THE INFLUENCE OF CYCLIC GMP ON POLYPEPTIDE SYNTHESIS IN A CELL-FREE SYSTEM DERIVED FROM WHEAT EMBRYOS

by

G.A.Lanzani
Ist.di Chimica Agraria,Fac.di Agraria,via Celoria,2,Milan

M.Giannattasio
Istituto di Botanica,Fac.di Agraria,80055 Portici,Naples

L.A.Manzocchi,R.Bollini,A.N.Soffientini
Laboratorio Virus e Biosintesi Vegetali del CNR,Milan

and

V. Macchia
Istituto di Patologia generale,II[a] Facoltà di Medicina,Naples

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SUMMARY

3',5'cyclic GMP stimulates polypeptide synthesis in a cell-free system derived from wheat embryos. The stimulatory effect is observed in presence either of crude or highly purified transfer factors. Attempts to elucidate the mechanism of cyclic GMP action indicated that this nucleotide influences the formation of the binary complex EF1-GTP and the conversion of the heavy form of EF1 into a lighter one.

INTRODUCTION

3',5' cyclic GMP (cyclic GMP) has been found to stimulate protein synthesis in hog thyroid and rat liver slices (1,2). Moreover, cyclic GMP stimulated polyphenylalanine synthesis in a rat liver cell-free system containing semipurified preparations of both transfer factors and ribosomes (2). The effect of cyclic GMP on polypeptide synthesis apparead quite specific since it was not produced by other nucleotides and was dependent on the concentration of the transfer factors (2). These results suggest that cyclic GMP can be involved in the mechanism of protein synthesis in eukaryotes at the translational step and that its action is correlated with transfer factors.

The present study, carried out with a cell-free system derived from wheat embryos (3), corroborates this possibility

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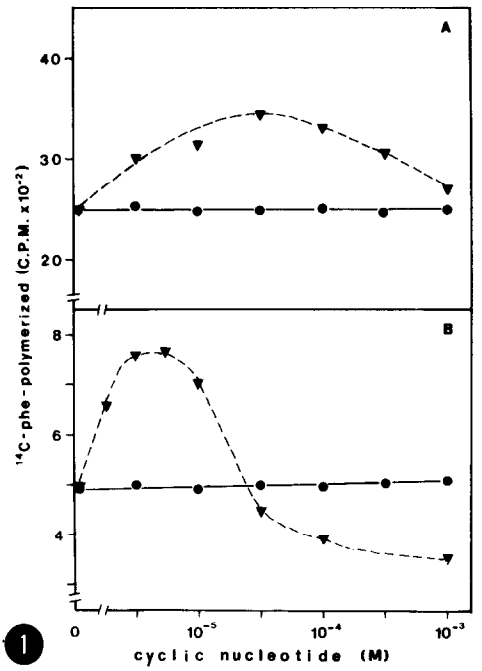

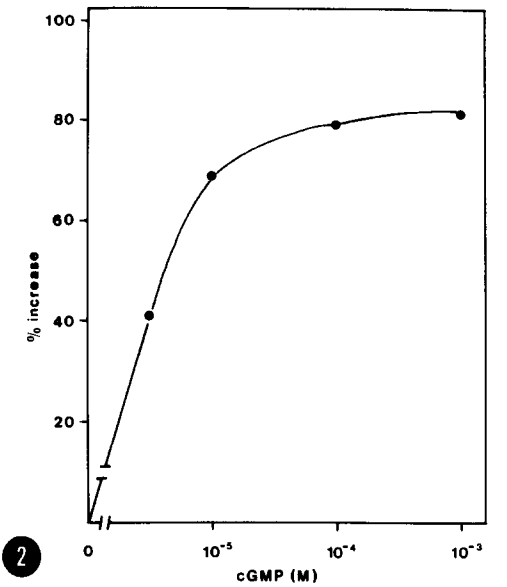

Fig.1. Influence of cyclic nucleotides on poly U directed polyphenylalanine synthesis. The reaction mixture (0.1 ml) contained: 50 mM Tris-HCl, pH 7.8; 7.5 mM $MgCl_2$; 25 mM KCl; 2 mM 2-mercaptoethanol; 0.5×10^{-3} M GTP; poly U (0.02 µmoles P); 1 A_{260} unit of (^{14}C)phe-tRNA; 3 A_{260} units of ribosomes and cyclic nucleotides as indicated. Experiments in A contained crude transfer factors (200 µg protein per test); experiments in B contained 10 µg of the heavy form of EF1 and 10 µg of EF2 per test. After incubation at 32° for 30 min, the hot TCA-insoluble material was collected and washed on nitrocellulose filters. ▼--▼ cyclic GMP added; ●——● cyclic AMP added.

Fig.2. Influence of cyclic GMP on EF1-GTP complex. The reaction mixture (0.1 ml) contained: 10 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; 50 mM NH_4Cl; 1mM DTT; 10^{-5} M(^{3}H)GTP (spec.act. 1,000 µCi/µmole); 10 µg of the heavy form of EF1 and cyclic GMP as indicated. After 5 min incubation at 0°C, the tests were diluted with 3 ml of cold diluting buffer (10 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; 50 mM NH_4Cl) and filtered on nitrocellulose filters. Filters were washed with 10 ml of diluting buffer, dried and counted.

and suggests that one target of the cyclic GMP action is the elongation factor EF1.

MATERIAL AND METHODS

Preparation of ribosomes. Ribosomes were pelletted at 100,000

x g from the postmitochondrial supernatant of 300 g of wheat embryos homogenized in Waring blendor with 600 ml of extracting buffer (0.25 M sucrose; 50 mM Tris-HCl, pH 7.8; 10 mM $MgCl_2$; 25 mM KCl; 5 mM 2-mercaptoethanol). The ribosomal pellet was washed once with extracting buffer, resuspended in the same buffer without sucrose and stored in liquid nitrogen. Crude ribosomes were purified from contaminating transfer factors by chromatography on DEAE-cellulose column, as already described (4).

Preparation of crude transfer factors. The wheat embryo post-ribosomal supernatant was precipitated with ammonium sulphate at 80% saturation. The pellet was dissolved in 10 mM Tris-HCl, pH 7.8, 1 mM 2-mercaptoethanol and desalted on a Sephadex G25 column (4 x 80 cm) equilibrated in the same buffer.

Preparation of EF1 and EF2 factors. EF1 and EF2 factors were separated by gel-filtration on a Sephadex G200 column (10 x 90 cm) in 20 mM Tris-HCl, pH 7.5; 8 mM Mg acetate; 200 mM KCl; 1 mM 2-mercaptoethanol, as already described(3). The EF1 peak was precipitated with ammonium sulphate (80% saturation) and purified by two hydroxyapatite chromatographies; the resulting EF1 preparation was one band on disc-gel electrophoresis and was represented by the heavy form of the enzyme (3). EF2 factor was purified according to the method of TWARDOWSKI and LEGOCKI (5).

(^{14}C)phe-tRNA was prepared by aminoacylating crude wheat germ tRNA and extracting it by the method of VOLD and SYPHERD (6). The product contained 20 pmoles (1,700 cpm) of (^{14}C) phe-t RNA per A_{260} unit.

Poly U directed polyphenylalanine synthesis was assayed as already described (3).

EF1-(^{3}H)GTP complex was performed according to ALLENDE et al. (7).

(^{14}C) phenylalanine and (^{3}H) GTP were purchased from NEN; ATP, GTP, cyclic GMP and cyclic AMP from Boehringer; poly

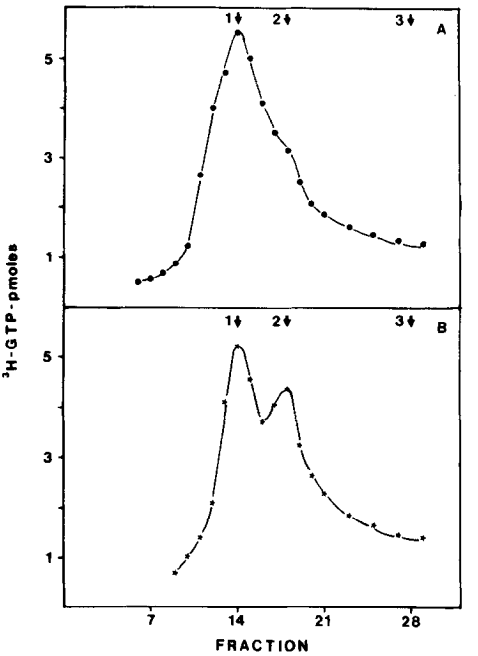

Fig.3. Sephadex G200 gel filtration of EF1. The column (1,5x15 cm) was conditioned with 50 mM Tris-HCl,pH 7.5;10 mM $MgCl_2$;60 mM NH_4Cl,1mM DTT. 200 µg of heavy form of EF1 were incubated in 0.5 ml of the same buffer containing 10^{-4} M cyclic GMP at 0°C for 5 min(Experiment B). The control was carried out by incubating without cyclic GMP (Experiment A). After incubation, the samples were applied to the column and eluted with the same buffer. Fractions of 0.5 ml were collected. EF1 activity was assayed on 200 µl aliquots by the formation of EF1-(^{3}H)GTP complex as described in Fig.2. Arrows indicate the elution volumes of dextran blue (1), catalase (2) and bovine serum albumin (3).

U from Miles. The nitrocellulose filters were Millipore H.A.W.P., 0.45 µ pore diameter. The other chemicals were analytical grade from Merck. Wheat germs were prepared with the methods of JOHNSTON and STERN (8).

RESULTS AND DISCUSSION

Cyclic GMP stimulates polypeptide synthesis in a cell-free system derived from wheat embryos (Fig.1,A). As with rat liver (2) the effect of cyclic GMP appears specific, since it is not produced by cyclic AMP (Fig. 1,A) or by 5'GMP(data not shown).

When purified elongation factors are used instead of the crude preparation, optimal stimulation by cyclic GMP occurs at a lower concentration and over a sharper range of concentration (Fig.1,B). This can be explained by the presence of phosphodiesterase activity toward cyclic GMP in the crude preparation (9). Cyclic AMP again has no effect.

Since in the rat liver system it was observed that transfer factors are involved in cyclic GMP effect (2), we studied the influence of cyclic GMP on the formation of EF1-GTP complex. It is well known that in wheat, as well as in other eukaryotes, EF1 is present in different molecular forms (3,10,11,12,13). The heavy species links GTP to form the binary complex EF1-GTP (3,10) as well as GDP (10) or guanosine 3'-diphosphate,5'-diphosphate (ppGpp) (14). Unlike the competitive inhibitory effect of ppGpp (14), cyclic GMP increases the amount of GTP bound to the heavy form of EF1 (Fig.2).

The heterogeneity of the elongation factor EF1 of wheat seems the result of the conversion of the heavy species into lighter forms during the formation of the binary and the ternary complex (3,10). GTP, GDP and aminoacyl-tRNA have been found to regulate this transformation (3,10). In the present study we have investigated the influence of the presence of cyclic GMP on the transformation of the heavy form of EF1. As it is revealed by the elution pattern on Sephadex G200 of EF1 after its incubation with cyclic GMP (Fig.3),the nucleotide induces the conversion of the heavy form of EF1 into a lighter species, whose elution volume is approximately the same as catalase (Fig.3).

The data presented above further support the possibility that cyclic GMP has a role in protein synthesis of eukaryotes and indicate that one target of cyclic GMP action is the elongation factor EF1. Although the biological significance of the transformation of EF1 is at present obscure, we can speculate that cyclic GMP stimulates the polypeptide synthesis by affe-

cting the conversion of the heavy form of EF1 into a lighter form and that this transformation makes EF1 more active in binding GTP.

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