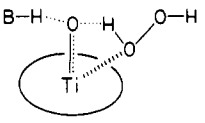

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transfer from hydrogen peroxide to the oxo group in the transition state.

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Registry No. $[Ti^{IV}O(tmpyp)]$, 96503-16-1; $[Ti^{IV}O(tmpyp)]I_4$, 96503-17-2; MES, 4432-31-9; MOPS, 1132-61-2; TAPS, 29915-38-6; H_2O_2, 7722-84-1; HOAc, 64-19-7.

Supplementary Material Available: Figure S1, data for the determination of pK_a values of MES, MOS, and TAPS, Figure S2, visible absorption spectra of **1** and **2**, Figure S3, dependence of $\{k_{0(H)} - (k + k_H[H^+])\}$ on the concentration of taps$^-$, and Figure S4, pressure dependence of rate constants k and k_H (4 pages). Ordering information is given on any current masthead page.

Contribution from the Departments of Chemistry, Emory University, Atlanta, Georgia 30322, and Georgia State University, Atlanta, Georgia 30303

Interactions of a Copper Antitumor Drug with DNA: [1-(α-Pyridylmethylene)-2-(α-pyridyl)hydrazine]copper(II) Chloride Does Not Intercalate

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The interaction of the copper metallodrug [1-(α-pyridylmethylene)-2-(α-pyridyl)hydrazine]copper(II) chloride with DNA (calf thymus, salmon sperm, Col E_1 plasmid) was selectively examined by UV–vis spectroscopy (binding constants, spectral changes, and flow dichroism), viscometry, and NMR spectroscopy (^{31}P NMR and Redfield ^1H NMR of the imino H). None of the physical measurements were consistent with an intercalation binding mode. The data were most consistent with an outside binding mode involving interaction of the drug with the phosphodiester group. The drug proved to be remarkably effective at broadening the ^{31}P NMR signal of DNA. In contrast, Cu^{2+} ion selectively broadened the GN(1)–H signal of DNA, consistent with proposals in the literature that Cu^{2+} binds to GC base pairs in DNA. Regardless of binding mode, the bound form of the drug is protonated at the noncoordinated hydrazine nitrogen. Although informative, this study did not provide a rationale for the very potent cytotoxicity of the drug toward certain tumor cell lines.

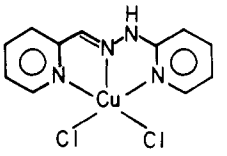
Introduction

The interactions of metal complexes with DNA are important both for their medicinal effects and as probes of DNA interactions and dynamics. A variety of drugs of such varied structure as *cis*-dichlorodiammineplatinum(II) (cisplatin),[1] bleomycin,[2] and metallocenes[3,4] of titanium and vanadium have shown wide spectrum antineoplastic action with DNA as the proposed biological target. In addition, other antitumor drugs such as the anthracyclines, which are thought to act at least partially at the DNA level in vivo, form metal complexes that have a pronounced effect on their DNA interactions.[5]

Metal complexes with planar aromatic bases have been used extensively as probes of DNA structure. Copper complexes of 1,10-phenanthroline, for example, cause DNA degradation in the presence of oxygen and a reducing agent, and the rate of degradation is dependent on the helical conformation (A, B, or Z).[6] Barton et al. have shown that Δ- and Λ-[tris(phenanthroline)-ruthenium(II)](2+) cations exhibit enantiomeric selectivity in intercalative binding to left- and right-handed helical forms of DNA.[7] Dervan and co-workers have synthetically coupled an EDTA derivative to the intercalator methidium cation.[8] This new agent, when complexed with iron, can also degrade DNA and has been extensively used in "footprinting" experiments to establish binding-site specificity for a variety of compounds that interact with DNA.

Porphyrin compounds that have no or easily removed axial ligands can also bind to DNA, presumably by intercalation, and are useful probes of DNA dynamics.[9-11] Lippard and co-workers have conducted an extensive study of metallointercalators containing Pt(II) and have carefully defined the structural requirements for intercalation to occur.[12]

It has recently been shown that the copper complex of the tridentate ligand 1-(α-pyridylmethylene)-2-(α-pyridyl)hydrazine (PCPH) inhibits DNA synthesis in neoplastic cells in tissue culture



CuPCPH

at concentrations similar to or less than those normally used for cisplatin and bleomycin.[13,14] The copper complex also causes dramatic inhibition of tumor growth in vivo in mice and, at the dosages used, produces no observable toxic side effects in the animals. CuPCPH, for example, gives 50% inhibition of DNA synthesis in cultured human melanoma cells at a concentration of only 0.05 ng/mL or several thousand times less than the

† Emory University.
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amounts of cisplatin and bleomycin required for the same level of inhibition.

The CuPCPH complex is quite different than other metal complexes whose interaction with DNA has been studied in detail, and because of its excellent biological activity, we have conducted a detailed investigation of its interaction with DNA.

Experimental Section

Materials. DNA Solutions. For the binding and viscometric studies, calf thymus DNA (Worthington) was sonicated to 600 base pairs and analyzed according to Wilson et al.[15] Col E_1 plasmid closed circular supercoiled DNA (ccs DNA) was prepared as previously described by Jones et al. (1979).[16] For the NMR studies, salmon sperm DNA (Worthington) was sonicated to ~200 base pairs.[15] Calf thymus DNA (Worthington) for the flow dichroism study was used without sonication; the average DNA length was 9000 base pairs.

The prepared DNA solutions were dialyzed extensively against MES 1 buffer (0.01 M 4-morpholineethanesulfonic acid adjusted to the desired pH with NaOH).

Methods. Synthesis of CuPCPH. The ligand, PCPH, from Aldrich and copper chloride from Fisher Scientific Co. were reagent grade and used without further purification.

[1-(α-Pyridylmethylene)-2-(α-pyridyl)-hydrazine]copper(II) chloride (CuPCPH) was synthesized according to the methods of Lions and Martin.[17]

A melting point of 230 °C was observed (lit.[17] mp 220–226 °C). Anal. Calcd for $C_{11}H_{10}N_4CuCl_2$: C, 39.72; H, 3.03; N, 16.84. Found: C, 39.36; H, 3.34; N, 16.70. This sample has a visible spectrum identical with one provided by Dr. Pickart.

Spectrophotometric Methods. 1. pK_a Determination. A 1-L solution of MES 1, adjusted to pH 3.00 with reagent grade HNO_3, was titrated with NaOH. The pH was monitored with a Fisher Accumet 825 MP pH meter. At each desired pH, and 8-mL aliquot of solution was removed and a CuPCPH solution (10 μL) was added to a final concentration of 2×10^{-5} M. The pH of each CuPCPH-containing aliquot was recorded, and spectra between 600 and 320 nm were obtained on a Cary 219 spectrophotometer (1-cm quartz cuvette).

2. Binding Constant (K) and Extinction Coefficient (ε) Determination. The binding constants and the extinction coefficients of both free and bound species were determined as a function of pH. The DNA solution was dialyzed extensively against MES 1 at pH 5.10, 5.60, and 7.00. For the determination of ε of the free CuPCPH species (ϵ_{free}), a 10-cm cylindrical quartz cell was used. For ε of the bound CuPCPH–DNA species (ϵ_b), a 1-cm quartz curvette was used with a typical DNA–phosphate concentration of ~1 mM. For determination of the apparent ε (ϵ_{app}), where the fraction of ligand bound to DNA is between 0.2 and 0.8, a 5-cm cylindrical quartz cell was used.

Extinction coefficients (ϵ_{free}, ϵ_b, ϵ_{app}) were determined by titrating a CuPCPH stock solution into a quartz cell containing either buffer or DNA. The CuPCPH stock solutions were prepared by first drying the CuPCPH under vacuum at 117 °C for 24 h; the dried compound was then weighed and dissolved in an MES 1 buffer solution.

3. Flow Dichroism. All measurements were made on the Cary 17D spectrophotometer at room temperature. The flow device consisted of a 0.1-cm cell with openings at the top and bottom to which Tygon tubing was attached. A syringe pump (Sage Instruments) connected to one end of this tubing maintained a constant flow rate of about 13.3 mL/min. Light was polarized either parallel or perpendicular to the direction of sample flow by placing a commercially mounted and calibrated Perkin-Elmer MPF44A polarizer between the light source and the sample. A solution containing unsonicated calf thymus DNA, MES 1 (pH 5.60), and 10^{-2} M EDTA was dialyzed extensively against MES 1 (pH 5.60) to remove the EDTA. The final concentration was adjusted to 8 mM in DNA-P. CuPCPH was added as a solid and dissolved by a tissue homogenizer designed to minimize the overall reduction in the DNA length.

4. Solution Study of CuPCPH Formation. Spectral changes of PCPH upon complexation with copper(II) ion were observed with a Cary 17D spectrophotometer using a 1-cm cuvette. An aqueous stock solution of PCPH (4×10^{-4} M) contained 10% methanol to increase the ligands solubility and then was diluted to 2×10^{-5} M with 0.01 M PIPES buffer at pH 7.00. Aqueous stock 10 mM solutions of copper chloride or copper nitrate were used.

Viscometric Methods. For viscometric titrations, 600 base pair calf thymus DNA and the Col E_1 plasmid ccs DNA solutions in MES 1 (pH 5.60) were used. Viscometric titrations were performed with Ubbelohde viscometers in a thermostated water bath at 30.7 °C. Several time readings were obtained at each titration point, and the standard deviation was no greater than ±0.03 s.

NMR Methods. Proton NMR spectroscopy was carried out by using a modified ^{1}H-Redfield 21412 pulse sequence on a 360 MHz Nicolet NMR spectrometer with NMC-1280 software.[18] To minimize the dynamic range problem, three sets of 2000 transient FID's at 20-bit words were collected on a given sample. The three sets of FID's were block averaged in double-precision mode (40-bit words) and Fourier transformed with 3-Hz line broadening prior to converting the averaged data back to 20-bit words. Each set of data was collected with 4K data points, 7042-Hz spectral window, and a carrier frequency at 15.5 ppm.

The NMR samples contained 200 base pair salmon sperm DNA prepared as described above. For the ^{1}H NMR studies, the DNA (0.04 M P) contained 10% D_2O and TSP as an internal reference. An external reference of dilute *tert*-butyl alcohol in CCl_4 contained in a sealed capillary tube was inserted into the 5-mm NMR tube and centered with a Teflon collar. Aliquots from aqueous stock solutions of known concentrations of CuPCPH and copper nitrate were lyophilized and DNA solutions were added.

^{31}P NMR spectra of salmon sperm DNA (0.012 M P) in D_2O were accumulated with quadrature detection on either a JEOL FX 60 Q (24.15 MHz) or an IBM WP-200 SY (81.01 MHz) NMR spectrometer. Trimethyl phosphate was the internal reference. 10-mm NMR tubes with a Teflon vortex plug inserted and a total sample volume of 1.8 mL were used. Line broadening of 1 Hz was used in the Fourier transformation. At 24.15 MHz the parameters used were 8192 time domain points, 90° pulse, 12-s delay time, broad-band proton decoupling, typically 5000 transients, 22 °C. At 81.01 MHz the parameters used were spectral window of 2000 Hz, 45° pulse, no delay time, proton decoupling, typically 8000 transients, 25 °C.

Another ^{31}P NMR sample containing extra PCPH ligand was prepared by adding a slightly acidic stock solution of 1 mM PCPH to a solution of 1.2×10^{-5} M CuPCPH and 1.2×10^{-2} M DNA-P. The final concentrations of the CuPCPH and the PCPH were 10^{-5} and 10^{-6} M, respectively. The pH after addition of PCPH remained at 5.60.

Results

pK_a and Visible Spectrum of CuPCPH. The relative intensities of the two bands in the visible spectrum of CuPCPH are pH sensitive. At pH ~3 and ~8 the most intense band was at 375 and 456 nm, respectively. A titration with NaOH from pH ~3 to ~8 yielded isosbestic points at 336 and 402 nm (see the supplemental material). The absorption data at either 375 or 456 nm were used to determine the pK_a of CuPCPH with eq 1, where

$$\mathrm{pH} = \mathrm{p}K_a + \log\left(\frac{A_A - A_{APP}}{A_{APP} - A_B}\right) \quad (1)$$

A_A and A_B are the absorption of the protonated and deprotonated CuPCPH species, respectively, and A_{APP} is the absorption at any pH value. A plot of pH vs. log $[(A_A - A_{APP})/(A_{APP} - A_B)]$ from the absorption data at 456 nm yields a line with a line with a pK_a value of 5.5 (Figure 1). From a plot of the absorption data at 375 nm (not shown), a similar pK_a value was determined.

The spectra from 600 to 300 nm of pH 7.00 solutions of PCPH (prepared as described above) were examined as a function of added $CuCl_2$ or $Cu(NO_3)_2$. On addition of copper salts, the absorption band at 337 nm decreased and a 456-nm band appeared. At a 1:1 ratio (2×10^{-5} M), ϵ_{456} was 1.7×10^3 M^{-1} cm^{-1} for solutions with either salt. This is the same value obtained with isolated CuPCPH.

DNA Binding and Visible Spectra. Spectral changes were also observed when calf thymus DNA was titrated into a buffered CuPCPH solution at either pH 5.74 (Figure 2) or 6.88 (see the supplemental material). With each addition of DNA there is a concurrent absorption decrease at 456 nm and an absorption increase at 375 nm. The spectral changes were very similar to those accompanying protonation of CuPCPH. However, the isosbestic points (at 341 and 406 nm) differed by 4 nm. With

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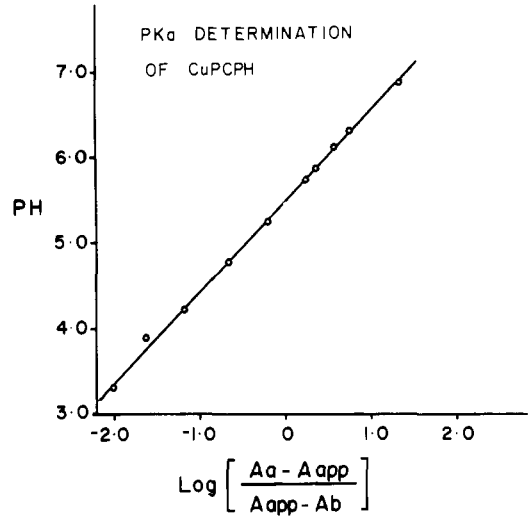

Figure 1. pH vs. log $[(A_A - A_{APP})/(A_{APP} - A_B)]$. The p$K_a$ is determined from the intercept, log $[(A_A - A_{APP})/(A_{APP} - A_B)] = 0$ at 456 nm.

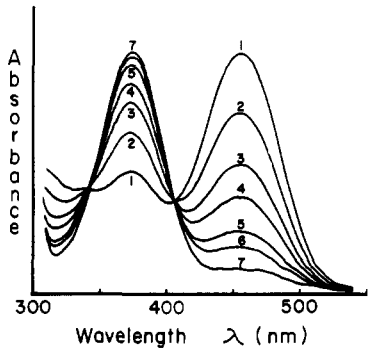

Figure 2. Spectrophotometric titration of CuPCPH with calf thymus DNA at pH 5.74. Concentrated DNA solution was added to CuPCPH at the molar ratios of (1) no DNA added, (2) 0.24, (3) 0.12, (4) 0.06, (5) 0.047, (6) 0.034, (7) 0.014.

Table I. Extinction Coefficients (ϵ) and Binding Constants (K_{app}) of CuPCPH with Calf Thymus DNA (600 Base Pairs) at 456 nm

pH	ϵ_f	ϵ_b	$10^{-5}K_{app}$, M^{-1}	n
5.10	7670	400	2.7	1.3
5.60	11400	1000	1.1	1.2

each subsequent titration of DNA, the absorption changes at the same concentration of pH 5.74 are greater than those at pH 6.88.

At constant pH, spectral changes of CuPCPH on addition of DNA are less as the ionic strength is increased. With the spectrophotometric methods described, it was not possible to accurately determine a value for the relatively weak binding constant of CuPCPH to DNA at pH 7.00 even at low ionic strength. The apparent binding constants at pH 5.10 and 5.60 were determined with DNA solutions at several DNA concentrations. The absorption changes at 456 nm were recorded, and the results were analyzed with the site exclusion model of McGhee and von Hipple[19]

$$\nu/c = K(1 - n\nu)[(1 - n\nu)/(1 - (n - 1)\nu)]^{n-1} \qquad (2)$$

where ν is the molar ratio of CuPCPH bound per base pair of DNA, c is the concentration of free CuPCPH, K is the intrinsic binding constant, and n is the size of the binding site in base pairs. Best fit values of n and K were obtained by a nonlinear least-squares computer program (Figure 3). In each case studied, $n \approx 1$. These results are summarized in Table I. From the pK_a value of CuPCPH, it can be calculated that the fraction of protonated CuPCPH decreased from 0.72 to 0.44 as the pH is changed from 5.1 to 5.6. The significant drop in binding constant over this pH range suggests that the protonated species of CuPCPH binds much better to DNA than the unprotonated species. From the pK_a value, the two apparent binding constants in Table I, and the assumption that the amount of unprotonated CuPCPH bound to DNA is negligible, it can be calculated that the binding free energy of protonated CuPCPH is approximately −7 kcal/mol (depends on ionic strength but not pH).[20] Using this value with the pK_a indicates that the apparent binding free energy at pH 7 should be less favorable by approximately +2 kcal/mol. The observed very weak binding of CuPCPH at pH 7 agrees with these results and indicates that ionic interactions of CuPCPH with the DNA–phosphate groups contribute a major part to the binding free energy.

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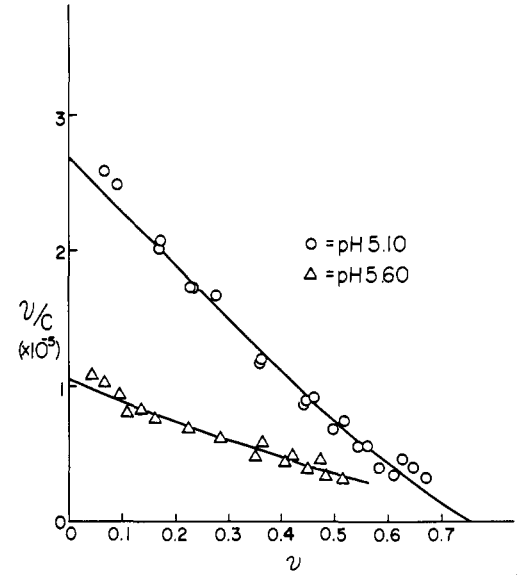

Figure 3. Scatchard plots of CuPCPH binding to calf thymus DNA as a function of pH. Each curve represents a set of three titrations at varying DNA concentrations. (See Table I for summary of the data.)

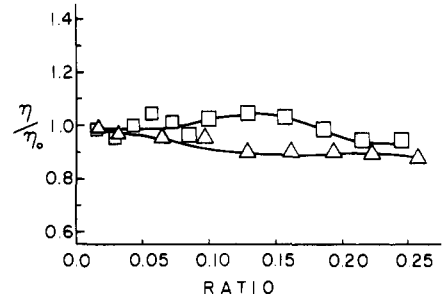

Figure 4. Viscometric titration of CuPCPH into a DNA–buffer solution of 0.01 M MES at pH 5.60. The relative reduced specific viscosity (η/η_0) is plotted as a function of the molar ratio of CuPCPH to DNA-P. Two types of DNA are used: linear 600 base pair calf thymus DNA (□), and ccs DNA (Δ).

Viscometry. The ability of CuPCPH to lengthen sonicated DNA or unwind ccs DNA was assessed viscometrically. The relative reduced specific viscosity (η/η_0) was calculated and plotted as a function of the molar ratio of drug to DNA–phosphate (ν)[21]

$$\eta/\eta_0 = \frac{(\eta_{sp}/C)_\nu}{(\eta_{sp}/C)_{\nu=0}} \qquad (3)$$

where η_{sp} is the specific viscosity of DNA (i.e. $\nu = 0$) or drug with DNA (i.e. ν varies). At pH 5.60 in MES 1, CuPCPH does not significantly affect the viscosity of either linear or ccs DNA (Figure 4).

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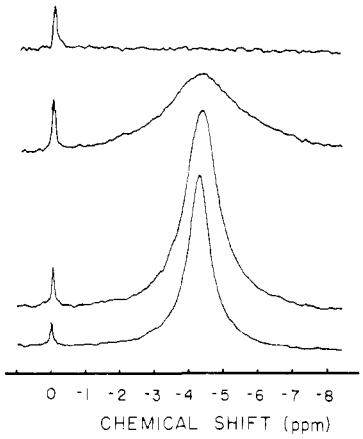

Figure 5. ^{31}P NMR spectra (81 MHz) of 200 base pair salmon sperm DNA (0.01 M) with [CuPCPH] as a function of ratio: 0, 10^{-4}, 10^{-3}, 10^{-2} (from bottom to top).

Flow Dichroism. Flow-dichroism studies of CuPCPH with DNA allow an assessment of the relative orientation of the copper complex to that of the flow-oriented DNA base pairs. This determination was made by calculating the reduced linear dichroism (^{red}D) of the drug–DNA complex:[22,23]

$$^{red}D = D/A_0 \quad (4)$$

A_0 is the absorption of the randomly oriented DNA. D is the linear dichroism where

$$D = A_{\parallel} - A_{\perp} \quad (5)$$

which is the absorption difference between parallel ($A_{\parallel}$) and perpendicularly ($A_{\perp}$) polarized light (relative to the direction of flow). At 260 nm, the 8 mM native calf thymus DNA gives a reduced linear dichroism of -0.015 ± 0.002 at pH 5.60. Upon addition of CuPCPH at molar ratios of 0.05 and 0.10, the dichroism remained unchanged within experimental error. There was no induced dichroism at either 375 or 456 nm.

NMR Results. The effect of the paramagnetic CuPCPH on the ^{31}P NMR signal of 200 base pair DNA (0.012 M) was studied as a function of CuPCPH concentration and compared with the effect of Cu^{2+} (Figure 5). In all cases, the signal observed was at −4.27 ppm. At 1.2×10^{-5} M CuPCPH the line width at half-height ($\Delta\nu_{1/2}$) increased to 207 Hz from 56 Hz for the uncomplexed DNA. By the addition of 1.2×10^{-4} M CuPCPH ($R = 10^{-2}$) the ^{31}P NMR signal was broadened into the base line. The addition of a reducing agent, β-mercaptoethanol, restored the signal. When 10% excess of PCPH ligand (1.2×10^{-4} M) was added to a CuPCPH–DNA ($R = 10^{-3}$) solution, there was no change in the line width at half-height.

Under identical conditions of DNA concentration, buffer, and pH, the addition of copper nitrate caused similar line width increases (Figure 6). However, the complexed copper of CuPCPH increased the ^{31}P NMR line width to a much greater extent than that of aqueous copper(II) ion.

The effect of CuPCPH on the ^1H imino signal of DNA (0.04 M) was some broadening of both the adenine–thymine and guanine–cytosine signals (Figure 7A). The area of the ^1H imino signals was determined relative to the external reference, *tert*-butyl alcohol. The signals were integrated for each titration point and compared with the area of the DNA alone. For CuPCPH the ^1H imino area was reduced by ~10% at the ratios of 0.10 and 0.20 (approximately equal to the percent error in determining the area). Essentially identical results were obtained at 0.1 M $NaNO_3$. Under low-[salt] conditions (Figure 7B), copper(II) ion brought about an increase in line widths and an area loss of ~30% for ratios of 0.005–0.10. The area loss for the guanine–cytosine peak

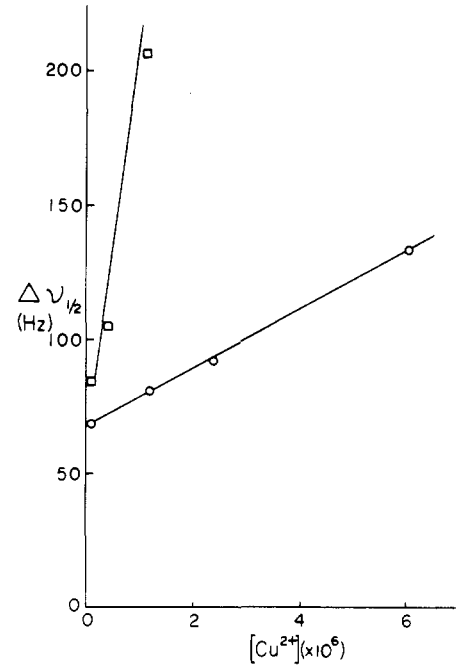

Figure 6. ^{31}P NMR (81 MHz) line width at half-height ($\Delta\nu_{1/2}$) vs. the molar concentration of copper for CuPCPH (□) and copper nitrate (○).

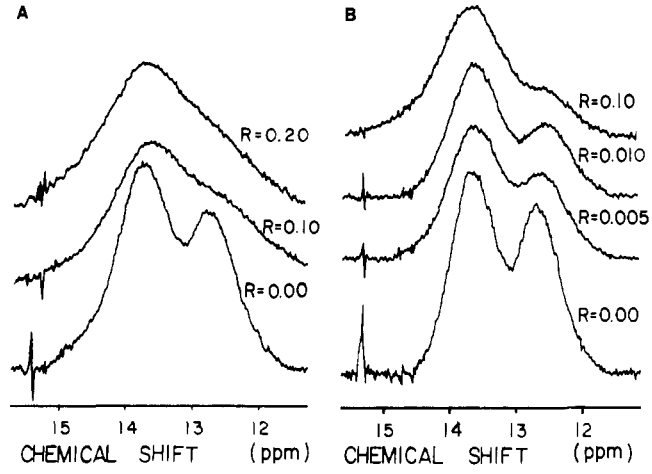

Figure 7. (A) ^1H NMR spectra of 200 base pair salmon sperm DNA (0.04 M) with [CuPCPH] as a function of ratio: 0, 0.10, 0.20 (from bottom to top). The upfield signal is for the GC base pairs. (B) ^1H NMR spectra of salmon sperm DNA (0.04 M) with [$Cu(NO_3)_2$] as a function of ratio: 0, 0.005, 0.01, 0.10 (from bottom to top).

was significant while the adenine–thymine peak was affected much less. At a ratio of 0.15:1 Cu^{2+}:DNA-P, the solution became cloudy and the titration was discontinued.

Discussion

For the purpose of this discussion, we will divide the possible binding modes of CuPCPH into three categories—intercalation, base binding, and outside (phosphate/electrostatic) binding. We will consider how our results relate to each binding mode. However, we must first consider the form of CuPCPH in solution.

Form of CuPCPH. Although Cl is a weak ligand, it was conceivable that Cl could remain bound to the Cu. Therefore, we prepared the CuPCPH complex in solution using both $CuCl_2$ and $Cu(NO_3)_2$ as starting reagents. Visible spectral comparisons of these samples, as well as comparisons to the isolated complex used in this study, revealed that the species formed were identical. Similarly, a pK_a determination on the solution prepared with $Cu(NO_3)_2$ gave a value of ~5.5, in excellent agreement with the value found for the isolated complex.

Previous work has established that the proton at the hydrazine function is titratable with a $pK_a = 5.5$. The spectral change, an

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intensity increase in the long-wavelength visible band at 456 nm and a decrease in the short-wavelength band at 375 nm, is characteristic of deprotonation. Thus at pH 5.60, we believe the form of the complex is $CuPCPH^{2+}$ (~44%) and $CuPCPH-H^{1+}$ (~56%). Water occupies any remaining coordination positions.

The visible spectral changes on binding of CuPCPH to DNA (Figure 2) are very similar to the changes that occur on protonation. This result strongly suggests that the DNA-bound form of CuPCPH is, in fact, $CuPCPH^{2+}$. This spectral change, indicative of an increased pK_a of CuPCPH, is easily understood as arising from the highly negatively charged polyelectrolyte environment of the bound CuPCPH. However, this result can be rationalized by using any of the three binding modes enumerated above. The spectral change, therefore, merely presents a convenient method for studying the DNA interaction.

Intercalation Binding Mode. The CuPCPH has the primary characteristics that could make it an intercalator—an aromatic group and positive charge. All well-established intercalators cause an increase in the viscosity of DNA and unwind ccs DNA.[24] The failure of CuPCPH to cause unwinding (Figure 4) is clear evidence against the intercalation binding mode. At $r = 0.21$ and 0.10 M $NaNO_3$, η/η^0 was found to be 0.87. Therefore, also under higher salt conditions, we observed no significant viscosity increase and therefore no evidence for intercalation.

The binding studies (Figure 3) lead to a value of $n = 1$, and the neighbor exclusion model for intercalation requires that n be 2 or greater.[25] Furthermore, there is no curvature in the plots in Figure 3, as would be expected by neighbor exclusion. Thus, the binding data are also inconsistent with an intercalation binding mode.

In flow-dichroism studies, the long DNA molecules become oriented in the direction of flow. Since the bases are oriented perpendicular to the DNA axis, dichroism is observed.[22,23,26] In the intercalation binding mode, the intercalator is oriented parallel to the DNA bases. Negative or positive induced flow dichroism should be observed depending on the direction of the transitions being monitored. The absence of flow dichroism in the visible region clearly implicates random orientation of CuPCPH with respect to the DNA bases, and these results also argue against intercalation by CuPCPH.

The aromatic moiety of the intercalators is usually aligned such that the imino H in AT and GC base pairs will experience an upfield ^1H NMR shift because of the ring current anisotropy of the aromatic groups.[27] Alternatively, the paramagnetic Cu(II) center should broaden out the resonances beyond detection. The Redfield ^1H NMR results show no appreciable shifts, and, although there is some broadening, in view of all the evidence against intercalation, this broadening is probably too small to be consistent with intercalation.

The ^{31}P NMR signal of DNA is broadened and shifted downfield by intercalators, even at very low ratios of added intercalator.[28] No shift is observed, and the broadening is consistent with the effect of the paramagnetic Cu(II) center since eventually the ^{31}P NMR signal disappears—an effect not observed with intercalators.

Outside Binding. Since it appears that intercalation can be clearly ruled out, other binding modes need to be considered. The majority of the evidence is in keeping with an outside binding mode. At the outset, it should be clear that it is difficult to distinguish between the following: a purely electrostatic interaction; a more specific outer-sphere binding mode where, for example, coordinated H_2O is hydrogen bonded to a mononegative phosphodiester group; and a direct bond between Cu and a phosphate oxygen.

The outside binding mode is compelling because all the data can be readily understood. The flow-dichroism study reveals random orientation. The ionic strength dependence of the binding constant (K) is consistent with an electrostatic interaction. The value of n from the Scatchard plots reveals charge neutralization. Most dramatically, CuPCPH appears to be very effective in broadening out the ^{31}P NMR signal, in comparison to Cu^{2+} (Figure 6). There is a total area loss at a ratio of 0.01! In contrast, Cu^{2+} ion has a much larger effect on the base imino proton resonances (vide infra).

CPK models of CuPCPH binding to DNA reveal considerable steric hindrance to base binding, but there are no steric problems associated with the binding to the phosphodiester backbone.

Base Binding. Much of the data can also be rationalized with Cu binding directly to the bases of DNA. For example, replacement of coordinated H_2O by an N-donor ligand could increase the pK_a of the CuPCPH by decreasing electron donation from the tridentate ligand to the Cu. In fact, at pH 5.60 imidazole and AMP cause similar spectral changes on addition to CuPCPH as does the addition of DNA although high ratios of ligand to Cu (~100:1) were needed and the isosbestic behavior at 341 and 406 nm is not fully preserved. The smooth Scatchard plot and n value of 1 obtained for binding suggest a large homogeneous class of binding sites, also more in keeping with phosphate than base binding.

The Cu^{2+} ion is known to bind preferentially to GC base pairs.[29,30] In contrast to CuPCPH, Cu^{2+} ion is much less effective at broadening the ^{31}P NMR signal and is more effective and much more selective in broadening the ^1H NMR signal of the guanine N(1)H resonance. This result adds further support to the base-binding selectivity of Cu^{2+} and presents a contrast to the NMR effects of CuPCPH which, in turn, are more indicative of outside (phosphate) binding than base binding.

Conclusion. The intercalative DNA binding mode for CuPCPH is clearly insignificant. Of the other two broad classes of binding, outside binding is most consistent with our data. The possibility of significant, even predominant, base binding cannot be fully excluded, but favorable base binding appears to be unlikely. It is possible that H_2O is coordinated to one or both axial positions of the Cu complex sufficiently strongly to prevent intercalation, an argument that has been used to explain the dependence of the proposed intercalation binding mode of tetrakis(4-*N*-methylpyridyl)porphine complexes on the nature of the metal center.[31] However, it has been proposed that metal phenanthroline complexes such as the tetrahedral Cu(II) complex or the octahedral Zn(II) and Ru(II) complexes can intercalate.[6,7,32] Thus, a nearly planar coordination geometry is not a requirement for metal intercalation.

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Supplementary Material Available: Figures of spectrophotometric titrations of CuPCPH by NaOH or DNA solutions (2 pages). Ordering information is given on any current masthead page.

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