complexes, as shown in Figure 5. A similar plot spanning a larger range of σ° values for a series of *fac*-(L)Re(CO)$_3$Cl complexes[53] is included for comparison. It is clear from Figure 5 that, while excellent linear correlations with σ° Hammett constants are exhibited by the absorption and emission energies of the *fac*-(L)-Re(CO)$_3$Cl complexes, considerable scatter occurs for the data relative to the *cis*-L$_2$Os(CO)Cl$^+$ complexes. Such scatter perhaps reflects inherent uncertainties in the band positions and the limited range of L for which complexes are synthetically accessible in the Os series.

A comparison of the *cis*-L$_2$Os(CO)Cl$^+$ and *fac*-(L)Re(CO)$_3$Cl complexes in Figure 5 yields ρ_{Os}(abs) = 5.83, ρ_{Os}(em) = 4.15 and ρ_{Re}(abs) = 12.07, ρ_{Re}(em) = 9.75, respectively, for these two series. These values are obtained from eq 15, where ν and ν° represent

$$\nu = \nu^\circ - (477\ \text{cm}^{-1})\rho_M\sigma^\circ \qquad (15)$$

the transition energies in cm^{-1} for the unknown and reference (L = bpy) complexes, respectively. ρ_M is the sensitivity parameter for complexes of metal M, and the remaining terms are defined at 25 °C analogous to eq 6 or 14. The large positive ρ_M value in each case is consistent with charge generation processes occurring near the L substituents, as expected for these transitions. The factor of ca. 2 difference between ρ_{Re} and ρ_{Os} can be accounted for by symmetry differences between the two families of complexes.[41] Comparable values of ρ_M are obtained after application of the necessary correction consistent with the proposed identical natures of the transitions (i.e., MLCT) for each family of complexes.

(52) (a) The two sets are rather similar, showing significant differences only for powerful +M groups such as NR$_2$ and OR. Such variations reflect the fact that resonance contributions to σ values are dependent on the electronic demands of a reaction.[52b] Thus, while the original Hammett σ constants, obtained by designating the ionization, in water at 25 °C, of meta- and para-substituted benzoic acids (i.e. a weak acceptor system) as the standard reference reaction, tend to emphasize the mesomeric effects of the donor groups, the unbiased values σ° provide a better fit for systems with nonconjugated reaction centers. Since it is not always possible to determine a priori the electronic demands of a reaction (σ^+ and σ^- scales have also been proposed for strong acceptor and strong donor systems, respectively), the best approach remains to choose whichever scale produces the best fit. (b) Reynolds, W. F.; Dais, P.; Taft, R. W.; Topson, R. D. *Tetrahedron Lett.* **1981**, *22*, 1795.

(53) Della Ciana, L.; Sullivan, B. P.; Meyer, T. J. *J. Am. Chem. Soc.*, submitted for publication.

Conclusions

We summarize our findings concerning the family of *cis*-L$_2$Os(CO)Cl$^+$ complexes as follows:

(1) *cis*-L$_2$Os(CO)Cl(PF$_6$) complexes can be synthesized from the reaction of *cis*-L$_2$OsCl$_2$ (L $\neq$ (NEt$_2$)$_2$bpy) with CO in ethylene glycol in a straightforward extension of literature methods.[16] For L = (NEt$_2$)$_2$bpy, *cis*-((NEt$_2$)$_2$bpy)$_2$Os(CO)Cl$^+$ can be produced by reflux of a *cis*-((NEt$_2$)$_2$bpy)$_2$Os(η^1-phosphine)Cl(PF$_6$) intermediate in glycerol. Preliminary experiments indicate that the importance of this new pathway to the *cis*-L$_2$Os(CO)Cl$^+$ species is a strong function of the basicity of the Os(II) center.

(2) The *cis*-L$_2$Os(CO)Cl(PF$_6$) complexes are assigned the cis configuration on the basis of results of ^{1}H NMR experiments. Using spin-decoupling and NOE experiments, it is possible to completely assign (note supplementary material) the proton resonances of the complexes. This represents the first application of the NOE technique to our knowledge in the study of luminescent (α-diimine)Os^{II} complexes. The configuration of the L about the Os(II) center can be assigned unambiguously by using chemically intuitive arguments based on the NMR results.

(3) Ground- and excited-state properties of the *cis*-L$_2$Os-(CO)Cl(PF$_6$) complexes are similar to those observed for related complexes.[16,17] MLCT excited-state deactivation is dominated by nonradiative processes via CO stretch acceptor vibrations. Correlations of IR, electrochemical, and spectral properties of the complexes with Hammett substituent constants are observed. Control of these properties by the L substituent in this family of complexes permits their estimation for any new member through knowledge of its Hammett constant.

Acknowledgment. Financial support for this work by the University of Fribourg, Swiss National Science Foundation, Ministero della Pubblica Istruzione, and Consiglio Nazionale delle Ricerche of Italy is gratefully acknowledged. We thank Drs. F. Fehr and T. A. Jenny for assistance in the acquisition and interpretation of all ^{1}H NMR data. We also thank Drs. B. P. Sullivan and T. J. Meyer for preprints of the spectral data for the *fac*-(L)Re(CO)$_3$Cl complexes.

Supplementary Material Available: Discussion of structure assignment and ^{1}H NMR analysis, tables of data for elemental and ^{1}H NMR analysis, and a structure of the *cis*-L$_2$Os(CO)Cl(PF$_6$) complexes (12 pages). Ordering information is given on any current masthead page.

Contribution from the Department of Chemistry,
Purdue University, West Lafayette, Indiana 47907-3699

Photophysical Studies of Copper Phenanthrolines Bound to DNA

Ramasamy Tamilarasan and David R. McMillin*

Received November 14, 1989

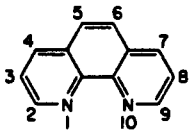
Electronic absorption and emission studies have been carried out for Cu(phen)$_2^+$, Cu(dmp)$_2^+$, and Cu(bcp)$_2^+$ interacting with a range of DNA duplexes, where phen denotes 1,10-phenanthroline, dmp denotes 2,9-dimethyl-1,10-phenanthroline, and bcp denotes 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline. Hypochromism is observed in the visible absorption bands of all three copper(I) complexes, but the bcp complex is unique in that binding to DNA causes the appearance of a measurable luminescence signal at room temperature. At low DNA-P/Cu ratios Cu(bcp)$_2^+$ appears to bind to DNA polymers in an aggregated form, but at high DNA-P/Cu ratios the complex binds as a monomer. The adduct of the bcp complex with DNA must be relatively rigid in the vicinity of the copper center because solvent-induced, exciplex quenching of the excited state is strongly inhibited. This suggests that the complex binds by intercalation, and emission polarization data support this model. The limiting emission intensity varies with the type of DNA used and is greater with poly(dA-dT)·poly(dA-dT) than with poly(dG-dC)·poly(dG-dC), possibly because propeller twisting facilitates intercalation. Interaction probably occurs within the major groove since T4 DNA, which is glycosylated in the major groove, does not induce monomer emission. None of the duplexes studied induced emission from Cu(dmp)$_2^+$, presumably because the dmp complex binds by another mechanism. Yeast tRNA was also effective at inducing emission from Cu(bcp)$_2^+$; hence the complex is also capable of intercalating into an A-form duplex.

Introduction

Metal complexes containing polypyridine and/or 1,10-phenanthroline (phen) ligands have been shown to be useful probes of DNA polymers because of binding interactions that occur in aqueous media.[1-3] Various chemical and physical methods can be used to characterize these systems. For example, photophysical techniques are being used to obtain information about DNA

(1) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180-186.
(2) Barton, J. K. *Science* **1986**, *233*, 727-734.

0020-1669/90/1329-2798$02.50/0



phen

binding interactions involving luminescent ruthenium(II) systems.[2,4-8] In contrast, $Cu(phen)_2^+$ is nonluminescent and serves as a convenient chemical probe.[1,9] Thus, in the presence of molecular oxygen and a reducing agent, $Cu(phen)_2^+$ acts as an efficient artificial nuclease, catalyzing the oxidative cleavage of ribose links within the DNA host. Hydrogen peroxide is an important intermediate in the process, which involves several steps. Scheme I represents a minimal reaction sequence,[1,10] where Red. and Ox. denote the reduced and the oxidized forms of a sacrificial reagent such as a thiol and where $Cu(phen)_2^+|DNA$ represents a noncovalently bound adduct of the copper complex with DNA. Formation of an adduct is invoked to account for the efficiency and the sequence dependence of cleavage as well as the sensitivity to substituents on the phen ligand.[1] In light of what is known about the chemistry of the copper center as well as hydrogen peroxide, eq 4 is expected to occur via an inner-sphere mechanism that leads to a reactive metal–oxo or metal–hydroxyl intermediate.[9,11]

Scheme I

$$2Cu(phen)_2^+ + O_2 + 2H^+ \rightarrow 2Cu(phen)_2^{2+} + H_2O_2 \quad (1)$$

$$Cu(phen)_2^{2+} + \text{Red.} \rightarrow Cu(phen)_2^+ + \text{Ox.} \quad (2)$$

$$Cu(phen)_2^+ + DNA \rightleftharpoons Cu(phen)_2^+|DNA \quad (3)$$

$$Cu(phen)_2^+|DNA + H_2O_2 \rightarrow \text{cleavage products} \quad (4)$$

Despite the effort that has been devoted to the investigation of the $Cu(phen)_2^+$ system, the factors that determine the DNA binding interaction(s) have yet to be defined. On the basis of a variety of cleavage studies, Sigman has proposed that $Cu(phen)_2^+$ binds at the surface of DNA within or about the minor groove.[1,9,10] The key observation made by Sigman and co-workers[1,12] is that cleavage of the sugar is initiated by a hydrogen atom abstraction reaction that occurs regiospecifically at the C1′ or C4′ carbon, which lies within the minor groove. Also relevant is the demonstration that scission of the central AATT region of a synthetic deoxyoligonucleotide is inhibited when Netropsin is bound along the minor groove.[13] Finally, 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.[14] On the other hand, Williams et al. have carried out systematic cleavage studies of oligonucleotides containing mismatched strands, and their data indicate that $Cu(phen)_2^+$ binds preferentially near bulged regions where intercalative binding is thought to be favored.[15] Still different behavior has been observed in studies involving $tRNA^{Phe}$ where Murakawa et al. have shown that $Cu(phen)_2^+$ preferentially cuts in the single-stranded loop regions rather than stem regions where the groove structure exists.[16]

Even though the interpretation of the cleavage results requires that some type of binding occur, this kind of experiment does not necessarily provide direct information about the phenomenon. One reason is that multiple modes of binding may occur, not all of which need give rise to productive cleavage. Also, if the putative cupryl intermediate has a finite lifetime, it may diffuse along the polymer, in which case the cleavage pattern reflects the inherent reactivity of DNA functionalities and/or binding equilibria involving the cupryl species.

In order to obtain more information about the key binding step, eq 3 in Scheme I, we have carried out DNA-binding studies involving copper(I) complexes of the dmp (2,9-dimethyl-1,10-phenanthroline) and bcp (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) ligands. The $Cu(dmp)_2^+$ and $Cu(bcp)_2^+$ complexes are useful spectroscopic probes because they exhibit relatively positive reduction potentials due to the influence of the methyl substituents.[11] As a result, they do not catalyze the cleavage of DNA efficiently; thus, we can isolate the binding equilibrium in Scheme I.

Table I. Effect of ST DNA on the Absorbance at 20 °C

complex	λ_{max}, nm: DNA-P/Cu = 0	λ_{max}, nm: DNA-P/Cu = 20	$\Delta\epsilon$,[a] %
$Cu(phen)_2^+$	433.5	434.6	5
$Cu(dmp)_2^+$	455.6	457.6	10
$Cu(bcp)_2^+$[b]	474.4	477.7	7

[a] In all cases the apparent molar absorptivity decreased when DNA was present. [b] In 33% MeOH aqueous buffer.

Experimental Section

Materials. The compounds $[Cu(dmp)_2]X$ ($X = Cl^-$ or BF_4^-), $[Cu(phen)_2]BF_4$, and $[Cu(bcp)_2]X$ ($X = Cl^-$, HSO_4^-, or BF_4^-) were prepared by variations of a previously reported procedure.[17] Trisma base, Trisma·HCl, salmon testes (ST) DNA, T4 phage DNA, and yeast tRNA were purchased from Sigma Chemical Co. Poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) were purchased from P. L. Biochemicals Inc. Covalently closed supercoiled $\phi X174$ RF I DNA from *Escherichia coli* was purchased from New England Biolabs. The synthetic oligonucleotide was a gift from Steven Schroeder of Purdue University. It was actually a mixture of byproducts isolated during the chemical synthesis of the 21-mer with the sequence 5′-dA-dA-dT-dT-dG-dT-dT-dA-dT-dC-dC-dG-dC-dT-dC-dA-dC-dA-dA-dT-dT-3′. With three mismatches this chain is not palindromic, but it does form a duplex in solution. The average chain length of the mixture is estimated to be 15. ST DNA was purified twice by precipitation with ethanol; the other biopolymers were used without further purification. Except for the synthetic oligonucleotide system (15-mer), DNA samples were syringed four times through a 0.22 gauge needle in order to reduce the viscosity and decrease the average chain length. Electrophoresis grade agarose was supplied by Bethesda Research Laboratories. All other chemicals used were reagent grade.

Methods and Instrumentation. 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 band-pass, and the emission slit was set for a 5-nm band-pass. A 525-nm long-wave-pass filter was inserted between the sample and the emission monochromator. The emission spectra were recorded with 474-nm excitation, and the emission quantum yields were determined by the method of Parker and Rees.[18] Emission polarization was measured in the "L" configuration by means of polaroid filters and was corrected for the instrumental response.[19] Luminescence lifetimes were measured with a nitrogen-pumped dye laser

(3) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107–1136.
(4) Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 2081–2088.
(5) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051–3058.
(6) Kelly, J. M.; Tossi, A. B.; McConnell, D. J.; OhUigin, C. *Nucleic Acids Res.* **1985**, *13*, 6017–6034.
(7) Tossi, A. B.; Kelley, J. M. *Photochem. Photobiol.* **1989**, *49*, 545–556.
(8) Barton, J. K. *Chem. Eng. News.* **1988**, *66* (39), 30–42.
(9) Sigman, D. S.; Chen, C.-H. B. In *Metal-DNA Chemistry*; Tullius T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989; pp 24–47.
(10) Thederahn, T. B.; Kuwabara, M. D.; Larsen, T. A.; Sigman, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 4941–4946.
(11) Tamilarasan, R.; McMillin, D. R.; Liu, F. In *Metal-DNA Chemistry*; Tullius T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989; pp 48–58.
(12) Goyne, T. E.; Sigman, D. S. *J. Am. Chem. Soc.* **1987**, *109*, 2846–2848.
(13) Kuwarbara, M.; Yoon, C.; Goyne, T.; Thederahn, T.; Sigman, D. S. *Biochemistry* **1986**, *25*, 7401–7408.
(14) Yoon, C.; Kuwabara, M. D.; Law, R.; Wall, R.; Sigman, D. S. *J. Biol. Chem.* **1988**, *263*, 8458–8463.
(15) Williams, L. D.; Thivierge, J.; Goldberg, I. H. *Nucleic Acids Res.* **1988**, *16*, 11607–11615.
(16) Murakawa, G. J.; Chen, C. B.; Kuwabara, M. D.; Nierlich, D. P.; Sigman, D. S. *Nucleic Acids Res.* **1989**, *17*, 5361–5375.
(17) McMillin, D. R.; Buckner, M. T.; Ahn, B. T. *Inorg. Chem.* **1977**, *16*, 943–945.
(18) Parker, C. A.; Rees, W. T. *Analyst (London)* **1960**, *85*, 587–600.
(19) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1983; Chapter 5.

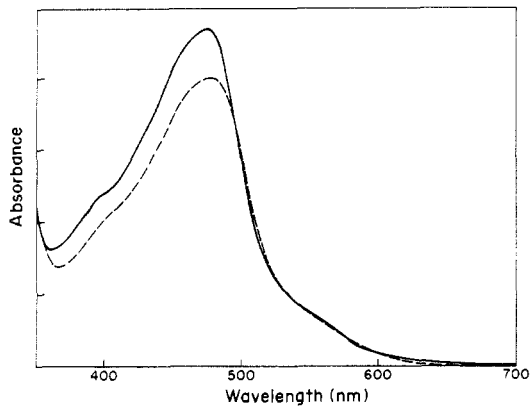


Figure 1. Absorption spectra of $Cu(bcp)_2^+$ in the absence (—) and the presence of ST DNA (– – –) in the 1/3 by volume MeOH/aqueous buffer. The DNA-P/Cu ratio was 50, the temperature was 20 °C, and the copper concentration was 25 μM.

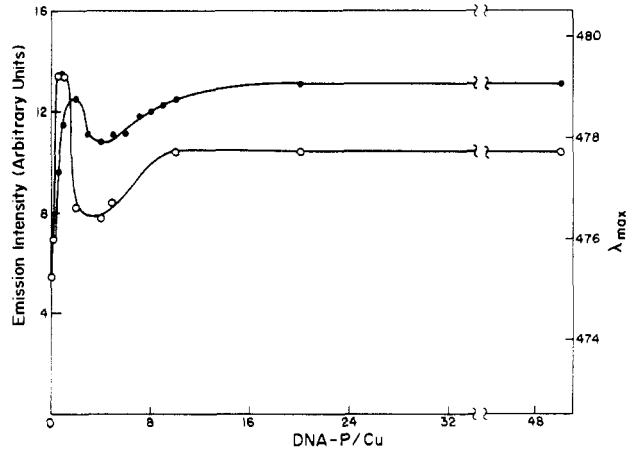


Figure 2. Dependence of the CT emission intensity (●) and the CT absorption maximum (○) of $Cu(bcp)_2^+$ on the DNA-P/Cu ratio in the 1/3 by volume MeOH/aqueous buffer. The temperature was 20 °C, and the DNA was from salmon testes. The copper concentration was 26 μM for the emission studies and 33 μM for the absorption work.

system as described previously.[20] All the spectral measurements were made with a copper concentration of 20–30 μM in 0.025 M pH 7.8 Tris buffer. For solubility reasons we used a mixed solvent containing MeOH and 0.025 M pH 7.8 Tris buffer for experiments pertaining to the bcp complex. Samples were typically prepared by combining aliquots of a DNA solution and a copper-containing stock solution, each of which had previously been diluted into the 33% MeOH aqueous buffer. The concentrations of the nucleic acid solutions were determined from the reported molar absorptivity values at 260 nm (6600 M^{-1} cm^{-1} for ST DNA,[21] poly(dA-dT)·poly(dA-dT),[22] and 15-mer; 8400 M^{-1} cm^{-1} for poly(dG-dC)·poly(dG-dC)[23]; 7700 M^{-1} cm^{-1} for yeast tRNA[24]; 8000 M^{-1} cm^{-1} for T4 DNA[25]).

Dye gel electrophoresis of the circular DNA was carried out in 1% agarose by the method of Espejo et al.[26] in a mini slab (horizontal) gel electrophoresis apparatus with the complex dissolved in the bath buffer and the gel matrix. The diffusion time was 30 min against 50 V of applied voltage.

Results

Absorption and Steady-State Luminescence. As was the case with $Ru(phen)_3^{2+}$,[2,5] interaction with DNA induced a small red shift in the charge-transfer absorption envelope of each copper complex as well as a decrease in the molar absorptivity at the absorption maximum. This effect is illustrated in Figure 1, and the results are summarized in Table I. Uniquely in the case of $Cu(bcp)_2^+$, introducing DNA also led to a dramatic increase in the luminescence intensity. In the absence of DNA the luminescence signal from $Cu(bcp)_2^+$ was barely detectable, while the intensity increased about 12-fold when excess DNA was added. As can be seen in Figure 2, the emission intensity and the absorption maximum varied in a similar, but complicated, way with the DNA-P/Cu ratio, i.e., the DNA phosphate to copper ratio in solution. These plots reveal that distinct binding interactions involving $Cu(bcp)_2^+$ occurred at high and at low DNA-P/Cu ratios. As argued below, a high DNA-P/Cu ratio favors binding of the monomeric form of the copper complex, and most of the data were collected under these conditions. To ensure solubility, all of the studies involving $Cu(bcp)_2^+$ were carried out in 33% MeOH. Although smaller alcohol concentrations could be used when DNA was present, the 33% MeOH composition was chosen so that appropriate control experiments could be run in the absence of DNA. Circular dichroism measurements have shown that the DNA conformation is hardly affected by the presence of alcohol in solution until much higher levels are attained.[27]

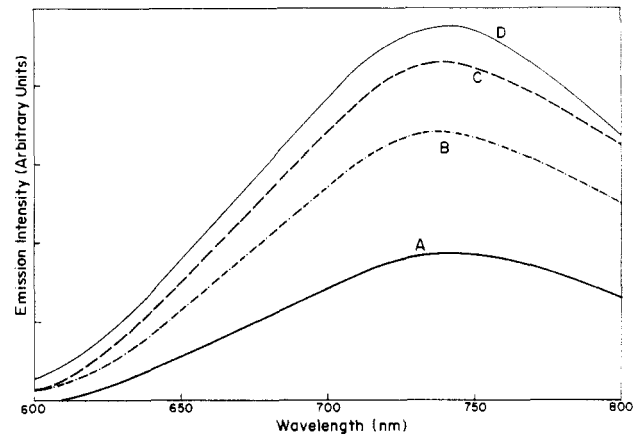


Figure 3. Corrected emission spectra from $Cu(bcp)_2^+$ in the 1/3 by volume MeOH/aqueous buffer at a DNA-P/Cu ratio of 20: (A) poly(dG-dC)·poly(dG-dC); (B) ST DNA; (C) poly(dA-dT)·poly(dA-dT); (D) yeast tRNA. The copper concentration was 8.7 μM, and the temperature was 20 °C.

Table II. Relative Quantum Yields and Lifetimes for $Cu(bcp)_2^+$ in 33% MeOH Aqueous Buffer with DNA-P/Cu = 50

DNA	ϕ^a	τ,[a] ns
ST	1.0	69
15-mer	1.0	64
poly(dG-dC)·poly(dG-dC)	0.6	65
poly(dA-dT)·poly(dA-dT)	1.3	67
yeast tRNA	1.4	67

[a] Estimated experimental uncertainties are ±10%.

All but one of the biopolymers employed produced an increase in the emission intensity from $Cu(bcp)_2^+$. Representative emission spectra are plotted in Figure 3. Integrations of the corrected spectra yielded the relative emission efficiencies listed in Table II. Within experimental error, the excitation spectrum obtained from $Cu(bcp)_2^+$ in the presence of ST DNA is congruent with the visible absorption spectrum. Introduction of T4 DNA, which is glycosylated in the major groove,[28] had almost no effect on the emission spectrum. The T4 DNA was also unique in that it yielded a precipitate when combined with $Cu(bcp)_2^+$ in 7% MeOH, even at a DNA-P/Cu ratio greater than 20.

The fraction of the emission that retained the polarization of the excitation was calculated according to eq 5. where $I_\parallel$ denotes

$$\rho = (I_\parallel - I_\perp)/(I_\parallel + I_\perp) \quad (5)$$

(20) Gamache, R. E., Jr.; Rader, R. A.; McMillin, D. R. *J. Am. Chem. Soc.* **1985**, *107*, 1141–1146.
(21) Felsenfeld, G.; Hirschman, S. Z. *J. Mol. Biol.* **1965**, *13*, 407–427.
(22) Inman, R. B.; Baldwin, R. L. *J. Mol. Biol.* **1962**, *5*, 172–184.
(23) Wells, R. D.; Larson, J. E.; Grant, R. C.; Shortle, B. E.; Cantor, C. R. *J. Mol. Biol.* **1970**, *54*, 465–497.
(24) Morrison, W. R. *Anal. Biochem.* **1964**, *7*, 218–224.
(25) Tissières, A. *J. Mol. Biochem.* **1959**, *1*, 365–374.
(26) Espejo, R. T.; Lebowitz, J. *Anal. Biochem.* **1976**, *72*, 95–103.
(27) Mokul'skii, M. A.; Kaiptarova, K. A.; Mokul'skaya, T. D. *Mol. Biol. (Moscow)* **1972**, *6*, 714–731.
(28) LePecq, J.-B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87–106.

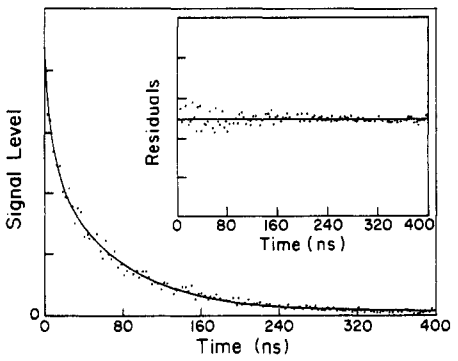


Figure 4. Luminescence decay for $Cu(bcp)_2^+$ in the 1/3 by volume MeOH/aqueous buffer containing ST DNA at a DNA-P/Cu ratio of 50. The solid line is the weighted fit of the experimental data to a double exponential decay. The temperature was 20 °C, and the copper concentration was 26 μM. Insert: Residual plot showing the quality of the fit.

the emission intensity sampled in a plane parallel to that of the electric vector of the excitation beam and $I_\perp$ is the emission intensity sampled in a perpendicular plane. For the emission from $Cu(bcp)_2^+$ in CH_2Cl_2 solution, $\rho = 4 \times 10^{-4}$, but $\rho = 0.41$ in buffer containing ST DNA at a DNA-P/Cu ratio of 50. In a control experiment the polarization of the emission from ethidium bromide was monitored in the presence of DNA, and excellent agreement with the literature[29] was obtained.

Luminescence Decay. In the 33% MeOH aqueous buffer the luminescence signal from $Cu(bcp)_2^+$ was too weak for use to obtain a reliable estimate of the lifetime unless DNA was present. When DNA was present, a biphasic decay was observed with a shorter-lived component that consistently resolved with a lifetime of 5–10 ns. Since this component was indistinguishable from the tail of the instrumental pulse, it was attributed to scattered light. The lifetime of the longer-lived component was about the same for all types of DNA (Table II). A representative decay curve and a residual plot, which indicates the goodness of fit, are depicted in Figure 4.

Electrophoretic Measurements. The presence of $Cu(bcp)_2^+$ had a pronounced effect on the mobility of the covalently closed supercoiled (CCS) form of *φX174* DNA. At a DNA-P/Cu ratio of 50 the mobility of the plasmid appeared to decrease by 30–45%; however, a comparable effect was observed for nicked DNA in the sample. Since the mobilities of the CCS and the nicked fractions were similarly affected, the experiments provided no insight into the question of whether or not intercalative binding occurs. An artifact, such as aggregation of the complex within the gel matrix, may have confused the results.

Discussion

Aggregation at Low DNA-P/Cu Ratios. The results in Figure 2 indicate that $Cu(bcp)_2^+$ interacts with DNA by at least two fundamentally different mechanisms. Emission similar to that observed at low DNA-P/Cu ratios can be induced by a variety of polyelectrolytes, and it has been attributed to an aggregated form of the copper complex.[30] The formation of aggregated species is not too surprising because hydrophobic molecules, such as $Cu(phen)_2^+$, are subject to self-association in aqueous media.[31] Moreover, polymers with pendant charged groups, including biopolymers,[32] are known to be able to stabilize charged colloidal particles. And DNA itself has been shown to combine with the surface of crystalline calcium phosphate,[33] possibly via ionic interactions with exposed cations. Since DNA wraps around histone proteins in the cell nucleus, it is not difficult to imagine that DNA can conform to the surface of aggregates or particulates in solution as well. Although there must be many avenues to explore in the chemistry of such aggregates, we have focused our present efforts on the binding that occurs at high DNA-P/Cu ratios where the copper complex can be assumed to be dispersed along the polymer.

Monomeric $Cu(bcp)_2^+$. As can be seen from Figure 2, at DNA-P/Cu ratios of 20 or larger the charge-transfer absorption maximum and the luminescence intensity achieve limiting values that we ascribe to the bound form of $Cu(bcp)_2^+$ monomer. Below we argue that the luminescence data indicate that the $Cu(bcp)_2^+$ monomer binds—at least in part—by intercalation into B-form DNA. Before the luminescence results can be appreciated, however, we must review some of the novel characteristics of the excited states involved.

Luminescent copper(I) complexes, like $Cu(bcp)_2^+$ and $Cu(dmp)_2^+$, with low-lying metal-to-ligand charge-transfer excited states are exquisitely sensitive to the solution environment. The reason is that the excited state formally contains a copper(II) center which tends to favor a coordination number of 5 or 6.[34] When the complex suffers nucleophilic attack by a solvent molecule, the excited-state potential energy surface is squeezed closer to that of the ground state, and curve crossing to the ground state is strongly facilitated. Thus, $Cu(dmp)_2^+$ has an excited-state lifetime of about 100 ns in the weakly basic solvent CH_2Cl_2, but the lifetime is reduced to 2 ns in the donor solvent CH_3CN.[35] For the same reason the luminescence quantum yield is greatly diminished in the donor solvent as well. In essence, the excited state undergoes a form of exciplex quenching. Solvent-induced, exciplex quenching of the excited state is attenuated or avoided altogether in a complex with a sterically crowded coordination environment about the copper center[36,37] because expansion of the coordination number is inhibited. On the other hand, a quenchable excited state becomes long-lived in a rigid matrix, e.g., a low-temperature glass.[34,38]

Because water and methanol are excellent nucleophiles, emission from the copper(I) systems is barely detectable in the buffer solutions, but the results reported in Figures 2 and 3, the lifetime data, and the excitation spectrum established that $Cu(bcp)_2^+$ is emissive when DNA is present.[39] Since binding to DNA obviously hinders solvent-induced quenching, conformational flexibility within the coordination sphere must be highly restricted in the DNA adduct. Simple surface contact mediated by hydrophobic and/or Coulombic interactions is unlikely to promote such rigidity. On the contrary, the complex has to be tightly wedged into the DNA in some way, and this suggests that $Cu(bcp)_2^+$ binds by intercalation.

Emission polarization data support this hypothesis. Because the complex is a small molecule, virtually all information about the polarization of the excitation beam is lost in a solvent like CH_2Cl_2 to rotational reorientation, which occurs during the lifetime of the excited state. When DNA is present, however, the emission retains significant polarization in solution. This is consistent with intercalative binding because the effective rotational correlation time of the adduct corresponds to that of the macromolecule. This assumes, of course, that the mean residence time in the intercalation site is at least of the order of the excited-state lifetime; however, this is likely to be the case. Otherwise, solvent-induced quenching would be more prevalent.

Structural Considerations. In crystals, *N*-methyl-3,5,6,8-tetramethyl-1,10-phenanthrolinium cation has been found to intercalate between base pairs of iodoCpG minihelices, with the nitrogen atoms of the guest directed toward what in a higher

(29) Travers, F.; Michelson, A. M.; Douzou, P. *Biochim. Biophys. Acta* **1970**, *217*, 1–6.
(30) Tamilarasan, R.; Ropartz, S.; McMillin, D. R. *Inorg. Chem.* **1988**, *27*, 4082–4084.
(31) Anson, F. C.; Lee, C.-W. *Inorg. Chem.* **1984**, *23*, 837–844.
(32) Dameron, C. T.; Reese, R. N.; Mehra, R. K.; Kortan, A. R.; Carroll, P. J.; Steigerwald, M. L.; Brus, L. E.; Winge, D. R. *Nature* **1989**, *338*, 596–597.
(33) Tullius, T. D.; Dombroski, B. A. *Science* **1985**, *230*, 679–681.
(34) McMillin, D. R.; Kirchhoff, J. R.; Goodwin, K. V. *Coord. Chem. Rev.* **1985**, *64*, 83–92.
(35) Palmer, C. E. A.; McMillin, D. R. *Inorg. Chem.* **1987**, *26*, 3167–3170.
(36) Dietrich-Buchecker, C. O.; Marnot, P. A.; Sauvage, J. P.; Kirchhoff, J. R.; McMillin, D. R. *J. Chem. Soc., Chem. Commun.* **1983**, 513–515.
(37) Palmer, C. E. A.; McMillin, D. R. *Inorg. Chem.* **1987**, *26*, 3837–3840.
(38) Buckner, M. T.; McMillin, D. R. *J. Chem. Soc., Chem. Commun.* **1978**, 759–761.
(39) For a discussion of the results at a low DNA-P/Cu ratio, see ref 30.

oligomer would be the major groove.[40] By analogy with this structure, we might expect $Cu(bcp)_2^+$ to dock in the major groove with one of its ligands (partially) inserted into the stack of base pairs. However, the bcp ligand is constrained to be nonplanar because of unfavorable steric interactions involving ortho hydrogens of the phenyl substituents and the C5 and C6 hydrogens behind the center ring. In the solid state the average dihedral angle subtended by the phenyl groups in $[Cu(bcp)_2]BF_4 \cdot CH_3OH$[41] is about 45° and is well removed from the 0° angle that would be desired for a classical intercalator. Indeed, Gabbay et al. have argued that due to nonplanarity, the *N*-methyl-4,7-diphenyl-1,10-phenanthrolinium ion does not intercalate into DNA like other phenanthrolinium ions.[42] On the other hand, Wilson and co-workers have recently demonstrated that molecules with unfused phenyl substituents can intercalate into DNA, albeit with a binding affinity that is somewhat compromised.[43] And in studies of related ruthenium systems Barton and co-workers have concluded that $Ru(dip)_3^{2+}$, where dip denotes 4,7-diphenyl-1,10-phenanthroline, shows a strong tendency to intercalate into DNA,[44] in spite of the fact that the phenyl groups of each ligand are constrained to be out of the phenanthroline plane.[45] In order to minimize strain within the dip ligand, they suggest that the complex may approach the DNA molecule along the major groove such that one of the dip ligands inserts into DNA in an asymmetric fashion, with only one of the phenyl substituents passing between the base pairs.[45] A somewhat similar mode of partial intercalation is one of the possibilities that Veal and Rill have suggested for the binding of $Cu(phen)_2^+$ to DNA, except they propose that the interaction occurs within the minor groove of DNA.[46]

For several reasons, however, we favor the idea that the interaction occurs via the major groove. One is the analogy with the crystallographically characterized system cited earlier.[40] Another reason is that, compared with the minor groove, the base pairs are more exposed and sterically more accessible in the major groove. Finally, we do not observe a significant enhancement of CT luminescence from $Cu(bcp)_2^+$ in the presence of T4 phage DNA where the access to the major groove is hindered by glycosylation.[27]

One other piece of structural information, albeit one concerning the electronic structure, that is of interest concerns the location of the electron in the π^* orbital of the ligand system. There is some evidence in the literature that the excited electron localizes on only one of the phenanthroline ligands in solution.[47] Thus, if $Cu(bcp)_2^+$ is intercalated into DNA, the electron can be localized in the ligand sandwiched between base pairs or in the ligand directed toward the aqueous environment. The formally anionic ligand of the excited complex should be a very strong electron donor, while the DNA base pairs are well suited to the role of acceptor in view of the density of electronegative nitrogen and oxygen atoms contained therein. Accordingly, to the extent that intercalation can be viewed as a donor/acceptor interaction, the excited electron might be expected to localize on the intercalated bcp ligand. The fact that the dioxygen molecule does not readily quench the emission of $Cu(bcp)_2^+$ in DNA-containing solutions is consistent with this hypothesis. Note that in methylene chloride, where the excited-state orbitals are fully accessible, dioxygen is an effective quencher.

In principle, differences in the donor/acceptor properties of the participating base pairs could explain the variations in emission intensity observed with different DNA samples. However, differences in steric effects, the local rigidity within the DNA structures, and the local electric field may obscure the trend. Of the DNA samples investigated, poly(dA-dT)·poly(dA-dT) best preserves the luminescence from $Cu(bcp)_2^+$. This means that the complex binds most intimately with this type of DNA. Tossi and Kelley have reported similar results for the interaction of $Ru(phen)_3^{2+}$.[7] Propeller twisting of the base pairs is believed to be relatively facile for a dA-dT sequence,[48] and this could alleviate steric effects associated with the nonintercalated ligand(s) about the metal and/or with the unfused phenyl substituent. The effectiveness of the yeast tRNA at preserving the emission is quite striking. this suggests that $Cu(bcp)_2^+$ is capable of intercalating into segments of A-form double helix, but further work will be required to establish this point. An example of a metallointercalator that binds within A-form structures is $(terpy)Pt(HET)^+$.[49]

Although variations in the rigidity or the strength of the adduct may occur, the hallmark of an intercalator is that it binds in a variety of DNA sequences. In fact, Sigman has suggested that the lack of sequence specificity in the DNA cutting induced by $Cu(dip)_2^+$ may trace to the fact that the complex binds by intercalation.[10] In contrast, $Cu(phen)_2^+$ gives a much less even cutting pattern, and Sigman has argued that the phen complex binds in a sequence-dependent fashion within the minor groove.[1] Our luminescence results reveal that $Cu(dmp)_2^+$ forms a much less rigid adduct than $Cu(bcp)_2^+$ since DNA has no significant effect on the CT luminescence intensity. This is consistent with groove binding, since simple surface contact with a DNA molecule would not appreciably affect the conformational flexibility of the $Cu(dmp)_2^+$ moiety. Accordingly, solvent-induced quenching or quenching mediated by basic sites on the DNA molecule itself would be very efficient. In the strictest sense, however, the luminescence data only require that $Cu(bcp)_2^+$ form a much more rigid adduct than $Cu(dmp)_2^+$; partial intercalation of the dmp complex is not ruled out so long as exciplex formation is facile. The viscometry data of Graham and Sigman provide the best evidence that $Cu(dmp)_2^+$ is a nonintercalator.[50]

As noted by Wilson and co-workers,[51] the factors that favor intercalation versus groove binding can be very subtle. For $Cu(phen)_2^+$ or $Cu(dmp)_2^+$ introducing phenyl substituents in the 4,7-positions of the ligands apparently alters the preference from groove binding to intercalation. Modification of the DNA host could also have important repercussions. For example, introduction of a bulged lesion in the DNA substrate could switch the preferred mode of binding of $Cu(phen)_2^+$. This hypothesis could form the basis of one explanation of the cutting results of Williams et al.[15] The complexities that can arise are illustrated by $Ru(phen)_3^{2+}$, which acts as a groove binder as well as an intercalator.[2,52]

Acknowledgment. This research was supported by the Public Health Service via Grant No. GM 22764 and by the National Science Foundation via Grant No. CHE-8719638. We thank Fang Liu for carrying out several electrophoresis experiments.

(40) Jain, S. C.; Bhandary, K. K.; Sobell, H. M. *J. Mol. Biol.* **1979**, *135*, 813–840.
(41) Klemens, F. K.; Fanwick, P. E.; Bibler, J. K.; McMillin, D. R. *Inorg. Chem.* **1989**, *28*, 3076–3079.
(42) Gabbay, E. J.; Scofield, R. E.; Bater, C. S. *J. Am. Chem. Soc.* **1973**, *95*, 7850–7857.
(43) Wilson, W. D.; Strekowski, L.; Tanious, F. A.; Watson, R. A.; Mokrosz, J. L.; Strekowska, A.; Webster, G. D; Neidle, S. *J. Am. Chem. Soc.* **1988**, *110*, 8292–8299.
(44) Barton, J. K.; Raphael, A. L. *J. Am. Chem. Soc.* **1984**, *106*, 2466–2468.
(45) Goldstein, B. M.; Barton, J. K.; Berman, H. M. *Inorg. Chem.* **1986**, *25*, 842–847.
(46) Veal, J. M.; Rill, R. L. *Biochemistry* **1989**, *28*, 3243–3250.
(47) McGarvey, J. J.; Bell, S. E. J.; Gordon, K. C. *Inorg. Chem.* **1988**, *27*, 4003–4006.
(48) Sarai, A.; Mazur, J.; Nussinov, R.; Jernigan, R. L. *Biochemistry* **1989**, *28*, 7842–7849.
(49) Barton, J. K.; Lippard, S. J. *Biochemistry* **1979**, *18*, 2661–2668.
(50) Graham, D. R.; Sigman, D. S. *Inorg. Chem.* **1984**, *23*, 4188–4191.
(51) Wilson W. D.; Tanious, F. A.; Watson, R. A.; Barton, H. J.; Strekowska, A.; Harden, D. B.; Strekowski, L. *Biochemistry* **1989**, *28*, 1984–1992.
(52) Barton, J. K. *Comments Inorg. Chem.* **1985**, *3*, 321–348.