DNA–Porphyrin Adducts. Five-Coordination of DNA-Bound VOTMpyP(4) in an Aqueous Environment: New Perspectives on the V=O Stretching Frequency and DNA Intercalation

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VOTMpyP(4) (vanadyl *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin) exists as five-coordinate (**5C**) and six-coordinate (**6C**) forms in nonaqueous solvents with V=O stretching bands at 996–1005 and 957–977 cm^{-1}, respectively. The V=O bands provided two types of information about adducts formed between VOTMpyP(4) and biopolymers, particularly nucleic acids, in aqueous solution. First, the appearance of two V=O bands (at 989–993 and 956–964 cm^{-1}) attributable to the **5C** and **6C** forms, respectively, allowed an assessment of the amount of each form. Second, the frequency dependence of the forms allowed us to conclude that the solvent environment around polymers such as DNA has an acceptor number similar to that of water and that both the **5C** and **6C** forms were bound. These results provide the first direct observation of **5C** VOTMpyP(4) in an aqueous environment and the first strong evidence that a **6C** form of a coordinatively fluxional metalloporphyrin can bind to DNA. The V=O stretching frequency of ~996 cm^{-1} estimated from acetonitrile/water and dimethylformamide/water mixtures for **5C** VOTMpyP(4) is rather different from the 970 cm^{-1} previously predicted by extrapolation of data in methanol/water mixtures. However, the value of 989–993 cm^{-1} found for the DNA-bound species was similar to the 996-cm^{-1} value found here. The DNA binding interactions were also probed by visible spectroscopy in the Soret region and by CD spectroscopy of porphyrin and DNA bands. These methods were used to compare the DNA binding of VOTMpyP(4) with the binding of porphyrins with known, but diverse, binding properties, namely TMpyP(4), NiTMpyP(4), and ZnTMpyP(4). These studies clearly showed that VOTMpyP(4) and ZnTMpyP(4) bind in a similar manner. ZnTMpyP(4) is an established outside binder which does not increase DNA viscosity and which selectively binds to AT over GC regions. Likewise, VOTMpyP(4) demonstrated no increase in DNA viscosity and an AT preference. In particular, the visible absorption and CD spectra of VOTMpyP(4) in the presence of 1:1 mixtures of poly(dA–dT)·poly(dA–dT) ([poly(dAdT)]$_2$) and poly(dG–dC)·poly(dG–dC) ([poly(dGdC)]$_2$) are nearly identical to those with [poly(dAdT)]$_2$ alone and very different from those of [poly(dGdC)]$_2$ alone. Thus, VOTMpyP(4) is not an intercalator, and there is no evidence to suggest that it is a partial intercalator. The species is thus a useful paramagnetic probe selective for AT regions of DNA.

Introduction

Interactions of the water-soluble, cationic porphyrins and their metal derivatives with nucleic acids have recently been studied extensively.[1-8] This interest arises mainly from the possible medical applications of porphyrins and their metal derivatives.[1] First, porphyrin–nucleic acid interactions can be used as models for anti-tumor drug–nucleic acid interactions.[1,9] Second, some porphyrins can inhibit the AIDS virus, HIV-1.[10,11] Third, many porphyrins are photosensitive and can be used in photodynamic therapy of tumors.[12-15] At least three different binding modes of porphyrins (or their metal derivatives) to nucleic acids have been proposed: intercalation, outside binding, and outside self-stacking.[1] In particular, it is believed that *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin (Figure 1), TMpyP(4), intercalates into GC-rich regions and binds on the outside at AT-rich regions. Moreover, metal derivatives of TMpyP(4) without axial ligands, such as Ni(II), Cu(II), or Au(III), can also intercalate at GC-rich regions.[16,17]

Resonance Raman, NMR, and optical spectroscopy have been used in our laboratories to study nickel porphyrins, especially NiTMpyP(4) and NiTMpyP(2) [nickel *meso*-tetrakis(2-*N*-methylpyridiniumyl)porphyrin].[1-3,18-21] In those studies, we investigated the binding of nickel porphyrins with calf thymus DNA (CT DNA) and several synthetic nucleic acid polymers: poly(dA–dT)·poly(dA–dT) ([poly(dAdT)]$_2$), poly(dG–dC)·poly(dG–dC) ([poly(dGdC)]$_2$), and poly(U), as well as other anionic polymers. Raman spectra of the nickel porphyrins investigated have core-size-sensitive bands characteristic of four-coordinate (**4C**) species and corresponding bands for the diaqua six-coordinate (**6C**) species (Scheme I). In aqueous solution, the ratio of **4C** to

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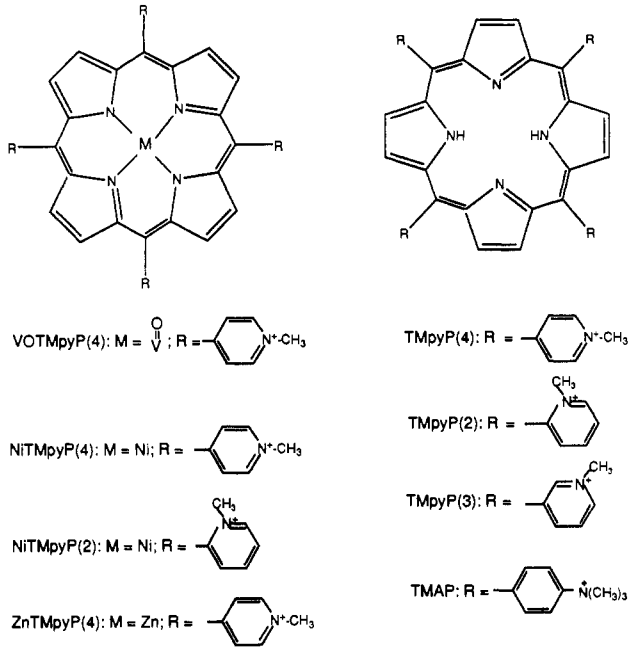
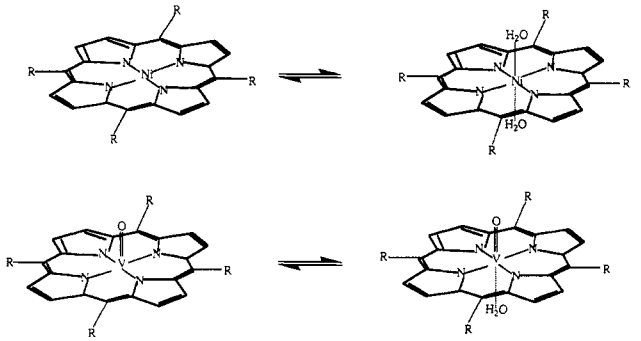


Figure 1. Structures of porphyrins referred to in the present study.

Scheme I.



6C species was found to be sensitive to the environment and can thus be used to probe the binding modes and binding sites of the nickel porphyrins to DNA and anionic polymers.[3] In solutions of DNA polymers, the characteristic bands of the **6C** species either disappear completely (for NiTMpyP(4)) or are greatly reduced (for NiTMpyP(2)), indicating that binding is dominated by a relatively unperturbed **4C** species. There is no direct evidence from Raman spectroscopy that the **6C** species can be bound. However, at high porphyrin to anionic polymer ratios, circular dichroism measurements indicated that an unperturbed **6C** NiTMpyP(4) is bound to poly(U) and dextran sulfate. On the other hand, no **6C** form can be observed when NiTMpyP(4) is bound to $[poly(dAdT)]_2$, in general agreement with Nakamoto.[22]

Like the corresponding nickel porphyrins, the vanadyl porphyrins also have two fluxional coordinations; vanadium can be either five-coordinate (**5C**) or six-coordinate (**6C**) (Scheme I). Vanadyl porphyrins are of particular interest because of an additional band due to V=O stretching in the resonance Raman spectrum.[23] Since it was suggested that the frequency of this mode for the **5C** vanadyl porphyrin depends linearly on the acceptor number of the solvent,[23] an estimate of the acceptor number of the binding environment can thus be obtained from monitoring the frequency of this mode upon binding of the porphyrin to nucleic acids. Therefore, we investigated the influence of solvent environment and polymer-binding on the V=O stretching mode and other coordination-sensitive modes.

In previous studies, only the **6C** modes were observed in the Raman spectrum of aqueous VOTMpyP(4). The frequency of the **5C** V=O stretching mode in water was found by extrapolation of mixed-solvent data. Our data show that when VOTMpyP(4) binds to DNA polymers, bands of both the **5C** and the **6C** species can be observed directly in aqueous solution. Analysis of the spectra provide the first compelling Raman spectroscopic evidence for DNA binding by a **6C** metalloporphyrin that can have different fluxional coordination environments. Such Raman results, as well as optical and viscometric studies, are used to assess DNA binding mode and binding selectivity. A previous report concluded that VOTMpyP(4) could be an intercalator or partial intercalator;[24] this conclusion contradicts the generally accepted belief that axially ligated metalloporphyrins are not intercalators.[1] Therefore, we utilized a range of spectroscopic and biophysical methods to assess the binding interactions of VOTMpyP(4) with DNA and with other polymers.

Experimental Section

Calf thymus DNA (CT DNA) was obtained from Worthington and prepared as described previously.[2,3] CT DNA was stored in PIPES 00 buffer [0.01 M PIPES (piperazine-*N*,*N*'-bis[2-ethanesulfonic acid] (Sigma)), deionized water, pH 7]. $[poly(dAdT)]_2$, $[poly(dGdC)]_2$, and poly(U) were purchased from Pharmacia and stored in 0.01 M NaCl solution. Extinction coefficients used to determine the polynucleotide concentrations in base pairs were as follows: CT DNA, $\epsilon_{260} = 1.32 \times 10^4$ M^{-1} cm^{-1};[25] $[poly(dAdT)]_2$, $\epsilon_{262} = 1.32 \times 10^4$ M^{-1} cm^{-1};[26] $[poly(dGdC)]_2$, $\epsilon_{254} = 1.68 \times 10^4$ M^{-1} cm^{-1}.[27] The extinction coefficient used for poly(U) per base was $\epsilon_{260} = 9 \times 10^3$ M^{-1} cm^{-1}.[28] Dextran sulfate, heparin, phosvitin, and poly(D-glutamic acid) were obtained from Sigma and used without further purification. The concentration of the charged units, e.g., sulfate in dextran sulfate, was calculated by the method described previously.[3] Drug/polymer ratios R are defined as follows: for DNAs, R = [porphyrin]/[DNA bp]; for poly(U) and non-nucleic acid polymers, R = [porphyrin]/[anionic unit].

The chloride salt of VOTMpyP(4) was obtained from Midcentury and purified by gel filtration chromatography, using Sephadex LH-20 as the stationary phase and a 1:1 solution of methanol and 0.01 M aqueous $NaHCO_3$ as the eluant. The purity of VOTMpyP(4) was checked by gel electrophoresis.[3] VOTMpyP(4) was dissolved in spectral grade solvents.

Visible absorption experiments were performed with a Perkin-Elmer 3A or a Cary 3 UV–visible spectrophotometer. Circular dichroism (CD) spectra and titrations were monitored with a JASCO 600 spectropolarimeter. Titrations of VOTMpyP(4) with different polymers were conducted as described previously.[3] The porphyrin concentration was $\sim 1 \times 10^{-5}$ M in the visible absorption and CD titrations. In the nonaqueous solvent experiments, the porphyrin was dissolved directly in the solvent. In the experiments with THF, pyridine, and acetone, $VOTMpyP(4)(BPh_4)_4$ was obtained by adding $NaBPh_4$ to an aqueous solution of the chloride salt of VOTMpyP(4); the precipitate obtained was dried and dissolved directly in the solvent.

The extinction coefficient of VOTMpyP(4) ($\epsilon_{438} = 2.07 \times 10^5$ M^{-1} cm^{-1}) was determined by dilution of a porphyrin stock solution with water; the value agrees well with another determination.[29] Beer's law was followed. Stock solutions were prepared by dissolving the dried porphyrin in deionized water.

Viscosity experiments were conducted with a Cannon-Ubbelohde semimicrodilution capillary viscometer in a temperature-controlled circulating water bath maintained at 30.0 °C. Typically, PIPES 10 buffer (1.0 mL) was added to the viscometer and a reading was obtained. A small amount of DNA stock solution was added to give a final DNA concentration of ~40–90 μM. The viscosity of the DNA solution was then measured. Small aliquots of porphyrin stock solution were added with frequent mixing. The viscosity was measured after each addition until three readings (typically ~100 s) were obtained with a deviation of less than ±0.1 s. The resulting solution reduced viscosity (SRV, the ratio of the viscosity of porphyrin/DNA solution to that of the DNA solution) vs R was analyzed.

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Table I. Soret Maximum of VOTMpyP(4) in Different Solvents

solvent	λ_{max} (nm)	shoulder (nm)	ϵ (M^{-1} cm^{-1})
water	438.5		2.07
MeOH	435		2.85
EtOH	437		2.28
imidazole	443		1.68
DMF	437	418	
i-prOH	437.5	420	
CH_3CN	437	421	
THF	433		
acetone	424	430	
pyridine	444		

Resonance Raman spectra were measured with excitations at either 406.7 nm from a krypton ion laser (Coherent Innova 100) or 457.9 nm from an argon ion laser (Spectra Physics Model 164). Power at the samples in melting point capillaries was kept below 20 mW to avoid heating and/or photolysis. Typical concentrations of porphyrin were either 60 or 180 μM. Raman signals were recorded at 90° to the excitation laser beam to a triple monochromator (Spex Model 1877 triplemate) with a photodiode array detector system consisting of a Model IRY-1024 detector and a Model ST-120 controller from Princeton Instruments which is interfaced to an IBM-AT microcomputer. Calibrations were done for each measurement using known Raman lines of toluene. Peak positions were reproducible to within ±1–2 cm^{-1} between different runs. Typical resolution was 6 or 8 cm^{-1}.

For Raman studies at different temperatures, a specially designed sample holder for a melting point capillary was connected to a refrigerated bath/circulator (ENDOCAL Model RTE-100) from NESLAB Instruments. Temperature stability of the circulator was ±0.1 °C.

Results

Viscosity Measurements. No SRV increases were observed when VOTMpyP(4) was added to CT DNA, $[poly(dGdC)]_2$, or $[poly(dAdT)]_2$. Instead, the SRV decreased slightly to ~0.93 and 0.91 for CT DNA and $[poly(dGdC)]_2$ at R = 0.25, respectively. For $[poly(dAdT)]_2$, at R = 0.25, the decrease was greater, i.e. to ~0.77.

Visible and Raman Measurements. Solvent Behavior. As mentioned above, in solution, vanadium in VOTMpyP(4) can be either **5C** or **6C** (with a solvent molecule as the sixth ligand). Previous studies found that only the **6C** VOTMpyP(4) can be detected in aqueous solution.[23] No Raman band attributable to the **5C** species in a totally aqueous environment has been detected. We first compared the visible and Raman spectral changes over varying concentrations and solvent conditions. In aqueous solution, the **6C** species can be easily observed by a slightly asymmetric Soret peak at 439 nm in the visible spectrum. The Soret maximum of VOTMpyP(4) was also monitored in other solvents (Table I). In methanol, ethanol, THF, pyridine, and 1 M aqueous imidazole, only one predominant Soret band was observed, just as for the aqueous solution. In DMF, an additional small shoulder due to the presence of a small amount of **5C** species was observed at 418 nm. In *i*-PrOH, the shoulder was observed at 420 nm, and in CH_3CN, two bands were observed in the Soret region, with maxima at 421 and 437 nm. In acetone the Soret maximum was observed at 424 nm and a shoulder was observed at 430 nm, indicating more **5C** than **6C** species.

The resonance Raman spectra of VOTMpyP(4) were measured in various solvents. Figure 2a shows a typical spectrum of VOTMpyP(4) in 0.01 M NaCl aqueous solution between 900 and 1700 cm^{-1} using 406.7-nm excitation. Most of the bands are similar or identical to those of H_2TMpyP(4) or NiTMpyP(4),[3,30] with the differences arising from shifts in core-size-sensitive modes. These modes are sensitive to the presence and type of metal ion.[30] In addition, there is a broad band at ~957 cm^{-1}, which has been assigned to V=O stretching.[23] As expected, this band is sensitive to the coordination number of vanadium.

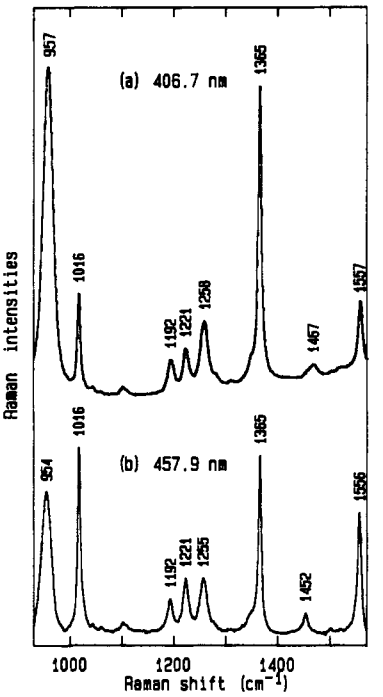

Figure 2. Raman spectra of VOTMpyP(4) (65 μM, ambient temperature, 0.01 M aqueous NaCl) with (a) 406.7-nm and (b) 457.9-nm excitation.

We use the same methodology to assign a particular Raman band to a given coordination species as we used previously in a resonance Raman study of NiTMpyP(4) binding to polymers.[3] In general, the Raman bands due to the **5C** species are selectively enhanced by 406.7-nm excitation relative to 457.9-nm excitation. The reverse, however, may not be true; that is, excitation at 457.9 nm gives less enhancement to the **6C** bands relative to excitation at 406.7 nm. For example, the intensity of the V=O stretching band at 957 cm^{-1} in aqueous solution, which can be assigned to the **6C** species (see below), is higher with 406.7-nm excitation than with 457.9-nm excitation, as compared to an internal sulfate standard (data not shown). Note that the intensity of the adjacent porphyrin mode at 1013 cm^{-1} is also sensitive to excitation; this mode is relatively enhanced with 457.9-nm excitation (contrast parts a and b of Figure 2). However, since the **5C** bands are generally not observable with 457.9-nm excitation, Raman bands observed with this excitation can be assigned to the **6C** species. Similarly, 406.7-nm excitation is necessary to detect small amounts of the **5C** species.

Since the amount of **5C** species is too small in aqueous solution for the corresponding V=O stretching mode to be observed, other solvents must be used to observe the band.[23] For example, in acetonitrile, where a new Soret peak at 421 nm corresponds to the **5C** species, a **5C** V=O stretching mode at 1006 cm^{-1} can be observed in the Raman spectrum, in addition to the **6C** band at 978 cm^{-1} (Figure 3a). The absence of the 1006-cm^{-1} peak with 457.9-nm excitation confirms our assignment that this peak is due to the **5C** species (data not shown). In addition to the changes in the relative amount of **5C** to **6C** species in different solvents, the frequencies of the V=O stretching bands of the two species also depend on the solvent.[23] For example, the **6C** band at ~957 cm^{-1} in aqueous solution (Figure 2a) gradually shifts to 978 cm^{-1} in 95% acetonitrile (Figure 3a). Note that the intensities of observable **5C** bands decreased with an increase in the amount of water until no **5C** species V=O stretching band was observed in water itself.

Since the **5C** V=O stretching band cannot be directly observed in a 100% aqueous environment, its frequency was previously estimated to be 970 cm^{-1} from methanol/water mixtures.[23] However, our visible spectral studies suggested that relatively little **5C** species exists in methanol. We used the mixed solvent

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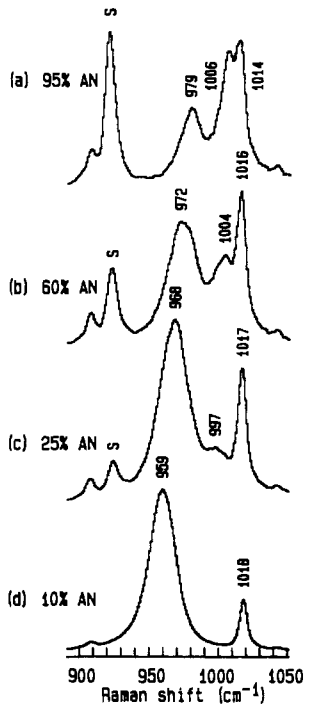

Figure 3. Raman spectra of VOTMpyP(4) (28 μM, ambient temperature, 406.7-nm excitation) in (a) 95%, (b) 60%, (c) 25%, and (d) 10% acetonitrile, AN.

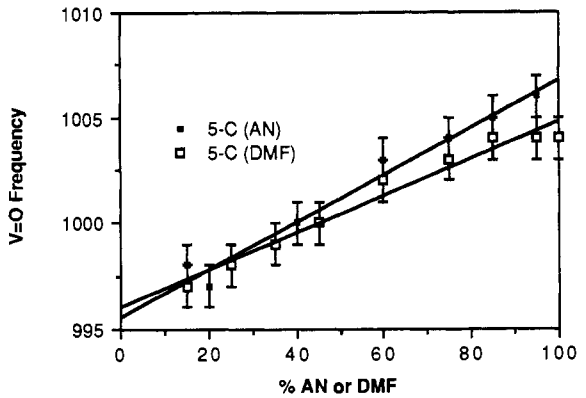

Figure 4. The **5C** V=O stretching frequency as a function of percentage of AN or *N,N*-dimethylformamide (DMF). Both sets of data were fit with straight lines. Bars indicate standard error of the data.

approach but with aqueous acetonitrile and with aqueous DMF. Figure 4 shows the frequency of the **5C** V=O band as a function of the percentage of acetonitrile or DMF. Both sets of data can be fit with straight lines yielding the same intercept for 100% water at ~996 cm^{-1} for the **5C** V=O stretching frequency. Since the peaks in this region are overlapping (consisting of at least three peaks, the **5C** and **6C** V=O stretching modes and a porphyrin mode; for example, see Figure 3a), a nonlinear least-squares fitting program using Lorentzian line shapes was used to obtain more accurate peak positions. This procedure is necessary, especially for solutions with low water content, where the band due to the **5C** species is small. The frequency of the **6C** V=O stretching band also shows a linear dependence on the percentage of either acetonitrile or DMF.

The frequency of the **5C** V=O stretching band in other solvents was also monitored. Figure 5 shows this frequency as a function of the acceptor number of the solvents used.[31] These results generally confirm the findings of Spiro and his group, who found these trends with several vanadyl porphyrins in a wide range of solvents. We have concentrated our efforts on VOTMpyP(4)

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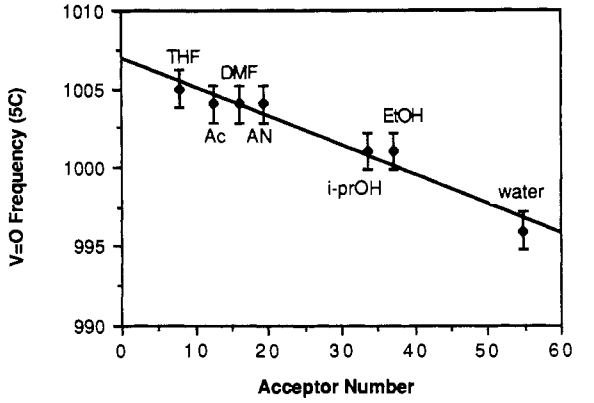

Figure 5. The **5C** V=O stretching frequency as a function of the acceptor number of solvents. The data were fit with a straight line. Bars indicate standard error of the data. Abbreviations: THF, tetrahydrofuran; Ac, acetone; *i*-prOH, 2-propanol; EtOH, ethanol.

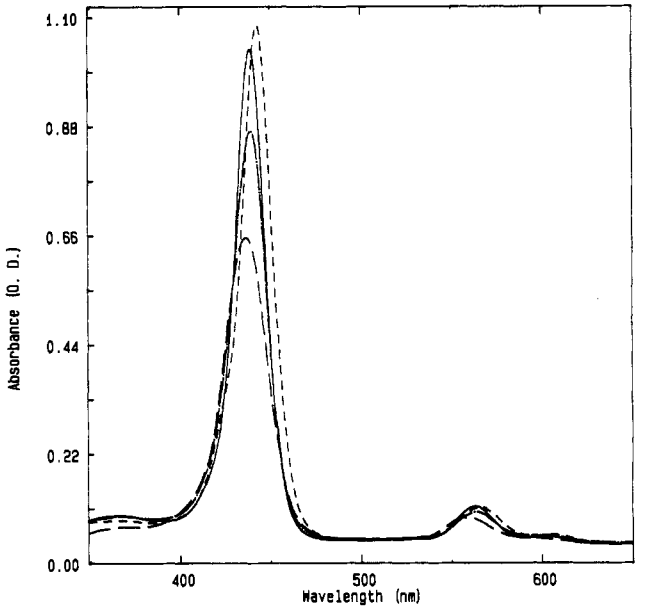

Figure 6. Visible absorption spectra of VOTMpyP(4) (9.5 μM) in (a) 0.01 M NaCl (—) and with (b) [poly(dAdT)]$_2$ (- - -), (c) [poly(dGdC)]$_2$ (– –), and (d) dextran sulfate (– · –).

because of its interactions with nucleic acids. This more in-depth study of VOTMpyP(4) was needed to define the V=O stretching mode more precisely for use in our polymer studies.

Effects of Polymer Binding. The interactions of VOTMpyP-(4) with CT DNA, [poly(dGdC)]$_2$, [poly(dAdT)]$_2$, poly(U), and dextran sulfate in 0.01 M NaCl (pH 7) solution were assessed by monitoring the Soret bands. Figure 6 shows the effect at the final R value of $\leq$0.05.

The effects of sequential additions of CT DNA and [poly-(dAdT)]$_2$ on the VOTMpyP(4) Soret bands were similar, with a decrease in intensity of the VOTMpyP(4) Soret band at high R values ($R > 0.25$). The greatest hypochromicity (ΔH) observed for [poly(dAdT)]$_2$ was 5% (λ_{max} at 440 nm) at $R = 0.5$. For CT DNA, this was 10% (λ_{max} at 441 nm) at $R = 0.25$. Further addition of [poly(dAdT)]$_2$ or CT DNA increased the Soret band intensity. By $R = 0.05$ (the final ratio), a small hyperchromicity of 2% and a 3-nm red shift were observed for both DNAs. A small shoulder at ~420 nm was observed at low R values ($R <$ 0.1) in both cases. The increase in absorption in the low-wavelength region upon binding to all DNA polymers is indicative of an increased percentage of the **5C** species.

Effects observed with [poly(dGdC)]$_2$ were different from the other DNA polymers at 0.01 M salt. A large hypochromicity (40%) and a 3-nm *blue* shift were observed at $R = 0.05$. The peak became highly asymmetric with very significant increase in

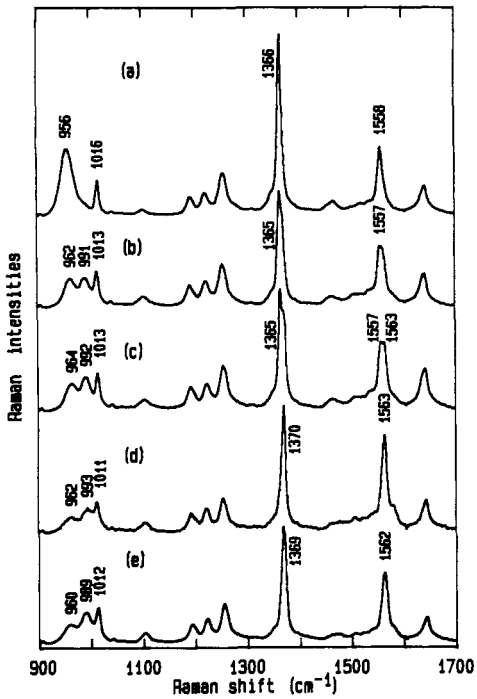

Figure 7. Raman spectra of VOTMpyP(4) (60 μM) with (a) dextran sulfate, (b) CT DNA, (c) $[poly(dAdT)]_2$, (d) $[poly(dGdC)]_2$, and (e) poly(U). Samples were at ambient temperature, in 0.01 M aqueous NaCl, with R = 0.01. Excitation was 406.7 nm.

absorption on the low-wavelength side (Figure 6). In 0.1 M NaCl, however, when $[poly(dGdC)]_2$ was titrated into VOTMpyP(4), only a small hypochromicity (12%) and no blue shift were observed at the same R value (data not shown).

Addition of poly(U) and dextran sulfate to VOTMpyP(4) gave similar effects. At $R > 0.25$, the addition of poly(U) or dextran sulfate decreased the intensity of the Soret band (ΔH = 30% for poly(U) and 45% for dextran sulfate, at $R = 0.25$). A small blue shift of 3 nm was observed in both cases. Further addition of poly(U) or dextran sulfate increased the Soret band intensity. The hypochromicity at $R = 0.025$ was 22% and 20% for poly(U) and dextran sulfate, respectively, and the Soret maximum shifted back to 438 nm at $R = 0.025$ in both cases.

The Raman spectra of VOTMpyP(4) with $[poly(dAdT)]_2$, CT DNA, $[poly(dGdC)]_2$, and poly(U) (0.01 M NaCl, at $R = 0.01$, 457.9-nm excitation) are almost identical showing characteristic bands due to the **6C** vanadyl porphyrin (data not shown). The only difference is in the frequency of the V=O stretching mode. On the other hand, with 406.7-nm excitation, the Raman spectra of VOTMpyP(4) bound to the various DNA polymers have peaks due to both the **5C** and **6C** species (Figure 7). For example, when VOTMpyP(4) is bound to CT DNA (Figure 7b), the 962-, 1365-, and 1557-cm^{-1} bands can be assigned to the **6C** vanadyl porphyrin and the 991-, 1371-, and 1564-cm^{-1} bands (which are absent with 457.9-nm excitation) to the corresponding **5C** species. All the peaks that are changed upon binding to the polymers are summarized in Table II. For overlapping peaks, a nonlinear least-squares fit with Lorentzians was used to obtain more accurate peak positions. Note that the relative intensities of the **5C** to the **6C** bands depend on the polymers, with $[poly(dGdC)]_2$ and poly(U) showing more intense **5C** peaks in general (Figure 7). This polymer dependence is particularly evident with the V=O stretching mode.

In the Raman spectra of VOTMpyP(4) solutions with CT DNA with increasing R using 406.7-nm excitation (Figure 8), the intensity of the **5C** V=O stretching mode decreased, whereas that of the corresponding **6C** mode increased. Moreover, the **6C** band shifted substantially whereas the **5C** band was unshifted. This general behavior is expected at high R values since an increasing amount of porphyrin is probably not bound. A nonlinear least-squares fit in the spectral region between 930 and 1030 cm^{-1} was performed to obtain better estimates of the frequencies and intensities of the **5C** V=O stretching band and the relatively invariant porphyrin band at ~1017 cm^{-1}. The changes in the amount of bound **5C** and bound **6C** species as a function of R, however, are not due solely to the presence of unbound **6C** species; i.e., the behavior cannot be fit by assuming that the ratio of bound **5C** to bound **6C** species is independent of R. On the contrary, a weak dependence of the ratio of **5C** to **6C** species on R is suggested, with the **5C** species preferred at lower R. Changing the concentration of porphyrin from 60 to 400 μM had little or no effect on the binding of VOTMpyP(4) to CT DNA (R = 0.01).

We have also studied the temperature dependence of the two V=O stretching bands for the VOTMpyP(4) CT DNA adduct with 406.7-nm excitation at $R = 0.05$. We define relative intensity, RI, to be the ratio of the intensity at a certain frequency to that of the porphyrin band at ~1017 cm^{-1}. The amount of bound **5C** and bound **6C** species can then be assessed by the RI's at the frequency of the corresponding V=O stretching mode at each temperature. Relative RI's were then obtained by dividing the RI's at higher temperatures by that at 10 °C. The **6C** V=O stretching frequency of VOTMpyP(4) in the absence of DNA and the corresponding relative RI was also monitored as a function of temperature (Figure 9). There is a small shift in the frequency of this band in the absence of DNA from 957 cm^{-1} at 60 °C to 963 cm^{-1} at 10 °C. It is clear that the relative RI of the bound **5C** band increased, whereas that of the **6C** band decreased as temperature increased, indicating an increase in the bound **5C** species and a concomitant decrease in the bound **6C** species.

Furthermore, the binding of VOTMpyP(4) to various anionic, non-nucleic acid polymers was studied. There was no indication of any significant interactions of phosvitin, poly-d-glutamic acid, and heparin with the vanadyl porphyrin. In contrast, upon porphyrin binding to dextran sulfate, there was a small decrease in the intensity of the V=O stretching mode due to the **6C** species with a corresponding increase in the intensity of the **5C** mode at ~992 cm^{-1}.

CD Measurements in the Visible Region. Addition of $[poly(dAdT)]_2$ to VOTMpyP(4) induced a CD signal in the visible region (Figure 10). At high R values, $R \geq 0.5$, there were two positive bands at 450 and 431 nm and a negative band at 441 nm. At $R = 0.5$ the CD signal reached its maximum value, with the molecular ellipticities (Θ) of 3.8×10^5 deg cm^2 $dmol^{-1}$ at 450 nm, 2.3×10^5 deg cm^2 $dmol^{-1}$ at 431 nm, and -4.8×10^5 deg cm^2 $dmol^{-1}$ at 441 nm. At lower R values ($0.25 > R > 0.15$), the negative band disappeared and the two positive bands began to merge, and at $R < 0.15$, only one positive band was observed at ~442 nm. At $R = 0.05$, the CD signal is at 444 nm with the $\Theta = 1.2 \times 10^5$ deg cm^2 $dmol^{-1}$.

Titration with CT DNA gave results very similar to those with $[poly(dAdT)]_2$, but the induced CD signal was smaller at high R values. The molecular ellipticities were 1.0×10^5 deg cm^2 $dmol^{-1}$ at 451 nm, -2.9×10^4 deg cm^2 $dmol^{-1}$ at 441 nm, and 4.0×10^5 deg cm^2 $dmol^{-1}$ at 432 nm at $R = 0.5$. At $R = 0.05$, only one positive CD band was observed at 443 nm with $\Theta = 1.1 \times 10^5$ deg cm^2 $dmol^{-1}$, a value very similar to that with $[poly(dAdT)]_2$.

Titration of $[poly(dGdC)]_2$ into VOTMpyP(4) induced a very weak CD signal in the visible region throughout the titration. At high R values ($R > 0.5$) two positive bands at 452 and 424 nm with $\Theta = 1.2 \times 10^4$ and 5.3×10^3 deg cm^2 $dmol^{-1}$, respectively, were observed as well as a negative band at 439 nm with $\Theta = -1.8 \times 10^4$ deg cm^2 $dmol^{-1}$ at $R = 1$. On further addition of $[poly(dGdC)]_2$, the negative band became smaller. At low R values (<0.15), a conservative band was observed at the visible region, with the positive maximum at 442 nm and the negative maximum at ~421 nm. At $R = 0.05$ Θ values were 7.4×10^3 and -7.0×10^3 deg cm^2 $dmol^{-1}$, respectively. These CD changes were much weaker than with either $[poly(dAdT)]_2$ or CT DNA (Figure 11).

Table II. Peak Positions for the 5C and 6C V=O Stretching Bands upon Binding to the Polymers[a]

polymer	6C	5C	porphyrin	6C	5C	6C	5C
none	956 (956.7, 22.6, 3.06)		1017 (1016.5, 7.8, 1.00)	1366		1558	
dextran sulfate	956 (957.7, 32.4, 1.96)	(992.2, 29.9, 0.23)	1016 (1016.0, 10.1, 1.00)	1366		1558	
CT DNA	962 (961.5, 26.4, 0.85)	991 (991.0, 24.2, 0.84)	1013 (1013.8, 11.6, 1.00)	1364.5	1371.2	1556.6	1564.0
$[poly(dAdT)]_2$	964 (962.2, 26.1, 0.74)	992 (991.1, 23.6, 0.93)	1014 (1014.2, 10.7, 1.00)	1364.6	1371.7	1556.6	1564.5
$[poly(dGdC)]_2$	962 (960.9, 38.5, 0.60)	993 (993.0, 24.0, 0.74)	1011 (1011.7, 13.2, 1.00)		1370		1563
poly(U)	960 (956.9, 31.6, 0.55)	989 (989.0, 26.6, 0.92)	1012 (1012.3, 12.3, 1.00)		1369		1562

[a] The fitted data are listed in parentheses. They are peak positions, half-widths, and relative intensities of the porphyrin ring bands, respectively.

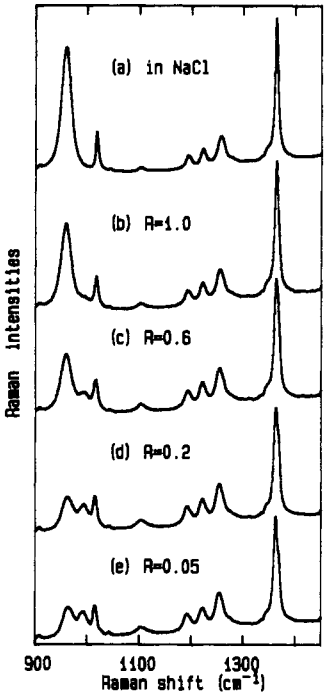

Figure 8. Raman spectra of VOTMpyP(4) (65 μM) bound to CT DNA at various R values. Excitation was 406.7 nm.

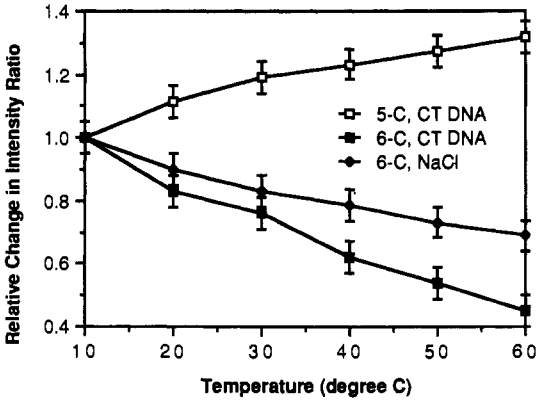

Figure 9. Relative change of the relative intensity ratios of the V=O stretching band to the porphyrin ring band at ~1017 cm^{-1} as a function of temperature and using data at 10 °C as reference. The following V=O stretching bands are plotted: (a) 5C bound to CT DNA; (b) 6C in 0.01 M NaCl; (c) 6C bound to CT DNA. Excitation was 406.7 nm. The concentration was 65 μM, with R = 0.05.

Titration of poly(U) into VOTMpyP(4) induced a weak conservative band at high R value ($R > 0.25$) with the positive maximum at 425 nm ($\theta = 4.29 \times 10^4$ deg cm^2 $dmol^{-1}$, $R = 0.5$) and negative maximum at 438 nm ($\theta = -2.29 \times 10^4$ deg cm^2 $dmol^{-1}$). On further addition of poly(U), the conservative band was transformed into a positive band at 438 nm, with $\theta = 1.67 \times 10^4$ deg cm^2 $dmol^{-1}$ at $R = 0.05$.

CD Measurements in the UV Region. VOTMpyP(4). Interesting changes in the CD spectra in the UV region were observed in a series of titrations of DNA with VOTMpyP(4). $[poly(dAdT)]_2$ has a positive band at 262 nm with a shoulder at ~275 nm and a negative band at ~246 nm. On addition of VOTMpyP-

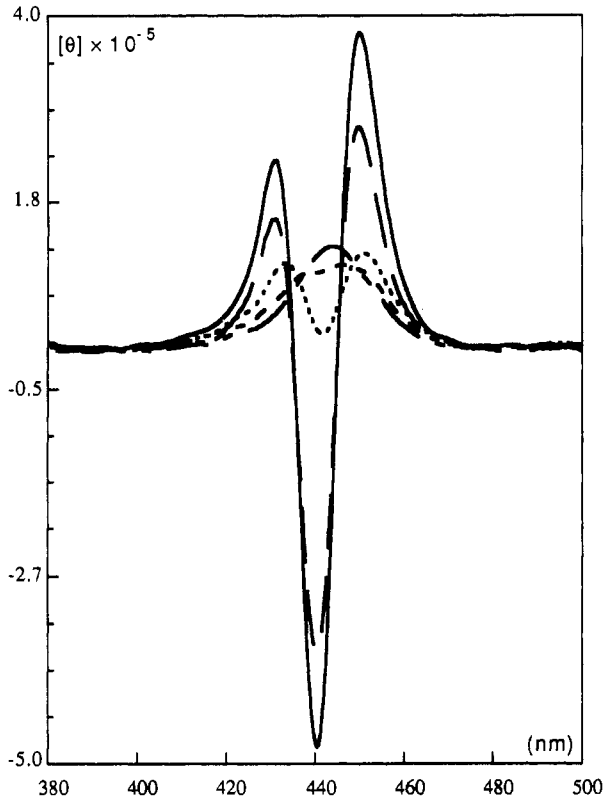

Figure 10. Induced CD spectra of VOTMpyP(4) (10 μM) with $[poly(dAdT)]_2$ at pH 7.0, 0.01 M NaCl: R = 1 (– –); R = 0.5 (—); R = 0.25 (···); R = 0.15 (- - -); R = 0.05 (- –).

(4), the shoulder region of the positive band was reduced and at higher R value ($R > 0.24$) developed into a negative band at 275 nm, while the negative band at 246 nm was reduced as R increased. Also, a weak, broad, positive band at longer wavelength was observed at higher R. At $R = 1$, the ellipticities for this negative–positive–negative pattern of bands at 276, 259, and 246 nm were -4.7×10^4, 2.0×10^4, and -1.7×10^4 deg cm^2 $dmol^{-1}$, respectively (Figure 12).

Similar changes in the UV region were observed in titrations of CT DNA with VOTMpyP(4). CT DNA has a conservative band with the positive band at 276 nm and the negative band at 245 nm. With the addition of VOTMpyP(4), the positive band at 276 nm was reduced, and as R increased to 0.24, a new negative band was observed at 276 nm. As R was increased further, the negative band became more intense. At R = 0.75, the CD spectrum of the VOTMpyP(4) bound CT DNA showed a negative–positive–negative pattern also: 276 nm ($\theta = -2.2 \times 10^4$ deg cm^2 $dmol^{-1}$); 258 nm ($\theta = 1.3 \times 10^4$ deg cm^2 $dmol^{-1}$); 244 nm ($\theta = -6.4 \times 10^3$ deg cm^2 $dmol^{-1}$) (Figure 13).

Changes observed when VOTMpyP(4) was titrated into $[poly(dGdC)]_2$ were significantly smaller (Figure 14). Ellipticity changes were observed following the addition of VOTMpyP(4) to poly(U), but the pattern of the CD spectrum of poly(U) was not changed.

Other Porphyrins. Titrations of DNA in 0.01 M NaCl solution with different porphyrins (TMpyP(4), ZnTMpyP(4), NiTMpyP-

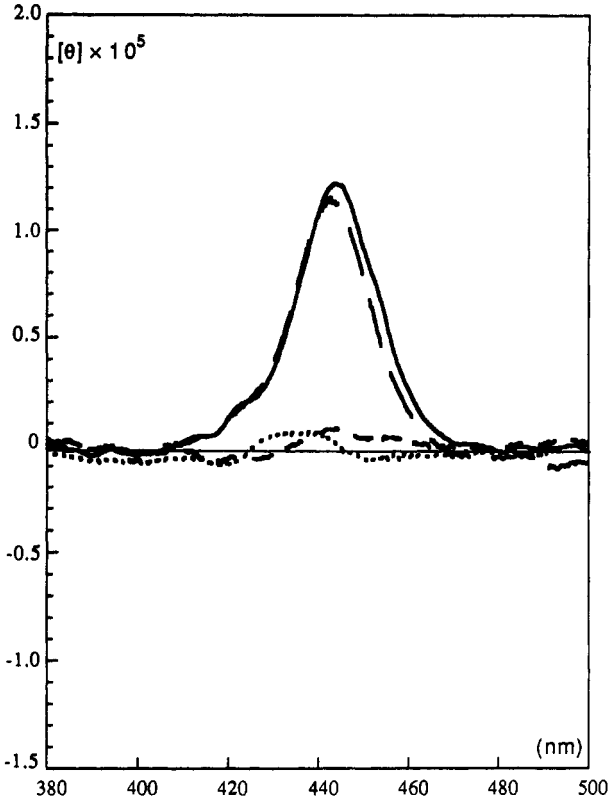

Figure 11. Induced CD spectra of VOTMpyP(4) (9.7 μM, in 0.01 M NaCl, pH 7.0) with [poly(dAdT)]$_2$ (—), [poly(dGdC)]$_2$ (- - -), CT DNA (– –), and poly(U) (···), R = 0.05.

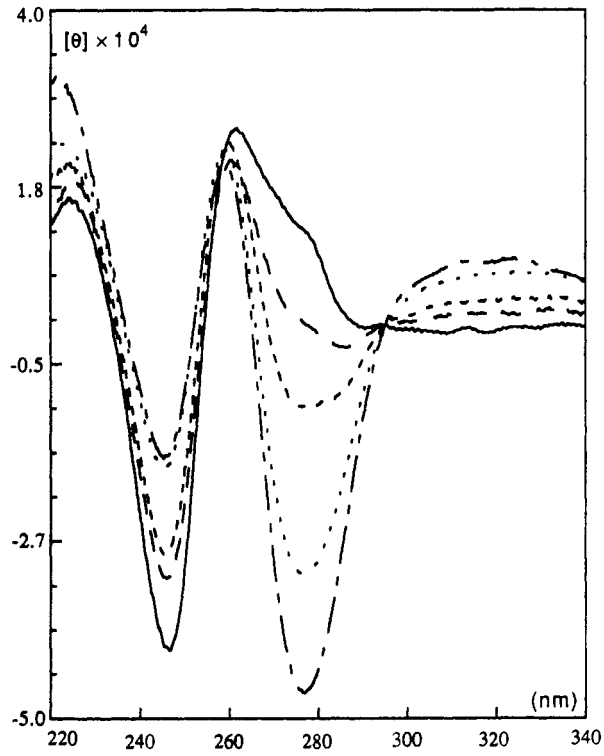

Figure 12. Ultraviolet CD spectra of [poly(dAdT)]$_2$ (34 μM) in 0.01 M NaCl, at pH 7.0 (—), with VOTMpyP(4): R = 0.1 (– –); R = 0.25 (- - -); R = 0.5 (···); R = 1.0 (- –).

(4)) were performed to compare the DNA CD changes caused by these porphyrins, which have known binding properties.[1,3,20,32]

As ZnTMpyP(4) was added to [poly(dAdT)]$_2$, the positive band, particularly in the shoulder region, was reduced; continuing

(32) Strickland, J. A.; Marzilli, L. G.; Gay, K. M.; Wilson, W. D. *Biochemistry* **1988**, *27*, 8870–8878.

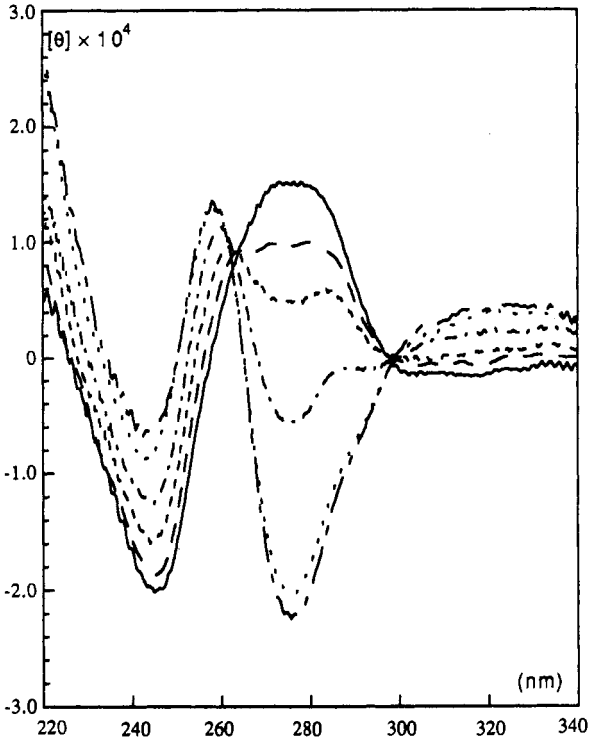

Figure 13. Ultraviolet CD spectra of CT DNA (37 μM) in 0.01 M NaCl at pH 7.0 (—), with VOTMpyP(4): R = 0.05 (– –); R = 0.1 (- - -); R = 0.25 (- · –); R = 0.5 (···); R = 0.75 (- –).

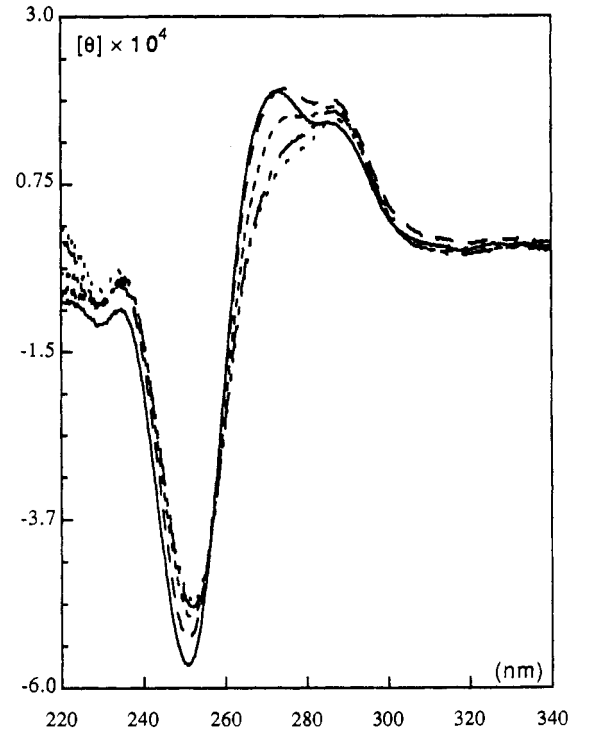

Figure 14. Ultraviolet CD spectra of [poly(dGdC)]$_2$ (32 μM) in 0.01 M NaCl, at pH 7.0 (—), with VOTMpyP(4): R = 0.1 (– –); R = 0.25 (- - -); R = 0.5 (– - –); R = 1.0 (···).

addition of ZnTMpyP(4) inverted the positive shoulder at ~275 nm into a negative band. At the same time, the negative band at 246 nm became less and less intense with added ZnTMpyP(4). At $R > 0.25$, a positive band emerged at 258 nm. These changes are very similar to those caused by VOTMpyP(4). At R = 1 the CD pattern of the ZnTMpyP(4)-bound [poly(dAdT)]$_2$ is very similar to the VOTMpyP(4)-bound [poly(dAdT)]$_2$ (Figure 15), except that the positive band is more intense with ZnTMpyP(4). However, titration of [poly(dAdT)]$_2$ with TMpyP(4) gave different results: at low R values ($R \leq 0.375$), the band at 275

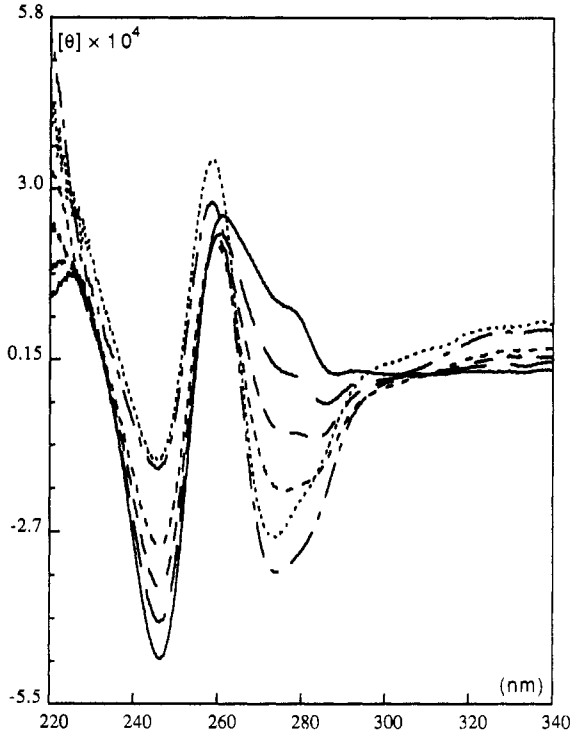

Figure 15. Ultraviolet CD spectra of $[poly(dAdT)]_2$ (40 μM) in 0.01 M NaCl, at pH 7.0 (—), with ZnTMpyP(4): R = 0.05 (– –); R = 0.1 (– – –); R = 0.25 (– · –); R = 0.5 (– · · –); R = 1.0 (···).

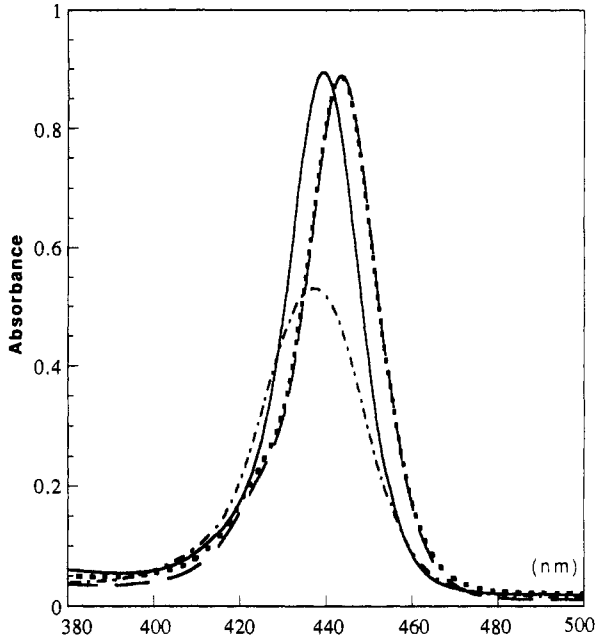

Figure 16. Visible absorbance spectra of VOTMpyP(4) (9.0 μM) in 0.01 M NaCl, pH 7.0 (—), (a) with $[poly(dGdC)]_2$ (90 μM), R = 0.1 (– · –), (b) with $[poly(dAdT)]_2$ (90 μM), R = 0.1 (– –), and (c) with $[poly(dGdC)]_2$ (90 μM) + $[poly(dAdT)]_2$ (90 μM) (– · –). Spectra b and c are severely overlapped.

nm was inverted into a negative band, and the ellipticity of the 245-nm band was reduced. At high R values, the negative band at 275 nm became a broad band, and the negative band at 245 nm was inverted into a weak positive band. The changes caused by NiTMpyP(4) are very similar to those caused by TMpyP(4).

Addition of ZnTMpyP(4) to CT DNA decreased the ellipticities of both the positive and the negative band of CT DNA at low R. Isosdichroic points were observed at 262 and 298 nm. At higher R ($R > 0.15$), the positive band at 275 nm had inverted into a negative band and a new positive band at 257 nm was observed. At R = 1, the ZnTMpyP(4)-bound CT DNA had a negative–positive–negative CD pattern similar to that for VOTMpyP(4)-bound CT DNA. Addition of TMpyP(4) to CT DNA at low R values decreased the ellipticities of both the positive and negative bands; at higher R values, the positive band at 275 nm had inverted into a negative band, and the negative band at 245 nm was much less intense. When $R > 0.5$, mainly a negative band was observed at 270 nm, but there is a weak positive band at ~290 nm. The CT DNA CD signal changes caused by NiTMpyP(4) fall between those of VOTMpyP(4)- or ZnTMpyP(4)-bound DNA and those for TMpyP(4)-bound CT DNA. At higher R values the NiTMpyP(4)-bound CT DNA has a negative–positive–negative pattern, but the ellipticities of the negative band at ~270 nm are much smaller than those caused by VOTMpyP(4), ZnTMpyP-(4), and TMpyP(4). The ellipticity of the positive band (at 255 nm) is much smaller than the related bands for the ZnTMpyP(4) and VOTMpyP(4) adducts. For TMpyP(4), this band is absent. However, as found for TMpyP(4) at high R, NiTMpyP(4) induced a weak positive band at ~290 nm.

Addition of ZnTMpyP(4) to $[poly(dGdC)]_2$ slightly increased the ellipticity of the positive band at 280 nm at low R values ($R < 0.1$); at higher R values the ellipticity of this positive band decreased. The ellipticity of the negative band at 252 nm decreased as ZnTMpyP(4) was added to the solution. Titration of $[poly(dGdC)]_2$ with TMpyP(4) gave different results: the positive band of $[poly(dGdC)]_2$ increased significantly as TMpyP-(4) was added. The ellipticity of the negative band at 252 nm decreased somewhat at the same time. An isosdichroic point was observed at 298 nm. Both bands shifted slightly to the red. Titration of $[poly(dGdC)]_2$ with NiTMpyP(4) also increased the ellipticity of the positive band and decreased the ellipticity of the negative band, but the changes in the positive band were not as significant as those caused by TMpyP(4). Also, at $R > 0.5$, a ~7-nm red shift of both bands was induced by NiTMpyP(4); this shift was greater than that caused by TMpyP(4).

In summary, changes in the DNA CD signal induced by VOTMpyP(4) are more similar to those induced by ZnTMpyP-(4) than those of TMpyP(4). NiTMpyP(4) induced changes intermediate between those induced by VOTMpyP(4) or ZnT-MpyP(4) and by TMpyP(4).

Competitive Binding to $[poly(dAdT)]_2$ and $[poly(dGdC)]_2$. Addition of $[poly(dGdC)]_2$ to VOTMpyP(4) (R = 0.1) in 0.01 M NaCl solution caused a decrease of the Soret band intensity and small blue shift (Figure 16). However, addition of the same amount of $[poly(dAdT)]_2$ to this VOTMpyP(4)/$[poly(dGdC)]_2$ solution induced a red shift of the Soret band and increased the intensity; the resulting spectrum was almost identical to that of VOTMpyP(4) with $[poly(dAdT)]_2$ at R = 0.1. In contrast, when $[poly(dGdC)]_2$ was added to a VOTMpyP(4)/$[poly(dAdT)]_2$ R = 0.1 solution, no change was observed in the visible spectrum.

When $[poly(dAdT)]_2$ was added to a VOTMpyP(4)/$[poly-(dGdC)]_2$ solution, giving a characteristically very weak CD signal in the visible region, a large positive CD band with the maximum at 440 nm was observed (Figure 17). This positive CD band was nearly identical to that obtained with VOTMpyP(4)/$[poly-(dAdT)]_2$ solution at the same R value. Adding $[poly(dGdC)]_2$ to a VOTMpyP(4)/$[poly(dAdT)]_2$ solution did not change the CD signal induced by $[poly(dAdT)]_2$. These results indicate that VOTMpyP(4) binds to $[poly(dAdT)]_2$ much more strongly than to $[poly(dGdC)]_2$.

Discussion

In an earlier study,[3] we found that the equilibrium between the **6C** and the **4C** NiTMpyP(4) is shifted essentially entirely toward the **4C** form upon binding to DNA polymers, suggesting such equilibrium can be used to probe DNA binding. The possibility that the **4C** nickel porphyrin species can intercalate in GC-rich regions complicates the analysis. Unlike the nickel porphyrins, the oxo group in vanadyl porphyrins ensures that these coordinatively fluxional metalloporphyrins have at least one axial ligand (cf. Figure 1). Consequently, in binding to DNAs, the vanadyl

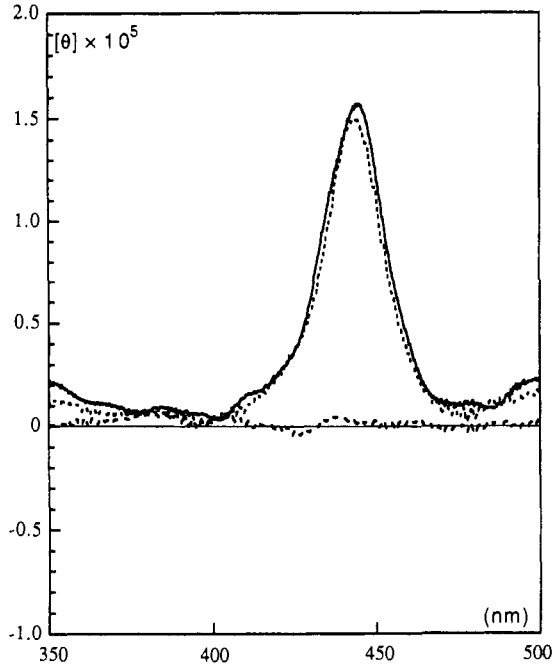

Figure 17. Induced CD spectra of VOTMpyP(4) (9.0 μM) in 0.01 M NaCl, at pH 7.0 with $[poly(dGdC)]_2$ (90 μM), R = 0.1 (- - -), with $[poly(dGdC)]_2$ (90 μM) + $[poly(dAdT)]_2$ (90 μM) (···) and with $[poly(dAdT)]_2$ (90 μM), R = 0.1 (—).

porphyrins are not expected to intercalate fully but can only partially intercalate or bind on the outside. Therefore, vanadyl porphyrins are less complicated than Ni porphyrins to probe the relationship of coordination number to binding interactions with nucleic acid polymers.

Although VOTMpyP(4) would seem to be a better candidate for study, intercalation has been suggested to occur, as mentioned briefly above.[24] Dougherty conducted electron paramagnetic resonance (EPR) studies of thin porphyrin–CT DNA films. Since intercalation of a molecule ensures a fixed orientation of the plane of the molecule with respect to the axial direction of the DNA helix, the EPR spectrum of such a DNA complex film will depend critically on the orientation of the film relative to the magnetic field. The EPR spectrum of the VOTMpyP(4)–DNA thin film depended on the orientation of the film with respect to the field. Dougherty concluded that although other binding modes may also exist, VOTMpyP(4) intercalated into CT DNA.

From the small frequency shifts of most bands in the Raman spectrum of NiTMpyP(4) upon DNA binding, we concluded that the structure of bound NiTMpyP(4) was relatively unperturbed from that of the **4C** form in solution. The major change upon binding to DNA polymers was a shift in the intensities of the coordination-sensitive modes, due to the formation of the dominant bound **4C** species. In different solvents, the coordination-sensitive bands for NiTMpyP(4) changed intensities but did not shift. In contrast, VOTMpyP(4) has a broad band due to V=O stretching in the resonance Raman spectrum, and the frequency of this stretching mode for the **5C** vanadyl porphyrin depends linearly on the acceptor number of different solvents, as suggested by pioneering studies on several vanadyl porphyrins.[23] Resonance Raman spectroscopy enables us to investigate the influence of solvent environments and of polymer binding on this and other coordination sensitive modes.

In aqueous solution, absorption spectroscopy shows that the **4C** species dominates for NiTMpyP(4), whereas the **6C** species dominates for NiTMpyP(2) and VOTMpyP(4). Resonance Raman spectra support these observations. In particular, most coordination-sensitive Raman bands in the spectrum of VOTMpyP(4) (e.g. 1365 and 1557 cm^{-1} in Figure 2a) correspond to the **6C** vanadyl porphyrin, including the V=O stretching mode at ~957 cm^{-1}.

Our data (Figure 5) confirm the report[23] that the frequency of the V=O stretching mode (for the **5C** species) is sensitive to the solvent environment. In addition, we have found that the V=O stretching frequency of the **6C** form is also sensitive to solvent; indeed, this response is greater than for the **5C** form. The frequency of the **5C** species increases by only ~10 cm^{-1} from 996 cm^{-1} in water to 1005 cm^{-1} in THF, whereas that of the **6C** species band increases by 20 cm^{-1} for the same solvent range. This greater sensitivity is probably due to the different solvent molecules coordinated as the sixth ligand. Furthermore, our data (cf. Figure 5) confirm an earlier report[23] on the inverse linear relationship between the frequency of the V=O stretching mode for the **5C** vanadyl porphyrin and the solvent acceptor number. However, the dependence on acceptor number is much weaker than in the previous study; i.e., the magnitude of the slope is smaller. Nevertheless, the general trend is confirmed: the stronger acceptor solvents weaken the V=O bond and thus lower the frequency.

As mentioned above, in aqueous solution, bands due to the **5C** species cannot be observed in the 900–1050-cm^{-1} region; the frequency of the **5C** V=O stretching cannot be determined directly. However, the behavior of this mode for aqueous mixtures of acetonitrile or DMF (Figure 4) strongly indicates that the **5C** V=O stretching frequency in aqueous solution is ~996 cm^{-1}, rather than 970 cm^{-1}.[23]

In contrast to previous studies,[2,3] both coordination forms of DNA-bound VOTMpyP(4) can be observed. Binding of VOTMpyP(4) to the various nucleic acid polymers and to dextran sulfate produced major changes in two spectral regions of the Raman spectra: 1300–1600 cm^{-1}, where several coordination-sensitive peaks are found, and 900–1050 cm^{-1}, where the V=O stretching band is observed (cf. Figure 7). In particular, the peaks at ~1365 and 1557 cm^{-1} in aqueous solution (Figure 2a) have been assigned to the core-size- (and coordination-) sensitive C_α–N and C_β–C_β stretching, respectively.[30] In aqueous solution, these modes are for the **6C** species and the corresponding modes for the **5C** species are at 1369 and 1562 cm^{-1}, respectively, which can be observed in nonaqueous solutions like acetonitrile (data not shown).

The frequencies of these modes, however, are not very sensitive to binding to DNA polymers. For example, only the **5C** bands of VOTMpyP(4) can be observed in this spectral region with 406.7-nm excitation when either $[poly(dGdC)]_2$ or poly(U) is present. The frequencies of these two coordination sensitive modes are within 1 cm^{-1} to those of the unbound **5C** VOTMpyP(4) in nonaqueous solutions. On the other hand, two pairs of overlapping peaks can be observed in this region in VOTMpyP(4) solutions containing $[poly(dAdT)]_2$ or CT DNA. The Raman bands at ~1365 and 1557 cm^{-1} can be assigned to the **6C** vanadyl porphyrin, and the bands at ~1370 and 1563 cm^{-1}, to the corresponding **5C** species. These frequencies are identical to those of the corresponding **5C** and **6C** bands for VOTMpyP(4) in nonaqueous solvents. Although a change in the relative amounts of **5C** to the **6C** species is evident, from the data in this region alone it is not clear whether the **6C** species is bound. However, the results appear to eliminate partial intercalation; intercalation might lead to more significant changes in the Raman spectrum, since the meso substituents would have to become more coplanar with the porphyrin.

The existence of a bound **6C** species, however, can be evaluated in the spectral region between 900 and 1050 cm^{-1}. For all nucleic acid polymers studied, three overlapping bands can be observed (Figure 7). Two of these peaks can be assigned to the V=O stretching modes for the two coordination forms. The third peak at ~1016 cm^{-1} is due to the porphyrin ring. Table II lists the frequencies of these peaks for the various nucleic acid polymers and dextran sulfate; it is clear that the **5C** and **6C** V=O stretching modes are different from 957 cm^{-1}. Thus both **5C** and **6C** species are bound to the polymers.

It is interesting to compare the V=O stretching frequencies

for both the **5C** and **6C** bound forms to those of the unbound forms in an aqueous environment (**5C**, 996 cm^{-1}; **6C**, 957 cm^{-1}). The frequency of this mode for the bound **5C** species is lower than that in water for all DNA polymers studied, indicating a weakening of the V=O bond. In contrast, the frequency for the bound **6C** species is higher than that in water, suggesting a stronger V=O bond.

The relative amount of the two bound species and the frequency shifts for the bound species are different for the different DNA polymers. For example, more **5C** species can be observed when VOTMpyP(4) binds to poly(U) or $[poly(dGdC)]_2$. In fact, the changes observed upon binding to CT DNA are very similar to those upon binding to $[poly(dAdT)]_2$, suggesting that AT sites are preferred over GC sites. From Table II, an order of the effectiveness of the polymers in the conversion of **6C** to **5C** species can be established: poly(U) > $[poly(dGdC)]_2$ > $[poly(dAdT)]_2$ > CT DNA > dextran sulfate. It is interesting to note that this order is different from the corresponding one for the nickel porphyrins.[3] The difference may arise from the fact that both coordination forms of VOTMpyP(4) can bind to the polymers, whereas this may not be true for NiTMpyP(4).

From the temperature studies of VOTMpyP(4) bound to CT DNA, on the other hand, it is clear that the relative change in intensity ratio of the **5C** V=O stretching band increased, whereas that of the **6C** band decreased as temperature increased (cf. Figure 9). This result suggests that the binding of VOTMpyP(4) to CT DNA favors five-coordination at higher temperature. Furthermore, there is a shift to higher frequency for the V=O stretching mode for both the **6C** form in solution and the **5C** and **6C** forms bound to CT DNA with increasing temperature, indicating a stronger V=O bond.

VOTMpyP(4) binds very selectively with $[poly(dAdT)]_2$ compared to $[poly(dGdC)]_2$. Figures 16 and 17 clearly show that VOTMpyP(4) binds with $[poly(dAdT)]_2$ much more strongly than $[poly(dGdC)]_2$. The difference between $[poly(dGdC)]_2$ and the other DNAs was suggested to depend on the conformational flexibility.[1] CT DNA and $[poly(dAdT)]_2$ are more flexible and appear to be more readily distorted by porphyrin binding.

The induced CD band in the visible region changed from a weak positive band at low R values to a strong, split band at high R values. Fiel[34] suggested that the splitting of the positive CD band reflects interactions between the porphyrins, whereas the single positive band at low R reflects outside binding of the porphyrin with the polynucleotide. The induced visible CD spectra are thus fully consistent with outside binding.

Distinct CD signal changes in the UV region were observed for CT DNA and $[poly(dAdT)]_2$ but not for $[poly(dGdC)]_2$ on titration with VOTMpyP(4). DNA CD signal changes upon binding of cationic porphyrin have been reported by Fiel.[33,34] Among the different cationic porphyrins studied, only TMpyP-(4) caused a remarkable change of the CT DNA CD signal. At high R values, the negative peak at 245 nm of CT DNA was reduced, and the positive peak at 275 nm was split into a distinct positive–negative–positive pattern. At even higher R values, the positive band of CT DNA at 275 nm was inverted to a negative band. TMpyP(2), TMpyP(3), and TMAP caused only intensity decreases. In contrast, inversion in the CD spectra of $[poly(dAdT)]_2$ was caused not only by TMpyP(4) but also by TMpyP-(3) and TMAP; the inversion was suggested to reflect conformational changes of the polynucleotide.

In order to understand the changes in the DNA CD spectra induced by VOTMpyP(4) binding, we studied additional porphyrins: the intercalators, TMpyP(4) and NiTMpyP(4), and the outside binder, ZnTMpyP(4). Some differences were observed when these porphyrins bound to DNA. First, we discuss changes in the $[poly(dGdC)]_2$ spectrum, which has a positive band at 270 nm with a shoulder at ~285 nm and a negative band at 252 nm (Figure 14). TMpyP(4) and NiTMpyP(4) caused an increase of the ellipticity of the positive band, while both ZnTMpyP(4) and VOTMpyP(4) first increased and then decreased the ellipticity of the positive 270-nm band. Second, all porphyrins altered the CD spectrum of $[poly(dAdT)]_2$, which has a conservative CD spectrum with the positive band at longer wavelength, 262 nm. All of the porphyrins studied converted the positive band into a negative band (cf. Figure 12). At high R, TMpyP(4) and NiTMpyP(4) induced a nonconservative spectrum with a broad and more intense negative band. In contrast, ZnTMpyP(4) and VOTMpyP(4) induced a negative–positive–negative pattern even at $R = 1$. For CT DNA, at high R values, TMpyP(4) induced only one negative CD band in the UV region; VOTMpyP(4) and ZnTMpyP(4) induced a negative–positive–negative pattern similar to those observed in $[poly(dAdT)]_2$ titrations. NiTMpyP(4) has an intermediate effect. From these comparisons, we concluded that VOTMpyP(4) and ZnTMpyP(4) bind to DNA in a similar manner. NiTMpyP(4) binding is most similar to that of TMpyP-(4); this conclusion is consistent with our previous resonance Raman study[3] of NiTMpyP(4) since NiTMpyP(4) becomes four-coordinate even when it is an outside binder. The overall shape of NiTMpyP(4) is thus similar to that of TMpyP(4).

Binding of all the porphyrins studied caused inversion of the positive CD band of CT DNA and $[poly(dAdT)]_2$. This inverted CD pattern is very similar to that of the X-DNA CD pattern reported by Vorlickova.[35] X-DNA was first observed with $[poly(dAdT)]_2$ in aqueous solution[36] but under very stringent conditions (6 M CsF). Later, other DNA sequences such as poly(dAdC)·poly(dGdT)[37] were reported to adopt the X conformation in the presence of a high concentration of CsF. In contrast, $[poly(d2aminoAdT)]_2$[38] can adopt the X conformation in high salt or alcohol solutions. In this study, we found that $[poly(dAdT)]_2$ and CT DNA can exhibit the CD pattern of the X conformation even at low porphyrin concentration (3.5×10^{-5} M).

Conclusions

The V=O stretching frequency of **6C** VOTMpyP(4) is sensitive to solvent. The **6C** form does bind to DNA, but some of the VOTMpyP(4) is converted to a bound **5C** form; this form has not been previously directly observed in an aqueous environment. The amount of the **5C** form depends on the polymer. The V=O stretching frequency of the **5C** form in H_2O is ~996 cm^{-1}, not ~970 cm^{-1} as previously estimated.

The Raman spectra are not consistent with partial intercalation of VOTMpyP(4). Viscosity and CD studies also do not support intercalation. An extensive comparison of CD patterns in the UV region suggests that this method may be a valuable additional spectroscopic probe to assess binding.

Competitive studies reveal a strong AT vs GC preference for VOTMpyP(4) binding. AT binding is accompanied by DNA structural changes that are similar to those induced by ZnTMpyP-(4)—an established outside binder.

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