EFFECT OF METAL CATIONS ON MISINCORPORATION BY E. COLI DNA POLYMERASES

Michiko Miyaki, Ikuko Murata, Michiko Osabe and Tetsuo Ono

Department of Biochemistry,
The Tokyo Metropolitan Institute of Medical Science,
3-18, Honkomagome, Bunkyoku, Tokyo 113, Japan

Received June 21,1977

SUMMARY: The effects of divalent metal cations on DNA polymerase activity of E. coli polymerase I, II or III and on their fidelities during DNA synthesis in vitro have been examined. Carcinogenic or mutagenic metal at the concentration which decreases polymerase activity increased misincorporation, whereas noncarcinogenic metal did not change the fidelity even at a concentration inhibiting polymerase. The change in 3'→ 5' exonuclease activity of polymerase by metal ions was not responsible for the increase in the misincorporation. The influences of metals on Km for the substrate and template-primer were also observed.

INTRODUCTION: Various metals have been shown to cause mutation in microorganisms (1-3), and to induce tumors in man and experimental animals (4-6). At present the mechanism of mutagenesis and carcinogenesis by metals is not understood, however, occurrence of chromosomal aberrations (7) and "rec-effect" (8) caused by metals indicate the significance of the effect of metals on the process of DNA replication or repair. A remarkable decrease in fidelity by manganese during DNA synthesis in vitro (9) has suggested that misreading during the replication of cellular DNA likely occured when the concentration of such a mutagenic or carcinogenic metal was near the replication point. In view of the possible relationship between the change in fidelity of DNA synthesis and mutagenesis or carcinogenesis, we examined the effect of metals on the polymerase reaction in vitro, and observed that carcinogenic or mutagenic metal cations cause a decrease in fidelity of DNA synthesis.

During the preparation of this manuscript, Sirover and Loeb reported similar effects of metals on the fidelity of DNA synthesis with polymerase from avian myeloblastosis virus (10, 11).

MATERIALS AND METHODS: E. coli DNA polymerase I and deoxyribonucleoside triphosphates (dNTP) were purchased from Boehringer. DNA polymerase II and III were prepared from E. coli P3478 as described by Kornberg et al. (12,13). Synthetic template-primers were obtained from P-L Biochemicals and radioactive nucleotides from Radiochemical Centre. The fidelity of DNA polymerase was determined by incorporation of correct nucleotides which were complementary to the template, and by also incorporation of nucleotides which were noncomplementary to the template. This was done in a separate assay using the same enzyme preparation. The reaction mixture (0.2 ml) was composed of 100 μM correct dNTP, 4 to 8 μM of incorrect dNTP, 100 μM phosphorus of template-primer, 5 mM $MgCl_2$ and/or other metal cations, 60 mM Tris-HCl, pH7.5,

ISSN 0006-291X

10 μg of bovine serum albumin and 0.3 unit (0.07 μg) to 3 unit (0.7 μg) of polymerase I. When the polymerase activity was inactivated by metal cations to 1/10 of control, the 10-fold enzyme protein was subjected to the assay in order to obtain the same level of correct incorporation as that of the control enzyme. Correct [^{3}H]dNTP of 70 mCi/mmol was used to measure the correct incorporation, and incorrect [^{3}H]dNTP of 11 to 40 Ci/mmol was used for the misincorporation. After the reaction mixture was incubated at 37° for 30 to 60 min, it was mixed with 100 μM of the cold incorrect dNTP. This treatment was effective to lower the background of radioactivity of misincorporation. The reaction mixture was placed on paper filter disc (Whatman 3MM) which was then washed twice with 10% CCl_3COOH containing 50 mM sodium pyrophosphate and three times with 5% CCl_3COOH and ethanol. The radioactivity was measured in toluene scintillator, and the incorporation without the template-primer or without the enzyme was subtracted from each assay. All assays were repeated more than three times. The reaction mixture for the assay of polymerase II or III contained the same components as that of polymerase I except for 10 mM Tris-HCl and 2 mM dithiothreitol. The reaction mixture was incubated at 30°. Metals were added to the reaction mixture as chlorides of divalent cations except for $ZnSO_4$ and $CdSO_4$ (Wako Pure Chemicals and Kanto Chemicals).

To measure only the 3'→ 5' exonuclease activity, Klenow's enzyme A (Boehringer) which has no 5'→ 3' exonuclease activity was used. The reaction mixture contained 19 μM poly(dA-dT)·poly(dT-dA) labeled with [^{3}H]dTTP, 50 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 50 μg bovine serum albumin and 5 mM $MgCl_2$ or 1 mM $MnCl_2$. The reaction mixture was incubated at 37° for 30 to 60 min and acid-soluble radioactivity was measured in toluene-Triton X100 scintillator. In order to estimate the activity ratio of nuclease to polymerase, each reaction mixture contained the same enzyme preparation.

Km for correct dNTP was measured with a different concentration of the correct [^{3}H]dNTP and other components were held constant in the reaction mixture of polymerase assay with 5 mM $MgCl_2$ or 1 mM $MnCl_2$. The Michaelis constants were estimated from the double reciprocal plots.

RESULTS AND DISCUSSIONS: Two distinct modes of inactivation of polymerase I by divalent cations were observed. The first group including Be, Ca, Cu, Zn, Cd and Hg exclusively inhibited polymerase reaction. These were unable to activate the polymerase (Fig. 1a, 1b). Be, Ca and Cu inhibited the reaction rather weakly, but Zn, Cd and Hg strongly inhibited the reaction at low concentrations. Metal cations of the second group such as Mn, Co and Fe had two activities, inhibition and activation of the polymerase, and they could substitute for Mg (Fig. 1c, 1d). These metals are transition elements which have the ability to form coordination compounds and are known to be carcinogenic.

Effect of metals on the fidelity of DNA sinthesis was examined at metal concentrations which inhibit DNA polymerase to 1/10 of the activity observed in the presence of 5 mM Mg alone (as metal). In the assay of the misincorporation, sulfhydryl compound often formed heavy metal sulfide accompanied by nonspecific coprecipitation of the incorrect labeled deoxynucleoside triphosphates which disturbed the results of the misincorporation. To avoid ambiguous misincorporation, E. coli DNA polymerase I was used because it does not require a sulfhydryl compound in the reaction mixture and is available commercially in a considerably pure form. Since high metal

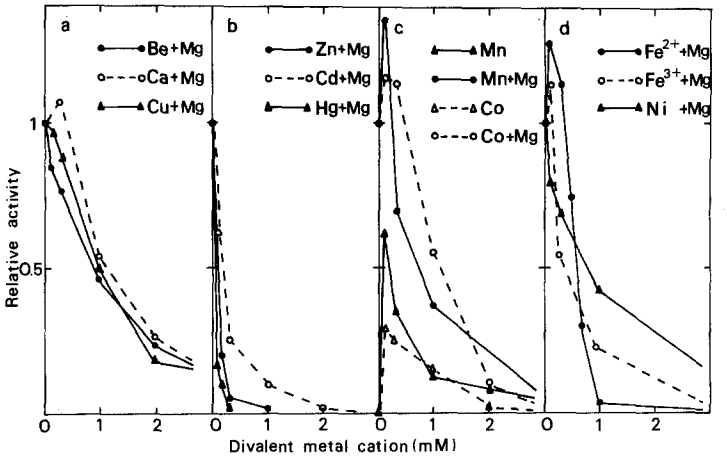

Fig. 1. Effect of metal cations on E. coli DNA polymerase I activity. Polymerase activity was measured with different concentrations of divalent metal cations as indicated in figures in the presence or absence of 5 mM $MgCl_2$. Activity at each concentration of metal cation is represented by the value relative to that observed in the presence of 5 mM Mg alone as metal.

concentrations also caused precipitations, every assay was done under the conditions producing no precipitation. The increase in the misincorporation was observed with respect to every dNTP (Table I) when 5 mM Mg was replaced by 1 mM Mn. The latter metal has been proven to be mutagenic for microorganisms (1-3) and mammalian cells (Akamatsu and Miyaki, manuscript in preparation), and also to be carcinogenic (6). As for the misincorporation, competition of Mg with Mn was slight, therefore, addition of 5 mM Mg to the reaction mixture containing 1 mM Mn incompletely inhibited the misincorporation observed in the presence of 1 mM Mn. However, Mn did not enhance misincorporation at a concentration of 0.1 mM which was optimum for activation of the polymerase (Fig. 1c). This indicated that binding of Mn at many inhibitory sites of enzyme (14) besides at an active center is necessary to cause remarkable change in the fidelity. Be, Fe, Co and Ni apparently increased misincorporation, and Cd, Hg and Zn had little effect, but Ca and Cu did not affect the fidelity at concentrations used in this experiment (Table I). The present results for divalent cations seem to be in agreement with the carcinogenesity previously reported. Be, Fe, Co, Ni, Cd, Hg and Zn are known to be carcinogenic (4-6), whereas Ca and Cu have not been reported to be carcinogens and they have been described to be negative for lung tumor production in mice (6). Trivalent cations, Cr^{3+} and Fe^{3+}, were not tested for effect on misincorporation because the precipitates formed in the reaction

TABLE I. Effect of divalent metal cations on misincorporation by E. coli DNA polymerase I

Metal ion (mM)	Incorrect dNMP	Template-primer	Error ($\times 10^{-5}$)[c]
Mg(5)	dAMP	dGdC[a]	6.4
"	dTMP	"	2.4
"	dGMP	$dAdT_{12-18}$[b]	0.3
"	dCMP	"	2.7 ± 0.7[d]
Mn(0.1)	dAMP	dGdC	4.5
"	dTMP	"	0.7
"	dCMP	$dAdT_{12-18}$	2.3
Mn(1)	dAMP	dGdC	45.1 *
"	dTMP	"	6.4 *
"	dGMP	$dAdT_{12-18}$	6.7 *
"	dCMP	"	16.0 *
Mn(1) + Mg(5)	dCMP	$dAdT_{12-18}$	9.8 *
Be(4) + Mg(5)	"	"	8.0 *
Ca(3) + Mg(5)	"	"	2.6
Cu(3) + Mg(5)	"	"	2.6
Zn(0.2)+ Mg(5)	"	"	3.0
Cd(1) + Mg(5)	"	"	4.4 *
Hg(0.1)+ Mg(5)	"	"	4.2 *
Fe(0.5)+ Mg(5)	"	"	13.0 *
Co(2)	"	"	6.4 *
Co(2) + Mg(5)	"	"	4.8 *
Ni(3) + Mg(5)	"	"	7.6 *

a) dGdC = poly(dG)·poly(dC), b) $dAdT_{12-18}$ = poly(dA)·$(dT)_{12-18}$

c) Error = misincorporation/correct incorporation(pmoles), mean of 2 or 3 experiments

d) mean of 8 experiments

*) statistically estimated to be significant

mixture disturbed the true misincorporation. Dichromate ion, $Cr_2O_7^{2-}$, inhibited the polymerase at a rather high concentration (50% inhibition was observed at 15 mM) but it did not affect the misincorporation although mutagenesity and carcinogenesity have been described to be significant.

Since DNA polymerase II and III are known to be concerned with DNA

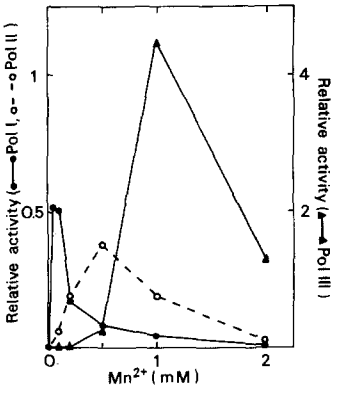

Fig. 2. Effect of Mn on activity of E. coli DNA polymerase I, II or III. Activity of polymerase at each concentration of $MnCl_2$ is represented by the value relative to that observed in the presence of 5 mM $MgCl_2$.

TABLE II. Effect of Mn on misincorporation of dCMP into $dAdT_{12-18}$ by E. coli DNA polymerase I, II or III

Polymerase	Error ($x10^{-5}$)	
	Mg (5 mM)	Mn (1 mM)
I	2.5	18.0
II	0.6	1.6
III	0.5	1.4

replication in E. coli, effect of metal cations on polymerase II and III were then examined. Maximal activities of all three enzymes were exhibited at 5 mM of Mg, however, optimal concentration of Mn differed according to the polymerase as shown in Fig. 2. The misincorporation by every polymerase in the presence of 1 mM Mn was higher than that with 5 mM Mg, even with polymerase III, despite the fact that 1 mM Mn was optimal for this enzyme. However, increases in the misincorporation with polymerase II and III by 1 mM Mn were less than with polymerase I (Table II). These results lead to an assumption that the number of Mn cations bound to the inhibitory sites of polymerase II or III were less than that bound to polymerase I, although interaction of Mn with dNTP and template was the same in all three polymerization reactions at the same 1 mM concentration.

The 3'→ 5' exonuclease activity of DNA polymerase is reported to excise noncomplementary nucleotides during polymerization (15). Effect of Mn on

TABLE III. Effect of Mn on 3'→ 5' exonuclease and polymerase activity

	Mg (5 mM)	Mn (1 mM)
dTMP released (pmol)	2.7	7.4
dTMP incorporated (pmol)	150.6	18.6
$\frac{\text{nuclease}}{\text{polymerase}} \times 100$	1.8	39.8

TABLE IV. Effect of Mn on Km for dNTP in polymerase reaction

dNTP	Km (μM) Mg (5 mM)	Mn (1 mM)
dATP [a]	0.2	0.4
dTTP [a]	1.8	42.0
dGTP [b]	0.4	1.7
dCTP [b]	0.7	0.8

a) Template-primer : poly(dA)·poly(dT)
b) " : poly(dG)·poly(dC)

3'→ 5' exonuclease activity was presently examined with Klenow's enzyme A (16) which has no 5'→ 3' nuclease activity. Substitution of 5 mM Mg by 1 mM Mn decreased polymerase activity but increased 3'→ 5' exonuclease activity (Table III). The increased ratio of nuclease/polymerase due to the presence of 1 mM Mn caused a decrease in the fidelity, and this suggested that a change in 3'→ 5' exonuclease activity was not responsible for the change in misincorporation in vitro. This result is consistent with that reported for avian myeloblastosis virus polymerase (10) but different from that described for micrococcus polymerase (17). The enhancement of nuclease by 1 mM Mn seemed to be caused by interaction of Mn with the substrate, poly(dA-dT)·poly(dT-dA), resulting in a lowering of Tm (18).

Km values for correct dNTP in the polymerase reaction were found to be changed by 1 mM Mn as shown in Table IV. These results suggest a decrease in the binding of the correct dNTP to the enzyme by substitution of 5 mM Mg with 1 mM Mn. The decreased strictness in the base selection during polymerization probably permits misincorporation of incorrect dNTP.

Effect of Mn on the affinity of polymerase for the template also differed from that of Mg. The Km value for poly(dG)·poly(dC) was 50 μM in the presence

of 5 mM Mg and 10 μM with 1 mM Mn. A smaller Km value in the presence of 1 mM Mn indicates a tighter binding of poly(dG)·poly(dC) to the enzyme by 1 mM Mn than by 5 mM Mg, while, Vmax was decreased by 1 mM Mn. The Km for poly(dA)·poly(dT) was unchanged by 1 mM Mn. These results did not explain the decreased fidelity with 1 mM Mn. On the other hand, interaction of Mn with the base moiety in dNTP and in the template has been described (18, 19) and increased mispairing between poly(I) and poly(C,U) or between poly(I) and poly(C,A) induced by Mn and Cd has been reported (20). Mn, Co, Ni and Zn have also been described to bind to the ligands of purine bases (21). These binding of metals may affect the base pairing during polymerization resulting in enhanced misincorporation.

REFERENCES:

1. Demerec, M. and Hanson, J. (1951) Cold Spr. Harb. Symp. Quant. Biol. 16, 215-228.
2. Lindegren, C.C., Nagai, S. and Nagai, H. (1958) Nature 182, 446-448.
3. Orgel, A. and Orgel, L.E. (1965) J. Mol. Biol. 14, 453-457.
4. Hueper, W.C. and Conway, W.D. (1964) Chemical Carcinogenesis and Cancers, p.379-402, Charles C. Thomas Publisher, Springfield, Ill.
5. Furst, A. and Haro, R.T. (1969) Progr. Expt. Tumor Res. 12, 102-113, Karger, Basel/new York.
6. Stoner, G.D., Shimkin, M.B., Troxell, M.C., Thompson T.L. and Terry, L.S., (1976) Cancer Res. 36, 1744-1747.
7. Paton, G.R. and Allisox A.C. (1972) Mut. Res. 16, 332-336.
8. Nishioka, H. (1975) Mut. Res. 31, 185-189.
9. Hall, Z.W. and Lehman, I.R. (1968) J. Mol. Biol. 36, 321-333.
10. Sirover, M.A. and Loeb, L.A. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2331-2335.
11. Sirover, M.A. and Loeb, L.A. (1976) Science 194, 1434-1436.
12. Kornberg, T. and Gefter, M.L. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 761-764.
13. Kornberg, T. and Gefter, M.L. (1972) J. Biol. Chem. 247, 5369-5375.
14. Slater, J.P., Tamir, L., Loeb, L.A. and Milvan, A.S. (1972) J. Biol. Chem. 247, 6784-6794.
15. Brutlag, D. and Kornberg, A. (1972) J. Biol. Chem. 247, 241-248.
16. Klenow, H. and Henningsen, I. (1970) Proc. Natl. Acad. Sci. U.S.A. 65, 168-175.
17. Luke, M.Z., Hamilton, L. and Hollocher, T.C. (1975) Biochem. Biophys. Res. Commun. 62, 497-501.
18. Luke, G. and Zimmer, C. (1972) Eur. J. Biochem. 29, 528-536.
19. Anderson, J.A., Kuntz, G.P.P., Evans, H.H. and Swift, T.J. (1971) Biochemistry 10, 4368-4374.
20. Murray, M.J. and Flessel, C.P. (1976) Biochim. Biophys. Acta 425, 256-261.
21. Collins, A.D., De Meester, P., Goodgame, D.M.L. and Skapsi, A.C. (1975) Biochim. Biophys. Acta 402, 1-6.