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Secondary structure specificity of the nuclease activity of the 1,10-phenanthroline–copper complex

(artificial DNase/oxidative nuclease)

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ABSTRACT **The artificial DNase activity of the 1,10-phenanthroline–cuprous ion complex [$(OP)_2Cu^+$] and H_2O_2 cleaves the A, B, and Z forms of DNA at different rates. The B structure, formed by most DNAs including poly(dA-dT) and poly(dA)·poly(dT), is the most susceptible to cleavage. It is completely degraded within 1 min by 40 μM 1,10-phenanthroline/4 μM Cu^{2+}/7 mM H_2O_2/7 mM 3-mercaptopropionic acid. The A structure, formed by RNA·DNA hybrids such as poly(rA)·poly(dT), is cleaved in both strands roughly 10–20% as rapidly as poly(dA-dT) under comparable conditions. In contrast, the left-handed Z structure, formed by poly(dG-dC) in 3.0 M NaCl, is completely resistant to cleavage even though the same copolymer in the B structure at 15 mM NaCl is readily degraded. Poly(dA-dT) is rendered acid soluble at both salt concentrations at similar rates. The basis for the secondary structure specificity of the DNA cleavage reaction most likely resides in the requisite formation of a productive complex between $(OP)_2Cu^+$ and DNA during the course of this reaction. Previous studies have suggested that strand scission is due to oxidative destruction of the deoxyribose by hydroxyl radicals produced by the oxidation of DNA-bound Cu^+ by H_2O_2. Apparently, the Z and A structures are unable to form a stable noncovalent complex with the same optimal geometry for cleavage as the B structure and are less susceptible to degradation. This artificial DNase activity may provide an approach to assess the formation of non-B-DNA structures in solution.**

Structural analysis using x-ray diffraction has shown that DNA can exist in three general helical forms designated A, B, and Z (1, 2). The specific structure a DNA adopts appears to depend on its primary sequence and environmental conditions such as salt content and solvent polarity (3, 4). Although variations in helical structure have been proposed to play a role in the regulation of genetic expression and may be important in directing a variety of ligands to specific regions of DNA (5–7), methods are not yet generally available to identify limited segments of alternative helical structures in a natural DNA.

Immunological approaches are promising (7–10), but physical techniques are not sufficiently sensitive to identify sequences in different secondary structure forms. Optical methods such as CD measure composite structures, and x-ray diffraction methods are not applicable to large segments of DNA. For example, the largest oligonucleotide that has been studied by single-crystal x-ray crystallographic analysis is a dodecamer (11, 12). As yet, no DNase has been described that can distinguish the various helical structures that might be characteristic of certain primary sequences. In this paper, we report that the 1,10-phenanthroline–cuprous ion complex [$(OP)_2Cu^+$] reacts differently with the three families of helical structure (A, B, and Z) that DNA forms in synthetic polynucleotides. This artificial DNase activity may therefore be a suitable tool to investigate the secondary structure of DNA.

We have previously shown that B-DNAs such as poly(dA-dT) and poly(dA)·poly(T) are readily degraded under conditions in which single-stranded DNAs such as poly(dT) are nonreactive (13–15). For example, the double-stranded DNAs indicated above are rendered completely acid soluble within 1 min by the [$(OP)_2Cu^+$] formed in a solution containing 40 μM 1,10-phenanthroline, 4 μM Cu^{2+}, 7 mM H_2O_2, and 7 mM 3-mercaptopropionic acid (14). Products of the reaction using poly(dA-dT) as substrate include the free bases adenine and thymine and oligonucleotides with 5′ and 3′ phosphorylated termini. The deoxyribose fragment generated by the presumed oxidative degradation has not yet been identified (15). In this communication, we report that the rate of cleavage of a poly(dT) strand that is complexed to a poly(rA) strand and therefore constrained to the A structure characteristic of RNA and RNA·DNA hybrids (16, 17) is cleaved at a rate that is 10–20% of that observed with the B-helical polymers poly(dA)·poly(dT) and poly(dA-dT). In addition, we report that poly(dG-dC) stabilized in the Z form by 3.0 M NaCl is resistant to cleavage, whereas the same polynucleotide in the B structure at lower salt concentrations is rapidly degraded.

MATERIALS AND METHODS

Materials. *Escherichia coli* DNA polymerase I was prepared to fraction VII according to the method of Jovin *et al.* (18).

E. coli RNA polymerase and nuclease S1 from *Aspergillus oryzae* were purchased from P-L Biochemicals and used without further purification. Avian myeloblastosis virus reverse transcriptase was obtained from Life Sciences (St. Petersburg, FL). Unlabeled NTPs and dNTPs were bought from Sigma, and radioactively labeled NTPs and dNTPs were purchased from ICN. The polymers poly([^{3}H]A), poly([^{3}H]dA), poly([^{3}H]dT), poly(dT), poly(dA), and poly(dA-dT) were purchased from Miles. The alternating copolymer poly(dG-dC) was from P-L Biochemicals.

Other chemicals obtained from commercial sources are Tris·HCl and Tris acetate (Sigma); 3-mercaptopropionic acid (Aldrich); NH_4OAc and $CuSO_4$ (Mallinckrodt); and 1,10-phenanthroline (G. F. Smith).

Preparation of Polymers. The alternating copolymer poly([^{3}H]dG-dC) was synthesized in 67 mM Tris·HCl (pH 8.2) with *E. coli* DNA polymerase I according to the method of Pohl and Jovin (4). The polymer was dialyzed with one change of dialysis buffer against 1 M KCl/10 mM sodium phosphate, pH 6.5/1 mM mercaptoethanol for 2 days and against 10 mM Tris·HCl (pH 7.0) for 2 days. Then, it was extracted with chloroform/isoamyl alcohol (24:1) and dialyzed for 2 days against 15 mM NaCl/10 mM Tris·HCl, pH 7.8.

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Abbreviation: $(OP)_2Cu^+$, 1,10-phenanthroline–Cu^+ complex.

The alternating copolymers poly(dA-[^{3}H]dT) and poly([^{3}H]dA-dT) and the homopolymer poly(dA)·poly([^{3}H]T) were synthesized with *E. coli* DNA polymerase I in 0.1 M Tris acetate (pH 7.0) as described (14). Unincorporated nucleotides were routinely removed by either extensive dialysis or ethanol precipitation of the polymer. The method of separation had no apparent effect on the rates of cleavage of the polymers by $(OP)_2Cu^+$. To eliminate possible differences due to buffer effects, poly(dA-[^{3}H]dT) was also synthesized and dialyzed using the buffers described for poly([^{3}H]dG-dC) synthesis.

A heteroduplex in which both strands were radioactively labeled poly([^{32}P]rA)·poly([^{3}H]dT) was synthesized using *E. coli* RNA polymerase and poly([^{3}H]dT) as template. Poly([^{3}H]dT) (2.3 μCi; 1 Ci = 37 GBq; 34.8 μg/ml) in 0.6 ml of 50% ethanol was dried under a stream of nitrogen. The polymer was then suspended in 1 ml of 0.04 M Tris·HCl, pH 7.9/0.15 M KCl/0.01 M MgCl/0.2 mM dithiothreitol containing bovine serum albumin at 1 mg/ml, unlabeled poly(dT) at 37.5 μg/ml, and [α-^{32}P]ATP (0.33 μmol/ml; 100 μCi/μmol). This mixture was equilibrated to 37°C and then 5 units of RNA polymerase was added. After 160 min at 37°C, the reaction mixture was treated with S1 nuclease as described below to remove single-stranded poly([^{3}H]dT). The solution was then made 1 M in NH_4OAc, and the polynucleotide was precipitated with 2 vol of ethanol. The pellet was dissolved in 0.1 M Tris acetate, pH 7.0/5 mM $Mg(OAc)_2$. Occasionally, proteins were removed by extraction with chloroform/isoamyl alcohol (24:1) prior to precipitation with ethanol. This had no apparent effect on the rate of cleavage by $(OP)_2Cu^+$.

The heteroduplex poly(rA)·poly([^{3}H]dT) was synthesized with reverse transcriptase using poly(rA) (52 μg/ml) as template. The reaction mixture (0.25 ml) contained oligo(dT)$_{12-18}$ (23 μg/ml) as primer, 50 mM Tris·HCl (pH 8.3), 10 mM $MgCl_2$, 20 mM dithiothreitol, 4 mM $NaPP_i$, 50 mM KCl, and 0.4 mM [^{3}H]dTTP (1,250 mCi/mmol). [^{3}H]dTTP (50 Ci/mmol, 1 mCi/ml) obtained in 50% ethanol was lyophilized to dryness and then resuspended by the addition of the remaining reaction components. The reaction mixture was kept on ice until the addition of 66 units of reverse transcriptase. The solution was mixed and incubated 20 min at 45°C. It was made 1 M in NH_4OAc and the hybrid was precipitated by the addition of 2 vol of ethanol. The pellet was suspended in 2 ml of 0.1 M Tris acetate, pH 7.0/5 mM $Mg(OAc)_2$ and dialyzed against the same buffer for 29 hr with one change of buffer. Immediately before use, the hybrid was treated with S1 nuclease as described below and precipitated with ethanol. Synthesis of all the above polymers was monitored by the incorporation of radioactivity into an acid-precipitable form as described (14).

Assay for DNA Scission. The assay for the cleavage of radioactively labeled polymers is based on the formation of acid soluble products and has been described (14). Variations to this procedure include changes in the buffer used as indicated in the figure legends.

To allow any conformational changes to reach equilibrium, polymers were incubated at 25°C at the salt and solvent concentrations indicated for the scission reactions at least 1 hr before the addition of 1,10-phenanthroline, Cu^{2+}, reducing agent, or H_2O_2. In these studies, mixtures of 1,10-phenanthroline, Cu^{2+}, and reducing agent were combined either at twice their final concentration in a buffer containing NaCl at the desired concentration or at 10-fold their final concentration in buffer alone. In the former case, 175 μl of the mixture was added to 175 μl of the solution of DNA. In the latter case, 40 μl was added to 350 μl of the DNA solution. H_2O_2 had been added to the DNA before the other reagents. The two methods gave similar results.

Polymers to be treated with S1 nuclease were diluted 1:2 with 80 mM NaOAc/0.4 M NaCl/20 mM $ZnSO_4$. The solution was equilibrated to 37°C and S1 nuclease (67 units/ml, 5 units/μg of polymer) was added. The reaction was monitored by following the production of acid-soluble products and was typically terminated after 60 min. A single-stranded polymer, poly([^{3}H]dA) or poly([^{3}H]dT) was routinely treated similarly as a control for nuclease activity. The single-stranded polymer was completely solubilized within 5 min.

Spectral Measurements. Absorption measurements at 260 nm to determine nucleic acid concentrations were made with a Gilford spectrophotometer model 250. CD measurements were made using a Durrum-Jasco spectropolarimeter, model J-10, and on an instrument constructed by J. Horwitz of the Jules Stein Eye Institute (University of California School of Medicine, Los Angeles). Solutions of poly(dA-[^{3}H]dT) and poly([^{3}H]dG-dC) were diluted into a buffer containing the desired concentration of NaCl to an A_{260} of 0.4–0.6 and were allowed to equilibrate 1 hr before the CD spectra were recorded.

RESULTS

A-DNA Cleavage. DNA·RNA heteroduplexes exhibit x-ray diffraction patterns and optical measurements characteristic of A helices (17, 19, 20) and provide convenient models of a DNA strand in that secondary structure in aqueous solution at physiological ionic strengths (21, 22). Fiber x-ray diffraction analysis has shown that, unless a synthetic DNA is paired to a polyribonucleotide, it adopts an A-helix conformation only at very low humidity or in the complete absence of salt (3, 23). However, DNA strands of the DNA·RNA hybrids are induced to form the A structure because the steric constraints associated with the 2′-hydroxyl group of the ribose prohibit the RNA strand from adopting a B form (16, 17).

The DNA·RNA heteroduplex poly(rA)·poly(dT) was prepared in two ways. With poly([^{3}H]dT) as template and [α-^{32}P]ATP as substrate, RNA polymerase was used to prepare poly([^{32}P]rA)·poly([^{3}H]dT). Alternatively, poly(rA)·poly([^{3}H]dT) was prepared using avian myeloblastosis virus reverse transcriptase with poly(rA) as template and [^{3}H]dTTP as substrate. The hybrids were treated with S1 nuclease to eliminate any single-stranded species that would be resistant to cleavage. As a control, B-DNA polymers were routinely digested with S1 nuclease. This treatment had no apparent effect on their rates of cleavage. The doubly labeled polymer poly([^{32}P]rA)·poly([^{3}H]dT) provides the additional opportunity of determining if a ribonucleotide has the same susceptibility as a deoxyribonucleotide to oxidative degradation by $(OP)_2Cu^+$. Both the RNA and DNA strands assume identical structures and therefore conformational effects are unlikely to mask intrinsic differences in reactivity. As shown in Fig. 1, we found that both strands of poly([^{32}P]rA)·poly([^{3}H]dT) are cleaved at equivalent rates but less extensively than poly([^{3}H]dA-dT) and poly(dA)·poly([^{3}H]dT), which adopt a B-helix structure (16, 23). In addition, since the heteroduplexes have been treated with nuclease S1, the sizes of the two strands are identical and acid solubilization provides an accurate measure of their relative susceptibility to degradation. No diffusible inhibitor is responsible for the observed difference between the two helical forms because parallel results were obtained when poly(dA-[^{3}H]dT) and poly([^{32}P]rA)·poly(dT) were cleaved in the same reaction mixture.

Strict kinetic comparison of the initial rates of solubilization of poly(dA-[^{3}H]dT) and poly([^{32}P]rA)·poly(dT) must be made cautiously because they will be biased in favor of the polynucleotide with the lower average molecular weight. Comparison of the extent of cleavage as a function of time gave a more reliable measure of the rates of cleavage. For example, 30% of the radioactive material in the A-helical

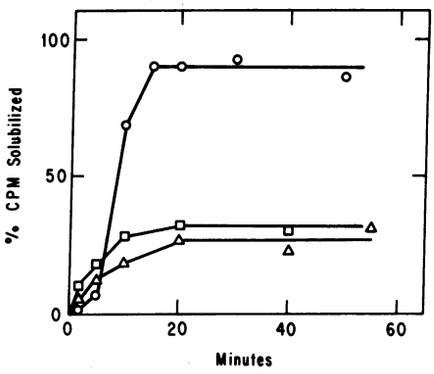


FIG. 1. Acid solubilization of A-helical poly([^{32}P]rA)·poly([^{3}H]dT) and B-helical poly([^{3}H]dA-dT) by $(OP)_2Cu^+$. Poly([^{3}H]dA-dT) (2.2 μg/ml) and poly([^{32}P]rA)·poly([^{3}H]dT) (2.2 μg/ml) were incubated in separate vessels with 40 μM 1,10-phenanthroline/4 μM $CuSO_4$/0.1 mM NADH/0.5 mM H_2O_2 at 37°C in 0.1 M Tris acetate, pH 7.0/5 mM $Mg(OAc)_2$. Aliquots (50 μl) were removed at the times indicated and spotted on Whatman glass fiber filters. The filters were washed in 5% trichloroacetic acid/1.5% $NaPP_i$ and dried; radioactivity was determined as described. ○, Poly([^{3}H]dA-dT) (^{3}H); △ and □, poly([^{32}P]rA)·poly([^{3}H]dT) (^{32}P and ^{3}H, respectively). Acid solubilization of B-helical poly(dA)·poly([^{3}H]dT) is indistinguishable from that of poly([^{3}H]dA-dT).

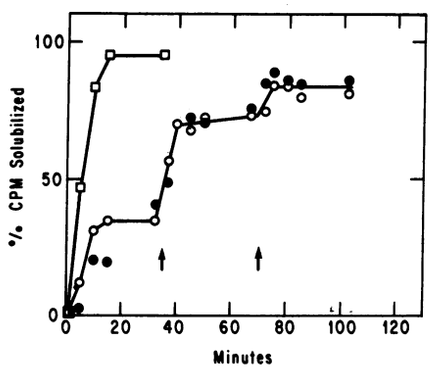
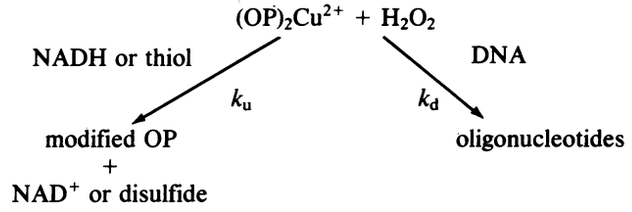


FIG. 2. Multiple additions of $(OP)_2Cu^+$ result in complete digestion of poly([^{32}P]rA)·poly([^{3}H]dT). Poly([^{3}H]dA-dT) ([^{3}H], □) and poly([^{32}P]rA)·poly([^{3}H]dT) (○, [^{3}H]; ●, [^{32}P]) (both at 2.2 μg/ml) were cleaved in separate vessels with 40 μM 1,10-phenanthroline/4 μM $CuSO_4$/0.1 mM NADH/0.5 mM H_2O_2 buffered with 0.1 M Tris acetate/5 mM $Mg(OAc)_2$, pH 7.0, at 37°C in 1-ml total volume. At the time indicated by the left arrow, 4 μl of 97 mM H_2O_2 and 21.4 μl of 3.6 mM NADH/1.52 mM 1,10-phenanthroline/0.152 mM $CuSO_4$ were added to and mixed with the reaction mixture remaining (0.750 ml). At the time indicated by the right arrow, 2.4 μl of 97 mM H_2O_2 and 12.8 μl of the same NADH/1,10-phenanthroline/$CuSO_4$ solution were combined with the remainder of the reaction mixture (0.450 ml).

polymers was solubilized after a 60-min incubation with $(OP)_2Cu^+$ (Fig. 1). Although this observation could be a reflection of the resistance to digestion of regions of the DNA·RNA hybrid, it could also be due to the competition of the degradation of the nucleic acid (k_d) with the unproductive consumption of the reagents (k_u) according to Scheme I.



Scheme I

In this view, A-form DNA is only partially digested under conditions in which B-form DNA is completely degraded because the k_d for the B form is greater than that for the A form. Several different processes have been shown to contribute to the unproductive consumption of reagents (i.e., k_u). They include the oxidation of thiol by oxygen and H_2O_2 catalyzed by $(OP)_2Cu^{2+}$ in the absence of DNA (24, 25) and the oxidation of the 1,10-phenanthroline by H_2O_2 to yield 2,2′-bipyridine-3,3′-dicarboxylic acid and 1,10-phenanthroline *N*-oxide (26, 27). Direct evidence for Scheme I is provided by the fact that poly(dA-dT) is not cleaved if it is added to a reaction mixture containing poly(dA-dT) that has already been cleaved in a 50-min incubation with 20 μM 1,10-phenanthroline, 2 μM $CuSO_4$, 7 mM mercaptopropionic acid, and H_2O_2 unless more 1,10-phenanthroline, reducing agent, and H_2O_2 are added. In addition, as shown in Fig. 2, the A-helical heteroduplex is made 85% acid soluble if the reaction mixture is supplemented with two additions of 1,10-phenanthroline, Cu^{2+}, NADH, and H_2O_2 at their original concentrations.

Z-DNA Cleavage. The susceptibility of Z-DNA to cleavage by $(OP)_2Cu^+$ was examined by generating this left-handed conformation in poly(dG-dC) with a high concentration of NaCl. This highly cooperative transition is characterized by a dramatic change in the CD spectrum and by a shift in the UV absorption spectrum to higher wavelengths (4). Fig. 3 shows that our preparation of poly([^{3}H]dG-dC) undergoes the expected change in its CD spectrum, whereas there are only slight differences in the CD spectra of poly(dA-[^{3}H]dT) in 15 mM NaCl and in 3.0 M NaCl (Fig. 4). Before testing the susceptibility of Z-helical poly([^{3}H]dG-dC) formed in high salt to scission by $(OP)_2Cu^+$, we examined the possibility that 3.0 M NaCl might block DNA cleavage by $(OP)_2Cu^+$ by interfering with the coordination and oxidation–reduction chemistry essential for the degradation of the nucleic acid. The best experimental test was to monitor the $(OP)_2Cu^{2+}$-catalyzed oxidation of thiol (e.g., mercaptopropionic acid) by H_2O_2 in the absence of nucleic acid. Using 5,5′-dithiobis-(2-nitrobenzoic acid) to monitor the consumption of thiol, we found that 3.0 M NaCl reduces the rate of oxidation of mercaptopropionic acid (7 mM) by H_2O_2 (7 mM) catalyzed by 1,10-phenanthroline (20 μM) and Cu^{2+} (2 μM) to 1/3rd of the rate observed in the presence of 15 mM NaCl. Increasing the

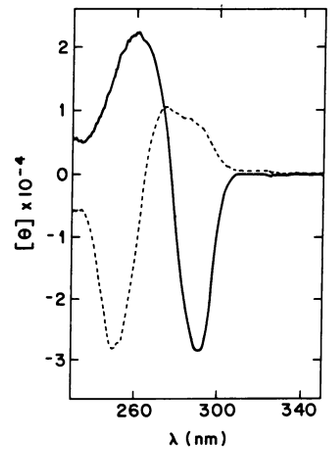


FIG. 3. Inversion of the CD spectrum of poly([^{3}H]dG-dC) in 3.0 M NaCl. Poly([^{3}H]dG-dC) was diluted to A_{260} = 0.590 in 10 mM Tris·HCl (pH 7.8) containing either 15 mM (----) or 3.0 M (——) NaCl. The solutions were equilibrated for 1 hr at 25°C before recording the CD spectra.

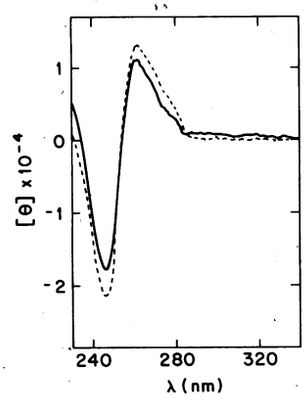


FIG. 4. CD spectra of poly(dA-[^{3}H]dT). Poly(dA-[^{3}H]dT) was diluted to A_{260} = 0.470 in 10 mM Tris·HCl (pH 7.8) containing either 15 mM (----) or 3.0 M (——) NaCl. Solutions were equilibrated for 1 hr at 25°C before recording the CD spectra.

1,10-phenanthroline and Cu^{2+} concentrations to 70 and 7 μM, respectively, makes the rate of mercaptopropionic acid oxidation in 3.0 M NaCl equivalent to the rate catalyzed by 20 μM 1,10-phenanthroline and 2 μM Cu^{2+} in 15 mM NaCl. Because the latter conditions are sufficient to completely degrade B-form DNA in 1 min, the higher concentrations of 1,10-phenanthroline (70 μM) and Cu^{2+} (7 μM) were used for cleavage in 3.0 M NaCl.

Although poly([^{3}H]dG-dC) is cleaved as rapidly as poly(dA-[^{3}H]dT) in 15 mM NaCl, in which it adopts the B-helix conformation, it is resistant to cleavage in 3.0 M NaCl, in which it adopts the Z-helix conformation. The same salt concentrations have little effect on the scission of poly(dA-[^{3}H]dT) by the complex (Fig. 5). Even with multiple additions of $(OP)_2Cu^+$, poly([^{3}H]dG-dC) is not solubilized in 3.0 M NaCl. Similarly, poly(dA-[^{3}H]dT) but not poly([^{3}H]dG-dC) is cleaved in the presence of 1.0 M $MgCl_2$. The midpoint for the B-to-Z transition of poly(dG-dC) is observed at 0.66 M $MgCl_2$ (4). The Z structure induced by ethanol (28) cannot be profitably studied because the high ethanol concentration (48%) required greatly inhibits the $(OP)_2Cu^{2+}$-catalyzed oxidation of thiol by H_2O_2.

The effect of NaCl on the scission of poly([^{3}H]dG-dC) with $(OP)_2Cu^+$ was compared with its effect on the B-to-Z transition as reflected by the CD measurements. The percentage of poly([^{3}H]dG-dC) remaining acid insoluble after 60 min of incubation with $(OP)_2Cu^+$ and the degree of transition (θ) (4) are plotted against NaCl concentration in Fig. 6. Inhibition

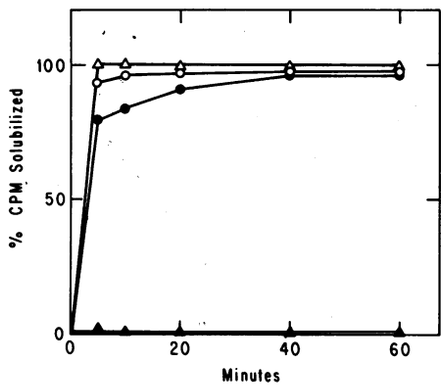


FIG. 5. Cleavage of poly([^{3}H]dG-dC) and poly(dA-[^{3}H]dT) in the presence and absence of 3.0 M NaCl. Poly(dA-[^{3}H]dT) (○, ●) and poly([^{3}H]dG-dC) (△, ▲) (both at 5.5 μg/ml) were cleaved with 7 mM H_2O_2/7 mM mercaptopropionic acid/70 μM 1,10-phenanthroline/7 μM $CuSO_4$ in 10 mM Tris·HCl (pH 7.8) in the presence of 15 mM NaCl (○, △) or 3 M NaCl (●, ▲).

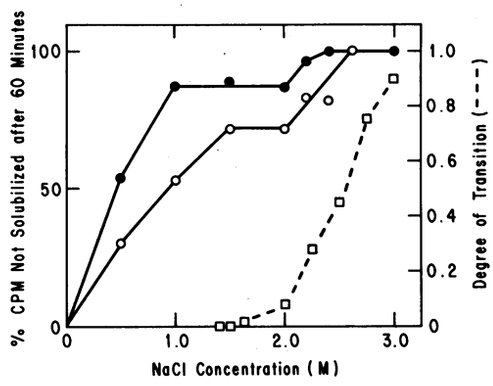


FIG. 6. Poly([^{3}H]dG-dC) (5.5 μg/ml) was incubated at 37° (○) or at 25°C (●) with 70 μM 1,10-phenanthroline/7 μM $CuSO_4$/7 mM H_2O_2/7 mM mercaptopropionic acid in 10 mM Tris·HCl (pH 7.8) containing the indicated concentrations of NaCl. The percentage of cpm remaining insoluble at the infinity point of 60 min was plotted versus NaCl concentration. For comparison, the degree of transition (θ) versus NaCl concentration (figure 5 of ref. 4) is also shown.

of scission by NaCl clearly occurs before the CD measurements indicate that the B-to-Z transition has occurred. No inhibition of scission of poly(dA-[^{3}H]dT) by $(OP)_2Cu^+$ and H_2O_2 was detected at any NaCl concentration under these conditions. The formation of an intermediate structure in the B-to-Z transitions provides a possible explanation for these observations. If this structure is right-handed (e.g., a C-type helix) (16, 29), it may not be readily detectable by CD measurement. If, in addition, this intermediate is not an effective substrate for the artificial DNase activity, the inhibition of the cleavage at lower salt concentrations than those that induce the spectroscopically observable B-to-Z transition can be understood.

DISCUSSION

The secondary structure specificity exhibited by $(OP)_2Cu^+$ and H_2O_2 in the cleavage of synthetic polynucleotides should be a useful guide in developing applications of this artificial nuclease in the study of nucleic acids. Previously, we reported that the reaction system is less specific than DNase I in the cleavage of a short piece of B-DNA (14). Here, we report that it is not equivalently efficient toward all types of secondary structures, indicating that regions of preferred nicking may be observed in large segments of DNA if the primary sequence dictates different secondary structures. Our earlier work had shown that single-stranded DNAs such as poly(dT) are unreactive under conditions in which poly(dA-dT) and poly(dA)·poly(dT) are rendered completely acid soluble (14). However, the recent report that $(OP)_2Cu^+$ degrades the self-complementing dinucleotide dC-dG (30) suggests that heterogeneous single-stranded DNAs in which base-paired regions can be formed may also be nicked. Our preliminary demonstration that the single-stranded DNA of phage M13 can be rendered more acid soluble than might be anticipated based on the fraction of bases involved in hairpin structures is consistent with this expectation.

The formation of a reactive noncovalent intermediate between the DNA and the coordination complex most likely is responsible for the specificity exhibited by $(OP)_2Cu^+$ and H_2O_2. The formation of a noncovalent complex analogous to a Michaelis complex in the cleavage of poly(dA-dT) has already been inferred based on (*i*) the reactivity of the cuprous complex of 1,10-phenanthroline relative to the reactivities of the cuprous complexes of bipyridine and 2,2′,2″-terpyridine (31, 32), (*ii*) the inhibition of the cleavage by intercalating agents (14), and (*iii*) the lack of reactivity of noncomplement-

agents (14), and (*iii*) the lack of reactivity of noncomplementing single-stranded DNAs such as poly(dT) (14). Although it is tempting to assume that the complex between a B-DNA and $(OP)_2Cu^+$ involves intercalation because of the structural similarity of the ligand to ethidium bromide, no firm evidence for this binding mode, as opposed to interaction with the major or minor groove, is available. However, the enantiomeric specificity of the binding of isomers of the 1,10-phenanthroline–zinc (3:1) complex to calf thymus DNA directly supports the hypothesis that coordination complexes interact with B-DNA in a stereochemically precise manner (33). In the same series of experiments, the $(OP)_2Zn^{2+}$ complex, which would be analogous to the $(OP)_2Cu^+$ complex proposed to be responsible for cleavage, also interacted with the DNA. However, it was bound less efficiently with unknown stereochemical precision. The 1:1 phenanthroline–Zn^{2+} complex also binds to DNA; the 1:1 phenanthroline–Cu^+ complex, however, does not cleave DNA (14).

Binding of the $(OP)_2Cu^+$ complex to the DNA therefore must be a necessary but not sufficient condition for cleavage. It is not yet clear whether the lack of reactivity of the Z-DNA is due to the low affinity of $(OP)_2Cu^+$ for Z-DNA, or to improper orientation of the reactive center of the coordination complex once bound to the polynucleotide (or both). Direct measures of ligand binding will be essential to resolve this question. Some ligands [e.g., ethidium bromide (34, 35)] bind more tightly to B-DNA than to Z-DNA while others (e.g., mithramycin) bind with equal efficiency to both conformations (35). Ligands capable of covalent modification of DNA also differ in their reactivity with the two forms of DNA. For example, aflatoxin strongly inhibits the salt-induced transformation of poly(dG-dC) to the Z-form (36) whereas 2-acetylaminofluorene facilitates it (37, 38).

Similarly, the slower rate of cleavage of DNA in the A structure cannot yet be interpreted in terms of binding or orientation effects. Nevertheless, the equivalent rates of cleavage of the RNA and DNA strands of the heteroduplex show that the intrinsic reactivity of the deoxyribose and ribose moieties to the postulated oxidative degradation is the same and that the 2′ position is not the likely initial site of attack in the reaction leading to depolymerization in either RNA or DNA. Because RNAs contain significant single-stranded regions and are also constrained to the A structure, the RNase activity of $(OP)_2Cu^+$ is expected to be less efficient than the DNase activity.

Examination of the cleavage patterns of RNAs and DNAs of known three-dimensional structure should provide a useful approach for identifying the structure of the essential noncovalent intermediate in the reaction pathway. These data might also suggest modified phenanthrolines whose cuprous complexes would exhibit valuable selectivity in cleavage of RNAs and DNAs.

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