ESCHERICHIA COLI TYROSYL-tRNA SYNTHETASE. INFLUENCE OF MAGNESIUM IONS ON THE ENZYMATIC ACTIVITY*

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1. Introduction

It is generally considered that magnesium ions are always required in enzymatic reactions which involve ATP. This is probably to help the binding of ATP to the enzyme and compensate for the negative charges in the transition state.

The aminoacyl-tRNA synthetases catalyze a two-step reaction:

(I) $E + ATP + AA \rightleftharpoons AA\text{-}AMP : E + PPi$
(activation reaction)

(II) $AA\text{-}AMP : E + tRNA \rightleftharpoons AA\text{-}tRNA + AMP + E$
(transacylation rection)

The requirement for Mg^{2+} in each of these two reactions has been studied in several laboratories, and depending on the enzyme considered, different results were obtained. Using rat liver threonyl-tRNA synthetase, Allende et al. [1] have shown that Mg^{2+} is indispensable for the formation of the threonyl-AMP: enzyme complex and for the transacylation of threonine onto tRNA. According to Grosjean et al. [2] the same requirement exists at least for the transacylation reaction using isoleucyl-tRNA synthetase from *Bacillus stearothermophilus*. On the other hand, Norris and Berg [3] have reported that in *E. coli* the transfer of isoleucine from the enzyme-substrate complex to tRNA does not require Mg^{2+}; Lagerkvist et al. [4] have found similar results with yeast valyl-tRNA synthetase. Takeda and Igarashi [5] have shown that at least leucine, isoleucine, phenylalanine and lysine can be charged with *E. coli* aminoacyl-tRNA synthetases onto the corresponding tRNAs in the absence of Mg^{2+}, but only if the charging mixture contains an organic polyamine such as spermine or spermidine.

The results presented in this paper indicate that *E. coli* tyrosyl-tRNA synthetase is able to catalyze the formation of tyrosyl adenylate in the absence of Mg^{2+}. Surprisingly, however, pyrophosphorolysis of the enzyme-bound tyrosyl adenylate does not occur in these conditions. The data reported also show that for the transacylation reaction with EDTA-dialyzed tRNA, spermidine is required.

2. Materials

Tyrosyl-tRNA synthetase, free of other aminoacyl-tRNA synthetases, was purified from *E. coli* B using the method of Calendar and Berg [6] with minor modifications [7].

E. coli B tRNA was purchased from General Biochemicals; ATP and AMP were from Pabst Laboratories; spermidine phosphate was purchased from Mann Research Laboratories; ^{14}C-tyrosine (121 mCi/mmole) was obtained from "Le Commissariat à l'Energie Atomique" and ^{3}H-ATP (7 Ci/mmole) from Schwarz Bioresearch. ^{3}H-Tyrosinyl-AMP (15 Ci/mmoles) was kindly prepared by Dr. J. Nunez from cold tyrosinyl-AMP, a gift from Drs. J.P. Waller and R. Boissonnas.

3. Methods

3.1. *Formation of tyrosyl-AMP:enzyme and tyrosinyl-AMP:enzyme complexes*

For the formation of the tyrosyl-AMP:enzyme complex, the incubation mixture contained in a total

* Part 2 of a series.

volume of 1 ml, 100 μmoles of Tris-HCl 7.4, 10 μmoles of $MgCl_2$, 10 μmoles of 2-mercaptoethanol, 4 μmoles of ATP, 50 μmoles of tyrosine and 0.2 mg of enzyme (about 60% pure) previously dialyzed for 2 hr against three changes of 500 volumes of 0.05 M Tris-HCl pH 7.4 containing 0.01 M 2-mercaptoethanol. After incubating for 10 min at 20°, the mixture was rapidly chilled, poured onto a Sephadex G-50 column (26 × 1 cm) and eluted. Previous equilibration of the column, and elution of the products were carried out with the same buffer as that used for dialysis [8].

Fractions of 0.6 ml were collected; aliquots were taken from each fraction for radioactivity determinations.

For the formation and isolation of the tyrosinyl-AMP:enzyme complex, the conditions were the same as above, except that tyrosinyl-AMP replaced tyrosine and that ATP was absent from the incubation mixture.

3.2. *Formation of tyrosyl-AMP:enzyme and tyrosinyl-AMP:enzyme complexes in the absence of Mg^{2+}*

The conditions for the formation and for the isolation of the complexes were the same as described above except that the enzyme was previously dialyzed for 2 hr against three changes of 0.05 M Tris-HCl pH 7.4 containing 0.01 M 2-mercaptoethanol and 0.01 M of EDTA pH 7.0. EDTA was present at this same concentration in the incubation mixture and in the elution buffer.

3.3. *Paper electrophoresis*

Paper electrophoresis was performed at room temperature on Arches paper no. 302 in 0.5 M formic acid for 90 min (20 V/cm). For the determination of the radioactivity, the paper was cut in 1.5 cm sections and counted.

3.4. *Transfer of tyrosine from the tyrosyl-AMP:enzyme complex to tRNA*

In a total volume of 0.25 ml, the incubation mixture contained: 25 μmoles of Tris-HCl pH 7.4, 2.5 μmoles of 2-mercaptoethanol, 2.5 μmoles of $MgCl_2$, about 1500 counts/min of complex and 0.2 mg of tRNA. The mixture was incubated for 10 min at 20°. The reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid (TCA) containing 2 mg/ml of ^{12}C-tyrosine. The TCA insoluble material was retained on Millipore filters and thoroughly washed with TCA solution. The radioactivity was determined after dissolving the filters in Bray's liquid scintillation mixture.

For experiments carried out in the presence of EDTA, the tRNA was previously dialyzed for 2 hr against three times 500 volumes of 0.01 M EDTA; where indicated, this tRNA was preincubated for 10 min at 20° with 0.01 M spermidine phosphate pH 6.9.

4. Results and discussion

4.1. *Activation reaction*

The study of the influence of Mg^{2+} on the activation reaction was undertaken in our laboratory when it was observed that the binding of tyrosinyl-AMP to tyrosyl-tRNA synthetase occurred with EDTA-dialyzed enzyme and in the presence of EDTA. Fig. 1A shows such an experiment in which nearly the same amount of ^{3}H-tyrosinyl-AMP (90%) was observed bound to the enzyme in the absence of Mg^{2+} as compared to that recovered in the presence of Mg^{2+}.

In fig. 1B, it can be seen that, starting with ^{14}C-tyrosine and ATP, virtually the same amount of ^{14}C-tyrosine was retained by the enzyme eluted from a Sephadex G-50 column, whether the incubation was carried out in the presence or in the absence of Mg^{2+}.

In order to determine if, in the absence of Mg^{2+}, the nucleophilic attack of the β,γ-anhydride bond of ATP by the carboxylic group of tyrosine still occurred, the complex, prepared with tyrosine and ^{3}H-ATP, was analyzed by paper electrophoresis. The results show that the radioactivity retained by the enzyme corresponds to tyrosyl adenylate (fig. 2).

The analytical methods used here were too slow to allow the detection of differences in the kinetics of the tyrosyl-AMP:enzyme complex formation under various conditions. Only physico-chemical methods will be appropriate for such studies.

As with several other aminoacyl-tRNA synthetases [3, 9, 10], tyrosyl-tRNA synthetase from *E. coli* B binds ATP alone, in the absence of tyrosine [11]. Rouget and Chapeville [12] have shown that *E. coli* B leucyl-tRNA synthetase requires Mg^{2+} for this reaction. Using EDTA-dialyzed tyrosyl-tRNA synthetase, we found that in the conditions allowing the

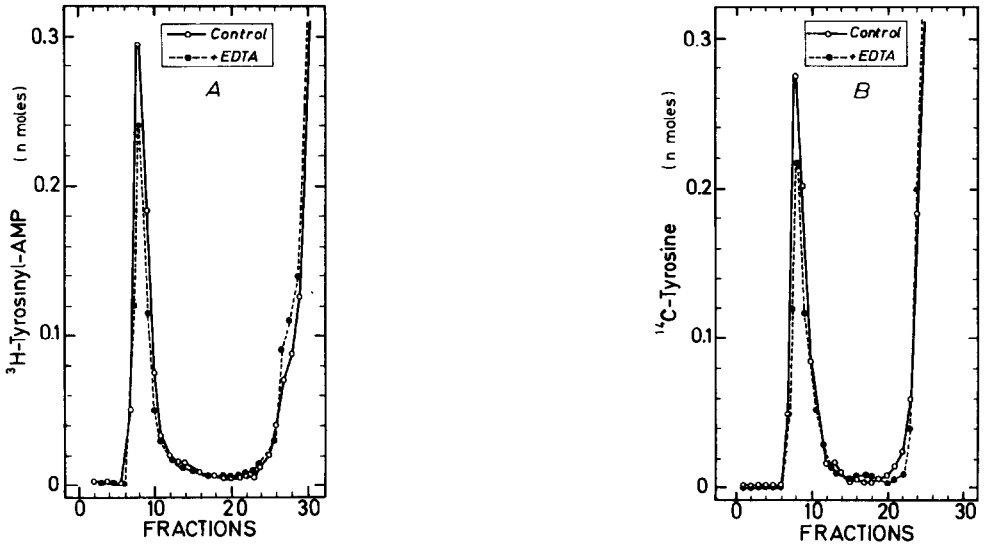

Fig. 1. Formation of enzyme-substrate complexes in the absence of magnesium. The enzyme was incubated in (A) with ^{3}H-tyrosinyl-AMP or in (B) with ^{14}C-tyrosine and ATP. Conditions are given in sect. 3. —○— ^{3}H activity (A) or ^{14}C activity (B); incubation in the presence of Mg^{2+}. - - ● - - ^{3}H activity (A) or ^{14}C activity (B); incubation in the absence of Mg^{2+}. For the sake of clarity, the absorbance at 280 mμ is not represented but in all cases it was checked that the peaks of radioactivity coincide with those of the absorbance.

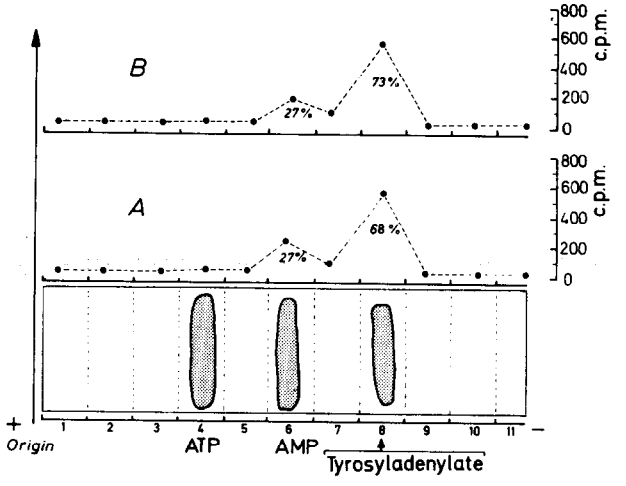

Fig. 2. Paper electrophoresis of enzyme-substrate complex prepared in the absence of magnesium. The complex was prepared as described in sect. 3 by incubating the enzyme with tyrosine and ^{3}H-ATP and analyzed by paper electrophoresis. (A) complex prepared in the presence of Mg^{2+}; (B) complex prepared in the absence of Mg^{2+}. The small amount of radioactivity in the ^{3}H-AMP spot corresponds to the spontaneous hydrolysis of tyrosyl adenylate.

formation of tyrosyl-AMP:enzyme complex in the absence of Mg^{2+}, enzyme:ATP complex is formed. Consequently, tyrosyl-tRNA synthetase behaves differently from leucyl-tRNA synthetase.

Since it is well known that in the presence of Mg^{2+}, the activation reaction is highly reversible as measured by the rate of ATP–PPi exchange, we studied this reaction in the absence of Mg^{2+}. Surprisingly, it was found that the rate of the exchange reaction was at least 4×10^{-5} times slower than in the presence of Mg^{2+}. This reaction was examined under various conditions and will be described in a later paper [11].

When Mg^{2+}-deprived tyrosyl-AMP:enzyme complex was incubated with PPi, only free AMP and tyrosine were released from the enzyme showing that the anhydride bond was not phosphorolyzed but hydrolyzed [11]. In the presence of Mg^{2+}, free ATP and tyrosine are obtained.

4.2. *Transacylation reaction*

The study of the influence of Mg^{2+} on the transacylation reaction is difficult because Mg^{2+} plays an important role in maintaining the secondary structure of tRNA. The results reported in the literature indicate that depending on the enzyme specificity or on the origin of the enzyme, Mg^{2+} is or is not required. For example, in the case of *E. coli* B isoleucyl-tRNA synthetase, Mg^{2+} seems not to be required, whereas in the case of the same enzyme from *B. stearothermophilus* Mg^{2+} is necessary.

In order to study the requirement for Mg^{2+} in the transacylation of tyrosine from the complex, the tRNA was dialyzed in such a manner as to contain no more that 0.5 atom of Mg^{2+} per tRNA molecule [11] and the transacylation reaction was performed in the presence of EDTA.

Table 1 shows that the transacylation reaction proceeds favorably in the absence of Mg^{2+}, provided the incubation medium contains spermidine. There is but little transfer if at all, in the absence of both Mg^{2+} and spermidine. These results suggest that, in physiological conditions, the role of divalent cations is only to maintain the secondary structure of tRNA. Several investigators reached the same conclusion for other aminoacyl-tRNA synthetases even though they did not examine the transacylation reaction alone but followed the charging of the amino acid on the corresponding tRNA in the presence of the amino acid,

Table 1
Transacylation reaction in the absence of magnesium.

Conditions	^{14}C-Tyrosine transferred (%)
(A) Tyrosyl-AMP:E + tRNA	50
(B) Tyrosyl-AMP:E + (EDTA dialyzed) tRNA	5
Tyrosyl-AMP:E + (EDTA dialyzed) tRNA + RNase	1
Tyrosyl-AMP:E + (EDTA dialyzed) tRNA + spermidine	55

Conditions are as indicated in sect. 3. In (A), the tyrosyl-AMP:enzyme complex was prepared in the presence of Mg^{2+} and incubated with untreated tRNA. In (B), the complex was prepared in the absence of Mg^{2+} and incubated with EDTA-dialyzed tRNA. Where indicated, 2 μg of bovine pancreatic ribonuclease or 0.1 μg spermidine were added.

ATP and catalytical amounts of the enzyme [5]. Spermidine seems to act only on the tRNA, since when this polyamine replaced Mg^{2+}, it was noticed [11] that it did not modify the activation reaction in any detectable manner.

The formation of tyrosyl-AMP:enzyme complex carried out with EDTA-dialyzed tyrosyl-tRNA and enzyme, and with AMP, was also examined [11]. As expected, this reaction occurred only in the presence of spermidine.

In conclusion, the results reported in this paper show that tyrosyl-tRNA synthetase is able to catalyze the formation of tyrosyl adenylate from tyrosine and ATP in the absence of Mg^{2+}. Tyrosyl-AMP remains bound to the enzyme and, in the absence of Mg^{2+} but in the presence of spermidine, interacts with $tRNA^{Tyr}$ to form tyrosyl-tRNA. The only cases in which aminoacyl adenylate formation was studied in the absence of Mg^{2+} are those of rat liver threonyl-tRNA synthetase and of *E. coli* B leucyl-tRNA synthetase: these enzymes were unable to catalyze the activation reaction. The results reported here demonstrate that tyrosyl-tRNA synthetase behaves differently at least from these two enzymes. Concerning the ATP-PPi exchange reaction, which requires the presence of Mg^{2+}, in all cases examined to-date, we are as yet unable to pinpoint at what stage of the reaction MG^{2+} becomes essential.

It should be emphasized, however, that although in the absence of Mg^{2+}, tyrosyl adenylate can be formed, it does not mean that in physiological conditions these ions are involved in this reaction. Their role could well be to enhance the rate of the reaction.

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