Spectral and Electrochemical Behavior of Copper(II)−Phenanthrolines Bound to Calf Thymus DNA. $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ (5,6-dimethyl-OP = 5,6-Dimethyl-1,10-phenanthroline) Induces a Conformational Transition from B to Z DNA

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The interaction of 1:2 copper(II) complexes of 1,10-phenanthroline (OP) and variously methyl-substituted phenanthrolines with calf thymus DNA has been investigated by viscometry and spectral and electrochemical techniques. Viscometry and competitive ethidium bromide (EthBr) emission studies reveal that substitutions at 4- and 4,7-positions confer the complex a reduced affinity for DNA via partial intercalative interaction of the middle ring of OP between the base pairs of DNA, while substitutions at 5- and 5,6-positions confer a weak affinity toward DNA. The tetramethyl substitution at 3,4,7,8-positions lead to an intermediate behavior for the complex. Circular dichroism spectral studies of the interaction disclose for the first time that, of all the complexes, the 5,6-dimethyl-OP complex is a unique and remarkable reagent in that it reversibly binds to DNA and effects the important conversion of right-handed B DNA to left-handed Z DNA even in the presence of EthBr, an allosteric effector of the B conformation of DNA. This novel conformational transition is unexpected of the low GC content of natural DNA. The ratios of the binding constants (K_+/K_{2+}) for DNA binding of the Cu(I) and Cu(II) forms of the redox active OP complexes rather than the Cu(II)/Cu(I) redox potentials (0.023 to −0.098 V vs SCE) are good measures of the substituent dependent DNA cleavage efficiency and rate. They also reveal that the Cu(I) form of OP and 4-methyl- and 5,6-dimethyl-substituted OP complexes displays enhanced affinity to bind but noncovalently to the minor groove of DNA. Attempts have been made to illustrate the cleavage rate and efficiency of nucleolytic reactions in the light of the relative binding constants of Cu(I) and Cu(II) forms of the OP complexes.

Introduction

The copper(I) complex of 1,10-phenanthroline (OP), $[(OP)_2Cu^I]^+$, with H_2O_2 as a coreactant, is a chemical nuclease that nicks DNA by a reaction mechanism that is sensitive to the conformation of the nucleic acid.[1] It has attracted considerable interest as probes of local DNA conformation and as reagents for "footprinting" sites of binding of proteins and other ligands on DNA. A precise understanding of the covalent chemistry of the reagent, its DNA binding affinity, and the cleavage mechanism would provide insight into the nature and structure of the reactive intermediate and would be potentially useful in the design of new site-specific scission reagents and chemical probes. The reaction is known to be funneled through a noncovalent intermediate composed of $[(OP)_2Cu^I]^+$ and DNA, and two alternative mechanisms (Scheme 1) for the cleavage reaction have been proposed.[2] In the first, the $[(OP)_2Cu^I]^+$ complex is formed in solution and diffuses to the minor groove where it is oxidized by H_2O_2 (eqs a and b). In the second one, it is the cupric complex that binds to the minor groove and then gets reduced before reacting with H_2O_2 (eqs c and d).

Sigman has demonstrated that the noncovalent binding of the tetrahedral $[(OP)_2Cu]^+$ in the DNA minor groove is the recognition event that determines the sequence-dependent nuclease activity.[3] On the other hand, equilibrium dialysis studies[4] have revealed that both the cupric and cuprous complexes of OP bind to DNA, lending support to both the mechanisms.[2] As the cupric OP complex is formed during the redox cycling required for the reaction to proceed,[5] its structure and mode and affinity of binding to DNA would be also expected to influence the nuclease activity. However, no attempt has been made so far to understand the factors governing the mode and extent of its binding interactions with the DNA host. So in our laboratory we have undertaken systematic studies to probe the DNA binding interactions of cupric complexes of OP and other related ligands.

Even though the effect of substituents on the DNA cleavage characteristics of copper(I)−OP complexes has been investi-

Scheme 1

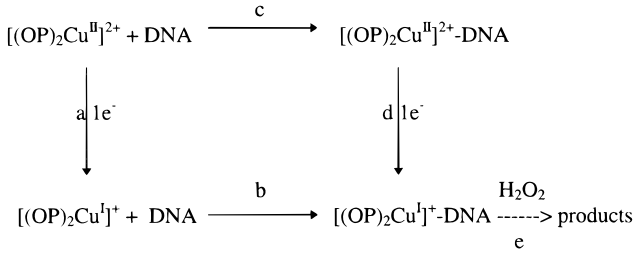

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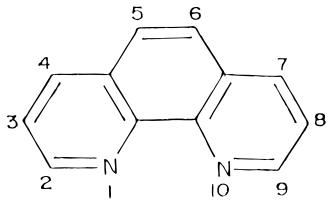
Table 1. Analytical Data[a] for Copper Complexes

complex	% Cu	% H	% N	% Cu
$Cu(OP)_2Cl_2 \cdot H_2O$	56.06 (56.20)	3.71 (3.54)	10.78 (10.92)	12.11 (12.39)
$Cu(4\text{-methyl-OP})_2Cl_2 \cdot H_2O$	57.56 (57.73)	4.32 (4.10)	10.08 (10.36)	11.56 (11.75)
$Cu(4,7\text{-dimethyl-OP})_2Cl_2 \cdot H_2O$	58.93 (59.11)	4.48 (4.61)	9.97 (9.85)	11.32 (11.17)
$Cu(5\text{-methyl-OP})_2Cl_2 \cdot H_2O$	57.92 (57.73)	4.28 (4.10)	10.21 (10.36)	11.83 (11.75)
$Cu(5,6\text{-dimethyl-OP})_2Cl_2 \cdot H_2O$	58.92 (59.11)	4.78 (4.61)	9.72 (9.85)	11.34 (11.17)
$Cu(3,4,7,8\text{-tetramethyl-OP})_2Cl_2 \cdot 2H_2O$	60.04 (59.76)	5.42 (5.64)	8.60 (8.71)	10.12 (9.88)
$Cu(5\text{-nitro-OP})_2Cl_2 \cdot H_2O$	47.63 (47.81)	2.82 (2.67)	13.76 (13.94)	10.39 (10.54)

[a] Calculated values are within parentheses.

Scheme 2. Phenanthroline Ligands



L1 – L7

gated,[2] no study has been carried out to evaluate the substituent effect on the binding structures of both copper(I) and copper(II) complexes of 1,10-phenanthrolines. We have recently studied the interaction of copper complexes of 2,9-dimethyl-1,10-phenanthrolines (2,9-dimethyl-OP) with CT DNA as nonreactive probes and evaluated for the first time the DNA binding parameters.[6] The present report deals with our systematic investigation on the interaction of the copper(II) bis-complexes of OP and variously substituted OP ligands (Scheme 2) with calf thymus (CT) DNA using viscometry and various spectral and electrochemical studies. It is our aim to understand the structure−reactivity relationship of the chemical nuclease activity. Also we are attempting to address the nature of forces which govern the DNA interaction so as to understand the role of the intrinsic redox chemistry due to the copper ion on the scission reactions and the extrinsic stereoelectronic and steric properties of OP complexes, which are important in the recognition event. During the course of this study we have discovered[7] that the copper(II) bis-complex of 5,6-dimethyl-OP effects the important B → Z transition in the natural CT DNA.

Experimental Section

Materials. Disodium salt of calf thymus DNA (SIGMA) was used as received. The solid Na^+ salt was stored at 4 °C. Solutions of DNA in buffer 1, 50 mM NaCl/5 mM Tris HCl in water−methanol (10:1, v/v, pH 7.1), gave the 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.[8] Concentrated stock solutions of DNA (10.5 mM) were prepared in buffer 1 and sonicated for 25 cycles, where each cycle consisted of 30 s with 1 min intervals. The concentration of DNA in nucleotide phosphate (NP) was determined by UV absorbance at 260 nm after 1:100 dilutions. The extinction coefficient, ϵ_{260}, was taken as 6600 M^{-1} cm^{-1}.[9] Stock solutions were stored at 4 °C and used after no more than 4 days.

The 1:2 Cu(II) complexes of 1,10-phenanthroline (OP, L1), 4-methyl-1,10-phenanthroline (4-methyl-OP, L2), 4,7-dimethyl-1,10-phenanthroline (4,7-dimethyl-OP, L3), 5-methyl-1,10-phenanthroline (5-methyl-OP, L4), 5,6-dimethyl-1,10-phenanthroline (5,6-dimethyl-OP, L5), 3,4,7,8-tetramethyl-1,10-phenanthroline (3,4,7,8-tetramethyl-OP, L6), and 5-nitro-1,10-phenanthroline (5-nitro-OP, L7) were prepared by adopting the procedure described for $Cu(OP)_2Cl_2 \cdot H_2O$.[10] All the present phenanthroline complexes were found to conform to the general formula $CuL_2Cl_2.nH_2O$ ($n = 1, 2$), on the basis of elemental analysis (Table 1).

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

Circular dichroic spectra of DNA were obtained by using 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

$$[\theta]_\lambda = [\theta_\lambda/cl] \times 100$$

where $[\theta]_\lambda$ is the molecular ellipiticity value at a particular wavelength expressed in degrees centimeter squared per decimole, c the concentration in moles of nucleotide phosphate per liter, l the length of the cell in decimeters, and θ_λ the observed rotation in degrees.

Emission intensity measurements were carried out by using a Jasco FP 770 spectrofluorometer. The Tris buffer was used as a blank to make preliminary adjustments. The excitation wavelength was fixed and the emission range was adjusted before measurements. DNA was pretreated with ethidium bromide (40 μM) in the ratio [NP]/[dye] = 1 for 30 min at 27 °C. The copper−OP complexes were added to this mixture and their effects on emission intensity were measured.

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 (area 0.34 cm^2) and the reference electrode a saturated calomel electrode. A platinum plate was used as the counter electrode. The supporting electrolyte was buffer 1. 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. Aqueous 0.1 M $NaClO_4$ solution of 1 mM hydroxymethylferrocene under identical experimental conditions gave an $\Delta E_p°$ value of 73 mV with $E_{1/2}$ 0.176 V vs SCE.

Results and Discussion

All the present cupric complexes do not exhibit any intense charge transfer (CT) or a sufficiently intense d−d band, suitable to monitor their interactions with DNA. So we have employed cyclic and differential pulse voltammetric techniques for the present study. In the study of metallointercalation and coordination of metal ions and chelates to DNA, these methods provide a useful complement to the previously used methods of investigation such as UV−visible spectroscopy. Recently we have successfully employed electrochemical techniques in discriminating the enantioselective interaction[11] of $[Ru(OP)_3]^{2+}$ with calf thymus DNA and for probing the interaction of copper

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complexes of certain bis(pyrid-2-yl)polythioether[12] and tridentate polypyridyl ligands,[13a] 2,9-dimethyl-1,10-phenanthrolines[6] and L-carnosine.[13b]

Structures of Copper−Phenanthroline Complexes and Their Interaction with DNA. The central role of the productive, noncovalent intermediate in catalysis means that the precise structures of $[(OP)_2Cu]^+$ and $[(OP)_2Cu]^{2+}$ complexes are crucial to understanding the specificity and reactivity of the nuclease activity. The cuprous ion is unique in its ability to form stable complexes of approximately tetrahedral geometry[14] with OP and its derivatives, which appears to be a requirement for the unique reactivity of the ion. The high site-specific sequence-dependent binding affinity and orientation of $[[OP)_2Cu]^+$ in the oxidatively sensitive minor groove[15] appear to be more important than any bond breaking or bond formation involving the complex. Despite intense study the exact mode of binding of the complex remains unknown. A series of viscometry and spectroscopic studies by Veal and Rill suggested partial intercalation of one of the OP rings with the second ring residing neatly in the minor groove.[16] However, strong evidence for a nonintercalative docking of the complex within the minor groove is available.[17] Structure−reactivity studies with a family of redox-active cuprous OP complexes (i.e., those complexes that do not have substituent ortho to the liganding nitrogens) do not require that the essential noncovalent intermediate have an intercalative component even though the physical studies of the binding do not disallow that possibility.[15]

The structure of the copper(II)−OP complex would also influence the nucleolytic reaction, as there should be no hindrance to configurational changes accompanying the oxidation of $[(OP)_2Cu]^+$ species to the divalent state, and thus $[Cu(2,9\text{-dimethyl-OP})_2]^+$, which shows reluctance to oxidation,[18] is a poor scission reagent. So a consideration of the structural features and mode of DNA binding of the Cu(II)−OP complex is essential for understanding the scission reaction. The crystal structures of $[Cu(OP)_2(H_2O)](NO_3)_2$[19] and $[Cu(OP)_2Cl]ClO_4$[20] reveal that the coordination geometry around copper(II) is distorted trigonal bipyramidal in which the water molecule or Cl^- ion occupies the trigonal plane. Spectral studies have suggested that these complexes assume a cis-octahedral configuration in aqueous solution, as the two OP ligands deviate from coplanarity owing to steric repulsion between the ortho 2- and 9 hydrogens.[21] Though this structure is an octahedral analogue of *cis*-$Pt(NH_3)_2Cl_2$ and is similar to $[Ru(OP)_2Cl_2]$, the complex is found to be optically inactive as expected of its susceptibility to optical inversion. As B-form DNA is a polyanion composed of two complementary, polymeric subunits

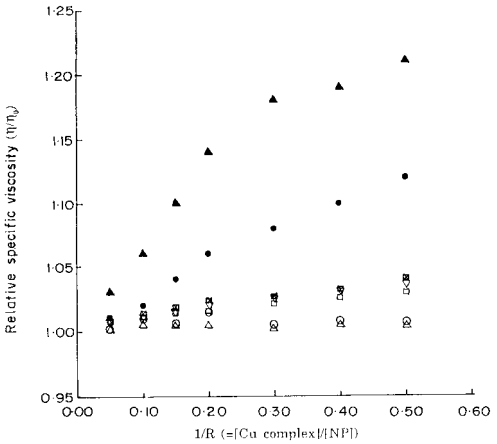

Figure 1. Effect of $[(OP)_2Cu]^{2+}$ (▲), $[(4\text{-methyl-OP})_2Cu]^{2+}$ (●), $[(4,7\text{-dimethyl-OP})_2Cu]^{2+}$ (□), $[(5\text{-methyl-OP})_2Cu]^{2+}$ (▽), $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ (△), $[(3,4,7,8\text{-tetramethyl-OP})_2Cu)]^{2+}$ (crossed boxes), and $[5\text{-nitro-OP})_2Cu]^{2+}$ (c) on the viscosity of calf thymus DNA; η_0 and η are the specific viscosity contributions of DNA in the absence and presence of complexes. The total concentration of DNA was 500 μM NP. The effect of ethidium bromide (*) is given for comparison.[16]

hydrogen bonded together in the form of a right-handed double helix, the present cationic Cu(I) and Cu(II) complexes bind to DNA, most likely by replacing a cation from the compact inner (Stern) layer or the diffuse outer layer surrounding DNA.[22] Because the OP ligands in this complex are coordinated to copper(II) in a nonplanar configuration, complete intercalation of the OP 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.[23] Thus, the forces responsible for binding interactions between $[(OP)_2Cu]^{2+}$ and DNA are both electrostatic and hydrophobic.

Viscometry Studies. Under appropriate conditions intercalation causes a significant increase in viscosity of DNA solutions due to the increase in separation of base pairs at intercalation sites, resulting in an increase in the overall DNA contour length. 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.[24] To understand the nature of DNA binding of the present Cu(II)−OP complexes, viscosity measurements were carried out on calf thymus DNA by varying the concentration of the added complexes. The values of relative specific viscosity (η/η_o), where η and η_o are the specific viscosity contributions of DNA in the presence and in the absence of complex, were plotted against $1/R$ (R = [NP]/[Cu complex]) (Figure 1). There is a small to large increase in viscosity of DNA for almost all the complexes and the ability of the complexes to increase the viscosity of DNA follows the order OP > 4-methyl-OP > 4,7-dimethyl-OP > 5-methyl-OP = 3,4,7,8-tetramethyl-OP = 5,6-dimethyl-OP = 5-nitro-OP. At first glance, we are tempted to ascribe the significant increase in viscosity of OP and 4-methyl-OP complexes to their intercalative binding to DNA. But the increase is very much less than that for the potential intercalator, viz., ethidium bromide (EthBr), or variously substituted *N*-ethyl-1,10-phenanthrolinium cations[25] in the same DNA concentration range. So

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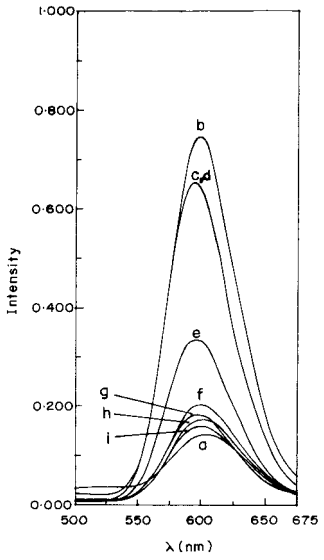

Figure 2. Emission spectra of 40 μM ethidium bromide in the absence (a) and presence of 40 μM NP (b), 40 μM NP + 40 μM [(5-methyl-OP)$_2$Cu]$^{2+}$ (c), 40 μM NP + 40 μM [5-nitro-OP)$_2$Cu]$^{2+}$ (d), 40 μM NP + 40 μM [(3,4,7,8-tetramethyl-OP)$_2$Cu)]$^{2+}$ (e), 40 μM NP + 40 μM [(4,7-dimethyl-OP)$_2$Cu]$^{2+}$ (f), 40 μM NP + 40 μM [(OP)$_2$Cu]$^{2+}$ (g), 40 μM NP + 40 μM [(4-methyl-OP)$_2$Cu]$^{2+}$ (h), and 40 μM NP + 40 μM [(5,6-dimethyl-OP)$_2$Cu]$^{2+}$ (i).

we suggest that [(OP)$_2$Cu]$^{2+}$ exhibits an affinity for the intercalative sites on DNA lower than EthBr or phenanthrolinium cations do. Further, the increase in viscosity of DNA caused by other complexes is less than that by [(OP)$_2$Cu]$^{2+}$, suggesting that the substitutents on the central OP ring lower their affinity toward the intercalative site to different extents. Thus, the methyl substitution at the 4-position slightly, while that at the 4,7-positions significantly, lowers the affinity. In contrast, all other complexes including [(5,6-dimethyl-OP)$_2$Cu]$^{2+}$ appear to exhibit very low affinity for intercalative sites. Thus, the present data are consistent with the involvement of the central ring of OP in partial intercalative interaction with DNA base pairs.

Competitive Binding Studies. Competitive ethidium bromide (EthBr) binding studies have been undertaken to gain support for the mode of DNA interaction inferred from the above viscometry results. They involve the addition of the present complexes to DNA pretreated with EthBr ([NP]/[EthBr] = 1) and then the measurement of emission intensities of EthBr. The observed enhancement in emission intensity of EthBr in the presence of DNA (Figure 2) is expected of its strong stacking interaction (intercalation) between the adjacent DNA base pairs.[26] The ability of the various phen complexes to hinder the DNA induced enhancement in EthBr emission decreases in the order OP $\approx$ 5,6-dimethyl-OP $\approx$ 4-methyl-OP $\approx$ 4,7-dimethyl-OP > 3,4,7,8-tetramethyl-OP > 5-methyl-OP $\approx$ 5-nitro-OP. The addition of [(OP)$_2$Cu]$^{2+}$ almost completely hinders the enhancement in emission; also the addition of DNA pretreated with [(OP)$_2$Cu]$^{2+}$ to EthBr fails to enhance the EthBr emission. These observations suggest that the complex efficiently competes with EthBr for the intercalative binding site, illustrating its higher affinity for the latter. While 4-methyl and 4,7-dimethyl substitutions do not appear to lower the affinity, 3,4,7,8-tetramethyl substitution lowers it significantly. The substitutions at the 5-position of the middle ring of OP as in 5-methyl-OP and 5-nitro-OP complexes confer still lower

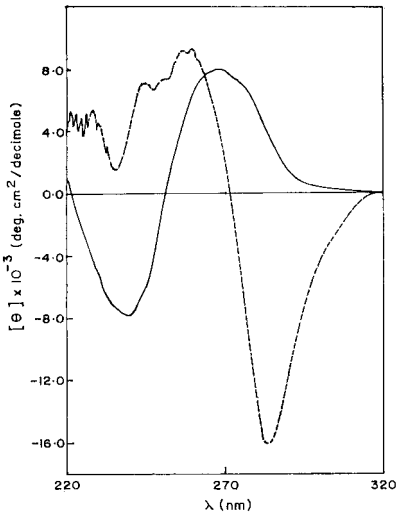

Figure 3. Circular dichroism spectra of calf thymus DNA in 5 mM Tris·HCl/50 mM NaCl water−methanol (10:1 v/v, pH 7.1) in the absence (—) and in the presence (---) of 0.5 mM [(5,6-dimethyl-OP)$_2$Cu]$^{2+}$.

affinities on them resulting in their failure to compete with EthBr for intercalative binding. All the observations are consistent with the above viscometry result that the central ring of phen is involved in intercalation. In contrast, interestingly, the 5,6-dimethyl-OP complex, which is shown by viscosity measurements not to be involved in intercalative binding, hinders the enhancement in emission of EthBr almost to the same extent as the unsubstituted [(OP)$_2$Cu]$^{2+}$ complex does. Further, on the addition of DNA pretreated with 5,6-dimethyl-OP complex to EthBr no enhancement in the emission is observed. Like the 5-substituted-OP complexes, this would be expected to display lower affinities for the intercalative site and to not interfere with the enhancement in EthBr emission. So the absence of enhancement in emission suggests that the complex binds to DNA in a nonintercalative mode but still prevents the intercalative binding of EthBr. All these observations are consistent with the conformational transition from the B to the Z form induced by this complex (cf. CD studies below); the Z helix, as it is a more rigid molecule, is unable to accommodate an intercalating drug like EthBr.[27]

Circular Dichroism. The observed CD spectrum of calf thymus DNA (Figure 3) consists of a positive band at 270 nm (UV: λ_{max} 259 nm) due to base stacking and a negative band at 240 nm due to helicity and is characteristic of DNA in the right-handed B form.[28] When incubated with the present copper−OP complexes, the DNA showed an additional positive feature (Table 2) around 296 nm with lower intensity for all of them, except 5,6-dimethyl-OP complex. One more positive band around 265 nm with an intensity almost the same as that of the 277 nm band is discernible for 5-methyl-OP and 4-methyl-OP complexes. These relatively weak additional positive ellipticity (Cotton effect) bands may be considered as relatively weak perturbation on DNA consistent with the binding of the OP chromophores (which have an absorption in the region 265−275 nm) of the complexes on the asymmetric surface of the DNA polymer. However, even when excess DNA was introduced into the solution containing the present complexes, no

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Table 2. CD Parameters for the Interaction of Calf Thymus DNA with Cu(II)

sample	molecular ellipticities[a] $[\theta]$/deg·cm^2·dmol^{-1}	
DNA	7116 (273)	−7632 (242)
DNA + 500 μM $[(OP)_2Cu]^{2+}$	4418 (295) sh	16486 (274)
	−7126 (245)	
DNA + 500 μM $[(4\text{-methyl-OP})_2]^{2+}$	3409 (298) sh	15530 (277)
	16028 (266)	−5796 (245)
DNA + 500 μM $[(4,7\text{-dimethyl-OP})_2Cu]^{2+}$	3526 (298) sh	19208 (277)
	−5012 (255)	−5164 (227)
DNA + 500 μM $[(5\text{-methyl-OP})_2Cu]^{2+}$	3430 (298) sh	15432 (277)
	18426 (265)	−5106 (245)
DNA + 500 μM $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$	−16972 (287)	8962 (263)
	5986 (247) sh	
DNA + 500 μM $[(3,4,7,8\text{-tetramethyl-OP})_2Cu]^{2+}$	3618 (305)	15428 (283)
	−2504 (253)	−6134 (234)
DNA + 500 μM $[(5\text{-nitro-OP})_2Cu]^{2+}$	13430 (270)	9826 (240)

[a] Wavelength at which the ellipticities are expressed is in parentheses.

CD signal is discernible in the visible region where d−d absorptions of the copper(II) complexes are located (745−760 nm). In contrast, 5,6-dimethyl-OP complex displays an interesting behavior. Upon the incremental addition of the complex to DNA, the intensities of both the negative and positive bands decrease and then a nearly inverted CD spectrum with a new positive band around 260 nm and a negative one around 285 nm is obtained.[7] The inverted spectrum is distinctly different[29] from the features induced by the other OP complexes but is similar to that observed earlier for poly(dG-dC) at high salt concentration,[30] a condition when the Z conformation is stabilized. The conformational transition from the right-handed B DNA to the left-handed Z DNA in solution has been widely studied[31] for the synthetic polynucleotides such as poly(dG-dC)·poly(dG-dC). A variety of environmental conditions including the presence of high salt concentrations,[30−32] transition metal complexes,[33,34] negative supercoiling,[35] and chemical modifications such as base bromination[36] and alkylation[37] have been shown to strongly influence the B ⇔ Z equilibrium. Since alternative purine−pyrimidine sequences are the only sequences capable[30,31,38] of the B ⇔ Z transition, studies have been limited to synthetic polynucleotides with alternating GC sequences. In natural systems such as CT DNA, however, such alternative GC stretches are of rather smaller length and so their Z form would normally escape detection.

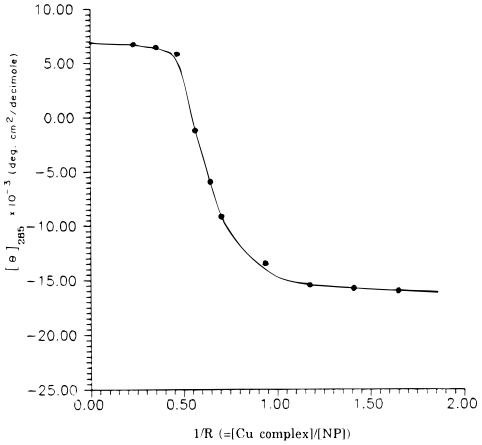

Figure 4. CD spectral titration of CT DNA with $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$; variation of θ_{285} with $1/R$.

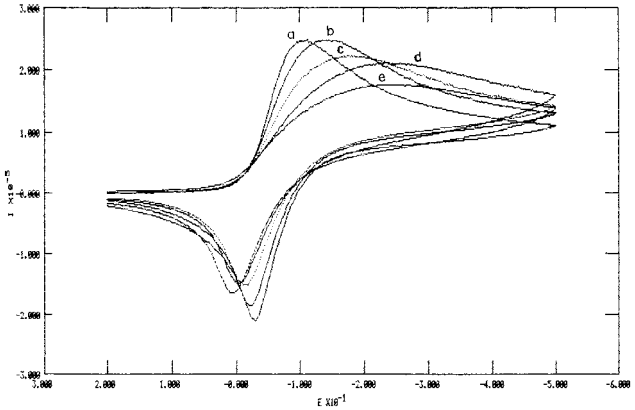

Figure 5. Cyclic voltammograms of 0.5 mM $[(OP)_2Cu]^{2+}$ in the absence (a) and in the presence of 25 μM NP (b), 50 μM NP (c), 75 μM NP (d), and 125 μM NP (e). Scan rate 10 mV/s. Supporting electrolyte, 5 mM Tris·HCl + 50 mM NaCl in water−methanol (10:1 v/v, pH 7.1).

Figure 4 shows that the B to Z transition induced by the complex is highly cooperative. The transition appears to start at $1/R$ (R = [NP]/[Cu complex]) of 0.5 and is completed when the $1/R$ value reaches unity. Contrastingly, the CD spectra of CT DNA obtained in the presence of copper(II) complexes of all the other OP ligands show a considerable increase in intensity of the positive band with only a slight perturbation of the

(29) It may be expected that $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ (UV 293 sh, 280, 230 nm), like the analogous $[Ru(phen)_2Cl_2]$ (Barton, J. K.; Lolis, E. *J. Am. Chem. Soc.* **1985**, *107*, 708), would bind to N7 of guanine, which is readily accessible in the major groove, and then, as is inversion labile, yield a certain net excess of the Λ enantiomer preferentially formed (Pfeiffer effect) giving rise to a positive cotton effect. But the observation of a negative Cotton effect eliminates the possibility of a Pfeiffer effect. Further, all the other copper−phen complexes, particularly the 5-substituted complexes, which give viscosity effects similar to 5,6-dmp complex, do not exhibit such an inversion in CD spectra. This is consistent with the failure of the 5-substituted complexes to inhibit the enhancement in DNA-bound EthBr emission.

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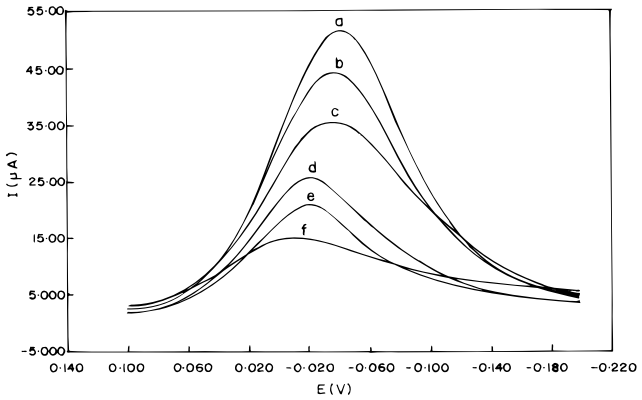

Figure 6. Differential pulse voltammograms of 0.5 mM $[(OP)_2Cu]^{2+}$ in the absence (a) and presence of 25 μM NP (b), 50 μM NP (c), 75 μM NP (d), 100 μM NP (e), and 125 μM NP (f). Scan rate 1 mV/s. Supporting electrolyte 5 mM Tris·HCl + 50 mM NaCl in water−methanol (10:1 v/v, pH 7.1).

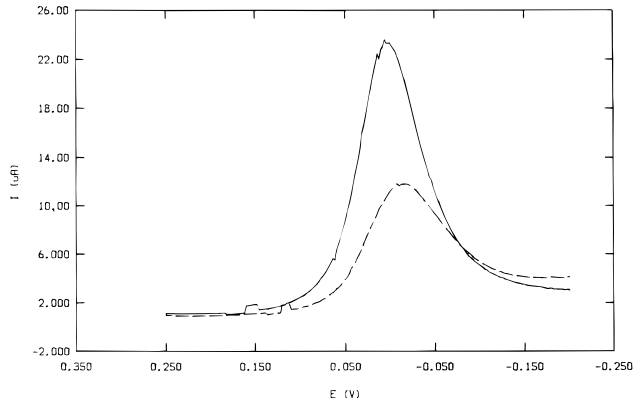

Figure 7. Differential pulse voltammograms of 0.5 mM $[(3,4,7,8\text{-tetramethyl-OP})_2Cu]^{2+}$ in the absence (—) and presence (---) of 125 μM NP. Scan rate 1 mV/s. Supporting electrolyte, 5 mM Tris·HCl + 50 mM NaCl in water−methanol (10:1 v/v, pH 7.1).

negative band and there is no inversion of the CD spectra. Thus $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ appears to be unique and selective in inducing the B to Z transition. When EDTA was added to DNA incubated with $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$, the original CD spectrum typical of native DNA was regenerated; this suggests that the induced B to Z transition is reversible and that the complex binds on the DNA surface to cause the transition. Further, when DNA pretreated with the complex is dialyzed against 50 mM NaCl buffer solution (pH 7.1) for 1 h, the retentate yielded a CD spectrum with decreased intensity for the longer wavelength CD band. On continuing the dialysis for 4 h more the retentate yielded the original CD spectrum typical of natural DNA. This confirms that the B to Z transition in natural CT DNA brought about by the complex is reversible.

Upon the addition of EthBr to CT DNA to which $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ is bound, the CD peak intensities were slightly reduced and the inverted spectrum did not revert to the original one. Since a strong intercalator like EthBr is well-known to convert Z DNA to an intercalated right-handed B form (allosteric effect) under solution conditions that would otherwise favor the Z conformation,[39] the present complex appears to be a strong reverse allosteric effector, shifting the equilibrium toward the Z conformation even in the presence of an allosteric effector. Further, when the complex is added to CT DNA pretreated with EthBr, the inverted CD spectrum characteristic of Z DNA is obtained. This suggests that EthBr fails to lock the DNA in the B-form and is unable to prevent the B → Z conversion effected by $[Cu(5,6\text{-dimethyl-OP})_2]^{2+}$. Competitive ethidium binding studies as illustrated above provide support for this observation. The substituents at 5,6-positions in $[Cu(5,6\text{-dimethyl-OP})_2]^{2+}$ would be expected to prevent the OP ring from intercalative interaction with the B or any other non-Z conformation as understood from the viscosity studies (Figure 1). It is clear that the binding of the present complex is extremely strong to resist the reversal of conformational transition by EthBr.

It is reasonable to expect that after the binding of the complex cation possibly to N(7) and O(6) of guanosine,[40] the hydrophobic methyl groups at 5- and 6-positions act to effectively place themselves between the phosphate groups at close proximity (5.9 Å)[41] with each other leading to the potentiation of Z DNA by decreasing the repulsion otherwise present between them in Z DNA and change the C_2'-endo, anti conformation of sugar pucker of deoxyribose into the C_3'-endo, syn conformation. Further, the effect appears to be similar to that by cytosine 5-methyl in poly(dG-m^{5}dC) in that the 5,6-dimethyl groups may effectively fill the pocket on the external surfaces, which might be hydrated in the major groove of the B form in the absence of the complex, and stabilize dG-dC base pairs in the Z form.

Redox Studies. In the absence of DNA, the limiting peak potential separations ($\Delta E_p°$, ΔE_p value extrapolated to zero scan rate) of 68−86 mV and the linearity of the i_{pc} vs $\nu^{1/2}$ plot passing through the origin indicated a fairly reversible 1e$^-$ redox process for $[(OP)_2Cu]^{2+}$ and the other substituted OP complexes. The number and position of the methyl groups do not appear to influence the observed trend in the redox potentials (0.023 to −0.098 V, Table 3) of the complexes. The latter are expected to illustrate the nuclease activity of copper−OP complexes, as their Cu(II) and Cu(I) forms are involved in the proposed cleavage mechanism (Scheme 1).[18] Thus, they are close to that of cleavage-active copper−OP complex (−0.080 V), suggesting that all of them should display significant nuclease activity. The ratio of cathodic to anodic peak currents, i_{pa}/i_{pc}, is greater than 1 (unity for reversible redox systems) for all the complexes, suggesting[42] that the copper(I) species are adsorbed on the glassy carbon electrode.

In the presence of 0.125 mM nucleotide phosphate (NP), significant changes in the CV waves are observed for all the complexes, except $[(5,6\text{-dimethyl-OP})Cu]^{2+}$, even at lower R (=[NP]/[Cu complex]) ≈ 0.25) values. Beyond $R \approx 0.25$, the redox waves tend to broaden out. Since the Cu(II)/Cu(I) couple becomes irreversible in the presence of DNA, as shown by the large increase in ΔE_p values even at low R values, no attempt was made to calculate the binding constants. Analogous to the treatment of association of small molecules with micelles[43] and DNA,[44] the ratio of the equilibrium constants, K_+/K_{2+}, for the binding of Cu(II) and Cu(I) forms of the present complexes to DNA can be estimated from the net shift in DPV[45] $E_{1/2}$ values,

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(45) $E_{1/2}$ values were determined from the DPV peak potential, E_p, by the relation $E_{1/2} = E_p + \Delta E/2$ where $E_{1/2}$ is the equivalent of the average of E_{pc} and E_{pa} in CV experiments and ΔE is the pulse amplitude (−50 mV) (Bard, A. J.; Faulkner, L. R. In *Electrochemical Methods*; Wiley: New York, 1980, p 194).

Table 3. Voltammetric Behavior of Cu(II) Complexes of Various Phenanthroline Ligands Bound to Calf Thymus DNA[a]

complex	R	E_{pc}/V	E_{pa}/V	ΔE_p/mV	$E_{1/2}$/V	i_{pa}/i_{pc}
$[(OP)_2Cu]^{2+}$	0.00	−0.124	−0.036	86	−0.080	1.3
	0.25	−0.420	0.028	448	−0.098	1.6
$[4\text{-methyl-OP})_2Cu]^{2+}$	0.00	−0.158	−0.048	110	−0.098	1.2
	0.25	−0.250	−0.028	222	−0.139	1.3
$[(4,7\text{-dimethyl-OP})_2Cu]^{2+}$	0.00	−0.096	−0.014	82	−0.055	1.5
	0.25	−0.198	0.016	214	−0.091	1.6
$[(5\text{-methyl-OP})_2Cu]^{2+}$	0.00	−0.114	−0.012	102	−0.063	1.4
	0.25	−0.250	−0.014	236	−0.132	1.6
$[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$	0.00	−0.036	0.076	112	0.020	1.1
	0.25	−0.030	0.080	114	0.025	1.1
$[(3,4,7,8\text{-tetramethyl-OP})_2Cu]^{2+}$	0.00	−0.062	0.058	120	−0.002	1.3
	0.25	−0.172	0.034	206	−0.069	1.3
$[(5\text{-nitro-OP})_2Cu]^{2+}$	0.00	−0.028	0.074	102	0.023	1.4
	0.25	−0.016	0.170	186	0.077	1.7

[a] Supporting eletrolyte, 5 mM Tris·HCl/50 mM NaCl in water−methanol (10:1 v/v, pH 7.1); scan rate, 50 mV/s.

Scheme 3

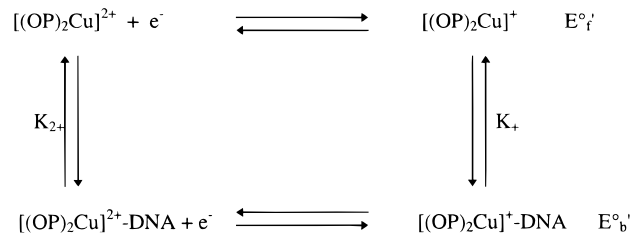

Table 4. DPV Behavior of Cu(II) Complexes of Various Phenanthroline Ligands Bound to Calf Thymus DNA[a]

complex	$E_f°$/V	$E_b°$/V	$(E_b° - E_f°)$	K_+/K_{2+}
$[(OP)_2Cu]^{2+}$	−0.066	−0.037	0.029	3.1
$[(4\text{-methyl-OP})_2Cu]^{2+}$	−0.087	−0.053	0.034	3.8
$[(4,7\text{-dimethyl-OP})_2Cu]^{2+}$	−0.034	−0.034	0.000	1.0
$[(5\text{-methyl-OP})_2Cu]^{2+}$	−0.065	−0.057	0.008	1.4
$[(5,6\text{-dmp})_2Cu]^{2+}$	−0.013	0.001	0.014	1.7
$[Cu(3,4,7,8\text{-tetramethyl-OP})_2]^{2+}$	−0.019	−0.033	−0.014	0.6
$[(5\text{-nitro-OP})_2Cu]^{2+}$	0.015	0.019	0.004	1.2

[a] Supporting electrolyte, 5 mM Tris·HCl/50 mM NaCl in water−methanol (10:1 v/v, pH 7.1); scan rate, 1 mV/s.

assuming reversible electron transfer (Scheme 3). Here $[(OP)_2Cu]^{n+}$−DNA represents the $[(OP)_2Cu]^{n+}$ complex bound to DNA.

Thus for a 1-e$^-$ process,

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

where $E_f°'$ and $E_b°'$ are the formal potentials of the Cu(II)/Cu(I) couple in the free and bound forms, respectively. The K_+/K_{2+} values (Table 4) for most of the complexes are >1 suggesting the preferential stabilization of Cu(I) forms. Interestingly, this is in contrast to our previous observation for the copper complexes of 2,9-dimethylphenanthrolines,[6] bis(pyrid-2-yl)polythioethers,[12] L-carnosine,[13] etc. that these values are less than 1, suggesting the preferential DNA interaction of these complexes in the copper(II) form. The variation of these values in the order $[(4\text{-methyl-OP})_2Cu]^{2+}$ > $[(OP)_2Cu]^{2+}$ > $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ > $[(5\text{-nitro-OP})_2Cu]^{2+}$ > $[(5\text{-methyl-OP})_2Cu]^{2+}$ > $[(4,7\text{-dimethyl-OP})_2Cu]^{2+}$ > $[(3,4,7,8\text{-tmphen})_2Cu]^{2+}$ represents the decreasing ability of DNA to stabilize the Cu(I) over the Cu(II) form. This is consistent with the space-filling binding model[2] for the docking of $[(5\text{-phenyl-OP})_2Cu^I]^+$ in the minor groove of DNA. The substituents at 5- and/or 6-position(s) permits the ready accommodation of the OP complex within the minor groove with minimum steric interference. In contrast, the symmetrical 3,4 and 7,8-positions of $[(OP)_2Cu]^+$ lie in close contact with the floor of the minor groove and so derivatization of the ligand at these sites would be expected to disrupt the minor groove binding. However, substitution only at the 4-position would permit positioning the unsubstituted, equivalent 7-position in the interior of DNA minor groove, leading to an interaction stronger than that involving the 4,7-dimethyl-OP complex.

To exhibit nuclease activity the $[(OP)_2Cu^I]^+$ complex has to be readily accommodated in the minor groove, without displacing it from close proximity to the C-1 hydrogen, its primary site of attack. So the above order of K_+/K_{2+} values, which are measures of the predominance of minor groove binding of the Cu(I) form over the partial intercalative interaction of the corresponding Cu(II) form, would reflect the decreasing order of DNA cleavage efficiency of the complexes. Thus, as expected, the copper complex of the 4-phenyl-OP complex generates a cleavage pattern comparable to that of the unsubstituted OP complex, but the rate is significantly reduced. For several 5-substituted complexes Sigman et al. have obtained the same digestion pattern as that of $[(OP)_2Cu]^+$, illustrating that there is a size-independent tolerance to substitution at the 5- and 6-positions of the OP ring.[2] Further, as the Cu(II) form of the 5,6-dimethyl-OP complex selectively and reversibly binds to Z-form DNA, which is not susceptible to cleavage[45] under conditions similar to those for the B form, the observation of the same cleavage pattern supports the a,b pathway in Scheme 1. Similarly, for the 4,7-dimethyl-OP complex a cleavage pattern not significantly different from that of $[(OP)_2Cu]^+$ but with a slight diminution in rate expected of its lower K_+/K_{2+} value has been actually observed.[2] The 3,4,7,8-tetramethyl-OP complex has a greatly reduced rate of cleavage and does not generate the cleavage pattern characteristic of the unsubstituted OP complex, as expected of its very low K_+/K_{2+} value. Similarly, the 2,9-dimethyl-OP complexes[6] with lower K_+/K_{2+} values lack DNA nuclease activity. All these observations lead to the conclusion that the K_+/K_{2+} value should be >1 for observable nuclease activity.

Conclusions

The present investigation provides strong and impressive evidence for the binding of copper(II)−phenanthrolines to DNA via partial intercalation of the middle ring of phenanthroline between the base pairs of DNA, the number and position of methyl groups on phenanthroline ring dictating the affinity of the complex for partial intercalative binding. Thus, substitutions at 4- and 4,7-positions lower the binding affinity, while that at the 5-position eliminates intercalative interaction; however, the

3,4,7,8-tetramethyl derivative exhibits an intermediate behavior. Interestingly, the results of viscometry and competitive binding studies of the 5,6-dimethyl-OP complex are not consistent with each other; this has been ascribed to the unique nonintercalative binding mode of the complex after inducing changes in the conformation of DNA target. Recent investigations have shown that outer-sphere binding of ammine complexes can significantly alter DNA conformation, even to the extent of reversing the helical direction.[37a,47,48]

Thus, among the present copper−phenanthroline complexes $[(5,6\text{-dimethyl-OP})_2Cu]^{2+}$ is a remarkable and unique reagent in selectively and reversibly effecting the complete conversion of B to Z conformation in CT DNA, though the Z form in such a natural DNA would normally escape detection. This observation constitutes the first report that a mixed sequence DNA can adopt the Z conformation. Further, such conformational microheterogeneity, which is necessarily associated with the formation of structural junctions, or interfaces between different DNA conformations have been suggested to play a role in key cellular processes.[49] The effect of the phenanthroline complexes on the conformational variation of poly(dG·dC) and poly(dA·dT) will constitute an interesting and important investigation.

From the shifts in redox potentials of all the phen complexes observed on the addition of DNA, the relative stabilities of their DNA bound copper(II) and copper(I) forms have been derived and correlated with the DNA cleavage efficiency of the complexes. Factors which significantly favor the noncovalent minor groove binding of the Cu(I) form over the partial intercalative major groove binding of its Cu(II) form appear to confer the cleavage efficiency on the complex.

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