peptide complexes, preventing the axial interaction of the appended pyridyl N or solvents. On the other hand, the axial interaction of side-arm pyridine *N*-oxide is strongly indicated.

The $E°$ values of copper(II,III) couples are more subject to ligand structural effects than those of nickel(II,III) complexes. The $E°$ values successively diminish as the number of imide anions increases. The side-arm substituents do not significantly affect the $E°$ values. The greater tetragonal distortion with dioxocyclam complexes with respect to oxo-free cyclam complexes is evidenced by the fact that the pyridine N remains uncoordinated with Ni(III) for the former while it binds with Ni(III) for the latter.

Cu(III) was once proposed[28] to be part of the active site of galactose oxidase, although this was later questioned.[29] Recently Cu(III) has been postulated to be a mild oxidizing species in the oxidation of benzyl groups with Cu(II)-peroxydisulfate complexes.[30] Ni(III) is also postulated to be involved in hydrogenase activities.[31] Our study shows macrocyclic oxo polyamines to be a suitable model for biological redox reactions and other metal-catalyzed reactions.

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Supplementary Material Available: Calculation procedure for metal complex formation constants for (pyridylethyl)dioxo tetraamines and trioxocyclam (3 pages). Ordering information is given on any current masthead page.

(28) Hamilton, G. A.; Adolf, P. K.; Jersey, J.; Dubois, G. C.; Dyrkacz, G. R.; Libbiy, R. D. *J. Am. Chem. Soc.* **1978**, *100*, 1899.
(29) Winkler, M. E.; Bereman, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 6244.
(30) Bhatt, M. V.; Perumal, P. T. *Tetrahedron Lett.* **1981**, *22*, 2605.
(31) Kojima, N.; Fox, J. A.; Hansinger, R. P.; Daniels, L.; Orme-Johnson, W. H.; Walsh, C. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 378.

Contribution from the Molecular Biology Institute and Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, California 90024

Zinc Ion in *Escherichia coli* DNA Polymerase: A Reinvestigation

DANIEL R. GRAHAM and DAVID S. SIGMAN*

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The demonstration that the inhibition of *Escherichia coli* DNA polymerase I by 1,10-phenanthroline is due to the nicking of DNA by the 1,10-phenanthroline–copper complex of DNA rather than the coordination of tightly bound zinc ion has prompted the reexamination of the metal ion content of *Pol*I and the "Klenow fragment". Both proteins were purified to greater than 95% purity and dialyzed against a Chelex-treated 50 mM Tris-HCl buffer, pH 7.4. Although both enzymes had high specific activities for polymerization, they only contained 0.08–0.20 mol of Zn^{2+}/mol of enzyme upon analysis using atomic absorption spectrophotometry. The 3′–5′ exonuclease activity was also independent of zinc content. The rec A protein of *E. coli* likewise lacks zinc ions. Although RNA polymerase and the restriction endonuclease *Eco*RI contain zinc ion, our findings are not consistent with zinc ion serving a unique function in enzymes that use DNA as a substrate. 1,10-Phenanthroline and its metal complexes bind to DNA. Even if the DNA scission reaction of the 1,10-phenanthroline–copper complex is suppressed, this interaction provides another mechanism of inhibition of DNA and RNA polymerase that is not related to the presence of a tightly bound metal ion.

Introduction

The inhibition of DNA and RNA polymerases by 1,10-phenanthroline and the presence of zinc ion in those enzymes available in sufficient amounts for trace metal analyses led to the generalization that zinc ion is an essential component in all these enzymes.[1,2] The potential significance of this correlation has been emphasized by the demonstration in model reactions for prebiotic syntheses of RNA that zinc increased the yield of the 3′–5′ phosphodiester bonds at the expense of 2′–5′ phosphodiesters in the template-directed polymerization of imidazoyl-AMP by poly(U).[3-7] The zinc ion catalyzed phosphorylation of 1,10-phenanthroline-2-carbinol by ATP forming 1,10-phenanthroline-2-carbinol phosphate and ADP indicated the metal ion could play a more direct role in forming the internucleotide bond.[7] In this simple reaction, the metal ion activates the carbinol for nucleophilic attack on the γ-phosphate of the ATP. By analogy, the zinc ion could coordinate the 3′-OH groups of the nascent RNA and DNA chains and enhance their nucleophilicity toward the α-phosphorus of an incoming nucleotide triphosphate.[8-10]

The first suggestion that 1,10-phenanthroline inhibition of polymerases did not reflect a central role of zinc ion was our demonstration that the kinetic effects observed with *Escherichia coli* DNA polymerase I[11] and DNA-dependent RNA polymerase and the RNA-dependent DNA polymerase of avian myeloblastosis virus[12] was due to the 2:1 1,10-phenanthroline–cuprous complex that can be formed from thiol and contaminating copper ion in assay mixtures. Subsequent studies have demonstrated that the inhibition of *E. coli* DNA

(1) Kornberb, A. "Supplement to DNA Replication"; W. H. Freeman: San Francisco, 1982; p 224.
(2) Mildvan, A. S.; Loeb, L. A. *CRC Crit. Rev. Biochem.* **1979**, *6*, 219–244.
(3) Lohrmann, R.; Bridson, P. K.; Orgel, L. E. *Science (Washington, D.C.)* **1980**, *208*, 1464–1465.
(4) Bridson, P. K.; Orgel, L. E. *J. Mol. Biol.* **1980**, *144*, 567–577.
(5) Lohrmann, R.; Orgel, L. E. *J. Mol. Biol.* **1980**, *142*, 555–567.
(6) van Roode, J. H. G.; Orgel, L. E. *J. Mol. Biol.* **1980**, *144*, 579–585.
(7) Inoue, T.; Orgel, L. E. *J. Am. Chem. Soc.* **1981**, *103*, 7666–7667.
(8) Sigman, D. S.; Wahl, G. M.; Creighton, D. J. *Biochemistry* **1972**, *11*, 2236–2242.
(9) Slater, J. P.; Tamir, I.; Loeb, L.; Mildvan, A. S. *J. Biol. Chem.* **1972**, *247*, 6784–6794.
(10) Springgate, C. F.; Mildvan, A. S.; Sbramson, R.; Engle, J. L.; Loeb, L. *J. Biol. Chem.* **1973**, *246*, 5987–5993.
(11) D'Aurora, V.; Stern, A. M. *Biochem. Biophys. Res. Commun.* **1977**, *78*, 170–176.
(12) D'Aurora, V.; Stern, A. M.; Sigman, D. S. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 1025–1032.

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polymerase I is due to the oxygen-dependent scission of the DNA used as the primer and/or template by the coordination complex.[13,14] The 3'-phosphorylated ends produced in the scission reaction are directly responsible for the inhibition of *Pol*I.[14] Presumably, the artificial nuclease activity of this coordination complex is responsible for the copper-dependent inhibition by phenanthroline of the other polymerases. However, these experiments only account for the inhibition of these enzymes when the assays are carried out by incubating the substrates with OP (1,10-phenanthroline) prior to the addition of polymerase to initiate the assay. They neither dispute that zinc ion is present in these enzymes nor refute the suggestion that 1,10-phenanthroline inhibition of *Pol*I observed when the ligand is incubated with the enzyme is due to the removal of a metal ion essential for catalysis.[10,13,15]

In our continuing studies on the role of zinc ion in the active sites of polymerase, we reinvestigated the metal ion content of *E. coli* DNA polymerase I. These experiments were prompted by our failure, contrary to literature reports,[13] to see any inhibition by 1,10-phenanthroline when the nucleic acid scission reaction was suppressed. We report here that stoichiometric levels of metals are not present in *E. coli* DNA polymerase I preparations that exhibited high specificity for polymerization and exonuclease activity. As a further clarification of the complex effects 1,10-phenanthroline can have on polymerases, we show that derivatives of 1,10-phenanthroline have affinity for DNA either as free bases or as metal complexes. These interactions could be responsible for additional mechanisms by which 1,10-phenanthroline can cause inhibition of polymerase activity without binding to a putative metal ion. Finally, we demonstrate that the rec A protein, which is central to recombinational repair of DNA,[16] does not contain zinc ion. These findings indicate that zinc ion is not essential for enzyme-mediated reversible formation of single-stranded regions of DNA catalyzed by enzymes that use nucleic acids as substrates.

While this work was in progress, two other groups had remeasured the zinc content of *Pol*I with similar results.[17,18] A preliminary report of our studies has been presented.[19]

Materials and Methods

Reagents. All chemicals were reagent grade unless otherwise noted. Tris, dATP, dTTP, dGTP, and dCTP were purchased from Sigma. ^{3}H-labeled nucleotides were purchased from ICN. OP and its derivatives were from G. F. Smith. All othr reagents were from Mallinckrodt.

Buffers. Buffers were made metal free by passage through a Chelex-100 column (Bio-Rad). Before use, the Chelex was cycled through a 1 N HCl, water, 1 N NaOH, and water wash as outlined by the manufacturer.

The HCl was distilled before use. The column was used until there was a detectable amount of Zn or Fe in a 20-μL sample and then recycled as described.

Metal Analysis. A Perkin-Elmer Model 603 atomic absorption spectrometer was used for all metal analysis. The machine was equipped with an HGA 5000 graphite furnace and a deuterium arc background corrector. The zinc analyses were performed on samples ranging in volume from 1 to 20 μL. The heating protocol was as follows: dry, 105 °C, 50 s; char, 500 °C, 45 s, 20-s ramp; atomize, 2100 °C, 15 s, 2-s ramp; clean, 2700 °C, 10 s.

The long atomization time was chosen to watch for late peaks. At 2100 °C zinc usually atomizes quite rapidly. However, we found that phosphate or MOPS (3-(*N*-morpholino)propanesulfonic acid) buffers could cause a delay of the zinc signal to as late as 9 s into the atomization cycle.

Iron analysis was performed under the following conditions: dry, 105 °C, 40 s, 20-s ramp; char, 900 °C, 40 s, 20-s ramp; atomize, 2700 °C, 11 s, maximum power.

Metal analysis was usually performed on small samples that had been transferred to polypropylene micro centrifuge tubes for transport to the instrument. This procedure invariably resulted in minor contamination of the sample by zinc, despite washing of tubes in EDTA containing dilute acid. In fact, washing tubes in anything except doubly distilled water or acid resulted in increased contamination of buffer as compared to stock. In order to bypass this problem, all final analyses of dialysis experiments were performed on material removed directly from dialysis bags.

Polymerase Purification. The polymerase was purified from a λ-lysogen containing the polA gene. The purification was essentially that of ref 20 with the addition of a DNA-Sepharose column[21] as the final step, which was necessary to obtain homogeneous enzyme. The purification yielded both holoenzyme and the large fragment of "Klenow" fragment. Protein concentration was determined routinely by the dye binding assay of Bradford with calibration by comparison against a sample of known concentration based on absorption at 280 nm.[22]

Polymerase Assay. The polymerase assays were routinely done with use of poly(dA)·oligo(dT) as template primer in 50 mM Tris–acetate, pH 7.4, 5 mM Mg^{2+}, 50 μM [^{3}H]dTTP, 10 μg/mL pdA, and 1 μg/mL dT_{10}. One hundred micromolar of EDTA was included for some assays. Activity was measured by following incorporation of counts into acid-insoluble material.[13]

Sonication and Purification of DNA for Viscometry and Equilibrium Dialysis. Salmon testes DNA (Sigma Chemical Co.) was dissolved to a final concentration of 2 mg/mL at pH 7.0 in 2 mM Hepes buffer containing 9.4 mM NaCl. A Branson W-350 sonic power sonifier equipped with a microtip extension was used at 115-W power to sonicate the DNA into fragments with a molecular weight on the order of 5×10^6.[23] The DNA was sonicated in 50-mL aliquots at 0–4 °C by using 20 pulses of 30-s duration with a 1-min cooling time between each pulse. The DNA solution was then purified according to the method of Marmur.[24]

Viscosity and Equilibrium Dialysis Measurements. Viscosity measurements were perfomed essentially by the method of Wakelin et al.[25] The capillary viscometer was maintained at 23 ± 0.03 °C. The buffer solution had a flow time of 116.3 s, and the DNA solution had an average flow time of 140.85 s with a standard deviation of 0.73 s.

For equilibrium dialysis experiments, 5 mL of salmon testes DNA was dialyzed at room temperature for 48 h vs. 1 L of dialysate. Solutions of $(2,9\text{-}Me_2OP)_2Cu^+$ were prepared by diluting stock solutions of the ligand and cupric ion into a 14 mM solution of mercaptopropionic acid that had been adjusted to pH 7.2.

Results and Discussion

1,10-Phenanthroline inhibits metalloenzymes by two mechanisms. In the first, it binds reversibly to available coordination sites of the tightly bound metal ions and blocks access of substrates to catalytically important metal ions. This mechanism is operative with horse liver alcohol dehydrogenase.[26,27] The most common mode of inhibition is the removal of the metal ion upon incubation with enzyme. This reaction usually has a half-life of greater than 5 min with millimolar OP. Enzymes inhibited by this mechanism include carbonic anhydrase, carboxypeptidase, and alkaline phos-

(13) Sigman, D. S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. *J. Biol. Chem.* **1979**, *254*, 12269–12272.
(14) Pope, L. E.; Reich, K. A.; Graham, D. R.; Sigman, D. S. *J. Biol. Chem.* **1982**, *257*, 12121–12128.
(15) Slater, J. P.; Mildvan, A. S.; Loeb, L. A. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 37–43.
(16) McEntee, K.; Weinstock, G. *Enzymes, 3rd Ed.* **1982**, *15*, 445–470.
(17) Walton, K. E.; Fitzgerald, P. C.; Hermann, M. S.; Behnke, W. D. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1353–1361.
(18) Ferrin, L. J.; Mildvan, A. S.; Loeb, L. A. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 723–728.
(19) Graham, D. R.; Sigman, D. S. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1983**, 42, 2147.
(20) Kelley, W. S.; Stumpf, K. H. *J. Biol. Chem.* **1979**, *254*, 3206–3210.
(21) Arndt-Jovin, D. J.; Jopvin, T. M.; Bahr, W.; Frischauf, A.; Marguadt, M. *Eur. J. Biochem.* **1975**, *54*, 411–418.
(22) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.
(23) Wakelin, L. P. G.; Wring, M. J. *Biochem. J.* **1976**, *157*, 721–740.
(24) Marmur, J. *J. Mol. Biol.* **1961**, *3*, 208–218.
(25) Wakelin, L. P. G.; Glanbiger, D.; Lannellakis, E. S.; Waring, M. J. *Biochemistry* **1978**, *17*, 5057–5063.
(26) Yonetani, T. *Biochem. Z.* **1963**, *338*, 300–316.
(27) Sigman, D. S. *J. Biol. Chem.* **1967**, *242*, 3815–3824.

Table I. Ligands That Fail To Inhibit *E. coli* DNA Polymerase I[a]

chelator	concn, mM	chelator	concn, mM
EDTA	1	pyridine dicarboxylic acid	5
bipyridine	1		
OP	10	diethyldithiocarbamate	2

[a] 10 μM *Pol*I was incubated in 50 mM Tris-acetate, pH 7.4, at 4 °C for 3 weeks. None of the sample lost activity relative to the control. (The control was at least 95% active after 3 weeks.)

Table II. Zinc Content of *E. coli* DNA Polymerase I[b]

protein	mol of Zn^{2+}/ mol of enzyme	mol of Fe^{2+}/ mol of enzyme	activity, %	3′–5′ exonuclease activity, %
polymerase I	2.2		100	
	1.0	<0.1	100	100
	0.2		102	100
polymerase I[c]	0.01	<0.1	90	
Klenow fragment	0.08		105	100
bovine carbonic anhydrase	0.94			
E coli rec A protein	0.01		>80[a]	

[a] rec A was assayed by measuring ATPase activity in the presence of single-stranded DNA.[48] [b] Dialysis conditions unless otherwise noted: Chelex treated, 50 mM Tris-HCl, pH 7.4. [c] Dialysis conditions as in footnote *b* except 10 mM EDTA was also added.

phatase.[28] Generally, 1,10-phenanthroline is more efficient than EDTA in removing tightly bound zinc ion from enzymes. 2,6-Dicarboxypicolinic acid has proved more effective than OP in removing metal in certain cases.[29]

During the course of investigating the thiol and copper dependent inhibition of *E. coli Pol*I by OP, we incubated the enzyme, which had been stored in 0.1 M EDTA, with 1,10-phenanthroline for 3 weeks at 4 °C. Contrary to published reports,[10,15] no progressive loss of polymerization activity was observed when aliquots were removed and assayed in the absence of thiol (Table I). Since the same reports have indicated that a rapid zinc exchange could be observed in the presence of Sephadex, the resin was added to the reaction mixture in order to determine if the zinc ion could be labilized and the enzyme made more susceptible to inhibition by 1,10-phenanthroline. This procedure failed to make the enzyme susceptible to OP inhibition. The inability of the other ligands indicated in Table I to cause inhibition prompted us to reinvestigate the metal ion content of *Pol*I.

*Pol*I and the "Klenow" fragment were purified to greater than 95% purity as analyzed by SDS gel electrophoresis.[30] After dialysis against Chelex-treated 50 mM tris-HCl buffer (pH 7.0), metal analysis were carried out with a Perkin-Elmer 603 atomic absorption spectrophotometer equipped with an HGA 2000 graphite furnace, a ramp generator, and a deuterium-arc background corrector. Although analysis of bovine carbonic anhydrase indicated the expected 0.94 mol of Zn^{2+}/mol of enzyme,[31] the metal ion content of both forms of *Pol*I contained contained only 0.08–0.20 mol of zinc ion/mol of enzyme (Table II). The specific activity of the polymerization and 3′–5′ exonuclease activities for the enzyme preparations analyzed for zinc ion corresponded to the previously reported values (Table II).[32,33] In contrast to published reports,[10,15] these results and those reported in Table I indicate that the zinc ion is not functionally important in *Pol*I. In order to test the possibility that the polymerase was picking up zinc from the assay mixture, some assays were performed in buffer containing 100 μM EDTA. Under these conditions the polymerase was at least 90% active, indicating that the explanation for full activity in the zinc-deficient enzyme is not the binding of adventitious zinc.

Table III. Properties of Nonfunctional High Affinity Zinc Site on *Pol*I[b]

free Zn^{2+} concn, μM	*Pol*I concn, μM	mol of Zn^{2+}/ mol of enzyme	free Zn^{2+} concn, μM	*Pol*I concn, μM	mol of Zn^{2+}/ mol of enzyme
"0"[a]	0.5	0.01	0.2	0.5	0.8
0.008	0.8	0.15	2.8	0.5	1.9

[a] 10 μM EDTA present. [b] From the data presented here, it appears that there are two zinc binding sites on *Pol*I. If one assumes that the two sites have roughly equal affinities, then a K_D of 50 nm can be derived. Because of the assumptions, this number merely demonstrates just how tightly an adventitious zinc ion can bind to a protein. (The standard curve for Zn^{2+} was linear and easily could be used to determine Zn^{2+} concentrations down to 1 nm.)

Table IV. Viscosity of DNA in the Presence of Phenanthroline

soln	flow time, s	helix extension, %
buffer	116.3	
1 mg/mL DNA	140.9	
1 mg/mL DNA + 66 μM OP	143.9	3.8
1 mg/mL DNA + 66 μM OP + 33 μM Cu^{2+}	142.9	2.8
1 mg/mL DNA + 66 μM 2,9-Me_2OP	143.3	3.1
1 mg/mL DNA + 66 μM 2,9-Me_2OP + 33 μM Cu^{+}	140.9	0

The data in Table III do indicate, however, that there is a binding site for zinc ion with an apparent dissociation constant of approximately 50 nm. Dialysis in the buffer containing $MgCl_2$ was carried out to determine if the biological function of the site might be to coordinate Mg^{2+}. Since the magnesium chloride had no reproducible effect on the zinc ion content of the protein, this hypothesis has no support.

The initial report of zinc ion in *Pol*I also included analytical data which suggested that 1 mol of iron was present in the protein although it was suggested that this metal ion was likely a contaminant.[10] In agreement with this conclusion, we have not found iron in our preparations of *Pol*I.

Interaction of Phenanthroline and Its Coordination Complexes with DNA. 1,10-Phenanthroline derivatives can cause inhibition of polymerase activity even when the nuclease activity of the copper complexes is absent. For example, 2,9-dimethyl-1,10-phenanthroline and its derivatives have been reported to inhibit yeast RNA polymerase I at concentrations of 2 mM[34] even though substitution ortho to the nitrogen destroys the nuclease activity of the copper complexes. We propose that a possible mechanism of this inhibition is the binding of the free base or its metal complexes to DNA. Other workers have demonstrated that zinc, platinum and ruthenium complexes of phenanthrolines bind tightly and stereospecifically to DNA.[35,36] We have used three methods to demonstrate

(28) Vallee, B. L.; Wacker, W. E. C. "The Proteins"; Neurath, H., Ed.; Academic Press: New York, 1970; Vol. V.
(29) Hirose, J.; Kidani, Y. *Chem. Pharm. Bull.* **1978**, *26*, 1768–1775.
(30) Laemmli, U. K. *Nature (London)* **1970**, *227*, 680–685.
(31) Lindskog, S.; Henderson, L.; Kannan, K.; Liljas, A.; Nyman, P. O.; Strandberg, A. *Enzymes, 3rd Ed.* **1971**, *5*, 587–629.
(32) Jovin, T. N.; Englund, P. T.; Bertsch, L. L. *J. Biol. Chem.* **1969**, *244*, 2996–3008.
(33) Brutlag, D.; Kornberg, A. *J. Biol. Chem.* **1972**, *247*, 241–248.
(34) Auld, D.; Atsuya, I.; Campino, C.; Valenzuela, P. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 548–554.

Table V. Equilibrium Dialysis of 1,10-Phenanthroline–Copper Complexes with DNA[a]

[total complex], μm	[inside], μm	[outside], μm
2,9-Me_2OP–Cuprous Complex		
25	183	17
37.5	360	28
50	558	38
OP–Cupric Complex		
50	1800	45

[a] Conditions: pH 7.2 Tris-acetate buffer; 4 °C; 1 mg/mL of salmon sperm DNA.

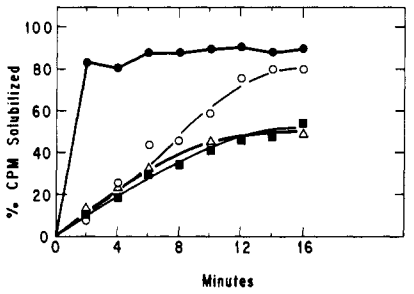

Figure 1. Inhibition of poly(dA-T) cleavage by intercalating agents (25 mM Hepes buffer (pH 7.2), 20 μM OP, 2 μM Cu^{2+}, 7 mM mercaptopropionic acid, 10 mM H_2O_2): (●) control; (○) 50 μM 2:1 2,9-dimethyl-1,10-phenanthroline–cuprous ion; (■) 12 μM ethidium bromide; (△) 5 μM Hoechst dye 33258.

the binding of 2,9-Me_2OP, OP, and their copper complexes to DNA. Viscometry data (Table IV) demonstrate that the free ligands OP and 2,9-Me_2OP bind to 1 mg/mL of salmon testes DNA at concentrations as low as 30 μm. Although both OP and 2,9-Me_2OP are equally effective in extending the helical length of the DNA, they increase the DNA length only 60% as efficiently as ethidium bromide and proflavine at a drug to base ratio of 0.06. The effect of the square-planar cupric complex of OP is about two-thirds that of the OP when the ligand serves as the concentration basis of the complex. The tetrahedral 2,9-dimethyl-1,10-phenanthroline–cuprous complex did not perturb the viscosity of the DNA. However, equilibrium dialysis demonstrated the binding of the phenanthrolines and their complexes to DNA. The data summarized in Table V indicate that both the tetrahedral Me_2-OP–cuprous complex and the square-planar OP–cupric complex bind at multiple sites. The free bases also bind to a significant extent. A more quantitative comparison is not possible since the absorption spectra of the DNA-bound metal-free phenanthrolines are significantly modified and accurate extinction coefficients could not be obtained.

An alternate measure of DNA binding is provided by the artificial nuclease activity of the 1,10-phenanthroline cuprous complex and is particularly useful in examining the interaction with poly(dA-T) which is commonly used in in vitro polymerase assays. The data in Figure 1 comparing the inhibition of the scission reaction by ethidium bromide, Hoechst dye 33258, and 2,9-dimethyl-1,10-phenanthroline–cuprous complex demonstrate that this coordination complex protects the poly(dA-T) almost as effectively as these two well-known intercalating agents.

Implications for Zinc Ion in RNA Polymerase. Zinc ion has been demonstrated in *E. coli* DNA-dependent RNA polymerase, T-7 RNA polymerase, RNA polymerase II from *Euglena gracilla*, and yeast RNA polymerase II.[37-41] Since these enzymes are not progressively inhibited upon incubation with OP, no data are available that correlate catalytic activity with zinc content. However, the possible importance of zinc ion in *E. coli* RNA polymerase is strongly supported by the demonstration that *E. coli* grown in a medium deficient in zinc ion but supplemented with cobalt incorporate the latter metal in the identical stoichiometry at the zinc ion.[42] The cobalt enzyme has catalytic properties similar to those of the zinc protein. Intriguing differences include its altered efficiency in transcribing certain T-7 promoters and a modified sensitivity to the catabolite activating protein in the presence of ATP in an in vitro transcription system.

On the basis of the data presented above, a central role for zinc ion in catalyzing phosphodiester bond formation is unlikely. However, two processes that are intrinsic to RNA polymerase but have no counterpart in *Pol*I are chain initiation and the reversible annealing of DNA base pairs. Since rec A protein catalyzes the latter process, it was examined for zinc ion content. The failure to find any zinc ion in rec A preparations that exhibited full ATPase activity (Table II) implies the metal ion is not absolutely essential for formation of single-stranded DNA even though zinc ion has been demonstrated to affect the melting point of DNA[43] and is required for the single-stranded nuclease activity of S-1.[44]

In summary, zinc ion is not an essential component in enzymes that use DNA as substrate. Although there is substantial support for the presence of metal in RNA polymerases and at least one restriction endonuclease,[45] no zinc is presented in *E. coli Pol*I. Inferring the presence of a metal ion in polymerases on the basis of inhibition studies with 1,10-phenanthroline requires the exclusion of several alternative explanations. In addition to problems of interpretation which arise for all enzymes such as the high affinity of OP for some enzyme active sites[46] and its participation in the copper-dependent oxidation of essential sulfhydryls,[47] possible mechanisms of inhibition unique to polymerases include the efficient nuclease activity of the cuprous complex and the DNA binding properties of 1,10-phenanthroline and its coordination complexes. Important positive evidence for the presence of metal ion would be the correlation of enzymatic activity with metal content or the direct demonstration of the reversible interaction of the ligands with the protein-bound metal ion.

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Registry No. *Pol*I, 9012-90-2; Zn, 7440-66-6; ethidium bromide, 1239-45-8; Hoechst dye 33258, 23491-45-4.

(35) (a) Barton, J. K.; Dannenberg, J. J.; Raphael, A. L. *J. Am. Chem. Soc.* **1982**, *104*, 4967–4969. (b) Barton, J. K. *Biochemistry* **1983**, *22* (15), 6A (Abstr. 7).
(36) Lippard, S. J.; Bond, P. J.; Wu, K. C.; Bauer, W. R. *Science (Washington, D.C.)* **1976**, *194*, 726–728.
(37) Speckhard, D. C.; Wu, F. Y.-H; Wu, C.-W. *Biochemistry* **1977**, *16*, 5228–5233.
(38) Falchuk, V. H.; Mazns, R.; Ulpino, L.; Vallee, B. L. *Biochemistry* **1976**, *15*, 4468–4475.
(39) Coleman, J. E. *Biochem. Biophys. Res. Commun.* **1974**, *60*, 641–648.
(40) Scrutton, M. C.; Wu, C. W.; Goldthwait, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 2497–2501.
(41) Wandizlak, T.; Benson, R. W. *Biochemistry* **1978**, *17*, 426–431.
(42) Wu, C.-W.; Wu, Y.-H.; Speckhard *Biochemistry* **1977**, *16*, 5449–5453.
(43) Shin, Y. A.; Eichhorn, G. L. *Biochemistry* **1968**, *7*, 1026–1032.
(44) Vogt, V. M. *Eur. J. Biochem.* **1973**, *33*, 192–200.
(45) Barton, J. K.; Basile, L. A.; Paranawithana, *J. Biol. Chem.* **1982**, *257*, 7911–7914.
(46) Anderson, B. M.; Reynolds, M. L.; Anderson, C. D. *Biochim. Biophys. Acta* **1966**, *113*, 235–253.
(47) Kobashi, K.; Horecker, B. L. *Arch. Biochem. Biophys.* **1967**, *121*, 178–186.
(48) Weinstock, G. M.; McEntee, K.; Lehman, I. R. *J. Biol. Chem.* **1981**, *256*, 8829–8834.