried out in total vol 0 05 ml and contained HEPES buffer 5 μmol (pH 8), ATP 0.2 μmol, $MgCl_2$ 0 3 μmol, 2-mercaptoethanol 0 1 μmol, amino acid 1–10 nmol, KCl 3 5 μmol, tRNA 0 05–0 1 mg, bovine serum albumin 10 μg, enzyme 1–100 μg Incubation was continued at 37°C for 1–8 min For the determination of kinetic parameters the volume of the assay mixture was 0 15 ml. The concentrations of all components were the same as in analytical assay Samples of 30 μl were taken off after 0 5, 1, 2 and 4 min, spotted on Whatman 3 MM paper discs, washed according to [13] and counted

The specific activity unit (U) is defined as no

nmol L-amino acid charged to a specific tRNA mg protein per min at 37°C.

3. Results and discussion

In the course of our studies on tRNAs from yellow lupin seeds (*Lupinus luteus*) we have obtained several pure species, two of which were sequenced, $tRNA^{Phe}$ [13] and $tRNA_1^{Met}$ [16]. Recently we have developed a method for the purification of plant aminoacyl-tRNA synthetases: MetRS [20], ArgRS and PheRS [21]. This procedure includes ammonium sulphate fractionation, Sephadex G-150 gel filtration, DEAE-cellulose and DEAE–Sephadex A-50 column chromatography. In table 1 we have reviewed data on aminoacyl-tRNA synthetases isolated from wheat germ and lupin seeds. In general structural parameters of all synthetases from both plants are very similar if not identical. Small differences in molecular weight could be the result of experimental error. The only exception is MetRS from wheat germ isolated in [23] which has different properties. These authors described two enzymes of mol. wt 75 000 and 110 000 with the capacity to charge $tRNA^{Met}$. In the case of ArgRS there are results which suggest that both monomer and dimer are active. From table 1 it is seen that kinetic data are similar. It is not possible to decide now which differences are caused by different experimental conditions and which reflect real differences between particular enzymes. In contrast to the bacterial enzymes, the specific activity of plant synthetases is ~50–200 units.

If we change our interest to tRNA, the picture is more clear. Figure 1 presents the nucleotide sequence of phenylalanine specific tRNAs so far sequenced. The structure of $tRNA^{Phe}$ from wheat germ [11] pea [12] and barley [14] is identical. No differences were found in major nucleosides and also in modified nucleoside pattern. In the case of $tRNA^{Phe}$ from yellow lupin seeds [13] the major subspecies differs from the common sequence by the presence of A–U instead G–C as the first base pair of TUC steam. The minor one (~20%) is again identical with the common sequence. Both subspecies were not resolved by column chromatography [13]. Complete conserva-

Table 1
Comparison of structural and kinetic parameters of aminoacyl-tRNA synthetases isolated from wheat germ and yellow lupin seeds

Wheat germ				Lupin seeds		
	Mol. wt and subunit structure	Michaelis constant (M^{-1})		Mol. wt and subunit structure	Michaelis constants (M^{-1})	
MetRS	165 000	Met	1.1×10^{-5} for $tRNA_1^{Met}$	170 000	Met	1.5×10^{-5}
	α = 74 000		1.3×10^{-5} for $tRNA_m^{Met}$	α = 85 000	$tRNA_1^{Met}$	0.7×10^{-7} 7.6×10^{-7}
	α_2			α_2	ATP	0.2×10^{-4} 2.9×10^{-4}
PheRS	250 000	Phe $tRNA^{Phe}$	6.6×10^{-6} 2.7×10^{-6}	260 000	Phe	0.9×10^{-6}
	α = 80 000 β = 50 000			α = 75 000 β = 59 000	$tRNA^{Phe}$ ATP	4.7×10^{-7} 0.3×10^{-4}
	$\alpha_2\beta_2$			$\alpha_2\beta_2$		
ArgRS	70 000	Arg	2×10^{-6}	140 000	Arg	4.8×10^{-6}
	α = 70 000			α = 70 000	$tRNA^{Arg}$	0.7×10^{-7} 1.1×10^{-7}
	α			α_2	ATP	0.2×10^{-4} 1.4×10^{-4}

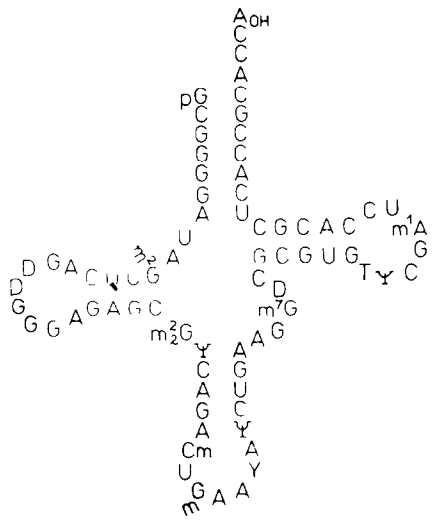

Fig 1 Primary structure of plant $tRNA^{Phe}$ from wheat germ [11], pea [12], barley [14] and lupin [13]

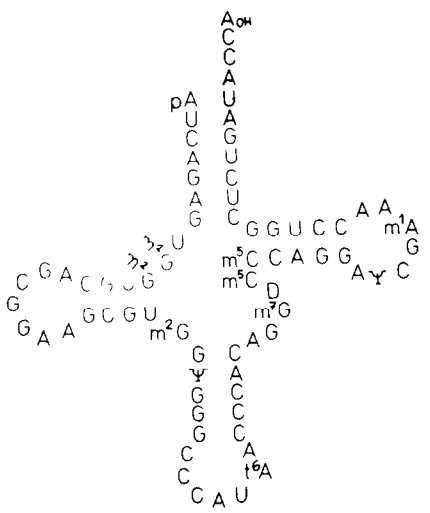

Fig 2 Primary structure of plant methionine initiator tRNA

tion of primary structure of $tRNA^{Phe}$ was observed in mammals, on the basis of comparison of 6 primary structures [24,25]

At present we have only small pieces of information on methionine-initiator tRNAs from plants The role of this tRNA species in the initiation of protein biosynthesis is well recognized, also in plants [26] Another $tRNA^{Met}$ is able to transport methionine into growing polypeptide chain Important similarities were found between $tRNA_i^{Met}$ so far sequenced, especially in mammals [27–31] The sequence of wheat germ $tRNA_i^{Met}$ was recently determined [15], fig.2 We have compared the fingerprints of yellow lupin $tRNA_i^{Met}$ and of the wheat germ initiator tRNA [16] This allows us tentatively to conclude that both sequences are identical

4. Conclusions

The above-presented information on structures and molecular properties of tRNAs and aminoacyl-tRNA synthetases concern several species of plants belonging to one family within each of the big subclasses, monocotyledons and dicotyledons (table 2). The structures of $tRNA^{Phe}$ are almost fully conserved between the two subclasses as concluded from 4 examples In the case of $tRNA_i^{Met}$ and aminoacyl-tRNA synthetases our data are not as good, but also give supoprt to the idea of structure conservation Some differences observed in the synthetases cannot be accounted for solely by experimental error Clearly, more data are needed on tRNAs and synthetases isolated from plants belonging to different systematic groups of Embryophyta One is nevertheless tempted to speculate that the higher

Table 2
Comparison of sources for isolation of tRNA and aminoacyl-tRNA synthetases from plants

Monocotyledons Graminae		Dicotyledons Papilionaceae	
AARS	tRNA	AARS	tRNA
Triticum sp		*Lupinus sp*	
MetRS	$tRNA^{Phe}$	MetRS	$tRNA^{Phe}$
ArgRS	$tRNA_i^{Met}$	ArgRS	$tRNA_i^{Met}$
PheRS		PheRS	
LeuRS		ValRS, TrpRS, SerRS [22]	
Hordeum sp		*Pisum sp*	
–	$tRNA^{Phe}$	–	$tRNA^{Phe}$

Only tRNA isoacceptors with known primary structure are included

conservation of tRNA structures as compared to synthetases might be the result of their multiple biological functions which in turn place greater constraints on their evolution.

Acknowledgements

This work was supported by the Polish Academy of Sciences within the project 09.3.1.

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