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INVOLVEMENT OF PROSTAGLANDIN-INDUCED PROTEINS IN THE INHIBITION OF HERPES SIMPLEX VIRUS REPLICATION

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Cyclopentenone prostaglandin(PG), Δ^7-PGA_1 was found to induce several polypeptides in human embryonic fibrobrast (HEF) cells which were noticed to be dose-related and appeared after 1 h of treatment with a peak at around 5 h and gradual disappearance after 12 h. PG-induced proteins were almost identical in terms of molecular weights with those induced by heat-shock at 42 °C. Regarding the mechanism of inhibition of herpes simplex virus (HSV) replication by PG in cell culture, dot blot hybridization has revealed that the level of immediate early (IE) mRNA of the virus was reduced after PG treatment with time dependence. And this delayed inhibitory effect of Δ^7-PGA_1 on HSV was shown to be associated with the production and accumulation of the induced polypeptides.

Cyclopentenone prostaglandins (PGs) such as PGA and PGJ have potent inhibitory activity for the growth of a variety of cultured cells (1,2). Narumiya and Fukushima (3) suggested that those PGs exhibit their activity by directly acting on nuclei rather than through the binding to cell surface receptor. Santro et al (4,5,6) have recently reported that the series of PGA and PGJ are potent inhibitors of the replication of animal DNA and RNA viruses. Our previous study (7) has shown that Δ^7-PGA_1, one of synthetic cyclopentenone PGs, is the

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most potent inhibitor for the replication of herpes simplex virus (HSV) among the nine PGs tested; this compound reduced the plaque formation of HSV by 50% at a concentration of 0.35 μg/ml at which protein synthesis of uninfected cells was not suppressed at all. Although the precise mechanism of the inhibition remained unclear, our data indicated that treatment with Δ^7-PGA_1 suppressed the level of immediate early mRNA of HSV. The objective of the present work was to clarify further the mechanism of inhibition of HSV reprication by Δ^7-PGA_1 and to identify the induced proteins by PGs in cell culture and compare with the heat-induced protein. We also tried to determine whether the reduction in the level of IE mRNA of HSV was associated with the production and accumulation of Δ^7-PGA_1 induced proteins.

MATERIALS AND METHODS

Human embryonic fibroblast(HEF) were prepared as described previously (8), grown in Eagle's essential minimal medium(MEM) supplemented with 10% fetal calf serum(FSC), and used for the experiments between passage 5 and 20. HSV-2 strain 186 was obtained from Dr. Fred Rapp, Pennsylvania State University College of Medicine, Penna.

Δ^7-PGA_1 was obtained from Teijin Ltd. Tokyo, and Δ^7-PGJ_2 from Fuji Chemical Industries Ltd. Takaoka Japan. PGs were stored as a 100% ethanolic stock solution (10mg/ml) at -20 °C and were diluted to appropriate concentration at the time of its use. Cycloheximide was purchased from Sigma chemical company. L-[^{35}S] Methionine (600 μCi/mmol) and [α-^{32}P]dCTP (410 μCi/mmol) were purchased from Amersham Laboratories.

Polyacrylamide slab gel electrophoresis(PAGE) was carried out by the method of Laemmli(9). Samples were prepared as described previously(7). The acrylamide concentrations were 8.5% for separating gel and 3% for staking gel.

Extracts of cytoplasmic RNA were prepared as described by White et al (10). HEF cells were pelleted by centrifugation (600 x g, 5 min). After suspension in 45 μl of ice-cold 10 mM Tris(pH 7.0), 1 mM EDTA, cells were lysed by addition of 10 μl aliquots of 5% Nonidet P-40 with 5 min of mixing on ice. Following pelleting of nuclei (15000 x g 2.5 mim), 50 μl of the supernatant was mixed with 30 μl of 20x SSC(0.15M NaCl, 0.015M sodium citrate) plus 20 μl of 37%(W/W) formaldehyde. The mixure was then incubated at 60 °C for 15 mim, and stored at -70 °C. For analysis, each sample was serially diluted, and each dilution was applied with suction to a 4 mm diameter spot on GENE SCREEN PLUS membrane purchased from New England Nuclear. Dot blot hybridization was performed as described previously(7). Cytoplasmic RNA was hybridized with plasmid (pACYC 184) containing HSV-2 Hind III digested D fragment that were shown to code immediate early (IE) protein (11).

RESULTS AND DISCUSSION

To examine the effect of Δ^7-PGA_1 on the protein synthesis of human embryonic fibroblasts(HEF), confluent monolayers of HEF were

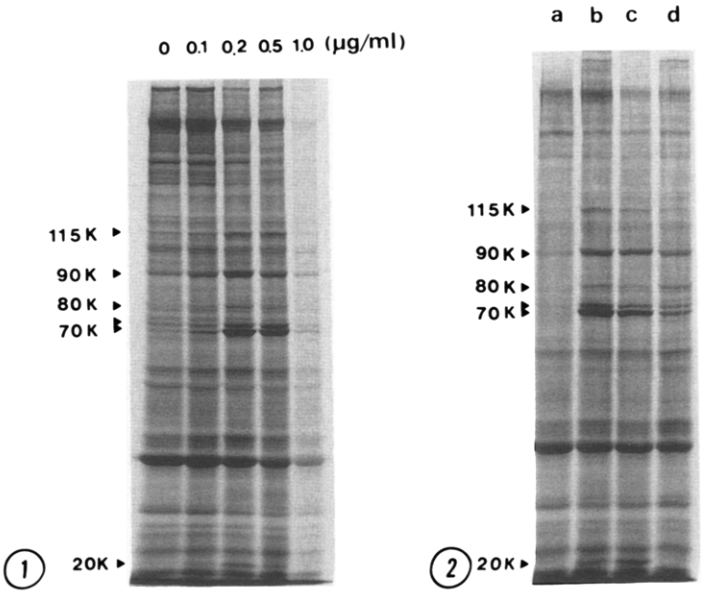

Fig.1. Effect of Δ^7-PGA_1 on the protein synthesis of human embryonic fibroblast(HEF). Confluent monlayers of HEF were treated for 5 h with various concentrations of Δ^7-PGA_1, and labelled with [^{35}S] Methionine for the next one hour. The radioactive polypeptide were analyzed by SDS-PAGE as described under MATERIALS AND METHODS. (▶) indicate new proteins induced by Δ^7-PGA_1.

Fig.2. The PAGE patterns of labelled proteins from PGs or heat treated cells. Confluent monolayers of HEF were treated without PG (a), for 5 h with 0.5 μg/ml of Δ^7-PGA_1 (b), for 5 h with 5 μg/ml of Δ^{12}-PGJ_2 (c) and for 4 h at 42 °C (d). (▶) indicate PGs- or heat-induced proteins.

treated for 5 h with various concentrations of the compound, and labelled with [^{35}S]methionine for the next one hour. As shown in Fig. 1, treatment with Δ^7-PGA_1 strikingly stimulated the production of several polypeptides. Under the conditions we have undertaken, the maximal stimulation was obtained at the concentrations ranging from 0.2 to 0.5 μg/ml, at which the overall protein synthesis was slightly suppressed. However treatment with 1 μg/ml of Δ^7-PGA_1 resulted in a marked inhibition of protein synthesis.

Since treatment of mammalian cells with some drugs have been known to induce heat shock proteins (12,13), we compared the PAGE patterns of labelled proteins from Δ^7-PGA_1-treated cells with those of cells incubated at 42 °C. The results are shown in Fig.2. The apparent molecular weights of Δ^7-PGA_1-induced proteins coincided with those of heat-induced proteins. In cells incubated at 42 °C, however, the induction of a protein with molecular weight of about 20 K was not

observed. Δ^{12}-PGJ_2, one of alkylidenecyclopentenone PG as well as Δ^7-PGA_1, also induced a set of polypeptides with the same molecular weight, but Δ^{12}-PGJ_2 required much higher concentrations to induce the proteins than Δ^7-PGA_1.

Fig.3 shows the time course of the production of induced proteins. In this experiment, cells were exposed to 0.5 μg/ml Δ^7-PGA_1 for 1 h at 37 °C, washed with cold PBS twice, and further incubated with maintenance medium at 37 °C in the absence of the drug. The cells were then pulse-labelled with [^{35}S]methionine for 1 h at various times after the beginning of treatment. The synthesis of PG-induced proteins was detectable at 1 h after treatment and increased gradually for the following 5 h. Following that the synthesis of PG-induced proteins decreased and became undetectable by 24 h after treatment. The results showed that exposure to 0.5 μg/ml of Δ^7-PGA_1 for 1 h gave rise to the optimal synthesis of induced proteins without significant suppression of constitutive host cell protein synthesis.

Our previous study (7) suggested that the inhibition of HSV replication by Δ^7-PGA_1 was primarily due to the inhibition of immediate early mRNA synthesis. The following experiments were thus performed to examine whether the level of immediate early viral mRNA is suppressed in the cells pretreated with Δ^7-PGA_1 without maintenance. Cells were treated with Δ^7-PGA_1 (0.5 μg/ml) for 1 h, washed with cold PBS three times, and further incubated with maintenance medium at 37 °C. At 1, 3 and 5 h after the beginning of treatment, the cells were infected with HSV at a multiplicity of 10 PFU/cell and incubated for 4 h in the presence of cycloheximide. Cytoplasmic RNA was then extracted and examined by dot blot hybridyzation using the Hind III D fragment as a probe which included immediate early genes. As shown in Fig. 4, no significant inhibition of IE mRNA synthesis was observed if HSV was infected at 1 h after treatment whith 0.5 μg/ml Δ^7-PGA_1. When infected at 3 and 5 after treatment, however, the obvious reduction of IE mRNA was demonstrated. The level of IE mRNA was reduced by more than 90% in the cells infected at 5 h after treatment.

In this study we demonstrated a delayed inhibitory effect of Δ^7-PGA_1 on the replication of HSV: when the cells treated with appropriate concentrations of the drug were throughly washed with PBS, the reduction in the level of IE mRNA was exhibited in the manner of time-dependence. This delayed effect was found to be associated with the production/accumulation of PG-induced proteins, the molecular weights of which were apparently identical to those of heat shock proteins. Although we don't know yet the mechanism how PG-induced

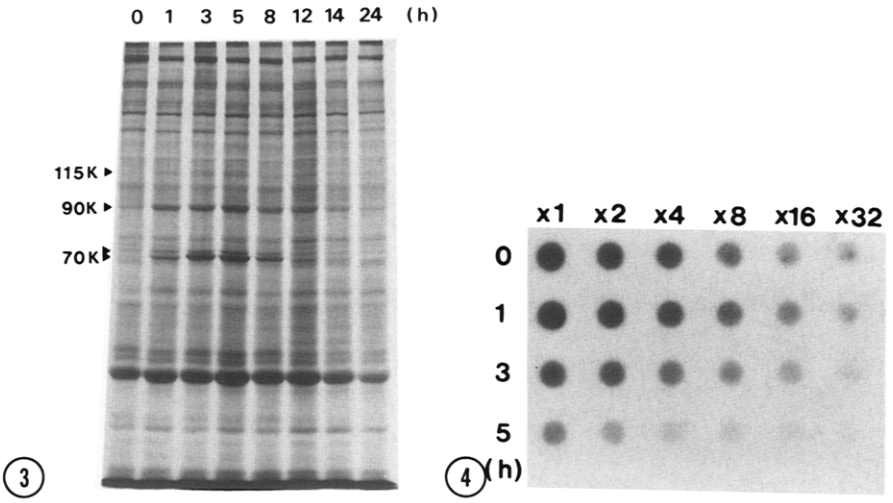

Fig.3. The time course of the production of $^{\Delta 7}$-PGA$_1$-induced proteins. HEF cells were treated with 0.5 μg/ml of $^{\Delta 7}$-PGA$_1$ for 1 h at 37 °C, washed with cold PBS twice, and further incubated with maintenance medium at 37 °C in the absence of PG. The cells were then pulse-labelled with [^{35}S] Methionine for 1 h at various times after the begining of treatment. (▶) indicate PG-induced proteins.

Fig.4. Inhibition of immediate early viral mRNA synthesis in the cells pretreated with $^{\Delta 7}$-PGA$_1$. Cells were treated with $^{\Delta 7}$-PGA$_1$ (0.5 μg/ml) for 1 h, with cold PBS three times, and further incubated with maintenance medium at 37 °C in the absence of PG. At various times after the beginning of treatment, the cells were infected with HSV and incubated for 4 h in presence of cycloheximide (100 μg/ml). Cytoplasmic RNA was diluted serially twofold. Extraction of cytoplasmic RNA and dot blot hybridization were performed as described in the MATERIALS AND METHODS section.

proteins work in the suppression of IE mRNA level, similar mechanism may be involved in the regulation of cell proliferation by PGs.

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