INTERRELATIONSHIP BETWEEN MAGNESIUM AND POLYAMINES IN A PSEUDOMONAD LACKING SPERMIDINE*

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SUMMARY: We have verified the report that Pseudomonas (Kim) contains no spermidine, but have found that this strain contains another polyamine, 2-hydroxyputrescine, previously unidentified in bacteria. As in Escherichia coli the concentration of magnesium in the S-100 fraction is the same as the extracellular concentration. Unlike Escherichia coli both ribosome-bound putrescine and ribosome-bound hydroxyputrescine vary inversely with extracellular concentration of magnesium, but the behavior of bound hydroxyputrescine in Pseudomonas (Kim) resembles that of bound spermidine in Escherichia coli.

We have reported that Escherichia coli can grow at optimal rates under conditions where the intracellular concentration of magnesium is as low as 4×10^{-5} M and where ribosome-bound magnesium is about 1/5 that required for protein synthesis in vitro. Ribosome-bound magnesium varied directly with extra- and intracellular concentration of magnesium while ribosome-bound spermidine varied inversely. Ribosome-bound putrescine was not affected by the intracellular concentration of magnesium (5). As a result of these findings and of several reports indicating that spermidine may replace Mg^{2+} at least partially in various aspects of protein synthesis (1, 3, 7, 8, 11, 12) we have postulated that spermidine may act as a polyfunctional ligand in vivo for properly aligning the components of the protein synthesizing complex (5).

The report that a strain of Pseudomonas lacked spermidine and contained only putrescine (6) was of obvious interest as a test of the above hypothesis. We have verified the finding that this strain contains no spermidine, but have found that it contains another polyamine, previously unidentified in bacterial strains.

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Since we have now identified this unknown polyamine as 2-hydroxyputrescine, it will be referred to as such in the following. Its identification will be described in another report (10). In this report we will describe the isolation of this polyamine and the effect of extracellular magnesium upon its binding to ribosomes in vivo.

METHODS: Minimal medium (2) was prepared in half concentration, to allow for centrifugation of the cells through silicone as described by Hurwitz et. al. (4). Magnesium was added as the chloride as indicated. The pellet of cells collected under silicones was disrupted by a French pressure cell. Magnesium in solution in the 104,000 x g supernatant fluid (S-100 fraction), magnesium bound to ribosomes, and polyamines bound to ribosomes were determined as described previously (5).

RESULTS: Cells were grown in minimal media containing three different concentrations of magnesium: 4×10^{-5}M, 4×10^{-4}M and 4×10^{-3}M. The growth rate was the same at each concentration of magnesium. The effect of extracellular magnesium on the concentration of magnesium in the S-100 fractions is shown in Table 1. As with E. coli ML35 (5), the concentration of magnesium in each S-100 fraction was the same as in the growth medium. It is possible, although not likely, that some accumulation of Mg^{2+} against a concentration gradient may have occurred at 4×10^{-5}M Mg^{2+} since this initial concentration of Mg^{2+} in the growth medium was reduced to 3.4×10^{-5}M at the time of collection of the cells.

The effect of extracellular magnesium on ribosome-bound magnesium is shown in Table 2. As extracellular magnesium increased from 4×10^{-5}M to 4×10^{-3}M the ratio of umoles Mg^{2+} to umoles RNA-ribose increased from .075 to 0.24, indicating that the increases in intracellular magnesium with increased extracellular magnesium results in increased binding of magnesium to ribosomes. The values are in good agreement with those obtained with E. coli ML35 over the same range of extracellular magnesium.

Bound polyamines were determined as described previously (5), using paper electrophoresis at 600 volts. The chromatogram in Fig. I confirms the pres-

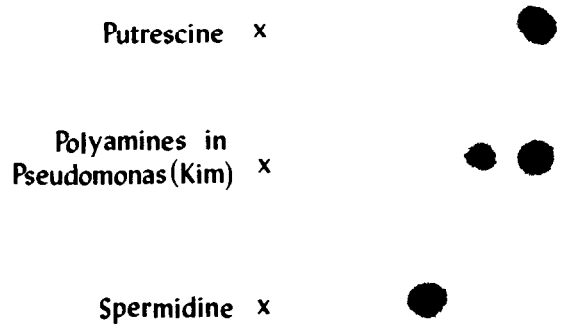

Fig. 1. Separation of the unknown ribosome-bound polyamine from spermidine and putrescine by paper electrophoresis.

For details of the extraction and electrophoresis of ribosome-bound polyamines see "Methods" and the legend under Fig. 2.

The migration velocities at 600 volts are 4.6 cm per hr. for spermidine, 6.0 cm per hr. for hydroxyputrescine, and 7.5 cm per hr. for putrescine.

TABLE I

Concentration of Magnesium

In Growth Medium	In Fraction S-100
M	M
4×10^{-5}	$4.2 \pm 0.5 \times 10^{-5}$
4×10^{-4}	$4.8 \pm 0.8 \times 10^{-4}$
4×10^{-3}	$5.1 \pm 0.4 \times 10^{-3}$

Pseudomonas (Kim) was grown out to 3×10^{8} cells per ml in one-half strength minimal medium containing the indicated concentration of magnesium. Fraction S-100 was isolated as described previously (5).

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ence of putrescine and the absence of spermidine, but reveals the presence of another polyamine having a migration velocity between those of putrescine and spermidine. Paper and column chromatography as used by Kim (6) fails to separate this compound from putrescine.

In *E. coli* ML35, spermidine bound to ribosomes has been shown to be inversely proportional to ribosome-bound magnesium while ribosome-bound putrescine was unaffected by Mg^{2+} (5). With *Pseudomonas* (Kim), the amount of both

TABLE II

Effect of Extracellular Mg^{2+} on Ribosome-bound Mg^{2+} and Putrescine

Concentration of Mg^{2+} in medium	umoles per umole RNA-ribose*			
	Mg^{2+}	Putrescine	Spermidine	Hydroxy-Putrescine
Pseudomonas (Kim)				
4×10^{-5}M	.075 ± .011	.145 ± .020	0	.038 ± .002
4×10^{-4}M	.130 ± .010	.071 ± .008	0	.022 ± .003
4×10^{-3}M	.240 ± .030	.024 ± .003	0	.013 ± .001
E. coli ML35**				
4×10^{-5}M	.090 ± .010	.071 ± .066	.027 ± .004	0
4×10^{-4}M	.138 ± .015	.059 ± .008	.014 ± .002	0
4×10^{-3}M	.240 ± .025	.063 ± .009	.007 ± .002	0
Pseudomonas fluorescens				
4×10^{-4}M	.147	.070	.018	0

* Calculated as umoles ribose in the RNA fraction of the ribosomes

** Values obtained from Hurwitz and Rosano (5).

ribosome-bound putrescine and hydroxyputrescine varied inversely with ribosome-bound magnesium (See Table 2 and Fig. 2). Within the range of extracellular magnesium from 4×10^{-5}M to 4×10^{-3}M, the ribosome-bound putrescine decreased approximately six-fold over a hundred-fold increase in concentration of extracellular magnesium while the ribosome-bound hydroxyputrescine decreased three-fold. Over this same range of extracellular magnesium, ribosome-bound magnesium increased approximately three-fold. The ratio of bound putrescine to bound hydroxyputrescine varied from 3.6 at 4×10^{-5}M Mg^{2+} to 1.6 at 4×10^{-3}M Mg^{2+}.

As seen from Fig. 2, the amount of ribosome-bound putrescine in Pseudomonas (Kim) appears to vary reciprocally with ribosome-bound magnesium, the sum of bound putrescine plus bound Mg^{2+} per umole RNA-ribose over the entire range of extracellular magnesium being .228 ± .027. In E. coli ML35, ribosome-bound putrescine remained constant at .064 ± .009 umole per umole RNA-ribose over the

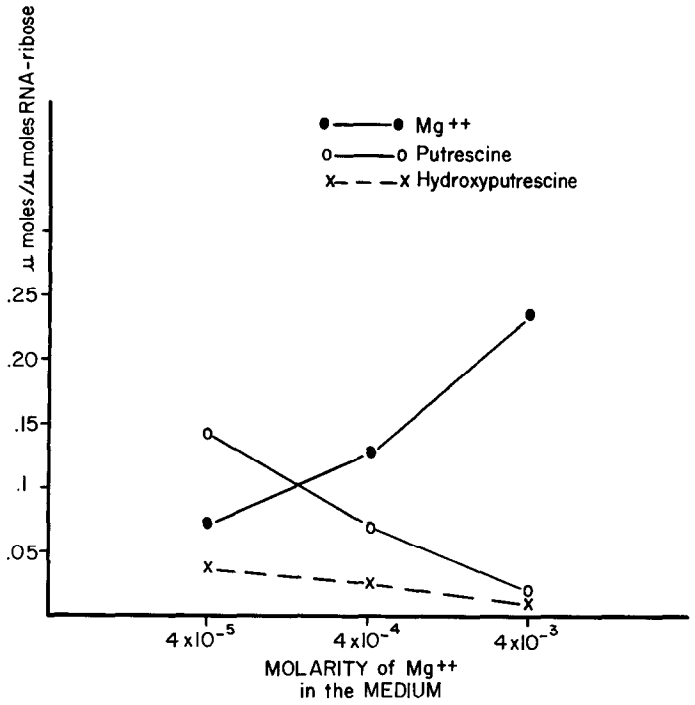

Fig. 2. Effect of concentration of magnesium in the growth medium on the ribosome bound magnesium and polyamines in Pseudomonas (Kim). Ribosomes were obtained from log-phase cells grown at indicated concentrations of magnesium. The procedures for collecting and disrupting the cells, as well as the procedures for extracting the ribosome-bound polyamines and magnesium, were described previously (5). Separation of polyamines by paper electrophoresis was performed at 600 volts for 60 minutes. The conducting solvent was 0.1M citrate buffer at pH 6.5.

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same range of external magnesium, while in Pseudomonas (Kim) the ribosome-bound putrescine decreased from .145 umole to .024. Unlike putrescine, the values for ribosome-bound hydroxyputrescine in Pseudomonas (Kim) are in reasonably good agreement with values for spermidine in E. coli (See Table 2). Perhaps even more striking is the finding that at 4×10^{-4} M extracellular magnesium, the umoles hydroxyputrescine per umole RNA-ribose in Pseudomonas (Kim) are about equal to the umoles spermidine per umole RNA-ribose in Pseudomonas fluorescens which contains no hydroxyputrescine.

DISCUSSION: We are presently investigating the possibility that hydroxyputrescine may play the same role in protein synthesis by Pseudomonas (Kim) as

postulated for spermidine in E. coli (5). The possibility is especially intriguing since, unlike putrescine, hydroxyputrescine has the same number of hydrogen-binding sites as does spermidine; and like spermidine in E. coli, hydroxyputrescine varies inversely with extracellular magnesium. We are presently preparing ^{3}H-hydroxyputrescine to study its binding characteristics.

Unlike E. coli, ribosome-bound putrescine in Pseudomonas (Kim) also varies inversely with concentration of magnesium. The physiological function of putrescine in protein synthesis is unknown. In E. coli, spermidine, but not putrescine, can partially substitute for magnesium in stimulating aggregation of ribosomal subunits (1). Moller and Kim (9) have found that in this spermidineless pseudomonad, putrescine at 10 mM stimulates aggregation of ribosomal subunits, although the pattern of aggregates obtained is not the same as when ribosomes are dialyzed vs. 10 mM Mg^{2+}. Aggregates formed in the presence of 1 mM Mg^{2+} plus 10 mM putrescine were less effective in cell-free protein synthesis than those formed in the presence of 10 mM Mg^{2+}, although addition of 10mM putrescine to 1 mM Mg^{2+} markedly stimulated the ability of the cell-free preparation to synthesize polyphenylalanine. It is also interesting to note that addition of 10 mM putrescine to 1 mM Mg^{2+} (but not to 10 mM Mg^{2+}) appeared to reduce misreading since poly U-mediated leucine incorporation into protein was decreased several-fold.

The reciprocal nature of the curves for ribosome-bound putrescine and magnesium suggests that in Pseudomonas (Kim) putrescine, like Mg^{2+}, may aid in neutralizing anionic charges in ribosomal subunits which might otherwise repel each other. Such a reciprocal relationship does not appear to operate in E. coli, where ribosome-bound putrescine is not affected by concentration of Mg^{2+}.

Spermidine and hydroxyputrescine appear to behave differently from putrescine. In E. coli, bound spermidine, but not bound putrescine varied with concentration of magnesium (5). In Pseudomonas (Kim) bound hydroxyputrescine varied with concentration of magnesium in a manner quite different from bound

putrescine, but similar to the behavior of bound spermidine in E. coli and in Ps. fluorescens.

These findings suggest that spermidine and hydroxyputrescine may play a more specific role than magnesium or putrescine in protein synthesis in vivo possibly by acting as polyfunctional ligands in aligning the components of the protein synthesizing complex.

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