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STUDIES ON METHIONYL-tRNA SYNTHETASE

II. EFFECTS OF DIVALENT AND MONOVALENT CATIONS ON METHIONYL-tRNA SYNTHETASE FROM *ESCHERICHIA COLI*

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SUMMARY

1. The influence of divalent and monovalent cations on the activity of methionyl-tRNA synthetase (L-methionine:tRNA ligase (AMP), EC 6.1.1.10) from *Escherichia coli* has been studied, and a comparison has been made with the corresponding enzyme from yeast.

2. Of the monovalent cations only NH_4^+ stimulates the rate of methionyl-tRNA formation. This effect is found with both homologous and heterologous tRNA. The magnitude of the stimulation varies with pH.

3. The results explain a phenomenon previously interpreted in terms of a "regenerating" enzyme in amino acid activation.

INTRODUCTION

In the preceding paper[1], the effects of different divalent and monovalent cations on yeast methionyl-tRNA synthetase (L-methionine:tRNA ligase (AMP), EC 6.1.1.10) were examined in the methionine-dependent PP_i–ATP exchange reaction and in the total reaction leading to synthesis of methionyl-tRNA. In this paper a detailed comparison is made between the yeast enzyme and methionyl-tRNA synthetase isolated from *Escherichia coli*.

MATERIALS AND METHODS

Strains and growth conditions

The *E. coli* and yeast strains used are described in the preceding paper[1]. *Aerobacter aerogenes* and *Pseudomonas fluorescens* were grown and harvested as described for *E. coli*[3], except that the temperature was kept at 30° and that the medium for *P. fluorescens* was supplemented with 0.8% Nutrient Broth (Difco).

Enzyme preparation

Methionyl-tRNA synthetase from *E. coli* K12, strain 30S0, was prepared, with some modifications, as described by BOMAN, BOMAN AND MAAS[4] for the arginyl-tRNA synthetase. 20 g of frozen cells were melted in 12 ml of 0.02 M $MgCl_2$ in 0.02 M Tris–HCl, pH 7.3, and disintegrated by pressing[5]. The resulting material was incubated at 37° for 15 min with about 0.1 mg of deoxyribonuclease. After centrifugation for 1 h at 100 000 × *g*, the supernatant fluid was dialysed overnight against 0.01 M Tris–HCl, pH 7.3. A volume containing 10 mg of protein of the dialysed solution was chromatographed on a column containing 100 ml of DEAE-cellulose, equilibrated with 0.04 M Tris–HCl, pH 7.4. The chromatogram was developed by stepwise elution with 0.23 M, 0.38 M and 1.0 M Tris–HCl, pH 7.4. The methionyl-tRNA synthetase activity was located in the region eluted by the 0.38 M buffer. The active fractions were pooled and concentrated by negative pressure dialysis against 0.01 M Tris–HCl, pH 7.6. The solution was stored with 50% ethylene glycol at −18°. It contained 25.6 mg of protein per ml and about 0.2 mg of nucleic acid per ml. This preparation is also a good source of arginyl-tRNA synthetase.

RNA preparations

The procedure for preparation of tRNA from *E. coli* and yeast has been described in the preceding paper[1]. tRNA from *A. aerogenes* and *P. fluorescens* was prepared as described for *E. coli*. tRNA from wheat germ was prepared as described by GLITZ AND DEKKER[6], except that the chromatography step using DEAE-cellulose was omitted.

TABLE I

EFFECT OF NH_4^+ AND K^+ ON METHIONYL-tRNA FORMATION WITH *E. coli* ENZYME AND tRNA FROM DIFFERENT ORGANISMS IN THE PRESENCE OF 5 mM Mg^{2+}

NH_4^+ and K^+ were added to a final concentration of 100 mM with the exceptions indicated in parentheses. The control values were obtained without added monovalent cations. In the case of *A. aerogenes* and wheat germ tRNA, the two controls are not identical, since they were obtained in different experiments with different amounts of enzyme present. The reaction mixtures with *P. fluorescens* tRNA were incubated at 30°, all others at 37°. For some of the reaction mixtures methionine labelled in the carboxyl group was used, thus eliminating the risk of supermethylation in a heterologous system.

Source of tRNA	*Reaction time (min)*	*Methionyl-tRNA formed (counts/min)*			
		Control	*Added NH_4^+*	*Control*	*Added K^+*
E. coli B	5	700	1300	700	700
S. cerevisiae	2	175	500 (80 mM)	175	100 (80 mM)
A. aerogenes	4	200	500	530	470 (75 mM)
P. fluorescens	10	90	210	90	40
Wheat germ	6	50	200	70	70 (75 mM)

Assays of enzyme activity

The methods used and the counting procedure are described in the preceding paper[1]. All incubations were performed at 37° except where otherwise stated. Methyl-labelled methionine was used except in some experiments reported in Table I.

Chemicals

L-[*Me*-^{14}C]methionine (17.7 and 29.5 mC/mmole) and [^{32}P]orthophosphate were obtained from the Radiochemical Centre, Amersham, England. DL-[1-^{14}C]-methionine (3.4 mC/mmole) was obtained from Volk Radiochemical Co., U.S.A.

A description of other chemicals is given in the preceding paper[1].

RESULTS

Influence of divalent metal ions on the formation of methionyl-tRNA

The effect of Mg^{2+} and Mn^{2+} on the rate of formation of methionyl-tRNA is shown in Figs. 1 and 2. In the homologous reaction, that is with *E. coli* tRNA as substrate (Fig. 1), the Mg^{2+} concentration for optimal rate is about 8 mM, while

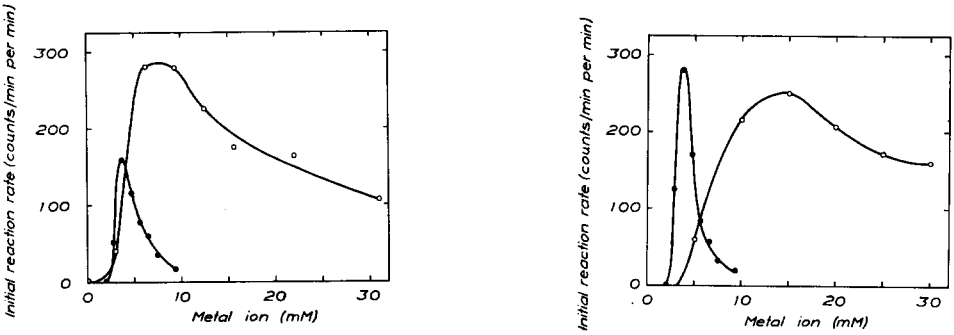

Fig. 1. Divalent metal ion dependence for the rate of formation of *E. coli* methionyl-tRNA. ○—○, Mg^{2+}; ●—●, Mn^{2+}. The reaction mixture contained 0.02 mg per ml of protein and 1.4 mg per ml of tRNA (*E. coli* B).

Fig. 2. Divalent metal ion dependence for the rate of formation of yeast methionyl-tRNA. ○—○, Mg^{2+}; ●—●, Mn^{2+}. The reaction mixture contained 0.07 mg per ml of protein and 1.2 mg per ml of yeast tRNA.

the corresponding Mn^{2+} concentration is 4 mM. When yeast tRNA is used as substrate (Fig. 2), the optimal rate concentration is shifted to 15 mM for Mg^{2+} but remains essentially the same for Mn^{2+}. It should be noted that at a concentration which is optimal for Mg^{2+} there is in both cases only a negligible activation with Mn^{2+}.

Influence of monovalent cations on the formation of methionyl-tRNA

With the yeast methionyl-tRNA synthetase it was found that the highest stimulatory effect by monovalent cations was achieved at suboptimal concentrations of divalent metal ions[1]. The study on the *E. coli* enzyme has therefore mostly been restricted to low concentrations of Mg^{2+} and Mn^{2+}, respectively. Fig. 3 shows the effect of added NH_4^+ and K^+ on the rate of formation of *E. coli* methionyl-tRNA. In the presence of 5 mM Mg^{2+} or 3 mM Mn^{2+} there is an appreciable stimulation by NH_4^+ in agreement with the results obtained with the yeast enzyme. On the other hand, the stimulatory effect exerted by K^+ on the yeast enzyme is not found with the *E. coli* enzyme.

In similar experiments it has been found that none of the ions Li^+, Na^+, Cs^+

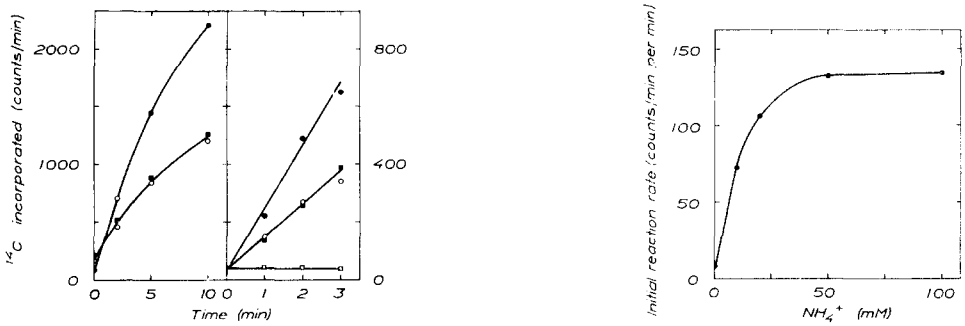

Fig. 3. Formation of *E. coli* methionyl-tRNA as a function of time in the presence of 5 mM Mg^{2+} (left part) and 3 mM Mn^{2+} (right part). ○—○, no monovalent cations added; ●—●, NH_4^+ added (100 mM in the left part, 50 mM in the right part); ■—■, K^+ added (100 mM in the left part, 25 mM in the right part). Blank experiments without any added tRNA were performed with Mn^{2+} both in the presence and absence of NH_4^+ and K^+. All these values are the same (□—□). The reaction mixture contained 0.02 mg per ml of protein and 1.4 mg per ml of tRNA (*E. coli* B). No corrections for background radiation were applied.

Fig. 4. The rate of formation of *E. coli* methionyl-tRNA as a function of ammonium ion concentration. The reaction mixture contained 3.5 mM Mg^{2+}, 0.02 mg per ml of protein and 1.7 mg per ml of tRNA (*E. coli* B).

or Rb^+, added to a final concentration of 50 mM in the presence of 4 mM Mg^{2+}, stimulates the rate of incorporation of methionine into tRNA.

Fig. 4 shows the magnitude of stimulation of the reaction rate with 3.5 mM Mg^{2+} present as a function of NH_4^+ concentration. The maximum stimulation is obtained with NH_4^+ in the concentration range between 50 and 100 mM. In this respect the *E. coli* enzyme behaves quite similarly to the yeast enzyme.

The stimulatory effect obtained with NH_4^+ is not restricted to the reaction

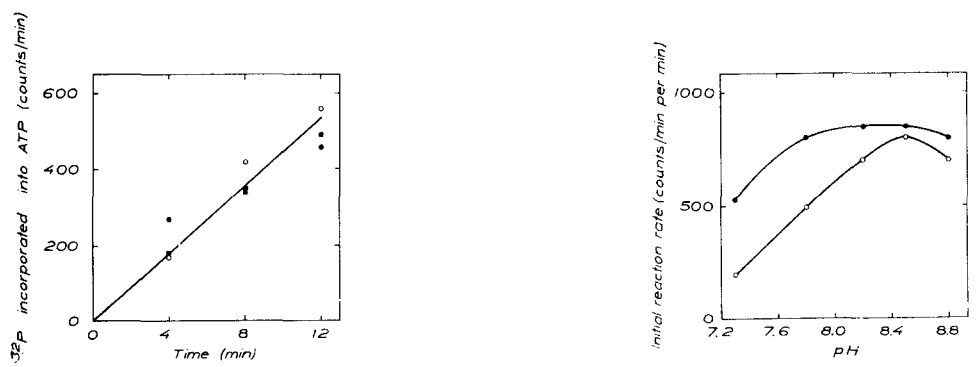

Fig. 5. Time curves for the methionine-dependent PP_i–ATP exchange in the presence of 0.5 mM Mg^{2+}. ●—●, 100 mM NH_4^+ added; ■—■, 50 mM K^+ added; ○—○, no monovalent cations added. The reaction mixture contained 0.05 mg per ml of protein. The values are corrected for a blank value amounting to about 600 counts/min.

Fig. 6. Rate of *E. coli* methionyl-tRNA formation in the absence (○—○) and in the presence of 100 mM NH_4^+ (●—●) as a function of pH. The reaction mixture contained 5 mM Mg^{2+}, 0.05 mg per ml of protein and 3.2 mg per ml of tRNA (*E. coli* B). The incubation temperature was 30°.

with homologous tRNA. Table I shows that the NH_4^+-dependent increase in rate occurs also with tRNA from yeast, Aerobacter, Pseudomonas and wheat germ. The table also shows that with none of these tRNA samples is there any stimulation of the reaction rate by added K^+; on the contrary, there is in some cases an inhibition.

Influence of monovalent cations on the methionine-dependent PP_i–ATP exchange

In experiments with the yeast methionyl-tRNA synthetase it was demonstrated that the monovalent cations, in concentrations up to 100 mM, do not alter the rate of methionyl-AMP formation, measured as a methionine-dependent PP_i–ATP exchange. Examination of the *E. coli* enzyme has revealed an identical mode of action. The rate of PP_i–ATP exchange in the presence of 2.5 mM or 0.5 mM Mg^{2+} is completely unaffected by the addition of 100 mM NH_4^+ or 50 mM K^+ (Fig. 5).

Effect of pH on the ammonium stimulation

The previous experiments were all performed at pH 7.3. Since the pH optimum is much higher, it was of interest to study the influence of pH on the NH_4^+-dependent increase in reaction rate. Fig. 6 shows the rate of formation of *E. coli* methionyl-tRNA as a function of pH in the absence and presence of 100 mM NH_4^+. It can be seen that over a pH interval ranging from 7.3 to about 8.2 the reaction rate with 5 mM Mg^{2+} is much enhanced by added NH_4^+. Around the pH optimum, however, the influence of NH_4^+ is rather small.

Other experiments have shown that when the Mg^{2+} concentration is raised as high as 10 mM there is practically no stimulation of the reaction rate by 100 mM NH_4^+ at any pH between 7.3 and 8.8.

DISCUSSION

Some characteristics of yeast methionyl-tRNA synthetase activation by divalent and monovalent cations were presented in the preceding paper[1]. These results together with the data on the *E. coli* methionyl-tRNA synthetase contained in this paper allow a comparison between the two enzymes.

The Mg^{2+} concentration for optimal rate of homologous methionyl-tRNA formation at pH 7.3 is for both enzymes higher (6–8 mM) than the corresponding Mn^{2+} concentration (4 mM). The Mn^{2+} dependence curves are rather narrow compared with the Mg^{2+} dependence curves. In a heterologous system, both enzymes need a much higher Mg^{2+} concentration for optimal reaction rate, 15 mM for the *E. coli* enzyme with yeast tRNA and 30 mM for the yeast enzyme with *E. coli* tRNA. However, only the yeast enzyme needs a higher Mn^{2+} concentration (7 mM) in the heterologous reaction. The *E. coli* enzyme has almost the same Mn^{2+} dependence curve whether *E. coli* or yeast tRNA is used.

The rate of methionyl-tRNA formation with the yeast enzyme was found to increase in the presence of NH_4^+, K^+ or Rb^+. This stimulation is especially pronounced at low concentrations of divalent metal ions. The reaction rate with the *E. coli* enzyme is stimulated in the same way by NH_4^+, but both K^+ and Rb^+ fail completely to give this effect. The results seem to be substantially the same whatever the kind of tRNA used in the reaction. The effect of K^+ is thus different for the two

enzymes in reactions with the same tRNA, indicating that the actual target of the monovalent cations is probably the enzyme and not the tRNA.

In the PP_i–ATP exchange reaction neither enzyme is stimulated by the presence of monovalent cations. For both the *E. coli* and yeast methionyl-tRNA synthetase, therefore, the stimulation by NH_4^+ concerns the second reaction only (Eqn. 2 in Ref. 1).

The magnitude of the stimulatory effect by NH_4^+ on the *E. coli* enzyme, exemplified in Fig. 4, fully explains the synergistic effect between a yeast methionyl-tRNA synthetase preparation and an *E. coli* arginyl-tRNA synthetase preparation reported earlier[2] and interpreted as a "regenerating" enzyme present in the *E. coli* enzyme preparation. The explanation is that the *E. coli* enzyme preparation also contained methionyl-tRNA synthetase, which was not detected as the reaction mixture did not contain more than 4 mM Mg^{2+}. Upon addition of the yeast enzyme preparation, which contained more than 0.1 M NH_4^+, the *E. coli* methionyl-tRNA synthetase was greatly stimulated, thereby contributing extensively to the rate of methionyl-tRNA formation.

For the sake of comparison, the experiments with both the yeast and the *E. coli* enzyme have been carried out at the same pH, 7.3, although the pH optimum is higher for the *E. coli* enzyme and lower for the yeast enzyme. The influence of pH on the rate stimulation by NH_4^+ shown for the *E. coli* enzyme in Fig. 6 indicates that there is a mutual interaction between cations and pH. Such an interaction has recently been demonstrated by MONDER[7] in a paper on glutamine synthetase from sheep brain. He found that different metal ions and even different concentrations of these have a pronounced effect on the pH optimum. Thus it is possible that the results obtained with the two methionyl-tRNA synthetases at pH 7.3 are not representative for other pH values.

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