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CHLOROPLASTIC METHIONYL-tRNA SYNTHETASE FROM WHEAT

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SUMMARY

Wheat chloroplastic methionyl-tRNA synthetase was isolated and appeared to be a monomer with a molecular weight of 75,000 daltons. Its catalytical properties in the aminoacylation for various isoacceptors $tRNA_s^{Met}$ from E. coli and wheat germ revealed a recognition of prokaryotic tRNAs and wheat cytoplasmic $tRNA_1^{Met}$, but not $tRNA_m^{Met}$. Using pI determinations and catalytical properties, it could be detected in non-chloroplastic quiescent wheat germ a form of methionyl-tRNA synthetase having the same properties as the chloroplastic one's.

INTRODUCTION

In higher plants, aminoacyl-tRNA synthetases involved in organellar protein biosynthesis system are coded in the nuclear genome, translated on cytoplasmic ribosomes and imported into the chloroplasts (1-4).

Two enzymes have been already isolated from Soybean seedlings and Euglena chloroplasts (5-6), but only few results were known about those that methionylate prokaryotic and/or organellar $tRNA_s^{Met}$ (7).

As cooperative activity of the nucleocytoplasmic and organellar systems appears to be of paramount importance for chloroplast development and function, we have studied the properties of the Met RS (E.C. 6.1.1.10), interesting due to its participation in the process of initiation of protein synthesis. In addition, the fact that in plant, the initiation process requires a formylated-methionyl-tRNA ($tRNA_f^{Met}$) in the chloroplast (8), and, on the contrary, a non-formylated methionyl-tRNA ($tRNA_i^{Met}$) in the cytoplasmic compartment is of particular interest for the regulation of cell plant protein biosynthesis.

The wheat germ cytoplasmic Met RS has previously been isolated and the molecular weight of this dimeric enzyme was found to be about 165,000 ;

Abbreviation : Met RS : Methionyl-tRNA synthetase.

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it was able to aminoacylate cytoplasmic $tRNA_m^{Met}$ and $tRNA_i^{Met}$ (9). In 1977, Rosa and Sigler (10) reported the isolation and properties of two cytoplasmic Met RS from wheat germ, both having a monomeric structure but distinct capabilities to aminoacylate wheat germ $tRNA_m^{Met}$. The discrepancy between the results reported by these authors and those obtained by us and other workers from Lupine seeds (11) led us to undertake the characterization of chloroplastic Met RS, to determine its properties in the aminoacylation of various $tRNA^{Met}$ isoacceptors and its possible presence among the cytoplasmic forms observed for this enzyme.

MATERIALS AND METHODS

Isolation of chloroplasts. Ten to twelve days old wheat seedlings, kept in dark for 48 hours, were harvested and cut into small bits, and homogenized (50 g for 300 ml) in a 0.05 M Tris-HCl buffer, pH 8, 0.3 M mannitol, 0.003 M EDTA, 0.001 M of reduced glutathione and 0.1 % BSA, in a Waring Blendor for 5 seconds at slow speed, and then, 7 seconds at high speed. The homogenate was twice filtered through a Nylon screen having 50 µ and 20 µ pore diameters respectively, and rapidly centrifuged at 2000 g for 90 seconds. The pellet was washed with the same buffer and collected by another centrifugation (2 500 g for 2 min.) and then frozen at - 25°C.

Preparation of tRNA. Chloroplastic tRNAs, extracted by the phenol process were partially purified by DEAE cellulose and deacylated (Methionine acceptance:110 pmole/OD). Wheat germ $tRNA_m^{Met}$ (485 pmole/OD) and $tRNA_i^{Met}$ (1005 pmole/OD) were obtained by chromatographies on benzoylated DEAE cellulose (12) and DEAE Sephadex A_{50} (13). E. coli $tRNA_S$ were obtained from Sigma Corporation, U.S.A. ; Boehringer Manheim or Laboratoire des Substances Naturelles, France.

Enzyme purification. The method utilized was adapted from that previously described for the purification of others aminoacyl-tRNA synthetases from wheat germ (9, 14) except phosphate buffer which only 10 mM. The chloroplastic Met RS, eluted at low phosphate concentration (≃ 50 mM) on hydroxyapatite column, was 280 fold purified and its specific activity was 126 nmol [^{35}S] methionyl-tRNA formed.minute^{-1} (mg protein)$^{-1}$ at 25°C. No cytoplasmic contamination of the preparation was detected (See results).

tRNA aminoacylation. Methionyl-tRNA synthetase activity was measured at 25°C in 0.1 ml of the following optima reaction medium : Hepes 55 mM, pH 8.2, 1.2 mM reduced glutathione, 30 mM KCl, 44 µM BSA, 15 mM $MgCl_2$, 10 mM ATP, L [^{35}S] methionine (210-295 mCi/mmol) 50 µM and no limiting amounts of $tRNA_S^{Met}$. After incubation (3 min. for initial rate conditions) the reaction medium was transfered to a 2.3 cm diameter Whatmann 3 MM filter paper circle treated according to the method of Mans and Novelli (15).

Polyacrylamide gel electrophoresis and electrofocusing. Non-denaturing electrophoresis in 4-8 % acrylamide gels in Tris-glycine pH 8.4 (16) was used for molecular weight determinations.

Small quantities of electrophoretically pure protein, obtained after its diffusion from the gel, served for structure determination in SDS gel electrophoresis (17).

Polyacrylamide gel electrofocusing (pI determinations) was carried out according to Righetti and Drysdale (18) on polyacrylamide gel plates (pH range 3.5 - 9.5). After a pre-electrofocusing (1/2 hour, 1 500 volts, 30 Watts),

20 µl of enzymatic preparation was deposited on a 1 cm Whatman 3 MM strip for a run of 1 hour. The gel, cut in small pieces of 0.25 cm, was eluted (2 hours, 4°C) in 100 µl of 55 mM Hepes buffer, pH 8.2. Twenty to 40 µl aliquots were used to determine enzymatic activity in the aminoacylation medium.

RESULTS AND DISCUSSION

Molecular weight - quaternary structure - pI. Whatever the enzyme preparation (crude extract or purified enzyme), sephadex G200 gel filtration (1.5 x 86 cm column) according to Andrews (19) led to the determination of a molecular weight about 70,000 ± 6,000 for the Met RS. On the other hand, electrophoresis in variable cross-linked gels (16) gave a value of 75,000 ± 5,000. Preliminary determination of structure by SDS gel electrophoresis (17), after dissociating treatment, enabled us to propose that chloroplastic Met RS was probably a monomeric enzyme. It thus appeared that chloroplastic Met RS was different in structure and in molecular weight from the cytoplasmic enzyme (165,000, β_2) (9).

pI determination of the purified chloroplastic Met RS by electrofocusing gave a value of 5.5 ± 0.1 (fig. 1e) which was different from that found in the native Met RS from of E. coli (5.0 ± 0.1), its trypsinized form (4.8 ± 0.1) (fig. 1f), or cytoplasmic wheat germ enzyme (5.1 ± 0.1).

Methionylation of $tRNA_s^{Met}$

From the results established on the basis of percentage of aminoacylation obtained in an heterologous system (using prokaryotic tRNA), comparatively to the homologous chloroplastic system, it was concluded that E. coli tRNA was a good substrate for the chloroplastic aminoacyl-tRNA synthetases (4, 6, 7, 20, 21). The study of the kinetic parameters (table 1) confirmed that it was also true for wheat chloroplastic Met RS ($K_m = 1.2.10^{-6}$ M for E. coli $tRNA_f^{Met}$ and $K_m = 2.5.10^{-7}$ M for chloroplastic $tRNA^{Met}$).

It could be observed that apparent K_m value for tRNA in the chloroplastic system was lower than in the cytoplasmic system, as previously described by others (5,6). For the initiator cytoplasmic $tRNA^{Met}$, K_m value was similar to that found in the homologous system ; this tRNA was as good a substrate for the chloroplastic Met RS as E. coli tRNA. On the contrary, cytoplasmic $tRNA_m^{Met}$ was not aminoacylated, whatever ionic and/or substrates concentrations or pH test conditions. This fact suggested that chloroplastic wheat $tRNA_m^{Met}$ was probably different from the cytoplasmic one's and, as the initiation process required a $tRNA_f^{Met}$ in chloroplasts, it should be confirmed on the basis of aminoacylation capabilities of the pure enzymes that the isoacceptors $tRNA_s^{Met}$ were distinct in the two compartments.

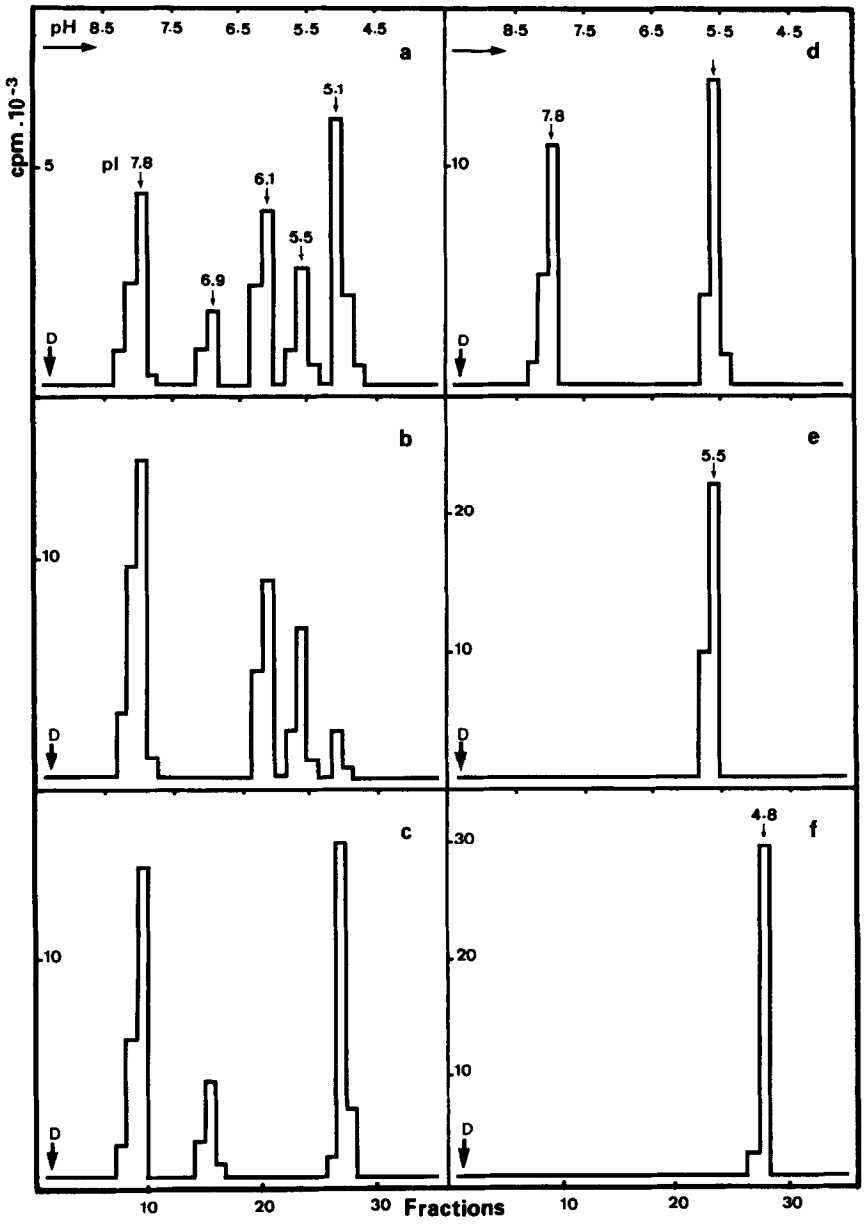

Figure 1 : Electrofocusing of Methionyl-tRNA synthetases on polyacrylamide gel plates.

- a, b, c, d : wheat germ crude extract enzymes.
  Aminoacylations were performed with : a) wheat germ $tRNA_s^{Met}$; b) wheat $tRNA_i^{Met}$; c) wheat $tRNA_m^{Met}$; d) E. coli $tRNA_f^{Met}$;
- e) chloroplastic Met RS.
- f) E. coli Met RS, trypsinized form.

D = Enzymatic deposits.

TABLE 1 - APPARENT K_m (μM) FOR $tRNAS_s^{Met}$

tRNA	Met RS	
	chloroplastic	cytoplasmic
chloroplastic	0.25	not charged
cytoplasmic		
Met_i	8.5	3.5
Met_m	not charged	2.6
E. coli (Met_f)	1.2	not charged

K_m values were obtained from Lineweaver-Burk plots.

As in the other eukaryotic systems cross aminoacylation was possible in the wheat cell between chloroplastic Met RS and cytoplasmic $tRNA_i^{Met}$, the specificity of recognition appeared only with the cytoplasmic $tRNA_m^{Met}$ that was not aminoacylated. A symmetrical result was observed with E. coli Met RS and wheat cytoplasmic $tRNA_s^{Met}$: only $tRNA_i^{Met}$ was recognized (22).

Selected physical and functional properties of some Met RS (table 2).

Chloroplastic Met RS from wheat appeared very different from all cytoplasmic Met RS previously described (9, 11, 23, 24, 25), but it resembled all proteolysed forms described in E. coli (25) wheat embryo (Quintard B., unpublished) sheep liver (26) and mammary gland (27).

Presence of prokaryotic Met RS identical to the chloroplastic one's in the subcellular fractions from wheat.

Several forms of Met RS were observed in the wheat cell :

- cytoplasmic high molecular weight pre-enzymatic complexes (28),
- cytoplasmic free enzyme (MW : 165,000, dimer) (9) and its proteolysed fragment (29),
- organellar enzymes : chloroplastic (75,000, α) and probably mitochondrial.

As plant aminoacyl-tRNA synthetases were encoded in nuclear DNA, synthesized in cytoplasm and segregated into different compartments following a post

TABLE 2 - PHYSICAL AND FUNCTIONAL PROPERTIES OF SOME Met RS

Organism	Localisation	Molecular weight	Q.S.	$tRNA^{Met}$ substrate	reference
B. stearothermophilus	cytoplasm	164,000	β_2	prokaryotic	23
Bakers'yeast	cytoplasm	110,000	α_2	eukaryotic	24
E. coli	cytoplasm	170,000	β_2	prokaryotic	25
E. coli	trypsinized enzyme	60,000	α	prokaryotic	25
Sheep liver	cytoplasm	78,000 (trypsinized enzyme)	α	eukaryotic	26
Sheep mammary gland	cytoplasm	78,000 (trypsinized enzyme)	α	eukaryotic	27
Lupine seeds	cytoplasm	165,000	β_2	eukaryotic	11
Quiescent wheat embryo	cytoplasm	165,000	β_2^-	m, i	9
		70,000 (proteolysed enzyme)	α	i	*
Wheat embryo	cytoplasm	105,000	β	m, i	10
		70,000	α	f, i	10
Wheat seedlings	chloroplast	75,000	α	prokaryotic chloroplastic cytoplasmic (i, not m)	

* Quintard, B. et al., unpublished

f : E. coli $tRNA_f^{Met}$; i : eukaryotic $tRNA_i^{Met}$; m : eukaryotic $tRNA_m^{Met}$.

translational event (30), we tried to determine if, in non-chloroplastic wheat germ, we could find a form of enzyme presenting the physical and fonctional properties of chloroplastic Met RS. In order to do that, we had used electrofocusing of enzymatic preparations and the ability to aminoacylate the different $tRNA_s^{Met}$.

If, in the crude chloroplast extract, we detected only one pI value (5.5) for Met RS (not shown), on the contrary the wheat germ crude extract electrofocusing let to the determination of several pI-distinct enzymatic forms. With wheat germ tRNA the pI observed for enzymatic activities (fig. 1a) were found to correspond respectively to :

- 7.8 : high molecular pre-enzymatic complexes and membrane-bound cytoplasmic Met RS which were able to aminoacylate all cytoplasmic $tRNA_s^{Met}$ (28,29).
- 6.9 : a variable partially proteolysed form of Met RS, which aminoacylate only $tRNA_m^{Met}$.
- 6.1 : a cytoplasmic proteolysed enzyme (70,000, α) always resulting from the proteolysis of the high molecular complexes and membrane-bound cytoplasmic Met RS (29).
- 5.5 : an enzymatic form of Met RS having the same properties of the chloroplastic enzyme.
- 5.1 : the cytoplasmic wheat germ Met RS (165,000, β_2) (9,29).

With the cytoplasmic $tRNA_i^{Met}$ only pI 7.8, 6.1, 5.5, 5.1 (fig. 1b) enzymatic activities were detected. In addition enzymatic form corresponding to pI 5.5 disappeared when aminoacylation was performed with cytoplasmic $tRNA_m^{Met}$ (fig. 1c), but it was detected again using E. coli $tRNA_f^{Met}$ (fig. 1d). The identical catalytic properties of the pI 5.5 wheat germ enzymatic form and chloroplastic Met RS (fig. 1e) enabled us to propose that a methionyl-tRNA synthetase could be detected in non-chloroplastic quiescent wheat germ, having the same properties as the chloroplastic one's. It was probably the same Met RS that the ligase B described by Rosa and Sigler (10).

The presence of an organellar enzyme in the wheat germ extract could result from the two following possibilities :

1) mitochondria and/or proplastids released during the extraction procedure their enzymatic content including Met RS ;
2) prokaryotic Met RS was normally present in the quiescent wheat germ cytoplasm before its importation into the organelles during plant cell development.

The possibility of an external microbial contamination of quiescent wheat germ leading to the appearance during extraction procedure of a proteolysed form of the prokaryotic enzyme was eliminated by the difference between the pI values of the chloroplastic Met RS and the trypsinized fragment of E. coli enzyme (fig. 1e and 1f).

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