COMPLEXES OF THE TRYPTOPHANYL tRNA SYNTHETASE WITH ADENOSINETRIPHOSPHATE AND TRYPTOPHANYLADENYLATE

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1. Introduction

Leucyl tRNA synthetase from *E. coli* [1] and threonyl tRNA synthetase from rat liver [2] were shown to form stable complexes with ATP in the absence of the two other substrates, i.e. the amino acid and tRNA. On the contrary, similar experiments with lysyl tRNA synthetase from *E. coli* [3] and with seryl tRNA synthetase from yeast [4] yielded negative results. In all these investigations the same method of gel filtration was used.

Here we describe the isolation and properties of the complexes of the tryptophanyl tRNA synthetase from beef pancreas with ATP and tryptophanyladenylate.

2. Materials and methods

Highly purified tryptophanyl tRNA synthetase was isolated from beef pancreas by the modified Davie-Lipmann method [5]. The procedure of purification and the characteristics of the enzyme will be published elsewhere. ^{3}H-ATP was obtained by the method of Osterman et al. [6]. tRNA aminoacylation experiments were carried out according to Muench and Berg [7], tryptophanylhydroxamate formation was followed by the method elaborated earlier in this laboratory [8]. The incubation mixture before gel filtration contained in a total volume of 0.8 ml: 50 μmoles of tris-HCl buffer, pH 7.5; 50 nmoles of L-tryptophan; 30 nmoles of ATP Na-salt; 2.5 μmoles of $MgCl_2$ and the enzyme preparation. Specific radioactivity of the ^{14}C-tryptophan was 37 mCi/mmole, that of the ^{14}C-ATP was 102 mCi/mmole. The mixture was incubated for 10 min at 37°, chilled and applied to the column of Sephadex G-50 fine (0.8 × 50 cm) equilibrated with 0.05 M tris-HCl buffer, pH 7.5. Separation was carried out at room temperature, 1 ml fractions were collected. 100 μl aliquots from the fractions were counted in the dioxane scintillator. In transfer experiments, 100 or 200 μl aliquots were taken from the Sephadex fractions and 1 mg of the total yeast tRNA or 0.3 mmoles of hydroxylamine were added. The mixtures were incubated at 37° for 1 and 5 min, respectively.

For analysis of ATP and tryptophanyladenylate bound to the enzyme, an aliquot from the Sephadex fraction was heated at 100° for 1 min and the precipitated protein removed. To reveal the elution positions of ATP, ADP and AMP during chromatography, carriers were added (2 absorbancy units at 260 nm of ATP containing ADP as contaminant and 2.5 units of AMP). The solution was applied to the column of DEAE-Sephadex A-25 coarse (0.5 × 20 cm) equilibrated with 0.1 M triethylammonium bicarbonate buffer, pH 7.5. The column was washed with the same buffer (both tryptophan and tryptophanyladenylate were eluted) and then the linear gradient of ionic strength was applied (0.1 and 0.7 M of the same buffer, 40 to 40 ml). Peak fractions were combined and dried. The residues were dissolved in water and the radioactivity counted.

3. Results

The tryptophanyl tRNA synthetase was incubated

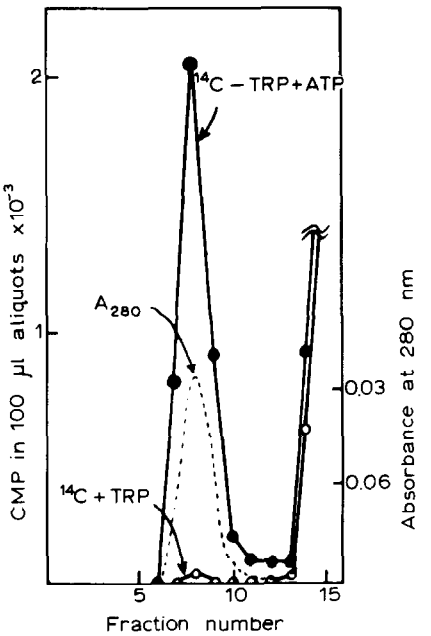

Fig. 1. Isolation of the complex of tryptophanyl tRNA synthetase with tryptophanyladenylate by gel filtration. ^{14}C-Tryptophan was used as a labelled substrate. Incubation mixture contained 0.11 mg of the enzyme preparation. Complete incubation mixture •—•, mixture without ATP ○—○, absorbance at 280 nm - - - - -.

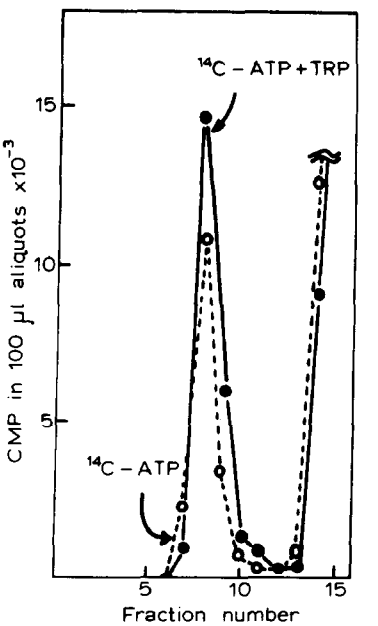

Fig. 2. Isolation of the complexes of tryptophanyl tRNA synthetase with ATP and tryptophanyladenylate. ^{14}C-ATP was used as a labelled substrate. Incubation mixture contained 0.19 mg of the enzyme preparation. Complete incubation mixture •—•, mixture without tryptophan ○ - - - ○.

with ^{14}C-tryptophan, ATP and Mg^{2+} and then passed through the Sephadex column. The radioactivity was found in the protein peak (fig. 1, full circles). In the absence of ATP the radioactivity in this peak was negligible: fractions 7–10 contained only 1% of the radioactivity found for the mixture containing ATP (fig. 1, open circles).

The tryptophan moiety from the protein fractions was found to be transferred to tRNA or NH_2OH forming Trp-tRNA and Trp-NHOH, respectively (table 1).

Maximum transfer to the tRNA was observed already after 1 min incubation. Table 1 shows that Mg^{2+} does not influence the percentage of the tryptophan transfer to the tRNA.

When the tryptophanyl tRNA synthetase was incubated with ^{14}C-ATP in the presence of unlabelled tryptophan, the protein peak from Sephadex, as would be expected contained radioactivity (fig. 2, solid line).

Table 1
Transfer of the tryptophan moeity from the tryptophanyladenylate-enzyme complex to tRNA and NH_2OH.

Complex added, cpm	Transferred to tRNA		Transferred to NH_2OH	
	cpm	%	cpm	%
2910	1240	42.6	1620	55.6
2910	1010*	38.1	–	–
1455	610	41.8	780	53.5

* In the presence of $MgCl_2$ (10^{-2} M).

Table 2
Dependence of the efficiency of the ATP-enzyme complex formation on the molar ratio ATP:enzyme.

Amount of protein (mg)	Amount of ^{3}H-ATP sp. act. 2 mCi/mmole (mg)	Molar ratio ATP:enzyme*	Radioactivity of the ATP-enzyme complex (cpm per mg of protein)
0.64	0.36	105	1630
0.64	0.18	52.5	1730
1.60	0.12	14	1770
0.64	0.03	8.7	1600

* Assuming the 100% purity of the enzyme preparation and mol. weight of the enzyme to be 110,000 [9].

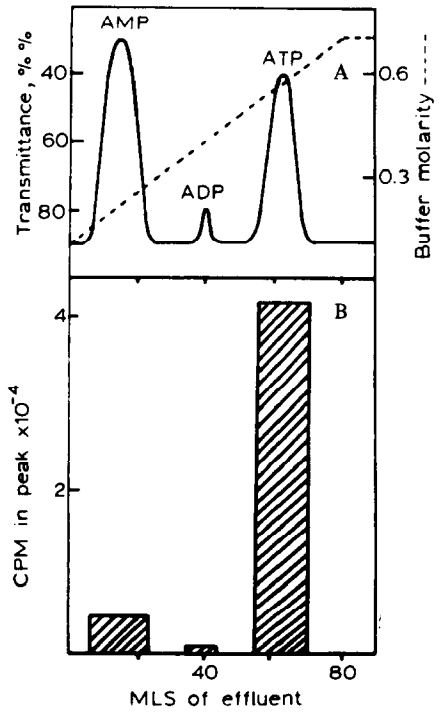

Fig. 3. Analysis of the ATP-enzyme complex on the DEAE-Sephadex column. 0.9 ml from fraction 8 (mixture without tryptophan, fig. 2, dotted line) were treated as described in Methods.
A: Distribution of the absorbance of carriers. —— transmittance at 254 nm, - - - ionic strength of the eluent.
B: Radioactivity of the combined peak fractions.

Calculations show that the protein peak contains 8.3 pmoles of ATP per 1 μg of protein. In similar experiments with ^{14}C-tryptophan under completely identical conditions the content of the amino acid in the same fractions was equal to 7.7 pmoles per 1 μg. Therefore, molar ratio tryptophan:ATP in the complex is close to 1. This fact as well as reactivity of the tryptophan residue (transfer to tRNA and hydroxylamine) prove that the substance isolated by gel filtration is the tryptophanyladenylate-enzyme complex.

If the tryptophanyl tRNA synthetase was incubated with ^{14}C-ATP and Mg^{2+} in the absence of tryptophan, the protein peak eluted from Sephadex contained 70% of the radioactivity found with the tryptophanyladenylate-enzyme complex (fig. 2,dotted line). Table 2 shows that the reduction of the molar ratio ATP: enzyme from 100 to 10 does not decrease considerably the radioactivity of the complex.

Analysis of the substance bound by the tryptophanyl tRNA synthetase in the absence of tryptophan has shown that most of the radioactivity ($\geqslant$ 90%) coincides with the ATP carrier (fig. 3). The small amount of AMP in the sample is probably due to the partial decomposition of the ATP during treatment before chromatography.

After incubation of the isolated ATP-enzyme complex with tryptophan, most of the radioactivity was found in the peaks corresponding to tryptophanyladenylate and AMP; only 25% remained in the ATP peak (fig. 4B). Comparion of this chromatographic pattern with that of the product formed by the enzyme in the presence of both substrates (fig. 4C) allows the conclusion that the tryptophanyladenylate-enzyme complex and the complex formed during the incubation of the ATP-enzyme with tryptophan are

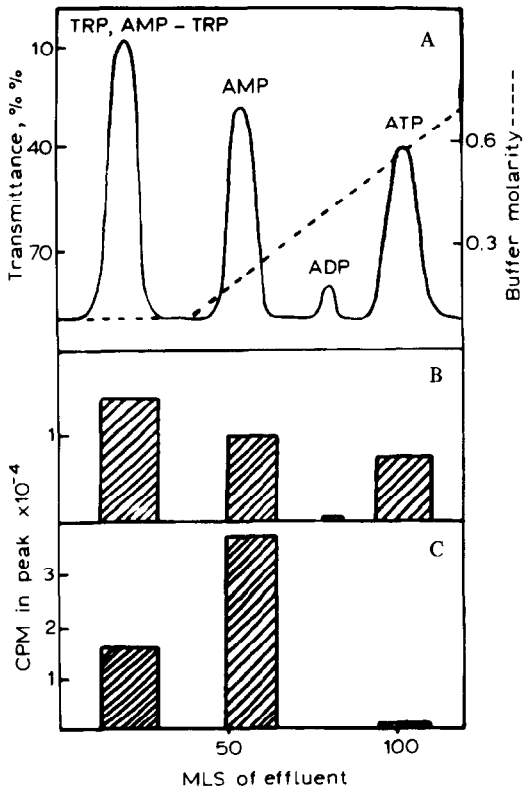

Fig. 4. Analysis of the complexes tryptophanyladenylate-enzyme (C) and ATP-enzyme incubated with tryptophan (B).
A: Distribution of the absorbance of carriers.
B: Radioactivity. 0.8 ml from the same fraction as in fig. 3 were mixed with 0.5 ml of L-tryptophan solution (5×10^{-2} M), incubated 30 min at room temperature and treated as indicated in the text.
C: Radioactivity. 0.9 ml from fraction 8 (complete incubation mixture, fig. 2, solid line) were treated as indicated in Methods. Tryptophan was added as a carrier before chromatography.

virtually identical. The relatively large AMP content is evidently due to the decomposition of the tryptophanyladenylate at neutral pH (cf. [10]) and during heating.

4. Discussion

Isolation of the stable tryptophanyladenylate-enzyme complex after incubation of the beef pancreas tryptophanyl tRNA synthetase with ATP, Mg^{2+} and tryptophan may serve in parallel with results obtained by other authors (cf. [11]) as a prooof of ability of the aminoacyl tRNA synthetases to form the aminoacyladenylate-enzyme complexes in the absence of tRNA.

ATP bound by the tryptophanyl tRNA synthetase participates in the formation of tryptophanyladenylate on the subsequent addition of the amino acid. This indicates that ATP is bound by the enzyme as a true substrate, i.e. at the active site. A high affinity of the enzyme for ATP is suggested because maximum binding of ATP is observed even at an ATP to enzyme molar ratio of approximately 10.

Fig. 1 shows that the complex between the enzyme and tryptophan cannot be isolated by the technique of gel filtration. However, Labouesse and coworkers [12] have shown that tryptophan protects this enzyme from thermal inactivation. Moreover, the preincubation of the tryptophanyl tRNA synthetase with tryptophan results in the appearance of a new enzyme form with a greater sedimentation constant [13]. These facts do suggest the existence of the tryptophan-enzyme complex which is probably able to dissociate after removal of the free amino acid in the course of gel filtration.

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