DNA-Binding Studies of $Cu(bcp)_2^+$ and $Cu(dmp)_2^+$: DNA Elongation without Intercalation of $Cu(bcp)_2^+$

Fang Liu, Kelley A. Meadows, and David R. McMillin*

Contribution from the Department of Chemistry, 1393 Brown Building, Purdue University, West Lafayette, Indiana 47907-1393

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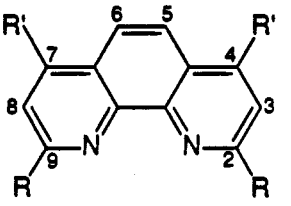
Abstract: We have used a variety of physical methods to investigate the ways by which copper phenanthrolines bind to B-form DNA. To vary the composition of the DNA, we have employed poly(dG-dC)·poly(dG-dC), *M. lysodeikticus* DNA, salmon testes DNA, and poly(dA-dT)·poly(dA-dT) as hosts. The specific copper complexes we have studied are $Cu(dmp)_2^+$ and $Cu(bcp)_2^+$ where dmp and bcp denote 2,9-dimethyl-1,10-phenanthroline and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, respectively. The results indicate that the dmp complex binds externally in a solvent-accessible site. However, the solvent cannot access the metal center as readily in the case of the bcp complex because $Cu(bcp)_2^+$ is emissive in the presence of DNA. Since the emission retains polarization, the complex does not rotate independently but tumbles with the macromolecule in solution. At low loadings of $Cu(bcp)_2^+$ the specific viscosity due to DNA molecules that are rich in adenine-thymine base pairs increases; however, the results are inconsistent with classical intercalative binding. They are more logically interpreted in terms of bridging structures in which one or more bcp complexes link DNA molecules together in solution. High loadings of $Cu(bcp)_2^+$ induce further condensation of the DNA and ultimately particulate formation. The techniques we have used in this work include absorption spectroscopy, circular dichroism, emission spectroscopy, emission polarization, and viscometry. The findings are relevant to the chemistry of copper complexes involving unmethylated phenanthrolines, systems widely being used to probe DNA structure.

Introduction

There is continuing interest in metal complexes that interact with DNA molecules in solution.[1-4] Complexes that reversibly bind to DNA through non-covalent interactions form an important subgroup of these systems. As a rule, members of this subgroup are cations, and they typically involve ligands that have extended hydrophobic regions or surfaces, e.g., metalloporphyrins with charged substituents on the periphery.[5] The gamut of d^6 Ru(II) or Rh(III) complexes with polypyridines or 1,10-phenanthroline ligands also falls into this category of DNA-binding agents,[2,6-8] where the structure, size, and relative disposition of the ligands in the coordination sphere of the metal ion help direct the binding. In particular, since DNA is a chiral molecule, specific binding sites may favor one or another enantiomeric form of the complex.[2,9]

Seminal work by Sigman and co-workers uncovered the first chemical nuclease which acts via an essential, non-covalently bound intermediate.[10,11] The actual scission is initiated by hydrogen atom abstraction from a deoxyribose residue, and the reaction requires hydrogen peroxide which is generally produced in situ. A series of rapid reactions is involved,[10] but the triggering event is a reaction between hydrogen peroxide and $Cu(phen)_2^+$ which is bound to the DNA.[12,13] (Here, phen denotes 1,10-phenanthroline; see Figure 1.) Sigma has proposed that $Cu(phen)_2^+$ binds at the surface of DNA, within or about the minor groove, since the hydrogen atom abstraction occurs regiospecifically at the C1′ or the C4′ carbon of the sugar.[1,10,14] Moreover, strong cutting sites on one strand are correlated with strong cutting sites offset in the 3′-direction on the opposite strand, consistent with interaction at the minor groove.[15] On the basis of studies with duplexes containing mismatched strands of oligonucleotides, Williams et al. have suggested that the copper complex may actually be partially intercalated into the DNA since cleavage occurs preferentially near the bulges where intercalation is thought to be favored.[16] Recently, Rill and co-workers have presented



	R	R'
phen	*H*	*H*
dmp	*Me*	*H*
bap	*H*	*Ph*
bcp	*Me*	*Ph*

Figure 1. Phenanthroline ligands.

* Author to whom correspondence should be addressed.

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viscometry data which can also be rationalized in terms of some type of partial intercalation.[17] However, Sigman and co-workers have introduced substituents in the 5 position of the phenanthroline ring and have shown that they have little influence on the cutting reaction.[12] These results are hard to reconcile with a binding model based on partial intercalation. On the other hand, in the case of the more hydrophic complex $Cu(bap)_2^+$, where bap denotes 4,7-diphenyl-1,10-phenanthroline, Sigman and co-workers have suggested that intercalative binding may be involved because DNA scission occurs at a significantly reduced rate and with much less sequence specificity by comparison with other copper phenanthroline complexes.[10,12]

We have been attempting to define the fundamental DNA-binding interactions of related copper(I) systems. Our approach has been to utilize the analogous copper complexes $Cu(dmp)_2^+$ and $Cu(bcp)_2^+$, where dmp denotes 2,9-dimethyl-1,10-phenanthroline and bcp denotes 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, respectively (Figure 1).[18-20] Because of the methyl substituents, these complexes have much higher reduction potentials such that solutions are stable in air. As a result, the complexes show little nuclease activity, and it is possible to focus on the non-covalent binding interactions with DNA. These copper(I) systems are characterized by metal-to-ligand charge-transfer (CT) excited states that fall in the visible region of the spectrum. Electronic absorption and luminescence studies reported to date have established that the two complexes bind to DNA in very different ways. Thus, hardly any luminescence can be detected from the bound form of $Cu(dmp)_2^+$ whereas $Cu(bcp)_2^+$ is luminescent when bound to DNA. This means that the DNA host somehow conformationally constrains the bcp complex and/or screens it from bulk solution because solvent-induced quenching of the charge-transfer excited state is normally very efficient in a polar solvent such as water or methanol.[21-25] To account for such intimate binding, we suggested that one of the ligands of $Cu(bcp)_2^+$ may intercalate between base pairs of DNA.[20] However, the problem with this interpretation is that steric interactions among hydrogen atoms of the ligand almost certainly cause the phenyl substituents to twist out of the plane of the phenanthroline core.[26] Intercalation would be more favorable with a planar ligand. To investigate this issue further, we have carried out viscometry studies as well as circular dichroism (CD) and additional luminescence studies which are described below.

Experimental Section

Materials. Preparation of $[Cu(bcp)_2]Cl\cdot 2.5H_2O$: $CuCl_2\cdot 2H_2O$ (2.6 mmol) was dissolved in 80 mL of water containing a few drops of a concentrated HCl solution to clarify the solution. It was then combined with 5.2 mmol of bcp in 60 mL of MeOH and stirred overnight. Then, sodium ascorbate was added as a reducing agent. Evaporation of the methanol produced a solid which was recrystallized from aqueous methanol, washed with water and diethyl ether, and finally dried in air. The product melted over the range of 156–158 °C. Calculated microanalytical results for $[Cu(bcp)_2]Cl\cdot 2.5H_2O$: C, 76.13; H, 4.93; N, 6.83; Cl, 4.32. Found: C, 72.17; H, 4.92; N, 6.72; Cl, 4.21. The chloride salt of $Cu(dmp)_2^+$ was prepared following a reported procedure.[27]

Commercially obtained $Na_5P_3O_{10}\cdot 6H_2O$ (94% pure, Fisher Scientific Co.) was recrystallized twice.[28] In this procedure an aqueous solution containing 12-15% by weight of the triphosphate salt was prepared and then filtered. The $NaP_3O_{10}\cdot 6H_2O$ crystallized out when ethanol was added slowly with stirring until the ratio of ethanol to water was about 1:4 by volume. The crystals were filtered, washed twice with a 1:1 mixture of ethanol and water by means of a buchner filter, and finally dried in air. The buffer solution was prepared by addition of $H_4P_2O_7$ (Pflaz and Bauer, Inc.) to a 0.01 M solution of triphosphate ion until the pH was 7.8.

Salmon testes DNA (ST DNA) was obtained from Sigma Chemical Co. and was first purified by precipitation with ethanol. Solutions of the redissolved DNA were sonicated for 5 min at 0 °C to yield an average chain length of 600 base pairs according to viscosity measurements. The highly polymerized *M. lysodeikticus* DNA (ML DNA) was also obtained from Sigma Chemical Co. and was purified by treatment with RNase which was a gift from M. Bina of Purdue University. The ML DNA was dissolved in 0.025 M pH 7.8 tris buffer, and the solution was sonicated for 20 min. Then, RNase was added and the solution was incubated overnight at 37 °C. After addition of an equal portion of a mixture of phenol and chloroform (4:1 v/v), the mixture was spun in a centrifuge and the top layer was retained. The DNA was then precipitated by adding 4 volumes of absolute methanol and subsequently rinsed with 70% methanol. After a second precipitation step the resulting white solid was dried in air.

Synthetic DNA with alternating base pairs, i.e., poly(dA-dT)·poly(dA-dT) or poly(dG-dC)·poly(dG-dC), was obtained from Pharmacia while covalently closed supercoiled pBR 322 DNA was obtained from Boehringer Mannheim, Inc. All were used without further purification. In the above dA, dT, dC, and dC represent the 5′-deoxyadenylate, 5′-deoxythymidylate, 5′-deoxyguanylate, and 5′-deoxycytidylate residues, respectively. Calf thymus topoisomerase I was obtained from Bethesda Research Lab. All other chemicals used were reagent grade.

Methods and Instrumentation. Samples were typically prepared by addition of an aliquot of a buffered DNA solution to a copper stock solution in methanol. Then, an appropriate amount of buffer was added to achieve a final composition of 33% methanol (MeOH). DNA concentrations were determined spectrophotometrically from the following molar extinction coefficients ($M^{-1}\,cm^{-1}$) at 260 nm: Salmon testes, 6600;[29] *M. lysodeikticus*, 6900;[29] poly(dA-dT)·poly(dA-dT), 6600;[30] and poly(dG-dC)·poly(dG-dC), 8400.[31]

For the gel mobility assay a sample of 1 μg of pBR 322 DNA in 10 μL of 2:1 (v/v) triphosphate/MeOH solution containing the copper complex was incubated with topoisomerase I at 37 °C for 3 h. After incubation, the copper compound was extracted with a 4:1 v/v mixture of phenol and chloroform, and the DNA plasmid was subjected to electrophoresis as previously described.[32]

Absorption spectra were recorded with a Perkin-Elmer Lambda 4C spectrophotometer. Emission spectra were recorded with an SLM/Aminco SPF-500C spectrofluorometer. The excitation slit was typically set for a 10-nm bandpass centered around 474 nm, and the emission slit was set for a 5-nm bandpass. A 525-nm long-wave-pass filter was inserted between the sample and the emission monochrometer. Emission polarization measurements were made by employing an "L-shaped" geometry polarization accessory from SLM/Aminco. CD spectra were obtained with a Jasco J-600 recording spectropolarimeter at room temperature.

Viscometric measurements were performed with a Cannon-Manning Semi-Micro 25 E16 viscometer (Cannon Instruments Co.). The viscometer was thermostated at 23 °C in a constant temperature bath. The DNA concentration was 500 μM in the triphosphate/MeOH solution and the flow times were determined with a manually operated timer.

Results

Absorption Spectra. For solubility reasons, previous DNA-binding studies in our laboratory were carried out in solutions

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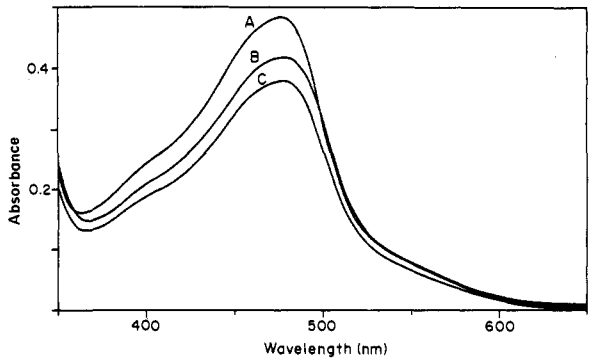

Figure 2. Absorption spectrum of a 25 μm solution of $Cu(bcp)_2^+$ at 25 °C: (A) in 2:1 (v/v) water/MeOH; (B) in 2:1 (v/v) 0.025 M pH 7.8 tris/MeOH containing ST DNA (DNA-P/Cu = 60); (C) in 2:1 (v/v) 0.01 M pH 7.8 triphosphate/MeOH containing ST DNA (DNA-P/Cu = 60).

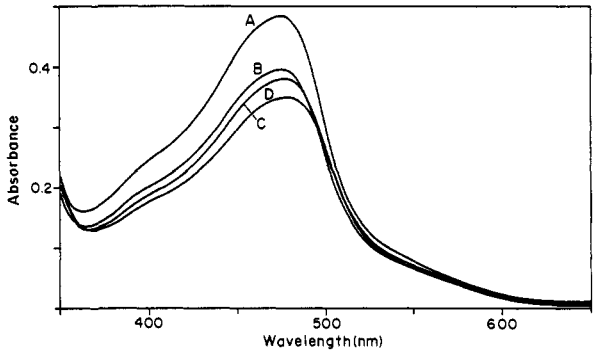

Figure 3. Absorption spectra of solutions containing $Cu(bcp)_2^+$ and ST DNA. DNA-P/Cu values are (A) 0, (B) 15, (C) 60, and (D) 80 in a triphosphate/MeOH solution at 25 °C.

Table I. The Influence of DNA on the Absorption Maximum of $Cu(bcp)_2^+$ at 25 °C in 2:1 Triphosphate/MeOH at DNA-P/Cu = 40

source of DNA	Δλ,[a] nm	Δε, %	AT,[b] %
poly(dA-dT)·poly(dA-dT)	4.1	16	100
ST	3.7	7	42
ML	4.1	5	28
poly(dG-dC)·poly(dG-dC)	5.6	*c*	0

[a] Red shift from the maximum at 474.4 nm in 2:1 water/MeOH. [b] Percentage of adenine-thymine base pairs in the DNA. [c] Sample absorbance decreased with time; see text.

prepared by combining 2 volumes of a 0.025 M pH 7.8 tris buffer with 1 volume of methanol. In an attempt to boost the ionic strength, we switched to a triphosphate buffer, once again in combination with 33% by volume MeOH. However, with this buffer some precipitate formed in the absence of DNA. Therefore, the spectra of samples which did not contain DNA were taken in 2:1 mixture of water and methanol. Figure 2 compares the absorption spectrum of $Cu(bcp)_2^+$ in water/MeOH with the spectrum obtained at a base-to-copper ratio of 60 (DNA-P/Cu = 60) in tris/MeOH as well as the spectrum obtained in triphosphate/MeOH at the same DNA-P/Cu value. In either buffer DNA induced a small red shift and a decrease in absorbance with a larger decrease in the absorption intensity in triphosphate buffer. Figure 3 shows how the red shift increased and the absorbance decreased with the addition of ST DNA as the DNA-P/Cu ratio ranged from 0 to 80. Representative absorption data obtained with different types of DNA are summarized in Table I. The data in Table I are incomplete because a unique complication occurred with poly(dG-dC)·poly(dG-dC). Upon addition of this type of DNA, there was a slow but steady decrease in the visible absorbance, and the rate of bleaching increased with the concentration of poly(dG-dC)·poly(dG-dC). In addition,

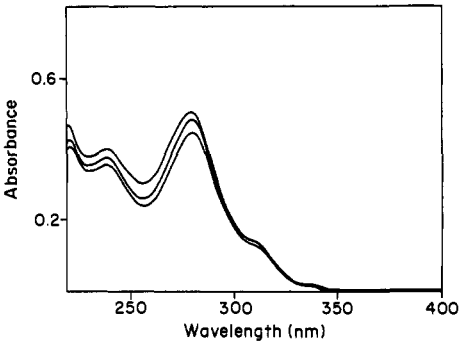

Figure 4. Effect of ST DNA on the absorption spectrum of bcp. The absorbance at 280 nm increases as DNA-P/Cu increases from 0 to 20 to 30 in triphosphate/MeOH solution at 25 °C. The bcp concentration was 12.5 μM, and the spectra have been corrected for the absorbance due to DNA.

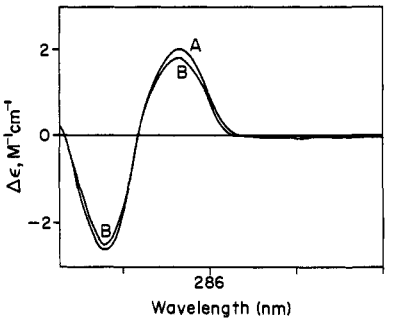

Figure 5. UV circular dichroic spectrum of ST DNA. Spectrum A was obtained in the absence of $Cu(bcp)_2^+$, while spectrum B was obtained at DNA-P/Cu = 31. The solvent was 2:1 (v/v) 0.025 M pH 7.8 tris/MeOH, and the temperature was 25 °C.

there was evidence of precipitate formation even at DNA-P/Cu > 20.

The binding interactions involved in solutions containing $Cu(bcp)_2^+$ and DNA may be complicated by the existence of several equilibria:

$$Cu(bcp)_2^+ \rightleftarrows Cu(bcp)^+ + bcp \quad (1)$$

$$Cu(bcp)^+ \rightleftarrows Cu^+ + bcp \quad (2)$$

$$Cu(bcp)_2^+ + DNA \rightleftarrows Cu(bcp)_2^+/DNA \quad (3)$$

$$Cu(bcp)^+ + DNA \rightleftarrows Cu(bcp)^+/DNA \quad (4)$$

$$bcp + DNA \rightleftarrows bcp/DNA \quad (5)$$

where for simplicity solvent molecules coordinated to copper are not explicitly indicated and where an adduct is designated with a slash between the two components. Since the absorption at the CT maximum of a solution containing ST DNA and $Cu(bcp)_2^+$ was unchanged in the presence of an excess of 2 equiv of bcp per copper ion, eqs 1, 2, and 4 can be ignored. To investigate eq 5, we measured spectra of solutions containing the bcp ligand and DNA. After the contribution from DNA was subtracted out, Figure 4 shows that a slight blue shift and an increase in the absorption intensity occurred, probably due to the dissociation of ligand aggregates.[33-35]

Circular Dichroism. Introduction of $Cu(bcp)_2^+$ into a solution of ST DNA induced a small change in the UV circular dichroic spectrum (Figure 5). The intensities of both the positive and negative ellipticity bands of DNA decreased, with the positive band being affected slightly more than the negative band. Although these changes could be due in part to the bcp

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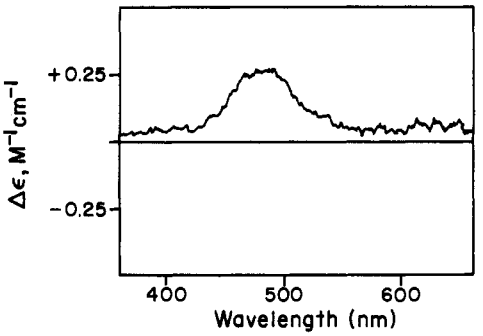

Figure 6. Induced circular dichroic spectrum of $Cu(bcp)_2^+$ in the presence of ST DNA at DNA-P/Cu = 31. Other conditions as in Figure 5.

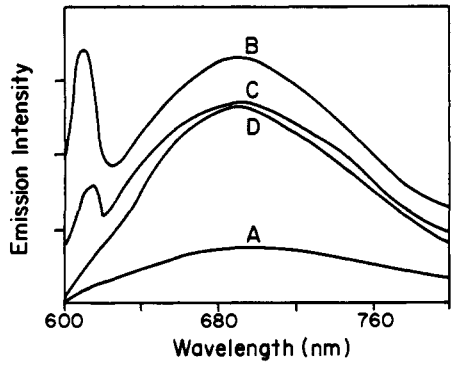

Figure 7. Uncorrected emission spectrum of $Cu(bcp)_2^+$. The DNA-P/Cu ratios were (A) 0, (B) 0.8, (C) 10, and (D) 40. The concentration of copper was 25 μm; excitation wavelength = 474 nm; excitation slit width = 10 nm; emission slit width = 5 nm. Except for the control solution, the solvent was 0.025 M pH 7.8 triphosphate/MeOH.

chromophore, which has an absorption band at 276 nm, the fact that distinct ellipticity changes were observed at both extrema in the spectrum of the DNA strongly suggests that the binding of $Cu(bcp)_2^+$ induces some type of structural change within the DNA molecule. The CD spectrum also exhibited a band with positive ellipticity at 480 nm (Figure 6) which is the location of the charge-transfer absorption band of $Cu(bcp)_2^+$. In contrast, no CD signal could be detected in this region when excess ST DNA was introduced into a solution containing $Cu(dmp)_2^+$.

Luminescence. The luminescence from $Cu(bcp)_2^+$ is barely detectable in pure MeOH but was enhanced more than tenfold in a tris buffer/MeOH solution containing ST DNA.[20] Figure 7 shows that the presence of ST DNA also enhanced the luminescence in a triphosphate/MeOH solution. Note that the complexity of the luminescence spectrum depended upon the DNA concentration. At DNA-P/Cu < 10 the emission spectrum consisted of a broad CT emission band which maximized around 700 nm as well as a much narrower signal centered at 610 nm. The 610-nm band faded out at higher DNA-P/Cu ratios while the CT emission approached a limiting intensity. Because the 610-nm signal could also be eliminated from the emission spectrum by placing a notch filter (nominally, a 485–520 nm band pass filter) between the excitation monochromator and the sample, this component can be assigned to stray light that passes through the excitation monochromator. In all likelihood, the particulates containing $Cu(bcp)_2^+$ that form at low DNA-P/Cu values[19,20] are responsible for scattering the light toward the emission monochromator. Consistent with this interpretation, at higher DNA-P/Cu ratios when the aggregated material is dispersed, the scattering artifact practically vanished. The enhancement in the CT emission intensity produced by excess ST DNA was about fourfold by comparison with the emission from a control solution of $Cu(bcp)_2^+$ in a 2:1 (v/v) mixture of water and methanol. Greater enhancements were observed when the DNA-containing samples were compared with methanolic solutions of $Cu(bcp)_2^+$. Since water is likely to be as good a quencher as methanol, this may suggest that the copper salt was not fully dissolved in the 2:1 (v/v) mixture of water and methanol. (Note that the aggregated form of $Cu(bcp)_2^+$ is emissive.[19])

When different types of DNA were compared at DNA-P/Cu = 50, the relative emission intensities induced by poly(dA-dT)·poly(dA-dT), ST DNA, ML DNA, and poly(dG-dC)·poly(dG-dC) were 1:0.93:0.75:0.47, respectively. In general the luminescence intensity decreased with the AT content of the DNA (Table I). It is also important to note that the emission that was observed retains significant polarization in the presence of DNA. We found that at DNA-P/Cu = 50, the room temperature emission bands exhibited average polarizations of 0.26 and 0.32 in the presence of ST DNA and poly(dA-dT)·poly(dA-dT), respectively. In contrast, the emission from $Cu(bcp)_2^+$ retained almost no polarization when dissolved in CH_2Cl_2 solution.

Viscometry Studies. As a means of further exploring the binding, we carried out two different types of viscosity studies. The first studies were based on the electrophoretic mobility of the pBR 322 plasmid which can be obtained in a covalently closed supercoiled (ccs) form that is compact and highly mobile. In contrast, the covalently closed relaxed form, which can be produced from the ccs form by treatment with topoisomerase I, has a larger effective size and decreased mobility. Several electrophoresis experiments and controls were carried out in which the ccs form of the plasmid was equilibrated wth $Cu(bcp)_2^+$ prior to treatment with topoisomerase I. At DNA-P/Cu = 30, the relaxed form of the DNA appeared to be the primary species present after workup. On the other hand, at DNA-P/Cu = 15, the electrophoresis pattern showed evidence of the ccs form and the fully relaxed form of the DNA as well as a range of species with intermediate mobilities.

The second type of viscosity measurement was based on the flow rate of a DNA solution through a capillary viscometer. In these experiments the specific viscosity contribution (η) due to the DNA was measured as a function of the concentration of $Cu(bcp)_2^+$ added. The advantage of this method was that a copper concentration could be used which was quite similar to that used in the spectral work. Therefore, we place more emphasis on these results.

Relative values η/η_0, where η_0 is the specific viscosity of the DNA sample in the absence of binding agent, are plotted as a function of the copper/base ratio in Figure 8. The results indicate that the presence of $Cu(dmp)_2^+$ had no effect on the viscosity of ST DNA, whereas the presence of $Cu(bcp)_2^+$ had a marked, but variable effect. Thus, the specific viscosity of the ST DNA sample increased with the addition of the bcp complex until there was approximately one copper ion present for every 20 bases; then the viscosity decreased and dropped below that of the control when there were fewer than 10 bases present per copper ion. When $Cu(bcp)_2^+$ was added to a solution containing ML, the viscosity decreased at all copper-to-base ratios investigated (Figure 8).

Discussion

Current Binding Models. The binding interactions between copper phenanthrolines and DNA molecules have generally been considered in the context of the docking of a relatively small molecule on a large biopolymer. Since hydrogen-bonding interactions are not likely to be important with ordinary phenanthroline ligands, the forces responsible for the binding can be roughly described as a combination of electrostatic and hydrophobic interactions. Electrostatics are important because B-form DNA is a polyanion composed of two complementary, polymeric subunits hydrogen bonded together in the form of a right-handed double helix.[36] The "atmosphere" surrounding the polymer can crudely be described in terms of two shells, or layers,

(36) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapter 11, pp 253–282.

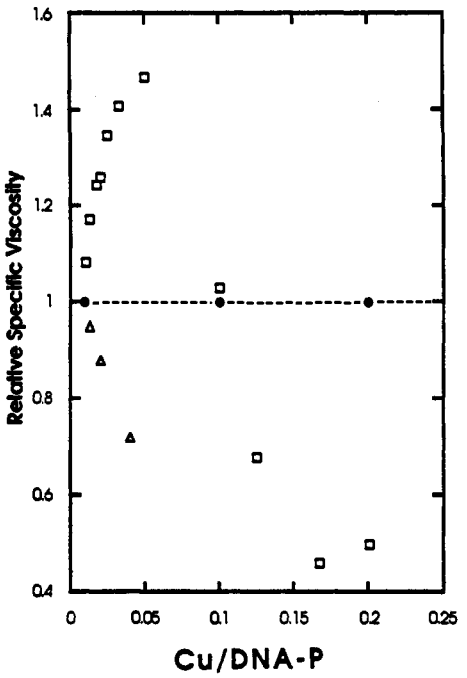

Figure 8. Ratio of the specific viscosity of DNA in the presence of copper complex to that of free DNA versus in Cu/DNA-P ratio Data are reported for ST DNA in the presence of $Cu(bcp)_2^+$ (□) or $Cu(dmp)_2^+$ (●) as well as for ML DNA in the presence of the bcp complex (△). The temperature was 23 °C, and the solvent was 2:1 (v/v) 0.025 M pH 7.8 triphosphate/MeOH. The total DNA base concentration was 500 μM.

containing appropriate counterions and solvent.[6] The inner shell (the Stern layer) is relatively compact and includes a sheath of counterions, enough to neutralize perhaps 50% of the ionized phosphodiester moieties in the polymer chains. Additional counterions are more loosely bound in a diffuse outer layer, which is analogous to the Guoy–Chapman layer at a solid electrode. When a cationic binding agent such as $Cu(phen)_2^+$ binds to DNA, it is likely to replace a cation from one or the other layer, hence the binding will be sensitive to the nature of the supporting electrolyte and the ionic strength. Steric effects and hydrophobic interactions are important as well. On the atomic scale the surface of B-form DNA is highly textured and features two continuous grooves, the major and minor grooves, that wind from one end of the molecule to the other. Chains of phosphate groups and sugar molecules condensed together in an alternating fashion rim the grooves, while the bulk of the 2′-deoxyribose units and the edges of the base pairs define the walls and the floors of the grooves, respectively. Small hydrophobic molecules or molecules with hydrophobic domains may migrate to sites within the grooves whereupon solvent molecules stationed in the groove region or on the surface of the binding agent are free to escape into the bulk solution. A more invasive type of binding, known as intercalation, occurs when the double helix unwinds enough to permit, for example, a fused-ring aromatic system to slide into the space created between a set of adjacent base pairs. Because the phenanthroline ligands in $Cu(phen)_2^+$ are roughly perpendicular to each other, complete intercalation is sterically impossible, but some type of partial intercalation involving one of the ligands can be envisioned.

External Binding of $Cu(dmp)_2^+$. The fact that the CT absorption band of $Cu(dmp)_2^+$ exhibits a bathochromic shift and hypochromism in the presence of DNA shows that the complex binds to the macromolecule.[18-20] The effects are not large, however, and under different conditions Veal and Rill could find no evidence for binding of the dmp complex.[17] In other studies, Graham and Sigman carried out equilibrium dialysis experiments which showed that $Cu(dmp)_2^+$ interacts with DNA.[37] Our results indicate that adduct formation has little influence on the DNA structure, because the specific viscosity of the DNA is unaffected by the presence of $Cu(dmp)_2^+$ (Figure 8). Moreover, since the CT emission from the complex is virtually completely quenched, the bound form must be quite solvent accessible. These findings are indicative of an external mode of binding to DNA and consequently very modest electronic coupling with the host. Groove binding is likely because it would provide for coulomic interactions with the phosphate diester moieties and a favorable entropy term due to the release of solvent molecules. Similar forces should influence the binding of $Cu(bcp)_2^+$, but classical groove binding is inadequate to explain all of the experimental results obtained with this system.

Nonclassical Behavior of $Cu(bcp)_2^+$. The spectral perturbation in the visible absorption spectrum of $Cu(bcp)_2^+$, as well as the induced CD signal, indicates that the bcp complex engages in a more intimate mode of binding with the DNA host. This is also evident from the persistance of the CT emission and the fact that the emission is strongly polarized. Previous work with copper phenanthrolines has shown that the charge-transfer absorption intensity is polarized along the axis which connects the metal ion to the center of mass of each ligand.[38,39] The emission is polarized along the same axis;[40,41] however, if the complex is free to tumble in solution, the information about the orientation in the laboratory frame of reference at the moment of absorption is lost since the excited state lifetime exceeds the rotational correlation time. On the other hand, when the complex is bound to DNA rigidly enough, the rotational correlation time approaches that of the macromolecule, and the polarization information is retained.

As pointed out previously,[18,20] the spectral results for the bcp complex are consistent with partial intercalation into DNA because this mode of binding would be quite likely to inhibit solvent-induced quenching, to prevent the complex from tumbling indpendently of the host, and to provide for intimate enough association to account for the comparatively strong hypochromism. This interpretation of the results also squares with the ideas of Barton and co-workers, who have concluded that $Ru(bap)_3^{2+}$ shows a strong tendency to intercalate into DNA.[42] However, our viscometry results present a problem.

The viscometry data show that there are at least two phases of binding between $Cu(bcp)_2^+$ and ST DNA (Figure 8). First, consider the higher loading regime, when the copper-to-base ratio exceeds a value of about 0.1. Under these circumstances the addition of $Cu(bcp)_2^+$ induces a decrease in the specific viscosity of the DNA. In principle, this could be explained by any of a number of effects including a change in the conformation, the flexibility, or the solvation of the DNA molecules. However, in view of the tendency toward particulate formation, vide supra, the most likely explanation is aggregation of the DNA. Extensive aggregation would sharply reduce the number of independently moving DNA molecules in solution and therefore the viscous drag that comes from molecules diffusing into each other. At high loadings of $Cu(bcp)_2^+$, similar behavior is observed with every type of DNA investigated, but with ST DNA or poly(dA-dT)·poly(dA-dT) the bcp complex induces an *increase* in the specific viscosity at low copper-to-base ratios.

At first glance, it is tempting to ascribe the viscosity increase to intercalative binding because this would cause the effective length of the DNA to increase. In essence the length of a linear piece of B-form DNA is given by the sum of the thicknesses of the base pairs that are stacked along the helix axis in van der Waals contact with each other. Introducing another aromatic molecule into the stack therefore increases the length. At the

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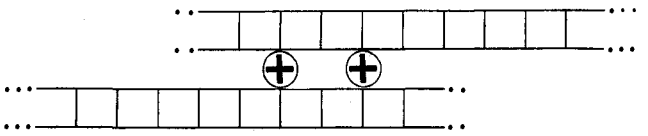

Figure 9. A schematic picture of a pair of B-form DNA duplexes bridged by $Cu(bcp)_2^+$ ions. The circumscribed crosses represent the copper complexes.

same time, some unwinding of the DNA helix has to occur in order to create the requisite space between two adjacent base pairs. Hiort et al. have, however, cautioned that other types of interaction may cause unwinding as well.[43] In particular, they have suggested that this may happen when bulky ruthenium phenanthrolines bind in the groove of DNA. Alternatively, agents which increase the rigidity of the DNA or induce appropriate conformational changes, e.g., by reducing the number of hairpin loops in poly(dA-dT)·poly(dA-dT), can cause an enhancement in the viscosity.[44]

Although any of these effects could be operative in our system, none seems adequate to explain the viscosity enhancements depicted in Figure 8. The problem is that the large viscosity increases occur at base-to-copper ratios of about 20 when only a fraction of the total number of lattice sites are occupied. For the sake of comparison, note that the viscosity ratio reaches a value of 1.4 at a base-to-drug ratio of about 5 for a classical intercalator like ethidium bromide.[17] To account for our results, each copper complex that binds would have to induce a long run of relatively rigid structure in the DNA molecule. This seems unlikely in view of the model of the DNA structure which is emerging from X-ray diffraction studies.[45] These studies show that DNA is highly adaptable and suggest that the influence of a small molecule bound to DNA is unlikely to propagate over a very great distance.

Our viscometry results can more readily be explained in terms of bridged structures such as the one depicted schematically in Figure 9. In this model the enhanced specific viscosity occurs because of the increase in the effective length of the species that has to diffuse through the medium. In a sense, this type of structure might be viewed as an outgrowth of the aggregates that are observed at the higher copper-to-base ratios. With the end-to-end approach illustrated in Figure 9 the two DNA duplexes can interact with one or more copper complexes while minimizing the coulombic repulsions between duplexes. The structure presented is, of course, highly schematic. The presence of multiple $Cu(bcp)_2^+$ ions between the DNA molecules would be expected to stabilize the bridged structure and to promote parallel extension of the duplexes in the overlap region. On average there are about 10 copper complexes available per double helix when the viscosity is maximum. This type of adduct would also be consistent with the emission data since interactions with two different biopolymers would be likely to protect the metal center from solvent attack.

How the bcp complex docks at a given duplex is unclear, but viscosity increases have only been observed with DNA that is rich in adenine and thymine base pairs. Runs of AT base pairs are believed to be relatively flexible because only two hydrogen bonds connect the base pairs. Thus, some degree of local melting of the DNA molecules may occur in order to maximize the hydrophobic interactions. Spontaneous unraveling of the double helix can even occur at the ends, particularly when AT base pairs are involved. Other workers have suggested that the uranyl ion is capable of bridging between duplexes.[46] With the uranyl ion, however, the binding involves innersphere complex formation via phosphate oxygens. In the case of the bcp complex hydrophobic interactions are clearly more important.

Perspective. We selected the dmp and bcp ligands with the idea that the results would provide information relevant to understanding the chemistry of the more redox-active copper(I) phenanthrolines that act as artificial nucleases. Because of the added bulk of the 2,9-dimethyl substituents, there may, however, be differences in some of the details regarding binding. The change of solvent may have an influence as well since we have used 33% MeOH to avoid solubility problems. Nevertheless, the results help identify factors that shape the binding phenomena. Thus, the fact that hydrophobic interactions are critically important even in aqueous alcohol emphasizes the major role they play. Along these lines, it would be interesting to determine whether the presence of alcohol has a significant effect on DNA cutting by $Cu(phen)_2^+$.

The connections between our work and DNA-binding studies involving ruthenium(II) phenanthrolines are less direct. The confounding effects are that the ruthenium complexes have a different coordination geometry (pseudooctahedral) and that they carry an extra positive charge. Barton and co-workers have suggested that the Δ isomer of $Ru(phen)_3^{2+}$ approaches DNA via the major groove and binds intercalatively with a phenanthroline ligand inserted between adjacent base pairs.[47] They have also concluded that the Λ isomer binds via a surface association in the minor groove. However, recent NMR data suggest that both isomers interact via the minor groove.[48] Moreover, contrary to what one would expect for intercalation, viscometry data indicate that $Ru(phen)_3^{2+}$ does not cause significant elongation of the DNA. On the basis of these findings, Dabrowiak and co-workers have proposed that there is limited penetration of the space between base pairs and that the binding of $Ru(phen)_3^{2+}$ may cause a local kinking of the helix axis.[49] As with the copper(I) systems hydrophobic interactions are quite important,[47] and the formation of the $Ru(phen)_3^{2+}$ adduct is an endothermic process.[49]

Aromatic molecules containing three or four fused rings are ordinarily good candidates for intercalative binding.[50] However, in the case of a metal complex like $Cu(phen)_2^+$ or $Ru(phen)_3^{2+}$ the intercalation of a phen ligand is probably inhibited by steric interactions involving the other phen ligands bound at the metal. In accord with this reasoning, analogous complexes containing a ligand with a more extended fused-ring system appear to be capable of intercalation.[51,52] Stereoelectronic effects have to be weighed carefully, however. Ligands with more extended π systems involving unfused rings, such as the bap ligand in $Ru(bap)_3^{2+}$, are not necessarily good candidates, even though $Ru(bap)_3^{2+}$ has been suggested to be an effective intercalator.[42] In view of our results with $Cu(bcp)_3^{2+}$, the $Ru(bap)_3^{2+}$ system should be reinvestigated. Viscometry studies would indicate whether it behaves like a classical intercalator or if it binds in another fashion, e.g., by forming bridged adducts such as $Cu(bcp)_2^+$.

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