Spectroscopic and Voltammetric Studies on Copper Complexes of 2,9-Dimethyl-1,10-phenanthrolines Bound to Calf Thymus DNA

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The interaction of copper(II/I) complexes of a few 2,9-dimethyl-1,10-phenanthrolines with calf thymus DNA has been investigated using absorption and circular dichroic spectral and electrochemical techniques and viscometry. The observation of the usual hypochromism and the novel hyperchromism in the absorption spectra of $[Cu^{I}(bcp)_2]^+$ [bcp = 2,9-dimethyl-4,7-diphenyl-1,10- phenanthroline] and $[Cu^{I}(dpsmp)_2]^{3-}$ [$dpsmp^{2-}$ = 2,9-dimethyl-4,7-bis-(sulfonatophenyl)-1,10-phenanthroline] respectively in the presence of DNA and the increase in viscosity of DNA at low loadings of both these complexes have been interpreted in terms of bridging of a pair of DNA duplexes by the complex species. These tetrahedral copper(I) complexes, which lack minor groove binding because of substituents at the 4- and 7-positions of phen ring, are efficient in bridging the duplexes. The electrochemical behaviors of $[Cu^{I}(dmp)_2]^+$ [dmp = 2,9-dimethyl-1,10-phenanthroline] and $[Cu^{I}(bcp)_2]^+$ bound to DNA have been compared with that of the analogous sulfonated complex $[Cu(dpsmp)_2]^{2-/3-}$. The DNA binding constants determined reveal that $dpsmp^{2-}$ complex is engaged in DNA binding less intimately than the bcp complex. While Coulombic interactions are clearly more important than other types of interactions for the former, nonclassical hydrophobic interactions for the latter. The Hill analysis of the absorbance data obtained as a function of added DNA reveals Hill coefficients greater than unity, which may be construed as evidence for cooperative binding of the copper complexes to B-DNA.

Introduction

A number of metal chelates have been used as probes of DNA structure in solution,[1] as agents for mediation of strand scission[2] of duplex DNA and as chemotherapeutic agents.[3] The interaction of a variety of d^6 Ru(II) and Rh(III) complexes of polypyridine or 1,10-phenanthroline (phen) ligands with DNA has been studied.[4] Copper complexes containing phen ligands have been shown to be useful probes of DNA duplexes.[5] They show antiviral activity by their interaction with nucleic acid templates and inhibit proviral DNA synthesis.[6] Sigman and his co-workers have shown that the cationic complex $[Cu^{I}(phen)_2]^+$, in the presence of molecular oxygen and a reducing agent, acts as an efficient nuclease with a high preference for double-stranded DNA.[7] Interpretation of data based on DNA cleavage by $[Cu^{I}(phen)_2]^+$ and design of efficient artificial nucleases requires a knowledge of the binding and kinetic mechanisms. So we became interested in defining and evaluating the key DNA-binding interactions of Cu(II/I) complexes of phenanthrolines and other related ligands like bis(pyridyl)-polythioethers.[8]

We recently investigated[9] the effect of methyl substitution on the nature of DNA binding of bis(phen)copper(II) complexes. We have discovered[10] the novel conversion of right-handed B to left-handed Z conformation on interaction of calf thymus (CT) DNA with $[Cu(5,6\text{-dmp})_2]^{2+}$ [5,6-dmp = 5,6-dimethyl-1,10-phenanthroline] though the Z form of a natural DNA such as CT DNA would normally escape detection. In the present report, we have investigated the interaction of copper complexes of 2,9-dimethyl-1,10-phenanthrolines with CT DNA. As the 2,9-methyl substituents stabilize the Cu^{I} over the Cu^{II} state, these complexes display little nuclease activity,[11] and so it is possible to focus on their noncovalent binding interactions with DNA. McMillin *et al.*[10] have made successful attempts to define the fundamental DNA-binding interactions of copper-phenanthroline systems like $[Cu^{I}(dmp)_2]^+$ and $[Cu^{I}(bcp)_2]^+$ (dmp = 2,9-dimethyl-1,10-phenanthroline, bcp = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline). Despite intensive study,[12] however, the

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exact mode and extent of binding of these complexes still remain unknown. We describe here the spectroscopic as well as voltammetric studies on the interaction of $[Cu^{I}(dmp)_2]^+$ and $[Cu^{I}(bcp)_2]^+$ with CT DNA in 33% MeOH solution. The addition of methanol, though, ensures solubility of these complexes, increases the hydrophobicity of the bulk solvent, which in turn decreases the DNA-binding ability of these complexes. In the present study, this difficulty has been overcome by using the water soluble sulfonated complexes $[Cu^{II}(dpsmp)_2(H_2O)]^{2-}$ and $[Cu^{I}(dpsmp)_2]^{3-}$ [$dpsmp^{2-}$ = 2,9-dimethyl-4,7-bis(sulfonatophenyl)-1,10-phenanthroline]. Such a ligand modification would also provide us an opportunity to obtain additional structural insight into the binding event.

Experimental Section

Materials. Disodium salt of CT DNA (Sigma) was used as received. The solid Na^+ salt was stored at 4 °C. Solutions of DNA in 50 mM NaCl/5 mM Tris-HCl (pH 7.1) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of ≈1.9, indicating that the DNA was sufficiently free of protein.[13] Concentrated stock solutions of DNA ([NP] = 10.5 mM) were prepared in respective buffers (Buffer I, 5 mM Tris-HCl/50 mM NaCl in 33% MeOH, pH 7.0; Buffer II, 20 mM Tris-HCl/50 mM NaCl in water, pH 7.2; Buffer III, 5 mM Tris-HCl/50 mM $MgCl_2$ in 33% MeOH, pH 7.0; Buffer IV, 20 mM Tris-HCl/50 mM $MgCl_2$ in water, pH 7.2; Buffer V, 20 mM Tris-HCl/50 mM NaCl in 33% MeOH, pH 7.2) and the concentration of DNA in [NP] was determined by UV absorbance at 260 nm, on 1:100 dilutions. The extinction coefficient, ϵ_{260}, was taken as 6600 M^{-1} cm^{-1}.[14] Stock solutions were stored at 4 °C and used after no more than 4 days.

$[Cu^{I}(dmp)_2]BF_4$[15] and $[Cu^{I}(bcp)_2]BF_4$[15] were prepared following the reported procedures. $[Cu^{II}(dpsmp)_2H_2O]^{2-}$ and $[Cu^{I}(dpsmp)_2]^{3-}$ were prepared in solution as reported earlier.[16]

Methods and Instrumentation. Absorption spectra were recorded on a Perkin-Elmer Lambda 3B spectrophotometer.

Circular dichroic spectra of DNA were obtained by using a JASCO J-20 automatic recording spectropolarimeter operating at 25 °C. The region between 220 and 320 nm was scanned for each sample. Molecular ellipticity values were calculated according to the formula

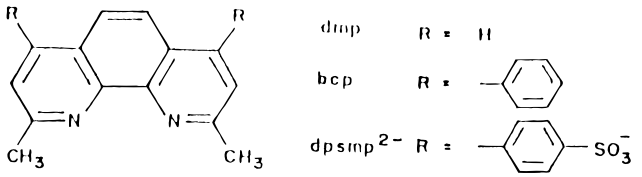
$$[q]_l = [q_l/Cl] \times 100$$

where $[q]_l$ is the molecular ellipticity value at a particular wavelength expressed in deg cm^2 $dmol^{-1}$, C the concentration in moles of nucleotide phosphate per liter, l the length of the cell in dm, and q_l the observed rotation in degrees.

For viscosity measurements the viscometer was thermostated at 25 °C in a constant temperature bath. The concentration of DNA was 500 mM in NP, and the flow times were determined with a manually operated timer.

All voltammetric experiments were performed in a single-compartment cell with a three-electrode configuration on a EG&G PAR 273 potentiostat/galvanostat equipped with an IBM PS/2 computer and a HIPLOT DMP-40 series digital plotter. The working electrode was a glassy carbon disk, and the reference electrode was a saturated calomel electrode. A platinum plate was used as the counter electrode. The supporting electrolytes were Buffers I and II. For the $dpsmp^{2-}$ complex Buffer II and for other complexes Buffer I were used. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to measurements; during measurements, a stream of N_2 was passed over the solution. All experiments were carried out at 25 ± 0.2 °C maintained by a Haake D8-G circulating bath.

Chart 1. Structure of Phenanthroline Ligands



Results and Discussion

Interaction of Copper Phenanthroline Complexes with DNA. B-form DNA is a polyanion composed of two complementary, polymeric subunits hydrogen bonded together in the form of a right-handed double helix.[17] When a cationic binding agent such as $[Cu(phen)_2]^+$ binds to DNA, it is likely to replace a cation from the compact inner (Stern) layer or the diffuse outer layer surrounding DNA and interact with the anionic phosphate residues[18] of DNA. Because the phen ligands in this complex are roughly perpendicular to each other, complete intercalation of the phen ring between a set of adjacent base pairs is sterically impossible, but some type of partial intercalation involving one of the ligands can be envisioned. Williams *et al*. have illustrated[19] the preferential cleavage by $[Cu(phen)_2]^+$ of DNA near the bulges where intercalation is favourable. Based on viscometry data, Veal and Rill have interpreted[20] that $[Cu^{I}(phen)_2]^+$ binds to DNA by partial intercalation. Thus the forces responsible for the DNA binding interactions of $[Cu(phen)_2]^+$ are both electrostatic and hydrophobic. However, the recognition event in the sequence dependent nuclease activity of this complex has been suggested to be its non-intercalative binding in the minor groove of DNA.[7,21] The intercalation of $[Cu^{I}(dip)_2]^+$ (dip = 4,7-diphenyl-1,10-phenanthroline) through 4,7-diphenyl rings has been proposed[22] to explain the lack of sequence specific cutting of DNA. Similarly, the introduction of phenyl substituents at the 4,7-positions of dmp in $[Cu^{I}(dmp)_2]^+$, as in $[Cu^{I}(bcp)_2]^+$, also leads to an intimate binding with DNA in the major groove through partial intercalation. Very recently McMillin[12] logically interpreted the increase in specific viscosity of salmon testes (ST) DNA at low loadings of $[Cu^{I}(bcp)_2]^+$, in terms of bridging structures in which one or more bcp complexes link a pair of DNA duplexes together rather than engage in classical intercalative binding. Luminescence[5] and viscometric studies[23] have revealed $[Cu^{I}(dmp)_2]^+$ to be a groove binder by surface or exterior association. Thus the introduction of phenyl groups in phen and dmp causes a subtle change in the preference of their Cu^{I} complexes from groove binding to partial intercalation. The present study aims at investigating the effect of introducing a SO_3^- group in these phenyl rings and also at the quantitative evaluation and understanding of the binding equilibria of all these complexes with CT DNA.

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Table 1. Effect of CT DNA on the Absorbance at 25 °C and Binding Constants

complex	buffer[a]/solvent	λ_{max}, nm DNA−P/Cu = 0	λ_{max}, nm DNA−P/Cu = 25	Δε (%)	binding constant 10^{-4} K_b (M^{-1})	Hill analysis 10^{-4} K_b (M^{-1})	Hill analysis t (h)
$[Cu(dmp)_2]^+$	**I**/33% MeOH	456.2	457.4	1.3			
$[Cu(bcp)_2]^+$	**I**/33% MeOH	475.0	477.8	20.0	9.7	15.2	1.9 ± 0.1
	III/33% MeOH	475.0	477.4	18.6	9.4	14.9	1.8 ± 0.1
$[Cu(dpsmp)_2]^{3-}$	**V**/33% MeOH	480.0	480.4	7.2	0.6	9.6	1.4 ± 0.1
	II/water	480.0	480.0	14.6	2.7	13.1	1.2 ± 0.1
	IV/water	480.0	480.3	19.6	5.6	15.3	1.2 ± 0.1

[a] **I**, 5 mM Tris-HCl/50 mM NaCl in 33% MeOH, pH 7.0; **II**, 20 mM Tris-HCl/50 mM NaCl in water, pH 7.2; **III**, 5 mM Tris-HCl/50 mM $MgCl_2$ in 33% MeOH, pH 7.0; **IV**, 20 mM Tris-HCl/50 mM $MgCl_2$ in water, pH 7.2; **V**, 20 mM Tris-HCl/50 mM NaCl in 33% MeOH, pH, 7.2.

Electronic Spectra. The binding of intercalative drugs to DNA helix has been characterised classically through absorption spectral titrations, by following the changes in absorbance (hypochromism) and shift in wavelength (red shift).[24] In the present investigation it has been used to monitor the interaction of $[Cu^I(dpsmp)_2]^{3-}$ in aqueous solutions and $[Cu^I(dmp)_2]^+$ and $[Cu^I(bcp)_2]^+$ in 33% aqueous methanol solutions, with calf thymus DNA.

The intense Cu(I) → N_{het}(p) metal-to-ligand charge transfer (MLCT) band of the Cu(I) complexes, observed in the range 455−480 nm (Table 1), was monitored as a function of added DNA. All the complexes except the sulfonated one show a decrease in molar absorptivity (hypochromism) as well as a slight red shift (2−3 nm) of this band, indicating their binding to DNA. Similar red shifts (2 nm) observed for tris-chelated Ru(II) complexes of phen ligands upon binding to DNA have been associated[25] with intercalative interaction, with partial insertion of one of the phen ring between adjacent base pairs on the DNA duplex. The very small change in the absorption intensity of MLCT band of $[Cu^I(dmp)_2]^+$ on introducing CT DNA, is consistent with its weak external contact with the duplex.[5] The appreciable decrease in absorption intensity and significant shift in wavelength of the MLCT band of $[Cu^I(bcp)_2]^+$ is similar to that observed for its interaction with ST DNA[5] and RNA[26] in aqueous methanol solution, suggesting that the complex binds to DNA strongly. In contrast, its sulfonated derivative $[Cu^I(dpsmp)_2]^{3-}$ shows an increase in molar absorptivity (hyperchromism) as well as an insignificant shift in position of the CT band (Figure 1). To understand whether the hyperchromism is due to formation of more of the complex (which may dissociate in dilute solutions) on the addition of DNA, spectral titrations were carried out by varying the concentration of $dpsmp^{2-}$. The percentage hyperchromism observed was found to be independent of the concentration of added $dpsmp^{2-}$, revealing that the origin of hyperchromism might lie in the mechanism of interaction of the complex with DNA. A similar hyperchromism has been observed for the soret bands of certain porphyrins when interacted with DNA[27] but has not yet been clearly explained. The present observation leads us to suspect that $[Cu^I(dpsmp)_2]^{3-}$ exhibits a strong interaction with DNA, with a mode different from that exhibited by $[Cu^I(bcp)_2]^+$. The intimate binding of $[Cu^I(dpsmp)_2]^{3-}$ is in contrast to the poor binding of anionic $[Fe(EDTA)]^{2-}$ [28] and $[Fe(CN)_6]^{4-}$ with DNA.[29] So it becomes evident that the negative charge on the ligand domain favors, while the residual negative charge on metal discourages, the extent of interaction of a metal complex with DNA.

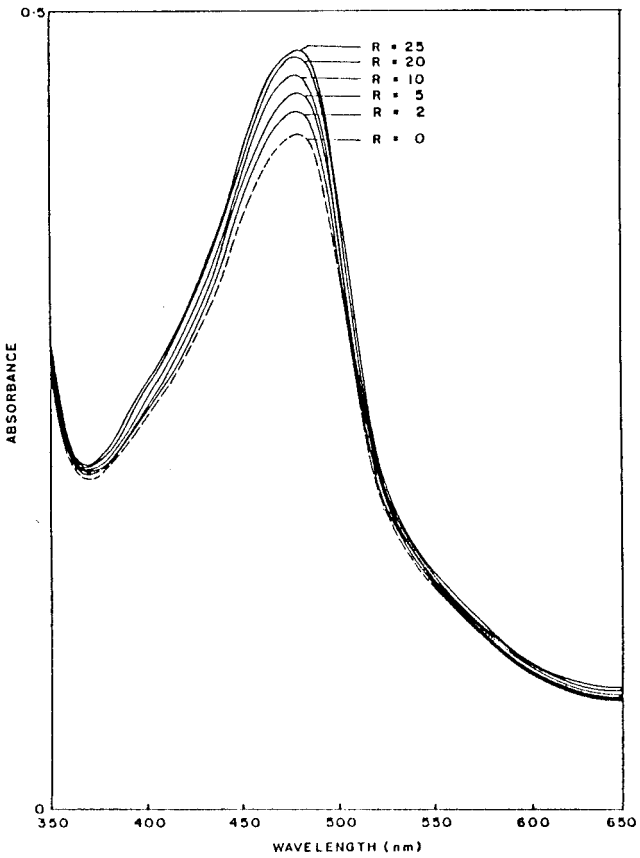

Figure 1. Charge transfer spectra of $[Cu^I(dpsmp)_2]^{3-}$ (0.035 mM) in the absence (-- -) and presence (—) of increasing amounts of CT DNA.

For the bcp and $dpsmp^{2-}$ complexes the intrinsic binding constant, K_b has been determined from the spectral titration data using the equation[25]

$$[DNA]/(\epsilon_A - \epsilon_f) = [DNA]/(\epsilon_B - \epsilon_f) + 1/K_b(\epsilon_B - \epsilon_f)$$

where ϵ_A, ϵ_f, and ϵ_B correspond to A_{obsd}/[Cu], the extinction coefficient for the free copper complex, and the extinction coefficient for the copper complex in the fully bound form respectively. In the plot (Figure 2) of $[DNA]/(\epsilon_A - \epsilon_f)$ *vs* [DNA], K_b is given by the ratio of the slope to intercept. The K_b values (Table 1) are lower than those observed for typical

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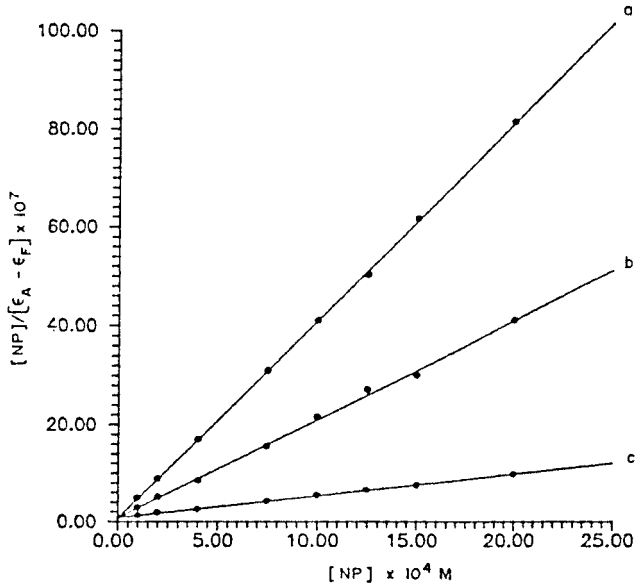

Figure 2. Typical plots of $[NP]/(\epsilon_A-\epsilon_f)$ vs [NP] for absorption titration of CT DNA with $[Cu^I(dpsmp)_2]^{3-}$ in buffers **IV** (a), **II** (b), and **V** (c).

classical intercalators (ethidium−DNA, 7×10^7 M^{-1} in 40 mM Tris-HCl buffer, pH 7.9,[30] and 1.4×10^6 M^{-1} in 40 mM NaCl−25 mM Tris-HCl;[31] proflavin with *Escherichia coli* DNA, 50% GC content, 4.1×10^5 M^{-1} in 0.1 M Tris-HCl)[32] with a proven DNA-binding mode involving the complete insertion of the planar molecules between the base pairs. This is indicative of binding of the complexes with DNA host with an affinity less than the classical intercalators.

The K_b value of $[Cu^I(dpsmp)_2]^{3-}$ is dramatically enhanced (Table 1, Figure 2a) in the presence of 50 mM $MgCl_2$. Further, an increase in methanol percentage decreases the percentage of hyperchromism observed for the complexes in aqueous solution (Table 1). Both these observations led us to suspect that the ligand SO_3^- group engages in ionic interaction with Na^+ on the surface of DNA. Thus, as expected, divalent Mg^{2+} enters into stronger ionic interaction with SO_3^-; however, interestingly, it fails to enhance the binding constant for $[Cu^I(bcp)_2]^+$ which involves no such ionic interaction. The hydrophobic interaction of the phenyl rings of $[Cu^I(bcp)_2]^+$ leads to a binding more intimate than the electrostatic interaction of SO_3^- groups of $[Cu^I(dpsmp)_2]^{3-}$ with DNA host; thus, the former exhibits a binding constant higher than the latter.

Circular Dichroism. The UV circular dichroic spectrum of CT DNA exhibits a positive band at 270 nm (UV: λ_{max} 260 nm) due to base stacking and a negative band at 239 nm (Figure 3, Table 2) due to helicity of B DNA.[33] Incubation of the DNA with all the present complexes induced small changes in the CD spectrum. The intensities of both the positive and negative ellipticity bands decrease, with the latter being affected slightly more than the other. This suggests that the DNA binding of the complexes induces certain conformational changes, such as the conversion from a more B-like to a more C-like structure within the DNA molecule. Interaction with cations effectively screens the negative charge on N(7) base sites as well as

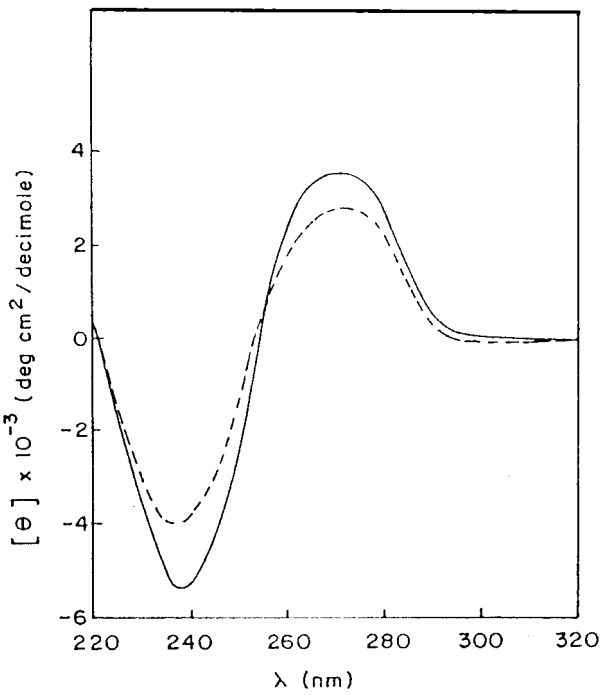

Figure 3. CD spectra of CT DNA in the absence (—) and presence (- - -) of 250 mM $[Cu^{II}(dpsmp)_2]^{2-}$.

Table 2. Effect of Copper Complexes on CD Spectra of CT DNA

samples	molecular ellipticities (deg.cm^2.dmol^{-1}) θ_{270}	θ_{239}
DNA 3564	−5498	
DNA + 250 mM $[Cu(dmp)_2]^+$	3285	−4806
DNA + 250 mM $[Cu(bcp)_2]^+$	3260	−4765
DNA + 250 mM $[Cu(dpsmp)_2]^{3-}$	3190	−4018
DNA + 250 mM $[Cu(dpsmp)_2]^{2-}$	2986	−4010

phosphate oxygens simultaneously, both along the deoxyribophosphate backbone and in the groove of the helix to promote such a transconformational change.[8] The CD spectral changes induced by $[Cu^{II}(dpsmp)_2]^{2-}$ are more significant than those by all the other Cu(I) complexes (Table 2), suggesting the importance of the electrostatic components of interaction in effecting transconformational changes. Further, no satisfactory indication of induced CD could be detected in the 300−700 nm region at all concentrations of the complexes. However, an induced CD band with positive ellipticity at 480 nm for the bcp complex bound to ST DNA has been reported by McMillin *et al.*[12]

Viscosity Measurements. As a means for further exploring the binding of the present copper complexes, viscosity measurements were carried out on CT DNA by varying the concentration of the added complexes. The experiment involves the measurement of the flow rate of DNA solution through a capillary viscometer. Under appropriate conditions, intercalation of drugs like ethidium bromide (EthBr) causes a significant increase in viscosity of DNA solutions due to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA contour length. By contrast, drug molecules that bind exclusively in the DNA grooves (e.g., netropsin, distamycin), under the same conditions, typically cause less pronounced (positive or negative) or no changes in DNA solution viscosity.[34] The values of relative specific viscosity (η/η_0), where η_0 and η are

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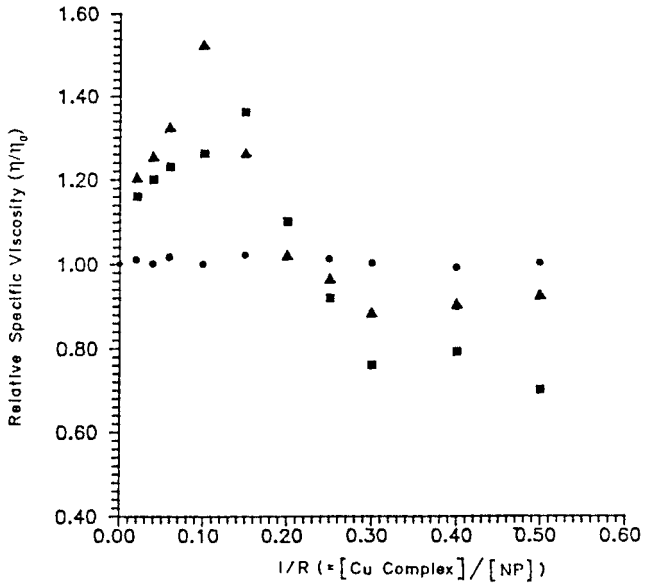


Figure 4. Effect of $[Cu^{I}(dmp)_2]^+$ (●), $[Cu^{I}(bcp)_2]^+$ (■), and $[Cu^{I}(dpsmp)_2]^{3-}$ (▲) on the viscosity of CT DNA; η_0 and η are the specific viscosity contributions of DNA in the absence and presence of complexes, respectively. The total concentration of DNA was 500 mM NP.

the specific viscosity contributions of DNA in the absence and in the presence of the present complexes, were plotted against $1/R$ (R = [NP]/[Cu complex]) (Figure 4). The results reveal that the presence of $[Cu^{I}(dmp)_2]^+$ had no effect on the viscosity of CT DNA whereas that of $[Cu^{I}(bcp)_2]^+$ and $[Cu^{I}(dpsmp)_2]^{3-}$ had a marked effect. With the addition of the latter two complexes, the specific viscosity of DNA first increased and then decreased. Similar behavior has been observed on the addition of $[Cu^{I}(bcp)_2]^+$ to ST DNA.[12] Since the increase is less than that observed for an intercalator like EthBr, interaction involving classical intercalation is ruled out for the present complexes. McMillin *et al*.[12] have hypothesized that there are at least two phases of binding for $[Cu^{I}(bcp)_2]^+$. The hydrophobic interaction of $[Cu^{I}(bcp)_2]^+$ with DNA occurs first, which is follwed by the bridging of duplexes involving both the ligands. We suggest a similar mode of interaction for $[Cu^{I}(bcp)_2]^+$ and also for $[Cu^{I}(dpsmp)_2]^{3-}$ with CT DNA. We suggest that the latter complex exhibits the electrostatic interaction of SO_3^- with Na^+ in the Stern layer accompanied by bridging. Further, the viscosity of DNA with added $[Cu^{I}(dpsmp)_2]^{3-}$ is not affected in the presence of Mg^{2+}. It is obvious that the presence of Mg^{2+} increases the extent of DNA interaction of the complex species but not the length of DNA. Interestingly, the addition of $[Cu^{II}(dpsmp)_2]^{2-}$ to CT DNA does not lead to any significant change in relative specific viscosity; however, the addition of a stoichiometric amount of a reducing agent like ascorbic acid to this solution increases the viscosity at low loadings of the complex. This implies that it is the tetrahedral Cu^{I} complex that is efficient in bridging the duplexes.

Redox Behavior. The application of electrochemical methods to the study of metallointercalation and coordination of metal ions and chelates to DNA provides a useful complement to the previously used methods of investigation, such as UV−visible spectroscopy. In the present study these methods have been used to understand the nature of DNA binding of the copper complexes.

The Cu(II)/Cu(I) redox potentials of the present complexes follow the trend $[Cu^{I}(bcp)_2]^{+/2+}$ > $[Cu^{I}(dmp)_2]^{+/2+}$ > $[Cu^{II}(dpsmp)_2]^{2-/3-}$ (Table 3); this is expected of the electron attracting ability of the phenyl rings, which tends to destabilize the Cu^{II} state and decrease on sulfonation. Thus, the introduction of methyl substituents at the 2- and 9-positions of phen ligand, as in the present case, tunes the redox potential to more positive values which does not favor the reaction of present cuprous complexes with H_2O_2 in the cleavage scheme proposed by Sigman *et al*.,[22] and hence there is no significant nuclease activity for these complexes under normal assay conditions. The limiting peak potential separation $\Delta E_p°$ of 70−85 mV reveals the fairly reversible behavior of the Cu(II)/Cu(I) couple. On the addition of DNA (R = [DNA-P]/[Cu] = 60), the dmp and bcp complexes experience negative shifts in $E_{1/2}$ of 49 and 26 mV, respectively and a decrease in ΔE_p at lower scan rates. The ratio of cathodic to anodic peak currents, i_{pc}/i_{pa} (≈1) decreases with increasing [NP], suggesting that the adsorption of the Cu(I) complexes on the GC electrode is enhanced in the presence of DNA. For $[Cu^{II}(dpsmp)_2]^{2-}$, the ratio i_{pa}/i_{pc} is greater than unity but decreases with increase in [NP], suggesting that the adsorption of the Cu^{I} product tends to be suppressed in the presence of DNA (Table 3, Figure 5).

Further, the shifts in $E_{1/2}$ with increase in R value suggests that both Cu(II) and Cu(I) forms of the present complexes bind to DNA but with different affinities. Analogous to the treatment of the association of small molecules with micelles[36] the ratio of the equilibrium constants, K_{2+}/K_+ for the binding of the Cu(II) and Cu(I) forms of complexes to DNA (Scheme 1) can be estimated from the net shift in DPV $E_{1/2}$, assuming reversibility and using the equation

$$E_b°' - E_f°' = 0.059 \log(K_+/K_{2+})$$

where $E_f°'$and $E_b°'$ are the formal potentials of the Cu(II)/Cu(I) couple in the free and bound species, respectively. For the present DNA-bound complexes, the K_{2+}/K_+ decreases in the order $[Cu^{I}(dmp)_2]^+$ (6.8) > $[Cu^{I}(bcp)_2]^+$ (2.8) > $[Cu^{II}(dpsmp)_2]^{2-}$ (1.3). For all of them, K_{2+} is higher than K_+, suggesting that B-DNA tends to stabilize the Cu^{II} over the Cu^{I} state by electrostatic interaction with these complexes even though all the present dmp ligands preferentially stabilize Cu^{I} in the absence of DNA. Because the preferred geometries of four-coordinate Cu(II) and Cu(I) complexes are planar and tetrahedral, respectively, the modification of such preferences in the presence of DNA is interesting. Such preferences for geometry may not be important for Co(III) or Fe(III) or Ru(III) complexes when binding to DNA because a change in charge does not alter their geometries substantially. Thus DNA provides a suitable template for planar Cu(II) rather than tetrahedral Cu(I) species through electrostatic interaction. Further, the order shown above illustrates how ligand variation makes subtle contribution to the binding and suggests that the ability of DNA to stabilize Cu^{II} over Cu^{I} decreases on introducing phenyl and sulfonyloxyphenyl groups. The smaller $[Cu^{II}(dmp)_2]^{2+}$, but not the larger $[Cu^{II}(dpsmp)_2(H_2O)]^{2-}$ with decreased positive charge on copper, binds strongly to DNA, illustrating that the DNA interactions are electrostatic in nature.

In addition to changes in the formal potential, the voltammetric peak currents decrease upon the addition of DNA to all

(35) Control DNA-binding experiments were carried out with $K_4[Fe(CN)_6]$ ($E_{1/2}$, 0.155 V vs SCE in the absence of DNA), the anion of which is expected not to interact with DNA because of the Coulombic repulsion it experiences with the negatively charged sugar-phosphate backbone of DNA. They show that the $E_{1/2}$ of the complex is not affected in the presence of DNA.

(36) Kaifer, A. E.; Bard, A. J. *J. Phys. Chem.* **1985**, *89*, 4876.

Table 3. Voltammetric Behavior of Copper Complexes of 2,9-Dimethyl-1,10-phenanthrolines in the Presence of DNA

complex	v (V s^{-1})	R	E_{pa} (V)	E_{pc} (V)	ΔE_p (mV)	$E_{1/2}$ (V)	i_{pc}/i_{pa}	i_{pa}/i_{pa} ($R = 0$)
$[Cu(dmp)_2]^{+/2+}$ [a]	0.05	0	0.476	0.392	86	0.435	1.07	1.00
		10	0.456	0.378	78	0.417	1.02	1.04
		50	0.442	0.352	90	0.397	0.89	1.20
		60	0.426	0.346	80	0.386	0.87	1.21
$[Cu(bcp)_2]^{+/2+}$ [a]	0.05	0	0.554	0.464	90	0.509	0.89	1.00
		10	0.556	0.460	96	0.508	0.80	0.87
		50	0.530	0.438	92	0.484	0.82	0.83
		60	0.524	0.442	82	0.483	0.81	0.82
$[Cu(dpsmp)_2]^{2-/3-}$ [b]	0.05	0	0.340	0.452	112	0.396	1.40[c]	1.00
		10	0.340	0.450	110	0.395	1.38	0.81
		50	0.342	0.436	94	0.389	1.36	0.70
		60	0.344	0.434	90	0.389	1.35	0.69

[a] Supporting electrolyte, 50 mM NaCl/5 mM Tris-HCl in 33% MeOH, pH 7.0. [b] Supporting electrolyte, 50 mM NaCl/20 mM Tris-HCl in water, pH 7.2. [c] i_{pa}/i_{pc}.

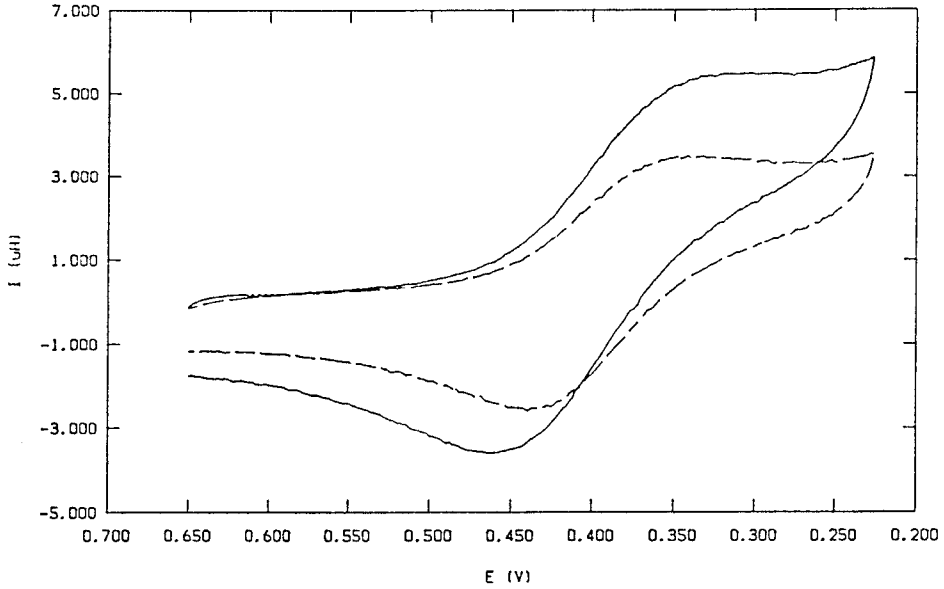
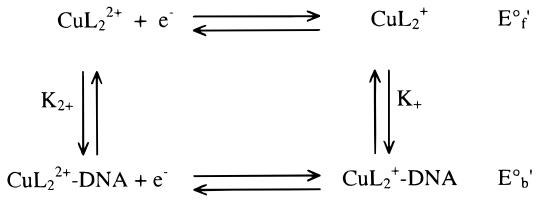


Figure 5. Cyclic voltammograms of 0.1 mM $[Cu^{II}(dpsmp)_2]^{2-}$ in the absence (—) and presence (- - -) of 6 mM NP. Supporting electrolyte, 50 mM NaCl + 20 mM Tris-HCl in water (pH 7.2).

Scheme 1



the complexes, except $[Cu^I(dmp)_2]^+$. The decrease in current can be explained in terms of the diffusion of an equilibrium mixture of free and DNA-bound metal complexes to the electrode surface. For $[Cu^I(dmp)_2]^+$, i_{pa} decreases at low R values but increases[37] beyond $R = 3$ in contrast to the other two complexes.

DNA Binding Model for $[Cu^I(dpsmp)_2]^{3-}$. DNA has been shown to combine with the surface of crystalline phosphate[38] possibly *via* ionic interactions with exposed cations and it also wraps around histone proteins in cell nucleus. So it is reasonable to imagine that the present Cu^I complexes replace the cations in the relatively compact inner shell (Stern layer) and/or in the loosely packed diffuse outer layer surrounding the B-DNA;[17] further, the sulfonato groups of $[Cu^I(dpsmp)_2]^{3-}$ interact with these cations and also possibly engage in hydrogen bonding with suitable donors on the base pairs; such an interaction would offset any possible repulsion of SO_3^- by phosphate backbone. The DNA double helix still possesses hydrogen-bonding recognition sites even after several of them are hidden by Watson−Crick base pairing.[39] The guanine N7 nitrogen and, to a lesser extent, adenine N7 are the potential hydrogen bonding acceptors on the DNA major groove. Specifically the minor groove of the host duplex presents a purine ring nitrogen (N3), exocyclic amino group of guanine (N2) as well as pyrimidine carbonyl group for potential hydrogen-bonding drugs. Such hydrogen-bonding interactions have been suggested for the binding of daunomycin to DNA[40] and the antineoplastic activity

(37) A strong binding to DNA would lead to a decrease in current, due to diffusion of the Cu complex−DNA adduct formed. However, diffusion of the free copper complex in a solution of increased viscosity or blockage of the electrode surface by an adsorbed layer of DNA that could possibly form at the electrode surface also contributes to the decrease in current (Welch, T. W.; Thorp, H. H. *J. Phys. Chem.* **1996**, *100*, 13829). This is revealed by the decrease in current (17%) for the redox-active control $K_4[Fe(CN)_6]$. Also the current from a bound, electrochemically reversible label may have interference from any freely diffusing species (Welch, T. W.; Thorp, H. H. *J. Phys. Chem.* **1996**, *100*, 13829). Thus, the observed slight decrease (or increase) in current would mean that absorption process compensates for the expected decrease in current.

(38) Tullius, T. D.; Dembroski, B. A. *Science* **1985**, *230*, 679.

(39) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer-Verlag: New York; 1983, p 193.

of cis-platin.[41] We have recently shown that hydrogen bonding interaction can be a driving force for the strong interaction of copper(II)−carnosine complex with CT DNA.[42]

It is obvious that the negatively charged sulfonato groups would sterically prevent the more invasive type of interaction *viz.* intercalation of the phenyl rings within the base-pairs of DNA. While the two $-SO_3^-$ groups on one of the ligands would be embedded on one duplex, those on the other one are embedded on a second duplex. Thus a pair of duplexes will be bridged by the sufficiently large cationic complex by the end-to-end approach illustrated by McMillin *et al.*,[12] and such a bridge also minimizes the Coulombic repulsions between the negatively charged duplexes. The presence of multiple $[Cu^I(dpsmp)_2]^{3-}$ ions between the duplexes would further stabilize the bridged structures. Such a bridged structure is similar to that already proposed for $[Cu^I(bcp)_2]^+$, which, however, binds to DNA but through hydrophobic interaction.[12] While the latter interaction is consistent with the hypochromism for the bcp complex, a different type of interaction, *viz.* the Coulombic one for the $dpsmp^{2-}$ complex with the novel hyperchromism is observed. Thus as the divalent Mg^{2+} stabilizes the bridged structure more than the monovalent Na^+ does, the hyperchromism is enhanced. Further, the introduction of SO_3^- on a bcp ligand of the copper complex does not alter the binding structure (bridging) but reduces the extent of interaction significantly.

The stacking of an aromatic heterocyclic surface into DNA base pair would hinder the MLCT transition, resulting in the observed hypochromism for $[Cu^I(bcp)_2]^+$. The presence of SO_3^- on $[Cu^I(dpsmp)_2]^{3-}$ would decrease the electron sink property of the phenyl ring (cf. redox studies), leading to less facile MLCT, but in the presence of CT DNA, the Coulombic interaction of SO_3^- with Na^+ ions in the Stern layer would decrease the electron density on phenyl ring, thus resulting in an increased ease of MLCT transition and hence the observed hyperchromism. This mode of interaction also explains the absence of any emission[43] for $[Cu^I(dpsmp)_2]^{3-}$ even in the presence of DNA. The negative charge on the SO_3^- group tends to stabilize the copper(I) state over the photoexcited copper(II) state, which is susceptible to exciplex quenching in aqueous environment. On binding to DNA, the ionic interaction of SO_3^- does not tend to stabilize the formally Cu^{II} exciplex, as also revealed by the relatively lower K_{2+}/K_+ value.

Moreover, the 4,7-positions of $[Cu^I(phen)_2]^+$ have been shown[22] to lie in the interior of the minor groove, and the presence of methyl groups in these positions has been shown[22] to decrease the DNA cleavage efficiency. Thus the introduction of sulfonatophenyl group in these positions should lead to unfavorable interactions on the floor of the minor groove, and we suspect that the present complex, as it is large in size, binds to the major groove rather than the minor groove. Similarly, $[Ru(dip)_3]^{2+}$ has been also suggested[25] to dock in the major groove.

Several studies on DNA structure[44] show that the influence of a small molecule bound to DNA is unlikely to propagate over a large distance. A large guest, like the present complexes, might contact only one or two base pairs, but sterically exclude the approach of other molecules to nearby sites. Further, a cationic guest like $[Cu(bcp)_2]^+$ or an anionic guest like $[Cu(dpsmp)_2]^{3-}$ would tend to repel another molecule of like charge attempting to bind nearby, but it will alter the local counterion (phosphodiester backbone), which means that the electrostatic free energy of the whole polymer becomes a variable in the analysis of the binding experiment. So the binding at one site along the nucleic acid helix may alter the probability of binding to neighbouring sites. To throw light on such possibilities, the present absorption spectral data were replotted according to Hill equation,[45]

$$\ln[Y/(1 - Y)] = h \ln[X] - \ln K_D$$

with $Y = \Delta A/\Delta A_\mu$, where ΔA_μ is the absorbance at the saturation point beyond which the addition of further DNA causes no appreciable change, [X] is the concentration of NP (host), K_D is the overall dissociation constant ($=1/K_b$), and h is the Hill coefficient, an index of cooperativity. (Non-cooperative systems exhibit $h \approx 1.0$, positively cooperative systems $h > 1.0$, and negatively cooperative systems $h < 1.0$). The bcp and $dpsmp^{2-}$ complexes gave satisfactory straight lines with slopes (h) in the range 1.2−1.9 (Table 1), suggesting the presence of a cooperative event in the binding of the present copper complexes, though the nature and mechanism of the cooperative event remain in question. Thus the DNA binding of the present complexes at a site a few base pairs away is enhanced, leading to cooperative effects. Further, interestingly, the K_b values (Table 1) obtained for them by Hill analysis agree reasonably well with those from equilibrium analysis. Similar cooperative binding of Cu(II) complexes has been observed previously.[8]

Conclusions

The docking of relatively small molecules like copper phenanthrolines on a biopolymer like DNA has been considered so far only in terms of intercalation or groove or surface binding. The present study supports the possibility of these complexes employing the bridged structures suggested by McMillin[12] to promote parallel extension of duplexes. Hydrophobic interactions are quite important for $[Cu^I(bcp)_2]^+$ in forming bridge structures with DNA. However, Coulombic interactions are important for $[Cu^I(dpsmp)_2]^{3-}$ in bridging DNA duplexes; it can be as dominant as but weaker than hydrophobic interactions. In the absence of such additional interactions involving the ligand domain, only a weak interaction results. Thus the substituents on the coordinated phenanthroline rings play a major role in governing the nature of binding with DNA. Further, the present results show that the tetrahedral Cu^I complexes are efficient in bridging the duplexes.

Electrochemical studies on DNA-bound copper 2,9-dimethyl-1,10-phenanthroline complexes reveal the significance of electrostatic interactions in dictating the binding phenomenon and hence the preference of DNA to interact with Cu(II) over Cu(I)

(40) Remeta, D. P.; Mudd, C. P.; Berger, R. L.; Breslauer, K. J. *Biochemistry* **1993**, *32*, 5064.
(41) Bruhn, S. L.; Toney, J. H.; Lippard, S. J. *Prog. Inorg. Chem.* **1990**, *38*, 477.
(42) Mahadevan, S.; Palaniandavar, M. *J. Inorg. Biochem.*, communicated.
(43) The complex $[Cu^I(dpsmp)_2]^{3-}$ was generated[14] by mixing aqueous solutions of $dpsmp^{2-}$ (disodium salt) and $Cu(BF_4)_2 \cdot 6H_2O$ in 2.2:1 molar ratio and then reducing it with less than the stoichiometric amount of ascorbic acid. Emission spectra were measured by keeping the excitation wavelength as 480 nm. Experiments were repeated by using different $dpsmp^{2-}$:copper ratios and various reducing agents like hydroxylamine hydrochloride, metallic copper, etc. For none of these solutions was emission was observed, even after deoxygenation by purging with N_2 gas. Emission was also not detectable even in the presence of large excess ($R = 50$) of CT DNA.
(44) Quintana, J. R.; Grzeskowiak, K.; Yanagi, K.; Dickerson, R. E. *J. Mol. Biol.* **1992**, *225*, 379.
(45) Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F.-T. *J. Am. Chem. Soc.* **1990**, *112*, 3860.

species. Further, the analysis of the absorbance data by Hill equation reveals that the influence of a small molecule bound to DNA is likely to propagate over a small distance (1−4 base pairs), *i.e.*, the binding phenomenon is cooperative and is relevant to the suggested bridge formation between two duplexes. All the present findings are obviously relevant to the chemistry of copper complexes of non-methylated phenanthrolines which are widely used as artificial nucleases.

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