Degradation of Deoxyribonucleic Acid by a 1,10-Phenanthroline–Copper Complex: The Role of Hydroxyl Radicals†

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ABSTRACT: Degradation of deoxyribonucleic acid (DNA) by 1,10-phenanthroline has been shown to require Cu(II), a reducing agent, and O_2. Other metal ions do not substitute for Cu(II), and degradation of DNA is inhibited by metal ions that can form stable complexes with 1,10-phenanthroline, such as Co(II), Cd(II), Ni(II), or Zn(II), as well as by chelators that can bind copper, such as triethyltetraamine, neocuproine, or ethylenediaminetetraacetic acid (EDTA). Neocuproine, a specific copper chelator, is more effective than EDTA in inhibiting the breakdown of DNA. The degradation of DNA shows a requirement for a reducing agent which can be satisfied by either ascorbate or a thiol. A free radical generating system, e.g., xanthine oxidase–hypoxanthine, can substitute for the reducing agent. DNA degradation, in the presence of either an organic reducing agent or xanthine oxidase–hypoxanthine, is inhibited by hydroxyl radical scavengers and by catalase, suggesting that hydroxyl radical is the reactive species in DNA degradation and that hydrogen peroxide is an intermediate in hydroxyl radical generation.

Virtually all nucleotidyl transferases (DNA[1] polymerase, RNA polymerase, reverse transcriptase, and terminal transferase) are reported to be inhibited by the metal chelator 1,10-phenanthroline (Chang & Bollum, 1970; Scrutton et al., 1971; Slater et al., 1972; Auld et al., 1974; Poiesz et al., 1974). This inhibition has been generally assumed to be the result of either the removal of zinc from the enzyme or the formation of a complex between the metal chelator and enzyme-bound zinc, since most nucleotidyl transferases have been found to contain zinc, and it has been demonstrated that zinc plays a functional role in nucleotide polymerization (Springgate et al., 1973; Mildvan, 1974).

We have recently demonstrated that 1,10-phenanthroline, in the presence of a reducing agent, a copper salt, and molecular oxygen, degrades double-stranded DNA to acid-soluble fragments (Downey et al., 1980). We have proposed that degradation of DNA is the mechanism responsible for the in vitro inhibition of DNA and RNA synthesis seen with this metal chelator. This is based on the observation that, under conditions usually employed to assay DNA and RNA polymerase activities, extensive degradation of DNA occurs. Recently, Sigman and his colleagues (Sigman et al., 1979) have also reported the oxygen-dependent cleavage of DNA by a 1,10-phenanthroline–Cu(I) (2:1) complex and postulated that the observed inhibition of DNA synthesis is the result of inhibition of DNA polymerase activity by the DNA cleavage products.

Since DNA degradation by a 1,10-phenanthroline–Cu(II) complex requires both a reducing agent and molecular oxygen, we have proposed that a reduced product of oxygen is responsible for the breakdown of DNA (Downey et al., 1980). In this paper we will present evidence that hydroxyl radical is the reactive species in the degradation of DNA.

Materials and Methods

[^{3}H]TTP was purchased from ICN Pharmaceuticals. 1,10-Phenanthroline and 2,9-dimethyl-1,10-phenanthroline (neocuproine) were obtained from K & K Chemicals. Unlabeled nucleotides and the "large fragment" of *Escherichia coli* DNA polymerase I were from Boehringer Corp. Chelex-100 was purchased from Bio-Rad Laboratories. Triethyltetraamine, EDTA, EGTA, 2,2′-bipyridine, 8-hydroxyquinoline, 2-mercaptoethanol, and 3-mercaptopropionic acid were purchased from Eastman Chemicals; L-cysteine, NADPH, glutathione, imidazole, xanthine oxidase (xanthine:oxygen oxidoreductase, EC 1.2.3.2), superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1), and catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) were from Sigma Chemical Co.; L-ascorbic acid, $ZnCl_2$, $CuSO_4$, $FeCl_3$, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, $CdCl_2$, $CoCl_2$, $NiCl_2$, and $CrCl_3$ were from Fisher Chemical Co.; $MnCl_2$, $BaCl_2$, sodium benzoate, sodium acetate, sodium chloride, and ammonium formate were from Mallinkrodt.

Distilled water and all solutions other than metal salts were Chelex-treated as previously described (Que et al., 1979). [^{3}H]Poly(dA-dT) was synthesized with the "large fragment" of DNA polymerase I by using 33 μM dATP and [^{3}H]TTP, 130 cpm/pmol, as described by Modrich & Lehman (1970).

Degradation of [^{3}H]poly(dA-dT) was followed by acid solubilization. The reaction mixture contained in a final volume of 0.1 mL 50 mM Hepes buffer, pH 7.4, 20 μM [^{3}H]poly(dA-dT), 1 mM 2-mercaptoethanol, 50 μM 1,10-phenanthroline, and 1.0 μM $CuSO_4$. After 30 min at 24 °C, the reaction was stopped by the addition of 0.01 mL of 1 M EDTA, pH 8.0, 0.1 mL of salmon sperm DNA (2 mg/mL), and 0.5 mL of 1 M perchloric acid. After 10 min at 0 °C the solution was centrifuged at 6700*g* for 10 min, and an aliquot of the supernatant was counted in 10 mL of Biofluor in a liquid scintillation spectrometer.

Results

Requirements for the Degradation of DNA by 1,10-

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[1] Abbreviations used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; NADPH, reduced nicotinamide adenine dinucleotide phosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid.

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Table I: Requirements for the Degradation of DNA[a]

components	% acid soluble
complete	53
minus Cu(II)	3
minus 2-mercaptoethanol	<1
minus 1,10-phenanthroline	<1
minus oxygen	2
minus Cu(II) and 1,10-phenanthroline	<1
minus Cu(II) and 2-mercaptoethanol	<1
minus 2-mercaptoethanol and 1,10-phenanthroline	<1

[a] The complete reaction mixture contained in a final volume of 0.1 mL 50 mM Hepes, pH 7.4, 18 μM [^{3}H]poly(dA-dT) (65 cpm/pmole), 1 mM 2-mercaptoethanol, 1 μM $CuSO_4$, and 50 μM 1,10-phenanthroline. After 30 min at 24 °C, acid-soluble radioactivity was determined as described under Materials and Methods. The minus oxygen experiment was carried out by bubbling N_2 through the reaction mixture.

Table II: Reducing Agent Specificity for DNA Degradation[a]

reducing agent	% acid soluble
none	<1
2-mercaptoethanol	86
3-mercaptopropionic acid	100
glutathione	75
L-ascorbate	100
NADPH	2

[a] DNA degradation was assayed as described under Materials and Methods except for the addition of the indicated reducing agents at 1 mM.

Table III: Effect of Metal Ions on the Degradation of DNA[a]

metal ion	% acid soluble	
	without Cu(II)	with Cu(II)
none	<1	94
Cd(II)	<1	<1
Co(II)	<1	4
Ni(II)	2	2
Zn(II)	<1	<1
Mn(II)	2	45
Mg(II)	2	81
Fe(II)	2	79
Fe(III)	3	74
Ba(II)	2	85
Be(II)	<1	100
Cr(III)	2	88

[a] DNA degradation was assayed as described under Materials and Methods except for the addition of different metal ions at 10 μM. $CuSO_4$ was present at 1.0 μM where indicated.

Phenanthroline. The degradation of [^{3}H]poly(dA-dT) by 1,10-phenanthroline is shown in Table I, where DNA degradation was followed by the conversion of [^{3}H]poly(dA-dT) to acid-soluble products. Degradation of DNA required, in addition to 1,10-phenanthroline, Cu(II), a reducing agent, and O_2. Omission of any of these components resulted in a very little or no degradation of DNA.

Sulfhydryl compounds such as 2-mercaptoethanol, glutathione, and 3-mercaptopropionic acid, as well as nonthiol reducing agents such as ascorbate induced extensive degradation of DNA in the presence of 1,10-phenanthroline and Cu(II) (Table II). No degradation was observed with NADPH. None of the reducing agents alone caused significant DNA degradation.

Effect of Metal Ions on the Degradation of DNA by 1,10-Phenanthroline. Cu(II) was the only metal found to be effective in causing degradation of DNA (Table III). However, metal ions which are able to form stable complexes with 1,10-phenanthroline, i.e., cobalt, nickel, cadmium, or zinc, markedly inhibited the degradation of DNA by 1,10-phenanthroline–Cu(II) when present in a 10-fold molar excess over Cu(II), possibly by preventing the binding of Cu(II) to 1,10-phenanthroline. Iron(II), which is required for degradation of DNA by bleomycin (Sausville et al., 1978a,b), had no effect with 1,10-phenanthroline. Metal ions alone at this concentration had no detectable effect on the breakdown of DNA.

Table IV: Effect of Metal Chelators on the Degradation of DNA[a]

metal chelator	% acid soluble	
	without 1,10-phenanthroline	with 1,10-phenanthroline
none	<1	83
neocuproine	<1	3
triethyltetraamine	<1	<1
EDTA	<1	11
EGTA	<1	68
2,2'-bipyridine	<1	83
8-hydroxyquinoline	<1	76
L-cysteine	<1	75
imidazole	<1	83

[a] DNA degradation was assayed as described under Materials and Methods except for the addition of metal chelators at 50 μM. 1,10-Phenanthroline was present at 50 μM where indicated.

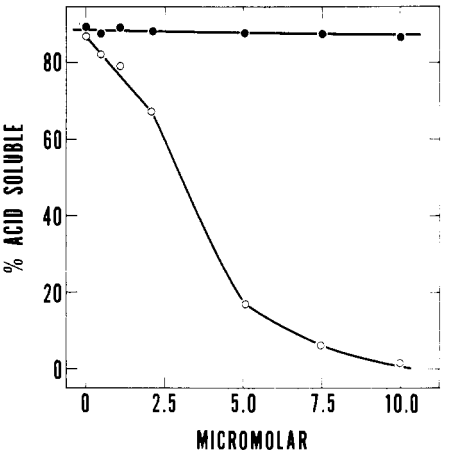

FIGURE 1: Effect of neocuproine (○) and EDTA (●) on DNA degradation by a 1,10-phenanthroline–Cu(II) complex. DNA degradation was assayed as described under Materials and Methods.

Effect of Other Metal Chelators on the Degradation of DNA by 1,10-Phenanthroline. 1,10-Phenanthroline was the only chelating agent found to be effective in inducing degradation of DNA in the presence of Cu(II) and a reducing agent (Table IV). Other chelating agents at equimolar concentrations, such as triethyltetraamine, EDTA, and neocuproine, a 1,10-phenanthroline analogue, inhibited degradation of DNA by preventing the formation of a 1,10-phenanthroline–Cu(II) complex. Neocuproine, which has a high affinity for copper, was considerably more effective in inhibiting the degradation of DNA than was EDTA, a nonspecific metal chelator (Figure 1). The degradation of DNA was inhibited >95% at 10 μM neocuproine, whereas a 5-fold higher concentration of EDTA was required to inhibit the reaction to the same extent. These results suggest that Cu(II) is the specific metal ion required for DNA degradation by 1,10-phenanthroline.

Effects of Superoxide Dismutase and Catalase on the Degradation of DNA. Degradation of DNA by 1,10-phenanthroline was also studied in the presence of a free radical generating system, i.e., xanthine oxidase–hypoxanthine, in which the reduced products of oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals are produced (Fong

Table V: Degradation of DNA by 1,10-Phenanthroline in the Presence of Xanthine Oxidase and Hypoxanthine[a]

conditions	acid-soluble radioactivity (cpm)	
	minus Cu(II)	plus Cu(II)
complete	28 000	59 150
minus 1,10-phenanthroline	900	2 100
minus hypoxanthine	800	800
minus 1,10-phenanthroline and hypoxanthine	800	600
plus superoxide dismutase (20 μg/mL)	1 830	3 900
plus catalase (10 μg/mL)	1 540	2 900
plus boiled catalase (10 μg/mL)	28 200	65 000
plus neocuproine (10 μM)	1 500	3 000

[a] The complete reaction mixture contained in a final volume of 0.1 mL 50 μM 1,10-phenanthroline, 50 mM Hepes, pH 7.4, 20 μM [^{3}H]poly(dA-dT), 50 μg of xanthine oxidase, and 0.1 mM hypoxanthine. $CuSO_4$ was present at 2.5 μM where indicated. Acid-soluble radioactivity was determined after 30 min at 24 °C as described under Materials and Methods.

Table VI: Degradation of DNA by 1,10-Phenanthroline–Cu(II) Complex in the Presence of either 2-Mercaptoethanol or Ascorbate[a]

conditions	acid-soluble radioactivity (cpm)	
	2-mercapto-ethanol	ascorbate
complete	96 000	95 000
plus superoxide dismutase (40 μg/mL)	91 000	90 000
plus catalase (10 μg/mL)	9 500	31 000
plus boiled catalase (10 μg/mL)	91 800	92 850

[a] DNA degradation was assayed as described under Materials and Methods.

et al., 1976). As shown in Table V, degradation of DNA in the presence of xanthine oxidase and hypoxanthine showed an absolute requirement for 1,10-phenanthroline and was enhanced by the addition of Cu(II). The significant degradation of DNA observed in the absence of added Cu(II) is probably due to the contamination of xanthine oxidase or other reagents with trace amounts of Cu(II), since the reaction is markedly inhibited by neocuproine. An organic reducing agent was not required for DNA degradation in the presence of xanthine and hypoxanthine.

Degradation of DNA in this system was strongly inhibited by either superoxide dismutase or catalase, suggesting that $O_2^{-}\cdot$ and H_2O_2, respectively, are required for the breakdown of DNA. These results further suggest that $O_2^{-}\cdot$ is not the primary reactive species; otherwise, the breakdown of DNA should have been inhibited by superoxide dismutase but not by catalase.

The effects of superoxide dismutase and catalase on the degradation of DNA by a 1,10-phenanthroline–Cu(II) complex in the presence of an organic reducing agent, either ascorbate or 2-mercaptoethanol, were also investigated (Table VI). In contrast to the inhibition of DNA degradation in the xanthine oxidase–hypoxanthine system, superoxide dismutase had no effect on the degradation of DNA in the presence of either 2-mercaptoethanol or ascorbate. However, catalase was effective in inhibiting the degradation of DNA in either system, again suggesting that H_2O_2 is required for the degradation of DNA.

Effect of Hydroxyl Radical Scavengers. The requirement for H_2O_2 generation for DNA degradation suggests the possibility that hydroxyl radical, a very potent oxidizing agent, may be the primary reactive species in the degradation of DNA. Also consistent with this suggestion is the requirement for 1,10-phenanthroline–Cu(II) for DNA degradation, since chelated transition metals have been shown to reduce H_2O_2 to hydroxyl radical (Fong et al., 1976). The effects of hydroxyl radical scavengers (Anbar & Neta 1967), i.e., formate, benzoate, and acetate, on the degradation of DNA in both the xanthine oxidase–hypoxanthine system and the thiol system are shown in Table VII. The degradation of DNA is markedly inhibited by these scavengers whereas an equivalent concentration of NaCl has little or no effect on DNA degradation. These results, together with the results of the experiments with catalase and superoxide dismutase, suggest that the proximate cause of DNA degradation is hydroxyl radical.

Table VII: Effect of Hydroxyl Radical Scavengers[a]

scavenger	concn (mM)	% inhibition	
		2-mercapto-ethanol	xanthine oxidase–hypo-xanthine
acetate	50	17	35
	100	47	50
benzoate	50	17	44
	100	54	88
formate	50	38	38
	100	67	43
NaCl	50	0	0
	100	7	0

[a] DNA degradation was assayed as described under Materials and Methods. For the 2-mercaptoethanol system, the reaction mixture was as described in Table I. For the xanthine oxidase–hypoxanthine system, the reaction mixture was as described in Table V. In each control reaction ~50% of the [^{3}H]poly(dA-dT) was rendered acid soluble.

Discussion

We have recently demonstrated that the metal chelator 1,10-phenanthroline, in the presence of a reducing agent, a copper salt, and O_2, causes the degradation of double-stranded DNA to acid-soluble fragments (Downey et al., 1980). The requirements for a reducing agent and oxygen for the degradation of DNA led us to propose that a reduced form of O_2, either superoxide anion or hydroxyl radical, is the reactive species. The present studies extend these observations and provide evidence that hydroxyl radical is the reactive species in the degradation of DNA.

The evidence obtained in the present studies suggests that the degradation of DNA is catalyzed by a phenanthroline–copper complex. Reagents which chelate copper, such as neocuproine, triethyltetraamine, or EDTA, as well as metal ions which can form a stable complex with 1,10-phenanthroline, such as Cd(II), Ni(II), Co(II), or Zn(II), inhibit the degradation of DNA by preventing the formation of a phenanthroline–Cu(II) complex.

Degradation of DNA is dependent on the presence of a reducing agent. This requirement can be satisfied by ascorbate or by thiols such as 2-mercaptoethanol, glutathione, or 3-mercaptopropionic acid. A free radical generating system, e.g., hypoxanthine–xanthine oxidase, can substitute for a reducing agent; however, even in the presence of a free radical generating system, both 1,10-phenanthroline and Cu(II) are required for the degradation of DNA.

The inhibition of DNA degradation by hydroxyl radical scavengers, either in the presence of xanthine oxidase and hypoxanthine or in the presence of an organic reducing agent,

suggests that hydroxyl radicals are the reactive species responsible for the breakdown of DNA. In the presence of a free radical generating system, the degradation of DNA is inhibited by either superoxide dismutase or catalase, suggesting that both superoxide anion and hydrogen peroxide are intermediates in the generation of hydroxyl radical. These data also indicate that superoxide anion itself is not the reactive species; otherwise, the degradation of DNA would have been inhibited by superoxide dismutase but not by catalase. Although hydroxyl radicals have been postulated to be the product of the direct interaction of superoxide anion and hydrogen peroxide, the Haber–Weiss reaction (Haber & Weiss, 1934), this is apparently a very slow reaction (Halliwell, 1976; McClune & Fee, 1976; Van Hemmen & Meuling, 1977); thus, some other pathway requiring H_2O_2 and $O_2^-\cdot$ may be responsible for the production of hydroxyl radicals. It is known that chelated iron can catalyze a reaction between $O_2^-\cdot$ and H_2O_2, thereby forming hydroxyl radicals:

$$O_2^-\cdot + Fe(III) \rightarrow O_2 + Fe(II) \quad (1)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + \cdot OH + OH^- \quad (2)$$

The above reactions have been shown to occur in the xanthine oxidase–hypoxanthine system (Fong et al., 1976). Furthermore, copper ion has been proposed to catalyze a similar reaction (Walling, 1975; Van Hemmen & Meuling, 1977). Thus, the following pathway is proposed for the generation of hydroxyl radicals by a 1,10-phenanthroline–Cu(II) complex [OP–Cu(II)] in the presence of xanthine oxidase and hypoxanthine.

$$O_2 + e^- \xrightarrow{\text{xanthine oxidase}} O_2^-\cdot \quad (3)$$

$$2O_2^-\cdot + 2H^+ \rightarrow H_2O_2 + O_2 \quad (4)$$

$$O_2^-\cdot + OP\text{–}Cu(II) \rightarrow OP\text{–}Cu(I) + O_2 \quad (5)$$

$$OP\text{–}Cu(I) + H_2O_2 \rightarrow OP\text{–}Cu(II) + \cdot OH + OH^- \quad (6)$$

$$\cdot OH + DNA \rightarrow \text{strand scission} \quad (7)$$

In this scheme, hydroxyl radical is generated by the interaction of H_2O_2 with OP–Cu(I) (reaction 6). Superoxide anion, which is required for the production of both H_2O_2 and OP–Cu(I), is produced by the reduction of O_2 by xanthine oxidase via the oxidation of hypoxanthine to xanthine (reaction 3). The superoxide anion thus generated not only produces the required H_2O_2 by spontaneous dismutation (reaction 4), but also it reduces OP–Cu(II) to OP–Cu(I) (reaction 5). Furthermore, the reduction of H_2O_2 by OP–Cu(I) also leads to the regeneration of OP–Cu(II) (reaction 6); thus, OP–Cu(II) acts catalytically in the generation of hydroxyl radical, being alternately reduced to OP–Cu(I) by superoxide anion and reoxidized to OP–Cu(II) by H_2O_2. Superoxide dismutase, which catalyzes the dismutation of superoxide anion to H_2O_2, would therefore inhibit DNA degradation by diverting all of the superoxide anion to H_2O_2 and preventing the reduction of OP–Cu(II) to OP–Cu(I).

In contrast to the xanthine oxidase–hypoxanthine system, degradation of DNA in the presence of either ascorbate or a thiol is inhibited by catalase but not by superoxide dismutase. This suggests that superoxide anion is not required for the generation of hydroxyl radicals in this system, except possibly as a source of H_2O_2. The following mechanism is proposed for the degradation of DNA in the presence of OP–Cu(II) and an organic reducing agent. This mechanism is based on the proposal of Misra (1974) for the generation of superoxide anion during air oxidation of thiols catalyzed by metal ions and on the observation of Kobashi & Horecker (1967) that a 1,10-phenanthroline–Cu(II) complex catalyzes the air oxidation of thiols at physiological pH.

$$HS\text{-}R\text{-}S^- + OP\text{–}Cu(II) \rightarrow HS\text{-}R\text{-}S\cdot + OP\text{–}Cu(I) \quad (8)$$

$$HS\text{-}R\text{-}S\cdot + O_2 \rightarrow \underset{R}{S\text{—}S} + H^+ + O_2^-\cdot \quad (9)$$

$$2O_2^-\cdot + 2H^+ \rightarrow H_2O_2 + O_2 \quad (10)$$

$$OP\text{–}Cu(I) + H_2O_2 \rightarrow \cdot OH + OH^- + OP\text{–}Cu(II) \quad (11)$$

$$\cdot OH + DNA \rightarrow \text{strand scission} \quad (12)$$

In the presence of an organic reducing agent, as in the xanthine oxidase–hypoxanthine system, hydroxyl radical is generated by the reduction of H_2O_2 by OP–Cu(I) (reaction 11), and H_2O_2 is produced by spontaneous dismutation of superoxide anion (reaction 10). However, in contrast to the oxidoreductase system, superoxide anion is produced by reduction of O_2 by thiyl radical (reaction 9), and OP–Cu(II) is reduced to OP–Cu(I) by an organic reducing agent (reaction 8) rather than by superoxide anion. Thus, the removal of superoxide anion by superoxide dismutase, which catalyzes the dismutation of superoxide anion to H_2O_2, would not inhibit the degradation of DNA in this system, since the reduction of OP–Cu(II) to OP–Cu(I), and consequently the generation of hydroxyl radicals, is not dependent on the availability of superoxide anion.

The inability of xanthine oxidase and hypoxanthine to cause significant DNA degradation in the absence of a 1,10-phenanthroline–Cu(II) complex suggests either that the generation of hydroxyl radicals in this system is inefficient and chelated copper is required to catalyze the formation of hydroxyl radicals or that the generation of hydroxyl radicals must occur at the site of susceptible bonds to effect efficient DNA degradation. It has been estimated that the very reactive hydroxyl radical travels only ~60 Å from its site of production to its site of reaction (Roots & Okada, 1975). Thus, the generation of hydroxyl radicals in solution may be inefficient in causing DNA degradation. 1,10-Phenanthroline is a planar, polynuclear, aromatic molecule, and it is likely that it can bind to DNA by intercalation. The binding of a 1,10-phenanthroline–Cu(II) complex to DNA would therefore lead to the generation of hydroxyl radicals in the vicinity of susceptible bonds, resulting in efficient DNA degradation. The binding of 1,10-phenanthroline and its copper chelates to DNA is currently under investigation in our laboratory. Recently, Sigman et al. (1979) reported that the cleavage of DNA by a 1,10-phenanthroline–cuprous ion complex is inhibited by catalase and proposed that DNA cleavage is the result of the synergistic action of H_2O_2 and the coordination complex. Our present studies suggest that hydroxyl radicals are the primary reactive species in the degradation of DNA.

The degradation of DNA by 1,10-phenanthroline appears similar to the degradation of DNA by the antitumor agent bleomycin; both are dependent on O_2 and a transition metal, i.e., Fe(II) for bleomycin (Sausville et al., 1978a,b) and Cu(II) for 1,10-phenanthroline, and the generation of hydroxyl radicals has also been reported with bleomycin (Lown & Sim, 1977; Oberley & Buettner, 1979). Furthermore, several other antitumor agents have been shown to cause DNA degradation in the presence of O_2 (Lown et al., 1977, 1978; Lown & Sim, 1976; Sim & Lown, 1978). Thus, the present system may provide a relatively simple model system to study the mech-

anism of action of a variety of structurally more complex antibiotics.

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Interaction of Aluminum Species with Deoxyribonucleic Acid†

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ABSTRACT: Interactions of aluminum with deoxyribonucleic acid (DNA) have been studied by thermal denaturation, circular dichroism, and fluorescent dye binding; a pH- and concentration-dependent alteration in the interaction of aluminum with DNA was observed. Three distinguishable complexes are produced when DNA is denaturated at pH 5.0–7.5 and in aluminum to DNA mole ratios of 0–0.7. Complex I appears at neutral pH and stabilizes a portion of DNA. Complex II is observed at acidic pH, destabilizes a fraction of the DNA double-helical molecule, and produces intrastrand cross-links. Complex III occurs at all pHs, is maximal at intermediate pH values, and is characterized by a noncooperative melting profile and cross-linking at low pH (<6.0). The DNA in complexes II and III can be renatured by treatment with either ethylenediaminetetraacetic acid (EDTA) or a high concentration of sodium chloride. The properties of complexes I and II are consistent with what could be expected for DNA complexes of $Al(OH)^{2+}$ and Al^{3+}, respectively. Complex III has intermediate properties that are consistent with a structure in which both ions bind the DNA simultaneously. The characteristics of complex III depend on the ratio of Al^{3+}/$Al(OH)^{2+}$ in solution. Aluminum–DNA complexes differ from other metal–DNA complexes in that melting profiles under many conditions are biphasic. Apparently more than one form of DNA can exist at any time in the presence of aluminum. The different DNA–aluminum complexes, which arise from the multiple species of aluminum in aqueous solution, lead to a variety of reactions with DNA.

Recent interest in the biological role of aluminum has been stimulated by the discovery that increased amounts of this metal occur in several human brain diseases. These conditions include Alzheimer's disease (Crapper et al., 1973, 1976; Trapp et al., 1978), dialysis encephalopathy (Alfrey et al., 1976; Arieff et al., 1979), and ALS-Parkinson dementia in Guam and Kii peninsula (Yase, 1977; Yoshimasu et al., 1976). Aluminum levels in normal human brain were also found to be significantly increased during aging (McDermott et al., 1979).

Alzheimer's disease is the most prevalent form of senile dementia and accounts for from 50 to 70% of such disorders (Butler, 1978; Terry, 1978). There is now substantial evidence that the disease is accompanied by localized depositions of aluminum in brain cells. Such deposition has been observed by histochemical techniques (DeBoni et al., 1974; Crapper &

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