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 tRNAsMet, thus contributing to the incorporation of methionine at both initiation and elongation steps of protein chains synthesis.

Similarly, two different eukaryotic tRNAsMet, 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. Mg2+ 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 (β2 type). Effect of pH, monovalent and divalent cations were studied in the ATP-PPi exchange 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 Met and tRNA 1 Met.

2. Materials and methods

2.1. Exchange reaction

Assays for ligase activity by ATP-PPi 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, MgCl2 (1 μmol), ATP (Na) (0.5 μmol), 32P-PPi (0.5 mCi/mm), 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, MgCl2 (1.5 or 1 μmol respectively in the presence of tRNA Met and tRNA 1 Met), ATP (Na) 1 μmol, L-[14 C]Methionine (2 to 8 nmol, 50 mCi/mm; K M for methionine were 1.1 X 10-5 M and 1.3 X 10-5 M, respectively in the presence of tRNA Met and tRNA 1 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 nmols 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-
Table 1
Purification procedure of Methionine-tRNA-ligase from wheat germ

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Buffer</th>
<th>Proteins (mg)</th>
<th>Specific activity (nmoles·mg⁻¹·min⁻¹)</th>
<th>Total activity (nmoles·min⁻¹)</th>
<th>Recovery (%)</th>
<th>Relative purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract after 20000 rev/min centrifugation</td>
<td>A 1020</td>
<td>0.58</td>
<td>592</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ammonium sulfate fractionation (50–65%)</td>
<td>A 131</td>
<td>1.50</td>
<td>196</td>
<td>33</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Sephadex G-75</td>
<td>B 39</td>
<td>2.5</td>
<td>96</td>
<td>16</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose pH = 7.2-elution between 0.03–0.12 M NaCl</td>
<td>B 7.7</td>
<td>5.5</td>
<td>42</td>
<td>7</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Hydroxylapatite pH = 7.2-elution between 0.1–0.15 M KH₂PO₄</td>
<td>B 0.4</td>
<td>87</td>
<td>35</td>
<td>6</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

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, MgCl₂ 0.01 M, GSH 0.001 M, KCl 0.06 M, 10% glycerol. (B): KH₂PO₄ 0.01 M pH 7.2, MgCl₂ 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 20000 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 Sacrina 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 $-20^\circ$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 $^{32}(P)$-PP$_i$ 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 from 100 to 200 mM. Similarly, it has been shown that these ions inhibit the ATP–PP$_i$ exchange catalysed by *E. Coli* ligase [13].

**Divalent cations (fig.2b).** The optimum ratio 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$_{\text{Met}}$ and tRNA$_{\text{Met}}^\text{m}$. 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 Mostafa [8]. Three buffers have been tested: HEPES, Tris–HCl, sodium cacodylate. HEPES buffer leads to the highest rate of aminoacylation for both tRNA$_{\text{Met}}$ and tRNA$_{\text{Met}}^\text{m}$. 

![Graph](image-url)
Effect of monovalent cations $K^+$ and $NH_4^+$ (fig. 3).
In the presence of $Mg^{2+}$ (15 mM for tRNA$_{Em}$ and 10 mM for tRNA$_{Met}$) 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 aminoaacylation of tRNA$_{Em}$ and tRNA$_{Met}$, but to a lesser extent than in the presence of higher $Mg^{2+}$ concentration. $K^+$ is unable to stimulate the aminoaacylation 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$_{Em}$ and tRNA$_{Met}$ (ratios unchanged for 5 and 10 mM ATP). $Mn^{2+}$ stimulates the aminoaacylation almost as well as $Mg^{2+}$. The optimum $Mn^{2+}$/ATP ratio was identical (1.5) for tRNA$_{Em}$ and tRNA$_{Met}$.

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$_1$ 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$_{Em}$ and tRNA$_{Met}$ 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$_{Em}$ can undergo aminoaacylation with E. Coli enzyme [14,15]. Nevertheless these differences cannot be correlated with the closely similar aminoaacylation conditions of tRNA$_{Em}$ and tRNA$_{Met}$.

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References
