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STUDIES ON METHIONYL-tRNA SYNTHETASE

I. EFFECTS OF DIVALENT AND MONOVALENT CATIONS ON METHIONYL-tRNA SYNTHETASE FROM *SACCHAROMYCES CEREVISIAE*

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SUMMARY

1. The activation of methionyl-tRNA synthetase (L-methionine:tRNA ligase (AMP), EC 6.1.1.10) from *Saccharomyces cerevisiae* by divalent metal ions has been investigated. It has been shown that the concentration for optimal reaction rate varies with the kind of cation and also with the kind of tRNA used as substrate.

2. In the presence of NH_4^+, K^+ or Rb^+ the rate of methionyl-tRNA formation is drastically increased, while the rate of methionyl-AMP formation is almost unaffected.

3. Some models for the mechanism of monovalent cation action are briefly discussed.

INTRODUCTION

The enzymatic formation of amino acyl-tRNA is known to proceed in two steps, both mediated by the same enzyme, amino acyl-tRNA synthetase[1]. The amino acid is first activated by ATP, forming an amino acyl-adenylate strongly bound to the enzyme (Eqn. 1). The amino acyl moiety is then transferred and esterified to the terminal adenylic acid of the tRNA chain proper (Eqn. 2):

$$\text{Amino acid} + \text{ATP} + \text{enzyme} \rightleftharpoons \text{amino acyl-AMP} \sim \text{enzyme} + PP_i \quad (1)$$

$$\text{Amino acyl-AMP} \sim \text{enzyme} + \text{tRNA} \rightleftharpoons \text{amino acyl-tRNA} + \text{AMP} + \text{enzyme} \quad (2)$$

It is a general finding that Mg^{2+} activates the amino acyl-tRNA synthetases when assayed for either PP_i–ATP exchange (Reaction I) or amino acyl-tRNA formation (Reaction 1 and 2). For certain enzymes of this type some other divalent metal ions have been reported to be able to replace Mg^{2+} (refs. 2–11). There are also a few observations in the literature on the action of monovalent cations on amino acyl-tRNA synthetases[12–17]. However, no systematic study on the action of cations on these enzymes has appeared to date.

In a previous paper[10] a synergistic effect between two enzyme preparations

was explained in terms of one methionyl-tRNA synthetase (L-methionine:tRNA ligase (AMP), EC 6.1.1.10) from yeast and one "regenerating" enzyme from *Escherichia coli*. Later research, however, revealed that the whole effect was due to a more than 10-fold stimulation of *E. coli* methionyl-tRNA synthetase by NH_4^+ present in the yeast enzyme preparation[18]. It turned out that both the yeast and the *E. coli* methionyl-tRNA synthetase could be greatly stimulated by NH_4^+. These results motivated a closer examination of the influence of cations on the enzyme activity. This paper contains data concerning the yeast enzyme, and an accompanying paper deals with the *E. coli* enzyme[19].

MATERIALS AND METHODS

Strains and growth conditions

Saccharomyces cerevisiae, strain C836, was used for preparation of yeast methionyl-tRNA synthetase and yeast tRNA. *E. coli* K 12, strain 30SO, obtained from Dr. W. K. MAAS[20], and *E. coli* B were used for preparation of *E. coli* tRNA.

The organisms were grown as described earlier[21] and harvested when still growing in the log phase to preserve the amino acid incorporating terminal of tRNA[22].

Enzyme preparation

Methionyl-tRNA synthetase from yeast was prepared as described by BERG[23]. Fraction AS-1, thoroughly dialysed against 0.01 M Tris–HCl, pH 7.3, was used in this study. A normal preparation contains about 20 mg of protein and 10–15 mg of nucleic acid per ml. It can be stored frozen at −18° for several months without any detectable loss of activity.

RNA preparations

tRNA was prepared from yeast in the following way. The frozen cells were thawed and centrifuged at 30 000 × *g* for 30 min. 50 g of the cell mass was suspended in 100 ml of water and shaken with 150 ml of freshly distilled 90% phenol for 2 h at room temperature. After centrifugation in the cold at 20 000 × *g* for 30 min, the water phase was carefully removed. To the solution was added potassium acetate to a final concentration of 2%, and the RNA was precipitated with twice the volume of absolute ethanol (−15°). After 1 h in the cold the precipitate was collected by centrifugation at 5000 × *g* for 30 min and dissolved in a small amount of water. The solution was made 1 M with respect to NaCl, and the precipitated rRNA was removed by centrifugation at 20 000 × *g* for 30 min. The tRNA in the supernatant solution was precipitated with ethanol, centrifuged and dissolved in 20 ml of water. The carbohydrates in the solution were removed by extraction with methyl cellosolve as described by GLITZ AND DEKKER[24]. The methyl cellosolve phase was dialysed against water for 20 h, and the tRNA was then precipitated with potassium acetate and ethanol. It was stripped free of amino acids by incubating in 0.1 M Tris–HCl, pH 9.0, for 1 h at 37°. Finally the solution was dialysed first against 0.01 M Tris–HCl, pH 7.5, and then against water for 30 h.

tRNA from *E. coli* was prepared in a slightly different way. 100 g of the frozen bacteria were thawed with 100 ml of 0.02 M $MgCl_2$ in 0.02 M Tris–HCl, pH 7.6, and shaken with 200 ml of freshly distilled 90% phenol for 2 h at room temperature.

The procedure is then identical with that described for yeast tRNA except that the stripping could be omitted.

Assays of enzyme activity

PP_i–ATP exchange. The reaction mixture for PP_i–ATP exchange contained 0.1 M Tris–HCl, pH 7.3, 2 mM ATP (disodium), 5 mM L-methionine, 1.25 mM sodium pyrophosphate containing 0.25 μC per ml of ^{32}P, and varying amounts of enzyme and metal ions. The incubation temperature was 37°. The assay volume was usually 1 ml, and aliquots of 0.2 ml were taken out at different times. The reaction was stopped with 0.5 ml of 7% $HClO_4$, and the washing procedure was performed according to BERG[23]. [^{32}P]pyrophosphate was prepared as described by BERG[23].

Methionyl-tRNA formation. The reaction mixture contained 0.1 M Tris–HCl, pH 7.3, 2 mM ATP (disodium), 0.1 mM EDTA (dipotassium), 5–10 $\mu\mu$M L-[*Me*-^{14}C]-methionine with a specific activity of about 16 mC/mmole, and different amounts of tRNA, enzyme and metal ions. The incubation temperature was 37°. Aliquots of 0.2 ml were taken out at different times, and the reaction was stopped with 2 ml of 0.01 M $La(NO_3)_3$ in 0.5 M $HClO_4$. The precipitate was washed as described by BOMAN, BOMAN AND MAAS[20].

Counting of radioactivity. The washed samples were transferred to planchets, dried and counted in a Nuclear Chicago windowless gas-flow counter. The background radiation amounted to 30 counts/min. All values given in counts/min are corrected for blank values unless otherwise stated. Values given in mμmoles are corrected for blank values, self absorption and counter efficiency. The probable error in the determination is 3% or lower.

Other methods

The concentration of RNA and protein was estimated from the absorbance at 260 and 280 mμ, respectively, (1-cm light path) in a Zeiss spectrophotometer. A solution of 1 mg per ml of tRNA gives an absorbance of about $A_{260\ m\mu} = 25$.

Chemicals

ATP (disodium), GSH and L-methionine were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Sigma 121 reagent grade Tris was used for preparation of Tris–HCl buffers. Sodium nucleinate from yeast, used as carrier RNA, was No. 6550 from E. Merck, Darmstadt, Germany.

The salt solutions were prepared from analytical grade chemicals. All were chlorides except $MgSO_4 \cdot 7\ H_2O$, $CoSO_4 \cdot 7\ H_2O$, $ZnSO_4 \cdot 7\ H_2O$, $NiSO_4 \cdot 6\ H_2O$, and $CdAc_2 \cdot 2\ H_2O$. There are no indications that the kind of anion in the concentration range used here is of any importance for the activity of the enzyme.

L-[*Me*-^{14}C]methionine (15.6 and 17.7 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., Skokie, Ill., U.S.A.

RESULTS

All experiments presented in this paper have been carried out with the crude

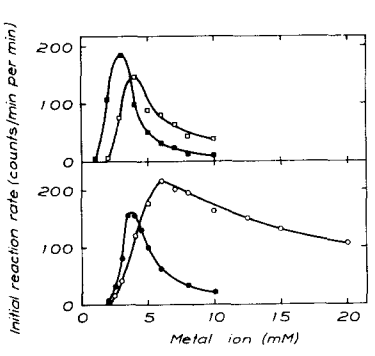
methionyl-tRNA synthetase preparation from yeast described by BERG[23]. With this enzyme it has previously been found that the rate of formation of methionyl-tRNA does not always show the simple kinetics expected with enzymes of this kind. In the heterologous system with tRNA from *E. coli*, the reaction rate decreases gradually with time, and the maximum incorporation of methionine is a function of the amount of enzyme added[25]. For this reason all data presented below have been carefully selected from those parts of the rate curves where the deviation from linearity could be neglected. They thus represent true initial rates.

It is a common feature to add reduced glutathione and an ATP-regenerating system to reaction mixtures with amino acyl-tRNA synthetases. The influence of these compounds on the reaction rate under the conditions used has been tested. No effect could be detected, and they were therefore omitted in the following experiments.

The Tris–maleate buffer, used in some previous work[10], has been found inhibitory to the yeast methionyl-tRNA synthetase. The inhibition is due to maleic acid, which in this study has been replaced by HCl. The inhibitory effect may be specific for yeast methionyl-tRNA synthetase, since other synthetases seem to function properly in the presence of maleate buffer[20,26,27].

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

The enzymatic synthesis of methionyl-tRNA is absolutely dependent on the presence of divalent metal ions. In Fig. 1 the reaction rate with yeast tRNA is shown



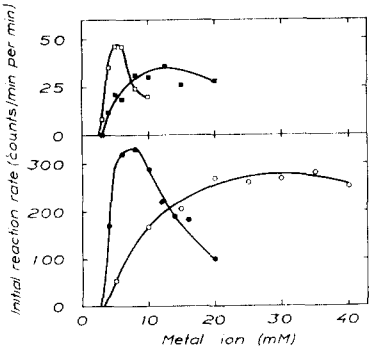


Fig. 1. Divalent metal ion dependence for the rate of formation of yeast methionyl-tRNA. ○—○, Mg^{2+}; ●—●, Mn^{2+}; □—□, Co^{2+}; ■—■, Ca^{2+}. The protein content was 0.17 mg/ml for Mg^{2+} and Mn^{2+} and 0.48 mg/ml for Co^{2+} and Ca^{2+}. The tRNA content was 2.0 mg/ml.

Fig. 2. Divalent metal ion dependence for the rate of formation of *E. coli* methionyl-tRNA. ○—○. Mg^{2+}; ●—●, Mn^{2+}; □—□, Co^{2+}; ■—■, Ca^{2+}. The protein content was 1.0 mg/ml for Mg^{2+} and Mn^{2+}, and 1.4 mg/ml for Co^{2+} and Ca^{2+}. The tRNA content was 1.6 mg/ml (*E. coli* B) for Mg^{2+} and Mn^{2+} and 0.6 and 0.8 mg/ml for Co^{2+} and Ca^{2+}, respectively, (*E. coli* K 12, 30SO).

as a function of the metal ion concentration for Mg^{2+}, Mn^{2+}, Ca^{2+} and Co^{2+}. The concentration for optimal rate varies from 3 mM for Ca^{2+} to 6 mM for Mg^{2+}. At concentrations above 10 mM only Mg^{2+} activates the enzyme.

With *E. coli* tRNA as substrate the picture is quite different, as can be seen from Fig. 2. The optimal rate concentrations are shifted to higher values for all the

metals. Most remarkable is the shift for Mg^{2+} from 6 mM with the yeast tRNA to about 30 mM with *E. coli* tRNA.

Since different amounts of enzyme were used for the curves in Figs. 1 and 2, the rates cannot be compared directly. Table I, however, summarizes the optimal rates expressed in comparable figures. The slower rate with heterologous tRNA compared with homologous tRNA is in accord with the results of ATTWOOD AND COCKING[3] and LAGERKVIST AND WALDENSTRÖM[28]. The highest rate in both the

TABLE I

RATES OF FORMATION OF METHIONYL-tRNA WITH DIFFERENT METAL IONS AT OPTIMAL CONCENTRATIONS

The reaction mixture for yeast tRNA contained 1.8 mg per ml of tRNA and 0.29 mg per ml of protein, and for *E. coli* tRNA 1.7 mg per ml of tRNA and 0.72 mg per ml of protein.

tRNA	*Metal ion*	*Optimal metal ion concentration (mM)*	*Methionyl-tRNA formed (mμmoles/min per mg protein)*	*Rate (%)*
Yeast	Mg^{2+}	6.0	0.37	100
Yeast	Mn^{2+}	4.0	0.22	60
Yeast	Ca^{2+}	3.0	0.15	40
Yeast	Co^{2+}	4.0	0.11	30
E. coli	Mg^{2+}	30.0	0.11	30
E. coli	Mn^{2+}	7.0	0.06	16
E. coli	Ca^{2+}	12.5	0.03	8
E. coli	Co^{2+}	5.5	0.05	13

homologous and the heterologous reaction is found with Mg^{2+}. It may be noted that with yeast tRNA, Ca^{2+} precedes Co^{2+}, whereas with *E. coli* tRNA this order is reversed.

Some other divalent metal ions have also been tested. With yeast tRNA as substrate, both Sr^{2+} and Ba^{2+} activate the enzyme. At the optimal concentrations, 5 and 10 mM, respectively, the reaction rate for both metals is about 10% of the optimal rate with Mg^{2+}. At a concentration of 4 mM, Cd^{2+} and Ni^{2+} activate the enzyme slightly, while Zn^{2+} does not give any significant activation.

The divalent metal ions probably activate the enzyme at the same site. This supposition is evidenced by the curves in Fig. 3, which shows the dependence on Mg^{2+} for the formation of yeast methionyl-tRNA at separate concentrations of Mn^{2+}. For the optima in these curves the following relation between the metal ion concentrations, given in mM, holds:

$$c_{Mg^{2+}} = 6.0 - 1.5\, c_{Mn^{2+}} \qquad (3)$$

The rates for the optimal concentrations according to Eqn. 3 decrease, in a non-linear fashion, with increasing Mn^{2+} concentration (Fig. 3). The explanation for this may be that Mn^{2+} is slightly inhibitory to the enzyme at a site different from

the site of activation, or that it is more strongly bound to the site of activation than Mg^{2+}, acting as a competitive inhibitor to Mg^{2+}.

Influence of monovalent cations on the formation of methionyl-tRNA

If NH_4^+ are added to the reaction mixture for incorporation of methionine into tRNA, there is a great increase in the rate of incorporation of radioactivity into the acid precipitate. Fig. 4 shows one such experiment with yeast tRNA and a suboptimal concentration of Mg^{2+}. In the presence of 100 mM NH_4^+ the rate is

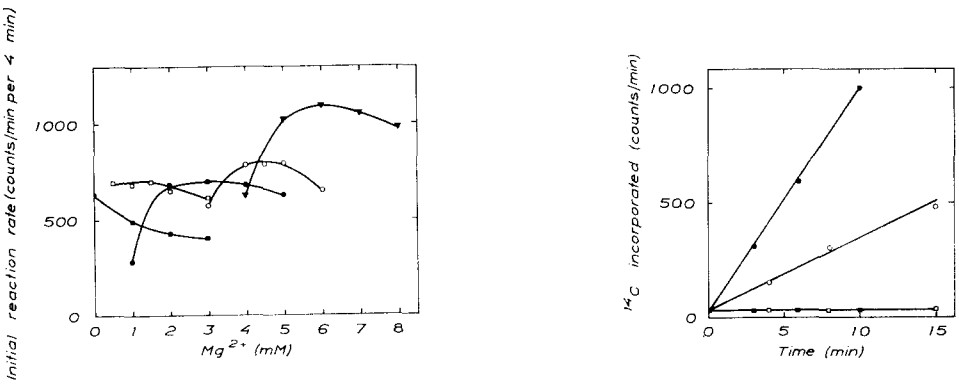


Fig. 3. The rate of formation of yeast methionyl-tRNA as a function of the magnesium ion concentration in the presence of manganese ions: ■—■, 4 mM Mn^{2+}; □—□, 3 mM Mn^{2+}; ●—●, 2 mM Mn^{2+}; ○--○, 1 mM Mn^{2+}; ▼—▼, no Mn^{2+}. The reaction mixture contained 0.27 mg per ml of protein and 1.8 mg per ml of tRNA.

Fig. 4. Formation of yeast methionyl-tRNA as a function of time in the presence of 3 mM Mg^{2+} (○—○) and 3 mM Mg^{2+} with 100 mM NH_4^+ (●—●). The reaction mixture contained 0.3 mg per ml of protein and 2 mg per ml of tRNA. Blank experiments without any added tRNA were performed with 100 mM NH_4^+ (■—■) and in the absence of NH_4^+ (□—□). All values are corrected for background radiation only.

increased 3-fold. That this increase is not an artifact but really represents a stimulation of the formation of methionyl-tRNA is strongly indicated by the following control experiments:

a. The two blank controls without tRNA in Fig. 4 show that all the radioactivity is incorporated into tRNA.

b. The same stimulatory effect with NH_4^+ is found if the methyl-labelled methionine is replaced by carboxyl-labelled methionine. This proves that the stimulation concerns the whole molecule and not only a part of it, *e.g.*, the methyl group[21].

c. "Stripping" of the reaction product at pH 9.0 removes the radioactivity completely from the tRNA.

d. NH_4^+ in the absence of divalent metal ions cannot sustain the reaction.

e. The maximum incorporation of radioactivity into tRNA is essentially the same whether or not NH_4^+ is present. This removes the suspicion that the ammonium-stimulated incorporation might be a coupling of methionine to the wrong chain of tRNA.

f. Since the buffer capacity of Tris–HCl is small at pH 7.3, it might be argued

that the addition of NH_4Cl to the reaction mixture could alter the pH of the solution, resulting in a change in reaction rate. It has, however, been shown by direct measurement that the pH of the reaction mixture is not changed by the addition of 0.2 moles per l of NH_4Cl.

The magnitude of the stimulation by NH_4^+ depends on the concentration. Fig. 5 shows the reaction rate with yeast tRNA as a function of NH_4^+ concentration

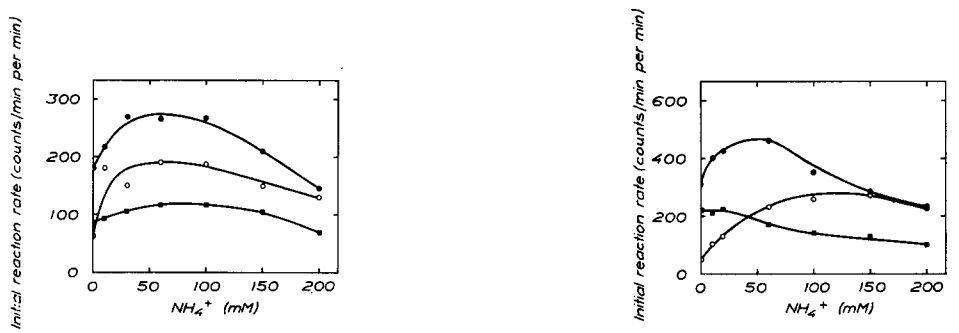


Fig. 5. The rate of formation of yeast methionyl-tRNA as a function of ammonium ion concentration with 3 mM Mg^{2+} (○—○), 6 mM Mg^{2+} (●—●), and 20 mM Mg^{2+} (■—■). The reaction mixture contained 1.4 mg per ml of tRNA and 0.16 mg per ml of protein.

Fig. 6. The rate of formation of *E. coli* methionyl-tRNA as a function of ammonium ion concentration with 5 mM Mg^{2+} (○—○), 25 mM Mg^{2+} (●—●) and 60 mM Mg^{2+} (■—■). The reaction mixture contained 1.0 mg per ml of tRNA (*E. coli* B) and 0.82 mg per ml of protein.

at three levels of Mg^{2+} concentration. At both the low, optimal and high Mg^{2+} concentrations the optimal NH_4^+ concentration lies between 50 and 100 mM. The relative increase in rate is 200% for 3 mM Mg^{2+} but only 50% for both 6 and 20 mM Mg^{2+}.

With *E. coli* tRNA as substrate the situation is rather similar, as shown in Fig. 6. The optima for NH_4^+ do not coincide, however, and at 60 mM Mg^{2+} there

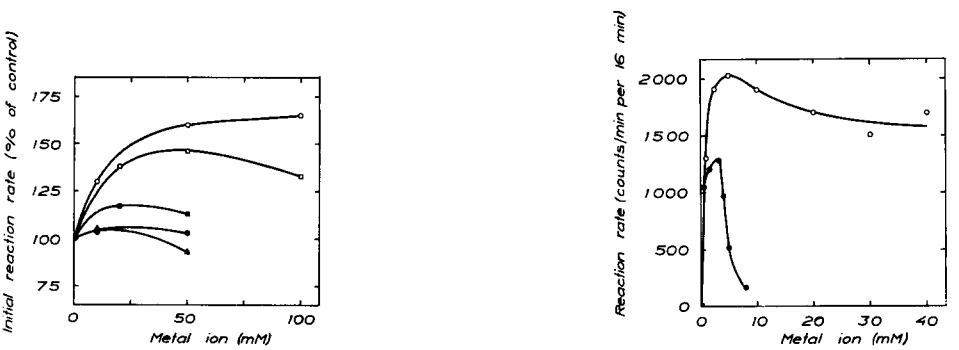


Fig. 7. The rate of formation of yeast methionyl-tRNA as a function of monovalent cation concentration. ○—○, K^+; □—□, Rb^+; ●—●, Na^+; ■—■, Cs^+; ▲—▲, Li^+. The reaction mixture contained 1.2–2.0 mg per ml of tRNA, 0.2 mg per ml of protein, and 6 mM Mg^{2+}.

Fig. 8. The rate of methionine-dependent PP_i–ATP exchange as a function of Mg^{2+} (○—○) and Mn^{2+} (●—●) concentration. The reaction mixture contained 0.62 mg per ml of protein.

is no stimulation by NH_4^+ at all, probably because of the high ionic strength. The relative increase in rate is 400% for 5 mM Mg^{2+} and 50% for 25 mM Mg^{2+}.

Among the other monovalent cations tested, K^+ and Rb^+ were also effective in stimulating the reaction rate. This is shown for yeast tRNA in Fig. 7. K^+ seems to behave quite as NH_4^+, while Rb^+ is somewhat less efficient. Cs^+ gives only a minor stimulation, and Li^+ and Na^+ do not influence the reaction rate when added in concentrations below 50 mM. At higher concentrations they tend to become inhibitory. With *E. coli* tRNA, K^+ and Rb^+ are also stimulatory while Na^+, Li^+, and Cs^+ are not.

A stimulation of the reaction rate by the monovalent cations NH_4^+ and K^+ is also found when Mg^{2+} is replaced by Mn^{2+}, Co^{2+}, or Ca^{2+}. Table II contains the results obtained with different combinations of divalent and monovalent cations.

TABLE II

STIMULATION OF THE RATE OF METHIONYL-tRNA FORMATION BY MONOVALENT CATIONS IN THE PRESENCE OF DIFFERENT DIVALENT CATIONS

All values were calculated from rate curves like those in Fig. 4. The figures are not comparable, since the amount of enzyme in the reaction mixtures varied. The increase in rate is given in per cent of the rate without any added monovalent cations.

tRNA	*Metal ion*	NH_4^+			K^+		
		M^{2+} *(mM)*	NH_4^+ *(mM)*	*Rate increase (%)*	M^{2+} *(mM)*	K^+ *(mM)*	*Rate increase (%)*
Yeast	Mg^{2+}	3	50	200	6	50	60
Yeast	Mn^{2+}	4	100	130	3	50	100
Yeast	Co^{2+}	3	50	120	3	50	100
Yeast	Ca^{2+}	2	50	20	2	50	20
E. coli	Mg^{2+}	5	100	400	5	50	200
E. coli	Mn^{2+}	7	100	200	6	100	90
E. coli	Co^{2+}	5	100	100	10	100	350
E. coli	Ca^{2+}	5	100	60	5	25	45

In almost all cases tested the increase in rate effected by the monovalent ions is of a notable and significant magnitude. The only exceptions are the two systems with yeast tRNA and Ca^{2+}. For these the stimulation is as small as about 20%. However, it should be emphasized that the figures in Table II do not represent maximum stimulation. It is thus possible that in some cases greater effects could be obtained with other choices of concentrations.

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

The formation of the enzyme-bound methionyl–AMP complex (Eqn. 1) was assayed as a methionine-dependent PP_i–ATP exchange. Fig. 8 shows that this reaction is absolutely dependent on divalent metal ions. The optimal Mg^{2+} concentration is about 5 mM. With increasing amounts of Mg^{2+} the reaction rate decreases very little. At 40 mM the rate is still about 80% of the maximum rate.

Mn^{2+}, on the other hand, shows a narrow peak with maximum at 3 mM and a rapid decrease as the concentration is increased.

At different concentrations of Mg^{2+} and Mn^{2+}, the effect of NH_4^+ and K^+ on the reaction rate has been studied. Fig. 9 shows two examples, one with 10 mM Mg^{2+} and the other with 2.5 mM Mn^{2+}. In both cases the addition of 100 mM NH_4^+ does not alter the rate. The same result is obtained if K^+ is added. At lower Mg^{2+} or Mn^{2+} concentrations, however, monovalent cations seem to inhibit rather than stimulate the reaction. For example, in the presence of 1 mM Mg^{2+} the reaction rate

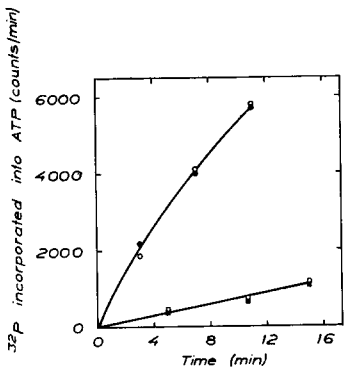


Fig. 9. Time curves for the methionine-dependent PP_i–ATP exchange in the presence of 10 mM Mg^{2+} (○—○), 10 mM Mg^{2+} and 100 mM NH_4^+ (●—●), 2.5 mM Mn^{2+} (□—□), 2.5 mM Mn^{2+} and 100 mM NH_4^+ (■—■). The reaction mixtures with Mg^{2+} and Mn^{2+} contained 0.44 and 0.71 mg per ml of protein, respectively.

is decreased by about 25% if 100 mM NH_4^+ is added, and with 0.25 mM Mn^{2+} the reaction is inhibited to 50% by 100 mM NH_4^+ or K^+. In no case has any stimulatory effect of monovalent cations been observed in the PP_i–ATP exchange reaction.

The effect of tRNA on the reaction rate.

In the present study, tRNA has always been added in excess to the reaction mixture for incorporation of methionine into tRNA. With different amounts above 0.6 mg/ml no change in the initial reaction rate has been observed. The magnitude

TABLE III

THE INFLUENCE OF tRNA ON THE STIMULATORY EFFECT OF NH_4^+ ON THE RATE OF YEAST METHIONYL-tRNA FORMATION

The reaction mixture contained 2.3 mM Mg^{2+} and 0.35 mg per ml of protein. The experimental error in the calculated rate increase is estimated to be about ±7%.

Amount of tRNA (mg/ml)	*Reaction rate (counts/min per 3 min)*		*Rate increase (%)*
	No NH_4^+	*125 mM NH_4^+*	
0.5	45	195	430
1.1	60	280	470
1.8	55	270	490

of the stimulation by monovalent cations also seems to be independent of the amount of tRNA. Table III shows that the increase in reaction rate with 125 mM NH_4^+ is almost the same for the three different additions of yeast tRNA.

DISCUSSION

Methionyl-tRNA synthetases have been partly purified from rat liver[29], wheat germ[30], *E. coli*[31] and yeast [23,32]. In his first paper on the yeast enzyme[23], BERG describes a fraction AS-1 with a specific activity of 15 (units per mg of protein) and a ribonuclease-treated fraction AS-2 with a specific activity of 17. In the second paper[32], the purification is extended to give a preparation with a specific activity of 42. For the present investigation fraction AS-1 has been used, partly because of its greater stability compared with the more purified fractions, and partly because the introduction of ribonuclease in the later steps seemed to be a potential risk.

The yeast enzyme has been shown to incorporate methionine into both yeast and *E. coli* tRNA in the presence of a variety of divalent cations (Table I). With yeast tRNA as substrate, the following metal ions were active:

$$Mg^{2+} > Mn^{2+} > Ca^{2+} > Co^{2+} > Sr^{2+}, Ba^{2+} > Cd^{2+}, Ni^{2+},$$

whereas Zn^{2+} was inactive. For comparison, the results in the literature on the activation of amino acyl-tRNA synthetases by different divalent metal ions are summarized in Table IV.

The active metal ions exhibit different concentration optima and different concentration ranges for activation in the reaction with tRNA (Figs. 1 and 2). This is also the case with Mg^{2+} and Mn^{2+} in the PP_i–ATP exchange reaction (Fig. 8).

TABLE IV

Literature reports on activation of amino acyl-tRNA synthetases by divalent metal ions besides Mg^{2+} and by monovalent cations. In the assay column, the abbreviations are: P, PP_i–ATP exchange; H, hydroxamate formation; T, amino acyl-tRNA formation.

Amino acid	*Source*	*Assay*	*Active cations*	*Inactive cations*	*References*
Ala	Pig liver	P, T	Co, Mg	Mn etc.	2
Ala	Tomato roots	P	Mg, Mn, Co		3
Tyr	Hog pancreas	P	Mg, Co, Ca		4
Pro	Mung bean	T	Mg, Mn		5
Pro	Rat liver	H	Mg, Mn, Ca		6, 7
Phe	*E. coli*	H	Mg, Mn, Co		8
		P	Mg, Mn	Co	
		T	Mg	Mn, Co	
Ser	Beef pancreas	H	Mg, Mn	Co, Ni	9
Arg	*E. coli*	T	Mg, Mn		10
Gly	Chick embryo	H	Mg, Mn	Ca	11
Tyr	Hog pancreas	P	K, NH_4, Rb	Na, Li	12, 13
Tyr	Guinea-pig liver	P	K		14
Tyr	Rat liver	P	K, NH_4, Rb	Na, Li, Cs	15
		T	K		
Phe	*E. coli*	T	K		16
Lys	*E. coli*	P	—	K, NH_4	17

In all these cases there is a striking difference between the curve for Mg^{2+} and the curves for the other metal ions. In Fig. 1, for example, it can be seen that neither Mn^{2+} nor Ca^{2+} allows any appreciable reaction rate at 10 mM, while Mg^{2+} at this concentration gives almost maximum rate. If similar differences exist for other amino acyl-tRNA synthetases, negative results with metal ions at one concentration only should be regarded with caution.

The optimal concentrations of the metal ions seem to be dependent on the nature of the tRNA involved in the reaction. The heterologous tRNA causes a shift to higher concentrations for maximum activity. If Figs. 1 and 2 are compared, it can be seen that the incorporation rate at 5 mM Mg^{2+} is almost maximum with yeast tRNA but very small with *E. coli* tRNA. It should also be noted that at 10 mM Mn^{2+}, the rate with yeast tRNA is negligible, while *E. coli* tRNA permits almost full activity. Such differences may have some bearing on results from experiments with heterologous systems performed at one fixed metal ion concentration. This has also been pointed out by JACOBSSON *et al.*[33].

The Mg^{2+} and Mn^{2+} dependence curves for PP_i–ATP exchange in Fig. 8 and for methionyl-tRNA formation in Fig. 1 show a notable difference at low concentrations. At 2 mM the PP_i–ATP exchange reaction has reached almost full rate, while the tRNA reaction does not proceed at all. The explanation for this may be that RNA present in the reaction mixture lowers the free metal ion concentration or that divalent cations are required for Reaction II in higher concentrations than for Reaction I. The first possibility does not seem to be relevant, since at low Mg^{2+} concentrations different amounts of tRNA do not notably influence the rate of methionyl-tRNA formation (Table III). The other possibility implies that Reaction II really has a requirement for divalent metal ions. There is some evidence for this. ALLENDE *et al.*[34] isolated the threonyl-adenylate–enzyme complex and have thus been able to study Reaction II independently. With tRNA that had been pretreated with EDTA they demonstrated an absolute dependence on either Mg^{2+}, Mn^{2+} or Ca^{2+}. With untreated tRNA, which may contain metal ions, addition of Mg^{2+} was unnecessary and even resulted in inhibition. However, NORRIS AND BERG[35] found no requirement for Mg^{2+} in Reaction II with an isolated isoleucyl-adenylate–enzyme complex, and LAGERKVIST, RYMO AND WALDENSTRÖM[36] have shown that an isolated valyl-adenylate–enzyme complex does not require metal ions for reaction with tRNA. Possibly some amino acyl-tRNA synthetases need metals in Reaction II while others do not.

Among the monovalent cations, K^+, NH_4^+ and Rb^+ were found to stimulate the incorporation of methionine into tRNA. Na^+, Li^+ and Cs^+ were almost indifferent or slightly inhibitory in the concentration range up to 100 mM. In Reaction I, however, no stimulation could be detected with either NH_4^+ or K^+. The effect of the monovalent cations therefore seems to be restricted to Reaction II. This is in contrast to the results of SCHWEET and collaborators[12–14] and HOLLEY *et al.*[15], who reported the stimulation of enzymes from different organisms by K^+ in the tyrosine-dependent PP_i–ATP exchange reaction (see Table IV).

The results with the hog pancreas[13] and rat liver[15] enzymes must be interpreted in terms of an absolute dependence on K^+ or Rb^+ (in addition to Mg^{2+}) for activity. The effect of K^+, NH_4^+ and Rb^+ on the rate of methionyl-tRNA formation, on the other hand, seems to be only a stimulation, that is, the enzyme has no absolute requirement for monovalent cations.

The reaction product with methionyl-tRNA synthetase has been tacitly assumed to be methionyl-$tRNA^{met}$, although this is by no means self-evident. In some heterologous systems, the amino acyl-tRNA synthetase from one organism has been found to attach the amino acid to the wrong species of tRNA from another organism[33,37–40]. However, the yeast methionyl-tRNA synthetase has been shown by BERG *et al.*[41] to incorporate methionine into one of the *E. coli* tRNA chains which normally does accept this amino acid. The possibility that a wrong product might be formed in the presence of non-physiological concentrations of some cations should be considered. Thus it has been reported by HARUNA AND SPIEGELMAN[42,43] that Mn^{2+} activation of a phage-induced RNA replicase, which is normally activated by Mg^{2+}, may cause the formation of a wrong product. Since there is a metal–protein–RNA interaction both in the case of the RNA replicase and in the case of amino acyl-tRNA synthetases, one has to consider the possibility that metal ions other than Mg^{2+} may cause the esterification of an amino acid to the wrong chain of tRNA or to the wrong place on tRNA.

The data now available do not permit any detailed discussion about possible mechanisms for the stimulatory effect of the monovalent cations. Only a few suggestions will therefore be presented below.

a. The displacement hypothesis. The monovalent ions might displace either an activating factor attached to tRNA or to a protein component in the enzyme preparation, or an inhibitor attached to the enzyme. Such a factor cannot be Mg^{2+}, since the stimulation is found even at and above optimal Mg^{2+} concentration (Figs. 5 and 6). Other divalent metal ions can also be excluded, since there is no change in the pattern of stimulation either with a base level of EDTA in the reaction mixture or with pretreatment of tRNA with EDTA. The hypothesis thus calls for a new component involved in amino acid activation, and it seems for this reason rather improbable.

b. Interaction with the active sites of the enzyme. The amino acyl-tRNA synthetases probably have different recognition sites for ATP (I), amino acid (II), the ACC-terminal of tRNA (III), and the identification site for the amino acid-specific tRNA chain (IV). The monovalent cations could facilitate the coupling of methionine to the adenylate moiety of tRNA either by direct interaction with the divalent metal ions at site I or II, or by increasing the affinity between tRNA and the enzyme at sites III or IV.

c. Conformational changes. Some divalent metal ions have been ascribed a stabilizing effect on the three-dimensional structure of tRNA[8,44]. The monovalent cations might exert their action in a similar way, thereby rendering the tRNA more rigid and facilitating recognition by the enzyme. Another possibility may be that the distance between sites III and IV on the enzyme is affected, favouring the attachment of tRNA or allowing the reaction to occur at a greater rate.

The physiological significance of the stimulatory effect of monovalent cations can only be a matter of speculation. LUBIN AND ENNIS[16], using *E. coli* systems, compared the magnitude of stimulation by K^+ on the rate of amino acyl-tRNA formation and on the rate of transfer of the amino acid to the ribosome. The transfer step was by far the most stimulated, and it was concluded that a possible K^+ control on protein biosynthesis must be located at this step and not at the activating step. Nevertheless, the stimulation in these two reactions might be comparable if lower Mg^{2+} concentrations were used. The yeast methionyl-tRNA synthetase has been

found to function properly with very low Mg^{2+} concentrations if K^+ or NH_4^+ are present in sufficient amounts. Since the intracellular concentration of K^+ in growing yeast can reach values as high as 0.2 M[45], the K^+ stimulation may, under special conditions at least, be of some physiological importance.

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