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https://doi.org/10.5109/23727

出版情報：九州大学大学院農学研究院紀要. 25 (4), pp.161-166, 1981-04. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 25(4), 161-166 (1981)

# Effect of Some Hydroxylamine-Metabolites on the Priming Activity for DNA Polymerase

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(*Received December 4, 1980*)

Mutagenic and virogenic hydroxylamine-metabolites, such as nitrite, hydroxylamine, oxime and hydrogen peroxide, stimulated more or less the priming activity of template DNA for DNA polymerase, although their actions were not so high as those of enediol reductones. Hydroxylamine and hydrogen peroxide were more effective for the stimulation than the others.

## INTRODUCTION

It was reported that some hydroxylamine-metabolites, such as nitrite (Yamafuji *et al.*, 1971 a), hydroxylamine (Yamafuji and Hashinaga, 1966, 1967; Yamafuji *et al.*, 1966, 1971 a; Omura and Yamaguchi, 1967; Omura *et al.*, 1973), oxime (Murakami and Yamafuji, 1970; Yamafuji *et al.*, 1971 a, b; Iiyama *et al.*, 1973; Omura *et al.*, 1973) and hydrogen peroxide (Yamafuji and Uchida, 1966, 1968) caused breakage of nucleic acids. These substances were found to be mutagenic and/or virogenic agent. Yamafuji (1970) presented a working hypothesis that some appropriate cleavage introduced in chromosomal nucleic acids might induce mutagenesis, virogenesis, carcinognesis or carcinostasis, as the initiating stage of these cellular anomalizations. Further studies elucidated that cleavage in DNA brought about some alteration of the priming activity for DNA-dependent RNA polymerase, stimulating or repressing according to the degree of the cleavage (Yamafuji *et al.*, 1972; Iiyama *et al.*, 1973; Omura *et al.*, 1973). On the other hand, antitumoric reductones induced changes of the priming activity for DNA polymerase as well as RNA polymerase in connection with DNA cleavage by them (Omura *et al.*, 1975; Tomita *et al.*, 1979). It is expected that hydroxylamine-metabolites may affect the priming activity for DNA polymerase, since they have abilities to cleave nucleic acids and to alter the priming activity for RNA polymerase.

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## MATERIALS AND METHODS

**Reagents**: Hydroxylamine hydrochloride (the special grade), sodium nitrite (the special grade) and hydrogen peroxide (the first grade) were purchased from Wako Chemical Co. Galactose oxime was kindly supplied by Dr. Iio (Kumamoto Women University). $^3$H-thymidine triphosphate ($^3$H-TTP, 20.9 Ci/mmol) was obtained from New England Nuclear.

**DNA**: DNA from calf thymus was purified according to the method reported in the previous paper (Omura *et al.*, 1975).

**DNA polymerase**: DNA polymerase was prepared from *E. coli* strain B after Richardson *et al.* (1964) by autolysis, ammonium sulfate fractionation, DEAE cellulose and phosphocellulose chromatographies, as described in the foregoing paper (Tomita *et al.*, 1979).

**Treatment of DNA with reagent**: DNA (100 μg/ml) was incubated with 1 μM or 1 mM reagent in 25 mM tris-HCl buffer (pH 6.8) at 37°C for 1 hr. After dialysis against 0.1 × SSC (0.15 M NaCl-0.015 M Na-citrate) at 4°C for 24 hr, the priming activity of the DNA was estimated.

**Priming activity assay**: The assay mixture (400 μl in total) contained 20 μmoles of tris-HCl buffer (pH 7.4), 2 μmoles of dithiothreitol, 2 μmoles of $MgCl_2$, 20 nmoles of dATP, dGTP, dCTP each, 14 pmoles of $^3$H-TTP (0.41 nCi), 20 μg of DNA and 12.5 μg of DNA polymerase preparation. An aliquot (50 μl) of the mixture incubated at 37°C was transferred into 2 ml of cold 0.5 N perchloric acid at 5 and 25 min after the beginning of the incubation and allowed to stand for 30 min. Acid-insoluble fraction was collected on glass filter paper (Whatman GF/C) and washed successively with 3 ml of cold 0.5 N perchloric acid, cold water and 95 % cold ethyl alcohol. Radioactivity of the dried filter paper was counted by a Beckman LS-250 liquid scintillation counter with 0.4 % PPO-toluene as scintillant.

## RESULTS

### (1) Hydroxylamine

The effect of hydroxylamine on the template DNA of DNA-polymerase was assayed. As described in Fig. 1, the priming activity was enhanced with a low concentration of 1 μM hydroxylamine.

### (2) Nitrite

DNA was treated with sodium nitrite as with hydroxylamine. Result in Fig. 2 indicates that the effect of 1 μM nitrite is not remarkable. However, a slight enhancement of the priming activity was observed with the treatment of 1 mM nitrite.

### (3) Galactose oxime

Galactose oxime showed a similar effect to that of nitrite for the priming activity, as seen in Fig. 3.

### (4) Hydrogen peroxide

DNA treatment with hydrogen peroxide was samely performed with hy-

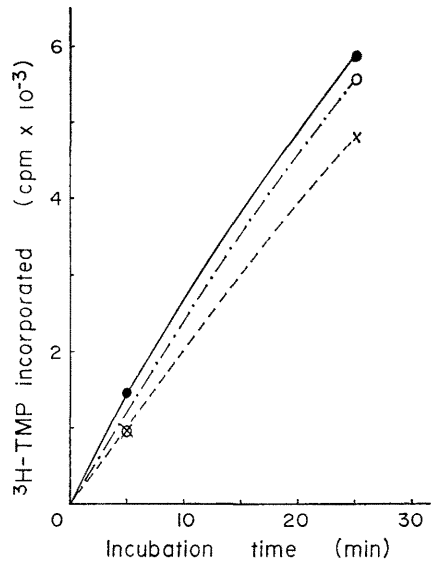


**Fig. 1.** Priming activity of DNA treated with $NH_2OH$. DNA (100 $\mu$g/ml) was incubated with 1 $\mu$M or 1 mM $NH_2OH$ in 25 mM tris-HCl buffer (pH 6.8) at 37°C for 1 hr. After dialysis against 0.1 × SSC (0.15 M NaCl-0.015 M Na-citrate) at 4°C for 24 hr, priming activity of the DNA was assayed. x---x: control, o-•-o: 1 $\mu$M $NH_2OH$, •——• : 1 mM $NH_2OH$.

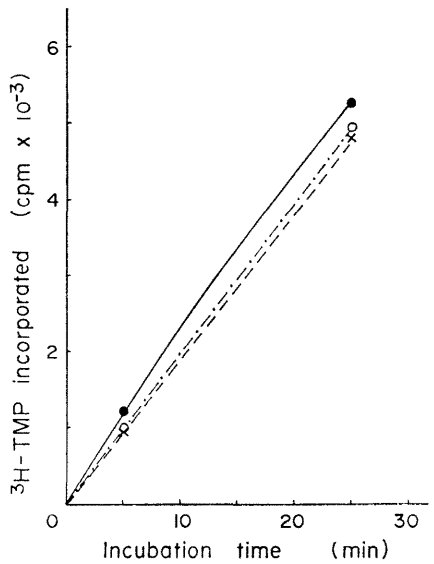


**Fig. 2.** Priming activity of DNA treated with $NaNO_2$. DNA treatment and the priming assay were described in the legend of Fig. 1, except that $NaNO_2$ was used instead of $NH_2OH$. x---x: control, o-•-o: 1 $\mu$M $NaNO_2$, •——•: 1 mM $NaNO_2$.

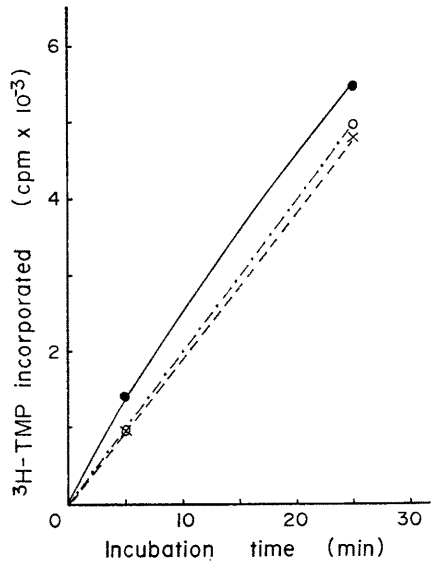


**Fig. 3.** Priming activity of DNA treated with galactose oxime. DNA treatment and the priming activity assay were described in the legend of of Fig. 1, except that galactose oxime was used instead of $NH_2OH$. x---x: control, o-•-o: 1 $\mu$M galactose oxime, •——•: 1 mM galactose oxime.

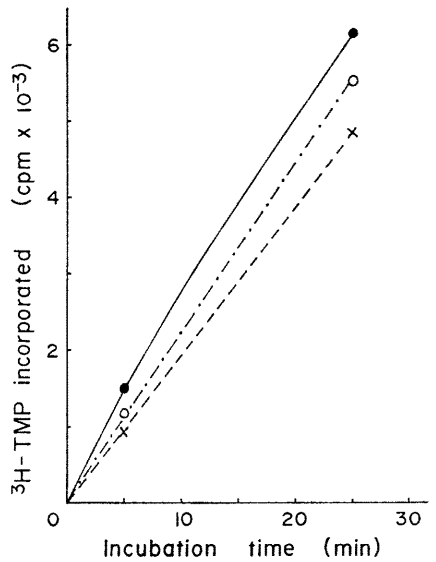


**Fig. 4.** Priming activity of DNA treated with $H_2O_2$. DNA treatment and the priming activity assay were described in the legend of Fig. 1, except that $H_2O_2$ was used instead of $NH_2OH$. x---x: control, o-•-o: 1 $\mu$M $H_2O_2$, •——•: 1 mM $H_2O_2$.

droxylamine, although peroxide is not hydroxylamine-metabolite. The effect of hydrogen peroxide on the template DNA is shown in Fig. 4. Hydrogen peroxide caused an enhancement of the priming activity by the treatment even with 1 $\mu$M of the concentration.

## DISCUSSION

The bacterial mutagenicity assay and animal experiments suggested the overlapping of mutagens and carcinogens. Yamafuji (1970) demonstrated that the cellular anomalization including mutagenesis, virogenesis, carcinogenesis and carcinostasis might be induced by some proper scissions in the chromosomal DNA. Along this working hypothesis, it was disclosed that the breakage of nucleic acid strands was brought about by some mutagens, virogens, carcinogens and antitumoric substances and that such breakages caused the alteration of RNA polymerase, DNA polymerase and protein synthesis. Hydroxylamine-metabolites are the first ones established as the chemical virogen as well as mutagen (Yamafuji und Shirozu, 1944; Yamafuji and Omura, 1950; Yamafuji and Yoshihara, 1950). As cited above, cleavage of nucleic acids and alteration of the priming activity for RNA polymerase by them were disclosed. In this study, an analogous effect is shown for DNA polymerase too. These results may provide an additional support to the Yamafuji's hypothesis.

All reagents tested increased more or less the incorporation of $^3$H-TMP into DNA, as shown in Figs. 1, 2, 3 and 4, although their effects were in general not so remarkable as those of ascorbic acid and its related substances (Omura *et al.*, 1975; Tomita *et al.*, 1975). Treatment with 1 mM reagents is more effective than with 1 $\mu$M. Galactose oxime and nitrite were relatively moderate for the effect compared with hydroxylamine and hydrogen peroxide, as especially seen in 1 $\mu$M treatment. In the case of ascorbic acid and its related substances, the enhancement of the priming ability was observed when single strand scissions were introduced into DNA strand, but double strand scissions which had been provoked in the much severer conditions decreased the ability. Within the range of concentrations applied, higher concentrations of reagents did not reduce the priming activity. Cleavage of nucleic acids by several DNA breaking reagents were accelerated in the coexistence of $Cu^{2+}$. Concerning with hydroxylamine-metabolites, cooperation of $Cu^{2+}$ should be examined. The precise mechanism for the increase of incorporation of $^3$H-TMP into DNA is not clear at present, but a possible interpretation may be that 3'-OH terminal groups, initiation sites for DNA synthesis (Mitra and Kornberg, 1966), increase as the strands are subjected to scissions, as shown by Iiyama *et al.* (1973) for RNA polymerase. Thus, mutagenic and virogenic hydroxylamine-metabolites may modulate the priming ability for DNA synthesis depending on the mode and degree of strand breakages. Such modulation for the replication and transcription may be related to anomalization of cell functions through affecting on protein synthesis.

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