METHIONINE-tRNA-LIGASE FROM WHEAT GERM: PURIFICATION AND PROPERTIES

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1. Introduction

Methionine-tRNA-ligase (AMP) EC 6.1.1.10, from various prokaryotic organisms has been extensively studied since it has been found to catalyse the aminoacylation of $tRNAs^{Met}$, thus contributing to the incorporation of methionine at both initiation and elongation steps of protein chains synthesis.

Similarly, two different eukaryotic $tRNAs^{Met}$, especially in plant cytoplasm are required for methionine incorporation at the initiation and elongation levels.

Wheat germ cytoplasmic methionine-tRNA-ligase was purified 150-fold. Mg^{2+} ions are required for conservation. Gel filtration through Sephadex G. 200 showed a mol. wt of 165 000. SDS-polyacrylamide gel electrophoresis indicates that the enzyme could probably be a dimeric protein (β_2 type). Effect of pH, monovalent and divalent cations were studied in the ATP-PP_i excange reaction and in the reaction leading to aminoacyl-tRNA formation. Particular emphasis was laid on the similarities and differences in the aminoacylation of both $tRNA_m^{Met}$ and $tRNA_i^{Met}$.

2. Materials and methods

$tRNA_m^{Met}$ and $tRNA_i^{Met}$ were extracted and purified according to a method following Gillam et al. [1] and Leis and Keller [2].

^{14}[C] Methionine and sodium ^{32}[P] pyrophosphate were obtained from the Commissariat à l'Energie Atomique (Saclay).

2.1. *Exchange reaction*

Assays for ligase activity by ATP–PP_i exchange were performed as previously described by Lemoine et al. [3], using 0.25 ml mixtures containing sodium cacodylate buffer (25 μmol) pH 7.5, $MgCl_2$ (1 μmol), ATP (Na) (0.5 μmol), ^{32}P-PP_i (0.5 mCi/mmol), L-Methionine (0.5 μmol) and enzyme preparations (20-50 μg). Incubation was carried out at 37°C for 15 min.

2.2. *Aminoacylation assay*

Attachment of L-Methionine to $tRNA^{Met}$ was determined following Mans and Novelli [4]. The reaction mixtures contained in a vol. of 0.1 ml, HEPES buffer 5.5 μmol, pH 8.2, KCl 3 μmol, GSH 0.12 μmol, $MgCl_2$ (1.5 or 1 μmol respectively in the presence of $tRNA_m^{Met}$ and $tRNA_i^{Met}$), ATP (Na) 1 μmol, L-^{14}[C] Methionine (2 to 8 nmol, 50 mCi/mmol; K_M for methionine were 1.1×10^{-5} M and 1.3×10^{-5} M, respectively in the presence of $tRNA_m^{Met}$ and $tRNA_i^{Met}$), tRNA 30 μg and limiting amounts of enzyme. Incubation was carried out at 37°C for 3 min.

Specific activity is defined as the number of nmoles of $tRNA^{Met}$ aminoacylated per mg protein and per min at 37°C.

2.3. *Other methods*

The protein concentration was determined by the method of Lowry et al. [5] of by 280/260 nm absorption quotient as described by Warburg et al. [6]. SDS-polyacrylamide gel electrophoresis was performed according to Weber and Osborn [7].

3. Results and discussion

3.1. *Purification of Met-tRNA-ligase*

All steps were carried out at 2–3°C. The following buffers were used throughout the purification proce-

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Table 1
Purification procedure of Methionine-tRNA-ligase from wheat germ

Purification step	Buffer	Proteins (mg)	Aminoacylation			
			Specific activity (nmoles-mg^{-1}. min^{-1})	Total activity (nmoles. min^{-1})	Recovery (%)	Relative purification
Crude extract after 20 000 rev/min centrifugation	A	1020	0.58	592	100	1
Ammonium sulfate fractionation (50–65%)	A	131	1.50	196	33	2.6
Sephadex G-75	B	39	2.5	96	16	4.2
DEAE-cellulose pH = 7.2-elution between 0.03–0.12 M NaCl	B	7.7	5.5	42	7	9.4
Hydroxylapatite pH = 7.2-elution between 0.1–0.15 M KH_2PO_4	B	0.4	87	35	6	150

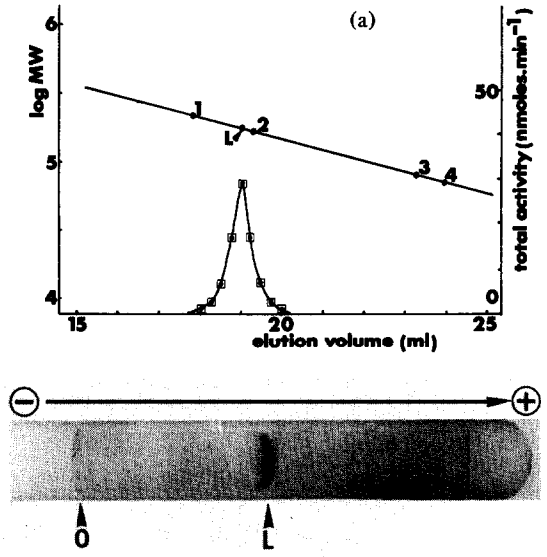

Fig.1(a) The molecular weight (MW) as determined by gel filtration (Sephadex G 200). (–●–●–) : log MW = f (elution volume) 1 : pyruvate kinase, 2 : alkaline phosphate, 3 : aldolase, 4 : BSA, L : Met-tRNA-ligase. (–□—■–) : total activity. (b) SDS-polyacrylamide gel electrophoresis of Met tRNA ligase. O : origin. L : Methionine-tRNA-ligase (See text).

dure: (A) : Tris–HCl 0.1 M pH 7.4, $MgCl_2$ 0.01 M, GSH 0.001 M, KCl 0.06 M, 10% glycerol. (B) : KH_2PO_4 0.01 M pH 7.2, $MgCl_2$ 0.01 M, β-mercaptoethanol 0.005 M, 10% glycerol.

10 g of wheat germ were ground in 50 ml of buffer (A) and centrifuged for 30 min at 20 000 rev/min. Table 1 summarizes the purification procedure. Substrate concentrations were saturating, leading to a maximum velocity. Specific activity of *E. Coli* enzyme [3] is about ten times that of wheat germ ligase. However, it must be considered that, unlike *E. Coli*, wheat germ is a quiescent organism, whereby probably no protein synthesis can occur, leading to a drastic failure of enzymatic activities. The relative purification (150-fold) seems to indicate that wheat germ is a rich source of Met-tRNA ligase [8]. Generally, low specific activities and also low relative purifications were found for plant seed aminoacyl-tRNA ligases, as showed by Jakubowski and Pawelkievicz [9]. As a comparison, Hahn and Brown [10] reported for *Sarcina Lutea* Met-tRNA ligase, a specific activity of 37.2 nmol of methionyl-tRNA formed per mg protein and per

minute and a 149-fold relative purification. Mg^{2+} ions, unlike *E. Coli* and other bacterial ligases, are required for conservation. In a buffer lacking Mg^{2+}, Met-tRNA-ligase loses 80% of its activity when stored for 3 days at −20°C.

3.2. *Characterization of purified enzyme preparations*

3.2.1. Molecular weight (Fig.1a)

The mol. wt of Met-tRNA-ligase was determined by gel filtration through Sephadex G-200 according to Andrews [11]. The elution vol. was compared to that obtained for BSA, alkaline phosphate, aldolase and pyruvate kinase. From this method, a mol. wt of 165 000 ± 10 000 was assigned to Met-tRNA-ligase. This is the same order of magnitude as for the *E. Coli* ligase (173 000 as determined by Lemoine et al. [3]).

3.2.2. Subunit structure

A single band (fig.1b) was observed, when the enzyme was submitted to SDS-polyacrylamide gel electrophoresis, which leads to the dissociation of protein into subunits [7]. Band mobility, compared to that of cytochrome *c*, chymotrypsin, ovalbumin, BSA, aldolase, catalase, in the same conditions, showed a mol. wt of 74 000 ± 5 000 instead of 165 000 ± 10 000, as determined by gel filtration. This result indicates that the enzyme might well be composed of two identical subunits; it has been reported by Koch and Bruton [12], that *E. Coli* enzyme is a dimeric protein.

3.3. *Ionic effects*

Ionic species strongly influence aminoacyl-tRNA-ligases activity. Optimal ionic conditions were detailed for the two reactions catalysed by wheat germ enzyme.

3.3.1. ATP–PP_i exchange reaction

Influence of pH. The effect of pH from 4 to 10, was studied using different buffers: Tris-HCl, sodium cacodylate, acetate and carbonate. The highest rate of ^{32}(P)-PPi incorporation was observed in the presence of sodium cacodylate, with an optimal pH in the range 7.2–7.9.

Influence of monovalent and divalent cations: K^+, NH_4^+, Mg^{2+}, Mn^{2+}: Monovalent cations (fig.2a). K^+ and NH_4^+ have almost identical inhibitory effects on the rate of ATP–PP_i exchange, for concentrations above 20–30 mM. Inhibition levels were constant

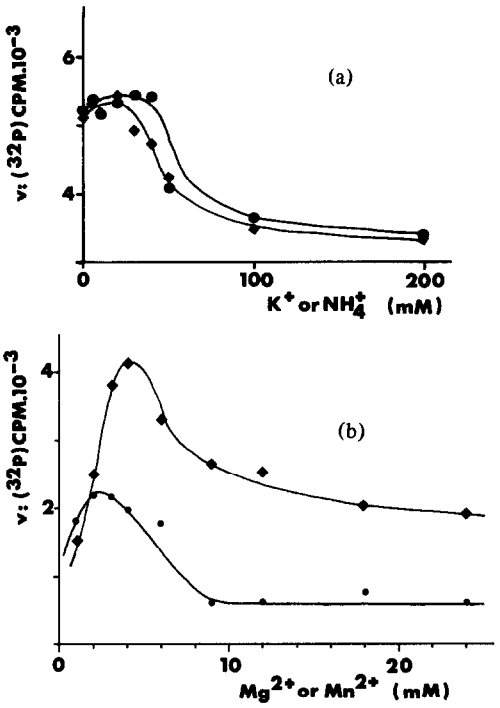

Fig.2(a) The effect of K^+ and NH_4^+ on ATP–PP_i exchange. (–◆——◆–) : K^+ ; (–●——●–) : NH_4^+. (b) The effect of Mg^{2+} and Mn^{2+} on ATP–PP_i exchange. (–◆——◆–) : Mg^{2+}; (–●——●–) : Mn^{2+}.

from 100 to 200 mM. Similarly, it has been shown that these ions inhibit the ATP–PPi exchange catalysed by *E. Coli* ligase [13].

Divalent cations (fig.2b). The optimum ration Mg^{2+}/ATP is 2 under standard assay conditions, whilst optimum Mn^{2+}/ATP is 1 in the same conditions. At optimal Mg^{2+} concentration the level of ^{32} [P] PP_i incorporated into ATP is about twice that obtained in the presence of optimal Mn^{2+} concentration.

Aminoacylation. Met-tRNA-ligase from wheat germ undergoes aminoacylation with isoacceptors $tRNA_i^{Met}$ and $tRNA_{in}^{Met}$. The former is the adaptor of methionine at the initiation stage of plant protein synthesis, and the latter at the elongation stage.

Effect of pH. Optimum pH for the two methionyl-tRNAs is in the range 8.1–8.4. This is consistent with Moustafa [8]. Three buffers have been tested : HEPES, Tris–HCl, sodium cacodylate. HEPES buffer leads to the highest rate of aminoacylation for both $tRNA_m^{Met}$ and $tRNA_i^{Met}$.

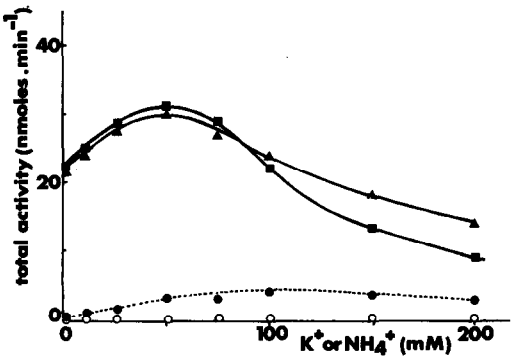

Fig.3. The effect of K^+ and NH_4^+ on aminoacylation in the presence of $tRNA_m^{Met}$ and 10 mM ATP. (-▲——▲-) : NH_4^+; (-■——■-) : K^+ for 15 mM Mg^{2+}; (-●——●-) : NH_4^+; (-○——○-) : K^+ for 0.5 mM Mg^{2+}. Similar curves are obtained for $tRNA_i^{Met}$

Effect of monovalent cations K^+ and NH_4^+ (fig.3). In the presence of Mg^{2+}(15 mM for $tRNA_m^{Met}$ and 10 mM for $tRNA_i^{Met}$) at pH 8.3, K^+ and NH_4^+ cause the rate of methionyl-$tRNA^{Met}$ formation to increase, then to decrease. At low Mg^{2+} concentration (0.5 mM), NH_4^+ alone stimulates the aminoacylation of $tRNA_i^{Met}$ and $tRNA_m^{Met}$, but to a lesser extent than in the presence of higher Mg^{2+} concentration. K^+ is unable to stimulate the aminoacylation in the absence of Mg^{2+} as seen in fig.3. These last two results are similar to those obtained by Lawrence et al. [13] on *E. Coli* enzyme.

Influence of divalent cations : Mg^{2+}, Mn^{2+} (fig.4). Optimum Mg^{2+}/ATP ratios were found to be 1.5 and 1, respectively in the presence of $tRNA_m^{Met}$ and $tRNA_i^{Met}$ (ratios unchanged for 5 and 10 mM ATP). Mn^{2+} stimulates the aminoacylation almost as well as Mg^{2+}. The optimum Mn^{2+}/ATP ratio was identical (1.5) for $tRNA_m^{Met}$ and $tRNA_i^{Met}$.

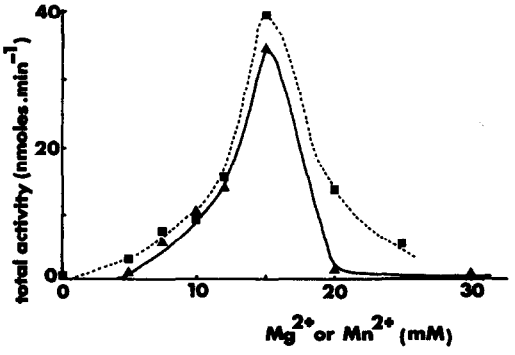

Fig.4. The effect of Mg^{2+} and Mn^{2+} on aminoacylation in the presence of $tRNA_m^{Met}$ and 10 mM ATP. (-■——■-) : Mg^{2+}; (-▲——▲-) : Mn^{2+}. Similar curves are observed for $tRNA_i^{Met}$; the only difference being in a shift of the optimum Mg^{2+}/ATP ratio (see text).

4. Conclusions

The last step of wheat germ Met-tRNA ligase purification leads to a ten-fold weaker specific activity than in the case of *E. Coli* enzyme. This might well be due to the physiological differences between bacterial and quiescent plant materials. ATP–PP_i exchange reactions showed similarities to exist between *E. Coli* and wheat germ enzymes, notably the inhibitory effect of K^+ and NH_4^+. Functional differences between $tRNA_i^{Met}$ and $tRNA_m^{Met}$ are evidenced by the fact that they allow the incorporation of methionine at various levels of protein synthesis. In addition, structural differences are suggested by their dissimilar chromatographic behaviors [2,14,15]. Moreover only $tRNA_i^{Met}$ can undergo aminoacylation with *E. Coli* enzyme [14,15]. Nevertheless these differences cannot be correlated with the closely similar aminoacylation conditions of $tRNA_i^{Met}$ and $tRNA_m^{Met}$.

Acknowledgements

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