Articles

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Metalloporphyrin DNA Interactions: Insights from NMR Studies of Oligodeoxyribonucleotides

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Received December 15, 1988

^{1}H and ^{31}P NMR spectroscopy were employed to investigate the binding to synthetic oligodeoxyribonucleotides (oligomers) of the following metalloporphyrin tetracations: nickel(II) *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin [NiTMpyP(4)]; palladium(II) *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin [PdTMpyP(4)]; zinc(II) *meso*-tetrakis(4-*N*-methylpyridiniumyl)-porphyrin [ZnTMpyP(4)]. Six self-complementary oligomers [5′ → 3′; d(TATATGCGCATATA)$_2$ (I), d(ATGCGCAT)$_2$ (II), d(TGCGCA)$_2$ (III), d(ACACACATGTGTGT)$_2$ (IV), d(AGAGAGATCTCTCT)$_2$ (V), d(TGTGCGCACA)$_2$ (VI)] were studied. Binding of NiTMpyP(4) or PdTMpyP(4) to I, II, or III induced NMR spectral changes characteristic of intercalative interactions, e.g. a sharp downfield ^{31}P NMR signal at ca. −1.0 ppm and an upfield imino ^{1}H NMR signal at ~11.5 ppm. The simplicity of the spectral changes and their similarities to the changes caused by *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin [TMpyP(4)] provide strong evidence that the porphyrin intercalates at the 5′CG3′ site at the C_2 center. Addition of NiTMpyP(4) or PdTMpyP(4) to IV and V produced no detectable new imino signals but caused significant broadening of the original signals and broad downfield ^{31}P signals noted at ca. −1.0 to −2.0 ppm, results consistent with some intercalative binding. Upon addition of NiTMpyP(4) to VI, simple spectra characteristic of intercalation at 5′CG3′ at the C_2 center were observed; therefore, we conclude that NiTMpyP(4) is selective for intercalation at 5′CG3′ sites over AC sites. In every case, addition of ZnTMpyP(4) to the oligomers produced upfield shifts and broadening of the ^{31}P signals *but no new downfield signals*. For II and III, the AT imino ^{1}H NMR signals shifted upfield and lost area, and the GC imino signals broadened considerably. These results, when combined with other studies, suggest that ZnTMpyP(4) binds selectively outside at AT regions. Addition of ZnTMpyP(4) to IV shifted and broadened the ^{1}H NMR imino signals for the interior base pairs, suggesting binding to the AT sequence at the C_2 center of the oligomer. Addition of ZnTMpyP(4) to V caused the GC imino signals to merge into one large GC imino signal. Differences in spectral changes observed on addition of ZnTMpyP(4) to IV and V can be attributed to the lower stability of the central four-base-pair region of IV compared to V. ZnTMpyP(4) binding to VI resulted in spectral changes similar to those seen with I, II, or III, suggesting a preference for the porphyrin to bind at the ends of the oligomer. The zinc porphyrin appears to induce a conformational change upon binding and prefers less stable areas of oligomers, i.e., ends or, in some cases, an AT region embedded in alternating GC and AT base-pair sequences.

Introduction

Many studies have been performed with native and synthetic polymeric DNAs to ascertain the binding mode and sequence selectivity of the tetracationic *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin [TMpyP(4)] species (Figure 1) and its tetracationic metalloderivatives [MTMpyP(4)] with nickel(II) [NiTMpyP(4)], palladium(II) [PdTMpyP(4)], and zinc(II) [ZnTMpyP(4)].[1-16] These studies have revealed that several types of binding occur. Some porphyrin cations are unusual in that they are almost equally capable of binding in an intercalative manner at some sites ("GC" or mixed "GC/AT") and in an outside manner at other sites (usually at AT-rich sites). Other porphyrin species bind outside regardless of base-pair composition.

Several studies have suggested that the intercalative binding mode of TMpyP(4), NiTMpyP(4), and PdTMpyP(4) is primarily observed for GC-rich DNAs.[1,11-14] Therefore, in an attempt to gain greater insight into intercalative porphyrin binding to a polymer, poly(dG-dC)·poly(dG-dC) (poly[d(G-C)$_2$]) was investigated by NMR spectroscopy.[17] Interestingly, addition of TMpyP(4) to poly[d(G-C)$_2$] resulted in a pronounced upfield shift of part of the imino proton NMR signals to ~11.7 ppm and in a new broad downfield ^{31}P NMR signal at ca. −1.0 ppm. Both NMR results[17] and stopped-flow studies[12,14] demonstrate that exchange at intercalation sites is relatively slow. Outside-binding porphyrins (as this study will also demonstrate) undergo rapid exchange,[17] and it is difficult to obtain direct insight into sequence selectivity.

The only possible intercalation sites in poly[d(G-C)$_2$] are 5′GC3′ and 5′CG3′. To determine which site was involved in binding, Marzilli et al.[17] performed a series of experiments on self-com-

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d(1 2 3 4 5 6 7 7 6 5 4 3 2 1)2

(I) T A T A T G C G C A T A T A

(II) A T G C G C A T

(III) T G C G C A

(IV) A C A C A C A T G T G T G T

(V) A G A G A G A T C T C T C T

(VI) T G T G C G C A C A

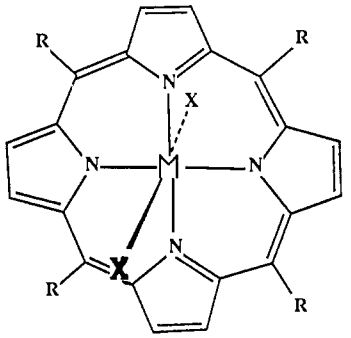

Figure 1. Oligomers and complexes used in the study. The top three oligomers (type 1) all contain the 5'GCGC3' sequence. The next two oligomers (type 2), IV and V, mimic poly[d(AC)(GT)] and poly(d-(AG)(CT)], respectively, and the type 3 oligomer, VI, combines characteristics of types 1 and 2. The general structure of the porphyrins used in this study is at the bottom, where M = Ni(II), Pd(II), or Zn(II) and X = an axial ligand.

plementary oligodeoxyribonucleotide (oligomer) duplexes that had either a 5'GC3' or 5'CG3' site at the C_2 center of the duplex. Addition of TMpyP(4) to d(TATATGCATATA)$_2$ resulted in broadening of the imino signals; no new upfield imino or downfield phosphorus signals were noted. However, on addition of TMpyP(4) to d(TATATGCGCATATA)$_2$ (I, Figure 1) a new ^{31}P signal located at ca. −1.0 ppm was observed. By ^{17}O labeling, this new signal was unambiguously assigned to the phosphate groups at the 5'CpG3' intercalation site at the C_2 center.[17] For d(TATATCGCGATATA)$_2$, where intercalation occurs at the 5'CG3' site off the C_2 center, two ^{31}P signals at ca. −1.0 ppm were observed, as expected, since the phosphate groups are not equivalent. Therefore, it was concluded that TMpyP(4) binds at the 5'CG3' sites of poly[d(G-C)$_2$].

Addition of TMpyP(4) to I resulted in a ~1.5 ppm upfield shift in the imino signal of GC(6), which is next to the CG(7) intercalation site at the C_2 center. This shift was attributed to the large anisotropic effects of the porphyrin ring.[17] At [porphyrin]/[DNA-duplex] (R) = 1.7, not all of the intercalated product was formed, as shown by the imino proton spectra.[17] Therefore, it was concluded that, although TMpyP(4) was highly selective for intercalation at 5'CG3' sites over 5'GC3' sites, competitive outside binding at AT sites prevented the exclusive formation of the intercalated product.[17] This result agrees with our recent finding that the observed equilibrium constant is approximately the same for TMpyP(4) binding to poly(dA-dT)·poly(dA-dT) (poly[d(A-T)$_2$]) and to poly[d(G-C)$_2$].[14]

While the study by Marzilli et al.[17] provided clear NMR evidence for 5'CG3' selectivity of TMpyP(4)–DNA binding to three oligomers, several issues remain unresolved. First, some concern has been expressed as to whether intercalating MTMpyP(4), e.g. NiTMpyP(4) and CuTMpyP(4), bind to 5'CG3' sites as selectively as TMpyP(4).[9,11,13,15] Alternatively, the metal could redirect the porphyrin to a different site, e.g., 5'GC3'. Second, several investigations have suggested that MTMpyP(4) without axial ligands may intercalate into poly(dA-dC)·poly(dG-dT) (poly[d(AC)-(GT)]), which has no 5'CG3' site.[1,9,13] NMR studies are essential for elucidation of the characteristics of this type of intercalation but are prohibitively expensive for poly[d(AC)(GT)]. Third, NMR spectroscopy is one of the best methods for assessing binding, but clear NMR evidence has not been found for intercalation by MTMpyP(4) lacking axial ligands. Fourth, since DNA structure depends on the sequence, there was some concern that the sequences studied were unusual and that the 5'CG3' selectivity observed might not be a general result.[9] Fifth, outside binding may have sequence selectivity, and the influence of sequence on such binding has not been fully evaluated.[15] Finally, a more detailed understanding of porphyrin binding requires the "isolation" in solution or solid of one species; insight into the means to achieve this goal, such as enhancing binding selectivity at one site, will only arise from detailed studies of a variety of porphyrins and sequences.

To address these issues, we carried out an extensive ^{1}H and ^{31}P NMR investigation of NiTMpyP(4), PdTMpyP(4), and ZnTMpyP(4) binding to several self-complementary oligomers both with 5'CG3' sites (in different sequences) and without 5'CG3' sites (Figure 1). To assess sequence and length effects, we examined oligomers consisting of several different sequences and oligomers with the same central sequence with different lengths of flanking AT regions.

Experimental Section

Instrumentation. ^{1}H NMR Spectra. Spectra were obtained on a Nicolet 360NB FT NMR spectrometer (NMC-1280 software) using a modified Redfield 21412 pulse sequence.[18,19] Two sets of 1000 transient free induction decays (FIDs) (20-bit words) were collected per sample to minimize the dynamic range problem. Each set was block-averaged in double-precision mode (40-bit words) and then converted back to 20-bit words. The data were Fourier transformed by using 0.5-Hz line broadening. Typically, the data were collected with 8K data points, a 7042-Hz spectral window, and a carrier frequency at ~15.4 ppm from sodium 3-(trimethylsilyl)propionate-d_4 (TSP).

^{31}P NMR Spectra. Spectra were accumulated with quadrature detection on an IBM WP-200SY (81.01 MHz) NMR spectrometer using a 2000-Hz spectral window, 45° pulse, no time delay between pulses and proton decoupling and collecting approximately 10 000 transient FIDs. Line broadening of 1 Hz was used in the Fourier transformation. Trimethyl phosphate (TMP) was used as the external reference. The spectra were obtained by inserting the ^{1}H NMR sample (5-mm tube) into a 10-mm tube containing PIPES 10 buffer [0.01 M piperazine-*N*,*N*'-bis-(2-ethanesulfonic acid) (PIPES, Sigma), 0.10 M NaCl, 0.001 M ethylenediaminetetraacetic acid (EDTA), and doubly deionized water]. The PIPES buffer was adjusted to pH 7.00 with a NaOH solution and passed through a 0.22-μm filter prior to use. A solution of PIPES buffer was lyophilized and redissolved in 99% D_2O for use in the 10-mm tube.

Materials and Methods. Oligomers. The oligomers were synthesized and purified as previously described.[20] NMR solutions were prepared by lyophilizing an aliquot from oligomer stock solutions of known concentration to make a 0.6-mL sample ~20 mM in DNA bases. The oligomers were then redissolved in PIPES 10 buffer (0.01 M PIPES, 0.10 M NaCl, 0.001 M EDTA) with 10% D_2O. Oligomer–porphyrin complex solutions were prepared by lyophilizing the appropriate amount of porphyrin from an H_2O stock solution and adding the oligomer solution. All NMR experiments were performed at 5 °C and in PIPES 10 buffer. Oligomer concentrations were determined in PIPES 50 buffer (0.01 M PIPES, 0.50 M NaCl, 0.001 M EDTA). The NMR samples were stored at 10 °C and in the absence of light to prevent photodegradation.

Porphyrins. Chloride salts of the porphyrins were obtained from Mid-Century Chemical Co. and used without further purification (Figure 1). Porphyrin concentrations were determined by using the following extinction coefficients: NiTMpyP(4), $\epsilon_{418} = 1.5 \times 10^5$ M^{-1} cm^{-1}; PdTMpyP(4), $\epsilon_{418} = 1.68 \times 10^5$ M^{-1} cm^{-1}; ZnTMpyP(4), $\epsilon_{436} = 2.04 \times$

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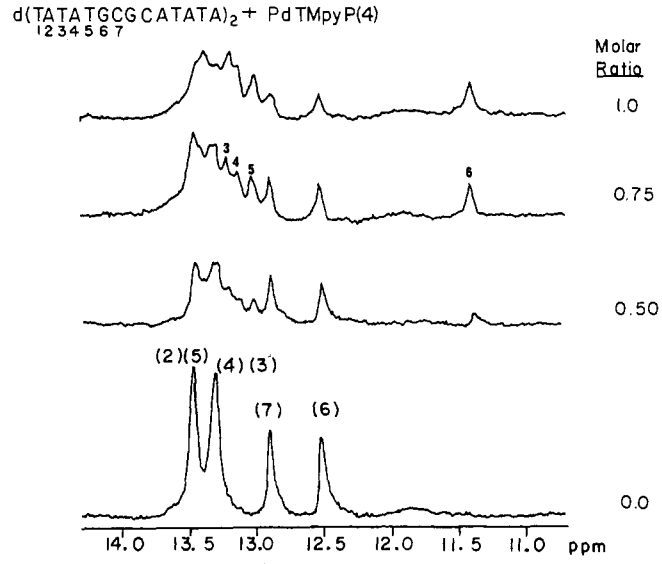

Figure 2. 361-MHz NMR ^{1}H imino proton spectra of d(TATATGCGCATATA)$_2$ (I) (0.7 mM duplex) at 5 °C in PIPES 10 buffer as a function of the ratio of PdTMpyP(4) to duplex. Peak assignments are given for the free oligomer. At R = 0.75, the likely assignments for the bound signals are given.[17]

10^5 M^{-1} cm^{-1}.[13] For simplicity, porphyrin species are abbreviated in this paper without designation of oxidation states, charge, counterions, or axial ligands.

Results

Oligomer Groups. Three groups of self-complementary oligomers, types 1–3, used in this study are shown in Figure 1. The first type contains three oligomers, I–III, which have a central 5′GCGC3′ moiety flanked by a different number of AT base pairs. This type was utilized to examine the effect of oligomer length on porphyrin selectivity and specificity. The second oligomer type, IV and V, which largely contains sequences similar to poly[d(AC)(GT)] and poly(dA-dG)·poly(dC-dT) (poly[d(AG)(CT)]), respectively, was used to examine porphyrin binding to several mixed GC/AT sites in DNA, i.e. 5′AG3′, 5′GA3′, 5′AC3′, and 5′CA3′. Finally, the third oligomer type, VI, allowed examination of the porphyrin binding to a central 5′GCGC3′ moiety flanked by mixed sites. Except for the extent of broadening, the spectra obtained upon adding NiTMpyP(4) or PdTMpyP(4) were similar. Therefore, representative spectra for only one of the porphyrin–oligomer studies for each type are shown below.

The ^{1}H NMR spectra of the oligomers used in this study include the imino signals at ~11–14 ppm relative to TSP. The GC imino protons resonate at ~12.8–12.2 ppm, while the AT imino protons resonate at ~13.9–13.2 ppm. Because of C_2 symmetry, there are at most half as many imino signals as base pairs. Each imino signal is specified by its base pair with the base closest to the 5′-end given first, e.g. CG(7) for the central base pair of I. The ^{31}P spectra of the oligomers used in this study consist of several signals centered at ca. −4.3 ppm relative to TMP. Assignment of each individual signal was not attempted.

Length Effects on Porphyrin Binding. Assignments of the imino proton signals by standard nuclear Overhauser effect (NOE) and/or melting experiments,[17] using the numbering scheme of Figure 1, follow (in ppm). For I: CG(7), 12.9; GC(6), 12.5; TA(5), 13.5; AT(4), 13.4; TA(3), 13.3; AT(2), 13.6. For (II): CG(7), 13.0; GC(6), 12.7; TA(5), 13.7; AT(4), 13.2. The TA(1) and AT(4) imino proton signals of I and II, respectively, are in rapid exchange with H_2O at 5 °C. The imino and phosphorus NMR assignments for III can be found in Wilson et al.[21]

Addition of NiTMpyP(4) or PdTMpyP(4) to a solution of I produced a new imino proton signal at ~11.7 ppm (Figure 2). A similar upfield signal observed upon addition of TMpyP(4) to I has been unambiguously assigned to the bound GC(6) imino proton. Further additions of either NiTMpyP(4) or PdTMpyP(4) increased the relative area of the 11.7 ppm signal.

While similar, the spectral changes for I upon addition of NiTMpyP(4) or PdTMpyP(4) exhibit some differences. For example, NiTMpyP(4) broadened the AT imino signals at lower R and all signals at higher R. In contrast, with PdTMpyP(4), well defined AT signals could be observed up to R = 1.0 (Figure 2).

On the basis of a previous study with I and TMpyP(4), intercalation of the porphyrin at the 5′CG3′ site should produce the following anisotropic[22] upfield shifts in imino signals (in ppm): CG(7), ~4.0; GC(6), ~1.2; TA(5), ~0.5.[17] While the CG(7) signal in the adduct cannot be observed for reasons discussed previously,[17] several other signals are clearly evident, especially at R = 0.75, and are assigned in Figure 2. At R = 1.0, the original CG(7) and GC(6) signals of "free" I can still be seen upon addition of PdTMpyP(4) (Figure 2) or NiTMpyP(4). A similar result was found with TMpyP(4). Therefore, these MTMpyP(4) bind at the 5′CG3′ site but do not exhibit a significantly greater selectivity for binding at 5′CG3′ sites than the parent TMpyP(4). Throughout, we will refer to the free or "unreacted" oligomer. However, these have rapidly exchanging outside-bound porphyrins in solutions containing MTMpyP(4).

Addition of NiTMpyP(4) to II at R = 0.5 produced a broad upfield signal at ~11.5 ppm, similar in shift to that seen with I. However, the AT and GC imino signals at ~12.5–14 ppm were broadened almost to base line. The signals on addition of PdTMpyP(4) to II were sharper. At R = 0.5, the following assignments for the free and bound imino signals (in ppm) can be made on the basis of ring current effects described above: $GC(6)_{bound}$ ~11.5; $GC(6)_{free}$, ~12.7; $AT(4)_{bound}$, ~12.9; $GC(7)_{free}$, ~13.0; $AT(4)_{free}$, ~13.1; $TA(5)_{bound}$, ~13.3; $TA(5)_{free}$, ~13.8. At R = 1.0, only broad signals centered at 13.3 and 11.5 ppm were observed.

The spectrum of III has overlapping CG(7) and GC(6) signals at ~13.2 ppm and a very broad TA(5) signal at ~11.7 ppm. Addition of PdTMpyP(4), as in the cases of I and II, resulted in the formation of a sharp signal located at ~11.7 ppm at R = 0.5. The analogous signal for NiTMpyP(4) solutions at 11.6 ppm was about twice as broad. From previous studies, we expect to observe only this imino signal in the intercalated adducts. In both cases, the GC imino signals of the "unreacted" III were broadened and shifted upfield. The shift was small for PdTMpyP(4) but ~0.5 ppm for NiTMpyP(4). At 5 °C and R = 1.0, all signals were broadened almost completely with NiTMpyP(4). PdTMpyP(4) at R = 1.0 broadened severely the small GC imino signal of unreacted III. The width of the larger GC(6) signal for the adduct was doubled. A slight upfield shift was also noted in both signals. While addition of NiTMpyP(4) and PdTMpyP(4) to II and III did result in new imino and phosphorus NMR signals at ~11.5 and −1.0 ppm, characteristic of intercalation, enhanced selectivity for the 5′CG3′ site in the shorter oligomers was not clearly indicated. For example, it is difficult to see any of the original GC(6) and CG(7) imino signals of II or III upon addition of either NiTMpyP(4) or PdTMpyP(4) at R = 1.0. In fact, all remaining signals of the shorter, less stable oligomers II and III are broadened almost completely to base line and the bound signals are also broad at 5 °C.[23] This phemomenon could mask a greater 5′CG3′ selectivity.

NiTMpyP(4) or PdTMpyP(4) binding to the type I oligomers resulted in a new downfield ^{31}P signal at ca. −1.0 ppm (Figure

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(23) Broadening of the imino spectra appeared to be enhanced in porphyrin–oligomer adducts of sequences containing a 5′ purine II, IV, V. These porphyrins stack better to purines than to pyrimidines. (Pasternack, R. F.; Gibbs, E. J.; Gaudemer, A.; Antebi, A.; Bassner, S.; De Poy, L.; Turner, D. H.; Williams, A.; Laplace, F.; Lansard, M. H.; Merienne, C.; Perrée-Fauvet, M. *J. Am. Chem. Soc.* **1985**, *107*, 8179.) This stacking phenomenon may result in greater aggregation of the oligomer, thus producing loss of signal.

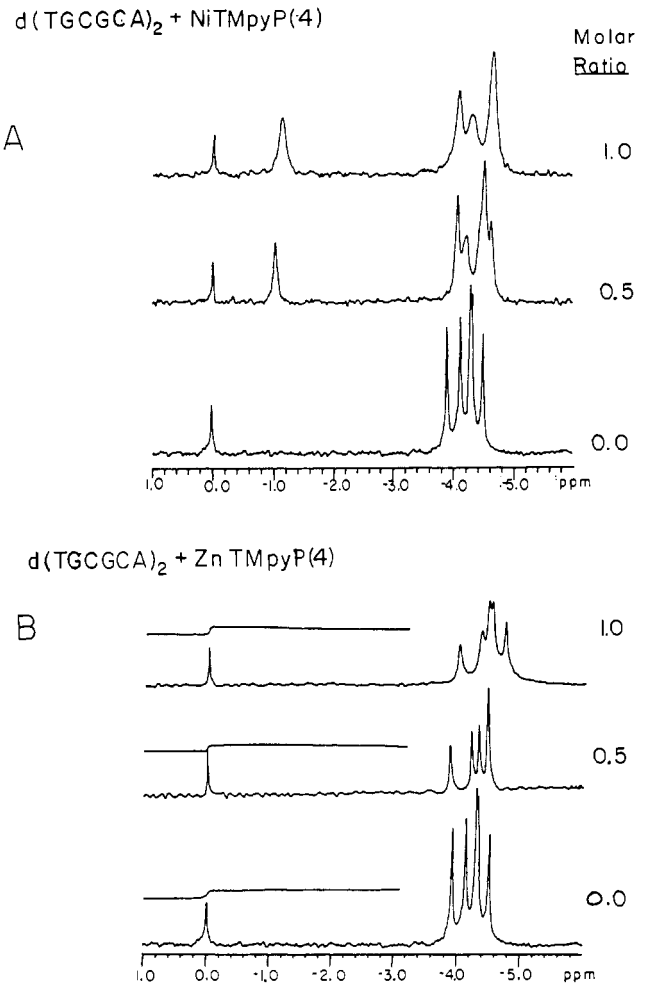

Figure 3. 81.01-MHz NMR ^{31}P spectra of $d(TGCGCA)_2$ (III) (1.67 mM duplex) at 5 °C in PIPES 10 buffer as a function of (A) the ratio of NiTMpyP(4) to duplex and (B) the ratio of ZnTMpyP(4) to duplex. The integration lines inserted above the spectra in (B) illustrate the absence of signal area at ca. −1.0 ppm.

3A). This new signal, which appears exactly at the shift found when TMpyP(4) was added to I, is assigned to the CpG phosphate group at the site of intercalation.[17] However, in all cases, the ^{31}P signals of the type 1 oligomers were broadened more upon the addition of NiTMPyP(4) than PdTMpyP(4). No downfield ^{31}P signal area was observed upon addition of ZnTMpyP(4) to the type 1 oligomers, at $R \leq 1.0$; however, upfield shifts and broadening of the signals were evident (Figure 3B). It is important to note that the downfield ^{31}P signal can be so broad that it is difficult to detect. In cases where the downfield area was not obvious, we found integration necessary to verify either the presence or absence of such signals.

Addition of ZnTMpyP(4) to I at $R = 0.5$ produced no upfield imino signal at 11.5 ppm; however, dramatic broadening and slight downfield shifting of the GC(6) imino signal were observed, while the CG(7) signal was little affected. At $R = 1.0$, all imino signals were broadened; the AT imino signals were clearly the most affected. The GC(6) signal sharpened somewhat, however.

Results similar to those observed in I were noted upon addition of ZnTMpyP(4) to II (Figure 4). For example, at $R = 0.5$, the GC(6) imino signal was shifted upfield very slightly, while the TA(5) signal was broadened and shifted upfield ~0.3 ppm. Also, the AT(4) imino signal was broadened almost completely, while the CG(7) imino signal did not shift. At $R = 1.0$, the CG(7) signal broadened but did not shift, while the other signals were shifted upfield and broadened further. Addition of ZnTMpyP(4) to III at $R = 0.5$ produced upfield shifting and broadening of the GC(6) signal as R increased. The GC(6) signal shifted upfield by 1.1 ppm at $R = 0.5$ and was broadened and shifted an additional 1.0 ppm upfield by $R = 1.0$. The GC(7) signal was shifted upfield

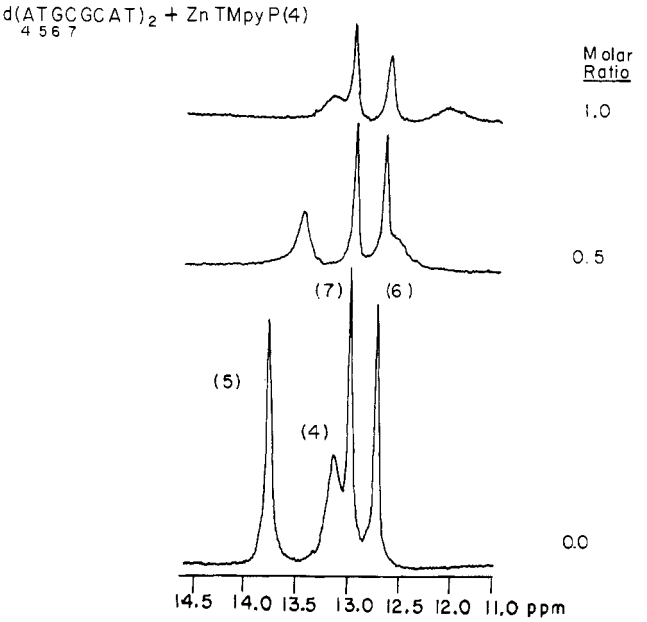

Figure 4. 361-MHz NMR ^1H imino proton spectra of $d(ATGCGCAT)_2$ (II) (1.25 mM duplex) at 5 °C in PIPES 10 buffer as a function of the ratio of ZnTMpyP(4) to duplex. Peak assignments are given for the free oligomer.

by 0.5 ppm at $R = 0.5$ and was broadened and shifted further upfield at $R = 1.0$.

It is important to note that NiTMpyP(4) and PdTMpyP(4) binding to the type 1 oligomers produced an upfield-shifted signal at 11.5 ppm and a downfield ^{31}P NMR signal at ca. −1.0, while addition of ZnTMpyP(4) to I, II, or III did not. In all the cases in this study, NiTMpyP(4) produced more broadening of the imino signals than either PdTMpyP(4) or ZnTMpyP(4).

Porphyrin Binding to Mixed Sites. Assignments of the imino proton signals of type 2 oligomers, based on the numbering scheme of Figure 1, follow (at 5 °C and in ppm). For IV: AT(7), 13.4; CG(6), 12.3; AT(5), 13.5; CG(4), 12.5; AT(3), 13.7; CG(2) 12.7. For V: AT(7), 13.9; GC(6), 12.8; AT(5), 13.7; GC(4), 12.7; AT(3), 13.6; GC(2), 12.5. The AT(1) imino proton of IV and V is in rapid exchange at 5 °C.

NiTMpyP(4) or PdTMpyP(4) binding to the type 2 oligomers, IV and V, did not result in clearly observable new imino proton signals; instead, the imino signal area decreased as R increased. Compared to PdTMpyP(4), NiTMpyP(4) clearly produced more broadening of the imino signals of IV and slightly more broadening of the signals of V; only PdTMpyP(4) data are shown (Figure 5).

Upon addition of the two MTMpyP(4) to IV, a very broad downfield ^{31}P signal area at −1.0 ppm was observed, with the area of this signal increasing as a function of R (Figure 6A). Addition of the two MTMpyP(4) to V resulted in at least two very broad downfield ^{31}P signals between ca. −1.0 and −2.0 ppm, as observed by integration. The area of these signals increased with R (Figure 6B). The ^{31}P signals located at −4.3 ppm broadened as R increased upon addition of NiTMpyP(4) or PdTMpyP(4) to either IV or V. However, as stated previously, the broadening was more pronounced, especially for IV upon addition of NiTMpyP(4) rather than PdTMpyP(4), a relationship also noted above for the imino signals.

Significant changes in the imino signals of IV and V occurred upon addition of ZnTMpyP(4) (Figure 7). In Figure 7A, it can be clearly seen that addition of ZnTMpyP(4) to IV at $R = 0.5$ resulted in broadening and downfield shifting of the CG(6) imino signal. The AT(7) signal shifted downfield and under the AT(5) signal to form one large signal at ~13.5 ppm. At $R = 1.0$, the GC(6) signal shifted under the GC(4) signal. The AT(7) signal shifted and broadened and the AT(5) signal broadened, whereas the AT(3) and GC(2) signals remained relatively sharp and unshifted throughout the titration. While ZnTMpyP(4) binding to V produced significant changes in the imino spectra (Figure 7B),

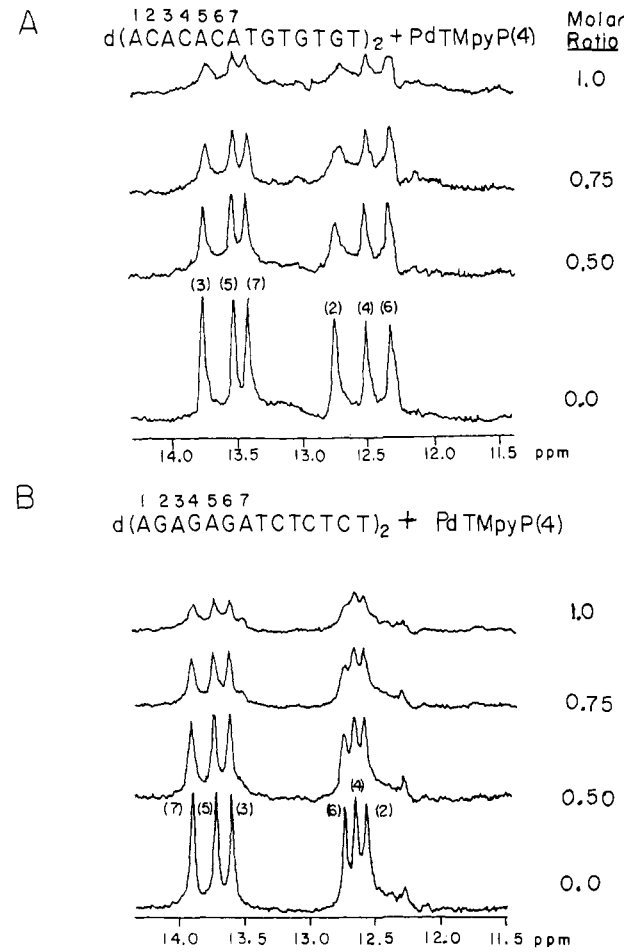

Figure 5. 361-MHz NMR imino proton spectra of (A) d(ACACACATGTGTGT)$_2$ (IV) and (B) d(AGAGAGATCTCTCT)$_2$ (V) (0.7 mM duplex at 5 °C in PIPES 10 buffer) as a function of the ratio of PdTMpyP(4) to duplex. Peak assignments are given for the free oligomers.

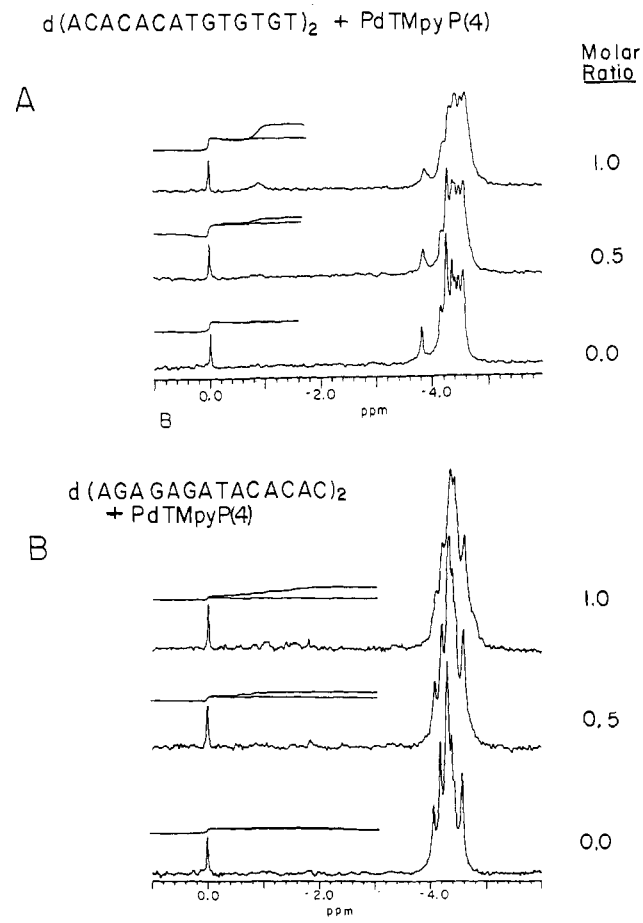

Figure 6. 81.01-MHz NMR ^{31}P spectra of (A) d(ACACACATGTGTGT)$_2$ (IV) and (B) d(AGAGAGATCTCTCT)$_2$ (V) (0.7 mM duplex at 5 °C in PIPES 10 buffer) as a function of the ratio of PdTMpyP(4) to duplex. The integration line, inserted above each spectrum, has a line drawn level with the TMP integral to aid in seeing the absence or presence of signal area at ca. −1.0 ppm.

the interaction with this oligomer appears to be quite different from that with IV. As R increased to 1.0, the GC imino signals of V shifted to form one large signal located at ~12.6 ppm (Figure 7B). No significant shifts were observed in the AT signals, although there was some loss of signal intensity. Thus, IV again seems to be more affected by porphyrin binding than V.

No downfield ^{31}P signal area was observed upon ZnTMpyP(4) binding to either IV or V, regardless of R; however, as R increased, some upfield-shifted ^{31}P signals were observed (data not shown). The effects were similar for both oligomers.

AC and GT Flanking Sequences. The third type of oligomer, VI, has the 5′CG3′ intercalation site at the C_2 center, as in the type 1 oligomers. However, it has flanking GC and AT base pairs instead of exclusively AT base pairs. Assignments of the imino signals of VI at 5 °C are as follows: CG(7), 13.0; GC(6), 12.8; TA(5), 13.7; GC(4), 12.6. The TA(3) imino proton signal is broad and shifted upfield to 12.1 ppm due to exchange with H_2O (Figure 8).

NiTMpyP(4) binding to VI at R = 0.5 resulted in the appearance of at least two new imino signals. The new signals at 11.5 and 13.2 ppm are consistent with 5′CG3′ intercalation and are assigned to GC(6)$_{bound}$ and AT(5)$_{bound}$, on the basis of the magnitude of ring current effects of the porphyrin (Figure 8).[17] Broadening of the new imino signals was observed as R increased. The signals of "unreacted" oligomer were greatly diminished in size, shifted slightly upfield at R = 0.5, and were not observed at R = 1.0. The ^{31}P spectra exhibited a new sharp signal at ca. −1.0 ppm (Figure 9) that increased in signal area as a function of R.

Addition of ZnTMpyP(4) to VI resulted in broadening of all imino signals; however, GC(4) was shifted upfield (~0.5 ppm) and broadened most significantly as R increased (Figure 10). Smaller shifts were seen in the TA(5) imino proton signal. Alternatively, no downfield signal was observed in the ^{31}P spectra, regardless of R, although the original ^{31}P signals did broaden and shift upfield as R increased (Figure 9).

Discussion

The information obtained by NMR spectroscopy on porphyrins binding to DNA has evolved through many stages. Initially, studies were performed on native DNAs.[16] However, due to the complexity of the system, i.e., 10 different types of dinucleotide binding sequences available and intercalative and/or outside binding modes possible, little information about single-site interactions or the mode(s) of binding could be obtained. For example, addition of TMpyP(4), NiTMpyP(4), or PdTMpyP(4) to salmon sperm DNA (SS DNA) resulted typically in an 80–90% loss in the imino NMR signals by [porphyrin]/[DNA bases] = 0.10.[1,13] In comparison, addition of ZnTMpyP(4) caused relatively little broadening of the DNA imino NMR signals. The ^{31}P NMR spectra revealed a broadening of the phosphorus signals upon addition of TMpyP(4), NiTMpyP(4), PdTMpyP(4), or ZnTMpyP(4). These results suggest multiple types of DNA–porphyrin interactions and/or aggregation at higher R values.

Although studies on polymers suggest that DNA binding of TMpyP(4) is similar to that exhibited by NiTMpyP(4) or PdTMpyP(4), i.e., intercalative binding at GC regions and outside binding at AT regions on the DNA, some differences do exist. For example, addition of poly[d(A-T)$_2$] to a NiTMpyP(4) solution induces a conservative CD spectrum in the Soret region, whereas addition of poly[d(A-T)$_2$] to a TMpyP(4) solution induces positive ellipticity.[11] Also, NiTMpyP(4) and PdTMpyP(4) have been shown to produce greater increases in the solution-reduced viscosity

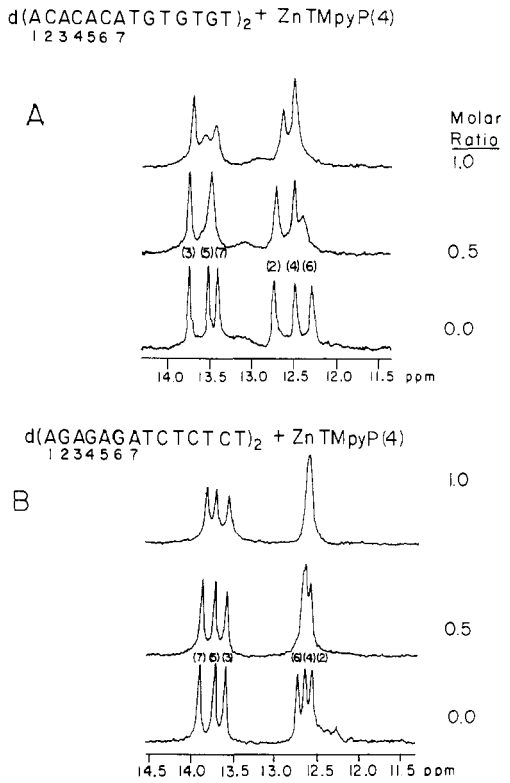

Figure 7. 361-MHz NMR imino proton spectra of 0.7 mM duplex at 5 °C in PIPES 10 buffer as a function of (A) the ratio of ZnTMpyP(4) to d(ACACACATGTGTGT)$_2$ (IV) and (B) the ratio of ZnTMpyP(4) to d(AGAGAGATCTCTCT)$_2$ (V). Peak assignments are given for the free oligomer.

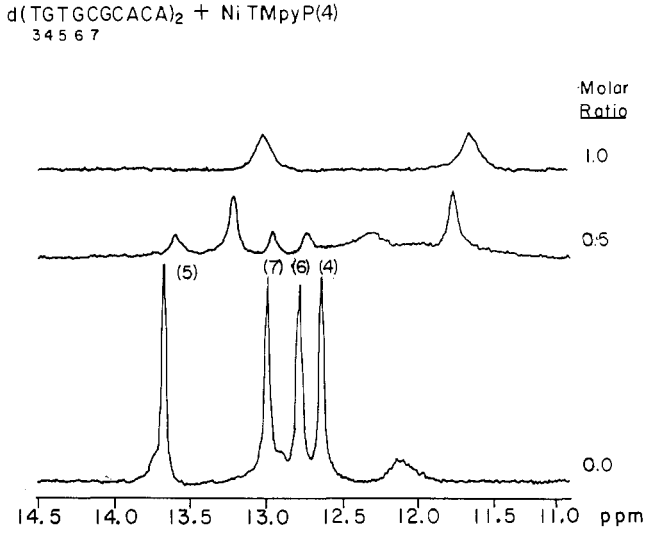

Figure 8. 361-MHz NMR imino proton spectra of d(TGTGCGCACA)$_2$ (VI) (1.0 mM duplex) at 5 °C in PIPES 10 buffer as a function of the ratio of NiTMpyP(4) to duplex. Peak assignments are given for the free oligomer.

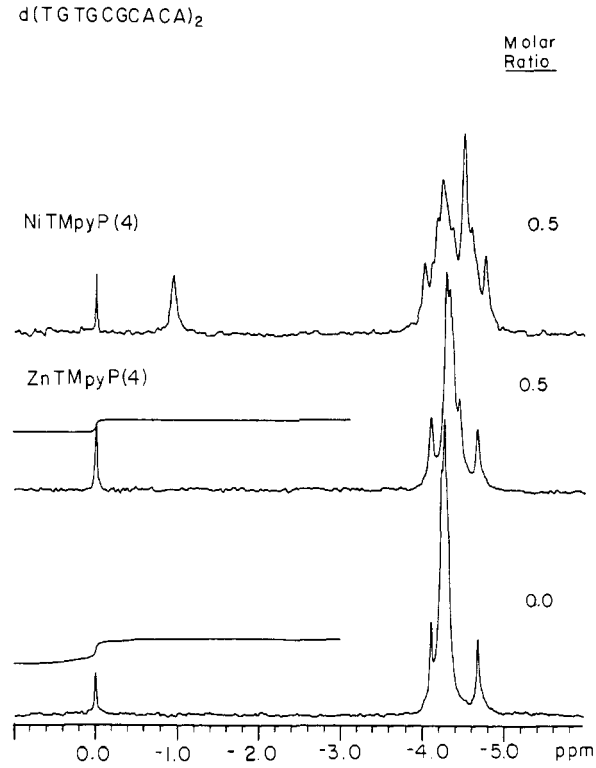

Figure 9. 81.01-MHz NMR ^{31}P spectra of d(TGTGCGCACA)$_2$ (VI) (1.0 mM duplex) at 5 °C in PIPES 10 buffer as a function of the R = 0.5 ratios of ZnTMpyP(4) and NiTMpyP(4) to duplex. The integration lines are inserted above the bottom two spectra.

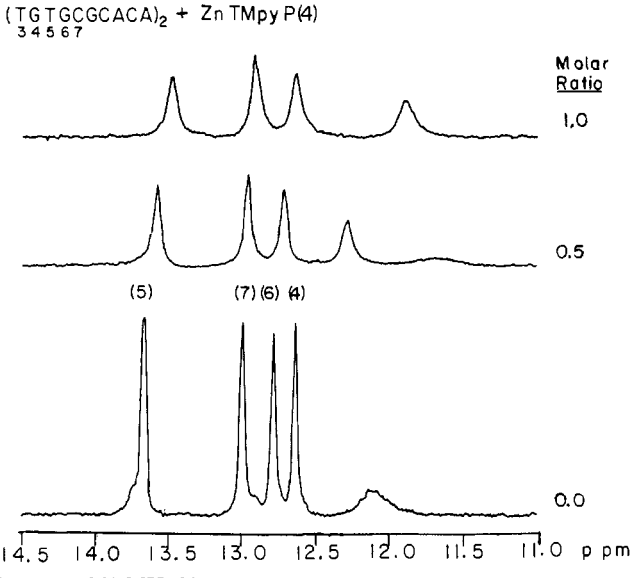

Figure 10. 361-MHz NMR imino proton spectra d(TGTGCGCACA)$_2$ (VI) (1.0 mM duplex) at 5 °C in PIPES 10 buffer as a function of the ratio of ZnTMpyP(4) to duplex. Peak assignments are given for the free oligomer.

(SRV) of calf thymus DNA (CT DNA) than TMpyP(4).[1,13] Clearly, the metal has an effect on the binding properties of the porphyrin.

For reasons enumerated in the Introduction, NMR experiments were performed to examine MTMpyP(4) binding to the type 1 oligomers (Figures 2–4). NiTMpyP(4) and PdTMpyP(4) produced spectral changes similar to those reported for TMpyP(4),[17] viz., a new imino and phosphorus NMR signal at ~11.5 and −1.0 ppm, respectively (Figures 2 and 3). Therefore, as with TMpyP(4), both NiTMpyP(4) and PdTMpyP(4) *do exhibit a selectivity for intercalation at 5'CG3' sites.* ZnTMpyP(4) exhibits different NMR behavior and will be discussed separately with each oligomer group.

Although these types of porphyrins have high binding constants to DNA,[14] for both NiTMpyP(4) and PdTMpyP(4) at R = 1.0, CG(7) and GC(6) imino signals of free I are still observable (Figure 2). Therefore, simultaneous outside binding of NiTMpyP(4) and PdTMpyP(4) must prevent the exclusive formation of a 1:1 complex. Comparison of the relative intensity of the GC(6) signal for unreacted I with the upfield GC(6) signal of the intercalation complex reveals, at best, ~20% greater selectivity for the PdTMpyP(4) over TMpyP(4). For NiTMpyP(4), enhanced selectivity is also not evident, although broadening makes the data less clear. These findings are in contrast with the results of footprinting studies,[15] where TMpyP(4) was found to bind less selectively than NiTMpyP(4) or CuTMpyP(4).

The d^8 NiTMpyP(4) species, which we find typically induces the greatest amount of broadening, is paramagnetic in its normal diaqua octahedral form.[24] Blom et al.[25] have observed resonance Raman bands characteristic of the four- and six-coordinate NiTMpyP(4) species and have reported that the bands for the four-coordinate NiTMpyP(4) species increase when interacting with CT DNA or with a CpG dinucleotide. The intercalating, square-planar NiTMpyP(4) species is diamagnetic and is not so effective as the outside-binding octahedral paramagnetic NiTMpyP(4) species in broadening NMR signals. Studies with I and with other oligomers discussed below reveal changes in shifts, changes in intensity, and broadening of the imino signals as the porphyrin concentration is increased. These phenomena can have several origins (solvent exchange, destacking, paramagnetic effects, conformational changes, binding site exchange, increases in correlation time, etc.).[26] Regardless of their origin, these spectral changes are quite revealing concerning the sequences most affected by porphyrin binding and provide insight into the binding selectivity of the porphyrins.

NiTMpyP(4) typically dissociates more slowly than ZnTMpyP(4) and binds both at AT and at GC sites.[11-14] In contrast to the 5'CG3' selectivity of NiTMpyP(4) and PdTMpyP(4), ZnTMpyP(4) binds selectively at AT regions in DNA as an external complex. As discussed above, PdTMpyP(4) and NiTMpyP(4) also form external complexes, but intercalative binding can also occur. With ZnTMpyP(4), the external binding is the only significant interaction with DNA.

Our results with the type 1 oligomers provide specific information on the differences between ZnTMpyP(4) and NiTMpyP(4) or PdTMpyP(4) interactions with DNA. The absence of new imino or ^{31}P signals at ~11.5 or −1.0 ppm, respectively, upon addition of ZnTMpyP(4) to either I, II, or III shows clearly that intercalation does not occur (Figures 3 and 4).

Outside binding by cations typically induces small upfield shifts in ^{31}P signals. Such shifts were observed here for the ZnTMpyP(4)–oligomer complexes (Figure 3). The imino proton signals also strongly indicated an external binding mode for ZnTMpyP(4) (Figure 4). For example, GC(6) is adjacent to the AT region and its imino signal is affected much more than CG(7), which is in the more stable central portion of the oligomer. The 5'GCGC3' sequence is the most stable sequence, in any case. The broadening of the GC(6) signal in I at $R = 0.5$ followed by sharpening at $R = 1.0$ can be attributed to exchange of the outside-bound ZnTMpyP(4). At $R = 1.0$, the oligomer should have about one porphyrin per duplex bound. For the shorter oligomers, II and III, the imino signal of GC(6) (toward the less stable ends of the duplex) was also affected much more than the CG(7) signal. For the two shorter type 1 oligomers, there was considerable upfield shifting of the AT imino proton signals. In contrast, although the AT signals of I were also affected, these were shifted slightly downfield. Thus, it is apparent that ZnTMpyP(4) prefers to bind to the weaker AT regions. With macromolecular DNA polymers, ZnTMpyP(4) binding to poly[d(A-T)$_2$] is approximately 200 times greater than that for poly[d(G-C)$_2$] (in 0.065 M Na^+ at 25 °C), and the AT interaction induces a cooperative conformational change in the DNA structure.[14]

In the following discussion, it is instructive to compare factors (base-pair composition, base sequence, oligomer length) leading to weaker total stacking energy (the total stabilizing energy of base-paired dimers arising from horizontal base pairing and vertical base stacking components)[27] with the loss of imino signal intensity and/or signal shifting. One significant suggestion for the preference of outside-binding porphyrins for AT vs GC regions is that the AT regions are more easily distorted, leading to better fits and more favorable interactions of the bulky porphyrin molecules.[11,12,15]

In a previous report, we found evidence for intercalative binding of NiTMpyP(4) and PdTMpyP(4) to poly[d(AC)(GT)];[13] however, results with this polymer are not so clear as those observed with poly[d(G-C)$_2$]. The binding of NiTMpyP(4) and PdTMpyP(4) to the type 2 oligomers is clearly different from that observed with the type 1 oligomers. For example, no significant upfield-shifted imino proton signal area at ~11.5 ppm or sharp downfield ^{31}P signal at ca. −1.0 ppm was observed with either IV or V upon addition of NiTMpyP(4) or PdTMpyP(4) (Figures 5 and 6). However, a broad downfield ^{31}P signal at ca. −1.0 to −2.0 ppm, which was observable only by integration at low R, was evident at higher R values. The broadness may be the result of partial intercalation and/or intercalation at several sites. Outside binding at multiple sites, as suggested by the upfield shifting of the ^{31}P signals, could also broaden the signals.

NiTMpyP(4) dissociates from poly[d(AC)(GT)] ~50 times faster than from poly[d(G-C)$_2$].[14,28] Our NMR data show that the imino signals of IV and V broaden and no new signals are observed upon addition of NiTMpyP(4) or PdTMpyP(4). Therefore, porphyrin exchange between sites appears to be faster for mixed sites than for 5'CG3' sites, thus complicating the interpretation of NMR data obtained with mixed-site oligomers. Nevertheless, the ^{31}P NMR data and published results with polymers[13] suggest that some form of intercalative binding probably occurs when NiTMpyP(4) or PdTMpyP(4) interact with the type 2 oligomers.

Since the stacking energies[27] (kcal mol^{-1}) of 5'AC3' (−10.5) and 5'CA3' (−6.6) sum to approximately the same value as the stacking energies of 5'AG3' (−6.8) and 5'GA3' (−9.8), one might expect little difference between the binding to IV and V. However, for NiTMpyP(4) and PdTMpyP(4) (Figure 5), the imino signals of the ends of the duplex appear to be more affected in IV but those in the center are most influenced in V. The central 5'GATC3' sequence of V is more stable than the 5'CATG3' sequence of IV by over 6 kcal mol^{-1}, since both oligomers have a 5'AT3' site (−6.6 kcal mol^{-1} stacking energy) at the center. Thus, NiTMpyP(4) and PdTMpyP(4) initially influence the signals for the most stable region of the duplex for both the type 1 and type 2 oligomers. However, for type 2 duplexes, differences due to stacking are not so marked as for type 1 duplexes. In contrast, our data with the ZnTMpyP(4)–IV adduct reveal shifting of the imino signals at and next to the weakly stacked AT sequence at the C_2 center of IV (Figure 7A). It is quite interesting that the total stacking energy of the 5'AC3' site at the end of IV is ~4 kcal mol^{-1} more stable than the 5'AG3' site at the end of V.[27] For V, the effects of ZnTMpyP(4) appear to be more uniform. As in the case of the type 1 oligomers, ZnTMpyP(4) binds preferentially at AT regions of the oligomer whether they are at the center or at the end of the duplex.

The type 3 oligomer was designed to examine competitive binding between different types of sites. If 5'CG3' sites are more favored than mixed sites, one would expect an imino ^1H NMR spectrum upon addition of NiTMpyP(4) similar to that observed with the type 1 oligomers, i.e., a new imino signal at ~11.5 ppm for GC(6) and a downfield ^{31}P signal at ca. −1.0 ppm. However, if mixed-site binding off the C_2 axis is preferred, addition of NiTMpyP(4) would be expected to produce small spectral shifts, broadening, and complexity of the imino signals, as well as a very broad ^{31}P signal at ca. −1.0 ppm. We observed spectral changes (Figures 8 and 9) similar to those seen with the type 1 oligomers (Figures 2 and 3). Therefore, we conclude that intercalative binding at 5'CG3' sites is favored over intercalation at mixed sites, although some undetectable binding at these sites is not precluded.

The GC(6) and AT(5) imino signals of the NiTMpyP(4)–VI adduct were not as broad as that of the AT(4) signal of the adduct or as the signals of the free VI. These results suggest that intercalation of the tetracationic porphyrin at the 5'CG3' site may

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prevent outside binding of a paramagnetic NiTMpyP(4) cation within several base pairs of the intercalation site.

The interaction of ZnTMpyP(4) with the type 3 oligomer exhibited NMR spectral properties suggestive of outside binding in that no NMR signals were observed at ~11.5 and −1.0 ppm, in the ^1H and ^{31}P spectra, respectively. However, the upfield shifts of the imino signals for base pairs located on the ends of VI suggest that ZnTMpyP(4), an outside binder, will bind preferentially to the AT-containing mixed sites over the more stable central GC sites. As in the other cases where a trend is evident, ZnTMpyP(4) prefers to bind to the weakest local region of the oligomer.

It is clear from the results presented here that PdTMpyP(4) and NiTMpyP(4) bind to DNA both by internal and external interactions. ZnTMpyP(4) is a purely outside-binding molecule and has high AT specificity reminiscent of the class of minor groove-binding molecules exemplified by netropsin,[29] distamycin,[29] and Hoechst 33258.[29,30,31] ZnTMpyP(4) is not structurally similar to these classical minor groove-binding agents, but footprinting results indicate that it does bind in the minor groove.[15] We will consider the factors involved in the minor groove AT-specific binding of known molecules and evaluate each factor in terms of its possible relevance to ZnTMpyP(4) binding.

The following factors have been identified as important in the interaction of groove-binding molecules with the minor groove of AT regions of DNA: (i) There is hydrogen bonding to the thymine carbonyl and adenine N(3). (ii) As Dickerson and co-workers[32,33] have pointed out, the minor groove is deeper and narrower in AT regions and forms an optimum binding site for several small unfused aromatic systems like netropsin. (iii) As Pullman and co-workers[34] have shown, the negative electrostatic potential is greater in AT regions of the minor groove, and species such as netropsin should be selectively attracted.[29] As indicated above, in analyzing the AT selective interaction of ZnTMpyP(4), we feel that the stacking energies must also be considered because of induced conformational changes.

Hydrogen bonding of TMpyP(4) derivatives is not possible through the porphyrin ring system. Hydrogen bonding to the base pairs through axially bound water ligands could make some contribution to the binding. Analysis of CPK models suggests that it is difficult to fit TMpyP(4) into the narrow minor groove in AT-rich regions of DNA and that the axial water is too remote from the DNA to participate in H-bonding. We have shown, however, that binding of ZnTMpyP(4) to poly[d(A-T)$_2$] is cooperative,[14] suggesting that the porphyrin induces an allosteric transition in the DNA that may widen the grooves and create an optimum binding site. Binding of the porphyrins in the major groove is also possible but would not require the allosteric transition. The electrostatic potential would also serve as a minor groove director for the cationic porphyrins. Compared to the minor groove of AT regions, the minor groove of GC regions is wider and has a less negative electrostatic potential, and the guanine NH_2 group sterically restricts deep penetration of groove-binding molecules.[29]

Our imino ^1H and ^{31}P NMR studies clearly indicate that ZnTMpyP(4) binds in the most AT-rich regions of DNA oligomers and induces a conformation change in the DNA structure. The results of ZnTMpyP(4) binding to IV and V are particularly instructive. Imino proton chemical shifts induced by ZnTMpyP(4) binding are largest at the center of IV but are largest at the ends of V. This correlates well with the weaker stacking energies of the 5′CATG3′ sequence in IV relative to the 5′GATC3′ sequence in V. This binding difference could also be influenced by the repeating purine and pyrimidine sequences of V relative to the alternating purine–pyrimidine sequence of IV and the other oligomers in Figure 1. However, in general, it appears that conformational flexibility of the weakly stacked regions could facilitate minor groove binding of the bulky porphyrin cations. Recently, Dabrowiak and co-workers[35] studied Mn(III) complexes of TMpyP(4) and a related porphyrin with a larger chain replacing all four methyl groups. Their results suggest these octahedral species bind in an end-on manner via the minor groove in a melted or partially melted region of a 139-base-pair restriction fragment. Such a binding mode is also consistent with our results. Additional oligomer sequences must be investigated to define in more detail the external binding of ZnTMpyP(4) with DNA. The ability to focus only on the external interaction when ZnTMpyP(4) is used is a significant advantage in investigations employing this metalloporphyrin.

Summary. With the exception of 5′AA3′ and 5′GG3′ sites, all possible combinations of two base-pair binding sites in DNA have been investigated in this study. NiTMpyP(4) and PdTMpyP(4) are very similar in their interactions with identical oligomers. The binding of NiTMpyP(4) and PdTMpyP(4) to the type 1 oligomers appears to be identical with that reported for TMpyP(4), i.e. selective intercalative binding at 5′CG3′ sites.[17] While partial or full intercalation at other sites is not precluded, our data unequivocally show that, regardless of flanking sequences or oligomer length, NiTMpyP(4) and PdTMpyP(4) prefer to bind selectively at 5′CG3′ sites.[36] However, binding selectivity at 5′CG3′ sites is not greatly enhanced relative to outside interactions at AT sequences either by shortening the flanking sequence length around the 5′CG3′ site or by changing the sequence of the flanking base pairs. NiTMpyP(4) and PdTMpyP(4) binding to mixed AT/GC sequences is more complex and, therefore, less clear. It appears that these porphyrins can intercalate, but they redistribute rapidly on the NMR time scale. Intercalative binding appears to be less favorable for these mixed sites than for 5′CG3′ sites. ZnTMpyP(4) does not intercalate, preferring to bind outside at AT regions of the oligomer. Our NMR results, when combined with previous footprinting[15] and binding analyses,[14] suggest that ZnTMpyP(4) probably binds in the minor groove and causes a structural distortion of the double helix at the binding site.

Acknowledgment. This work was supported by NIH Grant GM 29222 to L.G.M. and NSF Grant DBM 8603566 to W.D.W. The 360-MHz NMR spectrometer was purchased with partial support from the NSF through a departmental grant to Emory University.

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(36) A reviewer was concerned that the one sharp ^{31}P NMR signal located at −1.0 ppm we observed in several cases could be due to binding at an adjacent site. Such an explanation ignores the C_2 symmetry of the oligomer duplex and would require no downfield shift at the binding site. The intercalation of actinomycin D into type 1 oligomers is at the 5′GC3′ site and leads to *three* downfield-shifted ^{31}P NMR signals per adduct.[37] The two most downfield shifted signals are for phosphate groups at the intercalation site. Because this site is off the C_2 center, the two phosphate groups are inequivalent. We feel that the one downfield ^{31}P NMR signal in the multiple type 1 cases we have now studied cannot be explained in any other way than by binding at the 5′CG3′ site located at the C_2 center. Our ^1H NMR results are also totally consistent with binding at this site. The reader is referred to ref 17 for other sequences consistent with TMpyP(4) binding at 5′CG3′ sites.
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