Noncovalent DNA Binding of Bis(1,10-phenanthroline)copper(I) and Related Compounds†

James M. Veal‡ and Randolph L. Rill*

Department of Chemistry and Institute of Molecular Biophysics, The Florida State University, Tallahassee, Florida 32306-3006

Received April 11, 1990; Revised Manuscript Received September 7, 1990

ABSTRACT: The noncovalent DNA binding of the bis(1,10-phenanthroline)copper(I) complex [$(Phen)_2Cu^I$] was examined under anaerobic conditions by absorption and circular dichroism spectroscopy, and viscometry, as a function of phenanthroline concentration. Analyses according to the McGhee–von Hippel method indicated that binding exhibited both neighbor-exclusion and positive cooperativity effects, with a neighbor-exclusion parameter $n \approx 2$ and a cooperativity parameter $\omega \approx 4$. The association constant for $(Phen)_2Cu^I$ binding decreased with increasing concentration of phenanthroline in excess over that required to stoichiometrically generate $(Phen)_2Cu^I$, indicating that free phenanthroline was a weak competitive inhibitor of $(Phen)_2Cu^I$ binding. The maximal association constant for DNA binding of $(Phen)_2Cu^I$ in 0.2 M NaCl and 9.8% ethanol, extrapolated to zero concentration of excess phenanthroline, was 4.7×10^4 M^{-1} (DNA base pairs). The magnitude of the neighbor-exclusion parameter, the changes in spectral properties of $(Phen)_2Cu^I$ induced by DNA binding, and the increase in DNA solution viscosity upon $(Phen)_2Cu^I$ addition are consistent with a model for DNA binding by $(Phen)_2Cu^I$ involving partial intercalation of one phenanthroline ring of the complex between DNA base pairs in the minor groove as suggested previously [Veal & Rill (1989) *Biochemistry 28*, 3243–3250]. Viscosity measurements indicated that the mono(phenanthroline)copper(I) complex also binds to DNA by intercalation; however, no spectroscopic or viscometric evidence was found for DNA binding of free phenanthroline or the bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex. DNA binding of free phenanthroline may be cooperative and induced by prior binding of $(Phen)_2Cu^I$.

The monovalent cationic complex bis(1,10-phenanthroline)copper(I) [$(Phen)_2Cu^I$)][1] binds noncovalently in the minor groove of double-stranded DNA and, in the presence of molecular oxygen and a reducing agent, acts as an effective nuclease with a high preference for double-stranded DNA [reviewed by Sigman (1986)]. There has been considerable interest in the DNA binding and cleavage by this and other metal–phenanthroline complexes. $(Phen)_2Cu^I$ has been used as a small molecule pseudonuclease for examining the sequence specificities of DNA binding of a variety of ligands by "footprinting" techniques (Barton, 1986; Sigman, 1986; Francois et al., 1988a,b). Phenanthroline complexed with copper has also been used as the active moiety in synthetic restriction endonucleases constructed by covalently coupling phenanthroline to polynucleotides or sequence-specific DNA-binding proteins (Chen & Sigman, 1986, 1987; Sun et al., 1988; Francois et al., 1989). The $(Phen)_2Cu^I$ complex alone, as well as other metal–phenanthroline complexes, exhibits a significant degree of sequence selectivity in binding that is of interest as an indicator of local DNA conformation (Cartwright & Elgin, 1982; Drew & Travers, 1984; Barton, 1986; Sigman, 1986; Kazakovet et al., 1988; Williams et al., 1988; Veal & Rill, 1988, 1989; Guo et al., 1990).

Interpretation of data based on DNA cleavage by $(Phen)_2Cu^I$ and design of efficient artificial nucleases requires knowledge of the binding and kinetic mechanisms. $(Phen)_2Cu^I$ is known to bind preferentially or exclusively in the DNA minor groove (Drew & Travers, 1984; Kuwabara et al., 1986; Veal & Rill, 1988). DNA-strand cleavage is proposed to occur via proton abstraction from the deoxyribose C-4′ or C-1′ positions by active oxo species, which react within the immediate vicinity of the $(Phen)_2Cu^I$ binding site (Kuwabara, 1986; Goldstein & Czapski, 1986; Johnson & Nazhat, 1987; Goldstein et al., 1988; Thederahn et al., 1989). Because strand scission is localized, analogues of DNA sequencing techniques can be used to precisely map the sites of cleavage at the nucleotide level with DNA restriction fragments or synthetic oligonucleotides. We previously showed that $(Phen)_2Cu^I$ cleaves most preferentially at TAT triplets and that cleavage is significantly reduced at triplets differing from TAT by a single base transition, the order of preference being TAT > TGT > TAC > CAT (Veal & Rill, 1988, 1989). Studies using synthetic oligonucleotides containing C·G or C·I (I = inosine) base pairs showed that the guanine N^2-amino group, located in the minor groove, is primarily responsible for reduced cleavage at CAT and TGT sequences relative to TAT sequences (Veal & Rill, 1989). These and other data led us to propose a model for preferred $(Phen)_2Cu^I$ binding to DNA by partial intercalation of one phenanthroline ring between a T-3′,5′-A step, with the other phenanthroline ring lying within the minor groove approximately parallel to the helix axis (Veal & Rill, 1989). A similar model has been proposed by Stockert (1989) on the basis of modeling studies.

In order to test this model and obtain further insights into the cleavage mechanism, we have examined the *noncovalent*

† This work was supported in part by grant ER60588 from the Department of Energy and a grant from the Florida Division of the American Cancer Society.

* Corresponding author.

‡ Current address: Department of Chemistry, Georgia State University, Atlanta, GA 30303.

[1] Abbreviations: Hepes, *N*-2-(hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; MPA, mercaptopropionic acid; Phen, 1,10-phenanthroline; $(Phen)_2Cu^I$, bis(1,10-phenanthroline)copper(I).

0006-2960/91/0430-1132$02.50/0

binding of $(Phen)_2Cu^I$ and related compounds to random sequence DNA under anaerobic, reducing conditions. DNA binding by intercalation was strongly suggested by DNA-induced changes in the $(Phen)_2Cu^I$ visible absorption spectrum and the induction of a strong $(Phen)_2Cu^I$ circular dichroism (CD) spectrum, by a significant increase in DNA solution viscosity upon introduction of $(Phen)_2Cu^I$, and by a binding-site size consistent with the neighbor-exclusion principle. DNA binding of $(Phen)_2Cu^I$ was found to be positively cooperative, and analysis of the binding was complicated by competitive binding of free phenanthroline and the mono(phenanthroline)copper(I) complex. The DNA-binding constant of $(Phen)_2Cu^I$ was determined to be $\approx 5 \times 10^4$ M^{-1} (base pairs) in 0.2 M NaCl and 9.8% ethanol, comparable to that of other well-known intercalating agents. By contrast, no evidence was found for the binding of the bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex to DNA. The mono(phenanthroline)copper(I) complex appeared to bind DNA by intercalation, but no convincing evidence was found for DNA binding of free phenanthroline in the absence of $(Phen)_2Cu^I$.

Materials and Methods

Spectral Titrations. The general protocol required preparation of an anaerobic buffer solution in a sealed 10-cm cuvette containing 1 mM ascorbate and varying concentrations of copper, phenanthroline, and DNA. Standardized stock solutions of $CuSO_4$ in high-purity metal-free H_2O and 1,10-phenanthroline in ethanol were prepared from ACS-grade reagents and stored in the dark at 4 °C. Samples in 5 mM Hepes–200 mM KCl buffer containing 9.8% w/v ethanol, pH 8.0, were used for all measurements. Stock ascorbate solutions were prepared fresh daily in deaerated water to minimize oxidation.

Sheared salmon sperm DNA (Sigma Type III) was used for binding studies. DNA (500 mg/25 mL of buffer) was manually sheared to a size of approximately 3000 base pairs by using a 1-mL Hamilton syringe fitted with needles of decreasing bore sizes. After it was sheared, the DNA was extracted with phenol and chloroform, precipitated by addition of 2 volumes of 2-propanol, recovered by centrifugation, dried in vacuo, and redissolved in Hepes–KCl buffer. The final DNA solutions were extensively dialyzed against Hepes–KCl buffer. DNA concentrations were determined from the absorbance at 260 nm by using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide. DNA aliquots were saturated with argon and stored at 4 °C.

To avoid DNA degradation by $(Phen)_2Cu^I$, it was necessary that oxygen in solution be effectively removed. Prior to cuvette filling, 100 mL of Hepes–KCl buffer was transferred to a capped 125-mL glass beaker and vigorously saturated with argon. Appropriate volumes of the solution were immediately and gently transferred to matched 10-cm cuvettes that were than sealed with rubber septums. Remaining air in the cuvettes was purged with argon. All subsequent additions were made with Hamilton syringes in conjunction with an outlet needle for argon displacement.

After addition of ascorbate, preincubation for 30 min, and recording of a base-line spectrum, $Phen_2Cu^I$ was generated by addition of appropriate amounts of $CuSO_4$ and phenanthroline to the sample cuvette. The extinction coefficients of $(Phen)_2Cu^I$ at 435 and 440 nm were determined from the slope of an absorbance versus concentration plot. These measurements were made with phenanthroline in a 30–80 μM excess over the concentration required by the stoichiometry of $(Phen)_2Cu^I$. Spectra were obtained at 27 °C after equilibration for 15–20 min by using a Cary 14 UV/vis spectrophotometer.

The apparent extinction coefficients, ϵ_{app}, for solutions containing both $Phen_2Cu(I)$ and DNA·$(Phen)_2Cu^I$ were determined by titration. Usually small volumes of the concentrated stock DNA solution were titrated into a sample cuvette containing a fixed concentration of $Phen_2Cu^I$ (3–15 μM). The cuvettes were gently shaken and allowed to equilibrate for 15–20 min prior to absorbance measurements. DNA was also titrated into the reference cuvette to correct for any absorbance or light scattering due to the DNA itself. In some experiments the DNA concentration was fixed, and $CuSO_4$ and phenanthroline were titrated into the cuvette. Separate experiments were performed using different ratios of phenanthroline to copper ranging from the stoichiometric 2:1 ratio to as high as 10:1. The total phenanthroline concentration was in no case allowed to exceed 80 μM to minimize possible dimerization of $(Phen)_2Cu^I$, which appeared to occur at higher concentrations.

To test for possible DNA cleavage during titration, an anaerobic solution containing 8 μM $CuSO_4$, 16 μM phenanthroline, 1 mM ascorbate, and 200 μM DNA in Hepes–KCl buffer was prepared in a cuvette as above and allowed to stand for 1 h with occasional shaking. After addition of 2,9-dimethyl-1,10-phenanthroline to a concentration of 200 μM, the DNA was repurified and electrophoresed on a 1% agarose gel along with a control from the stock DNA solution. No differences in electrophoretic patterns were noted after ethidium bromide staining.

The extinction coefficients, ϵ_b, for $(Phen)_2Cu^I$ bound to DNA at different ratios of phenanthroline to copper were variable and were determined either by spectral titration in the presence of a large excess of DNA (120–400 nucleotides per copper) or by the method of Benesi and Hildebrand (1949) from the spectral titrations described above. At low ν values (moles of ligand bound per mole of DNA base pairs) the concentration of free DNA approximates the total DNA concentration, $[DNA]_{total}$, and

$$1/(\epsilon_f - \epsilon_{app}) = [1/(\epsilon_f - \epsilon_b)][1/(K_2[DNA]_{total}) + 1]$$

where K_2 represents the apparent equilibrium constant for $(Phen)_2Cu^I$ binding to DNA. A double-reciprocal plot yielded the inverse of the extinction coefficient of bound ligand from the intercept and K_2 from the slope. Both methods yielded similar extinction coefficients for bound $(Phen)_2Cu^I$.

The fraction of complex bound to DNA (f_b) was determined from the apparent extinction coefficient at 435 nm according to the equation $f_b = (\epsilon_f - \epsilon_{app})/(\epsilon_f - \epsilon_b)$. The fraction bound (in the range 0.2–0.8), the total $(Phen)_2Cu^I$ concentration, and the DNA concentration were then used to calculate ν. Binding data were fit by a simplex method to the McGhee–von Hippel site-exclusion model for binding of ligands to DNA to determine K_2, the binding-site size n, and the cooperativity parameter ω.

The binding to DNA of the nonreactive bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex was also examined. An anaerobic solution was prepared as before containing 9.5 μM $CuSO_4$, 39.1 μM 2,9-dimethyl-1,10-phenanthroline, and 1 mM ascorbate in Hepes–KCl buffer. DNA was titrated into the cuvette to a final DNA concentration of 80 μM, and the absorbance at 455 nm was recorded after each addition.

Circular dichroism spectra were obtained with a Jasco J500-C spectropolarimeter using 2-cm cells and anaerobic solutions prepared as described above containing 46.5 μM $Cu(NO_3)_2$, 93 μM phenanthroline, and 2 mM ascorbate. After the base line was recorded, DNA from a 68 mM stock solution

was titrated into reference and sample cuvettes (initial volume = 5.8 mL) to a final nucleotide concentration of 3.30 mM. Spectra were recorded after each addition.

Viscosity measurements were made with a Cannon Series 100 D1 capillary viscometer secured in a Sargent constant temperature water bath at 26.0 ± 0.1 °C. The viscometer was protected from atmospheric oxygen by connection to an argon buffer reservoir. Valves and tubes affixed to gas-tight Hamilton syringes were designed to permit solution preparation and mixing, argon saturation, and viscosity measurements under anaerobic conditions. Stock solutions of $CuSO_4$ and phenanthroline were prepared as described for spectroscopy experiments. DNA was sonicated to an average size of approximately 800 base pairs. The empty viscometer was vigorously purged with argon, and 18 mL of Hepes–KCl buffer was added with a 10-mL gas-tight Hamilton syringe. Ascorbate (0.58 M) was added to yield a concentration of 1 mM. Argon was then bubbled through the solution to displace all oxygen. Flow measurements, typically ≈100 s in duration, were made at least five times and accepted if they agreed within 0.20 s, although repeat measurements typically agreed within 0.10 s. Concentrated DNA was then added to the viscometer, and the solution was mixed by gentle bubbling with argon. The final DNA concentration was 200 μM. Flow times were then measured as for the buffer alone. $CuSO_4$ and phenanthroline were injected into the solution and mixed by argon bubbling, and the flow times were again determined. Individual measurements were made for approximately 20 μM increments of $CuSO_4$, and final concentrations as high as 200 μM were examined. Following the final titration, the viscosity of the solution was monitored for an additional 1–2 h with timings taken every 20 min. No changes were noted, indicating that the DNA was not degraded under these conditions.

Viscosity measurements were made for solutions containing 1:1, 2:1, and 4:1 ratios of phenanthroline to $CuSO_4$. Titrations were also performed with the known intercalator ethidium bromide as a control. In addition, titrations were performed with phenanthroline alone and with the bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex. All data were converted to reduced specific viscosities (η_{sp}). Minor corrections were made for the effect of addition of absolute ethanol and H_2O (the solvents for phenanthroline and $CuSO_4$) on solution viscosity.

Results

Effect of Mercaptopropionic Acid on the Absorption Spectrum of $(Phen)_2Cu^I$. Under aerobic conditions, the $(Phen)_2Cu^I$ complex is unstable and completely oxidizes to $(Phen)Cu^{II}$. The redox cycle leading to DNA cleavage is initiated by addition of a reducing agent to generate Cu(I). Mercaptopropionic acid (MPA) has been most commonly used as the reductant and was utilized in our previous studies of the sequence preferences of DNA cleavage (Sigman, 1986; Veal & Rill, 1988, 1989; Thederahn et al., 1989). The $(Phen)_2Cu^I$ complex has an intense visible absorption spectrum characterized by a major band (I) at 435 nm (ϵ = 7000–7250 M^{-1} cm^{-1}) and a weak band (II) appearing as a broad shoulder centered at about 530 nm (Smith & Frederick, 1954; Pflaum & Brandt, 1955). The expected spectrum was not generated when MPA was added in excess to solutions of $(Phen)_2Cu^{II}$ prepared from primary standard phenanthroline and $Cu(SO_4)$ in ≥2:1 stoichiometry.

A spectrum in good agreement with literature reports was obtained with ascorbate as the reductant. Ascorbate has been widely used in studies of Cu(I) complexes with 1,10-phenanthroline and other chelating agents and was used for all subsequent studies described here. When the $(Phen)_2Cu^I$ complex was prepared with 1 mM ascorbate as reductant and then excess MPA was added, the intense color and characteristic visible spectrum of the $(Phen)_2Cu^I$ complex disappeared, demonstrating that MPA disrupts the $(Phen)_2Cu^I$ complex (data not shown). The most likely occurrence is the replacement of one phenanthroline ring by MPA, which could also act as a chelate. Additional studies of this reaction will be described later (Veal and Rill, manuscript in preparation).

Factors Influencing Measurements of DNA Binding by $(Phen)_2Cu^I$. Measurements of $(Phen)_2Cu^I$ binding to DNA are complicated by the existence of two competing equilibria: the Cu(I) ⇌ Cu(II) redox cycle and the dissociation reactions:

$$(Phen)_2Cu^I \overset{K_1}{\rightleftharpoons} (Phen)Cu^I + Phen \qquad (1)$$

and

$$(Phen)Cu^I \overset{K_{1'}}{\rightleftharpoons} Phen + Cu(I) \qquad (1')$$

Use of an argon atmosphere, an excess of ascorbic acid (1 mM), and standard syringe techniques were found sufficient to perform spectroscopic titrations and viscosity measurements without oxidation and formation of $(Phen)_2Cu^{II}$ (see below). The alternatives of equilibrium dialysis or solvent extraction methods for determining binding constants could be performed only with significantly increased technical difficulties in preventing oxidation and were not attempted.

Ascorbate was an excellent reducing agent and oxygen scavenger but reacted [perhaps in combination with $(Phen)_2Cu^I$] with Tris, which was used as a buffer in previous experiments (Veal & Rill, 1988, 1989), to yield a yellow product with a spectrum partially overlapping that of $(Phen)_2Cu^I$. Hepes buffer at low concentrations proved to be relatively unreactive and was employed for all spectroscopy experiments.

The range of useful concentrations of $(Phen)_2Cu^I$ was restricted by the dissociation equilibrium (1) above and by the dimerization of $(Phen)_2Cu^I$ at high concentrations [reported previously; see Pflaum and Brandt (1955); Hodges and Arauzo (1982), and Lee and Anson (1984)], as well as by normal spectroscopic requirements. The association constant for the overall process

$$Cu(I) + Phen \rightleftharpoons (Phen)_2Cu^I \qquad (1'')$$

is large, $10^{15.8}$ (James & Williams, 1961); hence complete dissociation to form free Cu(I) was negligible under the conditions of these experiments. The dissociation equilibrium (1) was not negligible, however. A dissociation constant of $10^{-5.5}$ at pH 6 has been inferred from kinetic measurements (Hodges & Arauzo, 1982), and a similar value was estimated here from spectral titrations. Beer's law plots at 435 nm were obtained at a number of concentrations of Cu(I) and phenanthroline. Such plots were linear only when [Phen] was in modestly greater than 2-fold excess over [Cu^+] and yielded a constant value for the extinction coefficient (7200 M^{-1} cm^{-1}) in agreement with literature data only when [Phen] was in severalfold excess. The dissociation constant, K_1, for equilibrium 1 was estimated from spectral titration data to be $\approx 10^{-6}$, assuming the concentration of free Cu(I) was negligible. Since concentrations of $(Phen)_2Cu^I$ were restricted to approximately 2–15 μM in the spectral titrations with DNA, some dissociation of $(Phen)_2Cu^I$ occurred in situations where [Phen] was not in significant excess. The concentrations of $(Phen)_2Cu^I$ in individual titrations, therefore, were determined from the absorbancies at 435 nm prior to DNA addition. Neither the mono(phenanthroline)copper(I) complex nor

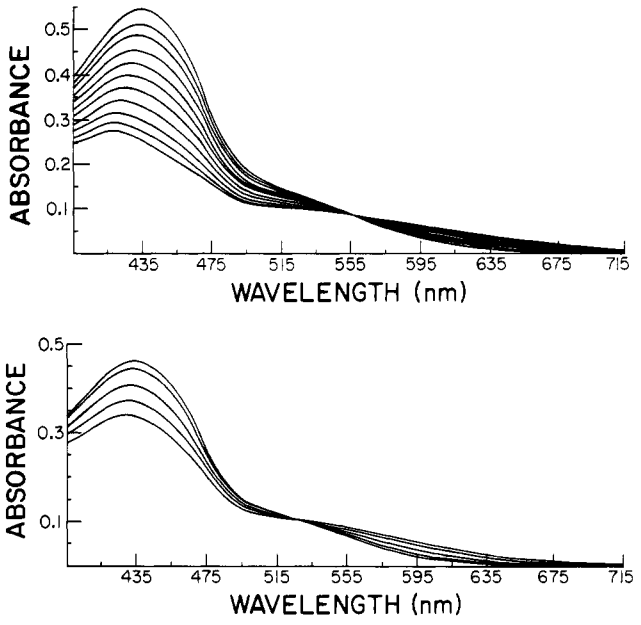


FIGURE 1: Spectral titration of $(Phen)_2Cu^I$ with DNA in the presence of excess phenanthroline. (A) $(Phen)_2Cu^I$ concentration was 7.6 μM; excess phenanthroline concentration was 7.0 μM; DNA concentrations range from 0 to 190 μM (nucleotides) (top to bottom). The isosbestic point occurred at 557 nm. (B) $(Phen)_2Cu^I$ concentration was 6.5 μM; excess phenanthroline concentration was 80 μM; DNA concentrations range from 0 to 507 μM (top to bottom). The isosbestic point occurred at 525 nm.

phenanthroline absorb in the visible region and hence did not interfere with absorbance measurements.

Effects of DNA on the Absorption and Circular Dichroism Spectrum of $(Phen)_2Cu^I$. Addition of DNA to a solution of $(Phen)_2Cu^I$ caused a blue shift and decrease in intensity of the major band (I) and a red shift and increase in intensity of the weaker band (II) (Figure 1). The blue shift of band I is atypical of intercalator complexes but may be the consequence of a change in the metal to ligand charge-transfer band due to distortion of the dihedral angle between phenanthroline rings in the complex (see below). Titrations of this type were performed by using several different ratios of $(Phen)_2Cu^I$ to excess phenanthroline. [The concentration of excess phenanthroline is defined as the concentration of total phenanthroline less the amount complexed in $(Phen)_2Cu^I$.] Each individual titration exhibited a clear isosbestic point (Figure 1), indicating that the total concentration of free and bound $(Phen)_2Cu^I$ species remained constant and that there was only one type of spectroscopically distinguishable binding site. Curiously, however, when the concentration of excess phenanthroline was increased, the position of the isosbestic point shifted to shorter wavelengths and the wavelength of maximum absorbance shifted to longer wavelengths. A similar dependence of the spectrum of the DNA-bound $(Phen)_2Cu^I$ on excess phenanthroline concentration was manifested when a DNA sample containing a constant concentration of DNA and Cu(I) was titrated with phenanthroline (Figure 2). Since the most extreme shifts in the $(Phen)_2Cu^I$ spectrum upon DNA addition were noted when the solution contained the lowest concentration of excess phenanthroline and the isosbestic point was not maintained when more phenanthroline was added, free phenanthroline must in some way modify the spectral properties of DNA-bound $(Phen)_2Cu^I$.

Intercalation of strong, but optically inactive, chromophores to double-stranded DNA via intercalation typically induces strong optical activity. The optically inactive $(Phen)_2Cu^I$, when bound to DNA, exhibited a circular dichroism spectrum in the visible region that was roughly the magnitude (in molecular ellipticity) of the UV spectrum of DNA (Figure 3). The spectrum was approximately "conservative", with two positive maxima at 344 and 415 nm, a crossover at 440 nm, and a strong negative maximum at 520 nm.

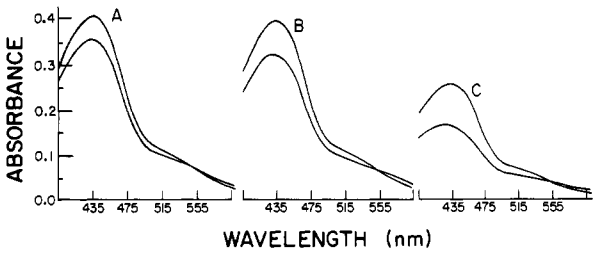


FIGURE 2: Effect of phenanthroline concentration on the absorption spectrum of the DNA·$(Phen)_2Cu^I$ complex. Phenanthroline was titrated into a cuvette containing 5.7 μM $CuSO_4$ and 0 μM (top spectrum) or 50 μM DNA (nucleotides) (bottom spectrum). The phenanthroline concentrations were (A) 57 μM, (B) 28 μM, and (C) 12 μM. Note that the intensities of the spectra decrease with decreasing concentration of phenanthroline due to a lower total concentration of $(Phen)_2Cu^I$ but that the differences between the top and bottom spectra increase with decreasing concentration of phenanthroline and the major band shifts to the blue due to increased formation of the DNA·$(Phen)_2Cu^I$ complex.

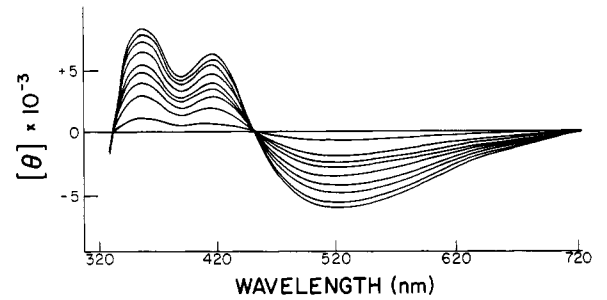


FIGURE 3: Effect of DNA on the circular dichroism spectrum of $(Phen)_2Cu^I$. The copper concentration was 46.5 μM, the phenanthroline concentration was 93 μM, and the ratios of DNA nucleotides to $(Phen)_2Cu^I$ were 0, 1.5, 3.1, 4.8, 6.8, 9.1, 12.8, 18.2, 25.2, and 35.5 (increasing with increasing spectral magnitude). Molecular ellipticities are expressed on a per mole of $(Phen)_2Cu^I$ basis.

Analyses of Equilibrium Binding Data. DNA binding by $(Phen)_2Cu^I$, expressed by the equilibrium

$$\text{DNA} + (Phen)_2Cu^I \overset{K_2}{\rightleftharpoons} \text{DNA}\cdot(Phen)_2Cu^I \qquad (2)$$

was quantitated on the basis of the changes in visible absorbance at 440 nm. Since the isosbestic point was dependent on the concentration of excess phenanthroline, determination of the extinction coefficient for the bound ligand was required for each titration condition. Extinction coefficients for bound ligand were determined by titrations utilizing a large excess of DNA and analyses of plots of $[1/\epsilon_f - 1/\epsilon_{app}]$ versus $1/[DNA]_{total}$ as described initially by Benesi and Hildebrand (1949). These plots also provided an initial estimate of the limiting K_2 (Table I). In all cases the plots were linear at low ligand concentrations, but the slopes and intercepts differed, indicating a decrease in the limiting K_2 and an increase in the extinction coefficient of the bound $(Phen)_2Cu^I$ at 440 nm with increasing concentration of excess phenanthroline (Figure 4).

Association curves plotted in the form of ν (moles of ligand per mole of DNA base pairs) versus log $[(Phen)_2Cu^I]$ deviated significantly from those expected for a simple binding isotherm and did not closely approach saturation (Figure 5). The curves were also shifted to higher (less negative) values of log $[(Phen)_2Cu^I]$ with increasing concentration of excess phen-

Table I: Association Constants, Neighbor-Exclusion Parameters, and Cooperativity Parameters for DNA Binding of $(Phen)_2Cu^I$ as a Function of Excess Phenanthroline Concentration

[Phen][a] (μM)	K_2(B-H)[b] $\times 10^{-4}$	K_2(M-V)[c] $\times 10^{-4}$	n[c]	ω[c]
7	3.2	2.7	2.0	4.6
14	2.4	2.2	2.0	3.6
21	1.6	1.5	2.0	3.0
31	1.3	1.2	2.1	3.1

[a] The concentration of free phenanthroline in excess of the concentration of $(Phen)_2Cu^I$. Different concentrations of $(Phen)_2Cu^I$ were used to increase the range of ν values while the fraction bound was kept in the range of 0.2–0.8. [b] Association constants (M^{-1} base pairs) estimated from the slopes of plots according to Benesi and Hildebrand (1949). [c] Parameters determined by a simplex analysis according to the theory of McGhee and von Hippel (1974). K_2 is the association constant (M^{-1} base pairs), n is the neighbor-exclusion parameter, and ω is the cooperativity parameter. Values differing from those cited by more than 10% yielded significantly poorer fits to the data as indicated by the standard deviations and examination of the predicted binding curves.

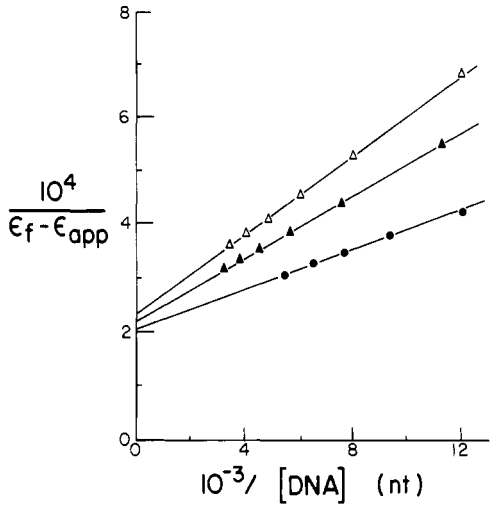


FIGURE 4: Dependencies of Benesi–Hildebrand plots for the DNA binding of $(Phen)_2Cu^I$ on the concentration of excess phenanthroline: (●) 14 μM; (▲) 21 μM; (△) 31 μM.

anthroline, suggesting that free phenanthroline is a competitive inhibitor of $(Phen)_2Cu^I$ binding (see also below). Plots of ν versus log $\{K_2[(Phen)_2Cu^I]\}$ were nearly superimposable, however (Figure 5B), an important result demonstrating the internal consistency of the titrations and data analyses. In addition, the superposition of these curves indicates that free phenanthroline, though competitive with $(Phen)_2Cu^I$, does not appreciably influence the intrinsic character of $(Phen)_2Cu^I$ binding. This conclusion was supported by analyses according to the method of McGhee and von Hippel (1974).

The McGhee–von Hippel theory treats the effects of both neighbor exclusion and cooperativity on the binding of a ligand to a linear lattice. The analysis is usually presented in the form of a Scatchard plot, i.e., ν/[A] versus ν, where [A] is the free ligand concentration; in this case [A] = $[(Phen)_2Cu^I]$. Scatchard plots of $(Phen)_2Cu^I$ binding were atypical, being nearly horizontal with slight upward concavity (Figure 6). As noted by McGhee and von Hippel, upward concavity is expected for positively cooperative binding. Moreover, the slope of the curve is expected to approach zero at low ν when the cooperativity parameter ω approximates $(n + {}^1/_2)$, where n is the number of lattice sites (base pairs) excluded by the ligand.

Data for $(Phen)_2Cu^I$ binding to DNA were fit to the McGhee–von Hippel equation by a simplex method to yield

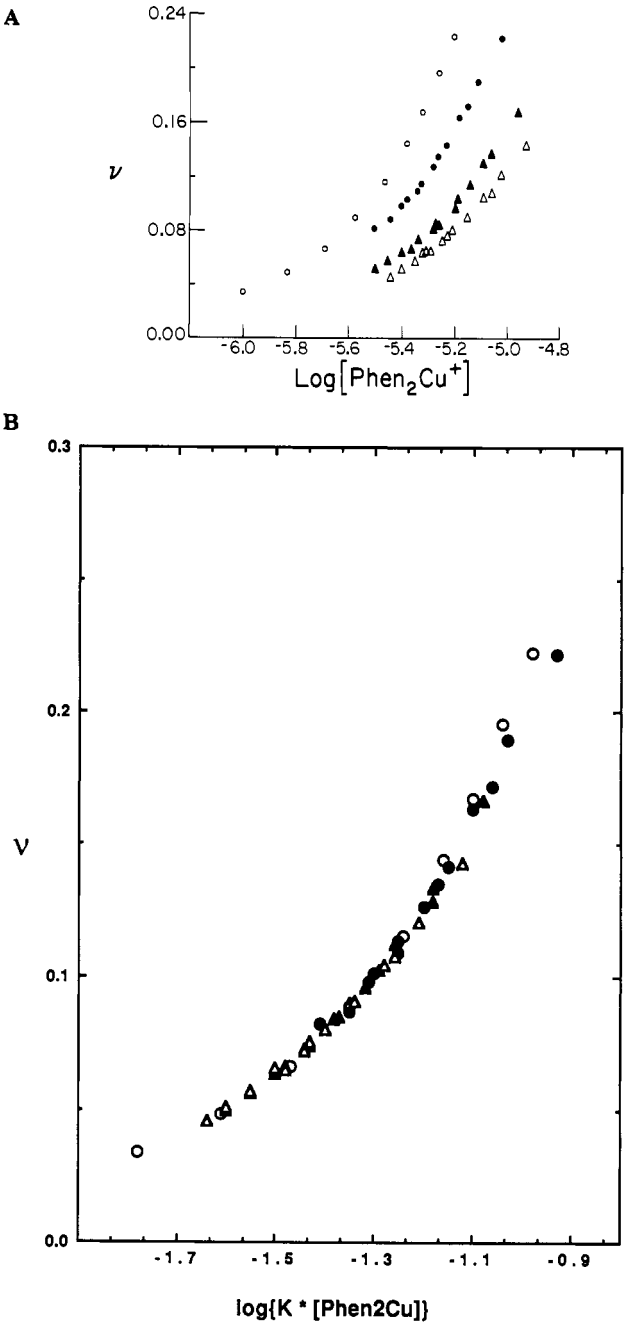


FIGURE 5: Isotherms for DNA binding of $(Phen)_2Cu^I$. (A) Plots of the fractional saturation (moles of ligand per mole of DNA base pairs) versus the log of free $(Phen)_2Cu^I$ concentration. The concentrations of excess phenanthroline were 7, 14, 21, and 31 μM (left to right). (B) The same data as in (A) replotted against log $\{[(Phen)_2Cu^I](Phen)\}$ to compensate for the effect of excess phenanthroline on the association constant for $(Phen)_2Cu^I$ binding. Excess Phen concentrations were 7 μM (○), 14 μM (●), 21 μM (▲), and 31 μM (△).

optimized estimates of K_2, n, and ω (Table I). The fits of these parameters to the binding data were examined graphically (Figure 6), and the influence of changes in parameters was examined by using a MATHCAD program. All titration data were best fit by both criteria with K_2 values on the order of 10^4, n values near 2.0, and ω values from 3.0 to 4.6, indicative of modest positive cooperativity. The association constant for $(Phen)_2Cu^I$ binding decreased moderately with increasing concentration of excess phenanthroline as did the cooperativity parameter ω. K_2 values obtained by the McGhee–von Hippel analysis were in good agreement with those obtained from the Benesi–Hildebrand plots (Table I).

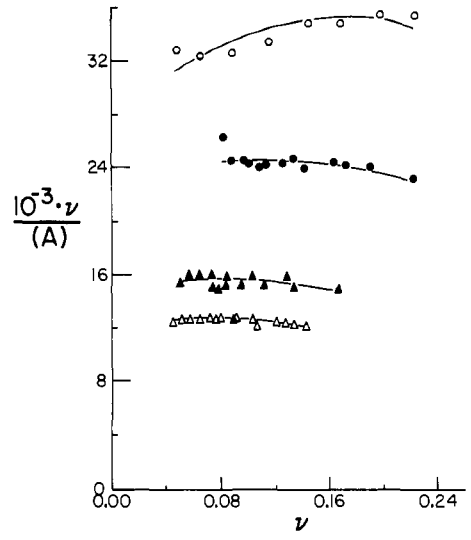


FIGURE 6: Scatchard plots of the data shown in Figure 5. Solid lines show the fits to McGhee–von Hippel theory (1974) for binding with neighbor exclusion and positive cooperativity. The parameters used are shown in Table I.

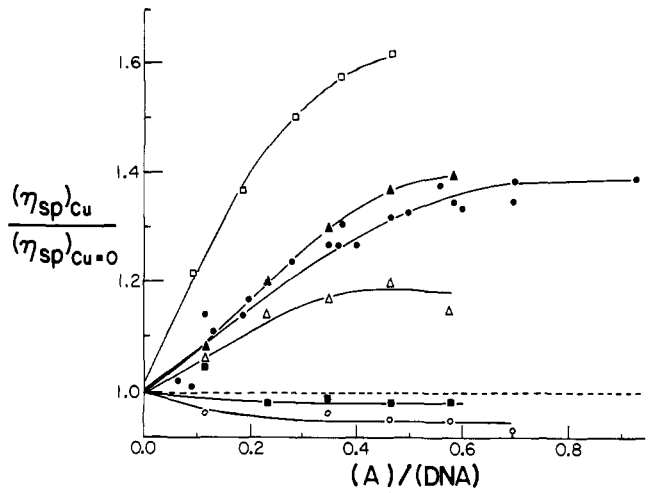


FIGURE 7: Effects of $(Phen)_2Cu^I$ and other potential ligands on the viscosities of DNA solutions. Ligands were titrated into a 200 μM (nucleotides) DNA solution at 26 °C. (□) Ethidium bromide; (●) 2:1 Phen:Cu; (△) 4:1 Phen:Cu; (▲) 1:1 Phen:Cu; (○) phenanthroline; (■) 2:1 2,9-dimethyl-1,10-phenanthroline:Cu. The *Y*-axis is the ratio of the specific viscosities of the DNA solutions in the presence and absence of ligand. The *X*-axis is the *total* concentration of ligand.

If there is simple competition for DNA binding between phenanthroline and $(Phen)_2Cu^I$ at low ν values, then the apparent association constant for $(Phen)_2Cu^I$ binding is related to the true association constant by $K_{2,app} = K_2/(1 + K_{phen}[phen])$, where K_{phen} is the association constant for phenanthroline binding, [phen] is the free phenanthroline concentration, and the denominator represents the correction to the free DNA concentration due to formation of the DNA·phen complex. Assuming that the concentration of free phenanthroline is essentially equal to the concentration of total phenanthroline at low ν values, a plot of $1/K_{2,app}$ versus [phen] should be linear, with an intercept of $1/K_2$ and slope of K_{phen}/K_2. Such a plot was fit by linear regression analysis and yielded values of $K_2 = 4.7 \times 10^4$ and $K_{phen} = 9.5 \times 10^2$, with a correlation coefficient of 0.99 (data not shown).

Effects of $(Phen)_2Cu^I$ and Related Ligands on DNA Solution Viscosity. Under appropriate conditions intercalation causes a significant increase in the viscosity of DNA solutions due to the increase in separation of base pairs at intercalation sites and hence an increase in overall DNA contour length (Lerman, 1961). By contrast, ligands that bind exclusively in the DNA grooves (e.g., netropsin, distamycin), under the same conditions, typically cause less pronounced changes (positive or negative) or no changes in DNA solution viscosity. Effects of $(Phen)_2Cu^I$ on DNA solution viscosity were compared to those of a known intercalator, ethidium, to the (predominantly) mono(phenanthroline)copper(I) complex, to the bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex, and to free phenanthroline. Addition of either ethidium, or $(Phen)_2Cu^I$ in the presence of modest excess phenanthroline, to linear, ≈700 base pair length DNA caused significant increases in the reduced specific viscosity consistent with intercalation by both ligands (Figure 7). A solution of Cu(I) and phenanthroline at a ratio of 1:1 also caused an increase in viscosity that was nearly equivalent to but slightly larger on a per mole total copper added basis than the increase caused by $(Phen)_2Cu^I$. Under these conditions, equilibrium 1 strongly favors the mono(phenanthroline)copper(I) species, and the magnitude of the viscosity increase cannot be attributed solely to the small amount of $(Phen)_2Cu^I$ present. Both $(Phen)_2Cu^I$ and $(Phen)Cu^I$, therefore, unwind DNA, though perhaps not to the extent per ligand as ethidium. (Precise comparison of the unwinding angles requires knowledge of ν versus [A] for each case. These data were not available. Note, however, that the DNA unwinding angle for ethidium is larger than for many other intercalators.)

By contrast to the behavior of $(Phen)_2Cu^I$ and $(Phen)Cu^I$, phenanthroline alone and the bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex caused a slight decrease and no change, respectively, in the DNA solution viscosity. The viscosity increase caused by $(Phen)_2Cu^I$ in the presence of excess phenanthroline (4:1 phen:Cu) was less than that observed for a stoichiometric ratio (2:1 phen:Cu), a result that is consistent with the reduced $(Phen)_2Cu^I$ binding caused by excess phenanthroline.

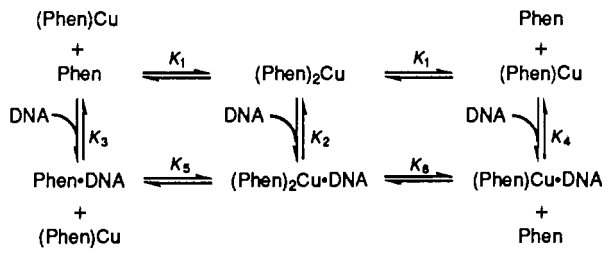
Spectroscopic Studies of DNA Interactions of Phenanthroline and Bis(2,9-dimethyl-1,10-phenanthroline)copper(I). The potential DNA binding of phenanthroline alone is of interest because of the inhibition of $(Phen)_2Cu^I$ binding by excess phenanthroline noted above. Bis(2,9-dimethyl-1,10-phenanthroline)copper(I) is an analogue of $(Phen)_2Cu^I$, but the dihedral angle between rings is locked near 90° by the methyl substituents and the copper(I) is not easily oxidized (Dobson et al., 1984; Berger et al., 1986).

Since phenanthroline is optically inactive and absorbs in the same ultraviolet region as DNA, circular dichroism rather than UV absorption spectroscopy was used to monitor binding. No change in the UV circular dichroism of a DNA solution was observed outside error upon addition of phenanthroline, even when a 10-fold excess of phenanthroline/base pairs was added at a DNA concentration 10 times that used for spectral titrations with $(Phen)_2Cu^I$. Absorption spectroscopy was used to monitor potential DNA binding of bis(2,9-dimethyl-1,10-phenanthroline)copper(I), which has an intense visible spectrum. No absorbance changes were observed when DNA was titrated into solutions of this complex.

Discussion

Binding Equilibria in the DNA–Copper–Phenanthroline System. Interpretation of the spectroscopic data for binding of $(Phen)_2Cu^I$ to DNA as described above could be significantly compromised, in principle, since both $(Phen)Cu^I$ and free phenanthroline [in the presence of $(Phen)_2Cu^I$] were found to bind to DNA. The DNA binding of $(Phen)_2Cu^I$ (equilibrium 2) and the dissociation of $(Phen)_2Cu^I$ to $(Phen)Cu^I$ +

Phen (equilibrium 1) can be formally written as parts of the following cycles.



Insufficient data were available about (Phen)Cu^I and Phen binding to DNA to completely describe the equilibria in this system. Some fundamental considerations suffice, however, to conclude that DNA binding by (Phen)$_2Cu^I$ is accurately represented by analyses previously described and to set limits on K_3 and K_4.

The existence of the dissociation equilibria represented by K_5 and/or K_6 is required by the observation of an isosbestic point in all titrations. In particular, at a ratio of phenanthroline to copper of 2:1, in the absence of DNA, the concentration of copper complexed as (Phen)$_2Cu^I$ calculated from the 435-nm absorbance was only 75–80% of the total copper concentration, indicating partial dissociation according to equilibrium 1. The resulting free (Phen)Cu^I is expected to act as a source of free (Phen)$_2Cu^I$ to replace that bound by DNA (equilibrium 2). Observation of an isosbestic point upon DNA addition requires that the total (free + bound) concentration of (Phen)$_2Cu^I$ remain constant (within limits of detection); hence a pathway for partial dissociation of the (Phen)$_2Cu^I$·DNA complex (equilibria 5 and 6) is required to maintain this constancy. The existence of equilibrium 4 was demonstrated by the viscosity studies by using 1:1 phenanthroline:copper, while the existence of equilibrium 3 was implied by the competitive effect of excess phenanthroline on (Phen)$_2Cu^I$ binding. The possibility of an additional equilibrium corresponding to free phenanthroline binding to an occupied (Phen)$_2Cu^I$ site on DNA is inconsistent with the observation that excess phenanthroline decreases the DNA binding affinity of (Phen)$_2Cu^I$, since formation of a ternary complex would cause a net increase in the concentration of bound (Phen)$_2Cu^I$. The association constant K_2 must be greater than either of the association constants K_3 or K_4; otherwise the concentration of total (Phen)$_2Cu^I$ would decrease and an isobestic point would not be observed. K_2 cannot significantly exceed the product K_3K_4, however, or the total concentration of (Phen)$_2Cu^I$ would increase with increasing degree of DNA saturation.

Given that K_4 is less than K_2, and titration experiments were conducted with phenanthroline in excess so that equilibrium 1 favored (Phen)$_2Cu^I$ formation, the influence of (Phen)Cu^I in these binding experiments is expected to be minor. (Similar arguments apply more stringently to the neglect of binding of free cuprous ions.) The presence of excess phenanthroline, on the other hand, clearly did influence spectral titrations, seemingly competing with (Phen)$_2Cu^I$ for DNA binding sites. The effects of the concentration of excess phenanthroline on the limiting slopes of Benesi–Hildebrand plots or the K_2's estimated by the McGhee–von Hippel method suggest that at low ν values there is a simple competition between phenanthroline and (Phen)$_2Cu^I$ for DNA lattice sites, where the intrinsic affinity of phenanthroline for a lattice site is significantly less than that of (Phen)$_2Cu^I$. This supposition is supported by the linear dependence of $1/K_{2,app}$ on the free phenanthroline concentration, which indicated that the association constant for phenanthroline binding is $\approx 10^3$, or about 0.02 times that of (Phen)$_2Cu^I$ binding. The superposition of plots of ν versus log [K_2(Phen)$_2Cu^I$] and the near constancy of the McGhee–von Hippel parameters n and ω also indicate that phenanthroline binding does not strongly influence the self-cooperativity of (Phen)$_2Cu^I$ binding. The dependence of the apparent absorption spectrum of the bound (Phen)$_2Cu^I$ on the ratio of excess phenanthroline to (Phen)$_2Cu^I$ remains to be reconciled, however.

Curiously, the spectrum of bound (Phen)$_2Cu^I$ depended on the concentration of excess phenanthroline but not on the concentration of DNA (i.e., an isosbestic point was maintained in each individual titration). Assuming that shifts in the apparent absorption spectrum of bound (Phen)$_2Cu^I$ reflect the influence of phenanthroline(s) immediately neighboring bound (Phen)$_2Cu^I$, constancy of the spectrum requires that the ratio of bound (Phen)$_2Cu^I$ with phenanthroline neighbors to those bound without phenanthroline neighbors remains approximately constant throughout the titration. Such a condition is inconsistent with a model where (Phen)$_2Cu^I$ binding is cooperative but phenanthroline binding is not, because cooperative binding of (Phen)$_2Cu^I$ would displace neighboring bound phenanthrolines and thereby alter the average spectrum of bound (Phen)$_2Cu^I$. The data seem consistent with a general model where (Phen)$_2Cu^I$ and phenanthroline binding are cocooperative in some way; e.g., a bound (Phen)$_2Cu^I$ facilitates binding of phenanthroline and (Phen)$_2Cu^I$. The suggestion that phenanthroline binding is facilitated near sites occupied by (Phen)$_2Cu^I$ is consistent with, but not proven by, the failure to spectroscopically observe any DNA interactions with phenanthroline alone. Since (Phen)$_2Cu^I$ is itself a dissociable complex, tests of this proposal are difficult. The cooperativity parameter for phenanthroline binding adjacent to (Phen)$_2Cu^I$ could not greatly exceed that for (Phen)$_2Cu^I$, however, or the average spectrum of bound (Phen)$_2Cu^I$ would be nearly independent of the excess phenanthroline concentration.

The data are most consistent with (Phen)$_2Cu^I$-induced binding of free phenanthroline to DNA. According to such a model, (Phen)$_2Cu^I$ binding to a DNA site creates a local environment more favorable for both (Phen)$_2Cu^I$ and phenanthroline binding. As the concentration of excess penanthroline is increased, the apparent association constant for (Phen)$_2Cu^I$ binding would decrease due to the loss of free lattice sites occupied by phenanthroline. The cooperativity parameter, ω, for (Phen)$_2Cu^I$ binding is also expected to be reduced, as observed, since the favorable energy gained in moving one bound (Phen)$_2Cu^I$ from an isolated lattice site to a site adjacent to another bound (Phen)$_2Cu^I$ would be reduced by the cost of displacing a bound phenanthroline.

Phenanthroline seems a reasonable ligand for DNA binding. The ring nitrogens could act as hydrogen-bond acceptors from a guanine N^2-amino group in the minor groove, and van der Waals contacts with the walls of the minor groove would promote binding. Partial charges at phenanthroline 2- and 9-positions, which are separated by 6.8 Å, could also interact in the groove in a manner similar to the interactions between the minor groove binding ligands netropsin, distamycin, and SN18071 and the CH2 positions of adenines in AT-rich tracts (Pullman, 1987; Breslauer et al., 1988). Positive cooperativity would occur if this interaction is favored by DNA unwinding at a (Phen)$_2Cu^I$ binding site.

Strength and Mode of DNA Binding by (Phen)$_2Cu^I$. We have shown that (Phen)$_2Cu^I$ binds strongly to double-stranded DNA. The limiting association constant, determined by either the Benesi–Hildebrand or McGhee–von Hippel method, and

extrapolated to correct for competitive phenanthroline binding, is $\approx 5 \times 10^4$ in 0.2 M supporting NaCl and 9.8% ethanol. This binding constant is within the range reported for a variety of intercalating, polycyclic aromatic cations at this relatively high ionic strength (Howe-Grant & Lippard, 1979; Wilson et al., 1985; Barton et al., 1986). The neighbor-exclusion parameter of $n = 2$ for $(Phen)_2Cu^I$ is also consistent with those of other intercalators. Binding of $(Phen)_2Cu^I$ is modestly cooperative, however. Positive cooperativity is uncharacteristic of the DNA binding of most simple intercalating ligands such as ethidium and proflavin but has been observed for some complex intercalators such as metalloporphyrins (Strickland et al., 1988). Moreover, positive cooperativity has not been observed for *N*-alkyl-1,10-phenanthrolinium ions (Gabbay et al., 1973) or for other metal–phenanthroline complexes binding to B-DNA (Howe-Grant & Lippard, 1979; Baron et al., 1986). The latter complexes are distinct from $(Phen)_2Cu^I$, however, in that they are not tetrahedral. Positive cooperativity is found for the binding of $(Phen)_3Fe^{3+}$ to Z-DNA, but this cooperativity is reconciled in terms of the Z- $\Rightarrow$ B-DNA transitions (Hard et al., 1987).

Previously we proposed that $(Phen)_2Cu(I)$ preferentially binds to double-stranded DNA by partial intercalation of one phenanthroline from the minor groove side, particularly at T-3′,5′-A steps, with the second phenanthroline lying in the minor groove, approximately with the long axis of the tricyclic ring system parallel to the helix axis and the ring plane passing through the positions occupied by the guanine N^2-amino groups in a C-3′,5′-G step [which shows low reactivity toward $(Phen)_2Cu^I$; Veal & Rill (1989)]. A similar model has been proposed by Stockert (1989). Such a binding model accounts for the relative preferences of $(Phen)_2Cu^I$ cleavage at pyrimidine–purine–pyrimidine steps and for the pattern of reactivities at specific nucleotides within the binding site (Veal & Rill, 1989). The observations that DNA binding by $(Phen)_2Cu^I$ causes a significant increase in viscosity of DNA solutions, results in a high degree of hypochromism of the visible spectrum of $(Phen)_2Cu^I$, and is consistent with the site-exclusion model provide additional evidence for binding by intercalation. The strong circular dichroism spectrum of $(Phen)_2Cu^I$ induced by DNA binding is consistent with, though not necessarily unique to, intercalation. Intercalation of copper–phenanthroline complexes has also been suggested recently by studies of cleavage of lesion sites in oligonucleotides (Williams et al., 1988) and by studies in which phenanthroline was covalently attached to poly(T), which binds to DNA via triple-helix formation (Francois et al, 1989). A proposal for nonintercalative, groove binding of $(Phen)_2Cu^I$, however, has also been made (Thederahn et al., 1989).

Assuming partial intercalation as the mode of binding, positive cooperativity of $(Phen)_2Cu^I$ binding can be rationalized in several ways. Positive cooperativity may be related to extensive displacement of the "spine" of hydration water that is well developed in the minor groove at AT-rich sequences (Dickerson & Drew, 1981) by the nonintercalated phenanthroline ring. An attractive alternate is that positive cooperativity is related to the unique twisted geometry of $(Phen)_2Cu^I$ and the influence of the second, nonintercalated phenanthroline ring of the complex. The dihedral angle between phenanthroline rings, though expected to be near 90° in solution, is not stringently fixed. Variations of the dihedral angle from $\approx$50° to 80° have been observed in crystalline $(Phen)_2Cu^+ \cdot X^-$ complexes, depending on the nature of X^- and the crystal symmetry (Fitzerald & Hathaway, 1984; Healy et al., 1985). These variations presumably reflect the influence of crystal-packing forces. Examination of space-filling models suggests that distortion of the dihedral angle from 90° may be required to minimize steric hindrances to DNA binding. In this regard the crystallographic observations that the minor groove is relatively narrow in the AT-rich tracts may be relevant. Positive cooperativity is expected if modest strain energy is required to distort the $(Phen)_2Cu^I$ dihedral angle from 90°. Since intercalation of one $(Phen)_2Cu^I$ must cause unwinding and opening of the groove, binding of a second $(Phen)_2Cu^I$ to a neighboring site may require less distortion of the dihedral angle and hence less strain energy cost. The strain energy need not be large to cause cooperativity. The cooperativity parameter ω can be expressed as the equilibrium constant for the process whereby a ligand moves from an isolated site to a site flanking a prebound ligand (McGhee & von Hippel, 1974). An $\omega \approx 3$ corresponds to a standard free energy of binding. $\Delta G° \approx 700$ cal/mol, or $\approx$70 cal mol^{-1} deg^{-1} for a 10° distortion of the $(Phen)_2Cu^I$ dihedral angle. Distortion of the dihedral angle may also assist in optimizing potential dipole $\leftrightarrow$ induced dipole interactions between partial charges at the phenanthroline 2-CH and 9-CH groups and, for example, the adenine 2-CH positions. Similar interactions are believed to stabilize complexes between DNA and groove binders that prefer AT-rich tracts (Braithwaite & Baguley, 1980; Kopka et al., 1985).

A binding model involving partial intercalation of one phenanthroline ring and active participation of the second phenanthroline ring is consistent with the failure to observe evidence for intercalation of bis(2,9-dimethyl-1,10-phenanthroline)copper(I). Bis(2,9-dimethyl-1,10-phenanthroline)copper(I) does not act as a nuclease (Sigman, 1986). Our viscosity and spectroscopic data support a previous report that this complex does not intercalate (Graham & Sigman, 1974). The DNA binding by bis(2,9-dimethyl-1,10-phenanthroline)copper(I) noted by Graham and Sigman (1984) clearly cannot be analogous to the binding of $(Phen)_2Cu^I$ [see also related work by Tamilarasan et al. (1988)].

The bis(2,9-dimethyl-1,10-phenanthroline)copper(I) complex is a reasonable model for the influence of the dihedral angle on DNA binding. Although the methyl groups limit the depth of penetration of the complex between base pairs, relative to $(Phen)_2Cu^I$, partial intercalation appears possible. The *N*-methyl derivative of 2,9-dimethyl-1,10-phenanthroline is known to bind DNA by intercalation more strongly than *N*-methyl-1,10-phenanthroline (Gabbay et al., 1973). The methyl groups do, however, preclude van der Waals interactions with the adenine 2-CH positions. Failure of bis(2,9-dimethyl-1,10-phenanthroline)copper(I) to bind DNA by intercalation may therefore be due to a combination of effects: insufficient π-orbital overlap, a large increase in strain energy required to distort the dihedral angle from 90°, and loss of van der Waals stabilization energy.

It now appears that van der Waals contacts in the minor groove of AT-rich tracts are important for DNA binding of classic "groove binders" such as netropsin and distamycin and particularly the compound SN18071, which is structurally similar to the latter drugs and binds in the minor groove but lacks potential for hydrogen-bond formation (Braithewaite & Baguley, 1980; Kopka et al., 1985a,b; Pullman, 1987; Breslauer et al., 1988). If the nonintercalated ring of $(Phen)_2Cu^I$ also participates in stabilizing the DNA complex via van der Waals interactions, then $(Phen)_2Cu^I$ is most properly regarded as a combination intercalator/groove binder in the classic senses. Such a unique binding mode may explain the high

degree of DNA sequence preference of the relatively simple $(Phen)_2Cu^I$ molecule. This degree of preference appears to approach that of 7-azidoactinomycin D (Rill et al., 1989; Veal, L. Su, and Rill, unpublished observations), a more complex combination intercalator/minor groove binder (Sobel & Jain, 1982).

ACKNOWLEDGMENTS

We thank Dr. David Graves (University of Mississippi) for providing the simplex program for analyzing binding data and Dr. Kenneth Goldsby (Florida State University) for helpful discussions of inorganic chemistry.

Registry No. Phen, 66-71-7; $(Phen)_2Cu(I)$, 17378-82-4; (Phen)-Cu(I), 100994-51-2; 2,9-dimethyl-1,10-phenanthrolinecopper(I), 21710-12-3.

REFERENCES

Barton, J. K. (1986) *Science 233*, 727–733.
Barton, J. K., Goldberg, J. M., Kumar, C. V., & Turro, N. J. (1986) *J. Am. Chem. Soc. 108*, 2081–2088.
Benesi, H. A., & Hildebrand, J. H. (1949) *J. Am. Chem. Soc. 71*, 2203–2207.
Berger, R. M., McMillin, D. R., & Dallinger, R. F. (1987) *Inorg. Chem. 26*, 3802–3805.
Braithwaite, A. W., & Baguley, B. C. (1980) *Biochemistry 19*, 1101–1106.
Breslauer, K. J., Ferrante, R., Marky, L. A., Dervan, P. B., & Youngquist, R. S. (1988) in *Structure and Expression, Vol 2: DNA and its Drug Complexes* (Sarma, R., Ed.) pp 273–290, Adenine, New York.
Chen, C. H., & Sigman, D. S. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7147–7151.
Chen, C. H., & Sigman, D. S. (1987) *Science 237*, 1197–1201.
Dickerson, R. E., & Drew, H. (1981) *J. Mol. Biol. 149*, 761–786.
Dobson, J. F., Green, B. E., Healy, P. C., Kennard, L. H. L., Pakawatchai, L., & White, A. H. (1984) *Aust. J. Chem. 37*, 649–659.
Drew, H., & Travers, A. (1984) *Cell 37*, 491–502.
Fitzerald, W., & Hathaway, B. (1984) *Acta Crystallogr., Sect. B 40*, 243–245.
Francois, J.-C., Saison-Behmoaras, T., & Helene, C. (1988a) *Nucleic Acids Res. 16*, 11431–11440.
Francois, J.-C., Saison-Behmoaras, T., Chassignol, M., Thuong, N. T., Sun, J.-S., & Helene, C. (1988b) *Biochemistry 27*, 2272–2276.
Francois, J.-C., Saison-Behmoaras, T., Chassignol, M., Thuong, N. T., & Helene, C. (1989) *J. Biol. Chem. 264*, 5891–5898.
Gabbay, E. J., Scofield, R. E., & Baxter, C. S. (1973) *J. Am. Chem. Soc. 95*, 7850–7857.
Goldstein, S., & Czapski, G. (1986) *J. Am. Chem. Soc. 108*, 2244–2250.
Goldstein, S., Czapski, G., Cohen, H., & Meyerstein, D. (1988) *Inorg. Chem. 27*, 4130–4135.
Graham, D. R., & Sigman, D. S. (1984) *Inorg. Chem. 23*, 4188–4191.
Guo, Q., Lu, M., Seeman, N. C. & Kallenbach, N. R. (1990) *Biochemistry 29*, 570–578.
Hard, T., Hiort, C., & Norden, B. (1987) *J. Biomol. Struct. Dyn. 5*, 89–96.
Healy, P. C., Engelhardt, L. M., Patrick, V. A., White, A. N. (1985) *J. Chem. Soc., Dalton Trans.*, 2541–2545.
Hodges, H. L., & Araujo, M. A. (1982) *Inorg. Chem. 21*, 3236–3239.
Howe-Grant, M., & Lippard, S. J. (1979) *Biochemistry 18*, 5762–5769.
Johnson, G. R., & Nazhat, N. B. (1987) *J. Am. Chem. Soc. 109*, 1990–1994.
Kazakov, S., Astashkina, T. G., Mamaev, S. V., & Vlassov, V. V. (1988) *Nature 335*, 186–188.
Kopka, M. L., Yoon, C., Goodsell, D., Pjura, P., & Dickerson, R. E. (1985a) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1376–1380.
Kopka, M. L., Yoon, C., Goodsell, D., Pjura, P., & Dickerson, R. E. (1985b) *J. Mol. Biol. 131*, 553–563.
Kuwabara, M., Yoon, C., Goyne, T., Thederahn, T., & Sigman, D. (1986) *Biochemistry 25*, 7401–7408.
Lee, C.-W., & Anson, F. C. (1984) *Inorg. Chem. 23*, 837–844.
Lerman, L. (1961) *J. Mol. Biol. 3*, 18–30.
McGhee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.
Pflaum, R. T., & Brandt, W. N. (1955) *J. Am. Chem. Soc. 72*, 2019–2022.
Pullman, B. (1987) in *Molecular Mechanisms of Carcinogenic and Antitumor Activity* (Pullman, B., Ed.) pp 3–30, Adenine, New York.
Sigman, D. S. (1986) *Acc. Chem. Res. 19*, 180–186.
Smith, G., & Frederick, C. A. (1954) *Anal. Chem. 26*, 1534–1538.
Sobell, H. M., & Jain, S. C. (1982) *J. Mol. Biol. 168*, 21–34.
Stockert, J. C. (1989) *J. Theor. Biol. 137*, 107–111.
Strickland, J., Marzilli, L., Gay, K. & Wilson, W. D. (1988) *Biochemistry 27*, 8870–8878.
Sun, J.-S., Francois, J.-C., Lavery, R., Saison-Behmoaras, T., Montenay-Garestier, T., Thuong, N. T., & Helene, C. (1988) *Biochemistry 27*, 6039–6045.
Tamilarasan, R., Ropartz, S., & McMillin, D. R. (1988) *Inorg. Chem. 27*, 4082–4085.
Thederahn, T. B., Kuwabara, M. D., Larsen T. A. & Sigman, D. S. (1989) *J. Am. Chem. Soc. 111*, 4941–4946.
Veal, J. M., & Rill, R. L. (1988) *Biochemistry 27*, 1822–1827.
Veal, J. M., & Rill, R. L. (1989) *Biochemistry 28*, 3243–3250.
Williams, L. D., Thivierge, J., & Goldberg, I. H. (1988) *Nucleic Acids Res. 16*, 11607–11615.
Wilson, W. D., Wang, Y.-H., Kusuma, S., Chandrasekaran, S., Yang, N. C., & Boykin, D. W. (1985) *J. Am. Chem. Soc. 107*, 4989–4995.