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Effect of Some Reductones on DNA and RNA Polymerases'

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Activities of DNA-dependent DNA and RNA polymerases were changed, when the primer DNA had been pre-treated with ascorbic acid, 5-methyl-3,4-dihydroxytetrone, scorbamic acid or 5-mercapto-4-hydroxycoumarin. The activity increased or decreased depending on the reaction condition, such as coexistence of Cu^{2+}, concentration of the reductones or reaction time. The study suggested that the degree of the variation seemed to relate with the breakage of the primer DNA due to the reductone structure.

INTRODUCTION

Reductones have strong reducing ability and take an important part in browning and formation of aroma or antioxidative activity during processing, storage or cooking of foods. Notwithstanding the peculiar chemical properties, their biological functions have not been elucidated except a few hormones such as epinephrine and norepinephrine or vitamin such as ascorbic acid. In our Institute, it was discovered that several reductones were able to break polynucleotide chains of DNA and RNA in *vivo* (Murakami *et* al., 1975. 1978a, b) as well as *in vitro* (Murakami and Yamafuji, 1970; Omura *et* al., 1974, 1975a, b, 1978a, b; Shinohara *et al.,* 1974, 1975, 1976; Shirahata *et al.,* 1977; Yamafuji *et al., 1970,* 1971a, b, 1972) and was demonstrated that the function was attributed to enediol (-(OH)C=C(OH)-), enaminol (-(OH)C=C(NH-)-) or thiolenol (-(OH) C=(SH)-) group in molecules.

As well known, nucleic acids play an essential roles in living organisms. When nucleic acids have been modified or injured in some way, functions of living organisms may be severely influenced. Strand breaks of nucleic acids were already evidenced by several mutagens, virogens, carcinogens, anticancer substances or hormones too. On the basis of many investigations about them, Yamafuji (1970) proposed a hypothesis called Yamafuji's effect that certain

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appropriate degradation of chromosomal DNA may be the initiating step in cellular differentiation and anomarization. It was also observed that retardation of growth of cancer cells (Yamafuji and Murakami, 1968; Yamafuji *et al.*, 1970, 1971; Omura *et al.*, 1974; Tomita *et al.*, 1974) or bacteria, pupation of silkworm (Yamafuji *et al.*, 1971) and mutation of certain strains of bacteria (Omura 1978a) were provoked by the action of some reductones. Although studies supporting the Yamafuji's effect are progressing, the steps succeeding to the degradation of DNA must be elucidated in more detail. According to the central dogma, DNA is replicated in the case of propagation of cells. The information in DNA is transferred to mRNA by transcription and then translated to protein. Therefore, some injury or modification of DNA may provoke variations of syntheses of DNA, RNA and protein. Since DNA-dependent DNA and RNA polymerases concern with these processes, thus, the effect of reductones on their activity was investigated as the next approach to confirm the Yamafuji's effect.

MATERIALS AND METHODS

1. DNA polymerase

Preparation of the enzyme: DNA polymerase was prepared from ***Escherichia coli*** B according to Richardson *et al.* (1964) with some modifications. ***E. coli*** B was grown in PY medium (1.0 % polypeptone, 0.1% yeast extract, 0.1% glucose, 50 mM NaCl, 1 mM $CaCl_2$ and 0.32 M KH_2PO_4) at 37°C in a jar fermentor at 1.6 atmospheric pressure (Marubishi MSJ) with 15 *l*/min aeration by agitating at 240 rpm, sometimes with adding 1 ml silicon emulsion (for fermentation) when the medium foams. Cells were collected with a continuous centrifuge at late log phase and washed. About 5g wet cells were harvested per 1 *l* medium and kept in a deep freezer at -20°C until used for preparation which was conducted at 4°C unless otherwise described. Partially thawed cells (about 50g wet weight) were mixed with 40 ml glycylglycine buffer (50 mM, pH 7.0) in a Waring blender and stirred slowly for 5 min. Into the suspension, 150g alumina were gradually added and stirred for 20 min at higher speed. An additional 140 ml of the same buffer were added and stirred further for 20 min as above. The homogenate was centrifuged at 12,000 rpm for 30 min. Alumina pellet was washed with 100 ml buffer and both the extract and the washings combined to give a final volume of about 200 ml. To the solution thus obtained were slowly added 200ml 50mM Tris buffer (pH 7.5) containing 1 mM EDTA and 28.8 ml 5 % streptomycin sulfate over a 1.5-minutes period with constant stirring. After 10 min, the suspension was centrifuged and the supernatant fluid was discarded. The precipitate was dissolved in 20 ml 50mM potassium phosphate buffer (pH 7.4) by slow stirring, adjusted to 50ml of total volume with the same buffer and left for about 12 hrs. Into the solution, 0.25 ml 0.3 M $MgCl_2$ were added and autolyzed by keeping at 30°C. At interval of several times, 5 μl-aliquots were taken, put into 4 ml 50 mM Tris buffer (pH 7.4) or 0.5 N perchloric acid and centrifuged at 2000 rpm for 10

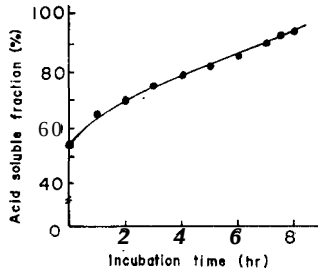

Fig. 1. Increase of acid-soluble fraction by autolysis.

min. Optical densities of both the supernatant fluids were determined at 260 nm and the ratio was estimated. As shown in Fig. 1, the ratio was increased with time and more than 90 % of the UV-absorbing material were rendered acid-soluble by 8 hrs. At this point, the autolysate was then chilled quickly to 0°C and centrifuged at 12,000 rpm for 5 min. To 25ml supernatant fluid were added glutathione and EDTA to be 1 mM of the final concentration respectively, and 7. 5 g ammonium sulfate were slowly added with stirring. The solution was centrifuged at 15, 000 ppm for 10 min to remove precipitate formed. Into the supernatant fluid, 2.9 g ammonium sulfate were additionally dissolved. The precipitate formed was collected and dissolved into 2.8 ml 20mM phosphate buffer (pH 7.2). The solution was mixed with 0.13 ml 1 M potassium phosphate buffer (pH 6.5) and then applied on a DEAE-cellulose column (Brown, 1.0 meq/g dry weight, 1.0 x 3.0 cm) equilibrated with 0.2 M potassium phosphate buffer (pH 6.5) containing 10 mM mercaptoethanol. The same buffer was passed through the column to adsorb nucleic acids and about 4ml of the initial fraction of eluate were collected. The fraction was then dialysed against the same buffer diluted 10 times. About the same volume of 10 mM mercaptoethanol were added to the dialysate and centrifuged tor emove insoluble portion and applied to the phosphocellulose column (Whatman P-11, 7.4 meq/g; 2. 0×12. Ocm) equilibrated with 20 mM potassium phosphate buffer containing 10 mM mercaptoethanol. After removing enough non-adsorbed materials by washing the column with the same buffer, a linear gradient elution was applied with 0. 02 M and 0. 3 M potassium phosphate buffer containing 10 mM mercaptoethanol. Active fraction eluted between 0.11 M and 0.18 M was collected and ammonium sulfate were added to recover the activity. The precipitate was centrifuged and dissolved again in 20 mM potassium phosphate buffer (pH 7.2) and concentrated by dialysis against 50 % (v/v) glycerin-20 mM potassium phosphate buffer (pH 7.2).

Assay of the activity: The activity of DNA polymerase was assayed by estimating the amount of ^{3}H-TMP incorporated into acid-insoluble material as follows. The reaction mixture shown in Table 1 was incubated at 37°C. At desired time, the 50 μl-aliquot was mixed with 3 ml cold 0. 5N perchloric acid and kept in cold for 30min. Acid-insoluble fraction was collected on glass filter (Whatman GF/C) and washed twice with 3 ml cold 0. 5 N perchloric acid, cold water and cold 95% ethanol, respectively. The precipitate was dried

Table 1. Components of DNA polymerase reaction system.

Tris-HCl (pH 7.4)	20 μmoles
dithiothreitol	2 pmoles
$MgCl_2$	2 μmoles
dATP, dGTP, dCTP	each 20 nmoles
^{3}H-dTTP*	0.014 nmoles (0.14 nCi)
DNA	20 μg
DNA polymerase	12.5 μg
Total	400 μl

* ^{3}H-dTTP employed : 29.1 mCi/mmole, New England Nuclear.

under an infrared lamp, put into a vial with 10 ml 0.4 % 2,5-diphenyloxazole (PPO)-toluene and counted in a Beckman LS-250 liquid scintilation counter.

2. **RNA polymerase**

Preparation of RNA polymerase: RNA polymerase was also prepared from *E. coli* B according to Burgess' procedure (Burgess, 1969) with a slight modification. *E. coli* B was grown in a modified PY medium, in which polypeptone and yeast extract had been increased to 1.6 % and 1.0 % respectively, and harvested as above. About 7.6 g wet cells per 1 *l* medium were obtained. About 100 g of frozen cell were finely ground and placed in a Waring blender. Previously chilled glass beads (about 100 μ diameter) of the same weight and 100 ml buffer solution A (50 mM Tris buffer, pH 7.5,—10 mM $MgCl_2$—0.2 M KCl-0.1 mM clithiothreitol-0.1 mM EDTA-5 % (v/v) glycerine) were added and homogenized at low speed for 2 min and at high speed for 5min. The homogenate was centrifuged at 10, 000 rpm for 10 min. The pellet of glass beads and cell mass was washed with 40ml buffer solution A and centrifuged. Both the supernatant fluid and the washings were combined and adjusted to 100 ml. Cell extract thus obtained was centrifuged at 28,000 rpm for 2 hrs using a centrifuge, Beckman L type with 30-rotor, to remove cell debris. Solid ammonium sulfate was dissolved in the supernatant fluid to give 30 % saturation, stirred for 30min and centrifuged at 9,000 rpm for 30 min. Concentration of ammonium sulfate in the supernatant fluid was then increased to 47% saturation. After stirring for 30 min, precipitate formed was obtained by centrifugation at 20,000 rpm for 20 min and resuspended in a buffer solution B (10 mM Tris buffer, pH 7.9-10 mM $MgCl_2$—0.1 mM EDTA-0.1 mM dithiothreitol-5% (v/v) glycerine) 42% saturated with ammonium sulfate. The suspension was stirred for 45 min and centrifuged at 15,000 rpm for 20 min. The precipitate was again suspended in the same buffer B-ammonium sulfate solution, stirred and centrifuged as above. The precipitate was dissolved in 50ml buffer solution B and dislysed against the same buffer for 8 hrs. Dialysate was applied on a DEAE-cellulose column (Brown, 1.0 meq/g dry weight; 2.5~ 10. Ocm) equilibrated with buffer B solution. The column was washed with 0.13 M KCl—buffer B solution and eluted with 200ml KC1 (containing buffer B solution) of linear gradient between 0.13 M and 0.3 M. The fraction at 0.18 M KCl, just before elution part of DNA, was collected and dialysed against buffer B solution. The dialysate was rechromatographed on DEAE cellulose

column and the active fraction of a single peak was concentrated by dialysis against the solution of 50 % (v/v) glycerine-10 mM Tris buffer, pH 7. 9-50 mM $MgCl_2$-50 mM KCl for 8 hrs. Specific activity was raised about 180 times from the initial extract and E_{280}/E_{260} to 1. 86 from 0.56.

Preparation of core RNA polymerase: From RNA polymerase thus prepared, core-enzyme solution was prepared according to the method of Burgess and Travers (1971) as follows. The RNA polymerase solution was dialysed against 5 % (v/v) glycerine-buffer C solution (150 mM Tris buffer, pH 7.9-50 mM KCl-- 0.1 mM EDTA-0. 1 mM dithiothreitol). The dialysate was adsorbed on a phosphocellulose column (Whatman P-11, 7. 4 meq/g dry weight; 1.1 ×7. Ocm) equilibrated with 5 % (v/v) glycerine-buffer C solution and a linear gradient elution was conducted with KC1 containing Tris buffer, pH 7.9, 0.1 mM dithiothreitol and 5 % (v/v) glycerine between 0.05 M and 0. 6 M. Core-polymerase fraction was eluted at about 0. 4 M KCI. The fraction was concentrated by dialysis against 50% (v/v) glycerine-buffer C solution eliminated EDTA and kept at -20°C until use.

Assay of the activity: The activity of RNA polymerase was assayed by estimating ^{14}C-UMP incorporated into acid-insoluble fraction. The reaction mixture shown in Table 2 was incubated at 37°C. At certain intervals, a 50 μl-aliquot of the mixture was chilled in an ice bath and mixed well with 1 ml ice-cold 10 % trichloroacetic acid-l % sodium pyrophosphate and 0.2 ml bovine serum albumin (2.5 mg/ml). After standing the mixture at 0°C for 30 min, it was filtered through a glass filter (Whatman GF/C) to obtain acid-insoluble precipitate. The precipitate was washed twice with 2 ml ice-cold 1 % trichloroacetic acid, 50 % ethanol and 95% ethanol respectively. After drying under an infrared lamp, the radioactivity of the precipitate was estimated in 0. 4 % PPO-toluene with a Beckman LS-250 liquid scintillation counter.

3. Chemicals

Ascorbic acid (AsA; special grade) was obtained from the Wako Pure Chemical Ind. Dehydroascorbic acid (DAsA) and 5-methyl-3,4-dihydroxytetrone (MDT) were prepared from AsA by oxidation with benzoquinone and by degradation of DAsA as reported in the foregoing paper (Tomita *et al.*, 1974). Scorbamic acid (ScA) and mercaptohydroxycoumarine (MHC) were synthesized (Omura *et al.*, 1978b) according to the method of Kurata *et al.* (1973) or Eisen-

Table 2. Components of RNA polymerase reaction system.

Tris-HCl (pH 7.8)	20 μmoles
dithiothreitol	1 μmole
$MgSO_4$	1 μmole
KC1	20 pmoles
$MnSO_4$	0.4 μmoles
ATP, GTP, CTP	each 0.1 μmoles
^{14}C-UTP*	5 nmoles (250 nCi)
DNA	20 μg
RNA polymerase	3 μg
Total	200 μl

* ^{14}C-UTP employed: 50 mCi/mmole, The Radiochemical Center Amersham.

hauer and Link (1954), respectively. Ascorbate-3-phosphate (AsA-3-P) was kindly supplied by the Takeda Pharm. Ind. Co., Ltd.

Calf thymus DNA was prepared in our institute (Omura et al., 1973, 1975b, 1978b) by the routine SDS-phenol method. However, sarcoma 180 DNA was prepared by the method of Saito and Miura (1963) as follows, since highly polymerized DNA responsible for the analysis by the sucrose density gradient centrifugation was hardly obtained by the usual method. Sarcoma 180 tumor cells were harvested by centrifugation at 15,000 xg for 10 min on 1 week after intraperitoneal inoculation with the tumor cells to mice. The cells were washed with SSC (0.15 M NaCl--0.015 M Na-citrate) and 0.1 ×SSC every 2 times and suspended in 1% SDS-O. 1 M Tris buffer (pH 9.0). The suspension was kept at room temperature for 20 to 30min. Into the lysed solution, an equal volume of water saturated with phenol was added. The mixture was stirred at 0°C for 20 min and centrifuged at 5,000~6,000×g for 20 min. After adding 0.1 volume of 3 M Na-acetate-l mM EDTA, the supernatant was mixed gently with the equal volume of isopropyl alcohol to precipitate DNA. Fibrous DNA was then washed successively with 70% and 90% ethyl alcohol. DNA was again dissolved in SSC-1 mM EDTA and the solution was incubated at 37°C for 30min with RNase A and RNase T_1. An equal volume of phenol was added to the reaction mixture, stirred for 10 min at 0°C and centrifuged at 5, 000~6, 000×g for 20 min to remove the denatured RNases. As above, the supernatant was mixed with 3 M Na-acetate-l mM EDTA and DNA was precipitated with isopropyl alcohol. Thus, fibrous sarcoma 180 DNA was obtained by washing and drying as usual.

4. **Treatment of DNA with reductones**

Reductone was incubated with DNA (100 μg/ml of the final concentration) in 25 μM Tris buffer, pH 6.8, at 37°C with or without Cu^{2+} and dislysed against 0.1 ×SSC at 4°C for 24 hrs for DNA polymerase, otherwise described. However, for RNA polymerase, 0.1 M Tris buffer, pH 7.2, or 10 mM Tris buffer, pH 7.8, was employed in reductone treatment on DNA or dialysis.

RESULTS AND DISCUSSION

DNA polymerase

Native calf thymus DNA was treated with 1 mM AsA, MDT, ScA or MHC in the presence of 10 μM Cu^{2+} at 37°C for 60min and the priming activity for DNA polymerase was estimated. The result is shown in Fig. 2. It was indicated that the priming activity was enhanced by the treatment of DNA with these reductones. However, coexistence of Cu^{2+} in the treatment decreased the activity, except the case of MHC, while no variation of the activity was observed with Cu^{2+} of the same concentration alone. On the contrary, combined action of MHC with Cu^{2+} also increased the priming activity of DNA.

As shown in Fig. 3, variation of the priming activity was brought about by the action of AsA with or without Cu^{2+} on sarcoma 180 DNA as well as calf thymus DNA in both the native double strand and the denatured single

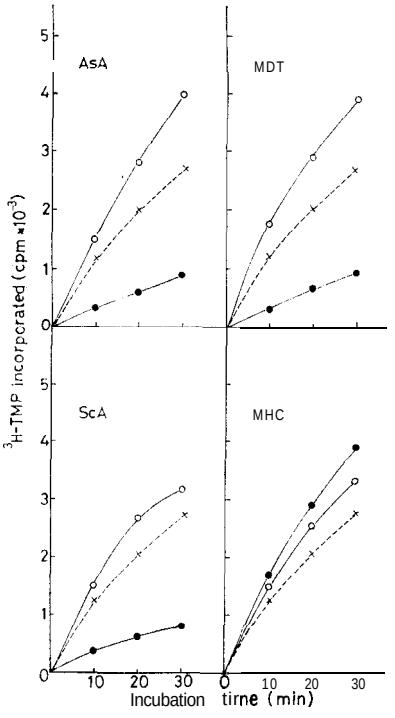

Fig. 2. Priming activity of DNA treated with reductone for DNA polymerase. DNA (100 μg/ml) was incubated with 1 mM reductone and 10 μM Cu^{2+} in 25 μM Tris buffer, pH 6.8, at 37°C for 60 min. After dialysis against 0. 1×SSC at 4°C for 24 hrs, DNA was employed for the assay of DNA polymerase. ×···× control; ○—○ reductone ; ●—● reductone and Cu^{2+}.

strand states. Change of the activity was observed for sarcoma 180 DNA too. In addition, the activity of the denatured DNA was increased by the action of AsA and Cu^{2+}, while that of the native one decreased. Similar variation was observed by the action of other enediol MDT (Fig. 4) and enaminol ScA (Fig. 5).

These variations of the activity were presumed to correlate with breakage of the primer DNA, as demonstrated already for RNA polymerase with several DNA breaking reagents (Yamafuji *et al.*, 1972; Omura *et al.*, 1973; Iiyama ***et al., 1973;*** Omura et al., 1975a). Regarding DNA polymerase too, the variation of the activity was brought about by the action of the DNA breaking reagents including some reductones (Omura ***et al.,*** 1975b; Iiyama ***et al., 1977).*** In the foregoing paper (Omura ***et al.,*** 1975b), it was reported that the activity was increased by the treatment with AsA between 10^{-4} and 10^{-6}M but decreased at 10^{-2}M. In the case of coexistence of Cu^{2+} with AsA, the priming activity was enhanced at lower concentration of AsA in the range of 10^{-6} to 10^{-8}M but decreased much highly at 10^{-2}M. This may show that the degree of the variation of the activity is related to that of the breakage of the primer DNA.

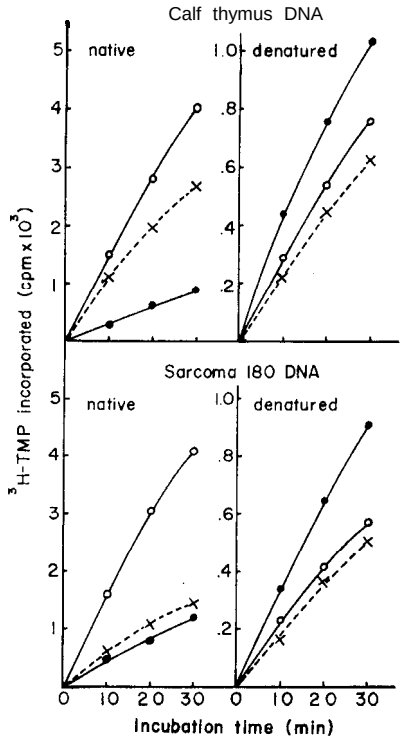

Fig. 3. Effect of AsA on the priming activity of DNAs. The same as in Fig. 2. except native and heat-denatured DNA of calf thymus or sarcoma 180 cell had been employed. ×···× control; ○—○ AsA; • -• AsA and Cu^{2+}.

In order to get additional proof of the presumption, DNA was treated with 1 mM AsA and 0.1 mM Cu^{2+} at 37°C for 5 or 60 min and the activity was estimated. The result is shown in Fig. 6. As expected, the priming activity was enhanced by some moderate action for 5 min, whereas in less extent by severe one for 60min. When reductones of low DNA-breaking ability had been employed, the increment of the activity may be induced by stronger condition. The primer DNA was treated with 1 μM or 1 mM AsA-3-P with or without Cu^{2+} (1/10 of them, respectively) at 37°C for 60min and the activity was assayed (Fig. 7). Similar result was obtained with MHC too (Fig. 8). On the other hand, the treatment of the primer DNA with 1 mM DAsA, the oxidized form of AsA, and 0.1 mM Cu^{2+} was conducted at 37°C for 30 or 120 min (Fig. 9). These phenomena infer that some proper scission of the primer DNA such as single-strand breakage increase the activity and excess ones such as double-strand breakage decrease. Enhancement of the priming activity of the denatured DNA by reductones with Cu^{2+} might be explained similarly.

In these experiments, the assay of the DNA polymerase activity was carried out in the absence of the reductone and Cu^{2+} after dialysis of the primer

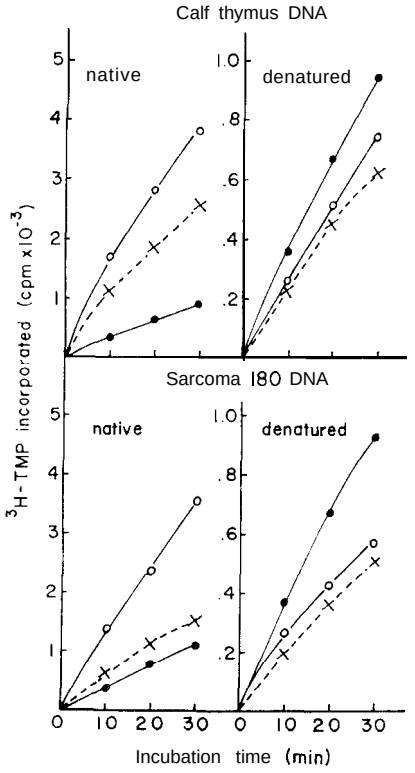

Fig. 4. Effect of MDT on the priming activity of DNAs. The same as in Fig. 3. ×···× control; ○—○ MDT; • -e MDT and Cu^{2+}.

DNA treated. Then, AsA of different concentrations was added into the reaction mixture using non-pretreated DNA and the activity was estimated. As shown in Fig. 10, the incorporation of ^{3}H-TMP was inhibited by AsA with increasing the concentration, attaining to about 50 % at 1 mM. The inhibition was also induced by DAsA or MDT (Fig. 11) to about the same extent, and by aromatic reductones such as epinephrine and adrenalone even though with less effective. Much higher concentration of them stopped the incorporation. In the experiment of Fig. 12, DAsA was added to the reaction mixture in 10 mM of the final concentration and almost complete inhihition was observed.

RNA polymerase

Similar to DNA polymerase, DNA takes a part in RNA polymerase as the template or the primer and its priming activity should be changed, when the primer DNA was modified to some extent. Indeed, some variations of RNA polymerase activity was observed with several DNA breaking reagents (Yamafuji et *al.*, 1972; Omura *et al.*, 1973; Iiyama *et al.*, 1973). Therefore, the effect of reductones on the priming activity of RNA polymerase was then investigated.

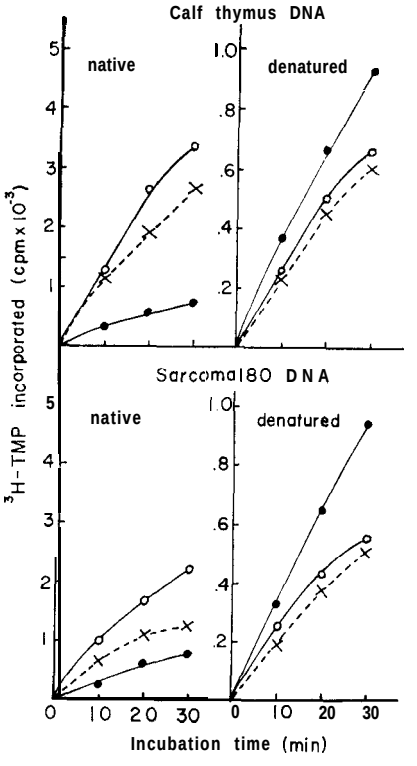

Fig. 5. Effect of ScA on the priming activity of DNAs. The same as in Fig. 3. ×···× control; o-o ScA; •—• ScA and Cu^{2+}.

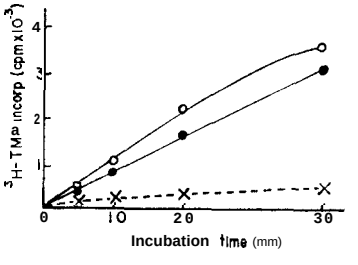

Fig. 6. Priming activity of DNA treated with AsA and Cu^{2+} for different lime. The same as in Fig. 2. ×···× control; ○—○ DNA treated for 5 min ; •—• DNA treated for 60min.

Calf thymus DNA was incubated with 10 μM AsA, MDT, ScA or MHC with and without 1 μM Cu^{2+} in 0.1 M Tris buffer (pH 7.2) at 37°C for 60 min and its priming activity for RNA polymerase was estimated. The result is shown in Fig. 13. The activity was enhanced by the action of these reductones, regardless of the presence of Cu^{2+}. However, the priming activity of DNA treated by AsA, MDT or ScA itself was higher than that treated by them with Cu^{2+}.

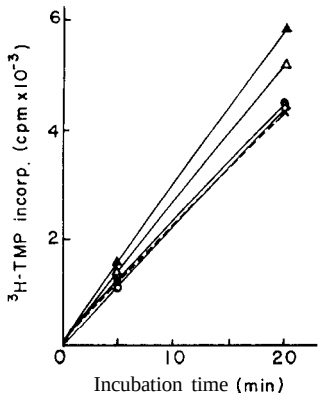

Fig. 7. Priming activity of DNA treated with AsA-3-P of different concentration. The same as in Fig. 2. ×···× control; o-o 1 μM AsA-3-P; • -e 1 μM AsA-3-P and 0.1 μM Cu^{2+}; ▵—▵ 1 mM AsA-3-P; ▲—▲ 1 mM AsA-3-P and 0.1 mM Cu^{2+}.

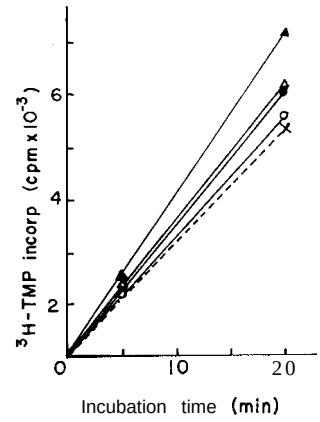

Fig. 8. Priming activity of DNA treated with MHC of different concentration. The same as in Fig. 2. ×···× control; o-o 1 μM MHC; •—• 1 μM MHC and 0.1 μM Cu^{2+}; ▵—▵ 1 mM MHC; ▲—▲ 1 mM MHC and 0.1 mM Cu^{2+}.

On the other hand, the latter was conversely higher than that of the former in the case of MHC having the weaker DNA breaking ability.

This observation suggests that the variation of the priming activity of DNA is concerned with the degree of scission in DNA. Therefore, DNA was treated with AsA of various concentrations between 10^{-8} and 10^{-2}M and the priming activity was estimated as usual. The result is shown in Fig. 14. The activity increased initially and then decreased with increasing the concentration of AsA, the peak being observed at 10^{-4}M under this condition. Regarding ScA and MHC too, the priming activity was higher when the DNA had been treated at 10 μM compared with that treated at 10 mM, as seen from the relative activity cited in Table 3. As often demonstrated, the reductone

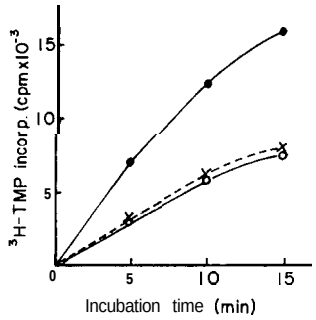

Fig. 9. Priming activity of DNA treated with DAsA and Cu^{2+} for different time. The same as in Fig. 6. ×···× control; o-o DNA treated for 30 min ; •—• DNA treated for 120 min.

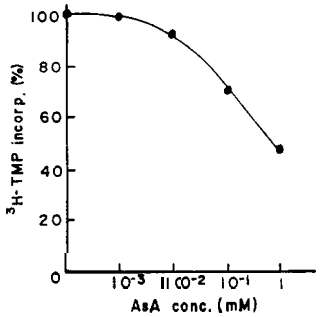

Fig. 10. Effect of AsA on assay of DNA polymerase activity. AsA was added into the reaction mixture using non-pretreated DNA and the DNA polymerase activity was estimated.

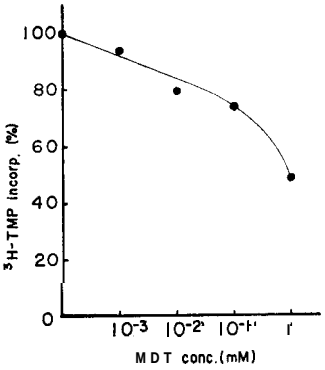

Fig. 11. Effect of MDT on assay of DNA polymerase activity. The same as in Fig. 10.

structures may play an essential role in the breakage of DNA and in the variation of its primer activity. Fig. 15 indicates that no variation was not

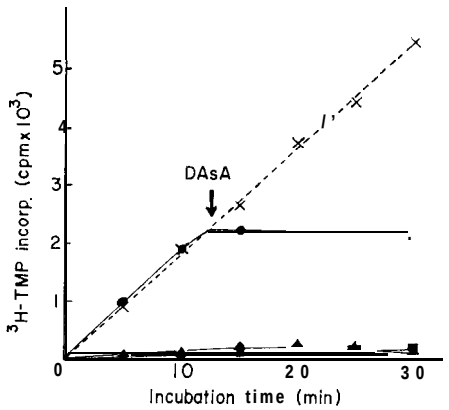

Fig. 12. Effect of DAsA on assay of DNA polymerase activity. The same as in Fin. 10. except 10 mM DAsA had been employed. ×···× control; ▪ -m DAsA added into the reaction mixture at 20min before starting the reaction; ▲—▲ DAsA added at 0 min; ●—● DAsA added at 12.5 min after starting the reaction.

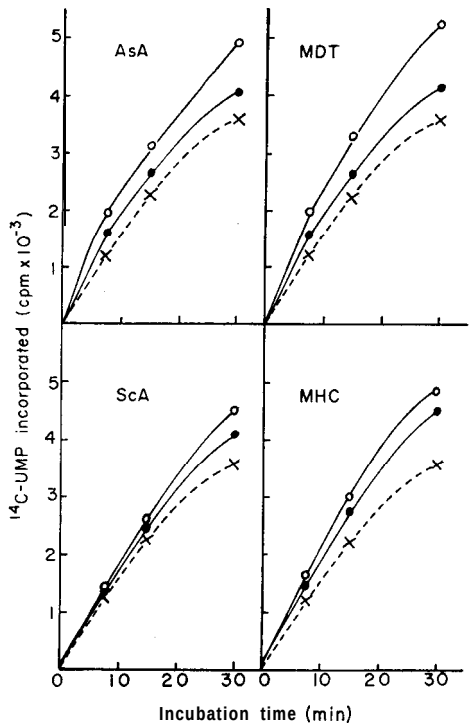

Fig. 13. Priming activity of DNA treated with reductone for RNA polymerase. The same as in Fig. 2. ×···× control; ○—○ reductone ; ●—● reductone and Cu^{2+}.

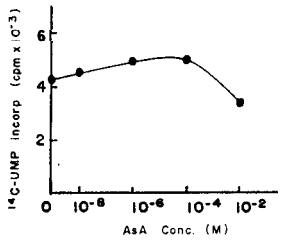

Fig. 14. Priming activity of DNA treated with AsA of different concentration for RNA polymerase. The same as in Fig. 13.

Table 3. Effect of ScA and MHC on the RNA polymerase reaction system.

Conc. of reductone	ScA	MHC --
0	100	100
10 μM	**160**	**150**
10 mM	120	120

Figures show the relative activity (incorporation of ^{14}C-UMP).

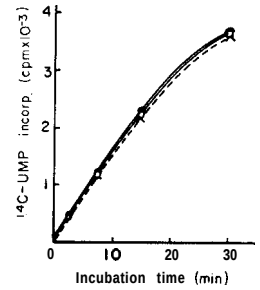

Fig. 15. Priming activity of DNA treated with AsA-3-P for RNA polymerase. The same as in Fig. 13. ×···× control; o - o AsA-3-P; .-. AsA-3-P and Cu^{2+}.

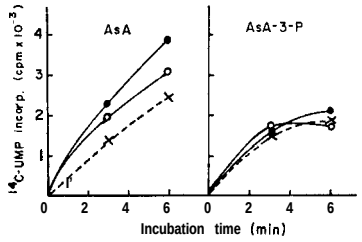

Fig. 16. Priming activity of DNA treated with AsA or AsA-3-P for core RNA polymerase activity. The same as in Fig. 13, except core RNA polymerase had been employed. ×···× control; ○—○ DNA treated for 30 min; ●—● DNA treated for 360min.

provoked by AsA-3P in which enediol group had been blocked.

Tiyama **(1974)** established that, when the core **RNA** polymerase lacking

σ factor, the recognizer of the initiation site of the reaction, was used instead of the holo enzyme, the incorporation of ^{14}C-UMP increased by the moderate treatment of the primer DNA with several DNA breaking reagents such as epinephrine, cortisone, glucose oxime, N-methyl-N'-nitro-N-nitrosoguanidine and mitomycin C. By comparing the variation with degree of the scission of the primer DNA, he demonstrated that some cleaved sites of DNA chain introduced by the single-strand scission may serve as the initiation sites for RNA synthesis. In order to ascertain whether or not the assumption is applicable to AsA, the primer DNA was treated with 0.5 mM AsA or AsA-3-P without Cu^{2+} at 37°C for 30 and 360min and used for the assay using the core enzyme. As shown in Fig. 16, the similar enhancement of the reaction was observed with AsA, but not with AsA-3-P.

Thus, it was confirmed that the reductones provoked some variation of the replication and transcription at least through the change of the primer activity of DNA for DNA polymerase as well as RNA polymerase due to certain scission of DNA chain by them.

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