METAL ACTIVATION OF DNA SYNTHESIS

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SUMMARY. We have determined that Co^{2+}, Ni^{2+} or Zn^{2+} may substitute for Mg^{2+} during DNA synthesis with E. coli DNA polymerase I, sea urchin nuclear DNA polymerase and the DNA polymerase from avian myeloblastosis virus (AMV). In addition, the frequency of non-complementary nucleotide incorporation using AMV DNA polymerase was increased using Co^{2+} or Mn^{2+} as the metal activator. These results suggest that the fidelity of DNA synthesis may be influenced by the metal activator used during catalysis.

The function of metal cations in DNA replication may be investigated by studying in vitro catalysis using purified DNA polymerases. Conversely, analysis of these reactions using different metals may help to define the detailed catalytic mechanism of these enzymes. DNA polymerases require an added divalent metal activator, Mg^{2+} or Mn^{2+}, for catalysis (1, 2) and recent evidence indicates that they are zinc metalloenzymes (3, 4). We now report that Co^{2+}, Ni^{2+} and Zn^{2+} can substitute for Mg^{2+} during catalysis with DNA polymerases from divergent organisms. Furthermore, the fidelity of DNA replication is reduced by the use of Co^{2+} or Mn^{2+} during catalysis.

Avian myeloblastosis virus (AMV) DNA polymerase and E. coli DNA polymerase I were homogeneous as determined by polyacrylamide gel electrophoresis (5, 6). Sea urchin nuclear DNA polymerase was partially purified and corresponded to Fraction V as previously described (7). Metals (at least 99.9% purity) were purchased as chloride salts from Baker Chemical Co. or from Fisher Scientific Co., and were added to reactions in aqueous solution.

RESULTS. All of the DNA polymerases tested were able to use Co^{2+} instead of Mg^{2+} during catalysis (Table 1). Synthesis of DNA by sea urchin

TABLE I

Effect of Divalent Metal Cations on DNA Replication

Source of Polymerase	Metal	Optimal Concentration (mM)	dTMP Incorporation (pmoles)	% of Mg^{2+} Incorporation
Sea Urchin Nuclei	Mg^{2+}	2.5	36	100
	Co^{2+}	4	21	58
	Ni^{2+}	6	10	29
	Mn^{2+}	0.5	8	21
	Zn^{2+}	3	3	9
	Sr^{2+}	-	<1	<1
Avian Myeloblastosis Virus	Mg^{2+}	5	200	100
	Mn^{2+}	0.5	84	42
	Co^{2+}	1	54	27
	Ni^{2+}	2	11	6
	Sr^{2+}	1	6	3
	Zn^{2+}	0.6	4	2
E. coli (Polymerase I)	Mg^{2+}	2	2,200	100
	Mn^{2+}	0.1	3,366	153
	Co^{2+}	1	1,260	57
	Zn^{2+}	0.6	82	4
	Sr^{2+}	0.2	10	<1
	Ni^{2+}	0.2	4	<1

The reaction mixtures (total volume 0.05 ml) contained 100 mM Tris-maleate (pH 8.0), 100 μM dGTP, 100 μM dATP, 100 μM dCTP, 100 μM α-^{32}P dTTP (200 dpm/pmole), 7.5 μg "activated" DNA, and 0.5 nM AMV DNA polymerase or 80 nM E. coli DNA polymerase I or 6 μg of sea urchin nuclear DNA polymerase. The incorporation of [α-^{32}P]dTTP by AMV DNA polymerase and by E. coli DNA polymerase I was measured at 37°C for 60 minutes and by sea urchin nuclear DNA polymerase at 37°C for 15 minutes. Metal cations were added separately at the indicated final concentrations. Samples were washed as previously described for complementary nucleotide incorporation[5]. All reactions were performed in duplicate. Optimal metal concentrations were determined in reactions in which the metal concentration was varied systematically from 0.1-60 mM. With Ba^{2+}, Be^{2+}, Ca^{2+}, or Cu^{2+}, incorporation was less than 0.1% of that obtained with Mg^{2+}.

nuclear DNA polymerase and by E. coli DNA polymerase I was approximately 60% of that incorporated using Mg^{2+}. Incorporation of dTMP by AMV DNA polymerase in the presence of Co^{2+} was 30% of that observed using Mg^{2+}. With sea urchin DNA polymerase, incorporation using Ni^{2+} was equal to 30% of that with Mg^{2+}; less incorporation was observed using Ni^{2+} with AMV DNA polymerase (6%) and E. coli DNA polymerase I (<1%). Zinc was effectively used by sea urchin DNA polymerase and by E. coli DNA polymerase I, but not by AMV DNA polymerase. None of the polymerases

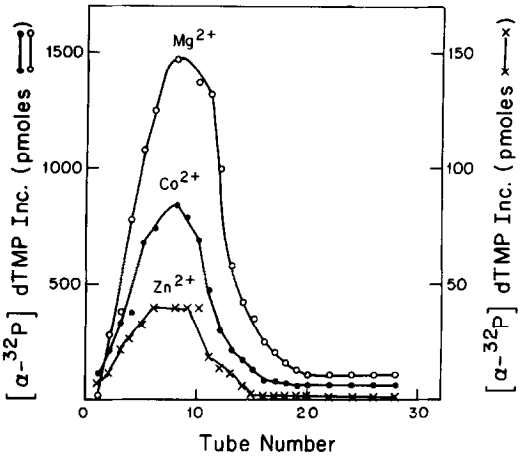

Fig. 1: Glycerol gradient centrifugation of E. coli DNA polymerase I. The polymerase (6 µg) was layered on a 10-35% glycerol gradient which contained 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM K_2EDTA (pH 7.0) and centrifuged at 30,000 rpm for 16 hrs. at 5°C. Fractions were collected from the top of the gradient. Metal dependent catalysis by E. coli DNA polymerase I was determined using Mg^{2+} (o), Co^{2+} (•), or Zn^{2+} (x), as described in Table I except that each reaction contained 25 µl of each fraction from the gradient.

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effectively used Sr^{2+}. In addition, Ba^{2+}, Be^{2+}, Ca^{2+}, or Cu^{2+} did not support synthesis with either AMV DNA polymerase or with E. coli DNA polymerase I.

E. coli DNA polymerase I was sedimented on a 10-35% glycerol gradient and polymerase activity was determined with Mg^{2+}, Co^{2+} or Zn^{2+} (Fig. 1). Catalysis with each of the metal cations was proportional to enzyme concentration throughout the gradient. Synthesis with Co^{2+} and Zn^{2+} was approximately 60% and 4% of that observed with Mg^{2+} and the results are comparable with those reported in Table 1. In addition, there was a constant proportionality in activity when AMV DNA polymerase was sedimented through a 10-35% glycerol gradient and activity was determined using Mg^{2+} and Co^{2+}. Furthermore, alkaline sucrose sedimentation analysis of the product synthesized using poly $(C)_{2500}$·oligo dG_{12-18} as the template with Co^{2+} and AMV DNA polymerase indicated that dGMP was incorporated into DNA during polymerization.

TABLE II

Reaction Requirements for Polymerization using Co^{2+}

Polymerase	Reaction Mixtures	dTMP Incorporation pmoles
AMV DNA Polymerase	Complete	138
	-Polymerase	2
	-dATP	9
	-poly [d(A-T)]	0
	$-Co^{2+}$	0
E. coli DNA Polymerase I	Complete	1,564
	-Polymerase	1
	-dATP	7
	-poly [d(A-T)]	0
	$-Co^{2+}$	0

The complete reaction mixtures (total volume 0.05 ml) contained 100 mM, Tris-maleate (pH 8.0), 1 mM $CoCl_2$, 100 μM dATP, 100 μM [α-^{32}P]dTTP (100 dpm/pmole), 10 μg poly [d(A-T)], and 0.5 nM AMV DNA polymerase or 80 nM *E. coli* DNA polymerase I. The incorporation of [α-^{32}P]dTMP by AMV DNA polymerase and by *E. coli* DNA polymerase I was measured at 37°C for 60 minutes.

The reaction requirements for polymerization using Co^{2+} are shown in Table 2. Using either AMV DNA polymerase or *E. coli* DNA polymerase I, DNA polymerization was dependent on Co^{2+}, the complementary deoxynucleoside triphosphates, the polymerase and the template. Elimination of any of these components reduced by greater than 10-fold the incorporation of dTMP into DNA. The requirements for catalysis using Co^{2+} were the same as with Mg^{2+} and were characteristic of DNA polymerization (5, 8). Furthermore, the dependence of dTMP incorporation on the presence of dATP with poly [d(A-T)] as a template indicated that the Co^{2+} - activated reaction did not proceed by terminal addition (9). Comparable reaction requirements were observed for polymerization by sea urchin DNA polymerase and by AMV DNA polymerase using Ni^{2+}. In addition, AMV DNA polymerase and *E. coli* DNA polymerase I catalyzed polynucleotide replication with a variety of ribonucleotide and deoxynucleotide template-primers using Co^{2+} instead of Mg^{2+} (unpublished results).

TABLE III

Effect of Metal Activators on the Fidelity of DNA Synthesis

Metal	Complementary Nucleotide (dTTP) Incorporation (pmoles)	Non-Complementary Nucleotide (dCTP) Incorporation (pmoles)	Error Frequency
5 mM Mg^{2+}	185	0.28	1/1321
1 mM Co^{2+}	179	0.33	1/1084
3 mM Co^{2+}	82	0.22	1/745
0.4 mM Mn^{2+}	205	0.64	1/640
2 mM Mn^{2+}	20	0.09	1/444

Error frequencies were determined by the simultaneous incorporation of complementary and non-complementary nucleotides. The reaction mixtures (total volume 0.05 ml) contained 100 mM Tris-maleate (pH 7.6), 100 µM dATP, 100 µM [α-^{32}P]dTTP (10 dpm/pmole), 100 µM [^{3}H-dCTP] (15,000 dpm/pmole); 10 µg poly [d(A-T)] and 0.5 nM AMV DNA polymerase. The reaction mixtures were incubated at 37°C for 60 min. Samples were washed as previously described for non-complementary nucleotide incorporation[5]. All reactions were performed in duplicate and the averages determined. Error frequency is defined as the ratio of non-complementary nucleotide incorporated to the total complementary nucleotide incorporated during synthesis.

The accuracy of DNA replication was then determined using Mg^{2+}, Co^{2+}, and Mn^{2+} with AMV DNA polymerase and poly [d(A-T)] as the template (Table 3). With 5 mM Mg^{2+}, AMV DNA polymerase incorporated 1 non-complementary nucleotide (dCMP) for every 1321 complementary nucleotides (dAMP and dTMP) polymerized. This error frequency was independent of Mg^{2+} concentration (5). Using 1 mM Co^{2+} the error frequency was increased to 1/1064; with 3 mM Co^{2+} it was elevated further to 1/745. Similar results were observed with Mn^{2+}. Using 0.4 mM Mn^{2+}, the error frequency was 1/640; with 2 mM Mn^{2+} it was increased to 1/430.

DISCUSSION. These results demonstrate that DNA polymerases may use several different metal cations during catalysis and that the accuracy of DNA synthesis may be determined by the metal cation. With each polymerase the maximal rate of catalysis may be inversely correlated with the stability with which divalent ions of the first transition series form metal complexes (Mg<Mn<Co<Ni)

(10). It is interesting to note that Co^{2+}, Ni^{2+} and Zn^{2+} have been reported to induce tumors in laboratory animals (11-14). At least one of these metals, nickel, may be a human carcinogen (15). It is unknown at present whether the use of these specific metals in DNA polymerization may be relevant to their mutagenicity (16) or carcinogenicity. As all activities associated with DNA polymerases require a metal activator, the use of Co^{2+}, Ni^{2+} or Zn^{2+} in these reactions may provide a means to delineate the mechanisms of catalysis by these enzymes.

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REFERENCES

1. Kornberg, T. and Kornberg, A. (1974) in The Enzymes (Ed. by Boyer, P.), 10, 119.
2. Loeb, L.A. (1974) in The Enzymes (Ed. by Boyer, P.) 10, 173.
3. Springgate, C.F., Mildvan, A.S., Abramson, R., Engle, J.L. and Loeb, L.A. (1973) J. Biol. Chem. 248, 5987.
4. Poiesz, B.J., Seal, G. and Loeb, L.A. (1974) Proc. Nat. Acad. Sci., USA 71 4892.
5. Battula, N. and Loeb, L.A. (1974) J. Biol. Chem. 249, 4086.
6. Slater, J.P., Tamir, I., Loeb, L.A. and Mildvan, A.S. (1972) J. Biol. Chem. 247, 6784.
7. Fansler, B.S. and Loeb, L.A. (1974) in Methods in Enzymology (Ed. by Grossman, L. and Moldave, K.) 29, 53.
8. Bessman, M.J., Lehman, I.R., Simms, E.S. and Kornberg, A. (1958) J. Biol. Chem. 233, 171.
9. Adler, J., Lehman, I.R., Bessman, M.J., Simms, E.S. and Kornberg, A. (1958) Proc. Nat. Acad. Sci., USA 44, 641.
10. Irving, M. and Williams, R.J.P. (1953) Jour. Chem. Soc. 637, 3192.
11. Gilman, J.P.W. (1962) Cancer Research 22, 158.
12. Health, J.C. (1956) Brit. J. Cancer 10, 668.
13. Thomas, J.A. and Thiery, J.P. (1953) Compt. Research Soc. de Biol. 236, 1387.
14. Van Esch, G.J., Van Genderen, H. and Vink, H.M. (1962) Brit. J. Cancer 16, 289.
15. Doll, R., Morgan, L.G. and Speizer, F.E. (1970) Brit. J. Cancer 24, 623.
16. Thomas, H.F., Herriott, R.M., Hahn, B.S., Wang, S.Y. (1976) Nature 259, 341.