Effect of Virogenic Hydroxylamine-Metabolites on Deoxyribonucleic Acid and RNA-Polymerase

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Effect of Virogenic Hydroxylamine-Metabolites on Deoxyribonucleic Acid and RNA-Polymerase

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Single strand scission in duplex DNA is caused by hydroxylamine, Na-nitrite or oximes of glucose and galactose of proper low molarities. The suitable nicking in the DNA-molecule brings about the elevation of its template nature. Excessive breakage of DNA, however, reduces its template activity. The results have been discussed in relation to an effect causing cyto-differentiation and -anomalization.

INTRODUCTION

Hydroxylamine is the first reagent which was successfully used for virus induction (Yamafuji et *al.,* 1944a; 1944b). Enzymatic studies on this amine have led us to the establishment of a cycle producing metabolic mutagens and virogens (Yamafuji, 1963 ; 1964 ; Yamafuji and Watanabe, 1964). The cycle includes nitrite and oxime. The present paper describes the effect of these three nitrogenous substances on the breakage of DNA-thread and the synthesis of mRNA.

MATERIALS AND METHODS

Cleavage of DNA-strand

DNA was prepared from calf thymus according to the method stated before (Yamafuji *et al.,* 1966 ; 1971b). To prove the single strand scission (nick) in the double helix-molecule, a mixture of 40 μg/ml DNA in SSC (0.15 M NaCl + 0.015 M Na-citrate, pH = 7) and hydroxylamine-metabolites of appropriate molarity was first incubated at 37" for 4 hrs., and a sucrose gradient centrifugation was then applied to the solution of pH=12.8 for 4 hrs. at 30,000 r.p.m. In the control, DNA was incubated without metabolites and centrifuged in the same way.

Alteration of RNA-polymerase

The preparation of enzyme from *E. coli* was carried out with the method of Ishihama and Kameyama (1967). In order to measure the enzymatic activity, 0.25 μCi ^{14}C-4-UTP was mixed with 0.08 μmoles ATP, 0.08 μmoles GTP, 0.08 μmoles CTP, 1.0 μmoles $MgSO_4$, 0.4 μmoles $MnSO_4$, 1.0 pmoles mercaptoethanol, 2.4 μmoles

Tris-HCl buffer (pH=7.8), 20 μg DNA and 150 μg polymerase ; the total volume was 200 μl. At regular intervals at 37°, a portion of the mixture was mixed with 10 % trichloroacetic acid. The precipitate was dissolved in 2 M NH_4OH and counted in Beckman LS 250-fluid scintillation counter. The polymerase action was thus expressed in cpm. To determine the template activity, the nicked DNA prepared by 30 min.-pretreatment with metabolic virogens was used in the test after dialyzing against water for 16 hrs. at 5". In the control, the DNA incubated in the SSC-solution without virogens was employed after the dialysis in the same manner.

RESULTS

Cleavage of DNA-strand

In the preceding investigation (Yamafuji ***et al.,*** 1971b), it was demonstrated that hydroxylamine or KNO, in co-operation with $CuSO_4$ breaks duplex DNA. We now investigated the effect in NH_2OH or $NaNO_2$ of much lower concentration on the DNA-breakage. As shown in Fig. 1, nicks produced in a 5 x 10^{-5} M amine or 5 x 10^{-5} M nitrite solution without copper.

It was also observed previously (Murakami and Yamafuji, 1970) that glucos- or galactosoxime + Cu cleaves native DNA. As illustrated in Fig. 2, we found here that single strand cleavages can be caused by 5 x 10^{-6} M oximes in the absence of Cu-salt.

The nick formation was further examined using DNA which had been pre-

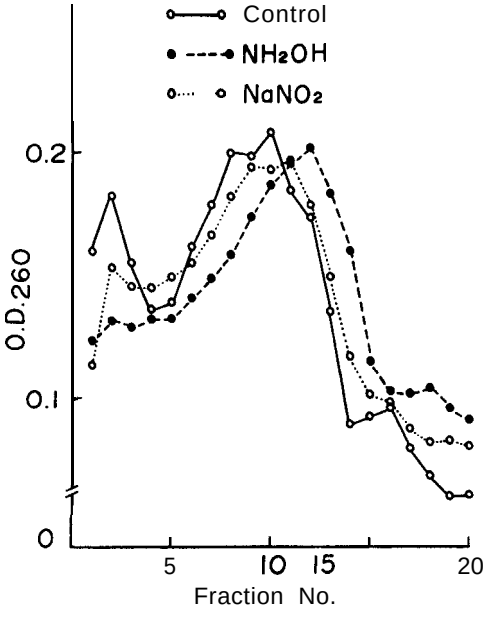

Fig. 1. Sedimentation pattern of DNA at pH=12.8 after treating with NH_2OH or $NaNO_2$.

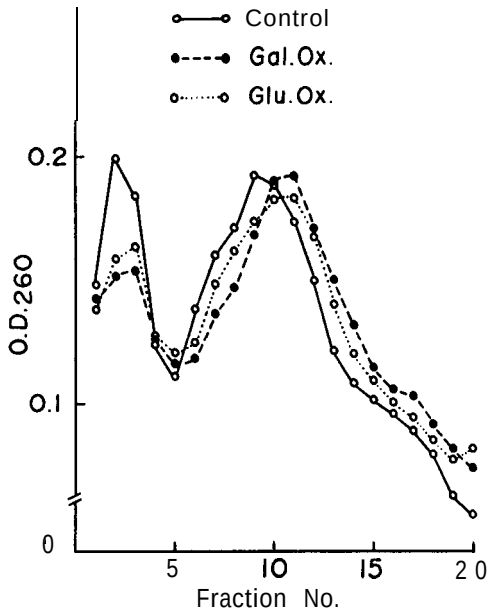

Fig. 2. Sedimentation pattern of DNA at pH = 12.8 after treating with glucos- or galactosoxime.

treated with hydroxylamine-metabolites for 30 min. The patterns obtained were the same as those in the above figures, but their peaks were pretty low.

Alteration of RNA-polymerase

In the previous paper (Yamafuji et *al.,* 1972), it was reported that the activity of RNA-polymerase is affected by the combined action of noradrenaline and $CuSO_4$. We now estimated the template potentiality of DNA pretreated with virogenic metabolites of relatively low and rather high molality without cooperation of Cu. The experiment disclosed that, as is evident from Fig. 3, an activation of enzyme occurs in the solution containing DNA treated with 5×10^{-5} M hydroxylamine. When the DNA was pretreated with 5×10^{-2} M amine, however, the polymerase was inactivated remarkably. This may be due to double strand-breaks of considerable amount. Taylor *et al.* (1970) proved that treatment of T7 DNA with 0.5 M NH_2OH inhibits transcription of this template by *coli*-RNA polymerase.

In the tests of DNA-splitting by hydroxylamine and $NaNO_2$, it was found that the nick-producing ability of the former is higher than that of the latter. As is obvious from Fig. 4, the template potency of DNA pretreated with diluted nitrite solution was lower than that of the one treated with the amine of the same concentration. The observation suggests that the RNA-polymerase activity is proportional to the nicking grade in DNA-molecules. It was further observed

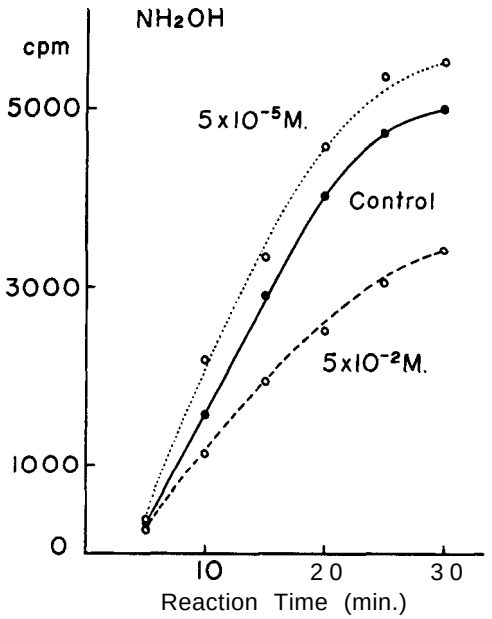

Fig. 3. RNA-polymerase activity in solution containing DNA pretreated with NH_2OH.

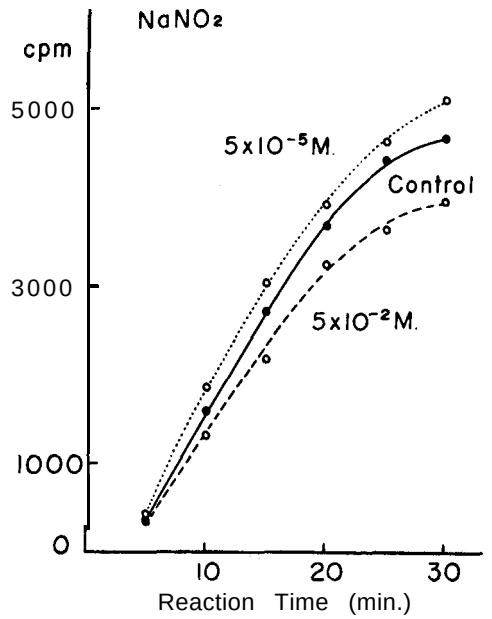

Fig. 4. RNA-polymerase activity in solutions containing DNA pretreated with $NaNO_2$.

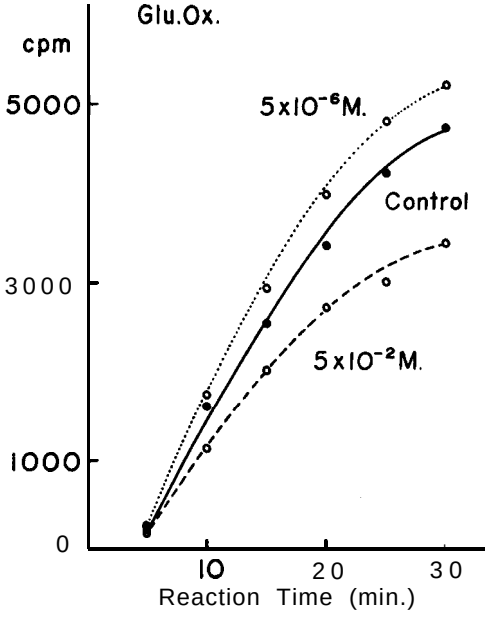

Fig. 5. RNA-polymerase activity in solutions containing DNA pretreated with glucosoxime.

that NO, of high normality also hinders the transcriptional capacity of DNA.

Glucosoxime has been regarded as one of the best polyhedral virus inducers and its toxic action is weak. As depicted in Fig. 5, the template capability of DNA was elevated by treating it with 5 x 10^{-6} M glucosoxime. The treatment of DNA with 5 x 10^{-2} M oxime, however, weakened its activity.

DISCUSSION

Cytoanomalizations including virogenesis would be primarily introduced by deviation of DNA-duplication and mRNA-synthesis. For instance, production of viral polyhedrosis necessiates intensive replication of special fragment of chromosomal DNA and rapid formation of specific protein by newly synthesized RNA. The activation of template nature of DNA by metabolic virogens should mean the synthesis of new RNA by polymerase. The formation of abnormal protein, therefore, can be the result of appropriate nicking in DNA-molecules. The present investigation may thus give a support for the formulation (Yamafuji et al., 1971a) of an effect causing cellular differentiation and anomallzation.

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