PURIFICATION AND PROPERTIES OF METHIONYL-tRNA-SYNTHETASE FROM YELLOW LUPINE SEEDS

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1. Introduction

In many eucaryotic organisms studied so far there are two methionine-specific tRNAs: initiator ($tRNA_i^{Met}$) and non-initiator ($tRNA_m^{Met}$). The primary structures of several $tRNA_s^{Met}$ have been elucidated and the existence of their great similarities established. Both $tRNA_s^{Met}$ are recognized by the same methionyl-tRNA synthetase (MetRS, EC 6.1.1.10) [1–3]. Purification and properties of MetRS from wheat germ have been published and differences have appeared [4–6].

In our studies on plant tRNA and aminoacyl-tRNA synthetases we have isolated $tRNA_s^{Met}$ and MetRS from yellow lupine seeds. In work on methionine-tRNA ligase we have found some interesting observations concerning purification and properties of this enzyme. The procedure for isolation consists of the ammonium sulphate fractionation, gel filtration on Sephadex G-150, DEAE-cellulose, DEAE–Sephadex A-50, Sephadex G-200 filtration and phosphocellulose column chromatography. We have obtained a 545-fold purification of the enzyme with the recovery of over 1.6% activity applied in the first step. Polyacrylamide gel electrophoresis and gel filtration method showed mol. wt 170 000. The yellow lupine MetRS has two subunits and the results look similar to those obtained for wheat germ [4,5] but different from [6].

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2. Experimental

Initiator tRNA of yellow lupine seeds was purified by chromatography of crude tRNA on benzoylated DEAE-cellulose, DEAE–Sephadex A-50 and Sepharose 4B [7]. L-[^{14}C]Methionine, (243 mCi/mmol) and L-[^{3}H]methionine, (169 mCi/mmol) were products of Radiochemical Centre, Amersham. Sephadex G-150, G-200 and DEAE-Sephadex A-50 were purchased from Pharmacia Fine Chemicals. DEAE-cellulose (DE-23) was a product of Whatman. Phosphocellulose was obtained from Serva Company.

The aminoacylation assay mixture in total vol. 0.05 ml contained: Hepes buffer 5 μmol, pH 8, ATP(Na_2) 0.2 μmol, $MgCl_2$ 0.3 μmol, 2-mercapto-ethanol 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 carried out at 37°C for 8 min.

The exchange reaction $^{32}PP_i$–ATP was carried out similar to [8]. The specific activity is defined as the number of nmol L-methionine charged to $tRNA_i^{Met}$/mg protein/min at 37°C. The protein concentration was determined by the Lowry and Ehresman methods [9,10]. Polyacrylamide gel electrophoresis was carried out as in [11].

3. Results and discussion

Preparation and purification of MetRS was performed in the cold room at 2–3°C. Milled yellow

Table 1
Purification of methionyl-tRNA ligase from yellow lupine seeds

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U)	Recovery of activity (%)	Relative purification (-fold)
1. Crude extract	105 500	9917	0.094	100	1
2. Ammonium sulphate 45–65%	18 900	3458	0.183	34.8	1.9
3. Sephadex G-150	1665	2297	1.38	23.2	14.6
4. DEAE-cellulose	122.9	1613	13.2	16.2	140.3
5. DEAE–Sephadex A-50					
Peak A	21.7	494.7	22.8	5.0	242.5
Peak B	4.7	222.3	47.3	2.2	503
6. Sephadex G-200					
Peak A	4.2	189	45.1	1.9	479
Peak B	3.1	159	51.3	1.6	545
7. Phosphocellulose					
Peak A	3.8	175.5	46.2	1.8	481

lupine seeds (1000 g) were extracted for 15 min with 4 l buffer A (Tris–HCl 0.1 M, pH 8.0, $MgCl_2$ 0.01 M, KCl 0.05 M, 2-mercaptoethanol 0.02 M and glycerol 10% v/v). The enzyme was precipitated with ammonium sulphate (45–65%), centrifuged and dissolved in 100 ml buffer B (Tris–HCl 0.05 M, pH 7.3, $MgCl_2$ 0.01 M, KCl 0.01 M, 2-mercaptoethanol 0.01 M and glycerol 10% v/v). The solution was dialysed against the same buffer for 2–3 h and than applied to the Sephadex G-150 column. The table 1 summarizes all purification steps. As can be seen from table 1 and fig.1, DEAE–Sephadex A-50 column chromatography shows two peaks A and B of MetRS activity. The selected fractions of the enzyme (fraction number 70, 95 and 110 in fig.1) were judged by polyacrylamide gel electrophoresis (fig.2). It is clearly seen (fig.2) that these fractions differ in molecular weight. The gel A on fig.2 represents peak A on fig.1 and shows two main bands, but gel B which represents peak B shows only one band identical with upper band on the gel A. The gel C represents fraction between the peaks A and B. Further purification of peak A from the DEAE–Sephadex A-50 column on Sephadex G-200 and phosphocellulose columns gave homogenous enzyme as checked by SDS gel electrophoresis (fig.3A). In fact MetRS activity was not retarded on the phosphocellulose column (data not shown). The peak B from fig.1 showed the homogenity just after the Sephadex G-200 column (fig.3A). From the

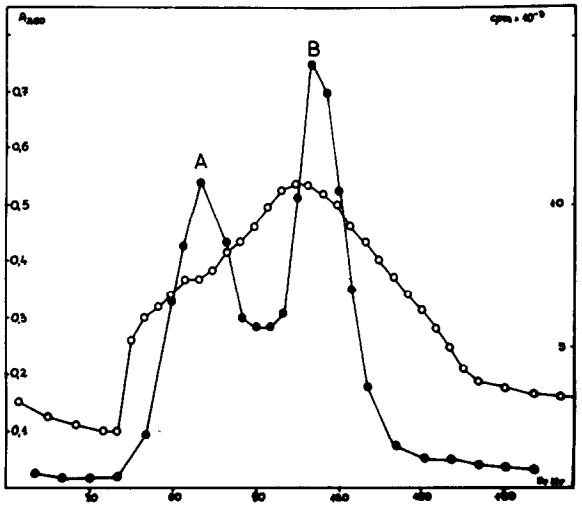

Fig.1. The DEAE-Sephadex A-50 column chromatography (2.3 × 40 cm) of methionyl-tRNA synthetase from yellow lupine seeds. The column was equilibrated with buffer containing 50 mM Tris–HCl, pH 7.0, 10 mM $MgCl_2$, 10 mM KCl, 10 mM 2-mercaptoethanol and 20% glycerol, and eluted with linear gradient of 10–120 mM potassium chloride in the above buffer. The fractions of 4 ml were collected at a flow rate 25 ml/h. A_{280} (–○–○–○–); MetRS activity (–●–●–●–).

Fig.2. Polyacrylamide gel electrophoresis of MetRS fractions: 70 (gel A), 95 (gel C) and 100 (gel B) from the fig.1.

Sephadex G-200 gel filtration method (fig.3B) mol. wt 170 000 was estimated for enzyme in peak B (on fig.1), which is supposed to be an intact active molecule of MetRS. SDS gel electrophoresis showed only one band with mol. wt 85 000 and suggests the presence of two identical subunits in MetRS (β_2 type). At the same time the enzyme in peak A (fig.1) showed a mol. wt ~ 85 000 after gel electrophoresis in native and denaturating conditions. One can conclude that this form of enzyme represents the active subunit of MetRS. Both forms of the enzyme showed a high

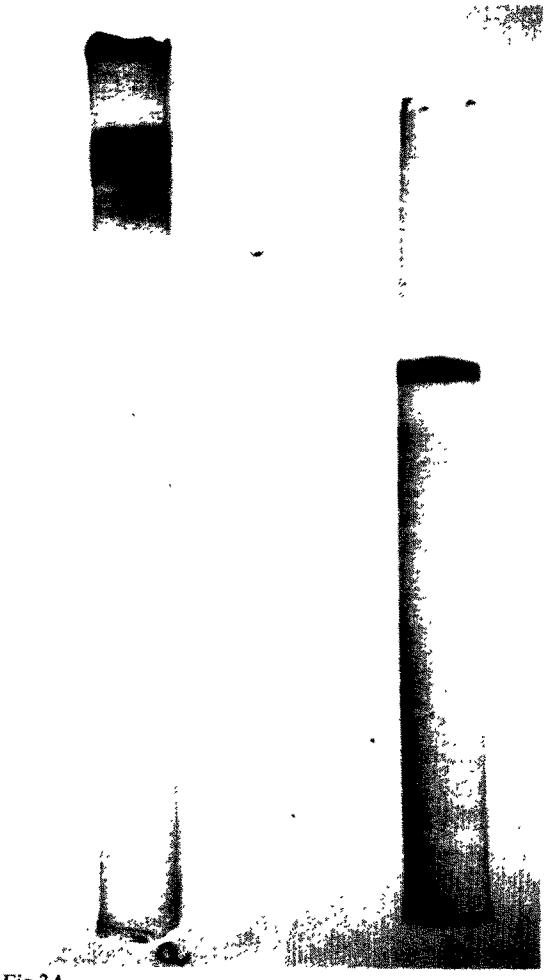

Fig.3A

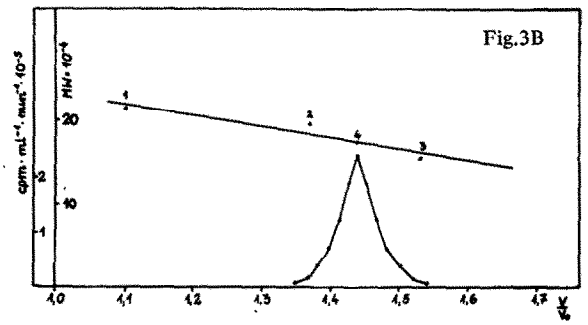

Fig.3A. Polyacrylamide gel electrophoresis of yellow lupine MetRS. 60 μg enzyme without SDS (a) and 20 μg enzyme in the presence of SDS (b). Fig.3B. Determination of molecular weight on the Sephadex G-200 column for MetRS [4]. The calibration curve was plotted for the standard proteins: 1, catalase (mol. wt 240 000); 2, bovine serum albumin, trimer (mol. wt 195 000); 3, aldolase (mol. wt 147 000).

activity of 50 units. The lower values of the activity were obtained when hydroxylapatite column chromatography in phosphate buffer was applied [12,13]. The total yield of methionyl-tRNA ligase was 7 mg from 1 kg lupine seeds. It is noteworthy that after concentration of pure enzyme, high molecular weight complexes of the MetRS are formed. We have found the specific activity for fractions with mol. wt 500 000, 340 000, 250 000, 180 000 and 80 000 during filtration of the concentrated MetRS on the Sephadex G-200 column (data not shown). The yellow lupine MetRS was characterized in aminoacylation and $^{32}PP_i$–ATP exchange reactions. The Michaelis constants for ATP, methionine and $tRNA_i^{Met}$ measured in charging reaction were 0.9×10^{-4}, 1.5×10^{-5} and 2.5×10^{-7}, respectively. The optimum Mg/ATP ratio was found to be 1.5 for $tRNA_i^{Met}$. Optimum pH has been determined to be 8. The pyrophosphate exchange reaction catalysed by MetRS is dependent only on methionine but not on tRNA. Optimum pH for Hepes and Tris buffers were 7.0–7.5 and 7.6–8.0, respectively (data not shown). Another MetRS from plant, wheat germ ligase, has a mol. wt 165 000 with two subunits (β_2 type) [4,5] and it is very similar to the *E. coli* enzyme (with mol. wt 173 000) and other MetRS [14,15]. According to [6] wheat germ contains two MetRS ligases called A and B with mol. wt 105 000 and 70 000, respectively.

4. Conclusions

The data presented in this paper have shown that lupine MetRS with mol. wt 170 000 is very similar to other known methionyl-tRNA synthetases [15]. It is composed of two active subunits with mol. wt 85 000. The activity of the enzyme is high (50 units) and it is stable in 20% glycerol at –20°C during at least 3 months.

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