EFFECT OF POLYAMINES AND DIVALENT METALS ON IN VITRO INCORPORATION OF AMINO ACIDS INTO RIBONUCLEOPROTEIN PARTICLES*

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A possible correlation between the biological action of polyamines and some divalent inorganic cations was suggested by the observation on the ability of spermine and related diamines to inhibit bacterial lysis induced under certain conditions by metal chelating substances (Mager, 1959). This concept was substantiated by the subsequent finding that Ca^{++} and Mg^{++} at relatively low concentrations (5×10^{-4}M) prevented, in a manner similar to that previously observed with spermine (10^{-4}M) (Mager, 1955), the respiratory decay of Pasteurella tularensis cells resulting from the leakage of essential cell constituents, which takes place in a suspending medium of low tonicity (Mager, to be published).

The present communication provides additional evidence attesting to a close relationship in the mode of action of polyamines and divalent metals.

Results and Discussion

The experiment illustrated in Fig. 1 shows that the reduced incorporation activity exhibited by the microsomal system at sub-

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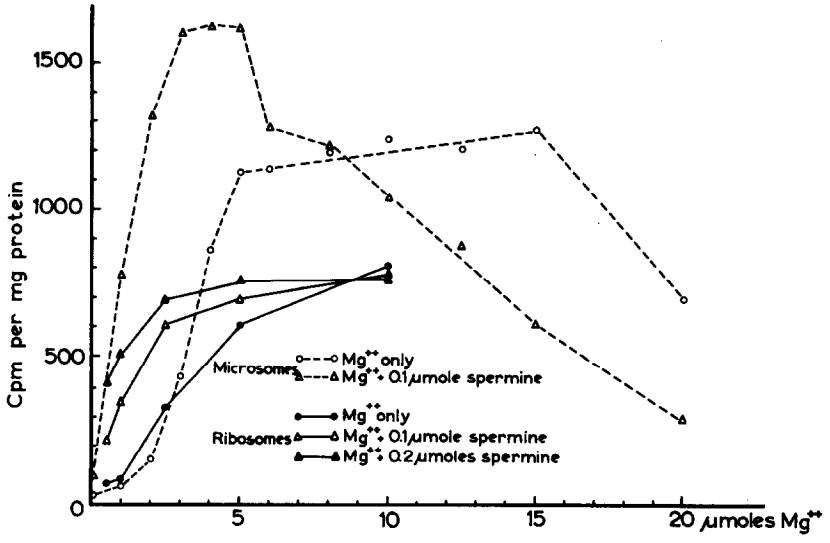

Fig. 1. Replaceability of Mg^{++} by spermine in the microsomal and ribosomal amino acid incorporation systems. The reaction mixture contained in 1 ml volume the following components: 50 μM Tris buffer, pH 7.5; 100 μM KCl; 1 μM ATP; 0.2 μM GTP; 1 μM $MgSO_4$; 10 μM 3-phosphoglycerate (potassium salt); 10 μM reduced glutathione (sodium salt); 0.2 μM DL-leucine-1-^{14}C (app. 700,000 cpm); 0.4 mg protein of dialyzed rabbit muscle extract, 52% - 72% $(NH_4)_2SO_4$ saturation-fraction (Cori et al., 1948); 1.5 mg of rat liver cell sap; 0.5 mg protein of rat liver microsomes (Keller and Zamecnik, 1956) or ribosomes (Kirsch et al., 1960) respectively. Incubation: 30 min. at 37°C. Assay procedures were the same as previously described (Mager and Lipmann. 1958; Izak et al., 1960).

optimal levels of Mg^{++} (cf. Keller and Zamecnik, 1956; Sachs, 1958) was strikingly enhanced by addition of spermine. The requirement of the incorporation system for added Mg^{++} as well as the extent of the stimulatory effect of spermine increased parallel to declining amounts of microsomes. The mutual replaceability of Mg^{++} and

spermine is indicated in Fig. 1 by the essentially reciprocal relationship prevailing between the varying concentrations of Mg^{++} and the degree of stimulation produced by adding a constant amount of spermine (10^{-4}M). It may be noted, however, that a combination of spermine and suboptimal amounts of Mg^{++} in suitable proportions resulted in an increase of the incorporation over and above the maximum level attainable with optimal concentrations of Mg^{++} alone. Furthermore, the additive nature of the activities mediated by Mg^{++} and spermine seems to be also reflected in the inhibitory effect exerted by spermine in the presence of optimal amounts of Mg^{++}; the latter phenomenon may be interpreted as a shift of the Mg^{++} excess-inhibition zone to lower levels of Mg^{++}.

Strictly analogous results were obtained when, under otherwise unaltered experimental conditions, the microsomal preparation was replaced by ribosomal particles.

The functional equivalence of Mg^{++} and spermine was further borne out by the ability of either agent to alleviate the inhibition of microsomal incorporation due to excessively high concentrations of monovalent cations, as well as by the capacity of spermine to obviate the disruptive action of chelating substances on the microsomal particles (cf. Sachs, 1958; Tabor, 1960).

Out of a selection of various polyamines tested, spermidine putrescine, cadaverine and agmatine (in decreasing order of potency) exhibited an activity which was essentially similar to, though considerably lower than, that observed with spermine; 1,3 - diaminopropane and 1,6 - diaminohexane proved to be totally ineffective. In a parallel survey of divalent metals, Ca^{++}, Mn^{++} and Co^{++} were found to duplicate to a varying extent of efficiency the incorporation promoting activity of Mg^{++}.

The described parallelism in the activity patterns of poly-

amines and divalent metals appears to be confined to a specific common site, located in the ribonucleoprotein particles. Thus, suitably designed experiments demonstrated the inability of spermine to substitute for Mg^{++} in the ATP regenerating system used in the incorporation experiments, as well as its failure to replace Mg^{++} in supporting the ATP-pyrophosphate exchange catalyzed by the soluble cytoplasmic fraction. Furthermore, spermine showed no appreciable effect on amino acid incorporation into S-RNA. On the other hand, Mg^{++}, Ca^{++} and spermine proved to substitute for each other in furthering the transfer of ^{14}C-leucine bound to S-RNA to the microsomal protein (Table I).

TABLE I

EFFECT OF Mg^{++}, Ca^{++} AND SPERMINE ON THE TRANSFER OF ^{14}C-LEUCINE FROM S-RNA TO MICROSOMAL PROTEIN

Additions /µmoles			Counts per minute
Mg^{++}	Ca^{++}	Spermine	per mg protein
2	-	-	66
8	-	-	634
2	2	-	522
2	-	0.1	643

Conditions and reaction mixture as in Fig. 1, but DL-leucine-1-^{14}C replaced by DL-leucine-1-^{14}C bound to 160 µg of S-RNA and containing 850 cpm (cf. Hoagland et al., 1958).

The results of the present investigation appear to link up with the well documented physicochemical notions, regarding the fundamental role of Mg^{++} and other divalent metals in preserving the structural integrity of the ribonucleoprotein particles (Chao and Schachman, 1956; Ts'o et al., 1958; Hamilton and Peterman, 1959). Recent publications, apart from demonstrating the natural occurrence of substantial quantities of polyamines in ribonucleoprotein preparations, have also revealed a stabilizing influence of these compounds on the ribosomal structure, closely resembling the action of Mg^{++} (Zillig et al., 1959; Cohen and Lichtenstein, 1960).

In the light of the above findings it appears reasonable to postulate that polyamines, by virtue of their stabilizing effect on the ribonucleoprotein particles, may play an important role in determining the rate of protein synthesis, according to the metabolically conditioned fluctuations in their intracellular content.

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