INCREASE OF DNA POLYMERASE ACTIVITIES IN HeLa CELLS TREATED WITH *N*-METHYL-*N'*-NITRO-*N*-NITROSOGUANIDINE

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1. Introduction

An inducible error-prone repair or replication enzyme system has been postulated to participate in the process of mutagenesis in prokaryotes [1,2]. It has also been suggested that mutagens and carcinogens may give rise to inducible repairs in eukaryotes [3,4].

Although the process of mutagenesis of carcinogenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) is not well known, it was demonstrated that MNNG mutagenesis resulted from both modification of DNA and induction or enhancement of mutagenic capacity of cells [5]. The participation of inducible change in DNA polymerase activities of *Escherichia coli* in the repair or mutagenesis by MNNG was suggested in [6]. Here, we have examined changes in the DNA polymerases α and β of HeLa cells during incubation after treatment with MNNG.

2. Materials and methods

2.1. *Cell culture and MNNG treatment*

HeLa S3 cells were plated at 2×10^5 cells/150 mm plastic petri dish and cultivated at 37°C in 20 ml Eagle's minimum essential medium (MEM) supplemented with 10% calf serum under 5% CO_2 in air. When the cells reached the proliferative phase, the medium was replaced by 20 ml Eagle's MEM, and 0.2 ml MNNG (Aldrich Chemical) solution in phosphate-buffered saline containing 0.15% dimethylsulfoxide was added to it. To the control cells, 0.2 ml of the same solution without MNNG was added. After incubation for 2 h at 37°C, the cells were rinsed with 20 ml Eagle's balanced salt solution and subsequently incubated for 16 h at 37°C in 20 ml Eagle's MEM supplemented with 10% calf serum.

2.2. *Measurement of DNA synthesis and repair synthesis*

To measure DNA synthesis, at 15 h after treatment with MNNG, the cells were incubated with 0.1 μCi/ml [^{3}H]thymidine (24 Ci/mmol, Radiochemical Centre) for 1 h. To measure repair synthesis, at 14 h after treatment with MNNG, the cells were pretreated for 1 h with 2 mM hydroxyurea (Sigma) then incubated for 1 h with 1 μCi [^{3}H]thymidine/ml in the presence of 2 mM hydroxyurea following the modified method in [7].

2.3. *Isolation of DNA polymerases*

About 0.3–1.0 ml packed cells was suspended in 4 ml of a mixture of 0.32 M sucrose, 1 mM potassium phosphate (pH 6.8), 2 mM $MgCl_2$ and 0.5 mM dithiothreitol (DTT), and the suspension was sonicated 6 times for 30 s. An equal volume of 4 M NaCl [8] was added to the disrupted cell suspension and the mixture was allowed to stand at 0°C for 1 h. It was then centrifuged at 105 000 $\times$ *g* for 18 h. The supernatant was dialyzed for 8 h against 0.35 M potassium phosphate (pH 7.7), 0.5 mM DTT–20% glycerol (v/v). To remove the cellular DNA, the dialysate was passed through DEAE-cellulose (Whatman DE 52) equilibrated with the same buffer, and the eluate was dialyzed for 8 h against the buffer containing 0.05 M KCl, 50 mM Tris–HCl (pH 7.7), 0.5 mM DTT–20% glycerol (v/v). The dialysate was adsorbed onto a phosphocellulose (Whatman P 11) column equilibrated with the same buffer. The DNA polymerases

were eluted with a 0.05–2.0 M KCl linear gradient of 14 bed vol. To obtain the nuclear fraction, the cell suspension was disrupted by a Dounce homogenizer and the isolated nuclei were treated as above for whole cells.

2.4. *Assay of DNA polymerases*

The 50 μl reaction mixture for the assay of DNA polymerase α or β contained 50 mM Tris–HCl (pH 8.0), 7.5 mM $MgCl_2$, 1 mM DTT, 500 μg/ml of bovine serum albumin (BSA), 250 μg/ml of activated calf thymus DNA [9], 0.1 mM of all 4 dNTPs (Sigma), d[^{3}H]TTP (37.5 cpm/pmol, Radiochemical Centre), and 10 μl enzyme solution. The 50 μl for the assay of DNA polymerase γ or β contained 50 mM Tris–HCl (pH 7.5), 0.5 mM $MnCl_2$, 2.5 mM DTT, 500 μg/ml of BSA, 20 μM poly(rA)$(dT)_{12-18}$ (P-L Biochemicals), 0.1 mM dTTP, d[^{3}H]TTP (37.5 cpm/pmol), and 10 μl enzyme solution. The reaction mixtures were incubated at 37°C for 30 min, and 40 μl portions were placed on filter paper discs (Whatman DE 81) and analysed as in [10].

3. Results and discussion

An induction of enhanced DNA repair capacity has been reported to require post-culture after treatment of the mammalian cells with carcinogens [3]. In MNNG-treated HeLa cells, the degree of DNA synthesis was maximal at 13–20 h incubation after treatment with MNNG, and repair synthesis was observed here throughout post-incubation. To permit inducible repair, the HeLa cells were incubated for 16 h after treatment with various concentrations of MNNG then examined for incorporation of [^{3}H]-thymidine and DNA polymerase activities. DNA synthesis and repair synthesis were investigated only for adhesive cells, although floating cells did appear during incubation of cells after treatment with MNNG at >40 μM. As shown in fig.1, DNA synthesis decreased as [MNNG] increased and a reproducible shoulder at 40–60 μM was observed. Repair synthesis increased in MNNG-treated cells and was maximal at 40–80 μM.

DNA polymerases were extracted from MNNG-treated or untreated HeLa cells and separated by phosphocellulose column. DNA polymerase α was eluted at 0.25–0.35 M KCl in phosphocellulose

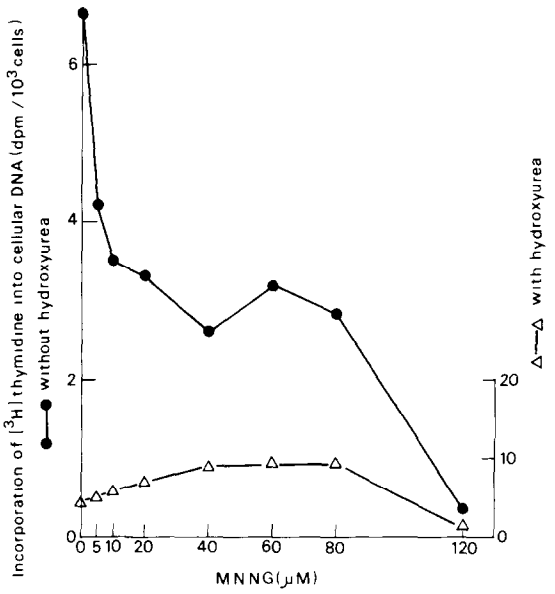

Fig.1. Effect of MNNG on DNA synthesis and repair synthesis. After treatment with MNNG for 2 h and subsequent cultivation for 15 h, the cells were incubated for 1 h with 0.1 μCi/ml of [^{3}H]thymidine in the absence of hydroxyurea or 1 μCi/ml of [^{3}H]thymidine in the presence of 2 mM hydroxyurea. The radioactivity incorporated into the cellular DNA was measured as in section 2.

chromatography, and polymerase β was eluted at 0.6–0.7 M KCl (fig.2A). The peak of polymerase γ could not be separated completely from that of polymerase α by this procedure, but polymerases γ and α were separated by subsequent DNA cellulose chromatography. The utilization of template-primer, elution patterns in the DEAE-cellulose chromatography and inhibition by *N*-ethylmaleimide of these polymerases were consistent with those in [8,11].

DNA synthesis, polymerase α and β activities in the control cells at 8 h–20 h of post-culture were the same as those of the cells before incubation for 2 h in MEM without MNNG. The integral of each DNA polymerase activity of MNNG-treated adhesive cells was compared with that of control cells after phosphocellulose chromatography of a cellular extract containing the same amount of protein as the control. The amount of protein extracted from MNNG-treated adhesive cells was almost the same as that from the control cells, although that from MNNG-treated floating cells was smaller than the control. The poly-

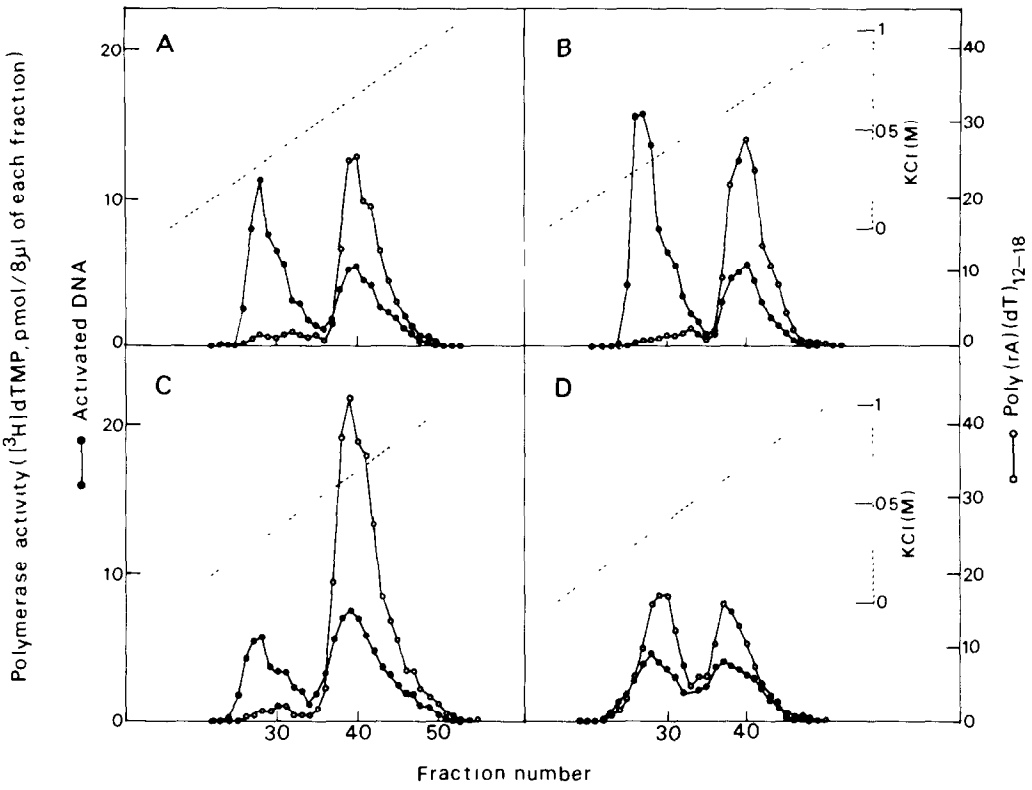

Fig.2. Phosphocellulose chromatographic patterns of DNA polymerases of control and MNNG-treated HeLa cells. HeLa cells were rinsed after treatment with MNNG for 2 h and harvested after 16 h incubation. Extracts containing 2 mg protein were adsorbed to 1 ml on phosphocellulose columns (0.6 × 3.6 cm), and the DNA polymerases were eluted with linear gradients from 0.05–2.0 M KCl containing 50 mM Tris–HCl (pH 7.7), 0.5 mM DTT and 20% glycerol (v/v). (A) Control cells; (B) 10 μM MNNG-treated adhesive cells; (C) 60 μM MNNG-treated adhesive cells; (D) 80 μM MNNG-treated floated cells.

merase γ activity could not be determined quantitatively because it was low and undistinguishable from the activity of polymerase α in its ability to utilize poly(rA)$(dT)_{12-18}$ [12].

Figs.2A–C show phosphocellulose chromatographic patterns of DNA polymerases from adhesive cells. The polymerase α activity was increased by treatment of the cells with a low concentration of MNNG such as 10 μM (fig.2B), but was decreased at high concentrations of MNNG (fig.2C). The polymerase β activity increased on treatment of the cells with a high concentration of MNNG such as 60 μM (fig.2C). In cells floated by treatment with high [MNNG], both polymerase α and β activities decreased. However, the polymerase γ activity increased (fig.2D), and 80% of the activity at 0.25–0.35 M KCl was estimated to be that of polymerase γ after separation of polymerases α and γ using a DNA-cellulose column [11]. The floated cells which appeared on treatment with other concentrations of MNNG revealed similar chromatographic patterns to those in fig.2D.

Fig.3 shows the change in polymerase α and β activities per cell on treatment of HeLa cells with various concentrations of MNNG. The polymerase α activity was increased at relatively lower concentrations of MNNG and significantly decreased at a high concentration of this chemical. Since it has been shown that polymerase α undergoes large variations during the cell cycle [8], it is uncertain whether the increased polymerase α activity in the cells treated with a lower concentration of MNNG was induced or not.

The polymerase β activities in fig.3 were deter-

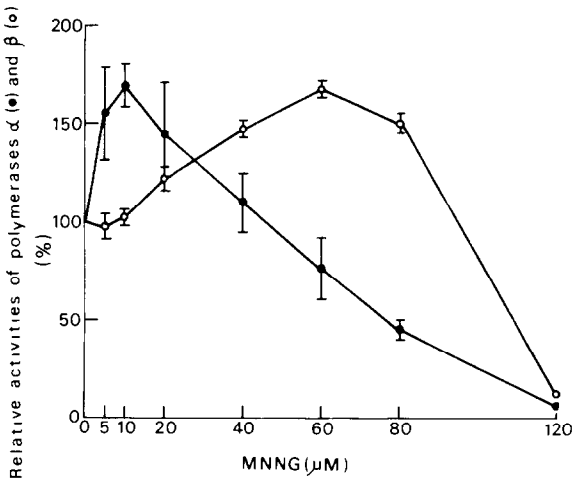

Fig.3. Variations in DNA polymerase α and β activities in adhesive HeLa cells treated with MNNG. Extracts from MNNG-treated HeLa cells containing 2 mg protein were chromatographed as in fig.2. The integral of the DNA polymerase α and β activities measured using activated DNA was calculated to activity per cell and was plotted as a relative activity. The value for control cells corresponded to 100%. The values indicated are means ± SE from 3 or 4 different expt.

mined on calf thymus activated DNA as template-primer, and similar results were obtained using poly(rA)$(dT)_{12-18}$. An enhancement of polymerase β was observed on 40–80 μM MNNG-treatment. When polymerase β was extracted from the isolated nuclei, the change in activity by MNNG was the same as that observed for the extract from whole cells as indicated in fig.3. The changes in polymerase β did not coincide with those of polymerase α, suggesting that the enhanced DNA polymerase β may play a role other than in normal DNA synthesis. Polymerase β has been reported to be essentially constant throughout the cell cycle [8,13] and to be relatively constant during different stages of the growth curve [14]. The cells used in the present experiments were always in the proliferative phase, so that any observed enhancement of polymerase β activity appears to have been induced by MNNG. Polymerase β also seems to participate in the repair of DNA damaged by MNNG at 40–80 μM based on the fact the enhancement of polymerase β activity coincided with increased repair synthesis at this concentration of MNNG.

Involvement of polymerase β has been reported in the case of repair synthesis after UV irradiation [15,16]. The induction of polymerase β in rats chronically fed with diethylnitrosamine has also been suggested [17]. These and the results in [6] demonstrate that similar types of DNA polymerase, polymerase β and polymerase II, were enhanced in mammalian and bacterial cells, although it is debatable whether these polymerases participate in error-free or error-prone repair. Results with aphidicolin have indicated that polymerase α could also play a role in DNA repair [18–20]. Examinations of the characteristics of the induced enzymes are currently being undertaken.

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