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Stimulation of valyl- and isoleucyl-tRNA synthetase reactions by polyamines

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Abstract. The aminoacylation of tRNA catalysed by valyl-tRNA synthetase (EC 6.1.1.9) and isoleucyl-tRNA synthetase (EC 6.1.1.5) from *Mycobacterium smegmatis* is dependent on the presence of divalent metal ions. Polyamines alone, in the absence of metal ions, do not bring about aminoacylation. In the presence of suboptimal concentrations of Mg^{2+}, polyamines significantly stimulate the reaction. Of the cations tested, only Mn^{2+}, Co^{2+} and Ca^{2+} can partially substitute for Mg^{2+} in aminoacylation, and spermine stimulates aminoacylation in the presence of these cations also. At neutral pH, spermine deacylates nonenzymatically aminoacyl tRNA. AMP and pyrophosphate-dependent enzymatic deacylation of aminoacyl-tRNA (reverse reaction) is also stimulated by spermine. The inhibitory effect of high concentration of KCl on aminoacylation is counteracted, by spermine. The low level of activity between pH 8·5-9·0 at 1·2 mM Mg^{2+} is restored to normal level on the addition of spermine. The inhibitory effect of high pH on aminoacylation in the presence of low concentration of Mg^{2+} is also prevntedvby spemine.

Keywords. *Mycobacterium smegmatis,* spermine stimulation, isoleucyl-tRNA synthetase, valyl-tRNA synthetase.

Introduction

Aminoacyl-tRNA synthetase reaction is conventionally represented as the sum of the two partial reactions (i) and (ii), to give the total reaction (iii).

(i) $AA + ATP \rightleftharpoons AA - AMP + PP_i$

(ii) $AA\text{–}AMP + tRNA \rightleftharpoons AA - tRNA + AMP$

(iii) $AA + ATP + tRNA \rightleftharpoons AA - tRNA + AMP + PP_i$.

However, controversy exists whether the reaction proceeds in a sequential (i and ii) or in a concerted manner in a single step. The influence of polyamines on aminoacylation of tRNA has been used as a supporting evidence for the concerted mechanism (Igarashi *et al.,* 1971; Loftfield, 1972). For instance, Igarashi *et al.* (1971) have shown that in the presence of polyamines ATP-PP_i exchange reaction (step i) was absent, whereas aminoacylation of tRNA proceeded at the same rate as in the presence of optimal concentration of Mg^{2+} supporting the concerted mechanism. However, it was shown subsequently (Chakrabartti *et al.,* 1975; Santi and Webster,

Abbreviations: AA, amino acid; AA-AMP, aminoacyl-adenylate; AA-tRNA, aminoacyl-transfer RNA.

1975; Thiebe, 1975), that polyamines could not stimulate aminoacylation in the complete absence of Mg^{2+}, and contaminating metal ions were associated with tRNA. When tRNA was completely freed of divalent cations, there was no amino-acylation in presence of polyamines alone (Santi and Webster, 1975). In contrast, Takeda and coworkers (Takeda and Ohnishi, 1975; Takeda and Ogiso, 1976; Takeda *et al.,* 1976), by using similar preparations of tRNA showed that poly-amines stimulated the amino acylation in the complete absence of added cations.

Most of the above studies were carried out with *Escherichia coli* isoleucyl-tRNA synthetase. In view of these conflicting reports and also because of the suggestion that the influence of polyamines on aminoacyl-tRNA formation differs from enzyme to enzyme (Kisselev and Favorova, 1974) and depends on the source of the enzyme (Igarashi *et al.,* 1978a, b), we have investigated the polyamine effect on aminoacyla-tion reactions using two enzymes from *Mycobacterium smegmatis.* In this paper, we report the effect of polyamines on the amino acylation in the total absence of Mg^{2+} and the probable mechanism of spermine action on the enzymatic and non-enzymatic deacylation of aminoacyl-tRNA.

Materials and methods

Spermine, spermidine and putrescine were from Sigma Chemical Company, St. Louis, Missouri, U.S.A. L-[^{14}C]-valine (sp. activity 280 mCi/mmol) and L-[^{14}C]-isoleucine (sp. activity 338 mCi/mmol) were from Radiochemical Centre, Amersham, England. [^{32}P] $Na_4P_2O_7$ was from Bhabha Atomic Research Centre, Bombay. Valyl- and isoleucyl-tRNA synthetases were homogenous preparations isolated from *M. smegmatis.* Valyl-tRNA synthetase was purified as reported earlier (Natarajan and Gopinathan, 1979) and isoleucyl-tRNA synthetase was purified to homogeneity by a similar procedure (unpublished results). Total tRNA isolated from *M. smegmatis* as already reported (Deobagkar and Gopinathan, 1978), was dialysed successively against a litre each of 2M NaCl + 1 mM EDTA, 1 mM EDTA and two changes of double distilled water. The Mg^{2+} content of this preparation was estimated in an atomic absorption spectrometer and found to be less than one mol per mol of tRNA. The tRNA dialysed only against water contained 6 mol of Mg^{2+} per mol of tRNA.

Enzyme assays

Aminoacylation of tRNA : This reaction was carried out in a volume of 125 μl containing 100 mM tris-HCl (pH 7·0), 2mM ATP, 50μg total tRNA, 10 mM KCl, 300 nM of L- [^{14}C] -amino acid, 4-5μg of enzyme and indicated amounts of Mg^{2+} and polyamines. The polyamines used were dissolved in water and the pH was adjusted to 7·0 with 2N HCl. The assay mixture was incubated at 37°C for 20 min; 0·1 ml samples were spotted on Whatman 3 MM filter discs and the tri-chloroacetic acid-precipitable radioactivity was estimated in a Beckman LS-100 liquid scintillation spectrometer.

ATP-PP_i exchange reaction : The assay was carried out according to Calender and Berg (1966). The reaction mixture contained, in a volume of 125μ1, 100 mM tris-HCl, pH 7·0, 2 mM ATP, 1·0-1·5 mM [^{32}P] $Na_4P_2O_7$, 2mM L-valine or

L-isoleucine, enzyme and indicated amounts of Mg^{2+}. The reaction mixture was incubated at 37°C for 20 min and the reaction was stopped with 0·5 ml of 15% perchloric acid. [^{32}P] ATP was adsorbed with 15 mg of activated charcoal, filtered through Whatman 3 MM circles, washed with 30 ml cold water and the radioactivity was estimated.

Isolation of [^{14}C]-*valyl- and isoleucyl-tRNA* : The aminoacylation assay reaction volume was scaled upto 5 0 ml and at the end of 60 min incubation, equal volume of 88% phenol was added and stirred for 30 min. After centrifugation the aqueous layer was re-extracted with phenol and aminoacyl-tRNA was precipitated with 2·5 volumes of alcohol. The precipitate was dissolved in water and dialysed against 500 ml each of 2 M NaCl + 1 mM EDTA, 1 mM EDTA and finally against two changes of distilled water.

AMP and PP_i dependent hydrolysis of aminoacyl-tRNA : The reverse reaction of aminoacylation of tRNA was carried out in a volume of 125 μ1 which contained 100 mM tris-HCl, pH 7·0,4 mM Mg^2+, 0·8 mM AMP, 0·8 mM $Na_4P_2O_7$, 3000 cpm of [^{14}C]-aminoacyl-tRNA and the enzyme. This was incubated at 37° C for 30 min. Samples (0·1 ml) were spotted on Whatman 3 MM filter papers and the trichloroacetic acid-precipitable radioactivity estimated. The [^{14}C]-aminoacyl-tRNA incubated with all the components of the reaction mixture, except the enzyme, was taken as control to calculate the extent of hydrolysis.

Results

Cation requirements

The results presented in figure 1 show the effect of increasing concentration of Mg^{2+} and spermine on valyl-tRNA formation. Aminoacylation was completely dependent on externally added Mg^{2+}. Practically there was no reaction upto 1 mM Mg^{2+}, but more than 65% of the maximal activity was obtained at 2 mM (figure 1a). Spermine showed stimulatory effect on aminoacylation especially at suboptimal concentrations of Mg^{2+}. For instance, at Mg^{2+} below 1 mM, spermine caused maximal stimulation, whereas at 6 mM Mg^{2+}, the polyamine had only a marginal effect. In the presence of spermine, the apparent K_m for Mg^{2+} was decreased by half but the V_{max} was not changed.

Even when the tRNA was dialysed against EDTA, a small amount of Mg^{2+}, equivalent to 0·6 mol/mol of tRNA was still associated with it; the final concentration of Mg^{2+} contributed by this tRNA was only 10 μM in the assay mixture. At this concentration of Mg^{2+}, spermine did not have any stimulatory effect on aminoacylation (figure 1b).

When tRNA contained bound cation (*i.e.*, not dialysed against EDTA), the concentration of Mg^{2+} contributed by it would be 100 μM, in the presence of which spermine showed some stimulatory effect. When 1·2 mM Mg^{2+} was added, the stimulatory effect of spermine was very evident. Hence it is clear that for spermine stimulation of aminoacylation, the presence of Mg^{2+} was necessary. Spermine (3 mM) in the presence of 1·2 mM Mg^{2+} catalysed aminoacylation at the same rate as in the presence of optimal Mg^{2+}. Spermidine and putrescine also stimulated valyl-tRNA formation in presence of Mg^{2+}, but to a lesser extent.

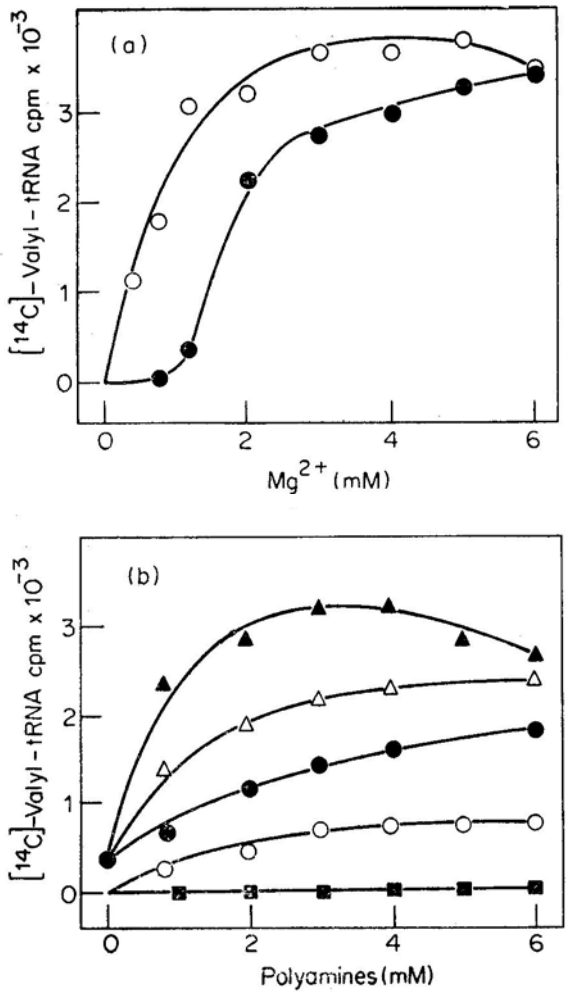

Figure 1. Effect of polyamines on valyl-tRNA formation.

The enzyme was assayed as described, in Materials and Methods. The tRNA dialysed against EDTA was used.

(a).Effect of increasing concentration of Mg^{2+}, ●—● No polyamine; O—O 2 mM spermine.

(b) Assays were carried out in the presence of 1·2 mM Mg^{2+} and concentration of polyamine was as indicated ▲—▲ Spermine; Δ — Δ Spermidine; ●—● Putrescine; ■—■ Spermine alone in the absence of added Mg^{2+}; O—O tRNA dialysed only against water (and not EDTA) was used and the activity was assayed in the absence of added Mg^{2+}. Spermine concentration was varied as shown.

The overall effect of spermine on isoleucyl-tRNA formation was also similar (figure 2). However, at higher concentrations of Mg^{2+}, spermine showed a slight inhibitory effect. In the presence of spermine, the apparent K_m for Mg^{2+} was decreased and the V_{max} was reduced.

When the cations were removed from tRNA by dialysis against EDTA, spermine, spermidine and putrescine could not stimulate isoleucyl-tRNA synthesis. Unlike

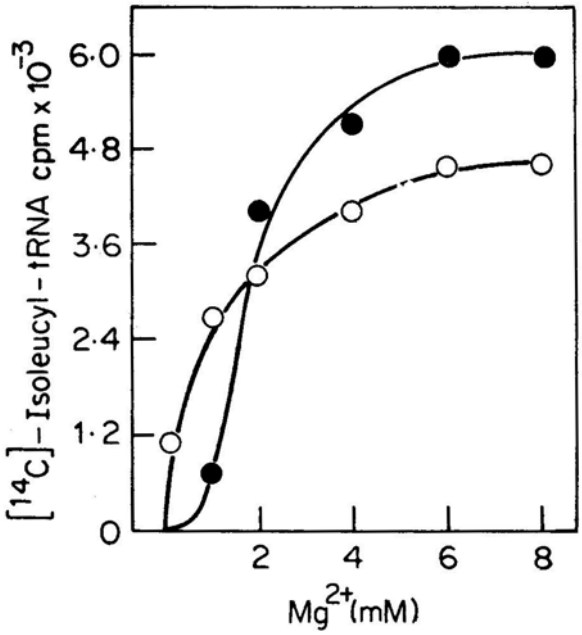

Figure 2. Effect of polyamines on isoleucyl-tRNA formation. Assay conditions as described under figure 1. The tRNA dialysed against EDTA was used. Effect of increasing concentration of Mg^{2+} is shown. ●—● No polyamine; O——O 2 mM spermine.

valyl-tRNA formation, however, spermine, spermidine and putrescine stimulated the isoleucyl-tRNA formation to a similar extent, in presence of 1·2 mM Mg^{2+}. Moreover, none of the polyamines could restore the activity to the level observed in presence of optimal Mg^{2+} concentration.

Other cations

In order to understand the specificity of Mg^{2+} in the formation of valyl-tRNA the effect of divalent cations and spermine on these cations were studied. The results are presented in figure 3. Mn^{2+} could substitute Mg^{2+} to a fair extent (upto 43% of activity in presence of Mg^{2+}). Co^{2+} and Ca^{2+} also showed upto 14 per cent activity at optimal concentrations. Ni^{2+} showed a marginal effect whereas Zn^{2+} and Fe^{2+} could not replace Mg_{2+} in the reaction.

Spermine stimulation was evident in the case of all these cations, including Zn^{2+}, Fe^{2+} and Ni^{2+} and in general the maximum effects were seen at low concentrations of the cations. The extent of polyamine stimulation depended on the nature of the divalent cation.

ATP-PP_i exchange reaction

Earlier studies showed that polyamine inhibited ATP-PP*i* exchange reaction and also pyrophosphorolysis of aminoacyl-AMP (Chakraburtty *et al.,* 1975). In our

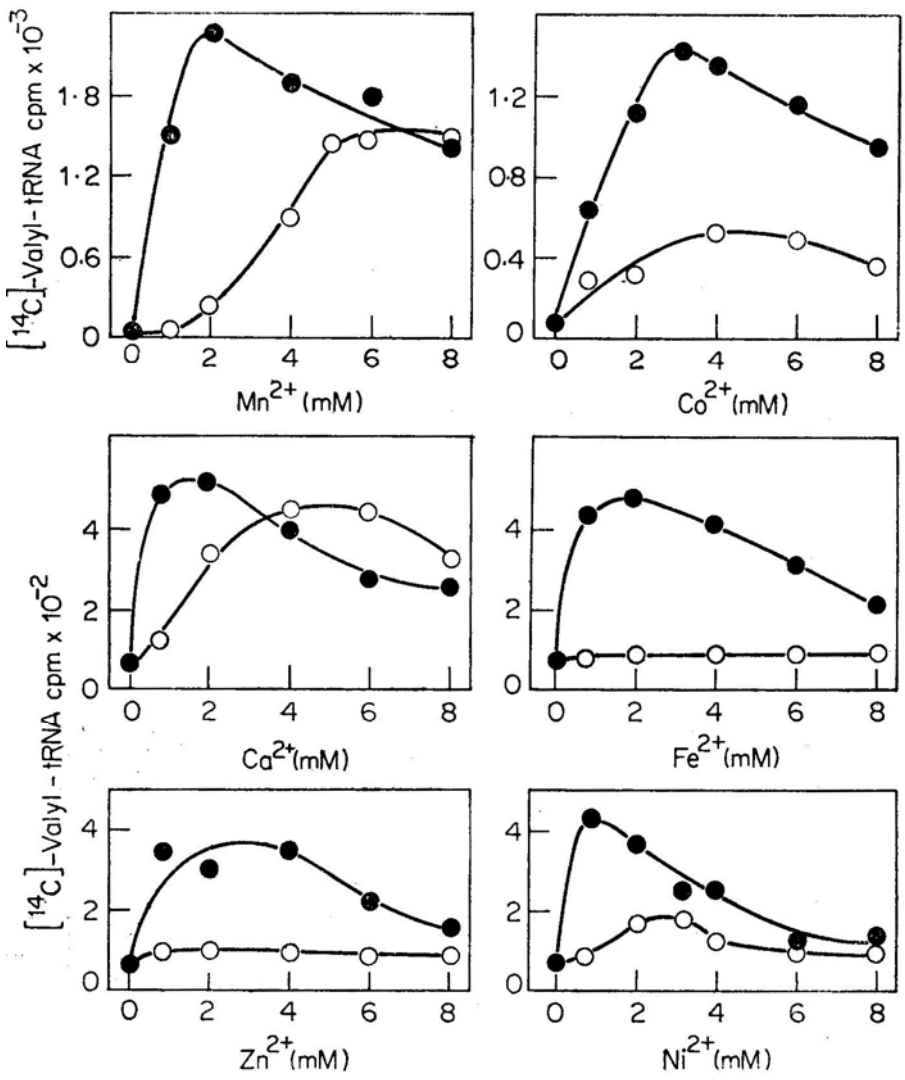

Figure 3. Valyl-tRNA formation in presence of various divalent cations and spermine. Mn^{2+}, Ca 2^{+}, Co^{2+} and Ni^{2+} were chlorides, and Zn^{2+} and Fe $^{2+}$ were sulphates. Blank values without any added cations were not subtracted.
O—O Divalent metal ion alone; ●—● Divalent metal ion plus 2mM spermine.

system, in the absence of added metal ions, spermine did not support ATP-PP_i exchange reaction. Furthermore, in the presence of 2 mM Mg^{2+}, it had no stimulatory or inhibitory effect (data not shown).

Reverse reaction

In order to determine whether spermine stimulation of aminoacyl-tRNA formation was the consequence of the inhibition of the reverse reaction of aminoacylation, the effect of polyamine on the reverse reaction was studied,

It is clear from figure 4 that the enzymatic hydrolysis of aminoacyl-tRNA takes place only when AMP and PPi were present indicating that non-enzymatic deacylation was negligible in the assay conditions used. In the presence of Mg^{2+} (4 mM) 45% hydrolysis was observed whereas spermine (2mM) caused 50% stimulation. Further increases in the concentrations of Mg^{2+} or spermine did not enhance the hydrolysis. Maximum hydrolysis of valyl-tRNA occurred in the presence of both Mg^{2+} and polyamine.

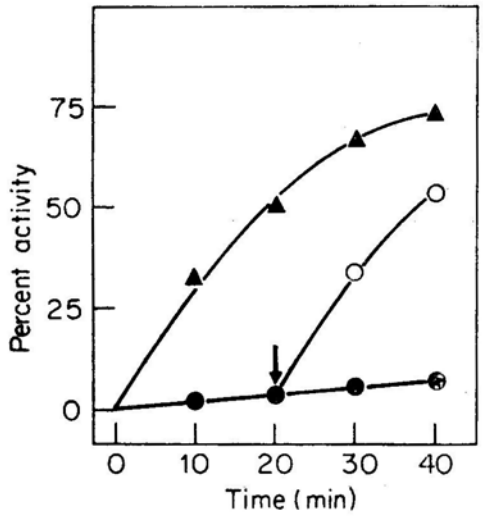

Figure 4. Effect of AMP and PP_i on the hydrolysis of [^{14}C]valyl-tRNA by vatyl-tRNA synthetase. Assays were done as given in Materials and Methods.
▲—▲ Complete system; ●—● Without AMP and PP_i at the time indicated by arrow, AMP and PP_i were added.

The stimulation of reverse reaction by spermine was also dependent on the presence of metal cation, because the polyamine-stimulated reaction was completely inhibited by EDTA (line 7 in table 1). A comparison of lines 2 and 6 in the table clearly shows that unlike the forward reaction, the amount of cation required was much lower for spermine to stimulate the reverse reaction (equivalent to the amount carried over by the dialysed tRNA) . However, spermine catalysed the hydrolysis of valyl-tRNA nonenzymatically to a significant extent (35%) whereas Mg^{2+} (upto 8 mM) did not have any effect in the absence of enzyme (table 1).

The nonenzymatic hydrolysis of valyl-tRNA by spermine was not dependent on AMP and PP_i, and the extent of hydrolysis was unaltered in the concentration range of 2-8 mM spermine. Similar results were obtained with isoleucyl-tRNA. Spermidine also catalysed partial hydrolysis nonenzymatically, whereas putrescine (upto 16 mM) did not have any effect.

Effect of KCl and pH on spermine stimulation

The effect of monovalent cation (K^+) on valyl-tRNA synthetase catalysed PP_i exchange reaction and total reaction (aminoacylation) was studied and the results

Table 1. Effect of spermine on the reverse reaction of valyl-tRNA synthetase and isoleucyl–tRNA synthetase.

Additions	Per cent hydrolysis*	
	Valyl-tRNA	Isoleucyl-tRNA
None (control)	0	0
Enzyme**	0	0
8 mM Mg^{2+}	0	0
8 mM Mg^{2+} + enzyme	43	80
8 mM Spermine	36	18
8 mM Spermine + enzyme	60	78
8 mM Spermine + enzyme + 2 mM EDTA	35	..

Assays were done as indicated in Materials and Methods.Control reaction contained 100mM Tris-HCl, pH 7·0, 0·8 mM AMP, 0·8 mM $Na_4P_2O_7$ and 2,540 cpm of [^{14}C]valyl-tRNA or 2450 cpm of [^{14}C]isoleucyl tRNA.

* The [^{14}C]aminoacyl-tRNA incubated with all the components of the reaction mixture except the enzyme, was taken as control to calculate the percent hydrolysis.

** Enzyme refers to valyl-tRNA synthetase and isoleucyl-tRNA synthetase respectively for the data in columns 1 and 2.

are presented in figure 5. Aminoacylation of tRNA was more sensitive than PP_i exchange reaction to the addition of KCl (figure 5a). Thus at 250 mM KCl, the

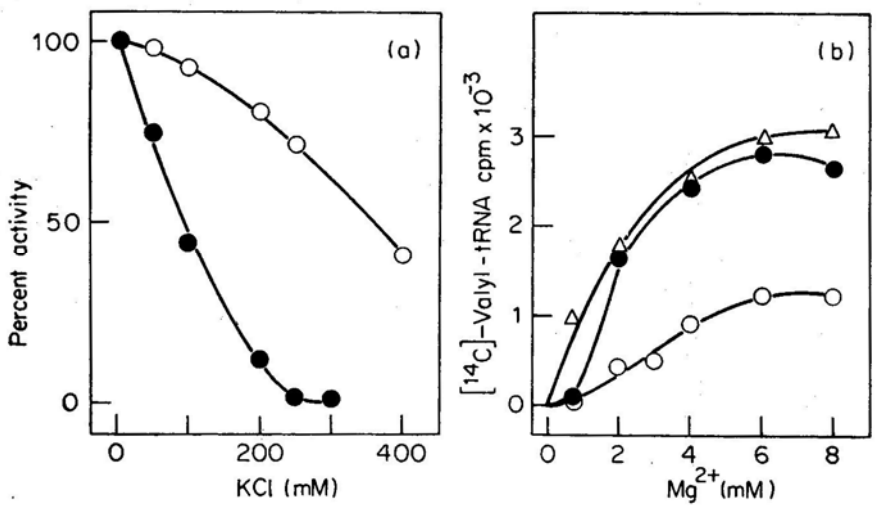

Figure 5. (a) Effect of KCl on valyl-tRNA synthetase reactions. 6mM Mg^{2+} wasused. ●——●Aminoacylation reaction; O——O pp_i exchange reaction.
(b) Effect of Mg^{2+} and spermine on the inhibition of valyl-tRNA formation caused by KCl. Mg^{2+} concentration was varied as shown. ●—● 10mM KCl; 100mM KCl; O——O 100mM KCl; Δ––Δ plus 2mM spermine.

total reaction was completely inhibited whereas the partial reaction was inhibited less than 30%. The effect of KCl was more obvious at lower concentrations of Mg^{2+} (figure 5b). The KCl inhibition was completely prevented by.spermine.

The results were similar with isoleucyl-tRNA formation in the presence of NaCl or KCl and spermine (table 2).

Table 2. Effect of spermine on NaCl and KCl inhibition of isoleucyl-tRNA formation.

Addition		Isoleucyl-tRNA formed (cpm)	
		− Spermine	+ 2mM Spermine
None		6540	5466
NaCl	100 mM	4053	6652
	200 mM	1661	3708
KCl	100 mM	3441	4847
	200 mM	1540	2738
	300 mM	580	1636

Assays were done as given in materials and methods. The final concentration of Mg^{2} was 6 mM in these assays.

The influence of pH on valyl-tRNA synthetase activity at suboptimal Mg^{2+} concentration is shown in figure 6. In N-2-hydroxyethyl piperazine N'-2-ethane sulfonic

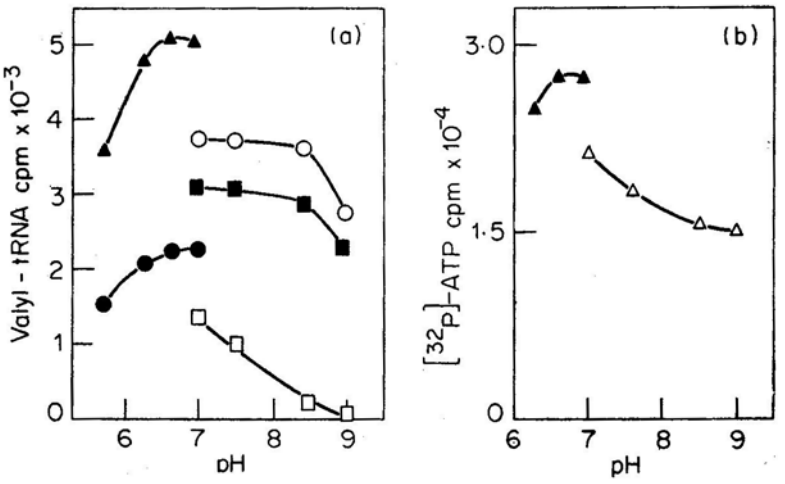

Figure 6. Effect of pH on spermine stimulation of aminoacylation.
(a) Aminoacylation of tRNA. ●—● HEPES buffer; 1·2mM Mg^{2+}. ▲—▲ HEPES buffer ; 1·2 mM Mg^{2+} plus 2mM spermine. ❑—❑ Tris-HCl buffer; l·2mMMg^{2+}. ■—■ Tris-HCI buffer; l·2mM Mg^{2+} plus 2mM spermine. O— O Tris-HCl buffer; 6mM Mg^{2+}
(b) PP_i exchange reaction. ▲——▲ HEPES; Δ——Δ Tris-HCl, Final concentration of Mg^{2+}, 2mM.

acid (HEPES) buffer the aminoacylation and PP_i exchange reactions were higher than inTris-HCl. Both reactions showed a pH optimum between 6·5 and 7. In the presence of 1·2 mM Mg^{2+}, spermine stimulated aminoacylation at all pH values tested. At pH 8·5–9·0, the low aminoacylation activity in the presence of 1 2 mM Mg^{2+} was restored to maximal levels on the addition of spermine or increasing the concentration of Mg^{2+} in the reaction mixture. These results were in contrast to the PP_i exchange reaction (figure 6b), which was affected much less at higher pH values; moreover, in the latter case, the observed reduction in activity at pH 8·5-9·0 was not restored by addition of polyamines.

These results clearly indicate that the pH and monovalent cation effects on enzyme activity were mostly mediated through the tRNA than directly on the enzyme.

Discussion

The results presented here with two pure amino acyl-tRNA synthetase preparations clearly show that there was no reaction in the presence of polyamine alone, but polyamines stimulated the reaction significantly at suboptimal concentrations of divalent cations. Moreover, they showed different effects on valyl- and isoleucyl-tRNA formation. Polyamines have been implicated in the *in vivo* aminoacylation of tRNA (Lovgren *et al.,* 1978). The differential effect of polyamines on *M. smegmatis* isoleucyl- and valyl-tRNA formation, together with the fact that the total concentration of Mg^{2+} can vary from 1·0-20 mM and polyamine from 0·1-10 mM in bacteria (Lovgren *et al.,* 1978), suggests that the polyamines may have a role in the regulation of formation of aminoacyl-tRNAs in bacteria.

Our studies with the reverse reaction show that spermine can influence this reaction also. The spermine-stimulation of aminoacylation is therefore, not attributable to the inhibition of reverse reaction. On the other hand, polyamine catalyses the hydrolysis of aminoacyl-tRNAs nonenzymatically at pH 7·0, suggesting that the actual synthesis of amino acyl-tRNA in a spermine stimulated reaction *in vitro* would have been more than what is experimentally determined. The nonenzymatic hydrolysis of N-acetyl [^{14}C] valyl-tRNA by spermidine at pH 8 ·8 has been reported (Schuber and Pinck, 1974); this has been explained by a polyelectrolyte model in which spermidine could bring about local pH changes around the ester bond leading to hydrolysis.

Available evidences suggest that polyamine may be expressing its effect by its action on both enzyme and tRNA. For instance, spermine inhibited the ATP-PP_i exchange reaction (Pastuszyn and Loftfield, 1972) and also pyrophosphorolysis of aminoacyl-AMP (Chakraburtty *et al.,* 1975) in which tRNA is not involved. Furthermore, spermine has been shown to have a binding site on isoleucyl-tRNA synthetase of *E. coli* (Holler, 1973). Two to three molecules of spermine have been found per molecule of tRNA isolated at low salt concentration (Sakai and Cohen, 1976). Four tightly bound Mg^{2+} and two spermine molecules have been located in the $tRNA^{Phe}$ crystal structure (Holbrook *et al.,* 1978; Quigley *et al.,* 1978) Spermine together with Mg^{2+} immobilises the anticodon loop and it has been suggested that both may be important in maintaining the three-dimensional fold of the tRNA molecule, recognised by the aminoacyl-tRNA synthetase (Quigley *et al.,* 1978). The monovalent cations like K^+, Na^+ and Li^+ are shown to have a

profound inhibitory effect on aminoacylation of tRNA than on ATP-PP_i exchange reaction in which tRNA is not involved and this has been attributed to the salt-dependent changes in tRNA (Smith, 1969; Deobagkar and Gopinathan, 1976). Our studies show that spermine or KCl (upto 100 mM) have no effect on partial reaction and hence, no direct effect on the enzyme. However, the total reaction was influenced by KCl at higher concentrations which was neutralised by spermine suggesting that whatever change has occurred on tRNA in presence of KCl could be prevented by spermine.

At low concentration of Mg^{2+} (1·2 mM), and pH 9·0 , no amino acylation was observed in the absence of spermine but the PP_i exchange reaction was not affected. Hence it is likely that the pH effect is also through tRNA rather than enzyme. Further, it is possible that the pH-mediated changes on tRNA structure could be prevented by spermine, or by increasing the concentration of Mg^{2+}. Recently Igarashi *et al.* (1978c) have shown that polyamine can also prevent the inhibitory effects of poly (G) and ribosome on aminoacylation.

Acknowledgement

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References

Calender, R. and Berg, P. (1966) *Biochemistry,* **5,** 1681.
Chakraburtti, K., Midelport, G. F., Steinschneider, A. and Mehler, A. H. (1975) *J. Biol. Chem.,* **250,** 3861.
Deobagkar, D. N. and Gopinathan, K. P. (1976) *Indian J. Biochem. Biophys.,* **13,** 24.
Deobagkar, D. N. and Gopinathan, K. P. (1978) *Can. J. Microbiol.,* **24,** 693.
Holbrook, S. R., Sassman, J. L. and Warrant, R. W. (1978) *J. Mol. Biol.,* **123,** 631.
Holler, E. (1973) *Biochemistry,* **12**, 1142.
Igarashi, K., Eguchi, K., Tanaka, M. and Hirose, S. (1978a) *Eur. J. Biochem.,* **82,** 301.
Igarashi, K., Eguchi, K., Tanaka, M. and Hirose, S. (1978b) *Eur. J. Biochem.,* **90,** 13.
Igarashi, K., Matsuzakai, K. and Takeda, Y. (1971) *Biochim. Biophys. Acta,* **254,** 91.
Igarashi, K., Tanaka, M., Eguchi, K. and Hirose, S. (1978c) *Biochem. Biophys. Res. Commun.,* **83,** 274.
Kisselev, L. L. and Favorova, O. O. (1974) *Adv. Enzymol.,* **40,** 141.
Loftfield, R. B. (1972) *Prog. Nucleic Acid Res. Mol. Biol.,* **12,** 87.
Lovgren, T. N. E., Peterson, A. and Loftfield, R. B. (1978) *J. Biol. Chem.,* **253,** 6702.
Natarajan, V. and Gopinathan, K. P. (1979) *Biochim. Biophys. Acta,* **568,** 253.
Pastuszyn, A. and Loftfield, R. B. (1972) *Biochem. Biophys. Res. Commun.,* **47,** 775.
Quigley, G. J., Teeter, M. M. and Rich, A. (1978) *Proc. Natl Acad. Sci. U.S.A.,* **75,** 64.
Sakai, T. T. and Cohen, S. S. (1976) *Prog. Nucleic Acid Res. Mol. Biol.,* **17,** 15.
Santi, D. V. and Webster, Jr. R. W. (1975) *J. Biol. Chem.,* **250,** 3874.
Schuber, F. and Pinck, M. (1974) *Biochimie,* **56,** 397.
Smith, D. W. E. (1969) *J. Biol. Chem.,* **244,** 896.
Takeda, Y. and Ogisco, Y. (1976) *FEBS Lett.,* **66,** 332.
Takeda, Y. and Ohnishi, T. (1975) *J. Biol. Chem.,* **250,** 3878.
Takeda, Y.,Ohnishi, T. and Ogiso, Y. (1976) *J. Biochem.,* **80,** 463.
Thiebe, R. (1975) *FEBS Lett.,* **51,** 259.
Thiebe, R. (1977) *FEBS Lett.,* **79,** 212.