Volume 275, number 1,2, 99–101 FEBS 09138 November 1990

An inhibitor of protein synthesis initiation from *Alhagi kirgisorum* S.

Salim K. Smailov[1], Bektas G. Mukhamedzhanov[1], Anatoly V. Lee[1], Bulat K. Iskakov[1] and Oleg N. Denisenko[2]

[1]*Ajtkhozhin Institute of Molecular Biology and Biochemistry, The Kazakh Academy of Sciences, Michurina 86, Alma-Ata, 480012, USSR* and [2]*Institute of Protein Research, The USSR Academy of Sciences, Pushchino, Moscow Region, 142292, USSR*

Received 18 June 1990; revised version received 25 September 1990

Polyproanthocyanidin - a plant phenolic compound from *Alhagi kirgisorum* S. effectively inhibited protein synthesis in rabbit reticulocyte and wheat germ cell-free systems. Poly-proanthocyanidin inhibited translation only at the level of initiation and not at the elongation level and aminoacylation of tRNA. The inhibitory effect of the phenolic compound is due to the blockage of the ternary complex formation of eIF-2 with GTP and initiator Met-tRNA.

Inhibitor of protein synthesis; Eukaryotic initiation factor 2; Cell-free translation; Plant biologically active compound

1. INTRODUCTION

Inhibitors of protein synthesis are widely used for elucidation of protein synthesis mechanisms and regulation. There are many highly specific inhibitors of translation, not only with respect to the step inhibited, but also with respect to the type of ribosome (70 S or 80 S) and to the species [1]. At present a number of inhibitors of polypeptide chain initiation of eukaryotes are known [2–4].

In this work we report that polyproanthocyanidin (referred to as PPA) from *Alhagi kirgisorum* S. also effectively inhibits protein synthesis at the initiation stage. Aminoacylation of tRNA and elongation stage of translation were not inhibited by PPA. The inhibition of translation initiation by PPA is due to the blockage of an early step – the formation of the ternary complex eIF-2 · GTP · Met-tRNA$_i$. This seems to be the first report on the specific interaction of this plant's phenolic compound with a key component of the protein synthesizing machinery.

2. MATERIALS AND METHODS

PPA was isolated from camel's thorn *Alhagi kirgisorum* S. by ethanol extraction. Fresh plant material (0.5 kg) was grinded and treated with benzol to remove lipid compounds. After complete evaporation of residual benzol 1 liter of ethanol was added to the plant material with gentle shaking for 3 h. An equal volume of acetone was then added to the ethanol fraction. The dark lower layer was collected and an equal volume of acetone was added again. After 4 h the precipitate of PPA appeared. This precipitate was collected, dried in a desiccator and stored under $CaCl_2$. The resulting brown powder of PPA was dissolved in water before use.

Correspondence address: S.K. Smailov, Ajtkhozhin Institute of Molecular Biology and Biochemistry, The Kazakh Academy of Sciences, Michurina 86, Alma-Ata, 480012 USSR

During the whole isolation procedure PPA was tested by reaction with vanillin in the presence of HCl [5]. Purified polyproanthocyanidin gives a characteristic spectrum with two maxima at 280 nm and 550 nm. The molecular mass of PPA was determined by gel-filtration on Sephadex G-50 (Pharmacia) in the presence of 0.1 M NaCl and was found to be about 10 000 Da.

Initiation factor 2 was isolated from wheat germ extract as described by Spremulli et al. [6], with slight modifications. The eIF-2 activity was assayed essentially as described in [7]. The reaction mixture (100 μl) contained 25 mM HEPES/KOH, pH 7.6, 10 mM 2-mercaptoethanol, 100 mM KCl, 10 pmol [^{35}S]Met-tRNA$_i$, 0.5 μg eIF-2. After incubation for 10 min at 30°C, ternary complex formation was measured by filtration through a nitrocellulose membrane (Millipore). [^{35}S]Met-tRNA$_i$ was prepared as described [8], using total tRNA from wheat germ, [^{35}S]methionine (250 Ci/mol, Isotop, USSR) and the enzymatic fraction from *E. coli* MRE 600.

The effect of PPA on protein biosynthesis in the cell-free system was tested using a reticulocyte lysate as described previously [9]. The incubation mixture (25 μl) contained 15 μl of rabbit reticulocyte lysate, 25 mM HEPES/KOH, pH 7.6, 120 mM K-acetate, 1 mM dithiothreitol, 10 mM creatine phosphate, 1 mg/ml creatinephosphokinase, 0.6 mM spermidine, 1 mM ATP, 0.2 mM GTP, 20 μM hemin. The incubation mixture contained also: (a) in case of endogenous mRNA translation, 19 amino acids (without leucine) 40 μM each, 10 μCi [^{14}C]leucine (260 Ci/mol, Amersham) and 1 mM Mg-acetate; (b) in case of poly(U) translation, 5 μM [^{3}H]phenylalanine (29 Ci/mol, Amersham), 10 μg poly(U)(Sigma) and 10 mM Mg-acetate.

In both cases, after incubation for 45 min at 34°C, 2 ml of a mixture of 1 M NaOH and 0.5 M H_2O_2 (1:1, v/v) was added and samples were incubated for 15 min at room temperature. Then trichloroacetic acid was added to a final concentration of 5% and samples were filtered through GFC filters (Whatman). Radioactivity of dried filters was measured in a liquid scintillation counter LS-100C (Beckman).

3. RESULTS AND DISCUSSION

We first examined the effect of PPA on the translation of endogenous mRNA and poly(U) in a rabbit reticulocyte system. The results shown in Fig. 1, demonstrate that PPA has no inhibitory effect on po-

Published by Elsevier Science Publishers B. V. (Biomedical Division)
00145793/90/$3.50

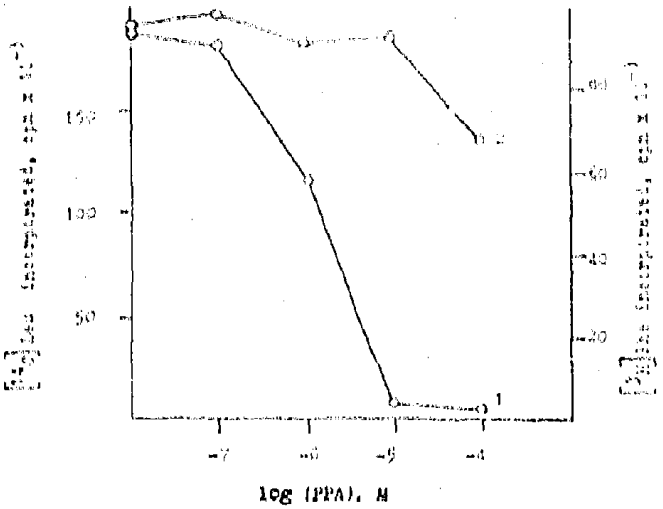

Fig. 1. Effect of PPA on the endogenous mRNA (1) and poly(U) (2) translation in a rabbit reticulocyte cell-free system.

ly(U)-dependent elongation at a 10 μM concentration (Fig. 1, curve 2) whereas the translation of natural mRNA is strongly inhibited (Fig. 1, curve 1). Some inhibition of poly(U)-dependent synthesis of polyphenylalanine was observed at a 100 μM concentration of PPA. Similar effects of PPA were observed while using a cell-free system from wheat germ (data not shown).

The selective inhibition of the initiation of translation by PPA was tested in rabbit reticulocyte system, where mRNA for *E. coli* β-galactosidase was translated. In this experiment 10 μM PPA was added to the incubation mixtures before, or 5 min after mRNA addition. The results are shown in Fig. 2.

When PPA was present in the incubation mixture from the start of translation, polypeptide was not synthesized (Fig. 2, track A). If PPA was added 5 min after

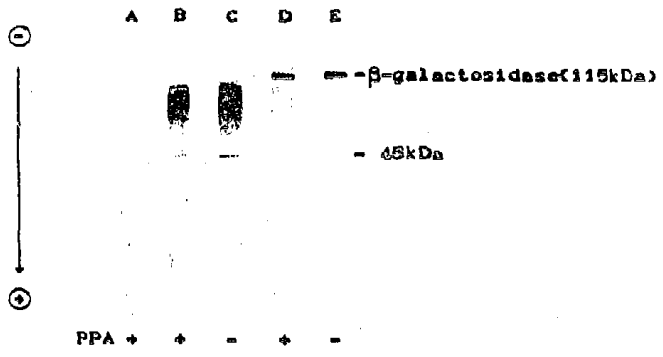

Fig. 2. Effect of PPA on LacZmRNA translation in a nuclease-treated rabbit reticulocyte lysate [9]. 10 μM PPA was added either at zero time of incubation with mRNA (A), or after 5 min (B,D). Incubation time was 10 min (B,C) and 45 min (A,D,E). Autoradiogram of [^{35}S]labeled products of translation. The reactions were incubated at 34°C, stopped by SDS-sample buffer addition and electrophoresed [11]. Gels were dried and exposed using RM-V film (Tasma, USSR).

the beginning of translation, the inhibition of polypeptide synthesis was not observed (Fig. 2, tracks B,D). Since the efficiency of mRNA translation was equal in the presence (Fig. 2, tracks B,D) and in the absence of PPA (tracks C,E), it may be concluded that only the initiation stage, but not the elongation was affected by PPA.

It is known that the overall process of initiation of translation in eukaryotic cells consists of at least 4 steps [10]: (1) formation of the ternary complex eIF-2 · GTP · Met-tRNA$_i$; (2) formation of the 43 S preinitiation complex; (3) formation of the 48 S preinitiation complex; (4) formation of the 80 S initiation complex.

To determine which step of the initiation process is inhibited by PPA we used a rabbit reticulocyte cell-free system lacking hemin. It is known that the translational activity of such a system depends upon exogenous eIF-2 addition [12]. Indeed, as shown in Fig. 3, significant stimulation of endogenous mRNA translation was observed in hemin-deficient cell-free systems in the presence of purified wheat germ eIF-2. But this stimulation was eliminated by PPA at concentrations of 10 μM. These results indicate that the eIF-2 dependent step of initiation may be inhibited by PPA. In order to verify this possibility, we examined the ternary complex formation of wheat germ eIF-2 with GTP and Met-tRNA$_i$ in the presence of different concentrations of several phenolic compounds: phenolic (caffeic) acid, M_r 18 016 (Sigma); quercetine, M_r 33 826 (Sigma); and PPA (Fig. 4). It is seen that only PPA has a strong inhibitory effect on the ternary eIF-2 · GTP · Met-tRNA$_i$ complex formation (Fig. 4, curve 3). It should be noted that the

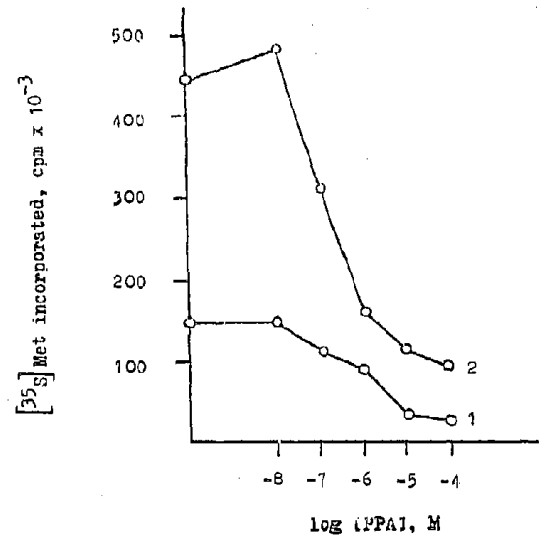

Fig. 3. Effect of PPA on endogenous mRNA translation in a hemin-deficient rabbit reticulocyte system in the presence (2) or absence (1) of 1.0 μg of purified wheat germ eIF-2. Experimental conditions were the same as described in section 2 but a mixture of 19 amino acids without methionine and 10 μCi [^{35}S]methionine was used.

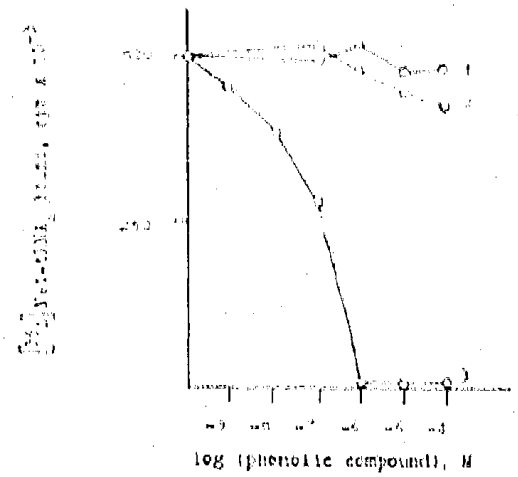

Fig. 4. Effect of different phenolic compounds on ternary complex formation. [^{35}S]Met-tRNA$_i$ binding was determined as described in section 2 with purified wheat germ eIF-2 and GTP. Curve 1, phenolic acid; curve 2, quercetin; curve 3, PPA.

effect of PPA on the activity of eIF-2 from rabbit reticulocytes was the same (data not shown).

Thus, PPA is found to be the specific inhibitor of eIF-2 function. Therefore total inhibition of protein synthesis initiation by PPA was due to the blockage of the ternary eIF-2·GTP·Met-tRNA$_i$ complex formation. Several non-enzymatic inhibitors of eukaryotic polypeptide chain initiation are known to block the formation of the ternary complex in cell-free systems: showdomycin (an analog of *N*-ethylmaleimide) [2–4]; adrenochrom and triphenylmethan dyes (such as aurintricarboxilic acid, Pyrochatechol violet, etc.) [2,3,13]; *N*-ethylmaleimide [2,3]. However the working concentrations of most of these are rather high (0.1–10 mM) [2] and they may interfere with other stages of translation [3] and processes other than protein synthesis [4]. Our results show that PPA at concentrations of 1–10 μM strongly inhibits the functioning of eIF-2 from rabbit reticulocytes and wheat germ, resulting in a blockage of protein synthesis in both cell-free systems. However, neither tRNA aminoacylation nor elongation and the termination steps of translation are affected by PPA.

It seems that PPA may serve as a useful tool in studying the mechanisms and regulation of translation in eukaryotic cell-free systems.

Acknowledgements: We are grateful to Prof. A.S. Spirin and Prof. J.H. Weil for useful discussions and critical comments on this manuscript.

REFERENCES

[1] Pestka, S. (1977) in: Molecular Mechanisms of Protein Biosynthesis (Weissbach, H., Pestka, S. eds) pp. 467–555, Academic Press, New York.
[2] Fresno, M., Carrasco, L. and Vazquez, D. (1976) Eur. J. Biochem. 68, 355–364.
[3] Vazquez, D. (1979) Inhibitors of protein biosynthesis, Springer, Berlin.
[4] Vazquez, D., Zaera, E., Dolz, H. and Jimenez, A. (1982) in: Protein Biosynthesis in Eukaryotes (Perez-Bercoff, R. ed) 41, 311–337, Plenum Press, New York.
[5] Hais, I.M. and Macek, K. (1959) in: Papirova chromatografie, Nakladatelstvi Ceskoslovenske Akademie VED, Praha.
[6] Spremulli, L.L., Walthall, B.J., Lax, S.R. and Ravel, J.M. (1977) Arch. Biochem. Biophys. 178, 565–575.
[7] Lax, S.R., Lauer, S.J., Browning, K.S. and Ravel, J.M. (1986) Methods Enzymol. 118, 109–128.
[8] Stanley Jr., W.M. (1974) Methods Enzymol. 29, 530–547.
[9] Denisenko, O.N. and Yarchuk, O.B. (1989) FEBS Lett. 247, 251–254.
[10] Pain, V.M. (1986) Biochem. J. 235, 625–637.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Safer, B. (1983) Cell 33, 7–8.
[13] Dettman, G.L., Stanly, W.M. (1972) Biochim. Biophys. Acta 299, 142–147.