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REPLACEMENT OF Mg^{2+} BY POLYAMINES IN THE AMINOACYLATION OF *t*RNA FROM *PHASEOLUS*

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; runner bean; *t*RNA; aminoacyl-*t*RNA-synthetases; polyamines; spermine; spermidine; putrescine.

Abstract—The ability of putrescine, spermidine and spermine to replace Mg^{2+} ions in the charging reaction of *t*RNA was estimated for seventeen amino acids. The polyamines promoted only the transfer reaction in the case of Leu, Ile, Val, Tyr and Arg. A synergistic effect was observed when spermine was added to a suboptimal concentration of Mg^{2+} (charging at only 5% of the optimal level). This synergistic effect was not observed for Ala, Asp-NH_2, His, Lys and Ser. Kinetic studies showed a slower aminoacylation rate in those experiments when spermine and Mg^{2+} (at 5% of the Mg^{2+} optimal concn) were used together than with Mg^{2+} (at the optimal concn) alone.

INTRODUCTION

Cohen [1] has pointed out the effects of polyamines on growth and biosynthetic processes. Aminoacyl-*t*RNA formation was stimulated by the three main polyamines (putrescine, spermidine and spermine) as effectively as by Mg^{2+} in *E. coli* [2]. In the presence of spermidine and a suboptimal concentration of Mg^{2+}, fixation of formylmethionine and Val in *E. coli* represented 80–85% of that obtained in presence of Mg^{2+} alone, at the optimal concentration [3]. In *E. coli*, the amino acid hydroxamate formation was not stimulated by spermine and there was no enzyme–aminoacyl–AMP complex formation in the first reaction of amino acid activation [4]. In this paper, we have tried to see if polyamines can replace Mg^{2+} *in vitro* for the aminoacylation of the *t*RNA of *Phaseolus vulgaris*.

RESULTS

Enzyme characterization

Optimal enzyme concentration. The amino acids could be classified in four groups according to the protein concentration necessary to reach maximum charging: His, Lys, Phe and Pro needed 0·2 mg of protein per ml of medium; Arg, Leu, Tyr and Val needed 0·4 mg; Ala, Asp–NH_2, Asp and Thr needed 0·8 mg and Glu, Gly, Ile and Ser needed 1·5 mg. When the protein concentration was greater than 0·2 mg/ml, a decreased incorporation was observed for lysyl-*t*RNA-synthetase.

Optimal Mg^{2+}/ATP ratios. The attachment of the 17 amino acids to the *t*RNA was studied using the corresponding enzymatic preparation, maintaining the ATP at 1 μmol in 0·1 ml and varying the Mg^{2+} from 0 to 8 μmol. The optimal Mg^{2+}/ATP ratio varied between 1 and 2·5. Leucyl-*t*RNA-synthetase and valyl-*t*RNA-synthetases were sensitive to high levels of Mg^{2+} ions, the inhibitions observed at a Mg^{2+}/ATP ratio of 8 were 50 and 80% respectively (Fig. 1).

Effect of various polyamines on aminoacyl-tRNA formation

Polyamines can only replace Mg^{2+} for 5 amino acids, Leu, Ile, Val, Arg and Tyr [5], and the extent of charging observed was respectively 60, 50, 30, 25 and 27% of the optimal charge obtained

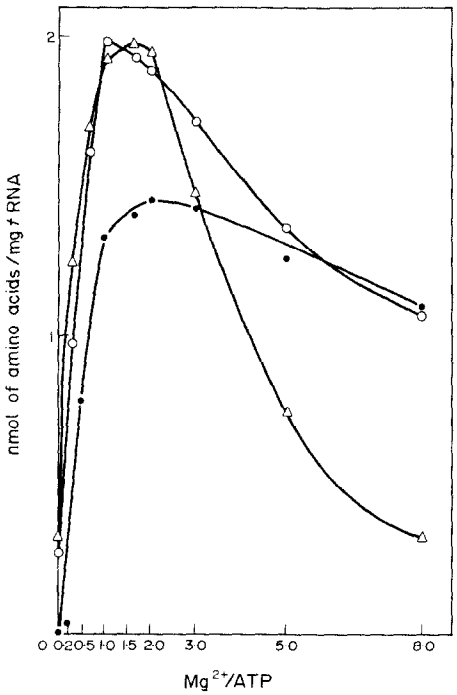

Fig. 1. Effect of increasing Mg^{2+}/ATP ratios on aminoacylation of *Phaseolus vulgaris* tRNA hypocotyls by homologous enzymes: (○) Leucine, (△) Valine, (●) Phenylalanine.

with Mg^{2+}. The polyamine concentration necessary for maximal rates were much lower than those needed with Mg^{2+}. The optimum concentration of spermine, spermidine and putrescine were 0·3–0·5, 2 and 5 mM, respectively.

Effect of spermine on aminoacyl-tRNA formation at different Mg^{2+} concentrations

As it appeared that in most cases spermine alone was not able to replace Mg^{2+}, this amine was combined with various concentrations of Mg^{2+}. A synergistic effect has been shown in rat liver for threonine, especially when a suboptimal concentration of Mg^{2+} was added to spermine [6]. The activation was up to 23 times at 1 mM Mg^{2+} and 1 mM spermine. For the amino acids Arg, Asp, Gly, Ile, Leu, Met, Phe, Thr, Tyr and Val, a synergistic effect was also observed in our system, particularly when a suboptimal concentration of Mg^{2+} (5% of the optimal level) was added to 0·5 mM spermine (Fig. 2). On the other hand, no effect was observed for Ala, Asp–NH_2, His, Lys, Pro and Ser. All incubations were made at 30° for 30 min. The importance of the synergistic effect was different for all the amino acids but it was always greatest at low Mg^{2+} concentrations.

Kinetic studies of the synergistic effect

Kinetic studies were made for those amino acids for which a synergistic effect was observed. These experiments were made with spermine at 0·5 mM and the suboptimal Mg^{2+} concentration giving the greatest effect. The plateau was only reached after 60 min. The activation was up to 3·5 times the level reached with the suboptimal Mg^{2+} concentration and was 80–90% of the optimal extent of charge. The reaction was slower than in the optimal conditions where the plateau was attained after 10 min.

DISCUSSION

Although in *E. coli*, polyamines alone seem able to replace Mg^{2+} [7], in *Phaseolus*, they could not replace Mg^{2+} with the same success. Since *in vivo* polyamines and Mg^{2+} are present together in plants [8–10], we tried to see if there was a synergistic effect of spermine and Mg^{2+}. For many amino acids (Arg, Asp, Gly, Ile, Leu, Met, Phe, Thr, Tyr and Val) an effect was observed, but for Ala, Asp–NH_2, His, Lys, Pro and Ser no effect

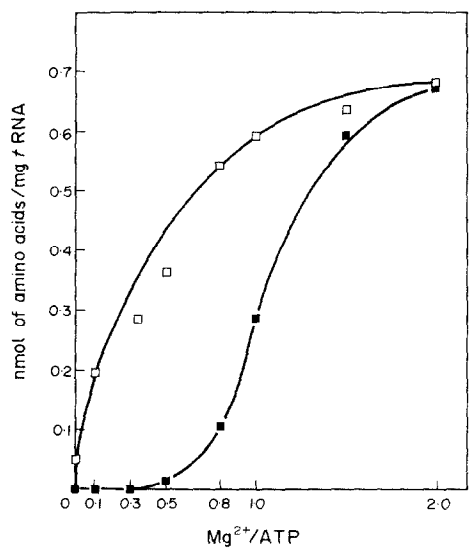

Fig. 2. Effect of spermine on methionyl-tRNA formation at different Mg^{2+} concentrations; (□) with 0·5 mM spermine, (■) without spermine.

was detected. The activation by spermine is more noticeable at low Mg^{2+} concentrations in the formation of aminoacyl-*t*RNA. These results suggest that small variations of Mg^{2+} concentration can have a dramatic effect on the ratios of the exchange vs transfer rates of aminoacyl-*t*RNA-synthetases when the polyamine effect is studied.

EXPERIMENTAL

Radioactive amino acids obtained from the Radiochemical Center, Amersham, and from the IRE Mol (Belgium) were adjusted to a sp act of 20 mCi/mmol.

Determination of proteins and tRNA concentrations. The spectrophotometric method of Ref. [11] was used to determine the protein concn. To determine the *t*RNA concn, the method of Ref. [12] (1 mg of *t*RNA in H_2O equivalent to an *E* value of 24 at 260 nm) was used.

Enzymes and tRNA preparations. To prevent phenolic oxidation, 0·4 M borate buffer pH 7·6 was used for extraction of aminoacyl-*t*RNA-synthetases [13,14]. Extraction of *t*RNA from *Phaseolus vulgaris* was made according to Ref. [15]. *t*RNA was extracted from 3-day-old, dark-grown hypocotyls and these were also used from preparation of aminoacyl-*t*RNA-synthetases. After the deproteinization step, the *t*RNA was purified by precipitation of high MW *r*RNA in M NaCl for 6 hr. After centrifugation *t*RNA was precipitated for 18 hr in cold EtOH and then purified on a DEAE-cellulose column. The *t*RNA was deacylated in Tris–HCl buffer pH 8·5 for 90 min at 37°. To remove bound Mg^{2+} from the *t*RNA preparation, the latter was dialyzed successively against a soln containing 1 mM EDTA and 2 M NaCl, against a soln containing 1 mM EDTA, and finally against H_2O. From 100 g of fresh bean hypocotyls, 10–15 mg of *t*RNA was obtained.

Standard assay for aminoacyl-tRNA formation. The standard reaction mixture (0·1 ml) for the aninoacylation of *t*RNA contained the following: 50 μmol cacodylate–HCl buffer pH 7·4, 10 μg bovine serum albumin, 10 μmol neutralized ATP, 3 μmol KCl, 0·1 μmol 2-mercaptoethanol, 16–25 μg *t*RNA, 10 nmol 1-^{14}C-aminoacids. The enzyme soln, $Mg(OAc)_2$, spermine, spermidine, putrescine were added at concns indicated. After incubating the reaction mixture at 30°, aliquots (80 μl) of each reaction mixture were placed at intervals on a Whatman 3 MM paper disc (25 mm diam), washed ×3 in 5% TCA for 10 min and ×3 in EtOH. Discs were dried and counted in a liquid scintillation counter.

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