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^{1}H NMR Studies of Nickel(II) Complexes Bound to Oligonucleotides: A Novel Technique for Distinguishing the Binding Locations of Metal Complexes in DNA

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The selective paramagnetic relaxation of oligonucleotide proton resonances of d(GTCGAC)$_2$ and d(GT*G*C*A*C)$_2$ by Ni(phen)$_2$(L)$^{2+}$ where L = dipyridophenazine (dppz), dipyrido[3,2-d:2′,3′-f]quinoxaline (dpq), and phenanthrenequinone (phi) has been examined to obtain structural insight into the noncovalent binding of these metal complexes to DNA. In the oligonucleotide d(GTCGAC)$_2$, preferential broadening of the G1H8, G4H8, T2H6, and C3H6 proton resonances was observed with Ni(phen)$_2$(dppz)$^{2+}$, Ni(phen)$_2$(dpq)$^{2+}$, and Ni(phen)$_2$(phi)$^{2+}$. In the case of the sequence d(GT*G*C*A*C)$_2$, where the central two bases are juxtaposed from the previous one, preferential broadening was observed instead for the A5H2 proton resonance. Thus, a subtle change in the sequence of the oligonucleotide can cause significant change in the binding location of the metal complex in the oligonucleotide. Owing to comparable changes for all metal complexes and sequences in broadening of the thymine methyl proton resonances, we attribute the switch in preferential broadening to a change in site location within the oligomer rather than to an alteration of groove location. Therefore, even for DNA-binding complexes of low sequence-specificity, distinct variations in binding as a function of sequence are apparent.

Introduction

Paramagnetic NMR spectroscopy has emerged as a valuable technique to probe the structural and magnetic properties of transition metal ions in biological systems.[1] Since only the protons proximate to the paramagnetic center are affected, a fingerprint of the metal ion environment relative to the macromolecule can be obtained. These hyperfine-shifted resonances and their nuclear relaxation times are very sensitive to the distance and the orientation of the proton relative to the paramagnetic metal ion. Thus, a great deal of structural and magnetic information can be obtained regarding the local environment of the paramagnetic metal center. In this study, we have used paramagnetic NMR experiments to map the binding locations of transitional metal complexes within an oligonucleotide and also to explore whether small changes in the ligand structure can affect binding orientation and location.

The DNA binding ability of inert chiral transition metal complexes has attracted considerable interest. Recent studies have shown that a variety of transition metal complexes have significant potential as probes for sequence- and structure-specific DNA binding.[2] Significant attention has centered upon metal complexes capable of binding DNA by intercalation,[3−6] and, in particular, due to their luminescent properties and strong DNA binding affinity, polypyridyl complexes of

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ruthenium(II). It has been shown that complexes such as Ru(bpy)$_2$(dppz)$^{2+}$ and Ru(phen)$_2$(dppz)$^{2+}$ (bpy = 2,2′-bipyridine, phen = 1.10 phenanthroline, and dppz = dipyrido[3,2-a:2′,3′-c]phenazine) act as "molecular light switches" for double helical DNA.[7] Dppz complexes of osmium and rhenium have been shown also to behave in a similar fashion.[8] Ru(phen)$_2$(dppz)$^{2+}$ has, in addition, been extensively utilized to study DNA-mediated long-range electron-transfer chemistry.[9] Ru(phen)(bpy′)(dppz)$^{2+}$ (bpy′= 4-butyric acid-4′-methylbipyridine) tethered covalently to oligonucleotides has been employed extensively in spectroscopic and biochemical experiments to probe oxidative damage to DNA from a distance.[10] Ruthenium complexes have also been utilized in electrochemical experiments to sensitively monitor DNA hybridization through oxidation chemistry. As a wide range of applications for the ruthenium(II) polypyridyl complexes in probing nucleic acids has emerged, it becomes important that a detailed description of their DNA binding interactions and intercalation geometry be established.

Ruthenium polypyridyl complexes generally bind to DNA without high specificity. As a result, any detailed characterization of the structures of the interacting metal complexes with the DNA helix either by NMR or X-ray crystallography becomes difficult. Initial studies of the parent ruthenium(II) polypyridyl complexes,[11] Ru(phen)$_3^{2+}$, which binds weakly to DNA, as well as the paramagnetic Δ and Λ Ni(phen)$_3^{2+}$ and Cr(phen)$_3^{3+}$, suggested the existence of two binding modes: a surface bound interaction in the minor groove and a major groove intercalated form. Eriksson et al.[12] proposed that both Δ- and Λ-Ru(phen)$_3^{2+}$ bind only in a nonintercalating fashion in the minor groove and display an AT binding preference. Complexes based on the dipyridophenazine ligand, such as Ru(phen)$_2$(dppz)$^{2+}$ and the tris(heteroleptic) Ru(phen)(bpy′)(dppz)$^{2+}$, have been shown unambiguously to bind strongly ($K_a > 10^6$ M^{-1}) to DNA by intercalation; however, their DNA binding orientation has been debated. From NMR studies, it was proposed that Ru(phen)$_2$(dppz)$^{2+}$ intercalates from the major groove.[4] Moreover, from the chemical shifts of dppz ligand proton resonances upon binding to DNA, it was suggested that Ru(phen)$_2$(dppz)$^{2+}$ isomers bind to the DNA helix with a population of intercalative geometries consistent with earlier structural models based upon luminescence studies. Photophysical studies of Δ- and Λ-Ru(phen)$_2$(dppz)$^{2+}$ bound to calf thymus (CT) DNA, T4 DNA, and several synthetic DNA polymers were then carried out,[13] and on the basis of the differences of the emission characteristics among those DNAs, it was concluded that Ru(phen)$_2$(dppz)$^{2+}$ isomers bind by intercalation instead from the minor groove of the DNA helix. This proposition was supported by an NMR study of the binding properties of Δ-Ru(phen)$_2$(dpq)$^{2+}$ (dpq = dipyrido[3,2-d:2′,3′-f]quinoxaline), a complex similar to Ru(phen)$_2$(dppz)$^{2+}$ that has been proposed to bind to DNA from the minor groove.[6] However, a parallel study supported the original assignment of ruthenium intercalation from the major groove of duplex DNA.[14] Furthermore, do these metallointercalators all bind in a similar fashion, or do small changes in ligand design lead to significant changes in binding orientation? Terpyridine complexes of platinum[15] and metal complexes based on the phi ligand[5,20] intercalate from the major groove side, but most small organic molecules prefer the minor groove.[21] The general question of why some molecules bind to DNA from a particular groove is important also to establish in the context of rational therapeutic design.

A second issue of importance is site location. Why do some metallointercalators bind preferentially at 5′-pyrimidine-purine-3′ sites, for example? Can site preferences be established even without significant hydrogen-bonding interactions directed by ancillary ligands?

In an attempt to reinvestigate and compare the binding of dppz- and dpq-containing metal complexes to oligonucleotides and more generally to develop methods to screen the binding location of the transition metal complexes that bind to DNA, isostructural nickel(II) analogues of the dppz and dpq complexes have been prepared (Figure 1), and paramagnetic NMR experiments have been conducted using two

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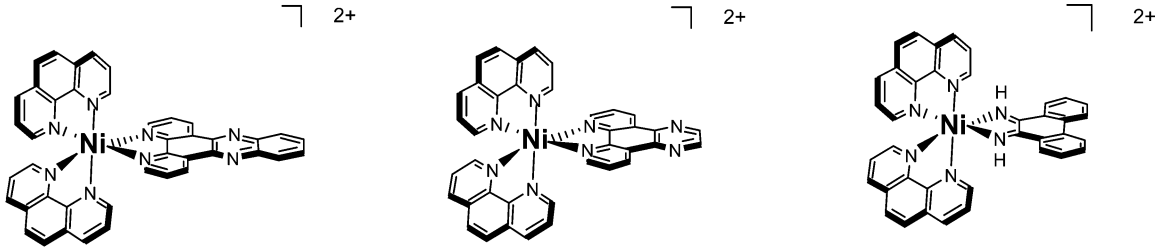

Figure 1. Paramagnetic metal complexes employed in this study.

different oligonucleotide systems. The comparison is also made in binding to the oligonucleotides by the phi complex of nickel(II). These results are consistent with a similarity in binding by the full family of complexes but reflect a remarkable sensitivity in binding for all complexes depending upon sequence.

Material and Methods

Synthesis of Metal Complexes. [Ni(phen)$_2$(dppz)](PF$_6$)$_2$·4H$_2$O. [Ni(phen)$_2$(dppz)](PF$_6$)$_2$ (phen = 1,10 phenanthroline; dppz = dipyrido[3,2-a:2′,3′-c]phenazine) was prepared according to the procedure reported in the literature.[16]

[Ni(phen)$_2$(dpq)](PF$_6$)$_2$·3H$_2$O. [Ni(phen)$_2$(dpq)](PF$_6$)$_2$ (dpq = dipyrido[3,2-d:2′,3′-f]quinoxaline) was synthesized in an analogous method. The complex was prepared starting from Ni(phen)$_2$Cl$_2$.[17] To a 25 mL ethanolic solution of Ni(phen)$_2$Cl$_2$ (245 mg, 0.5 mM) was added a 174 mg (0.75 mM) sample of dpq. The resulting solution was refluxed for 1 h and further stirred for 4−5 h under nitrogen. It was filtered, and the complex was precipitated upon addition of a saturated ethanolic solution of ammonium hexafluorophosphate. The complex was filtered and dried under vacuum before being recrystallized from acetone−ether. Yield: ∼76%. Anal. Calcd for $C_{38}H_{30}N_8O_3P_2F_{12}Ni$: C, 45.82; H, 3.01; N, 11.25. Found: C, 45.73; H, 3.13; N, 11.21. MALDI/ TOF MS(*m*/*z*): [M − 2PF$_6$ − 3H$_2$O]$^+$, 651; [M − phen − 2PF$_6$ − 3H$_2$O]$^+$, 470. UV−vis (water), λ_{max}, nm: 226, 268, 292, 320. The MALDI/TOF mass spectra were obtained from the Macromolecular Resources of Colorado State University.

[Ni(phen)$_2$(phi)](PF$_6$)$_2$·3H$_2$O. [Ni(phen)$_2$(phi)]Cl$_2$ was synthesized from Ni(phen)$_2$Cl$_2$. A 100 mg portion of Ni(phen)$_2$Cl$_2$ was dissolved in 20 mL of anhydrous DMF, and to it was added silver triflate. Stirring was continued for 8 h, and the resultant solution was centrifuged. The supernatant solution was added to a solution of 9,10-diaminophenanthrene amine (110 mg) in DMF and stirred overnight. DMF was evaporated off, and the residue was dissolved in 10 mL of ethanol. The complex was precipitated upon addition of a saturated ethanolic solution of ammonium hexafluorophosphate. The complex was filtered and dried under vacuum before being recrystallized from acetone−ether. Yield: ∼60%. Anal. Calcd for $C_{38}H_{32}N_6O_3P_2F_{12}Ni$: C, 46.95; H, 3.29; N, 8.64. Found: C, 46.87; H, 3.21; N, 8.59. MALDI/TOF MS (*m*/*z*): [M − 2PF$_6$ − 3H$_2$O]$^+$, 628; [M − phen − 2PF$_6$ − 3H$_2$O]$^+$, 447. UV−vis (water), λ_{max}, nm: 230, 255, 295, 330, 342, 525.

Each hexafluorophosphate salt was dissolved in a minimum amount of acetone, and a saturated solution of tetrabutylammonium chloride in acetone was added dropwise until the precipitation was complete. The water soluble chloride salts thus obtained were filtered, washed thoroughly with acetone, and vacuum-dried. Recovery was about 85% of the theoretical yield in each case. No separation of the enantiomers was attempted owing to the racemization rate of these complexes; therefore, only racemic mixtures were employed in the experiments.

Oligonucleotide Synthesis and Purification. Oligonucleotides were synthesized using standard phosphoramidite chemistry on an Applied Biosystems 392 DNA synthesizer with a dimethoxy trityl protective group on the 5′ end. The oligonucleotides were then purified on a reversed-phase Rainin Dynamax C$_{18}$ column on a Hewlett-Packard 1050 HPLC using 50 mM triethylammonium acetate and an acetonitrile gradient and deprotected by incubation in 80% acetic acid for 15 min. After deprotection, the oligonucleotides were purified again by HPLC and desalted in a Waters C$_{18}$ SepPak column and converted to the sodium salt using CM Sephadex C-25 (Sigma) equilibrated in NaCl and washed well with water. The concentration of the oligonucleotides was determined by UV−vis spectroscopy (Beckman DU 7400) using the extinction coefficients estimated for single-stranded DNA: ϵ(260 nm, M^{-1} cm^{-1}) adenine (A) = 15 400; guanine (G) = 11 500; cytosine (C) = 7400; thymine (T) = 8700. Single strands were mixed with equimolar amounts of complementary strand and were annealed in Perkin-Elmer Cetus thermal cycler by gradual cooling from 90 °C to ambient temperature in 90 min. Duplex formation was evaluated by examining its temperature-dependent absorbance at 260 nm.

Sample Preparation for NMR Analysis and Instrumental Methods. Deuterated D$_2$O (99.96%) and sodium 3-trimethylsilyl-[2,2,3,3-D$_4$]propionate (TMSP) were obtained from Aldrich. Other chemicals and biochemicals were of highest quality available commercially. ^{1}H NMR spectra were recorded in Varian Unity-*PLUS*-600 spectrometer with variable temperature control and pulsed-field gradients in three dimensions. DNA samples for NMR contained 0.5 mM duplex, 5 mM sodium phosphate buffer (pD 7.0), and 15 and 50 mM NaCl. Various temperatures and ionic strengths were examined in an effort to maximize the resolution of the aromatic region of ^{1}H NMR spectra of the oligonucleotides. Stock solutions of the metal complexes for NMR titrations were 0.2−0.5 mM in concentration. Samples were repeatedly freeze-dried from D$_2$O and finally made up in 99.96% D$_2$O. One-dimensional NMR and NOESY experiments were carried out at 4 °C. Spectra recorded in D$_2$O were collected with presaturation of the residual water signal. For the spectra taken in 90/10 H$_2$O/D$_2$O, WATERGATE gradient pulse water suppression[18d] was used. Typical instrument setting for acquiring one-dimensional spectra at 600 MHz were as follows: sweep width, 6492 Hz; number of scans, 128; relaxation delay, 1.3 s; spectral size, 4416 data points with −0.5 Hz line broadening. Two-dimensional phase sensitive NOESY spectra were recorded using 2048 points in t_2 for 512 t_1 values with a mixing time of 300 ms. Data were processed and analyzed using the VNMR software (version 6.1b) on a SUN workstation.

Results

Resonance Assignments. The assignments of DNA proton resonances were based on the presence of distinct base proton (H8, H6, H5, and TMe) to sugar proton sequential connectivities. Spectra of the duplex were unambiguously estab-

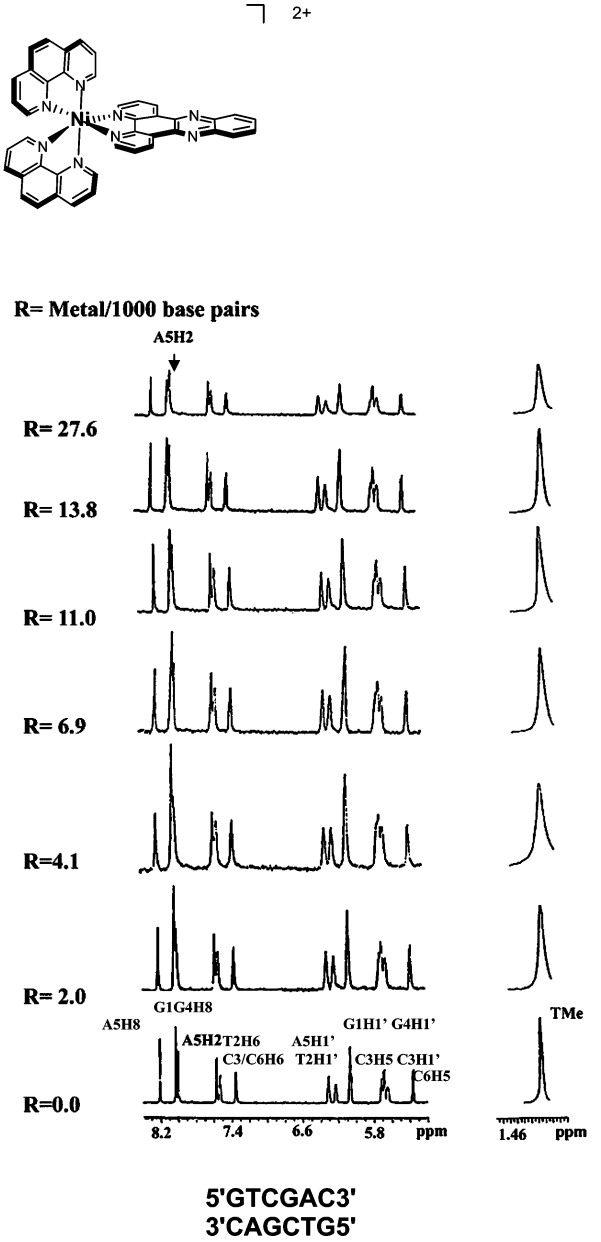

Figure 2. Effects of $[Ni(phen)_2(dppz)]Cl_2$ on the ^1H NMR spectrum of $d(GTCGAC)_2$ in D_2O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. Shown is the aromatic and sugar H1′ region. The DNA sample contained 0.5 mM duplex, 5 mM NaCl, 15 mM sodium phosphate, pD 7.0 in 100% D_2O. The free metal complex sample contained 0.2 mM of $[Ni(phen)_2(dppz)]Cl_2$. The chemical shifts are relative to TMSP at 4 °C. The broadening effect was most pronounced on major groove protons such as T2CH3, GIH8, and G4H8 while the minor groove proton A5H2 remained relatively unbroadened.

lished according to published methods.[18] Since the aromatic resonance of each base displays a stronger NOE cross-peak to its own sugar H2′2″ resonances than to the sugar of the 5′-flanking residue, the oligonucleotide may be described generally by a B-type conformation (e.g., with C2′-endo sugar puckering).

Titration of $d(GTCGAC)_2$ with $[Ni(phen)_2(dppz)]Cl_2$. The titration of the oligonucleotide (0.5 mM) in D_2O with the nickel complex was performed at 4 °C, and the one-dimensional ^1H NMR spectra as a function of Ni complex concentration are shown in Figure 2. The addition of the

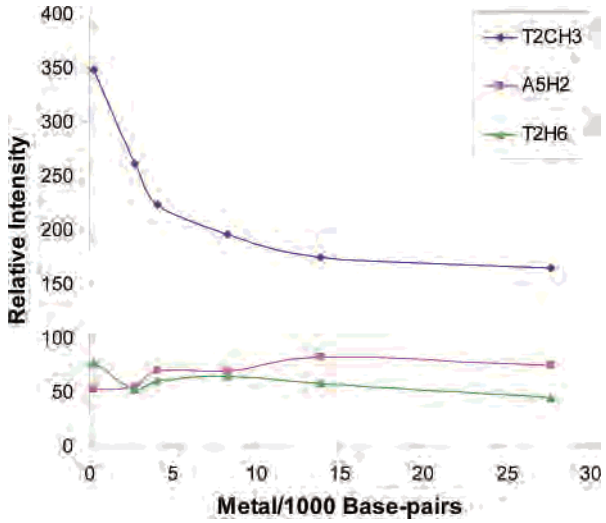

Figure 3. Paramagnetic relaxation of the proton resonances of $d(GTCGAC)_2$ with increasing amounts of $[Ni(phen)_2(dppz)]Cl_2$. The major groove protons, T2H6 and T2CH3, relax much faster as compared to the minor groove aromatic proton A5H2.

paramagnetic metal complex leads to the broadening of all of the signals. The preferential broadening of resonances associated with some protons on the oligonucleotide by the metal complex can nevertheless be observed. The loss of intensity is quantitated in Figure 3, and from these plots the selective broadening effects are evident. The broadening is most pronounced with the thymine methyl protons; other major groove protons T2H6, G1H8, and G4H8 are also broadened. Interestingly, the A5H2 proton remains relatively unbroadened even at high metal concentration where most of the other proton resonances of the duplex are extremely broad. The thymine methyl proton loses more than 55% of its peak intensity upon the addition of the metal complex, while the A5H2 proton loses less than 10% of its integrated area. The loss of intensity of the A5H2 proton due to paramagnetic broadening is therefore negligible. A point to note is that all of these protons discussed, other than the A5H2 proton, are positioned at the major groove of the oligonucleotide. Since the rate of longitudinal relaxation of a proton is dependent on its distance from the paramagnetic nucleus,[19] it appears that the A5H2 proton is not near the paramagnetic nucleus.

In order to verify whether titration experiments in D_2O led to an overemphasis of the major groove binding, we repeated the titration experiments in 90/10 H_2O/D_2O, keeping other conditions identical; under these conditions, the exchangeable amino and imino protons can be observed. Under low concentrations of the paramagnetic metal complex, no selective broadening is found in the case of the exocyclic amino protons of guanines (G1NH2 and G4NH2) which are positioned at the minor groove (Supporting Information). Some broadening of the amino protons was observed with higher concentrations of the metal complex. Furthermore, the broadening of the nonexchangeable aromatic protons in 90/10 H_2O/D_2O is very similar to that in D_2O.

We also considered whether some direct coordination of $Ni(phen)_2^{2+}$ to the bases from the major groove side might

be occurring, owing to some lability associated with the Ni(II) center. Titrations under comparable conditions using Ni(phen)$_2$Cl$_2$, however, yielded no selective broadening (data not shown).

The lack of broadening of the A5H2 proton also cannot simply be attributed to a lack of binding near the A5 base, since the complementary T2-methyl protons are significantly broadened. Indeed, these data support an overall sequence-neutrality of the complex. The strong paramagnetic broadening observed for all the protons that are directed toward the major groove may instead indicate that Ni(phen)$_2$(dppz)$^{2+}$ binds to the DNA duplex from the major groove side.

Titration of d(GTCGAC)$_2$ with [Ni(phen)$_2$(dpq)]Cl$_2$. Similar titration experiments were performed using the metal complex, [Ni(phen)$_2$(dpq)]Cl$_2$, at 4 °C (Figure 4). Broadening of the G1H8, G4H8, T2H6, C3H6, and A5H8 protons are observed as in the earlier case. The minor groove proton A5H2 remains relatively unbroadened (Figure 5). Most importantly, these data show a resemblence to that seen for the dppz analogue. Thus, these observations indicate that Ni(phen)$_2$(dpq)$^{2+}$ binds to the duplex d(GTCGAC)$_2$ in a fashion similar to Ni(phen)$_2$(dppz)$^{2+}$.

Titration of d(GTCGAC)$_2$ with [Ni(phen)$_2$(phi)]Cl$_2$. To further substantiate and calibrate this technique, a titration experiment was performed with [Ni(phen)$_2$(phi)]Cl$_2$. The isostructural complex Rh(phen)$_2$(phi)$^{3+}$ binds to DNA with some, albeit limited, sequence selectivity, and as a result, NMR studies have been used to establish binding of the parent rhodium complex to DNA from the major groove.[20] A titration was therefore performed using the Ni analogue at 4 °C (Supporting Information), and a plot of resonance intensity as a function of added Ni complex is shown in Figure 5 along with parallel data for the dpq complex. With increasing amounts of the metal complex, broadening is evident for the major groove protons (G1H8, G4H8, T2H6, C3H6, C6H6, C6H5) as in the earlier two cases, but not for A5H2. Again, therefore for the major groove binding intercalator, the behavior resembles that seen for the dpq and dppz complexes.

Titration of d(GT*GC*AC)$_2$ with the Nickel Complexes. To further explore this technique and behavior of the metal complexes, another oligonucleotide sequence was examined. In this sequence, only the position of the central C and G are interchanged. Three separate titrations of the oligonucleotide (0.5 mM) in D$_2$O with the nickel complexes [Ni(phen)$_2$(dppz)]Cl$_2$, [Ni(phen)$_2$(dpq)]Cl$_2$, and [Ni(phen)$_2$(phi)]Cl$_2$ were performed at 4 °C. The one-dimensional ^{1}H NMR spectra for the dpq and dppz complexes as a function of Ni complex concentration are shown in Figure 6. Interestingly, with this sequence the minor groove proton A5H2 is the one which is initially broadened out in comparison to the other major groove protons G1H8 and G3H8 in all three cases (see Supporting Information for the phi complex). In fact, the comparative loss of intensity due to paramagnetic broadening for A5H2 versus G1H8 and G3H8 is quite remarkable.

It is noteworthy here that higher levels of Ni(II) complex are also required to detect broadening compared to that seen with the other sequence; there is no observable paramagnetic broadening below $R = 75.0$ (R = metal/1000 base pairs). This reflects a lower binding affinity of all of the metal

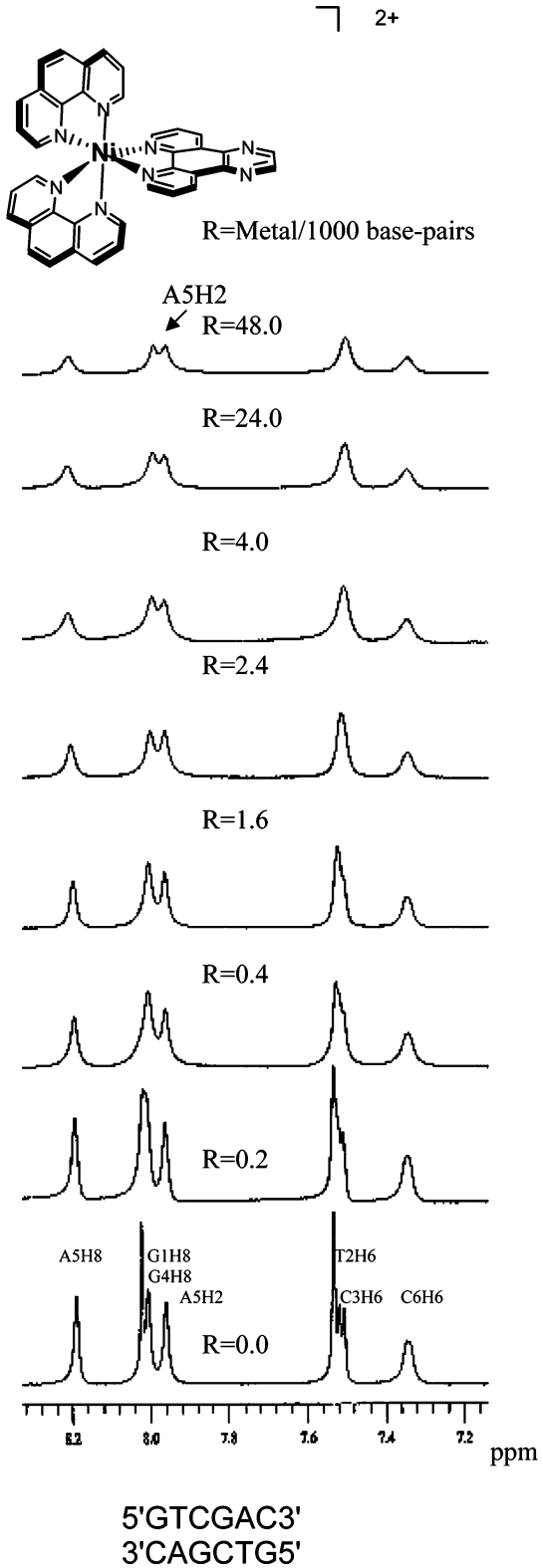

Figure 4. Effects of [Ni(phen)$_2$(dpq)]Cl$_2$ on the ^{1}H NMR spectrum of d(GTCGAC)$_2$ in D$_2$O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. The DNA sample contained 0.5 mM duplex, 5 mM NaCl, 50 mM sodium phosphate, pD 7.0 in 100% D$_2$O. The free metal complex sample contained 0.2 mM [Ni(phen)$_2$(dpq)]Cl$_2$. The chemical shifts are relative to TMSP at 4 °C.

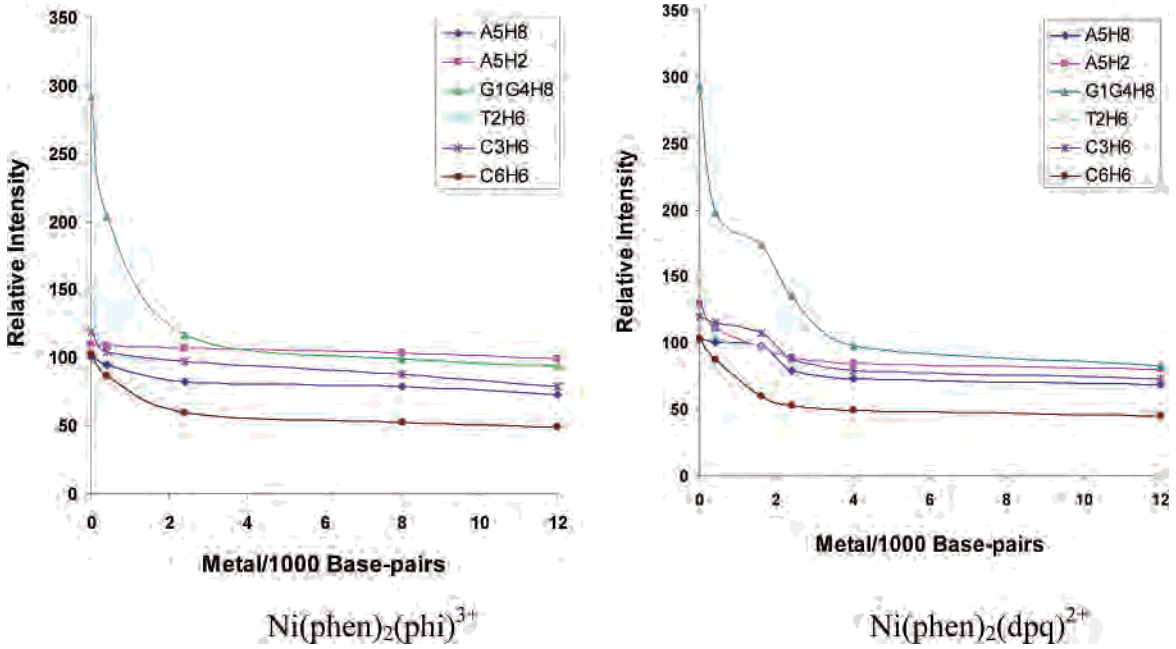

Figure 5. Paramagnetic relaxation of the proton resonances of $d(GTCGAC)_2$ with increasing amounts of $[Ni(phen)_2(phi)]Cl_2$ and $[Ni(phen)_2(dpq)]C1_2$.

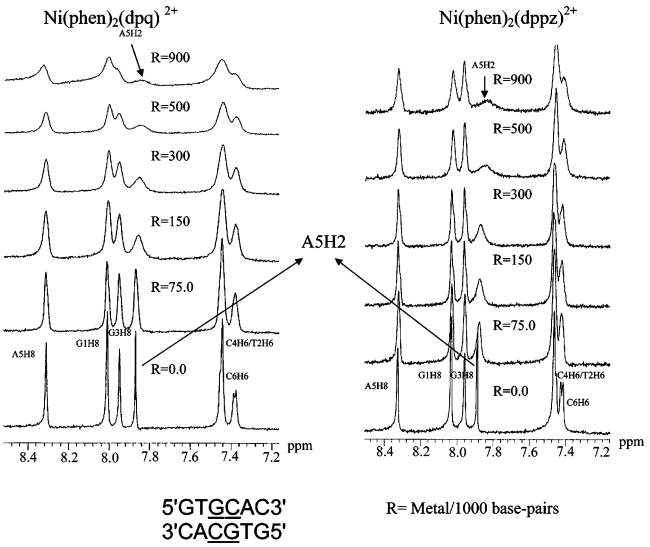

Figure 6. Effects of $[Ni(phen)_2(dpq)]CI_2$ and $[Ni(phen)_2(dppz)]Cl_2$ on the ^1H NMR spectrum of $d(GTGCAC)_2$ in D_2O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. The DNA sample contained 0.5 mM duplex, 5 mM NaCl, 50 mM sodium phosphate, pD 7.0 in 100% D_2O. The free metal complex sample contained 0.5 mM metal complexes. The chemical shifts are relative to TMSP at 4 °C. The broadening effect is most pronounced with the proton A5H2, located in the minor groove of the oligonucleotide.

complexes for this particular oligonucleotide. Again these data indicate a similarity in behavior among the complexes. It is interesting also that for all the complexes such a subtle change in the sequence (changing the central CG to GC) causes such a dramatic change in binding characteristics.

Whether these data can be ascribed to a change in groove preference for all the complexes rather than a change in site location can be examined through inspection for both sequences of the broadening of the thymine methyl proton resonances, located in the major groove. Figure 7 shows a plot of T2CH3 signal intensity as a function of metal/DNA ratio for all complexes in the two different sequences. What is apparent from these plots is the similarity in behavior among the metal complexes. In all cases, broadening of the T2CH3 proton resonances is observed. The change in sequence leads to a change in Ni(II) complex concentration required to achieve a given level of broadening. However, here for both sequences and all metal complexes, appreciable and comparable broadening is evident.

Discussion

Paramagnetic NMR studies provide a useful approach in delineating structural information regarding how metal complexes associate with DNA. In particular, for metal complexes that bind DNA with little sequence-specificity, conventional NMR methods and crystallography cannot be utilized. Despite fast exchange processes occurring among multiple binding sites, groove locations as well as site preferences on the helix can be identified through paramagnetic broadening and quenching. Thus, for transition metal complexes where the paramagnetic analogue can be prepared, paramagnetic NMR can offer a sensitive, alternative strategy in characterizing structural interactions.

Paramagnetic relaxation methods may not to be as useful in characterizing the binding mode of the complex with DNA. Whether the interaction involves intercalation, groove-binding, direct coordination, or some combination cannot be identified. In the case of $Ni(phen)_3^{2+}$, paramagnetic broadening had been observed both for major and minor groove resonances, and only through comparisons using enantiomers could more than one binding mode be identified.[11] Additionally, in cases where unpaired spin density is delocalized onto the intercalating ligand or coordinated base, the associated relaxation phenomena could be a complication. With the family of polypridyl complexes described here, such contributions are expected to be minimal. The extent of broadening observed also depends on the residence time of the complex on the helix, or indirectly, the strength of the binding interaction.

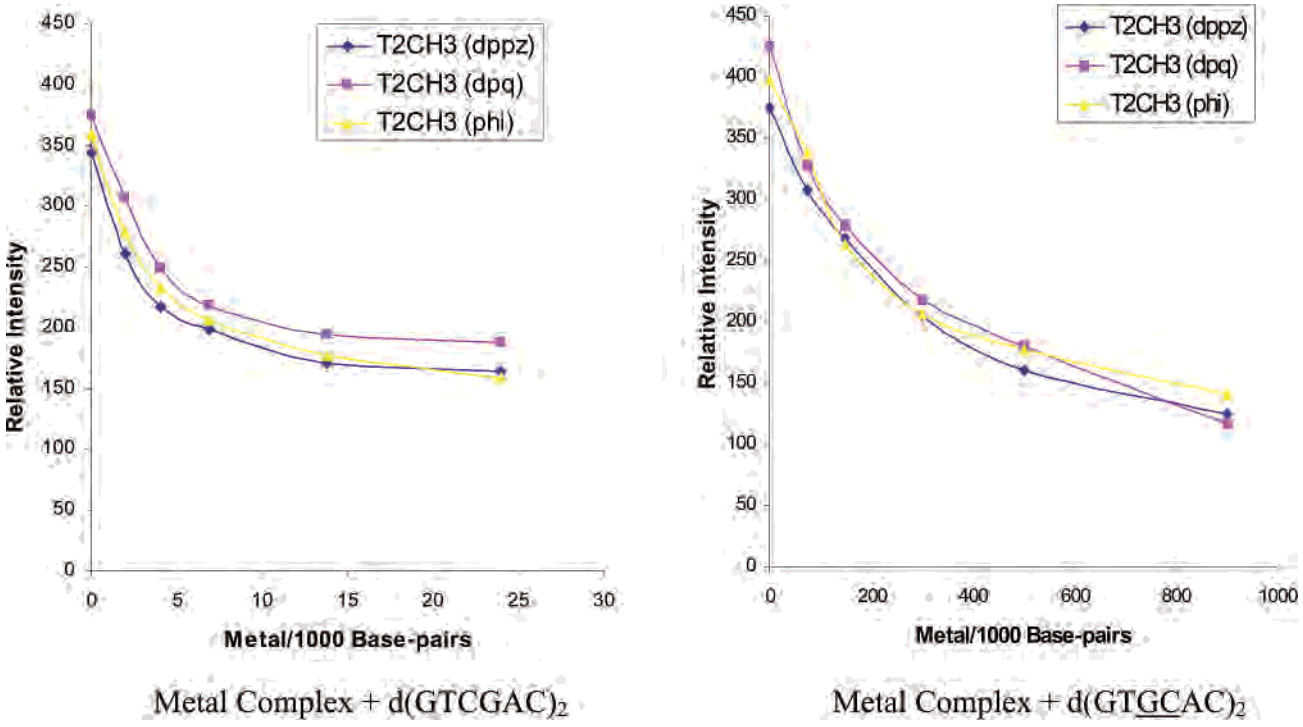

Figure 7. Comparative paramagnetic relaxation of the T2CH3 proton resonances of the two oligonucleotides, $d(GTCGAC)_2$ and of $d(GTGCAC)_2$, in the presence of the increasing amounts of $[Ni(phen)_2(dppz)]Cl_2$, $Ni(phen)_2(dpq)]Cl_2$, and $[Ni(phen)_2(phi)]C1_2$

Dppz[4] and phi[20] complexes are known to intercalate from the major groove of the DNA, while dpq complexes are reported to bind from the minor groove. Owing to the lack of sequence selectivity, however, the groove assignments for the dppz and dpq complexes are not definitive. What we observe in these sets of experiments is that paramagnetic relaxation leads to the broadening of the major groove protons for all the dppz, dpq, and phi complexes of nickel in the case of $d(GTCGAC)_2$, while that of the minor groove proton A5H2 in the case of the duplex $d(GTGCAC)_2$. Is this selective paramagnetic broadening the result of a switch in the preferential groove binding of these metal complexes to oligonucleotides, or do these results reflect a change in sequence preference of the metal complexes?

The similarity in behavior among the metal complexes and the broadening in all cases of the thymine methyl protons, located in the major groove, support the proposal that what we are observing does not reflect a groove preference but only a site preference. The notion that an intercalation geometry favors approach from one groove versus the other depending on sequence would be unprecedented. To propose instead that all the complexes bind in a similar fashion with a sequence preference for the pyrimidine/purine 5′-CA-3′ seems most reasonable. Broadening of the adenosine and cytosine aromatic protons (A5H8, C4H5, and C4H6) has been observed in the case of $d(GTGCAC)_2$. Preferences for intercalation within 5′-pyrimidine/purine-3′ steps have been noted previously.[5,6,20] For complexes that bind to DNA with little sequence preference, the ability to obtain detailed structural information is a challenge. However, comparing results among the family of complexes, notably including the phi complex, for which high-resolution structural information exists,[5,20] provides a useful strategy in making assignments. Moreover, since we observe wholly parallel behavior among the complexes, these results point to a similar intercalative binding interaction, notably from the major groove side, for all the complexes.

This technique of paramagnetic NMR provides a first order screening of the metal complex which preferentially binds to one location of an oligonucleotide versus another. The utility of paramagnetic NMR in establishing groove preference for metal complexes is not clear, owing to the central positioning of the metal within an intercalator complex, and the few protons available as structural probes of the minor groove. This technique may be particularly useful instead in establishing site preferences for the metal complexes, especially in the case of metal complexes that bind nonspecifically to oligonucleotides where the scope of conventional NMR techniques to obtain structural information is limited. It is clear that subtle and complex factors determine the binding location of the metal complexes in DNA. The challenge now is to more rationally predict binding locations and orientations.

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Supporting Information Available: ^1H NMR spectral titrations of $d(GTCGAC)_2$ with $[Ni(phen)_2(dppz)]Cl_2$ in 90/10 H_2O/D_2O and of $d(GTCGAC)_2$ and $d(GTGCAC)_2$ with $[Ni(phen)_2(phi)]Cl_2$ in D_2O. This material is available free of charge via the Internet at http://pubs.acs.org.

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