# Effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine on DNA polymerases $\alpha$ and $\beta$ in vitro

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DNA polymerases $\alpha$ and $\beta$ were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). The activities of the modified DNA polymerase were decreased and the degree of lowered activity varied with the template employed. However, no decrease in fidelity was observed in DNA synthesis by the MNNG-treated polymerases. It is suggested that direct modification of the DNA polymerase molecule by MNNG is not the cause of carcinogenesis.

*DNA polymerase* $\alpha$ *DNA polymerase* $\beta$ *Fidelity in DNA synthesis* *MNNG*

## 1. INTRODUCTION

DNA is a primary target in the modification of cellular components by carcinogens, and DNA syntheses on modified DNAs are found to be mutagenic [1]. Some DNA modifications have been said to induce error-prone repair systems in mammalian cells [2], and several DNA polymerases with decreased accuracy have been isolated from human leukemia cells [3], from rat liver after exposure to *N*-2-fluorenylacetamide [4] and from rat liver fibroblasts after treatment with mitomycin C [5]. Such altered polymerases induced by treatment of animals or cells with carcinogens appear to play a role in carcinogenesis.

Chemical carcinogens have also been suggested to interact directly with polymerases resulting in an altered base-selective function [6], and our previous experiments showed that modification of *E. coli* DNA polymerase by MNNG changed the fidelity of the enzyme [1]. We investigate here the effect of MNNG on the fidelity of mammalian DNA polymerases $\alpha$ and $\beta$.

## 2. MATERIALS AND METHODS

Ehrlich ascites carcinoma DNA polymerases $\alpha$ (Pol $\alpha$) and $\beta$ (Pol $\beta$) were prepared as in [7]. The enzymes were incubated with MNNG (Aldrich) at 30°C for 30 min in 10 mM potassium phosphate (pH 7.7) containing 200 mM KCl and 50% glycerol. The reaction mixture was thoroughly dialysed against 50 mM Tris–HCl (pH 7.2) containing 200 mM KCl and 50% glycerol to remove unreacted MNNG [1].

The error rate was determined from the ratio of non-complementary nucleotide to complementary nucleotide incorporated into the template-primer. These estimates were performed in separate assays with the same reaction mixture, except the labeled nucleoside triphosphate as in [8]. The 100 $\mu$l reaction mixture contained 50 mM Tris–HCl (pH 7.2–8.9), 100 $\mu$M poly(dA)$(dT)_{12-18}$ (1:1, P-L Biochemicals) or poly(dC)$(dG)_{12-18}$ (1:1, P-L Biochemicals), 100 $\mu$M complementary dNTP (Sigma), 2–13 $\mu$M non-complementary dNTP, 3–7.5 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mg/ml bovine serum albumin and 1.9–20.1 $\mu$g Pol $\alpha$ or 0.07–3.1 $\mu$g Pol $\beta$. Complementary [$^3$H]dNTPs (60 mCi/mmol) were used for correct incorporation, and non-complementary [$^3$H]dNTPs (7.7–50 Ci/mmol, Radiochemical Centre) for misincorporation. The reaction mixture was incubated at 37°C for 1 h, then mixed with 10 $\mu$l of unlabeled cold 10 mM non-complementary dNTP to lower the back-

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ground radioactivity, and placed on a Whatman 3 MM filter paper disc which was successively washed twice with cold 10% trichloroacetic acid, 4 times with 3% trichloroacetic acid and 3 times with ethanol. The radioactivity was measured in a toluene scintillator and the incorporation without template-primer was subtracted from the value for each assay.

## 3. RESULTS AND DISCUSSION

Incubation of Pol $\alpha$ and Pol $\beta$ with MNNG in vitro decreased their polymerase activities, the degrees of inactivation being different depending on the template-primer (fig.1,2). The activity of Pol $\alpha$ on poly(dA)$(dT)_{12-18}$ was inhibited by MNNG to a greater extent than that on poly(dC)$(dG)_{12-18}$. On the other hand, more marked inactivation of Pol $\beta$ by this carcinogen was observed on poly-(dC)$(dG)_{12-18}$ than on poly(dA)$(dT)_{12-18}$. This differential inactivation pattern did not appear to result from the thermolability of these enzymes, since the extents of inactivation of Pol $\alpha$ and Pol $\beta$ by heating at 30°C for 30 min without MNNG were about 7 and 50%, respectively, on all kinds of templates used (not shown).

The spontaneous error rates of Pol $\alpha$ and Pol $\beta$ were higher in the transition type than in the transversion type; i.e. the misincorporation of dCMP was greater than that of dGMP on poly(dA)$(dT)_{12-18}$ and the misincorporation of dAMP was greater than that of dTMP on poly-(dC)$(dG)_{12-18}$ (table 1). These results are comparable to those in [9] and to our previous data for *E. coli* polymerase [1]. The fidelities of Pol $\alpha$ and Pol $\beta$ were then measured after treatment of the enzymes with MNNG which reduced the correct incorporation to 10–15% of the controls. No decreased fidelity of Pol $\alpha$ and Pol $\beta$ was observed after treatment of the enzymes with MNNG (table 1).

In the case of *E. coli* polymerase, alteration of the fidelity of the enzyme was caused by the differential inactivation of polymerase activity and 3′ to 5′ exonuclease activity [1]. It appears reasonable to suppose that the fidelities of mammalian DNA polymerases are not lessened by this carcinogen since these polymerases are without exonuclease activity. It would be interesting to check whether the fidelity of a DNA polymerase such as $\delta$ [10], which possesses 3′ to 5′ exonuclease activity, is altered by treatment with MNNG.

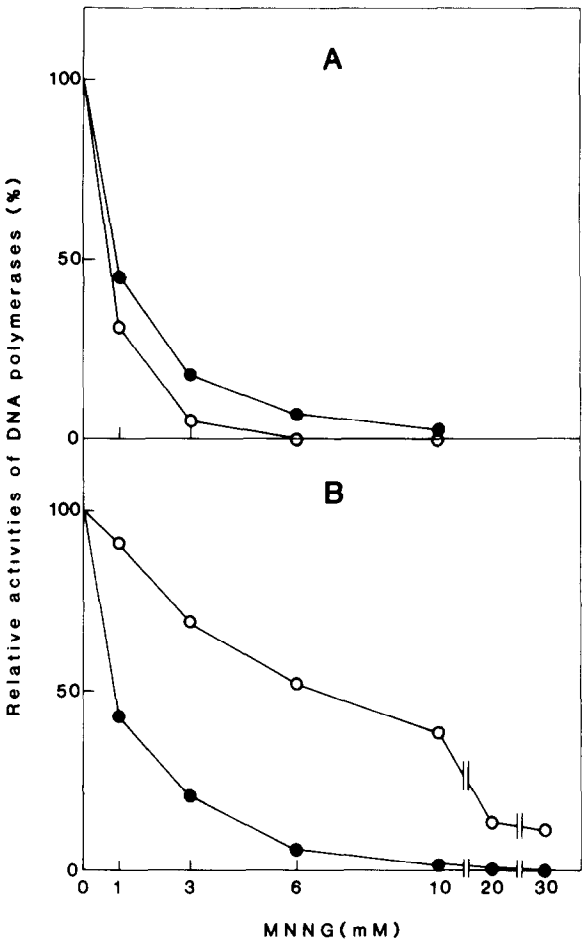


Fig.1. Effect of MNNG on DNA polymerase activities. Pol $\alpha$ and Pol $\beta$ were preincubated with or without MNNG at 30°C for 30 min and then assayed for correct incorporation as described in section 2. The pH of 50 mM Tris–HCl and concentration of $MgCl_2$ were 7.5 and 7.7 mM, respectively. (○) incorporation of [$^3$H]dTMP into poly(dA)$(dT)_{12-18}$, (●) incorporation of [$^3$H]dGMP into poly(dC)$(dG)_{12-18}$. (A) Pol $\alpha$. Incorporation of control enzyme treated without MNNG into poly(dA)$(dT)_{12-18}$ and poly(dC)$(dG)_{12-18}$ was 2400 pmol and 4400 pmol, respectively. (B) Pol $\beta$. Incorporation of control enzyme treated without MNNG into poly-(dA)$(dT)_{12-18}$ and poly(dC)$(dG)_{12-18}$ was 12700 pmol and 1300 pmol, respectively.

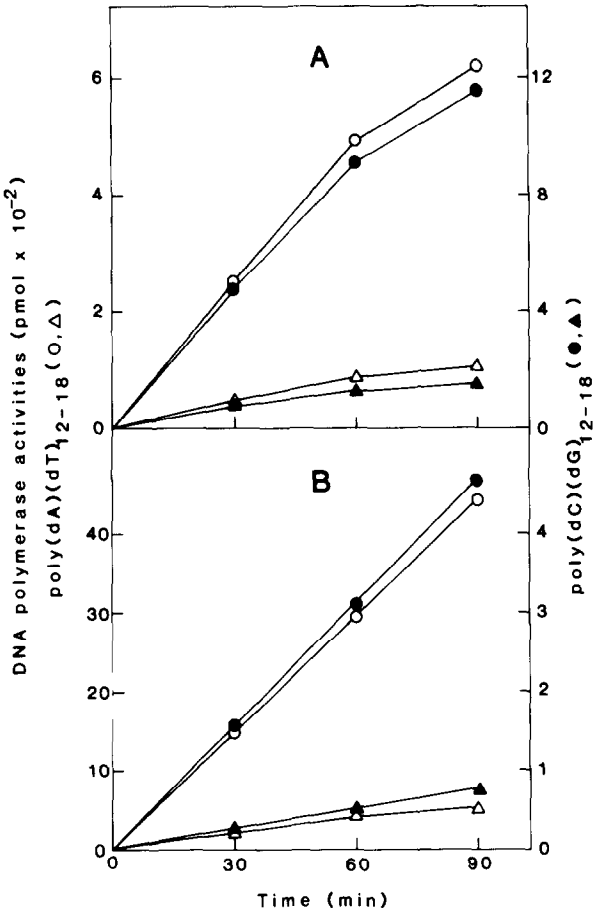


Fig.2. Time course of DNA polymerase activities after treatment of the enzymes with or without MNNG. After preincubation of Pol$\alpha$ and Pol$\beta$ with or without MNNG, Pol$\alpha$ and Pol$\beta$ were incubated under the same conditions as described in fig.1. Portions of 25 $\mu$l were withdrawn at the indicated times and the correct incorporations were measured. (○,△) incorporation of [$^3$H]dTMP into poly(dA)$(dT)_{12-18}$, (●,▲) incorporation of [$^3$H]dGMP into poly(dC)$(dG)_{12-18}$. (A) Pol$\alpha$. (○,●) control enzymes, (△) 2 mM MNNG treatment, (▲) 4 mM MNNG treatment. (B) Pol$\beta$. (○,●) control enzymes, (△) 20 mM MNNG treatment, (▲) 4 mM MNNG treatment.

It has been shown that replicationally essential proteins other than DNA polymerase participate in increasing the fidelity of DNA synthesis in phage T4 [11] and that an *E. coli* single-strand binding protein decreases the infidelities of Pol$\alpha$ and Pol$\beta$ [12]. Since MNNG modifies proteins more highly than DNA in vivo [13], the possibility remains that an enhancement of the error rate in DNA synthesis is produced when MNNG inactivates some proteins which have functions in bringing about high accuracy of the replication complex in mammalian cells.

Table 1
Misincorporation catalysed by Pol$\alpha$ and Pol$\beta$ after treatment with MNNG

| Template | Enzyme | MNNG (mM) | Correct incorporation (pmol) | Incorrect incorporation (pmol) | | Error rate | |
|---|---|---|---|---|---|---|---|
| | | | dTMP | dCMP | dGMP | dCMP/dTMP | dGMP/dTMP |
| Poly(dA)$(dT)_{12-18}$ | Pol$\alpha$[a] | 0 | 2040 | 0.173 | 0.029 | 1/12 000 | 1/70 000 |
| | | 2 | 1500 | 0.060 | 0.023 | 1/25 000 | 1/65 000 |
| | Pol$\beta$[b] | 0 | 2426 | 0.226 | 0.020 | 1/11 000 | < 1/100 000 |
| | | 20 | 1568 | 0.113 | 0.009 | 1/14 000 | < 1/100 000 |
| | | | dGMP | dAMP | dTMP | dAMP/dGMP | dTMP/dGMP |
| Poly(dC)$(dG)_{12-18}$ | Pol$\alpha$[c] | 0 | 1670 | 0.439 | 0.005 | 1/3 800 | < 1/100 000 |
| | | 4 | 1030 | 0.135 | 0.004 | 1/7 600 | < 1/100 000 |
| | Pol$\beta$[d] | 0 | 3170 | 2.484 | 0.138 | 1/1 300 | 1/23 000 |
| | | 4 | 3050 | 0.808 | 0.092 | 1/3 800 | 1/33 000 |

Pol$\alpha$ and Pol$\beta$ were preincubated with MNNG which decreased the activities to 10–15% of control enzymes, dialysed, and assayed for correct and incorrect incorporation as detailed in section 2. The control enzymes were preincubated without MNNG and were dialysed. The pH and concentration of $MgCl_2$ in the reaction mixture were: (a and c) pH 7.2, 3 mM; (b) pH 8.0, 7.5 mM; (d) pH 8.9, 4.5 mM, respectively.

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