NECESSITY OF POLYAMINES FOR MAXIMUM ISOLEUCYL-tRNA FORMATION IN A RAT LIVER CELL-FREE SYSTEM

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SUMMARY: From a study of the effect of polyamines on aminoacyl-tRNA formation of nine amino acids in a rat liver cell-free system, it is shown that isoleucyl-tRNA formation in the presence of polyamines is much greater than that in the presence of Mg^{2+}. The data suggest that polyamines may play an important role in protein synthesis by regulating aminoacyl-tRNA formation.

The replacement of magnesium ions by polyamines in polypeptide synthesis in cell-free system of various organisms (1-10) has been reported by many workers.

To elucidate further the role of polyamines in polypeptide synthesis, it is necessary to investigate in detail the effect of polyamines on various steps of polypeptide synthesis. In this respect, we have reported that polyamines can stimulate some aminoacyl-tRNA formation in an E. coli cell-free system without adding Mg^{2+} (11, 12).

In this communication, the effect of polyamines on aminoacyl-tRNA formation in a rat liver cell-free system has been studied. Evidence is presented that isoleucyl-tRNA formation in the presence of polyamines is much greater than that in the presence of Mg^{2+}. The physiological roles of polyamines in rat liver protein synthesis are discussed also.

MATERIALS AND METHODS

Preparation of aminoacyl-tRNA synthetase - All praparation steps were performed at 4°C, unless indicated otherwise. Rat liver

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S100, prepared as described previously (10), was used as the starting material. After RNA was precipitated by purified protamine (13) and removed by centrifugation, saturated ammonium sulfate solution (pH 7.5) was added to the supernatant fluid to obtain 30% saturation with ammonium sulfate. The suspension was centrifuged for 15 min at 20,000 x g and the precipitate was discarded. To the supernatant solution, saturated ammonium sulfate solution (pH 7.5) was added to obtain 70% saturation. The precipitate collected by centrifugation was dissolved in Buffer I (10% glycerol, 0.01M Tris-HCl pH 7.5, 0.05M KCl, and 0.006M 2-mercaptoethanol) and dialyzed overnight against the same buffer. The dialyzed ammonium sulfate fraction (50mg of protein) was applied to a column of DEAE-Sephadex (2 x 35cm) equilibrated with Buffer I. After the column was washed with 200ml of Buffer I, the enzymes were eluted stepwise with 200ml of Buffer II (10% glycerol, 0.01M Tris-HCl pH 7.5, 0.15M KCl, and 0.006M 2-mercaptoethanol) and 200ml of Buffer III (10% glycerol, 0.01M Tris-HCl pH 7.5, 0.25M KCl, and 0.006M 2-mercaptoethanol) successively. The major portion of both peaks was collected separately and concentrated by ultrafiltration. These two fractions were designated as Fr. I (0.15M KCl eluate) and Fr. II (0.25M KCl eluate), respectively. Fr. I and Fr. II were dialyzed overnight against Buffer I and stored at -80°C until used.

Standard assay procedure for aminoacyl-tRNA formation - The standard reaction mixture (0.1ml) for the aminoacylation of tRNA contained the following: 0.05M Tris-HCl pH 7.5, 0.01M NH_4Cl, 0.006M 2-mercaptoethanol, 0.0025M ATP, 100µg of rat liver tRNA, 0.1µCi of ^{14}C-amino acid, and enzyme solution (100µg of Fr. I or 120µg of Fr. II). Magnesium acetate and polyamines were added at the concentration as indicated in the tables. Rat liver tRNA was made from S100 by the method of Zubay (14) except that the isopropanol treatment

was omitted. The specific activities of the ^{14}C-amino acids used in the experiments were as follows: arginine, 175μCi/μmole; glycine, 99.7μCi/μmole; isoleucine, 279μCi/μmole; leucine, 50μCi/μmole; lysine, 216μCi/μmole; methionine, 59μCi/μmole; phenylalanine, 382μCi /μmole; tyrosine, 50μCi/μmole; and valine, 50μCi/μmole. After the reaction mixture was incubated at 30°C for 30 min, a 0.08ml aliquot of each reaction mixture was placed on a paper disc (25mm diameter) and cold trichloroacetic acid insoluble radioactivity was counted by a Beckman liquid scintillation spectrometer. The counting efficiency was 1.2×10^6 cpm per μCi.

RESULTS

Effect of spermine and magnesium acetate on nine aminoacyl-tRNA formations - The fact that Mg^{2+} is essential for the aminoacyl-tRNA formation is now well established (15, 16). We have recently reported the replacement of magnesium ions by polyamines in some aminoacyl-tRNA formations in an E. coli cell-free system (11, 12). In the rat liver cell-free system, nine aminoacyl-tRNA formations were examined using partially purified aminoacyl-tRNA synthetases (Fr. I or Fr. II) either in the presence of Mg^{2+}or spermine. As shown in Table 1, aminoacyl-tRNA formations can be classified into three types: (a) aminoacylation at 10mM Mg^{2+} is greater than that at 2mM spermine (arginyl-, glycyl-, phenylalanyl-, and tyrosyl-tRNA formation); (b) aminoacylation at 10mM Mg^{2+} is similar to that at 2mM spermine (lysyl-, methionyl-, and valyl-tRNA formation); and (c) aminoacylation at 10mM Mg^{2+} is less than that at 2mM spermine (isoleucyl-, and leucyl-tRNA formation). To obtain a greater understanding of the function of polyamines in the formation of aminoacyl-tRNA, the isoleucyl-tRNA and phenylalanyl-tRNA systems, as representatives of the groups (a) and (c) just described, were studied in greater detail.

Table 1. Effect of spermine and magnesium acetate on aminoacylation of tRNA with various amino acids.

Amino acids used for aminoacylation	Fr. used	Aminoacyl-tRNA formed (CPM)* Mg^{2+} (10mM)	Spermine (2mM)
Arg	I	4315	1653
Gly	II	1034	591
Ile	II	218	690
Leu	II	415	1076
Lys	II	2002	1876
Met	I	498	468
Phe	I	3844	290
Thr	I	1917	1342
Val	I	418	401

*Radioactivity adsorbed to a paper disc of a sample without ATP was subtracted from each value.

Effect of spermine on isoleucyl- and phenylalanyl-tRNA formations in the presence of various concentration of Mg^{2+} - The experimental results in Table 2 indicate that isoleucyl-tRNA formation in the presence of 2mM spermine and various concentrations of Mg^{2+} is greater

Table 2. Effect of spermine and magnesium acetate on isoleucyl-tRNA and phenylalanyl-tRNA formation.

Ions (mM) Mg^{2+}	Spermine	Aminoacyl-tRNA formed (CPM) Ile	Phe
1	0	92	1240
1	2	662	1363
2	0	84	2145
2	2	682	2214
5	0	198	3412
5	2	660	3398
10	0	240	3584

The conditions of the experiment were as indicated in Table 1.

than that in the presence of 10mM Mg^{2+}. These data are consistent with the observation that isoleucyl-tRNA formation at 10mM Mg^{2+} is less than that at 2mM spermine (Table 1). In contrast, spermine stimulates phenylalanyl-tRNA formation very slightly and only in the presence of low Mg^{2+} (1mM and 2mM).

Effect of other polyamines on isoleucyl- and phenylalanyl-tRNA formation - As shown in Table 3, spermidine and putrescine stimulated isoleucyl-tRNA formation in the presence of 1mM or 2mM Mg^{2+}. Although the degree of stimulation by spermidine and putrescine was almost the same as the stimulation by spermine, the optimal concentrations for stimulation by spermidine and putrescine were higher.

Table 3. Effect of different polyamines on isoleucyl-tRNA and phenylalanyl-tRNA formation.

Ions (mM)				Aminoacyl-tRNA formed (CPM)	
Mg^{2+}	Spermine	Spermidine	Putrescine	Ile	Phe
1	-	-	-	115	988
1	1	-	-	530	989
1	3	-	-	689	1172
1	-	5	-	535	1085
1	-	10	-	520	1061
1	-	-	10	471	993
1	-	-	20	519	997
2	-	-	-	86	1954
2	1	-	-	452	2012
2	3	-	-	673	1949
2	-	5	-	528	2073
2	-	10	-	524	2016
2	-	-	10	436	1967
2	-	-	20	521	1911
10	-	-	-	286	3712

The conditions of the experiment were indicated in Table 1.

It has been reported that in some aminoacyl-tRNA formations certain monovalent cations can replace Mg^{2+} either completely or partially (17-19). In isoleucyl-tRNA formation, monovalent cations could not substitute for polyamines, although they could stimulate isoleucyl-tRNA formation slightly (unpublished data).

DISCUSSION

Polyamines have been implicated in numerous growth processes (20), although their physiological role is not fully understood. During our studies of the effect of polyamines on protein synthesis, we have proposed that Mg^{2+} usually is replaceable by polyamines in protein synthesis. In addition, we have recently found in both E. coli and rat liver cell-free systems that polyamines not only have a sparing effect on the Mg^{2+} requirement for polyphenylalanine synthesis but also a stimulating effect which can not be fulfilled by any amount of Mg^{2+} in the absence of polyamines (21). However, the stimulatory effect by polyamines in the rat liver cell-free system was less than that in the E. coli cell-free system. Therefore, we have looked for another regulatory step in polypeptide synthesis by polyamines.

In an E. coli cell-free system, polyamines can replace Mg^{2+} in some aminoacylations but can not produce an overall stimulation of aminoacyl-tRNA formation (11, 12). In the present studies of the rat liver cell-free system, we have found that isoleucyl- and leucyl-tRNA formations in the presence of polyamines are greater than those in the presence of Mg^{2+}.

These results infer that polyamines may play an important role in protein synthesis by regulating aminoacyl-tRNA formation. Further purification of the isoleucyl-tRNA synthetase is now in progress so that the properties of this enzyme can be studied in detail.

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