Interferons induce accumulation of diadenosine triphosphate (Ap_3A) in human cultured cells

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Abstract After incubation of human monocytes J96 and human myeloid leukemia HL60 cells with interferons (IFN) α or γ, the Ap_3A concentration considerably increases in parallel with accumulation of tryptophanyl-tRNA synthetase (TrpRS, EC 6.1.1.2) The Ap_3A formation in response to IFNs is catalysed by an excessive amount of TrpRS. Although the Ap_3A function still remain unknown its accumulation may imply the Ap_3A involment in the IFN-signalling pathway.

Key words: Interferon; Tryptophanyl-tRNA synthetase; Ap_3A synthesis; Human cultured cell

1. Introduction

Interferons are a family of regulator proteins that bind to surface receptors of responsive cells inducing an antiviral and antiproliferative states and a variety of immune responses [1–3]. Induction of gene transcription is an essential part of the cellular response to IFNs. Promoters of the IFN-responsive genes contain specific DNA sequences termed ISRE and GAS [4,5]. One of the genes stimulated by IFNs is a tryptophanyl-tRNA synthetase (TrpRS, EC 6.1.1.2), the enzyme involved in protein synthesis [6]. The amino acid sequence and the exon–intron organisation of the TrpRS gene are known [7,8]. The gene contains in the 5′-regulatory region the ISRE and GAS elements [8] and TrpRS is induced by IFNs α and γ in cultured cells [9–11]. Contrary to the majority of the aminoacyl-tRNA synthetases, mammalian TrpRS in a wide range of the in vitro reaction conditions is unable to catalyse diadenosine tetraphosphate (Ap_4A) synthesis [12].

However, in the presence of L-Trp, ATP-Mg^{2+} and ADP the TrpRS catalyzes the Ap_3A formation [12]. Based on the above data, it was suggested that accumulation of TrpRS in response to IFNs may cause an alteration of the Ap_4A/Ap_4A ratio [13].

The objective of this work was to obtain an evidence of Ap_3A accumulation in response to IFNs α and γ action on cultured human cells and to show that the increase of the Ap_3A level is due to the TrpRS induction in IFN-treated cells.

2. Experimental

The human monocytes J96 and human myeloid leukemia HL60 cells were grown on MEM and RPMI 1640 media, respectively, with 10% of fetal calf serum and penicillin (100 IU/ml). Before addition of IFNα (1000 IU/ml) or IFNγ (100 IU/ml), cells were grown 24 h with [^{32}P]orthophosphate (50 μCi/ml). For extraction of diadenosine oligophosphates, 10% ice-cold percloric acid was mixed with (25–30) 10^6 cells.

After 10 min on ice with gentle shaking, the mixture was centrifuged 10 min at 1000×*g* and the supernatant was neutralized with 5 M K_2CO_3. The pellet was discarded and the neutralized extracts were treated with *E. coli* alkaline phosphatase (1.25 μg/ml in 25 mM Tris–HCl, pH 8.0 and 10 mM $MgCl_2$) 3 h at 37°C. After 4-fold dilution with water, the extract was applied to a DEAE-cellulose column (0.8×10 cm) and nucleotides were eluted with a linear 0.2–0.8 M gradient of NH_4HCO_3, pH 8.2 (12 ml/h).

Thin-layer chromatography of Ap_nA was performed on Silufol (Kavaler, Czechoslovakia) and PEI-cellulose (Merck, Germany) plates with isopropanol/ammonia/water (7:3:1, v/v/v and 0.85 M LiCl, pH 4.2, respectively. For autoradiography, the dried plates were kept in contact with X-ray film (Kodak X-Omit AR) for 48 h. In order to estimate the amount of the labeled nucleotides, the correspondent areas were removed from plates and counted in the toluene scintillation liquid on SL-30 (Intertechnique, France) counter.

The TrpRS activity in cell extract was measured as described earlier [14]. 20 μl of cell lysate (protein concentration 5 mγ/ml) was incubated with 80 μl of 20 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 30 mM KCl, 0.1 mM dithiotreitol, 2.5 mM ATP, 30 μg of total yeast tRNA, 8 μM [^{14}C]Trp for 3 min at 37°C. The radioactivity was measured in TCA-insoluble fraction.

Ap_3A synthesis in cell extract was measured as described earlier [12].

3. Results

3.1. TrpRS activity in human cultured cell extracts

We measured the TrpRS activity in cells extracts prepared from untreated and IFNα/γ-treated human monocytes J96. It appeared that after 24-h incubation with IFNα the TrpRS activity increased 5.5-fold. The 24-h incubation with IFNγ had no effect, but after 36 h the TrpRS activity increased 2.7-fold and after 48 h 1.8-fold (Fig. 1, 1–5).

We also measured the TrpRS activity in human myeloid leukemia HL60 cell extracts. The TrpRS activity in the IFN-untreated (control) cells was higher than that in the J96 cells (115 pmol/mg protein and 74 pmol/mg protein, respectively). After 22-h incubation with IFNα, the TrpRS activity increased 3.6-fold (Fig. 1, 6 and 7). Probably, IFNα is more efficient inducer of TrpRS in human blood cells than IFNγ at variance with the human fibroblast FS_4 cells [10], where IFNγ is a more efficient inducer.

3.2. Ap_3A synthesis in IFN-treated cultured cells

Human cells were incubated in the presence of IFNα or γ and [^{32}P]orthophosphate. After extraction with percloric acid, the acid-soluble fraction was treated with alkaline phosphatase to dephosphorylate the labeled nucleotides, then the mixture was subjected to chromatography (Fig. 2). The data obtained demonstrate that the Ap_3A concentration in the J96 cells treated with IFNs significantly increased and reached the level of 0.6 pmol/10^6 cells (for IFNα) and 0.13 pmol/10^6 cells (for IFNγ).

The Ap_3A concentration in HL60 cells, incubated with IFNα for 15 and 22 h, increased 1.8- and 3.2-fold, respectively

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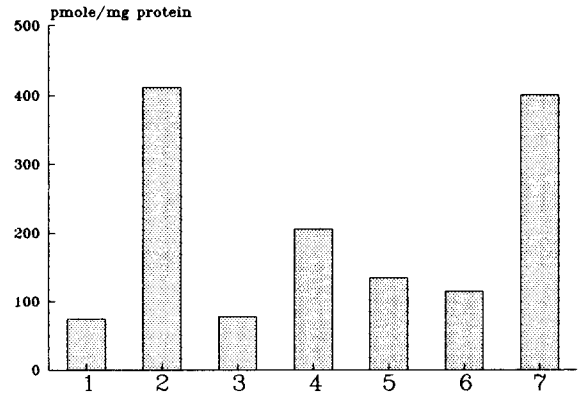

Fig. 1. TrpRS activity in J96 (1-5) and HL (6 and 7) cell lysates. Control without IFN (1); IFNα, 24 h (2) IFNγ: 24 h (3), 32 h (4), 48 h (5), Control (6), IFNα, 22 h (7).

(Fig. 3), over the control cells (4.8 pmol/10^6 cells). In both cell lines that have been investigated, the level of Ap_4A was considerably lower (0.46 pmol/10^6 cells in HL60 cells and 0.05 pmol/10^6 cells in J96 cells) than the level of Ap_3A.

The results obtained are comparable with the known data: e.g. the Ap_4A concentration in human carcinoma A549 was 0.03 pmol/10^6 cells [14].

3.3. *Inhibition of Ap_3A synthesis in cell extracts by tryptophan analogues*

To ascertain that Ap_3A formation is catalysed by an excessive amount of TrpRS the cell extracts prepared from untreated and IFN-treated J96 and HL60 cells were incubated with [α-^{32}P]ATP as described in [12] in the presence of tryptamine (10 K_i, $6 \cdot 10^{-4}$ M, data not shown) or β-indolilpyruvic acid (3 and 10 K_i, $1.5 \cdot 10^{-3}$ M and $5 \cdot 10^{-3}$ M, respectively) the competitive inhibitors of TrpRS preventing tryptophanyl adenylate formation [14]. The data obtained demonstrated that the level of Ap_3A considerably decreased (Fig. 4) in the presence of tryptophan analogues: consequently, the Ap_3A accumulation results from the TrpRS activity.

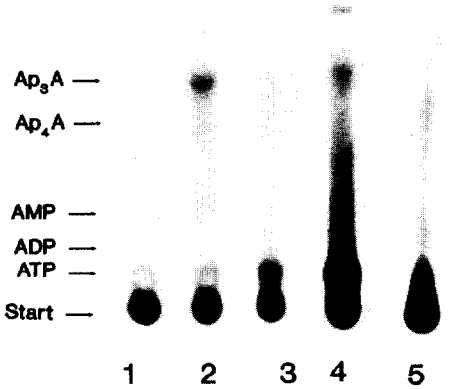

Fig. 2. Ap_nA synthesis in J96 cells. TCL of labeled nucleotides extracted from IFN-treated cells on Silufol plates. Control (1), IFNα, 24 h (2) IFNγ: 24 h (3); 32 H (4); 48 h (5).

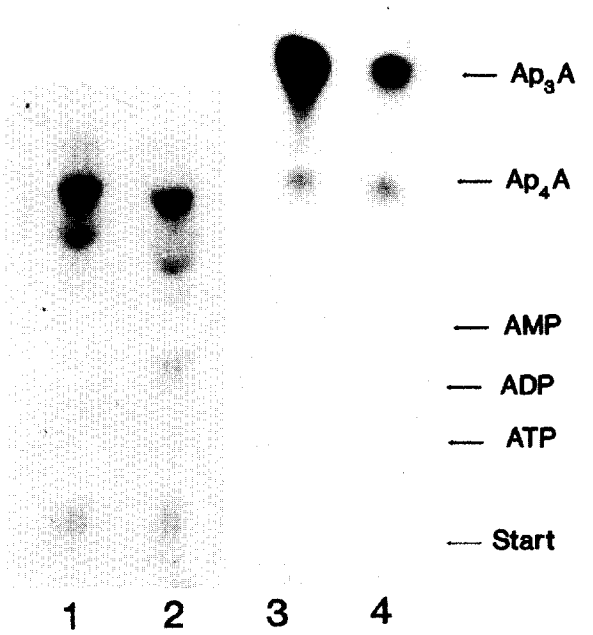

Fig. 3. Ap_nA synthesis in HL60 cells. TLC of labeled nucleotides on Silufol plates. IFNα: 15 h (1); 22 h (3). Control (2 and 4).

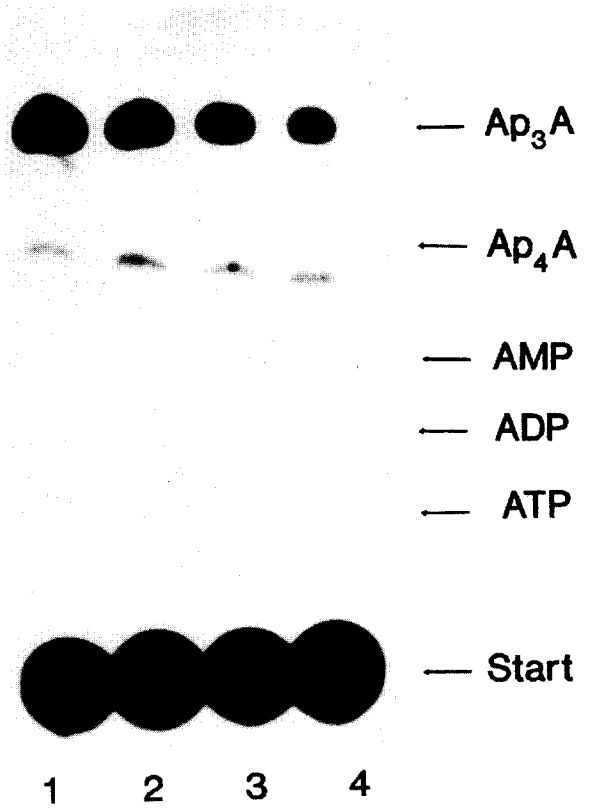

Fig. 4. Ap_nA synthesis in IFN-treated HL60 cell lysates in the presence of β-indolylpyruvic acid. IFNα, 24 h (1) Control, without IFN (4). IFNα, β-indolylpyruvic acid (3 K_i) (2). IFNα, β-indolylpyruvic acid (10 K_i) (3).

4. Discussion

After incubation of cell extracts with IFNα or γ, the TrpRS activity significantly increased in human J96 and HL60 cells (Fig. 1). We have shown that this IFN-induced accumulation of the enzyme is accompanied by a parallel accumulation of Ap_3A (Fig. 2 and 3), a cellular substrate identified long ago but still with unknown function [15,16]. This observation points to the coupling between the IFNα/γ action and the Ap_3A function not discussed before in the literature. The Ap_3A accumulation in cells could be related to cell growth retardation induced by IFNs. This possibility could be examined by measuring the Ap_3A/Ap_4A ratio in cell cultures before and after the IFN treatment. The elevated levels of Ap_4A were observed in cell-contact growth-inhibited cells [16]. We failed to measure the Ap_3A/Ap_4A ratio in the J96 cells because of very low Ap_4A concentration, but in the HL60 cells the Ap_3A/Ap_4A ratio increased about 3× in response to IFNα. Some data available in the literature indirectly confirm our observation. In several mammalian primary cultures, the Ap_4A concentration was insignificantly changed upon mitogenic stimulation by growth factors, although protein and DNA syntheses manifested an active proliferation [17]. The Ap_4A concentration was not changed under conditions leading to a cellular death [17]. The highest Ap_4A concentration (28 μM) was reported for sperm, which is composed of completely quiescent cells [18].

It is also possible that the high level of Ap_3A induced by IFNs stimulates the transcription of several genes. Finally, the Ap_3A function could be mediated via interaction with proteins and, therefore, it may be interesting to look for Ap_3A-binding protein(s). The occurrence in cells of Ap_3A-specific hydrolase indirectly points to some biological role for Ap_3A [19].

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