Effects of Adrenaline, Cortisone, Glucose Oxime, N-methyl-N'-nitro-N-nitrosoguanidine and Mitomycin C on DNA Polymerase and DNA Ligase

Iiyama, Satoru
Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Omura, Hirohisa
Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Murakami, Hiroki
Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Narazaki, Yoko
Food Chemistry Institute, Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/23499

出版情報：九州大学大学院農学研究院紀要. 21 (4), pp.181-190, 1977-07. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 21, 181-190 (1977)

Effects of Adrenaline, Cortisone, Glucose Oxime, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and Mitomycin C on DNA Polymerase and DNA Ligase

Satoru Iiyama, Hirohisa Omura, Hiroki Murakami, Yoko Narazaki and Kazuo Yamafuji

Food Chemistry Institute, Faculty of Agriculture, Kyushu University 46-09, Fukuoka 812

(Received February 28, 1977)

In the course of studies to confirm the hypothesis that nucleic acid-cleavage is a prerequisite for cellular differentiation and anomalization, it has been found that appropriate DNA-scission caused by carcinogen, virogen, catecholamine, steroid hormone etc. stimulates RNA synthesis and the nick region of DNA can serve as initiative site in RNA polymerase reaction. We have now advanced our investigation to the influence of nucleic acid-breaking reagents such as adrenaline, cortisone, glucose oxime, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and mitomycin C on DNA polymerase and ligase. As a result, DNA polymerizing reaction had in general an inclination to be stimulated by these chemicals. On the other hand, joining activity was fluctuated with the species of chemicals. In case of some treatments, marked enhancement of enzymatic activity was not observed, indicating that cells may have sufficient potency to restore to the competent state without notable change of enzymatic activity, even if they were injured by mutagen, carcinogen etc. The present findings may thus give an additional support for the formulation of the Yamafuji effect causing cyto-differentiation and cytoanomalization.

INTRODUCTION

Catecholamine-hormonal adrenaline (Yamafuji et al., 1972a), steroid-hormonal cortisone (Yamafuji et *al.*, 1971), virogenic glucose oxime (Omura et al., 1973), carcinogenic *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG, Yamafuji et *al.*, 1972 b), and carcinostatic mitomycin C (MC, Otsuji, 1968) were proved to induce the single and double strand-scission of DNA and also to decompose RNA, so that they were termed nucleic acid-breaking reagents (Iiyama *et al.*, 1973). The mechanism of phosphodiester-breakage in nucleic acid has been studied using deoxypurine- and deoxypyrimidine-oligonucleotides (Iiyama *et al.*, 1973). Furthermore it was confirmed that nick region of DNA can serve as initiative site in RNA polymerase reaction (Yamafuji *et al.*, 1972a, Iiyama et *al.*, 1973, Omura et ***al.,*** 1973).

The present paper describes the alteration of DNA polymerase and DNA ligase activities by bacterial treatments with these reagents.

MATERIALS AND METHODS

Materials

Adrenaline, cortisone, MNNG and MC were purchased from E. Merck AG., Sigma Chemical Co., Tokyo Kasei Kogyo Co. Ltd. and Kyowa Hakko Kogyo Co. Ltd., respectively. Glucose oxime was prepared in our laboratory (Tsutsumi *et al.*, 1969).

Cultivation of bacteria

Over night culture (3 ml) of *B. subtilis* MU12-U17 was inoculated to 100 ml of PY medium (1 % polypeptone, 0.1% glucose, 0.1% yeast extract, 50 mM NaCl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$ and *0.32* mM KH_2PO_4, pH 7.0) and was shaken at 37°C for 165 min first, followed by the addition of chemicals and an additional shaking for 15 min, distilled water being used instead of chemicals in the control sample. After rapid centrifugation at 6, 000 rpm for 5 min, cells were resuspended in an equal volume of fresh medium. Ninety minutes later (unless otherwise specified), cells were harvested and were applied to enzyme preparation as follows.

Preparation of crude enzyme

Cells were washed with 10 mM Tris-HCl, pH 8.0 and suspended in 0.8 ml of 25 % sucrose-10 mM Tris-HCl, pH 8. O-l mM ethylenediaminrtetraacetic acid. After the addition of 0.2 ml of a lysozyme solution (Worthington Biochemical Corp., 5 mg/ml in 0. 25 M Tris-HCl, pH 8. 0), the suspension was kept at 37°C for 5 min and then was quickly frozen in an acetone-dry ice mixture and thawed, and was repeated 3 times. The suspension was mixed with 1 ml of a lytic mixture (1 % "Brij-58"-2 mM Tris-HCl, pH 7.3-30 mM $MgSO_4$) and was immersed in an ice bath for 5 min. The lysate was centrifuged at 8,000 rpm for 5 min and the supernatant was used as a crude enzyme mixture of DNA polymerase and DNA ligase. On enzyme activity estimation, protein concentration in the extract was determined with Folin-reagent (Lowry *et al.*, 1951) and adjusted when the concentration of each lot was unequal.

Assay of DNA polymerase

The enzyme activity was measured by the conversion of ^{3}H-labeled deoxyribonucleoside triphosphate into an acid-insoluble product.

The incubation mixture (200 μl) contained 13.3 μmoles of Tris-malate, pH 8.2, 0.2 μmoles of 2-mercaptoethanol, 1.33 μmoles of $MgCl_2$, 6 nmoles of each of dATP, dGTP, dCTP, 8.6 pmoles of ^{3}H-dTTP (17.5 Ci/mmoles, Schwarz Bioresearch Inc.), 10 μg of *B. subtilis* DNA and 100 μg (calculated as protein) of crude enzyme. After incubation at 37°C at different intervals of time, the reaction was terminated by adding 1 ml of ice cold 10 % trichloroacetic acid (TCA) containing 1 % sodium pyrophosphate (Na-PPi). Precipitate formed was collected on a glass filter disk (Whatman GF/C) and then washed with ice cold 1 % TCA containing 1 % Na-PPi, ethanol and ether, successively. Dried disk was put into tolucne-PPO (2, 5-diphenyloxazole) scintillator and the radioactivity

was determined by the use of Beckman LS 250 scintillation counter.

Assay of DNA ligase

The assay was carried out according to Weiss *et al. (1968)* with minor modifications. It measures the conversion of "*P-labeled 5′-end in nicked DNA molecule to a resistant form to alkaline phosphatase.

The incubation mixture (200 μl) contained 2 μmoles of Tris-HCl, pH 8.0, 2 μmoles of dithiothreitol, 0.8 μmoles of $MgCl_2$, 2 μmoles of NH_4Cl, 20 μg of bovine serum albumin, 2 nmoles of nicotinamide adenine dinucleotide, 2 μg of *E. coli* tRNA, 2 μg of ^{32}P-DNA and 25 μg (calculated as protein) of crude enzyme. Transfer RNA was added to inhibit cndonuclease (Lehman *et al.*, 1962).

After incubation at 37°C for 15 min, 0.2 ml of calf thymus DNA (250 μg/ml), 0.5 ml of ice cold 10 % TCA and 2.0 ml of ice cold water were added in succession. After centrifugation at 10,000 rpm for **10** min, the supernatant was discarded. Two ml of ice cold 10 mM HCl was added, mixed and recentrifuged. The precipitate was dissolved in *0.5* ml of 0.1 M NaOH. The solution was adjusted to pH 8.0 with 50 μl of 0.2 M Tris-1.1 M HCl. Alkaline phosphatase (0.35 unit) was added and reaction mixture was incubated at 65°C for 30 min. After chilling, 0.5 ml of ice cold 10 % TCA and 2.0 ml of ice cold water were added and precipitate was collected on a glass filter disk, washed with 10 mM HCl and dried. The radioactivity was determined as in the case of DNA polymerase assay.

5′-^{32}P (phosphoryl)-DNA

5′-^{32}P-DNA, the substrate of DNA ligase reaction was prepared according to Lindahl *et al.* (1968), as follows.

B. subtilis DNA was partially degraded with pancreatic DNase (Sigma Chemical Co.) to introduce about 6 single strand-breaks per original strand and reulted phosphomonoesters were hydrolyzed by the treatment with alkaline phosphatase (Garen *et al.*, 1960) purified from *E. coli* K12(λ). Each 5′-hydroxyl group was labeled with ^{32}P transferred from [γ-^{32}P]-ATP (16.4 Ci/mmole, The Radiochemical Center, Amersham) by the use of polynucleotide kinase (Richardson, 1965), purified from T4 phage infected *E. coli* B. The substrate contained 6 μCi of ^{32}P per milligram of DNA, with **20%** *of* the label at repairable single strand-breaks.

RESULTS

A. Alteration of DNA polymerase

Exponentially grown *B. subtilis* MU12-U17 was treated with 50 μM or 250 μM of adrenaline at 37°C for 15 min, and after additional 90 min shaking DNA polymerase was prepared as described in Materials and Methods.

As being evident from Fig. 1, the enzyme derived from bacteria treated with adrenaline incorporated more substrates into DNA than control. Especially in the case of 50 μM, incorporation was about twofold. This indicates that the amount of DNA synthesis in bacteria increased significantly by treatment with adrenaline.

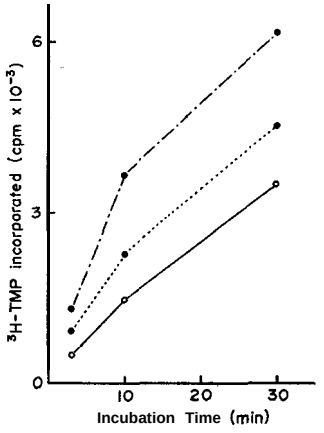

Fig. 1. Stimulation of DNA synthesis by adrenaline. Exponentially grown B. *subtilis was* treated with 50 μM or 250 μM of adrenaline at 37°C for 15 min and was shaken for further 90 min after the elimination of adrenaline. DNA polymerase preparation from the treated bacteria and DNA synthesizing reaction were carried out as described in Materials and Methods. o-o, control ; •—·—•, 50 μM adrenaline ; •---•, 250 μM adrenaline.

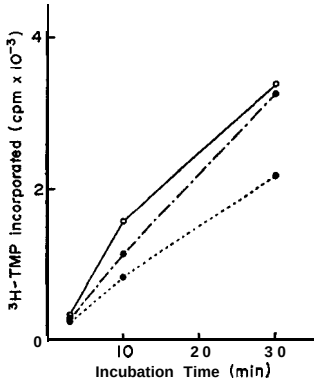

Fig. 2. Suppression of DNA synthesis by cortisone. See the legend of Fig. 1 for other conditions. o-o, control; •—·—•, 50 μM cortisone; • ---•, 250 μM cortisone.

On the other hand, treatment with 50 μM or 250 μM of cortisone decreased enzyme activity (Fig. 2).

In the case of 0.2 mM or 2 mM of glucose oxime, it appears to be slightly activated (Fig. 3).

The results of the test with 50 μM or 250 μM of MNNG showed no change in polymerizing potency (Fig. 4).

Figure 5 shows treatment with 6 μM of MC, followed by further 60min or 120 min shaking instead of 90 min, after elimination of the above-mentioned reagent. Very low concentration of MC remarkably enhanced the DNA synthesis

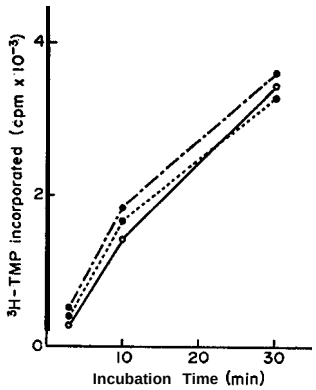

Fig. 3. Effect of glucose oxime on DNA synthesis. See the legend of Fig. 1 for other conditions. ○—○, control ; ●—·—●, 0.2 mM glucose oxime ; ●---●, 2 mM glucose oxime.

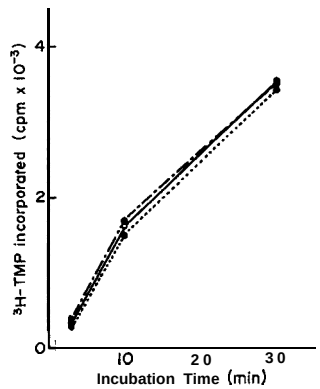

Fig. 4. Effect of MNNG on DNA synthesis. See the legend of Fig. 1 for other conditions. ○—○, control; ●—·—●, 50 μM MNNG; ●---●, 250 μM MNNG.

in the bacteria.

In the above experiments, native DNA was employed as the template of polymerase reaction. As these chemicals are capable of splitting nucleic acid, cellular DNA might be properly broken by the treatment. Therefore, it was inferred pertinent to use DNA treated with these chemicals as template of enzyme reaction ***in vitro.***

Bacterial DNA (250 μg/ml) was preincubated with 50 μM of cortisone in 10 mM Tris-HCl, pH 7.6 at 37°C for 6 hr and dialysed, while enzyme was prepared from the cells treated with 10 μM or 100 μM of cortisone at 37°C for 30 min after the elimination of the reagent. In the control, DNA and enzyme were handled in the same way with distilled water instead of cortisone. The combined reaction of those prepared template and polymerase gave the results shown in Fig. 6. In comparison with the preceding test (Fig. 2), in which

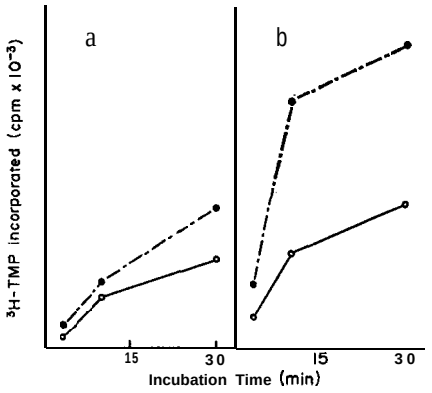

Fig. 5. Stimulation of DNA synthesis by MC. Exponentially grown *B. subtilis was* treated with 6 μM of MC at 37°C for 15 min, and after the elimination of MC shaking was continued for 60 or 120 min. Enzyme preparation and reaction are same as in Fig. 1. a, 60 min shaking; b, 120 min shaking; o-o, control; •—·—•, 6 μM MC.

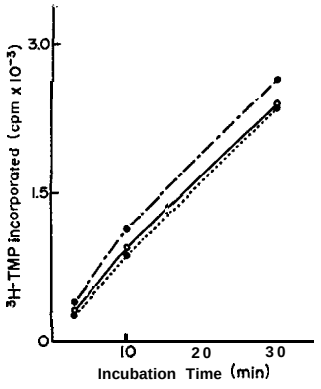

Fig. 6. Effect of cortisone on DNA synthesis when assisted by scissioned DNA. Template DNA was preincubated with 50 μM of cortisone in 10 mM Tris-HCl, pH 7.6 at 37°C for 6 hr and dialysed, while enzyme was prepared from the bacteria shaken at 37°C for 15 min with 10 μM or 100 μM of cortisone and for further 30min after the removal of cortisone. o—o, untreated enzyme paired with untreated DNA; •—·—•, 10 μM cortisone treated enzyme paired with 50 μM cortisone treated DNA; •---•, 100 μM cortisone treated enzyme paired with 50 μM cortisone treated DNA.

template was native DNA, activity was recovered to the same extent as control.

The enzyme treated with 10 μM or 100 μM of glucose oxime thus increased DNA synthesis when paired with the template, which had been preincubated with 50 μM of glucose oxime (Fig. 7).

In the previous paper (Yamafuji *et al.*, 1972b), we have succeeded in inducing a replicative phage from *B. subtilis* treated with MNNG continuously, so now the influence of this reagent on DNA synthesis was examined under the

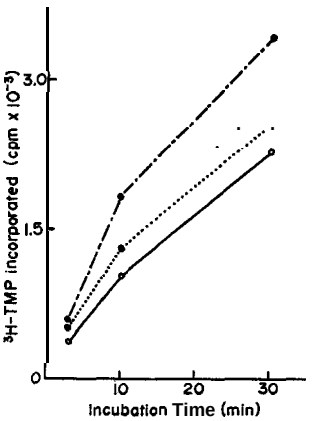

Fig. 7. Stimulation of DNA synthesis by glucose oxime when assisted by scissioned DNA. See the legend of Fig. 6 for other conditions. o-o, untreated enzyme paired with untreated DNA ;●—·—●, 10 μM glucose oxime treated enzyme paired with 50 μM glucose oxime treated DNA ;●---●, 100 μM glucose oxime treated enzyme paired with 50 μM glucose oxime treated DNA.

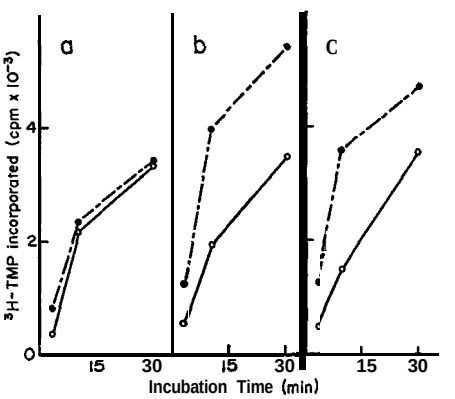

Fig. 8. Stimulative effect of prolonged MNNG treatment on DNA synthesis. B. *subtilis* was shaken in yeast extract peptone medium containing 0.7 mM MNNG at 37°C for 24 hr and 10% of this culture was inoculated daily to the same medium including MNNG. After lst, 5th and 10th transfer, enzymes were prepared and polymerizing activity was assayed. a, 1 day; b, 5 days; c, 10 days ;o—o, control ;●—·—●, 0.7 mM MNNG.

same conditions as those of phage induction.

Culture of MU12-U17 was shaken in yeast extract peptone medium containing 0.7 mM MNNG at 37°C for 24 hr and 3 ml was transferred daily to 30 ml of the same medium containing MNNG. After 1, 5 and 10 days cultivation, enzymes were prepared and polymerase activity was assayed by using native DNA as template (Fig. 8). The activity was enhanced by the factor of 1.6 after 5 days and this elevation was continued for additional 5 days, A replica-

tive phage appeared after 10th to 16th transfer in the previous experiment (Yamafuji *et al.*, 1972b). These results indicate that phage production will be initiated by appropriate DNA-breaks, followed by the augmentation of DNA synthesis.

B. Alteration of DNA ligase

The effects of these reagents on DNA ligase activity which cooperate with DNA polymerase in repair and replication were examined with the same manner as DNA polymerase.

B. subtilis at exponential phase was incubated with 25 μM of adrenaline at 37°C for 15min. After the removal of adrenaline, the culture was shaken for 30 or 50 min and enzyme was prepared from the bacteria.

Table 1. Effects of nucleic acid-breaking reagents on DNA ligase. Exponentially grown ***B. subtilis*** in PY medium was shaken with each reagent at 37°C for 15 min. After removal of reagents, the culture was shaken at the same temperature at different intervals of time as indicated. Enzyme preparation from these cells and assay of joining activity were carried out as described in Materials and Methods.

Treatments with reagents		^{32}P-incorporated (cpm)	
		Control	Test
25 μM Adrenaline	30min	1224	1305
	50 min	1443	1440
25 μM Cortisone	30 min	1200	908
	50 min	1415	1209
25 μM Glucose oxime	30 min	1309	1745
	50 min	1395	1420
680 μM MNNG	60 min	1643	1742
	120 min	1861	1815
6 μM MC	60 min	1304	2107
	120 min	1756	2284

As represented in Table 1, DNA joining activity was not affected by adrenaline. Cortisone (25 μM) reduced 15 to 20 % of its joining activity. It also shows the results of treatment with 25 μM of glucose oximc, ligase activity at 30 min was about 30 % higher, but at 50 min it decreased to the same level as control. The bacteria treated with 680 μM of MNNG were cultured for 1 or 2 hr after the removal of MNNG. Under these conditions MNNG showed no change on ligation. But in case of 6 μM MC, nick joining activity was raised significantly.

DISCUSSION

Recently we have proposed the hypothesis that the initiative process of cellular differentiation and anomalization should be an appropriate nucleic acid-

breakage (Yamafuji *et al.*, 1971). Cytodifferentiation would be brought about by deviation of RNA transcription and DNA replication. As a continuation of studying nucleic acid-breaks, we have elucidated previously that the nick region of DNA can serve as initiative site in RNA polymerase reaction (Iiyama *et al.*, 1973). Moreover, marked enhancement in template activity of nicked DNA was certified when employed core enzyme taken off σ factor from holo enzyme (unpublished). Thus appropriate DNA scission would start the mRNA transcription and may come in succession of protein synthesis necessary for each step of cytodifferentiation and cytoanomalization.

In the present paper, effects of adrenaline, cortisone, glucose oxime, MNNG and MC on DNA polymerase and ligase were investigated and it was proved that subcellular DNA polymerizing and joining activities were altered peculiarly by the treatments of bacteria with these nucleic acid-breaking reagents.

The cells may have sufficient capacity to repair the cleaved DNA into competent state and, in case of necessity, may replicate and/ or repair with the changes of both enzyme activities. The alterations of RNA polymerase, DNA polymerase and DNA ligasc activities which were caused by DNA-breakage should change cellular function and may result in differentiation and anomalization. Of course when surplus activity can compensate the repair or replication, remarkable changes of these enzymatic potency may not be necessary.

REFERENCES

Garen, A. and C. Levinthal 1960 A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. *Biochim. Biophys. Acta, 38: 470-483*

Iiyama, S., H. Murakami, M. Kuraki and H. Omura 1973 Action of DNA breaking reagents on RNA, deoxyoligonucleotide and RNA polymerase reaction. *J. Agr. Chem. Soc. Japan, 47*: *455-461*

Lehman, I. R., G. G. Roussos and E. A. Pratt 1962 The deoxyribonucleases of *Escherichia coli*. *J. Biol. Chem., 237: 829-833*

Lindahl, T. and G. Edelman 1968 Polynucleotide ligase from myeloid and lymphoid tissues. *Proc. Natl. Acad. Sci. U. S., 61: 680-687*

Lowry, 0. H., N. J. Rosebrough, A. L. Farr and R. J. Randall 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem., 193: 265-268*

Omura, H., S. Iiyama, T. Fujii and K. Yamafuji 1973 Effect of virogenic hydroxylamine-metabolites on deoxyribonucleic acid and RNA-polymerase. *J. Fac. Agr., Kyushu Univ.*, 17 : 181-186

Otsuji, N. 1968 Properties of mitomycin C-sensitive mutants of *Escherichia coli K32*. *J. Bacteriol., 95*: *540-545*

Richardson, C. C. 1965 Phosphorylation of nucleic acid by an enzyme from T4 bacteriophage-infected *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S., 54: 158-165*

Tsutsumi, M., M. Iio and H. Omura 1969 Preparation of fructose oxime. *J. Japan Soc. Food and Nutr., 22: 462-467*

Weiss, B., A. Jacquemin-Sablon, T. R. Live, G. C. Fareed and C. C. Richardson 1968 Enzymatic breakage and joining of deoxyribonucleic acid. IV. Further purification and properties of polynucleotide ligase from *Escherichia coli* infected with bacteriophage T4. *J. Biol. Chem., 243: 4543-4555*

Yamafuji, K., S. Iiyama and K. Shinohara 1971 Mode of action of steroid hormones on

deoxyribonucleic acid. ***Enzymol., 40: 259-264***

Yamafuji, K., S. Iiyama and H. Murakami 1972a Effect of noradrenaline on brain nucleic acid and RNA polymerase. ***Enzymol., 42: 439-447***

Yamafuji, K., S. Iiyama and H. Omura 1972b Induction of reproductive phage in B. *subtilis* by triethylenemelamine or methylnitronitrosoguanidine and their reaction with deoxyribonucleic acid. ***Enzymol., 43: 113-120***