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ENHANCING EFFECT OF MAGNESIUM ION ON CELL-FREE SYNTHESIS OF READ-THROUGH PROTEIN OF BACTERIOPHAGE Qβ

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SUMMARY: This report describes the enhancing effect of magnesium ion on the synthesis of read-through protein of bacteriophage Qβ in a cell-free protein synthesizing system from E. coli. At 6 mM of magnesium acetate, the major product was coat protein. At 12 mM of magnesium, it was replaced by read-through protein. This enhanced synthesis was substituted by the addition of 0.25 mM of spermine or 1 mM of spermidine to 6 mM of magnesium. These results suggest that magnesium or combination of magnesium and polyamines causes leaky termination at the end of the coat protein cistron of Qβ-RNA.

It is well known that read-through (A1 or IIb) protein of RNA phage Qβ is a product of improper translation of a termination codon UGA of coat protein cistron (1-4). When translation is terminated at the UGA codon of the coat protein cistron, coat protein is formed. If termination is suppressed at this position by appropriate factors, translation proceeds for another 600 nucleotides of intercistronic region between the coat and replicase subunit cistrons, resulting in read-through protein.

Since Qβ grows normally even in a non-suppressing strain of E. coli, in spite of the fact that read-through protein is an essential structural component of the Qβ virion (5), it has been suggested that leaky termination codon UGA may participate in read-through protein synthesis (4). However, the mechanism by which leaky termination takes place has remained obscure. In this report, we describe the enhanced synthesis of read-through protein accompanying an elevated concentration of magnesium ion. The results suggest that magnesium plays a role in causing leaky termination of the UGA codon.

MATERIALS AND METHODS

Purification of RNA phages and preparation of their RNAs: RNA phage Qβ, MS2, JP34, ST, and FI were separately grown on E. coli Q13 (Hfr, met$^-$, RNase

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I^-, Su^-) as described previously (6). Purification of RNA phages and preparation of their RNAs were performed by the methods of Pace et al. (7). RNAs were dissolved in TM buffer containing 10 mM of Tris-HCl (pH 7.5) and 5 mM of magnesium acetate, and then stored at -75°C until use.

Preparation and analysis of phage proteins synthesized in the cell-free system: Cell-free protein synthesis was carried out in a reaction mixture (0.2 ml) containing 50 mM of Tris-HCl (pH 7.8), various amounts of magnesium acetate (see figure legends), 60 mM of NH_4Cl, 7 mM of 2-mercaptoethanol, 4 mM of phosphoenolpyruvate, 1 mM of ATP, 0.2 mM of GTP, 25 μg/ml of pyruvate kinase, proper amount of [^{3}H]-lysine or [^{14}C]-amino acid mixture (see legends), 32 A_{260} units/ml of preincubated S-30 extract from E. coli Q13 (8, 9), and 100 μg/ml of phage RNA. After incubation for 45 min at 37°C, the reaction mixture was treated with alkali (9). The trichloroacetic acid-insoluble materials, which had been washed with a mixture of ethanol and ether (1:1), were solubilized, applied to a 12.5% polyacrylamide gel containing 0.1% Na dodecyl sulfate and 6 M of urea, and run at 2.5 mA/tube for 22 hr (10).

RESULTS AND DISCUSSION

During studies (11) on the biosynthesis of read-through protein of RNA coliphages, we observed that minor change in the concentration of magnesium quantitatively affected the read-through protein synthesis directed by Qβ-RNA in a cell-free system from E. coli. In order to confirm this observation, we further examined the effect of magnesium ion on read-through protein synthesis. As shown in Fig. 1, proteins synthesized in vitro were analyzed by polyacrylamide gel electrophoresis and three major peaks were identified as replicase subunit (peak I), read-through protein (peak II), and coat protein (peak III), respectively, according to various procedures (1, 4, 11, 12). At the same time, it can be seen that these proteins were differentially synthesized by changing the concentration of magnesium acetate, although 8 to 8.5 mM of magnesium acetate was optimum for overall protein synthesis in the

tRNA synthetases 370- and 140-fold pure, respectively. The final product is devoid of all other aminoacyl-tRNA synthetases.

An interesting aspect of the purification is the copurification of the small molecular weight enzymes. In addition to the procedures used in the purification, CM-agarose and preparative isoelectric focusing also failed to separate the activities. The association of lysyl- and arginyl-tRNA synthetases with each other has also been documented by other workers. In rabbit reticulocytes, Irvin and Hardesty (11) found these enzymes to be associated with ribosomes and with each other in a 14S enzyme complex. From rat liver, Goto and Schweiger (17) purified lysyl-tRNA synthetase 440-fold and found that arginyl-tRNA synthetase copurified during the procedure 260-fold. More recently, out of rat liver, Dang and Yang (9) were unable to separate the enzymes by affinity chromatography on a lysine-Sepharose column. Whether or not the two activities exist on the same or separate polypeptides cannot yet be stated. It must be noted, however, that one group (18) using rat liver has purified arginyl-tRNA synthetase 100-fold having only 3% lysyl-tRNA synthetase still present.

In order to determine purity and an apparent molecular weight, a sample of the purified synthetases was chromatographed on Sephadex G-200 as described in Figure 1. A single protein peak emerged from the column, and it coincides precisely with both enzyme activities. The specific activities of the enzymes were constant throughout the peak, with lysyl-tRNA synthetase varying from 13 to 16.1 units/mg and arginyl-tRNA synthetase from 4.8 to 5.5. units/mg, providing evidence for the homogeneity of both activities. The enzymes eluted from the column at an apparent molecular weight of about 240,000.

Further attempts to ascertain the purity of the enzymes by native gel electrophoresis 6% acrylamide showed no entry into the gels (data not shown). Kane et al. (19) were unable to get histidinyl-tRNA syn-

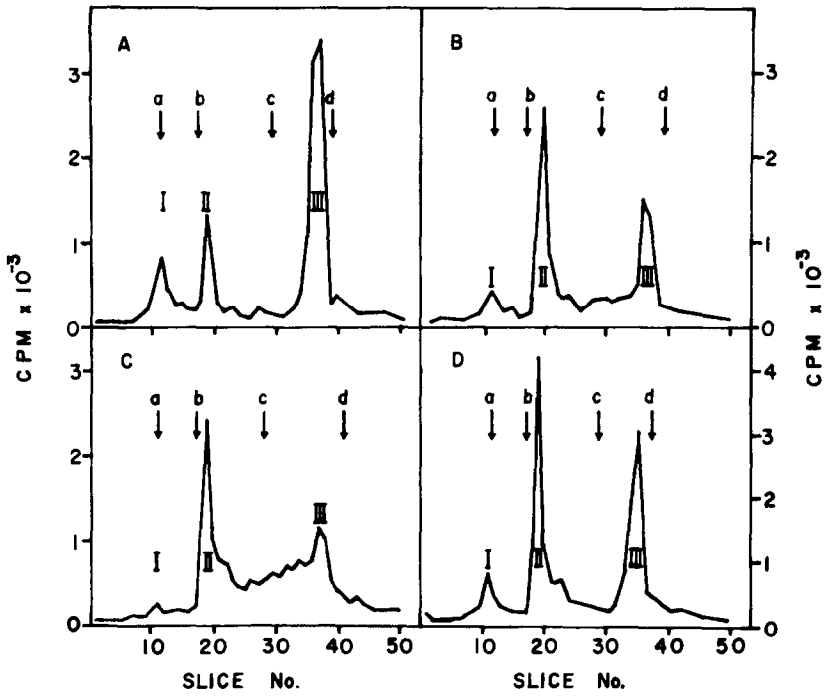

Fig. 2. Cooperation of magnesium and polyamine for read-through protein synthesis. The reaction mixture for protein synthesis was as described in Fig. 1, except for the concentrations of magnesium: A, 6 mM; B, 1mM spermidine was supplemented to A; C, 2 mM spermidine was supplemented to A; D, 0.25 mM spermine was supplemented to A. The letters and Roman numerals have the same meanings as in Fig. 1.

6 mM of magnesium the major product was again coat protein. However, by the addition of 1 mM (Fig. 2-B) and 2 mM (Fig. 2-C) of spermidine under these conditions, coat protein synthesis was strikingly decreased, and read-through protein synthesis was conversely increased. Furthermore, by the addition of 0.25 mM of spermine (Fig. 2-D), read-through protein was synthesized 2.6 fold of that in Fig. 2-A. From these results one can conclude that read-through protein is preferentially synthesized in the higher magnesium concentration.

The above fact was also observed in the biosynthesis of read-through protein directed by other phage RNAs, which are known to encode this protein (11, 13). As shown in Fig. 3-C to -D, ST-RNA (group III) and FI-RNA (group IV) directed quite large amounts of read-through (peak II) protein at 12 mM of magnesium acetate. While, MS2-RNA (group I) and JP34-RNA (group II), which do not encode read-through protein, did not produce a protein of such molecu-

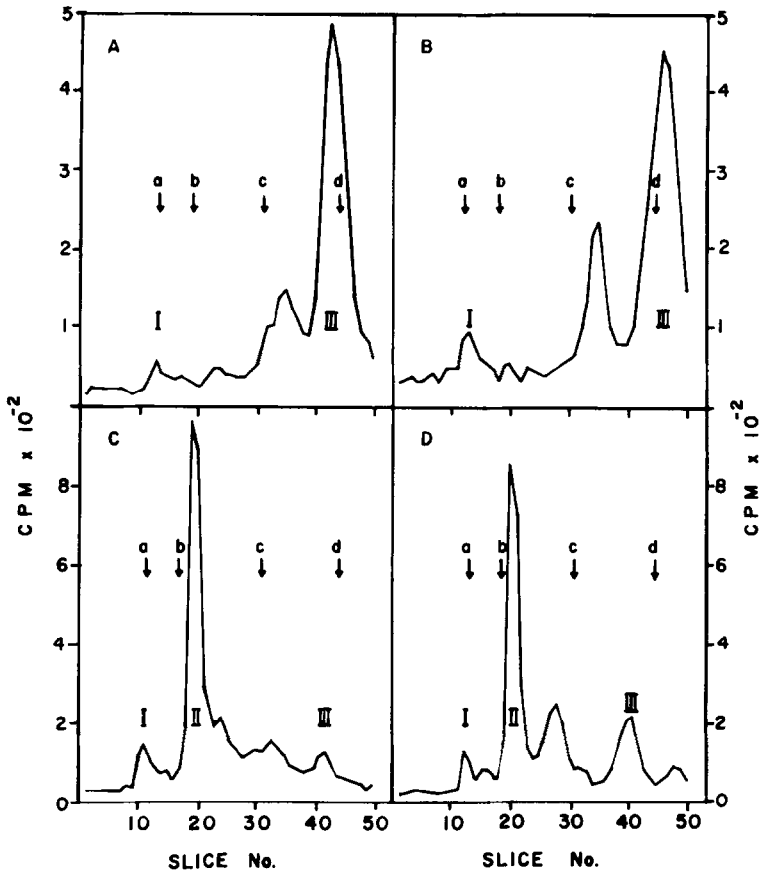

Fig. 3. Effect of higher magnesium ion on phage protein synthesis directed by other phage RNAs. The reaction mixture for protein synthesis was as described in the text, except that 2.5 μCi/ml of [^{14}C]-amino acid mixture (New England Nuclear, NEC-483), 25 μM of a mixture of cysteine, methionine, glutamine, asparagine and tryptophan, 12 mM magnesium acetate, and 50 μg/ml of phage RNAs were used. A, MS2-RNA; B, JP34-RNA; C, ST-RNA; D, FI-RNA. The letters and Roman numerals have the same meanings as in Fig. 2.

lar weight under the same conditions, although replicase subunit and coat protein were normally synthesized. However, it should be noticed that unknown protein of molecular weight 19000 was abnormally synthesized by MS2-RNA and JP34-RNA (Fig. 3-A and -B).

Since it has been shown that read-through protein appears to arise from the misreading of UGA codon as tryptophan codon at the end of the coat protein cistron (3, 4), it is likely that these cations play a role in such misreading. Although the mechanism which these cations are involved in the misread-

ing is not clear at present, it is possible that a high concentration of magnesium can lead to conformational changes of mRNA or tRNA.

Similar evidences have been reported by Cappechi (14) and recently Pelham (15), who described that polarity of f2 phage and UAG codon in tobacco mosaic virus RNA were suppressed by the addition of a high concentration of magnesium.

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