Volume 258, number 1, 163-165 FEB 07830 November 1989

Poly(ADP-ribose) polymerase activity is inhibited by 2',5'-oligoadenylates in mouse L-cells

Daniela Tornese Buonamassa, Maria Malanga+, Eliana M. Coccia*, Giovanna Romeo*, Elisabetta Affabris°, Benedetta Farina+ and Hisanori Suzuki†

*Centro Ricerche Sclavo, 53100 Siena, +Dipartimento di Chimica Organica e Biologica, Università di Napoli, 80134 Napoli, *Laboratorio di Virologia, Istituto Superiore di Sanità, 00161 Roma, °Istituto di Microbiologia, Università di Messina, Messina* and *†Istituto di Chimica Biologica, Università di Verona, 37134 Verona, Italy*

Received 23 August 1989

Down regulation of poly(ADP-ribose)polymerase (ADPRP) activity was observed in mouse LW-cells after treatment with 2'-5'oligoadenylates or with fibroblast interferon and poly(rI).poly(rC). The poly(rI).poly(rC)-induced inhibition of the enzymatic activity correlates with the observed increase of endogenous 2',5'-oligoadenylate cores which were reported to be potent inhibitors of ADPRP in vitro.

Oligoadenylate, 2',5'-; Poly(ADP-ribose)polymerase

1. INTRODUCTION

The molecular mechanisms by which interferons (IFNs) inhibit replication of many viruses and cell growth as well as produce regulatory effects on cellular physiology (such as changes in cell structure, modulation of differentiation and inhibition of cell growth) appear very intriguing and susceptible of continuous variations. Some IFN-induced proteins have been identified, including the double-stranded (ds) RNA-activated protein kinase and 2'-5'oligoadenylate (2-5A) synthetase which are able to reproduce in vitro the IFN-induced inhibition of mRNA translation. The 2-5A synthetase activated by dsRNA segments and by some cellular RNAs polymerizes ATP into ppp(A2'p)A oligomers [1-5]. The oligonucleotides bind an 80 kDa latent ribonuclease (RNase L) which is thereby activated and cleaves RNA without significant specificity for viral RNA in vitro [1-5]. Antiviral and antimitogenic effects of 2-5A and analogs (cores) were observed in some systems suggesting further that they represent one mechanism of IFN action. However, this is not always correlated with the RNA cleavage (i.e. in some cell variants lacking RNase L) probably because of the additional enzymes involved in the mechanism of action of IFN.

2. MATERIALS AND METHODS

2.1. *Cell cultures and interferons*

Mouse LW were grown in MEM supplemented with 5% fetal calf serum. Mouse fibroblast IFN was prepared as described by Cachard and De Mayer-Guignard [6]. Amounts of mouse IFNs are given throughout this paper in laboratory units, i.e. the amount of IFN reducing plaque production by vesicular stomatitis virus by 50%. This unit equals 4 research reference units of the National Institutes of Health Standard Preparation, code G-002-904-511, whose titer was 12000 IU/ml when reconstituted. Details about this standard preparation are reported in Research Reagents Note No. 15 (World Health Organization Standard, 1979).

2.2. *Poly(ADP-ribose)polymerase (ADPRP) assay*

ADPRP activity was determined measuring TCA-insoluble radioactivity of a reaction mixture containing 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 2 mM dithiothreitol, 80 μM [^{14}C]NAD (10 cpm/pmol) and extract from 1×10^6 cells rinsed twice with 140 mM NaCl, 35 mM Tris-Cl pH 7.5 and collected by scraping with a rubber policeman in the presence of a lysis buffer containing 20 mM Hepes pH 7.5, 0.25 M sucrose, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM dithiothreitol and 0.5% NP40. Incubation was carried out at 25°C for 5 min. About 2000 cpm were set up as 100% ADPRP activity.

2.3. *Measurement of cellular protein synthesis*

To measure protein synthesis, 1.8×10^6 cells/condition were labelled in growth medium containing ^{35}S-methionine (2 μCi/condition, 800 Ci/mol) at 37°C for the indicated times. At the end of incubation, cells were washed three times with saline phosphate buffer and lysed for 15 min at 37°C with a lysis buffer containing 10 mM Tris-HCl, pH 7.5, 1.5 mM MgOAc, 0.1% SDS, 20 μg/ml of DNase and 1 mM paramethylsulfonylfluoride. The whole samples were filtered on cellulose nitrate filters (Millipore, 0.45 μm) and the radioactivity of TCA-insoluble materials was measured in a beta-counter.

2.4. *HPLC analysis of 2-5A core levels in extracts from LW cells*

(2-5A)n core (n = 2-4) levels in cell extracts were determined as already reported [7]. Briefly, 100×10^6 cells were extracted with chilled 1 N perchloric acid and the extracts were immediately neutralized with 9 M KOH. After removing the precipitate, the supernatant was treated with nuclease P1 (Sigma, 1 U) at pH 8.0, and with alkaline phosphatase (Sigma, 1 U) at pH 9.4. The resulting 2-5A cores were separated by high-performance liquid chromatography (HPLC) using a column system composed of an ORD precolumn (4.5 × 45

Correspondence address: H. Suzuki, Istituto di Chimica Biologica, Facoltà di Medicina e Chirurgia, Università di Verona, Strada Le Grazie, 37134 Verona, Italy

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

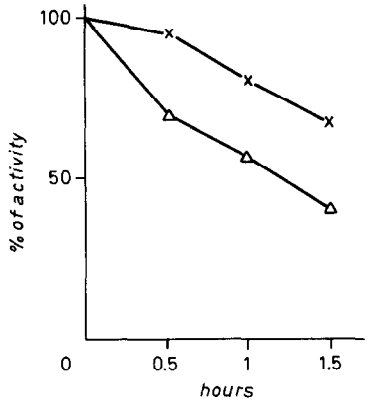

Fig.1. ADPRP activity in fibroblast IFN-treated LW cells after poly(rI).poly(rC) treatment. Mouse LW cells grown as described in section 2, were passaged the day before the experiments to give monolayers of equal age and density. Confluent LW cells treated for 20 h with 500 U/ml of fibroblast IFN (specific activity, 10^5 U/mg protein), were rinsed twice with MEM and added with MEM containing 50 μg/ml of poly(rI).poly(rC) and 800 μg/ml of DEAE dextran. At the indicated times, ADPRP activity was determined as described in section 2. (×—×), cells treated with poly(rI).poly(rC) (Δ—Δ), cells treated with IFN and poly(rI).poly(rC).

mm, Altex) and an Octyl column (4.5 × 150 mm, Altex). By a stepwise elution with 30 ml of 50 mM potassium phospate buffer, pH 7.0, 20 ml of 5% methanol and 10% methanol in the same buffer, at a flow rate of 1 ml/min, cores of $(2\text{-}5A)_4$, $(2\text{-}5A)_3$ $(2\text{-}5A)_2$ were eluted at 57.0 min, 58.6 min and 60.8 min, respectively. Oligonucleotide cores bigger than a tetramer were not detected in any analyzed sample. The amount of each core was estimated directly measuring the absorption area. The authenticity of each peak as a 2-5A core was checked by digesting it with snake venom phosphodiesterase. After digestion, the original peaks corresponding to each core were completely absent, while AMP and adenosine appeared in HPLC under the same conditions described above.

3. RESULTS AND DISCUSSION

We previously observed that 2-5A and its cores were potent non-competitive inhibitors of nuclear ADPRP activity in vitro [8]. Furthermore, inhibition of ADPRP activity was often observed in cultured cells after IFN treatment [9–11].

To investigate in more detail a possible effect of IFN on ADPRP activity, we have first determined ADPRP activity in fibroblast IFN- and poly(rI).poly(rC)-treated mouse LW cells. Fibroblast IFN is a mixture of alpha and beta IFN species and the synthetic double-helical polyribonucleotide was used as activator of 2-5A synthetase [12]. As shown in fig.1, a rapid decrease in ADPRP activity was observed in fibroblast IFN-treated cells after addition of poly(rI).poly(rC). After 1.5 h, the enzyme activity was decreased to about 50% of its initial value. Treatment with poly(rI).poly(rC) (fig.1) or fibroblast IFN alone (data not shown) induced a significant but less pronounced decrease in ADPRP activity.

Next, we have measured the concentration of $(2\text{-}5A)_n$ core ($n = 2$–4) in LW cells [13] under conditions where we have observed a marked reduction of the enzymatic activity. A definite increase of each of the 2-5A core species tested in fibroblast IFN-treated LW cells after 1 h of poly(rI).poly(rC) treatment have been observed (table 1). The concentrations of the $(2\text{-}5A)_2$ core, $(2\text{-}5A)_3$ core and $(2\text{-}5A)_4$ core in control LW cells were $0.03 + 0.01$, $0.19 + 0.03$ and $0.07 + 0.03$ pmol/10^6 cells (the mean + SD for three experiments), respectively. After 1.5 h of poly(rI).poly(rC) administration to fibroblast IFN-treated LW cells, the concentration of each (2-5A) core was increased 6-, 5- and 4.5-fold, respectively over the basal values. A lower but significant increase in 2-5A levels was also observed in LW cells treated only with poly(rI).poly(rC) (table 1) or fibroblast IFN according to the constitutive, but lower, presence of the enzyme in IFN-untreated cells. In each case, the trimer core level was the highest among the oligomers examined, being about 5- and 3-fold higher than the dimer and tetramer core, respectively. We have investigated, furthermore, whether LW cells treated with an exogenously added trimer core could show a decrease in ADPRP activity similar to that observed in the experiments described above (see fig.1). Fig.2a shows that trimer core (10 μM) added to the culture medium induced a prompt decrease of ADPRP activity in LW cells: 60 and 20% of initial enzymatic activity remained after 1.5 and 3 h of trimer core treatment, respectively.

Since both phosphorylated and unphosphorylated 2-5A seem to be potent inhibitors of protein synthesis

Table 1

HPLC analysis of 2-5A core levels in LW cells

Treatment	2-5A core, pmol/10^6 cells			
	A_2	A_3	A_4	Total
----	0.03	0.19	0.07	0.29
poly(rI).poly(rC)	0.09	0.50	0.14	0.73
Poly(rI).poly(rC) + fibroblast Interferon (500 U/ml)	0.18	0.91	0.31	1.40

Cells were treated as in the legend of fig.1. $(2\text{-}5A)_n$ core ($n = 2$–4) concentration in cells treated or not treated with poly(rI).poly(rC) or poly(rI).poly(rC) + IFN was determined as in section 2.

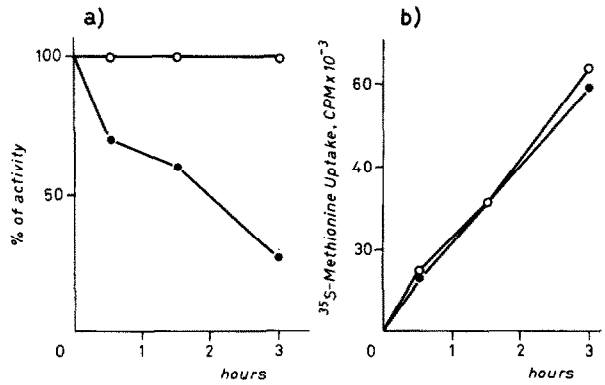

Fig.2. ADPRP activity and cellular protein synthesis in LW cells treated with trimer core. Mouse LW cells were grown as described in section 2, and passaged the day before the experiments to give monolayers of equal age and density. Confluent cells were treated with trimer core (10 μM) for the indicated times. (a) ADPRP activity. Enzyme activity was measured as described in section 2. (○—○), control cells; (●—●), cells treated with 2-5A. (b) Cellular protein synthesis. (○—○), control cells; (●—●), cells treated with 2-5A.

[13], their increased concentration in LW cells could determine an apparent inhibition of ADPRP activity due to protein synthesis inhibition. In order to examine this possibility, we have measured ^{35}S-methionine uptake by LW cells in the presence of 10 μM 2-5A trimer core. Under these conditions (where we observed inhibition of ADPRP) no detectable changes in ^{35}S-methionine uptake by the cells was observed (fig.2b). On the contrary, treatment of LW cells with poly(rI).poly(rC) (50 μg/ml) was followed by a drastic decrease in ^{35}S-methionine uptake. To exclude that inhibition of ADPRP activity was related to a specific protein synthesis inhibition, we have assayed the enzyme activity after treatment with a classic protein synthesis inhibitor. No variation in ADPRP activity could be detected after treatment of cells with cycloheximide (1 μg/ml) that induced more than 90% inhibition of protein synthesis (data not shown). These results strongly suggest that the observed decrease in ADPRP activity (Figs.1 and 2a) induced by poly(rI).poly(rC) cannot be related to the inhibition of protein synthesis. Moreover, the half-life of human placenta ADPRP (about 18 h, unpublished data) indicates further that the prompt decrease of ADPRP activity observed in our experimental system might be due to mechanisms different from protein synthesis inhibition. All the data presented here seem to indicate the possibility that a rapid accumulation of 2-5A core, especially trimer core, in LW cells induces inhibition of ADPRP activity.

Acknowledgements: This work was supported in part by grants of MPI 40%, CNR P.F. Oncologia 87.01332.44, Associazione Italiana Ricerca sul Cancro.

REFERENCES

[1] Knight, M., Cayley, P.J., Silverman, R.H., Wreschner, D.H., Gilbert, C.S., Brown, R.E. and Kerr, I.M. (1980) Nature 288, 189–192.
[2] Hersh, C.L., Brown, R.E., Roberts, W.K., Swyryd, E.A., Kerr, I.M. and Stark, G.R. (1984) J. Biol. Chem. 259, 1731–1737.
[3] Williams, B.R.G. and Kerr, I.M. (1978) Nature 276, 88–90.
[4] Kimchi, A., Shure, H., Lapidot, Y., Rapaport, S., Panet, A. and Revel, M. (1981) FEBS Lett. 134, 212–216.
[5] Baglioni, C. (1979) Cell 17, 255–264.
[6] Cachard, A. and De Maeyer-Guignard, J. (1981) Ann. Virol. 132, 307–312.
[7] Brown, R.E., Cayley, P.J. and Kerr, M. (1981) Methods Enzymol. 79, 208–216.
[8] Pivazian, A.D., Suzuki, H., Vartanian, A.A., Zhelkovsky, A.M., Farina, B., Leone, E. and Karpeisky, M.Y. (1984) Biochem. Int., 9, 143–152.
[9] Butt, T.R. and Sreevalsan, T. (1983) Exp. Cell. Res. 148, 449–459.
[10] Suhadolnik, R.J., Sawada, Y., Gabriel, J., Reichenbach, N.L. and Henderson, E.E. (1984) J. Biol. Chem. 259, 4764–4769.
[11] Leone, E., Suzuki, H., Farina, B., Pivazian, A.D. and Karpeisky, M.Ya. (1985) in: ADP Ribosylation of Proteins (Althaus, F.R., et al., eds) pp. 106–110, Springer Verlag.
[12] Lengyel, P. (1982) Annu. Rev. Biochem. 51, 251–282.
[13] Kerr, I.M. and Brown, R.E. (1978) Proc. Natl. Acad. Sci. USA 75, 256–260.