

INHIBITION OF PROTEIN SYNTHESIS BY EXOGENOUS A2'p5'A2'p5'A (2–5 A core) AND ITS BIS-PHOSPHORAMIDATE ANALOG IN INTACT MOUSE LYMPHOCYTES, HEPATOCYTES AND BONE MARROW CELLS

Michal JUROVČÍK and Jiří SMRT*

*Institute of Molecular Genetics, Czechoslovak Academy of Sciences, 160 20 Prague 6 and *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia*

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1. Introduction

Interferon-treated cells are capable of synthesizing at least 3 enzymes which are involved in normal cell proliferation and anti-viral immunity [1–4]. One of these enzymes, interferon- and dsRNA-dependent oligoadenylyl synthetase is responsible for a polymerization of ATP resulting in a series of (2' → 5')-linked oligoadenylic acid 5'-triphosphates. The major active species is the trimer, pppA2'p5'A2'p5'A (2–5 A), which is able to inhibit protein synthesis in cell-free system at subnanomolar levels [5–7]. This inhibition is mediated by activation of a ribonuclease which degrades cellular mRNA [8–10]. In [11] it was shown that (2' → 5')-oligoadenylylates and their bacterial alkaline phosphatase (BAP)-resistant cores (which do not contain triphosphate groups) were able to inhibit protein synthesis in hypertonically permeabilized cells, whereas in cell-free system these BAP-treated nucleotides were inactive [7].

In another case pppA2'p5'A2'p5'A co-precipitated with calcium phosphate showed inhibitory activity on protein synthesis in a variety of different cell types [12,13]. In [14], inhibition of DNA synthesis by (2' → 5')-oligoadenylylates was observed in lymphocytes stimulated by Con A.

Here, we report protein synthesis inhibition in intact mouse lymphocytes, hepatocytes and bone marrow cells by exogenous A2'p5'A, A2'p5'A2'p5'A and its bis-phosphoramidate analog.

2. Materials and methods

[U-¹⁴C]Leucine (spec. act. 8880 MBq/mM) was obtained from the Institute for Research, Production

and Application of Radioisotopes (Prague). Medium 199 (dried) was purchased from Borrough Wellcome Co. (London). Fetal calf serum was from Difco Labs. (Detroit MI). A2'p5'A was prepared by successive treatment of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) adenylyl(2' → 5')-2',3'-di-O-benzoyl-N,N-dibenzoyladenosine (2-chlorophenyl) ester by ammonia and tetrabutyl ammonium fluoride (TBF). A2'p5'A2'p5'A was prepared by analogous deblocking of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-adenylyl(2' → 5')-3'-O-(tetraisopropylidisiloxane-1-yl)-adenylyl(2' → 5')-2',3'-di-O-benzoyl-N,N-dibenzoyladenosine P¹,P²-bis-(2-chlorophenyl) ester [15]. The bis-phosphoramidate was prepared from the last compound by treatment with methanolic ammonia and TBF [16]. Chromatographically uniform compounds were used for testing.

Mouse liver, bone marrow and spleen cells were obtained from random bred animals (~14 days-old) by in vitro perfusion of organs with incubation medium through Nylal nylon sieve. Released cells were layered on 9.25% (w/v) sucrose buffered with 30 mM Hepes (pH 7.45) and centrifuged at 1000 rev./min for 7 min at 2°C. The sedimented cells were suspended at 4–8 × 10⁶ cells/ml in M 199 medium buffered with 30 mM Hepes and supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). Protein synthesis was followed by [U-¹⁴C]leucine incorporation (0.8 µCi/ml). Cultures of 1 ml were incubated for maximal 5 h. Aliquots of 0.1 ml were withdrawn and samples were processed by a slightly modified method using paper pieces of Whatman 3 MM [17,18]. Aliquots of cell suspension were soaked on the paper (2 × 3 cm) and strips were immediately immersed into cold trichloroacetic acid (7.5%). Washing with cold trichlo-

roacetic acid was repeated 4 times and the strips were finally washed with ethanol*. Radioactivity of dried strips was measured in toluene scintillation liquid.

3. Results

The inhibitors tested were added to the culture medium to 0.1 μ M simultaneously with [U- 14 C]leucine. The inhibitory effect of protein synthesis in all cell types studied was apparent after 1 h incubation. After 5 h, protein synthesis was inhibited by 64% in lymphocytes, in the presence of A2'p5'A2'p5'A, and few % less by its bis-amidate analog (fig.1).

Practically the same values of protein synthesis inhibition were observed in bone marrow cells (fig.2). On the other side, the inhibition of protein synthesis in hepatocytes reached 45% only (fig.3).

Levels of 0.1 μ M for both inhibitors was found to be optimal for observation. A 10-fold decrease of concentration led to negligible inhibition of protein synthesis (not shown). On the other hand, a 10-fold increase of both A2'p5'A2'p5'A and its bis-amidate

* Washing of samples with hot trichloroacetic acid decreased all values (including controls) by 9–10%, and therefore did not affect the degree of inhibition

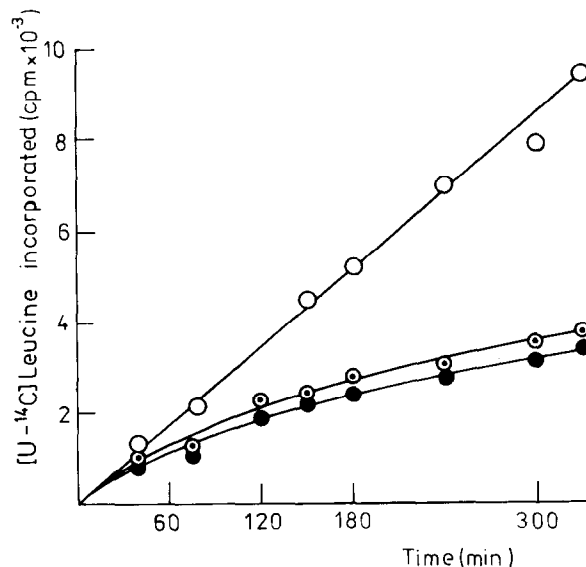


Fig.1. Inhibition of protein synthesis by exogenous 0.1 μ M/ml of A2'p5'A2'p5'A (●—●) and its bis-phosphoramidate analog (○—○) in spleen cells.

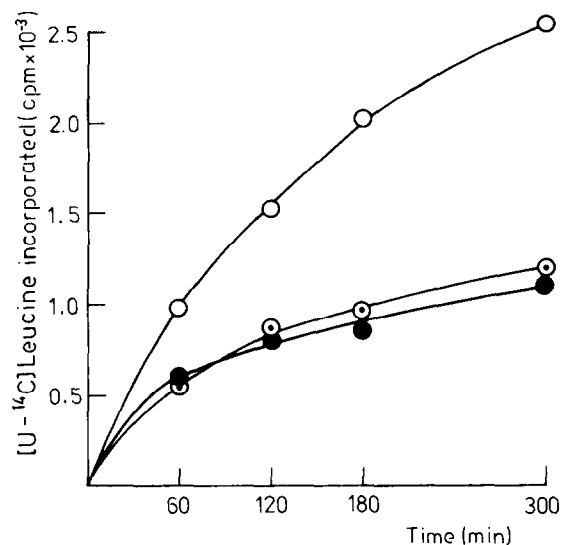


Fig.2. Inhibition of protein synthesis by exogenous 0.1 μ M/ml of A2'p5'A2'p5'A (●—●) and its bis-phosphoramidate analog (○—○) in bone marrow cells.

analog caused complete inhibition of protein synthesis in all cell types (not shown).

In the presence of 0.1 μ M A2'p5'A, protein synthesis inhibition was $\leq 10\%$ with all types of cells studied (not shown).

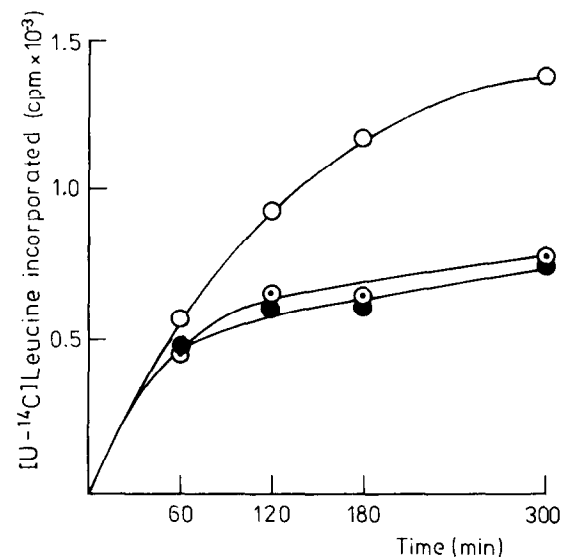


Fig.3. Inhibition of protein synthesis by exogenous 0.1 μ M/ml of A2'p5'A2'p5'A (●—●) and its bis-phosphoramidate analog (○—○) in hepatocytes.

4. Discussion

In [13] pppA2'p5'A2'p5'A was shown to be a potent inhibitor of protein synthesis in intact cells after its uptake was facilitated by calcium co-precipitation. In the absence of calcium salts, however, different concentrations of the compound did not affect protein synthesis *in vivo* due to the impermeability of cell membrane. Inhibition of protein synthesis in hypertonically permeabilized BHK-21 cells by pppA2'p5'A2'p5'A and A2'p5'A2'p5'A, which was presumably metabolized to active triphosphate derivative, was observed in [11].

Our task was to study the influence of exogenous (2' → 5')-oligoadenylates on protein synthesis in intact mouse cells. Considering that the triphosphate group-containing molecule could barely penetrate the intact cell membrane, we focused our interest on 'core' compounds lacking the triphosphate group.

At first, 3 substances of this type were studied, A2'p5'A, A2'p5'A2'p5'A and its analog containing (2' → 5')-phosphodiester amide bonds (A2'p5'A2'-p5'A bis-amide). Three types of functionally different mouse cells, the lymphocytes, hepatocytes and bone marrow cells, were selected on the basis of assumed differences in cell membrane properties.

Our findings led us to the conclusions:

- (i) A2'p5'A2'p5'A is able to penetrate the cell membrane of these intact mouse cells in spite of its negatively charged internucleotide bonds.
- (ii) An exogenous level of 0.1 μM/ml of this compound is about critical to create sufficient intracellular concentration and cause observable protein inhibition.

Results of parallel experiments with A2'p5'A2'p5'A bis-amide lacking charged internucleotide bonds led us to the conclusion that this neutral molecule, despite

its expected higher ability to penetrate the cell membrane, is apparently somewhat less efficiently metabolized to the active intracellular inhibitor.

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