ENHANCEMENT OF DNA POLYMERASE II ACTIVITY IN E. COLI AFTER TREATMENT WITH N-METHYL-N'-NITRO-N-NITROSOGUANIDINE

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SUMMARY: Treatment of E. coli P3478 (polA1, thy$^-$) with N-methyl-N'-nitro-N-nitrosoguanidine followed by incubation of cells in growth medium enhanced DNA polymerase II activity up to 5 times as high as that of control cells, in contrast to slight change in polymerase III activity. This enhancement was inhibited by chloramphenicol added to the post-treatment incubation medium.

INTRODUCTION: N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) is a potent mutagen (1) and carcinogen (2) which interacts with nucleic acids (3) and with protein (4,5). Chemical modofication of the base moiety in DNA is assumed to be the cause of mutation, but the process responsible for mutation fixation is not understood. Different mutagenic specificities in the different species treated with MNNG suggested participation of inherent cellular function in mutation (6). This chemical induced mutation selectively at the replication point (7), and Kondo et al. suggested that MNNG affects the replication machinery to cause error-prone repair or replication directly or indirectly (8). One possibility is direct modification of the pre-existing DNA polymerase, but treatment of DNA polymerase I, II and III *in vitro* resulted in little change in fidelity of DNA synthesis (Miyaki et al. submitted). Another possibility is induction of an enzyme system responsible for error-prone repair. In the course of examination of change in DNA polymerase we have found

ABBREVIATIONS: MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine, CM = Chloramphenicol

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the remarkable enhancement of DNA polymerase II activity after treatment of E. coli with MNNG in contrast to slight change in polymerase III activity.

MATERIALS AND METHODS: E. coli P3478 (polA1, thy$^-$) were grown at 37° for 4 hr to 7 × 10^8 cells/ml in L-broth (8g DIFCO Trypton, 4g Yeast Extract, 4g NaCl and 1g glucose in 1000 ml) supplemented with 20 µg/ml thymine. The cells were collected and suspended in 0.067 M phosphate buffer, pH 6.8 (1.4 × 10^{10} cells/ml) and the suspension was incubated at 37° for 1.5 hr in the absence or presence of 10 to 15 µg/ml of MNNG without shaking. After washing the cells were suspended in fresh L-broth (7 × 10^8 cells /ml) and cultured at 37° for 1 hr, and then harvested and frozen at -20°. Three to 8 g of the frozen packed bacteria were broken by stirring with glass beads. The disrupted mixture was centrifuged at 100,000 × g for 1 hr, and the supernatant was passed through a DEAE cellulose column (Whatman DE 52) at 0.3 M salt concentration to remove nucleic acids. When the eluate was adjusted to 55% saturation with ammonium sulfate about 93% total DNA polymerase activity was precipitated. The solution of the precipitates (30 mg protein) was dialyzed and applied to a DEAE cellulose column (1.5 × 17 cm) equilibrated with 0.02 M potassium phosphate buffer containing 10 mM mercaptoethanol and 20% glycerol, pH 6.5, and the column was eluted with a gradient of 0.02 to 0.3 M phosphate (total volume 200 ml). Two ml fractions were collected and the polymerase activity was assayed as follows: The reaction mixture (45 µl) contained 10 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 2 mM dithiothreitol, 10% ethanol, 500 µg/ml bovine serum albumin, 33 µM [^{3}H]dTTP (7.5 to 37.5 cpm/pmol), 20 µM poly(dA), 10 µM $(dT)_{12-18}$ and 5 µl of enzyme solution. The reaction mixture was incubated at 30° for 30 min and 40 µl of the reaction mixture was placed on filter paper disc (Whatman 3MM) which was then washed three times with 5% TCA and ethanol. The radioactivity was measured in toluene scintillator. The quantity of protein eluted in coincident with the peak of polymerase was also measured.

RESULTS: Treatment of cells for 1.5 hr in phosphate buffer with or without MNNG did not permit cell growth. After 1 hr post-treatment culture in L-broth, wet weight of the control cells increased to 1.8 times of that before the post-culture, and weight of MNNG-treated cells increased to 1.5 times. And also, cell filamentation was observed. Under these conditions the colony-forming ability of MNNG-treated cells was 2 to 12% of the control cells when scored at 18 hr after plating on agar.

DNA polymerase II (Pol II) and III (Pol III) in the control cells were eluted at 0.15 M and 0.2 M phosphate, respectively (Fig. 1a), and chromatographic behaviors of these enzymes on

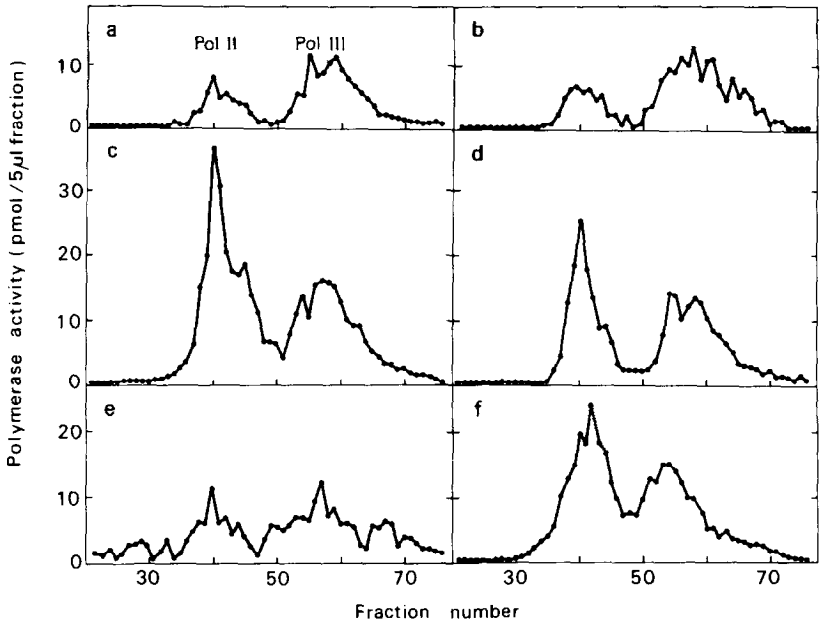

Fig. 1. Chromatography of DNA polymerase II and III from E. coli P3478.

a. Untreated or treated without MNNG;
b. Immediately after MNNG-treatment;
c. Cultured for 1 hr post MNNG-treatment;
d. Mixture of protein solution, (a)+(c)=1:1;
e. Cultured for 1 hr with Chloramphenicol (60 μg/ml) post MNNG-treatment;
f. Cultured for 2 hr after incubation with Chloramphenicol (30°, 18 hr) post MNNG-treatment.

phosphocellulose and sensitivities to thiol reagent were consistent with those described (9,10). Immediately after treatment of bacteria with MNNG in phosphate buffer, Pol II and Pol III activities were the same as those of control cells (Fig. 1b). The post-MNNG-treatment culture resulted in increase of Pol II activity up to 5 times as high as that of control cells as indicated in Fig. 1c, Pol II activity at 1 hr post-culture being higher than at 0.5 hr. Whereas Pol III activity was unchanged or increase was not more than 1.5 times. To examine if increase in Pol II activity results from change of a certain

factor affecting polymerase, the extracts from the control and MNNG-treated cells were previously mixed. Pol II activity in chromatography of the mixture exhibited the mean of activity in individual chromatography, indicating that there was no inhibitor for Pol II in the control or activator in the MNNG-treated cells (Fig. 1d). The enhancement induced by MNNG was inhibited by chloramphenicol (CM) as shown in the following. When CM was added to the post-treatment culture medium, no enhancement of Pol II(Fig. 1e) nor multiplication of cells was observed. After removal of CM, both enhancement of Pol II (Fig. 1f) and cell growth were initiated, but enhancement was less than in Fig. 1c because the cells were incubated with CM for a long period (18 hr) and increase in cell density of the post-culture was only 1.2 times in this experiment. On the other hand, in the control cells the peak of Pol II did not increase in spite of the 2.4 times increase in cell density after removal of CM, and the profile of elution (not shown) was similar to Fig. 1a.

DISCUSSION: Identity of the induced Pol II with the pre-existing Pol II in the control cells is not confirmed yet, and hence a possibility that the induced polymerase is an altered enzyme (Pol II' or Pol III') or a certain altered complex is not excluded. But the possibility of a new enzyme such as Pol IV will be ruled out since polymerase fraction other than Pol III was not detected in DEAE cellulose chromatography of the extract from MNNG-treated H10261 cells (polA1, polB1, $dnaE_{ts}$, thy^-) (data are not shown).

Enhancement of the polymerase activity may be induced from modification of a certain repressor protein (11) for the polymerase by MNNG, and the degradation products of modified DNA

is possibly involved in the induction. A striking result was that during the 18 hr incubation of the MNNG-treated cells with CM the cellular damage caused by MNNG was conserved, and increase in the polymerase was initiated at the time of removal of the antibiotic. It is concluded that enhancement of Pol II activity is caused via protein synthesis after MNNG-treatment of cells. Mutation to streptomycin-resistance induced by MNNG was also observed under the same treatment conditions as those which induced the enhancement of Pol II activity (data are not shown).

Requirement for protein synthesis of the post-replication repair responsible for UV-mutagenesis has been described (12) and this evidence has supported the prediction by Witkin that an inducible product is involved in the UV-mutagenesis (13). Witkin also proposed the "SOS-regulation" which being caused via activation of rec A^+ product resulted in simultaneous induction of many UV-inducible functions such as SOS-repair, UV-mutagenesis, prophage induction, and filamentous cell growth etc. Although participation of Pol II in DNA repair and/or mutagenesis is not clear yet, our present results seem to reflect a part of simultaneous induction of change in DNA polymerase, filamentous growth of cells, and, MNNG-mutagenesis etc. caused by MNNG which interacts with DNA and other cellular components probably in a manner different from UV. Characterization of induced Pol II and error-proneness is under investigation.

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