CHAPTER 4

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

Abstract

The selective paramagnetic relaxation of oligonucleotide proton resonances of $d(GTCGAC)_2$ and $d(GT\underline{GC}AC)_2$ 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 non-covalent 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 $d(GT\underline{GC}AC)_2$, 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 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 non-intercalating 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 ($Ka > 10^6$ 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 a 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). Clearly then, the unambiguous characterization of the binding modes of dppz-containing metal complexes is needed. 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.

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 complex have been prepared and paramagnetic NMR experiments have been conducted using two 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
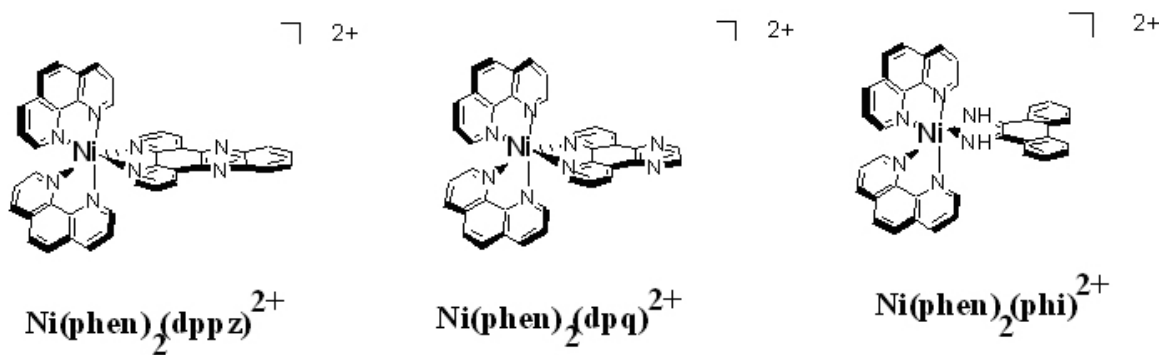
Figure 4.1.   Paramagnetic metal complexes employed in this study.

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 1hr and further stirred for 4-5 hrs 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$_8$O$_3$P$_2$F$_{12}$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-visible (water), $\lambda_{max,}$nm: 226,

268, 292, 320. The MALDI/TOF Mass Spectra was obtained from the Macromolecular

Resources of Colorado State University.

[Ni(phen)$_2$(phi)]Cl$_2$·3H$_2$O. [Ni(phen)$_2$(phi)]Cl$_2$ was synthesized from

Ni(phen)$_2$Cl$_2$. 100 mg of Ni(phen)$_2$Cl$_2$ was dissolved in 20 mL of anhydrous DMF and to

it silver triflate was added. Stirring was continued for 8 hrs and and the resultant solution

was centrifuged. The supernatant solution was added to 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 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$_6$O$_3$P$_2$F$_{12}$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-visible (water),

$\lambda_{max,}$nm: 230, 255, 295, 330, 342, 525.

Each hexafluorophosphate salt was dissolved in 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 performed and the 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 minutes. 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-visible 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) = 7,400; thymine (T) = 8,700. 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 mins. Duplex formation was evaluated by examining its temperature-dependent absorbance at 260 nm.

Sample Preparation for NMR Analysis and Instrumental Methods*:* 99.96% deuterated D_2O 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 50 mM NaCl. Stock solutions of the metal complexes for NMR titrations were 0.2 -0.5 mM in concentration. Samples were repeatedly freeze-dried from D_2O and finally made up in 99.96% D_2O. One-dimensional NMR and NOESY

experiments were carried out at 4 °C. Spectra recorded in D_2O were collected with presaturation of the residual water signal. Typical instrument setting for acquiring one dimensional spectra in D_2O at 600 MHz were as follows: sweep width, 6492 Hz; number of scans, 128; relaxation delay, 1.3s; 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 in D_2O were unambiguously established 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 ^{1}H NMR spectra as a function of Ni complex concentration is shown in Figure 2. The addition of the paramagnetic metal complex leads to the broadening of all
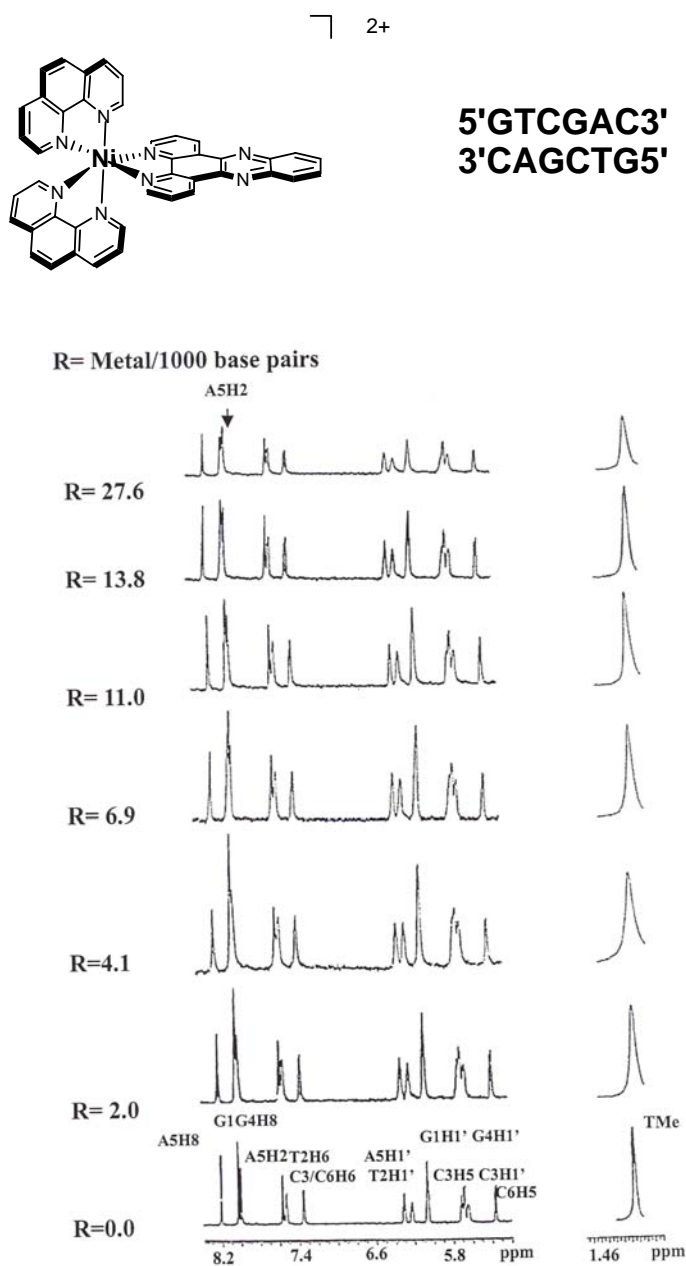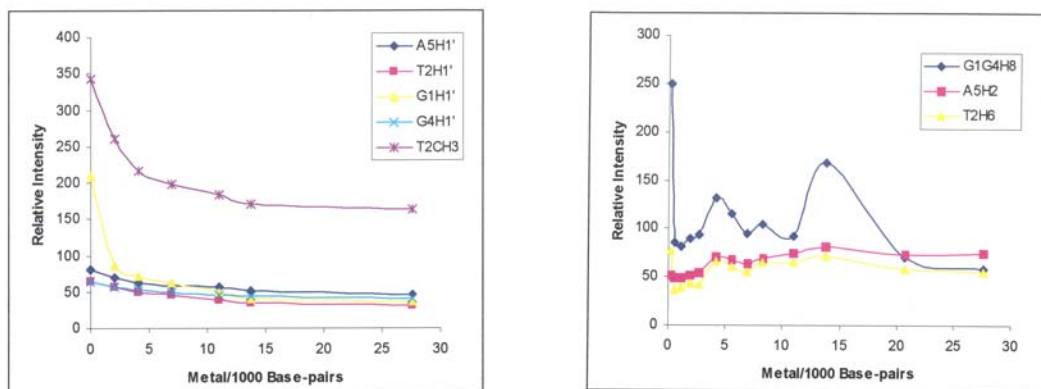
Figure 4.2. Effects of [Ni(phen)$_2$(dppz)]Cl$_2$ on the ^{1}H NMR spectrum of d(GTCGAC)$_2$ in D$_2$O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. Shown is the aromatic and sugar H1' region. The DNA sample contained 0.5 mM duplex, 5mM NaCl, 15 mM sodium phosphate, pD 7.0 in 100% D$_2$O. The 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 like T2CH3, GIH8 and G4H8 while the minor groove proton A5H2 remained relatively unbroadened.

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 apparent 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, T2H6, G1H8 and G4H8. 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.

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 $Ni(phen)_2Cl_2$, 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 towards 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).

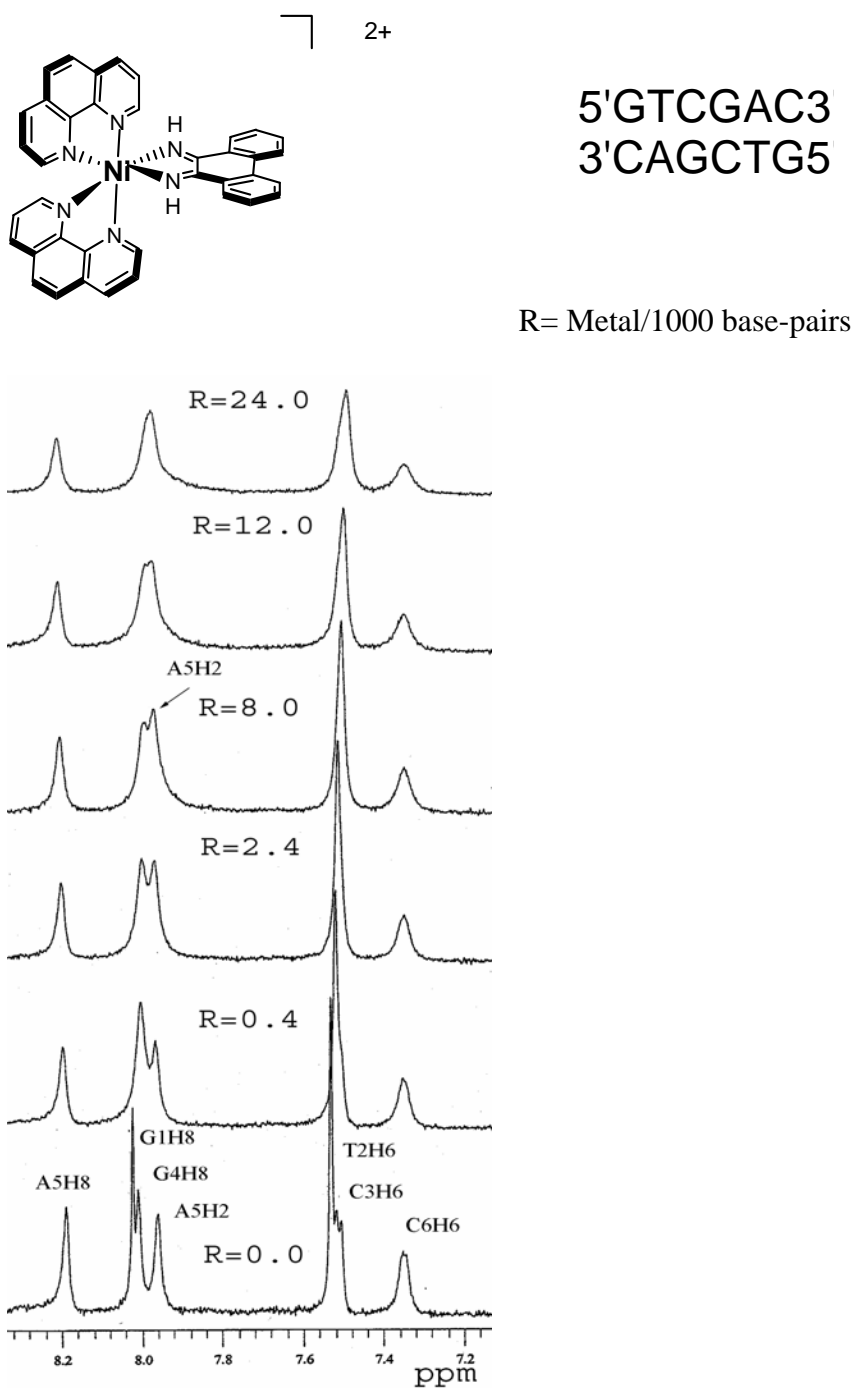**Figure 4.3.** Paramagnetic relaxation of the proton resonances of d(GTCGAC)$_2$ with increasing amounts of [Ni(phen)$_2$(dppz)]Cl$_2$. The major groove protons- GIH8, G4H8, T2H6 and T2CH3 relaxes much faster as compared to the minor groove aromatic proton A5H2.
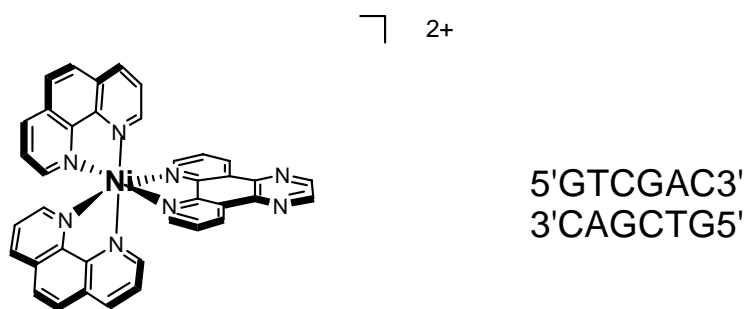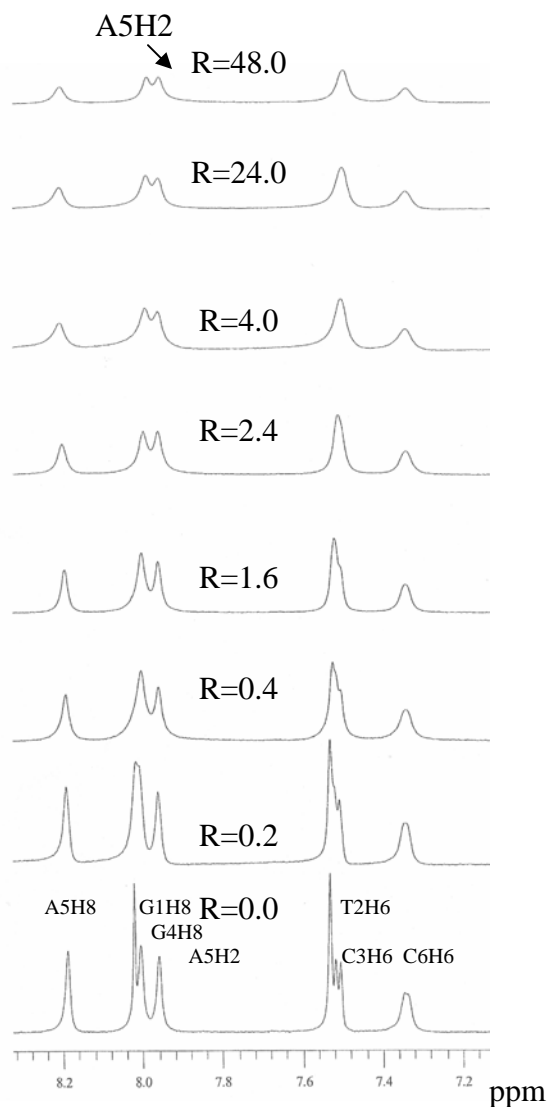
Figure 4.4. Effects of [Ni(phen)$_2$(dpq)]Cl$_2$ on the ^{1}H NMR spectrum of d(GTCGAC)$_2$ in D$_2$O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. The DNA sample contained 0.5 mM duplex, 5mM NaCl, 15 mM sodium phosphate, pD 7.0 in 100% D$_2$O. The free metal complex sample contained 0.2 mM [Ni(phen)$_2$(dpq)]Cl$_2$. The chemical shifts are relative to TMSP at 4 °C.

2+

5'GTCGAC3'
3'CAGCTG5'

R= Metal/1000 base-pairs

R=24.0

R=12.0

A5H2

R=8.0

R=2.4

R=0.4

G1H8

T2H6

A5H8

G4H8

C3H6

A5H2

C6H6

R=0.0

8.2   8.0   7.8   7.6   7.4   7.2
ppm

Figure 4.5.   Effects of [Ni(phen)$_2$(phi)]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. Shown is the aromatic region. The DNA sample contained 0.5 mM duplex, 5mM NaCl, 15 mM sodium phosphate, pD 7.0 in 100% D$_2$O. The free metal complex sample contained 0.2 mM [Ni(phen)$_2$(phi)]Cl$_2$ in the same buffer. The chemical shifts are relative to TMSP at 4 °C.

earlier case. The minor groove proton A5H2 remains relatively unbroadened (Figure 6).

Most importantly, these data shown 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 (Figure 5), and a plot of

resonance intensity as a function of added Ni complex is shown in Figure 6 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<u>GC</u>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 7.

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

Ni(phen)$_2$(phi)$^{2+}$ Ni(phen)$_2$(dpq)$^{2+}$
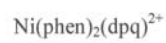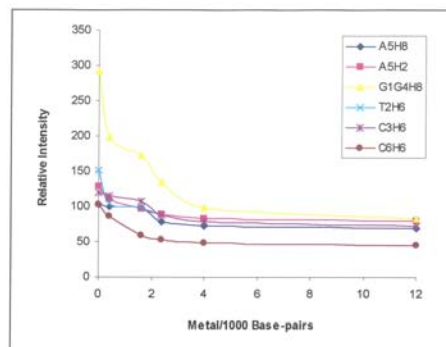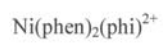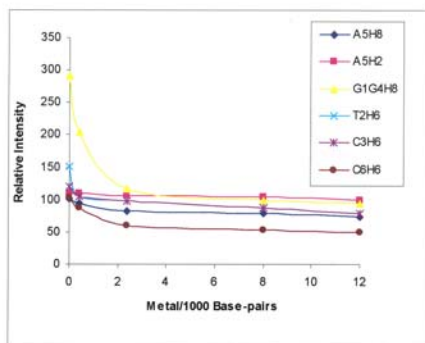
Figure 4.6. Paramagnetic relaxation of the proton resonances of d(GTCGAC)$_2$ with increasing amounts of [Ni(phen)$_2$(phi)]Cl$_2$ and [Ni(phen)$_2$(dpq)]C1$_2$.

in all three cases (Figure 8). 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 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 9 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
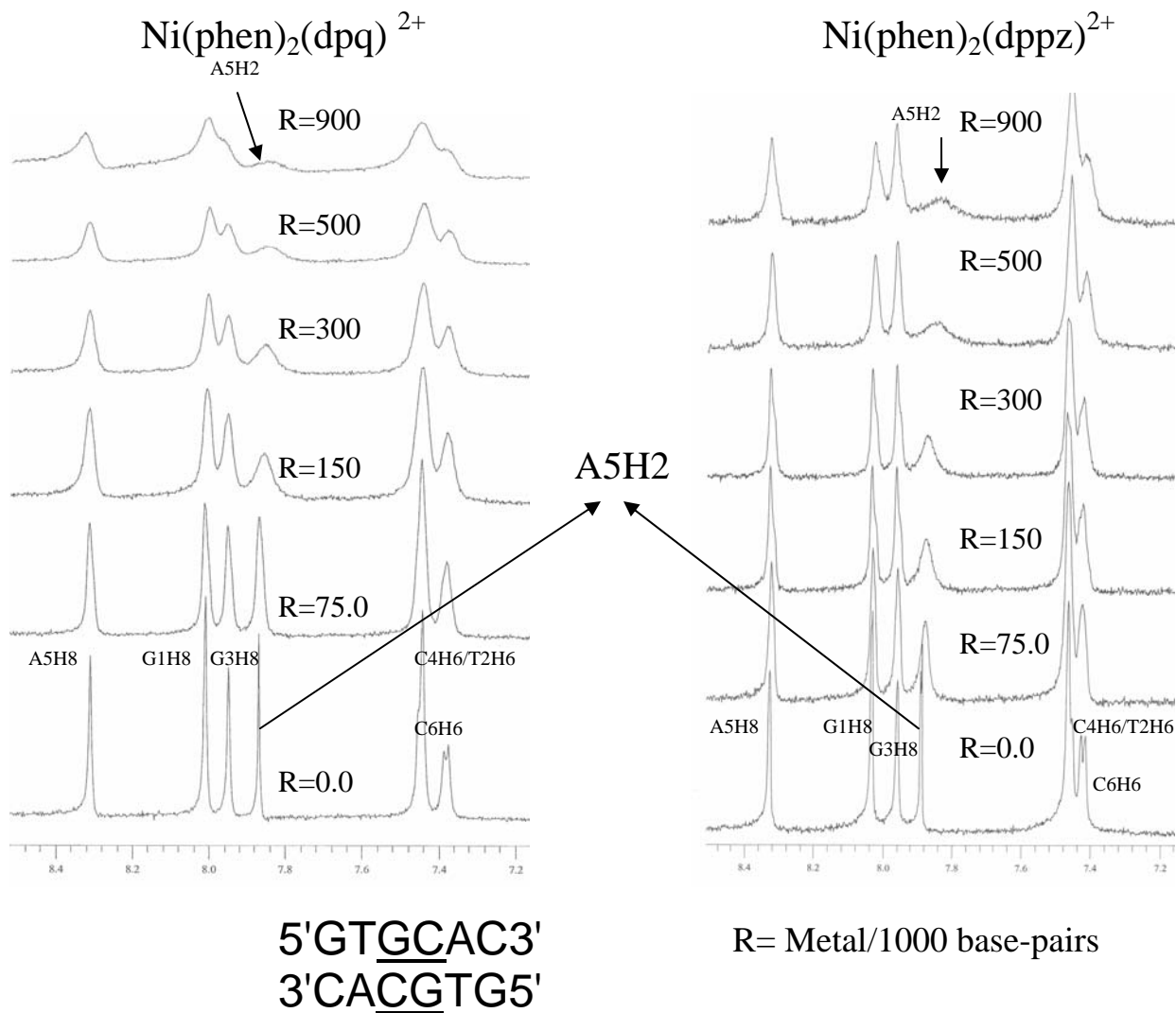
Figure 4.7. Effects of [Ni(phen)$_2$(dpq)]CI$_2$ and [Ni(phen)$_2$(dppz)]Cl$_2$ on the ^{1}H NMR spectrum of d(GT$\underline{GC}$AC)$_2$ in D$_2$O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. The DNA sample contained 0.5 mM duplex, 5mM NaCl, 15 mM sodium phosphate, pD 7.0 in 100% D$_2$O. The 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.

5'GTGCAC3'
3'CACGTG5'

R= Metal/1000 base-pairs
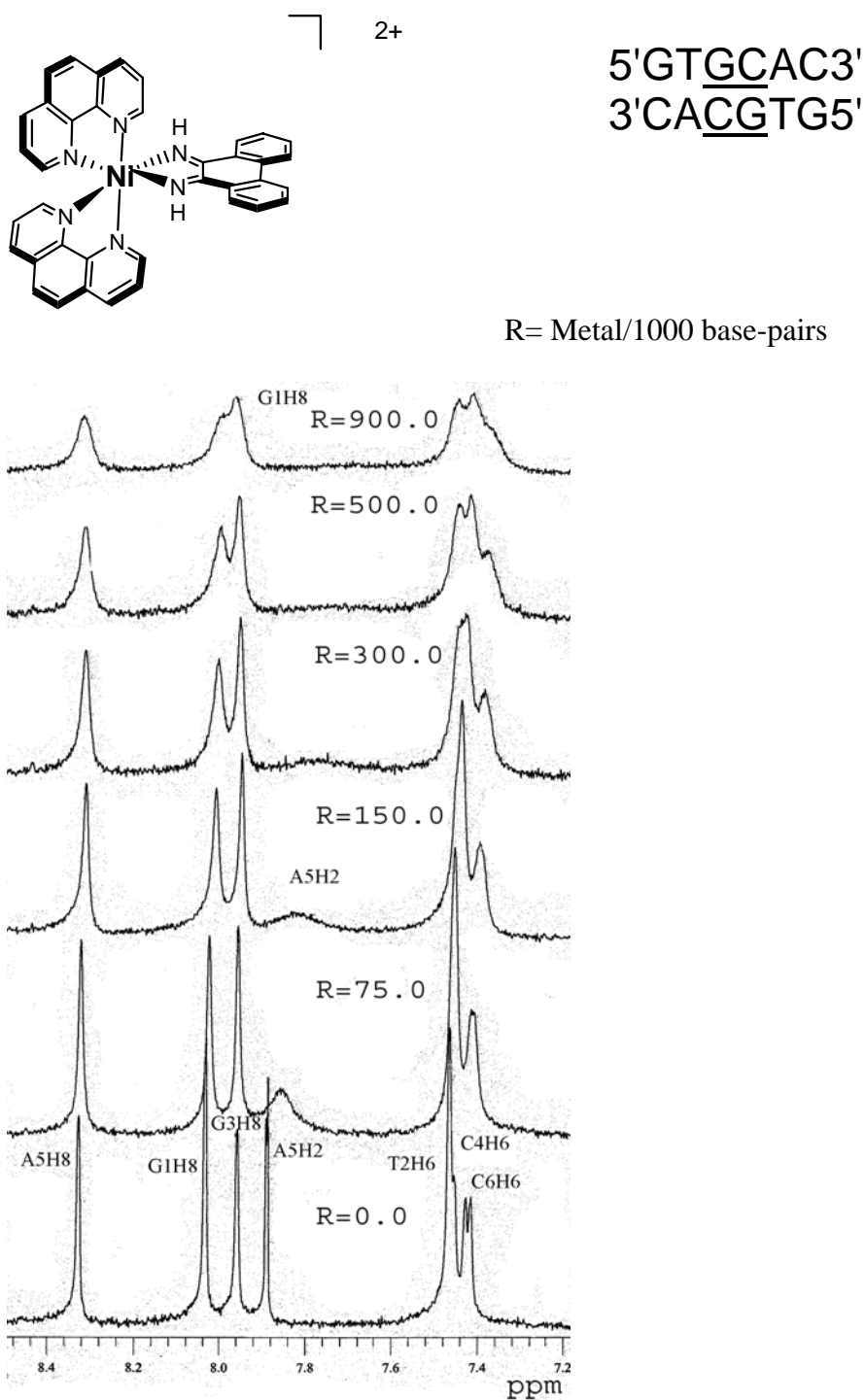
Figure 4.8. Effects of [Ni(phen)$_2$(phi)]Cl$_2$ on the ^{1}H NMR spectrum of d(GTGCAC)$_2$ in D$_2$O at 4 °C on a Varian Unity*PLUS*-600 NMR spectrometer. Shown is the aromatic region. The DNA sample contained 0.5 mM duplex, 5mM NaCl, 15 mM sodium phosphate, pD 7.0 in 100% D$_2$O. The free metal complex sample contained 0.5 mM of [Ni(phen)$_2$(phi)]Cl$_2$ in the same buffer. The chemical shifts are relative to TMSP at 4 °C.

Metal Complex + d(GTCGAC)₂

Metal Complex + d(GT_GC_AC)₂
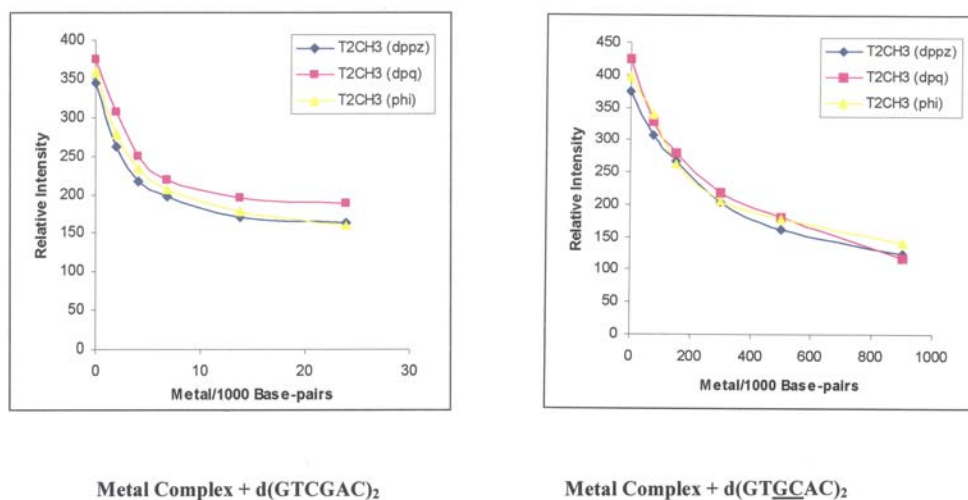
Figure 4.9. Comparative paramagnetic relaxation of the T2CH3 proton resonances of the two oligonucleotides, d(GTCGAC)$_2$ and of d(GT$\underline{GC}$AC)$_2$ in presence of the increasing amounts of [Ni(phen)$_2$(dppz)]Cl$_2$, Ni(phen)$_2$(dpq)]Cl$_2$ and [Ni(phen)$_2$(phi)]Cl$_2$

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 upon the residence time of the complex on the helix, or indirectly, the strength of the binding interaction.

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 set 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 case of d(GTCGAC)$_2$, while that of the minor groove proton A5H2 in case of the duplex d(GT<u>GC</u>AC)$_2$. 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 to favor approach from one groove versus the other depends upon 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 & C4H6) have been observed in the case of d(GT$\underline{GC}$AC)$_2$. 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, by 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. This technique is particularly important in the case of metal complexes that bind non-specifically 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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