is influential at pH 6.3. One possibility for *T.z.* is the locality defined by His58, with (nearby) conserved polypeptide lysines at positions 74 and 75, which are close to the active site (His73 is a ligand). In the case of *P.g.* there is no His at position 58 and the negatively charged Glu at 76 presumably makes this region less influential. The fact that $[Co(dipic)_2]^-$ does not give saturation kinetics rules out the possibility that there is a rate-controlling process involving the protein (P → P*) prior to oxidation. We see no reason to favor the so-called "dead-end" mechanism.[44]

Finally it is of interest to make further comparisons of the reactivities of *T.z.* and *P.g.* hemerythrins. Whereas rate constants for the reduction of octameric metHr by $[Co(sep)]^{2+}$ and $[Co(9\text{-}aneN_3)_2]^{2+}$ are very similar, those for $[Fe(CN)_6]^{3-}$ oxidation of deoxy (*P.g.*) are significantly slower ($\sim 10^3$ times at pH 8.2 and 500 times at pH 6.3). It is possible that the faster reaction of $[Fe(CN)_6]^{3-}$ with deoxy (*T.z.*) ensures that isomerization and not redox is rate-determining in the second stage (Scheme II).

Acknowledgment. We thank the Science and Engineering Research Council (U.K.) for support (to G.D.A.) and wish to acknowledge helpful discussions and correspondence from Professors D. M. Kurtz, Jr., and R. G. Wilkins.

Registry No. $[Co(dipic)_2]^-$, 71605-21-5; $[Ru(NH_3)_5(CH_3CN)]^{3+}$, 44819-54-7; $[Fe(CN)_6]^{3-}$, 13408-62-3; $[Co(sep)]^{3+}$, 72496-77-6.

(42) See e.g. sequences in: Sykes, A. G. *Adv. Inorg. Bioinorg. Mech.* **1982**, *1*, 156.
(43) Harrington, P. C.; Wilkins, R. G. *J. Inorg. Biochem.* **1983**, *19*, 334.
(44) See e.g. discussion in: Armstrong, F. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1978**, *100*, 7710.

Contribution from the Departments of Chemistry, Emory University, Atlanta, Georgia 30322, and Georgia State University, Atlanta, Georgia 30303

Metalloporphyrin Effects on Properties of DNA Polymers

James A. Strickland,† Debra L. Banville,† W. David Wilson,*‡ and Luigi G. Marzilli*†

Received January 14, 1987

Interactions of metallo derivatives of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin [TMpyP(4)] and *meso*-tetrakis(2-*N*-methylpyridiniumyl)porphyrin [TMpyP(2)] with several native and synthetic DNAs were studied by a variety of physical techniques: viscosity, flow dichroism (FD), and NMR (^{31}P and ^1H). The porphyrins were divided into two groups, group I and group III, based on the criteria suggested by Banville et al. Group I porphyrins include NiTMpyP(4) and PdTMpyP(4). Large negative reduced dichroisms (^{red}D) observed in FD studies indicated that the group I porphyrins were bound perpendicular to the axis of calf thymus DNA. ^{31}P NMR spectra of salmon sperm DNA with the group I porphyrins revealed a small broad downfield peak centered at ca. −1 ppm. No significant shifts were noted for the imino proton signals (ca. 12–14 ppm) of salmon sperm DNA on addition of the group I porphyrins. Comparison of the effects of the group I porphyrins on DNAs with different GC content revealed larger changes in solution viscosity with increased GC content. Viscosity changes of AT-rich DNAs were dramatically lower, and precipitation of the DNA–porphyrin adducts was often observed. For poly[d(A-C)(G-T)], a large increase was found in the solution viscosity upon treatment with NiTMpyP(4), raising the possibility of mixed GC/AT intercalation sites for NiTMpyP(4). In addition, NiTMpyP(4) increased the viscosity of calf thymus DNA (CT DNA) to a greater extent than PdTMpyP(4) or TMpyP(4). Contrary to previously reported studies, our viscosity data suggest that NiTMpyP(4) is less selective than TMpyP(4) in intercalative type binding to GC base pairs of DNA; i.e., NiTMpyP(4) also binds to mixed AT/GC sites. However, our viscosity results with PdTMpyP(4) are similar to those reported with TMpyP(4), indicating similar binding properties. Group III porphyrins include NiTMpyP(2), CoTMpyP(4), FeTMpyP(4), ZnTMpyP(4), and SnTMpyP(4). No viscometric increase, low ^{red}D values, and the absence of signals shifted downfield or upfield in ^{31}P and ^1H NMR spectra, respectively, indicate outside, randomly oriented binding. SnTMpyP(4) was shown to nick CCS DNA. This nicking requires the presence of light and may be a radical process dependent on oxidation–reduction of the porphyrin ring system. In any case, it is likely that previously reported changes in the viscosity of CCS DNA with group III porphyrins could be the result of nicking and not intercalation. Thus, all the results we have with group III porphyrins are suggestive of outside binding. The binding of group I porphyrins to DNA is complex, and decreases in signal area in both ^{31}P and ^1H NMR spectra are attributed to outside self-stacking, which leads to aggregation of the polymer at the high concentrations needed for NMR studies. Such signal loss is not usually observed for group III porphyrins, which cannot self-stack. However, in a few cases, some signal loss was evident, and this result was attributed to paramagnetic species. In general, the binding properties of metalloporphyrins appear to reflect those of nonmetalloporphyrins; i.e., the results with group I species are consistent with intercalation whereas those with group III species are most consistent with electrostatic interactions. However, it is not possible to extrapolate the results found with oligonucleotides to those found with these polymers.

Introduction

Binding between DNA and DNA constituents with tetracationic porphyrins and metalloporphyrins has been the subject of numerous recent investigations. Many different techniques (^1H NMR,[1-4] ^{31}P NMR,[1,3,4] circular dichroism,[5-10] viscosity,[1,3,5,6,8,11] fluorescence,[12] flow dichroism,[1] electrophoresis,[11,12,14] electron spin resonance,[15] melting studies,[6] UV–vis[2,6-9,13,16] and resonance Raman[17] spectroscopy, and kinetics[13]) have been applied to a multitude of different polymers and, to a lesser extent, to monomers[2,17] and oligomeric nucleic acid species.[4] However, physical methods used in studies with metalloporphyrins have largely been limited to electronic spectroscopy.

We have recently reported two detailed studies of *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin (TMpyP(4)) (Figure 1). These studies extended the range of nucleic acid polymers that have been studied and included the most extensive NMR

† Emory University.
‡ Georgia State University.

(1) Banville, D. L.; Marzilli, L. G.; Strickland, J. A.; Wilson, W. D. *Biopolymers* **1986**, *25*, 1837.
(2) Pasternack, R. F.; Gibbs, E. S.; Gaudemer, A.; Antebi, A.; Bassner, S.; DePoy, L.; Turner, D. H.; Williams, A.; Laplace, F.; Lamsurd, M. H.; Merienne, C.; Perree-Favet, M. *J. Am. Chem. Soc.* **1985**, *107*, 8179.
(3) Banville, D. L.; Marzilli, L. G.; Wilson, W. D. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 148.
(4) Marzilli, L. G.; Banville, D. L.; Zon, G.; Wilson, W. D. *J. Am. Chem. Soc.* **1986**, *108*, 4188.
(5) Fiel, R. J.; Carvlin, M. J.; Byrnes, R. W.; Mark, E. H. *Mol. Basis Cancer, Proc. Conf., 1984, Part B.* **1985**, 215.
(6) Fiel, R. J.; Howard, J. C.; Mark, E. H.; Datta Gupta, N. *Nucleic Acids Res.* **1979**, *6*, 3093.
(7) Carvlin, M. J.; Mark, E.; Fiel, R. J.; Howard, J. C. *Nucleic Acis Res.* **1983**, *11*, 6141.
(8) Carvlin, M. J.; Fiel, R. J. *Nucleic Acids Res.* **1983**, *11*, 6121.
(9) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406.

0020-1669/87/1326-3398$01.50/0

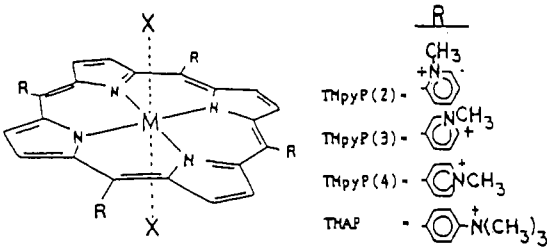

Figure 1. Metalloporphyrins used in this study. The axial substituent, X, may be present or absent depending on the metal, M.

study reported to date.[1] In a second study with oligonucleotides,[4] we found that TMpyP(4) had a selective interaction with 5′-CG-3′ sites which leads to an unusual downfield ^{31}P signal (ca. −1.0 ppm relative to trimethyl phosphate, TMP). Also, imino ^{1}H signals of bases located one to two base pairs away from the unique 5′-CG-3′ binding site are shifted upfield by ca. 0.5 to 1.5 ppm, consistent with the large magnetic anisotropy caused by the porphyrin ring current. The NMR shift changes, as well as other changes in physical properties on TMpyP(4) binding to DNA polymers, are consistent with intercalative binding.

Intercalation involves the insertion of the porphyrin ring between the adjacent base pairs in the DNA. However, demonstrating beyond question that intercalation has occurred requires more detailed structural information than is currently on hand. Studies aimed at obtaining structural details of the binding of TMpyP(4) itself to the 5′-CG-3′ site have been hampered by the presence of additional, different binding sites on nucleic acids and oligonucleotides. These additional sites may involve less specific outside binding by the cationic porphyrin species to the DNA, cooperative or anticooperative effects, etc.

One problem noted previously[1] was that viscosity increases of solutions of CT DNA (calf thymus DNA) induced by TMpyP(4) appear to be too large to be accounted for by binding to 5′-CG-3′ sites, which have an abundance of only 1.6%.[18] Furthermore, the NMR spectral changes found with native DNAs were not as consistent with intercalation as were those for oligonucleotides containing 5′-CG-3′ sites or for poly[d(G-C)$_2$].

Several studies in the literature indicate that metalloporphyrin derivatives of TMpyP(4), (M(TMpyP(4)), e.g. NiTMpyP(4)), are more selective for "GC" over "AT" binding sites.[9,13] We hoped that a more selective type of binding would be found with either NiTMpyP(4) or other M(TMpyP(4)) species. Such selectivity could lead to the presence of one oligonucleotide–porphyrin adduct, which then could be investigated in detail by 2D NMR methods. As a prelude to carrying out an extensive investigation of M(TMpyP(4)) binding with oligonucleotides, we have used several techniques to compare the effects of M(TMpyP(4)) species on native and synthetic polymeric DNAs with those of TMpyP(4). It was possible that the difficulties encountered in rationalizing the results for oligonucleotides with those for polymers, particularly the NMR results, could be overcome if the more selective binding suggested by electronic spectroscopy did indeed occur. Length effects and adjacent sequence effects on local structure are clearly evident in recent investigations of DNA properties.[19-22] Thus, studies on both polymers and oligonucleotides are required for an adequate understanding of DNA binding.

There are other reasons for studying metalloporphyrin binding to DNA. Some metalloporphyrins have been used as bleomycin analogues to cleave DNA.[23] CuTMpyP(4) promotes the B to Z conversion of (poly[d(G-C)$_2$]), whereas Mn^{III}TMpyP(4) does not.[24] M(TMpyP(4)) species where M = Co(III), Mn(III), or Fe(III) have been shown to cleave DNA in the presence of ascorbate, superoxide ion or iodosobenzene.[2] ^{111}In-labeled porphyrins have been used for lymph node imaging,[26-28] while other porphyrin ring systems have been employed to detect and destroy neoplastic cells by a variety of methods.[29]

Experimental Section

Materials. DNA Solutions. For viscometric, flow dichroism (FD), and NMR studies, the following native and synthetic DNA polymers were used: *Micrococcus Lysodeikticus* (ML DNA) and *Clostridium perfringens* (CP DNA) from Sigma; calf thymus (CT DNA) and salmon sperm (SS DNA) from Worthington; poly(dG-dC)·poly(dG-dC) (poly[d(G-C)$_2$]), poly(dA-dT)·poly(dA-dT) (poly[d(A-T)$_2$], poly(dA-dC)·poly(dG-dT) (poly[d(A-C)(G-T)]), and poly(dA)·poly(dT) (poly[d(A)·d(T)]) from P. L. Biochemicals. Col E$_1$ plasmid closed circular supercoiled DNA (CCS DNA) was prepared as previously described by Jones et al.[30]

For viscosity and NMR studies, the native DNAs used were dissolved and then sonicated to 600 and 200 base pairs, respectively, according to Wilson et al.[31] After sonication, the DNA was phenol and ether extracted, and alcohol precipitated. All DNA's were dissolved in PIPES buffer [0.01 M PIPES (Sigma), 10^{-5} EDTA, doubly deionized water] containing either 0.10 M $NaNO_3$ (PIPES 10) or no $NaNO_3$ (PIPES 00). The buffer solution was adjusted to pH 7.0 with NaOH and passed through a 0.22-μm millipore filter prior to use. The DNAs were then dialyzed extensively against the desired PIPES buffer. The synthetic polymers were used without sonication for the viscometric studies.

CT DNA used for FD studies was prepared by dissolving the DNA in PIPES 10 buffer containing an excess of EDTA (0.01 M). The solution was dialyzed extensively against PIPES 10.

Porphyrins. Salts of the following porphyrin cations were obtained from Mid-Century Chemical and used without further purification: Ni^{II}TMpyP(4), Ni^{II}TMpyP(2), Zn^{II}TMpyP(4)H_2O, Fe^{III}TMpyP(4)-ClH_2O, Co^{III}TMpyP(4)ClH_2O, Sn^{IV}TMpyP(4)Cl_2, Pd^{II}TMpyP(4), and TMpyP(4), where TMpyP(4) is *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin and TMpyP(2) is *meso*-tetrakis(2-*N*-methylpyridiniumyl)porphyrin.[7,32] For NMR studies, the Ni^{II}TMpyP(4) iodide salt was converted to the chloride form with an anion-exchange resin (Bio-Rad AG-1X8). All other porphyrins were received in the chloride form. For simplicity, porphyrin species are abbreviated in this paper without designation of oxidation states, charge, counterions, or axial ligands.

Porphyrin Concentration Determination. Porphyrin concentrations were determined by using the following extinction coefficients:

(10) Carvlin, M. J.; Datta-Gupta, N.; Fiel, R. J. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 66.
(11) Fiel, R. J.; Munson, B. R. *Nucleic Acids Res.* **1980**, *8*, 181.
(12) Kelly, J. M.; Murphy, M. J.; McConnel, D. J.; OhUigin, C. *Nucleic Acids Res.* **1985**, *13*, 167.
(13) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 5409.
(14) Musser, D. A.; Datta-Gupta, N.; Fiel, R. J. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 918.
(15) Dougherty, G.; Pilbrow, J. R.; Skorobogaty, A.; Smith, T. D. *J. Chem. Soc., Faraday Trans. 2* **1985**, *81*, 1739.
(16) Fiel, R. J.; Beerman, T. A.; Mark, E. H.; Datta-Gupta, N. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1067.
(17) Blom, N.; Odo, J.; Nakamoto, K.; Strommen, D. P. *J. Phys. Chem.* **1986**, *90*, 2847.
(18) *CRC Handbook of Biochemistry and Molecular Biology*, 3rd ed.; Fasman, G. D., Ed.; CRC: Boca Raton, FL, 1984; Vol. II.
(19) Tung, C. S.; Harvey, S. C. *J. Biol. Chem.* **1986**, *261*, 3700 and references therein.
(20) Wilson, W. D.; Wang, Y. H.; Krishnamoorthy, C. R.; Smith, J. C. *Biochemistry* **1985**, *24*, 3991 and references therein.
(21) Koo, H. S.; Wu, H. M.; Crothers, D. M. *Nature (London)* **1986**, *320*, 501 and references therein.
(22) Saenger, W. *Principles of Nucleic Acid Structure*; Cantor, C. R., Ed.; Springer-Verlag: New York, 1984; and references therein.
(23) Lown, J. W.; Sondhi, S. M.; Ong, C. W.; Skorobogaty, A.; Kishikawa, H.; Dabrowiak, J. C. *Biochemistry* **1986**, *25*, 5111.
(24) Pasternack, R. F.; Sidney, D.; Hunt, P. A.; Snowden, E. A.; Gibbs, E. J. *Nucleic Acids Res.* **1986**, *14*, 3927.
(25) Ward, B.; Skorobogaty, A.; Dabrowiak, J. C. *Biochemistry* 1986, *25*, 6875. See also: Bromley, S. D.; Ward, B. W.; Dabrowiak, J. C. *Nucleic Acids Res.* **1986**, *14*, 9133.
(26) Vaum, R.; Heindel, N. D.; Burns, H. D.; Emrich, S.; Foster, N. *J. Pharm. Sci.* **1982**, *71*, 1223.
(27) Foster, N.; Woo, D. V.; Kaltovich, F.; Emrich, J.; Ljungquist, C. *J. Nucl. Med.* **1985**, *26*, 756.
(28) Robinson, G. D.; Alavi, A.; Vaum, R.; Staum, M. *J Nucl. Med.* **1986**, *26*, 239.
(29) Van den Bergh, H. *Chem. Br.* **1986**, *22*, 430.
(30) Jones, R. L.; Davidson, M. W.; Wilson, W. D. *Biochem. Biophys. Acta* **1979**, *77*, 561.
(31) Wilson, W. D.; Keel, R. A.; Mariam, Y. H. *J. Am. Chem. Soc.* **1981**, *103*, 6267.
(32) Hambright, P., personal communication.

NiTMpyP(4), $\epsilon_{418} = 1.5 \times 10^5$ M^{-1} cm^{-1}; NiTMpyP(2), $\epsilon_{428} = 2.0 \times 10^5$ M^{-1} cm^{-1}; ZnTMpyP(4), $\epsilon_{436} = 2.04 \times 10^5$ M^{-1} cm^{-1}; FeTMpyP(4), $\epsilon_{424} = 1.02 \times 10^5$ M^{-1} cm^{-1}; CoTMpyP(4), $\epsilon_{434} = 2.15 \times 10^5$ M^{-1} cm^{-1}; SnTMpyP(4), $\epsilon_{423} = 4.3 \times 10^5$ M^{-1} cm^{-1}; TMpyP(4), ϵ_{424} 2.26 $\times 10^5$ M^{-1} cm^{-1}.[33-35] A value of $\epsilon_{418} = 1.68 \times 10^5$ M^{-1} cm^{-1} for PdTMpyP(4) was determined by titrating a PdTMpyP(4) stock solution into a quartz cell containing buffer. The stock solution was prepared by weighing and dissolving the dried compound in PIPES 10 buffer.

Gel Electrophoresis. Agarose gels[36] were prepared by dissolving agarose (1.0% w/v) (Fisher type M) in a hot solution of 0.04 M Tris buffer, (0.04 M Tris, 0.003 M sodium acetate, 0.001 M EDTA). After the agarose solution had cooled below 50 °C, ethidium bromide[37] was added until the final concentration was 0.5 μg/mL. The solution was poured on a horizontal electrophoresis apparatus. Samples consisted of 0.15 μg of CCS DNA and porphyrin at various R values, where R is the molar ratio of porphyrin to DNA-P. The samples, which were aliquots from solutions used in viscometric measurements, were adjusted to 8% sucrose and 0.025% Bromophenol Blue before being loaded on the gels. Electrophoresis was carried out for 2 h at a current of 20 mA at room temperature. The gel was placed in a cold aqueous ethidium bromide solution[37] for staining. It was then examined on a UV light table.

Flow Dichroism. FD instrumentation was used as described in Banville et al.[38] with a rectangular (0.025 × 1 × 3 cm) quartz cell. The DNA–porphyrin solution was made by weighing and adding dried porphyrin to 15 mL of an unsonicated CT DNA (7.5 mM) solution in PIPES 10. Due to the high viscosity of the DNA, the solution was stirred overnight at 40 °C to insure complete dissolution of the porphyrin. Data were obtained by recording the relative absorbance before, during, and after the flow. Only the results in which the absorbance was the same before and after the flow were used in the calculations. The linear dichroism, D, was calculated as the difference $(A_\parallel - A_\perp)$ at 260 nm or at the Soret peak. To determine the absorbance of the DNA solutions without flow (A_0), a 20-μL aliquot of solution was added to 3 mL of PIPES 00 buffer. PIPES 00 was used to insure minimal dissociation of the porphyrin from the DNA. The reduced dichroism (^{red}D) was determined as $(A_\parallel - A_\perp)/A_0$.

Viscometric Methods. Viscometric titrations were performed with Cannon-Ubbelohde viscometers in a thermostated water bath at 31 °C unless otherwise noted. Several time readings were obtained at each titration point. Each point represents at least two time readings with an average deviation of less than ±0.10 s for each titration. At least two separate titrations were performed at each R value. For each titration, a difference in the SRV (the solution-reduced viscosity, the ratio of the viscosity of the DNA–porphyrin complex to that of pure DNA) of two separate titrations of less than ±0.05 constituted reproducibility for this study.

NMR Methods. ^{1}H NMR. ^{1}H NMR spectroscopy was carried out by using a modified ^{1}H-Redfield 21412 pulse sequence on a 360-MHz Nicolet NMR spectrometer with NMC-1280 software.[39,40] Typically, three sets of 2000 transient FIDs (20-bit words) were collected on a given sample. The three FIDs were block-averaged in double-precision mode (40-bit words) and Fourier transformed with 3-Hz line broadening prior to converting the averaged data back to 20-bit words. Each set of data was collected with 8K data points, a 7042-Hz spectral window, and the carrier frequency at 15.5 ppm from TSP.

The NMR samples contained 200 base pair SS DNA prepared as described previously.[1] For the ^{1}H NMR studies, the DNA (0.04 M-P) solution contained 10% D_2O and TSP as an internal reference. Aliquots from aqueous stock solutions of known concentrations of porphyrins were lyophilized and DNA solutions were added.

^{31}P NMR. Spectra were accumulated with quadrature detection on an IBM WP-200SY (81.01 MHz) NMR spectrometer. Line broadening of 1 Hz was used in the Fourier transformation, with a 2000-Hz spectral window, 45° pulse, no time delay between pulses, proton decoupling, approximately 8000 transient FIDs, and a temperature of 22 °C. Trimethyl phosphate 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 (10% D_2O). For each porphyrin titration point, 0.04 M DNA solutions were added to lyophilized aliquots of porphyrin and stirred to ensure complete dissolution.

Results

To aid in the presentation of the results, the porphyrins were organized into three groups by using the criteria suggested by Banville et al.[1] Group I porphyrins induce changes characteristic of intercalation in DNA samples with greater than 40% GC base composition as follows: (i) the increase the linear DNA viscosity of CT DNA, ML DNA, and (poly[d(G-C)$_2$]),[1,3,5,6] (ii) they unwind CCS DNA,[5,8,11,12] (iii) they give large negative ^{red}D results in FD experiments,[1] and (iv) they induce downfield peaks or shoulders in ^{31}P NMR and extreme line broadening in ^{1}H NMR experiments with native DNAs.[1,3,4]

Group II porphyrins, i.e. *meso*-tetrakis(*p*-tri-*N*-methylaniliniumyl)porphyrin (TMAP),[1,7,8,10] are believed to be outside binders, but have additional self-stacking features that induce DNA aggregation.[1] TMAP has been shown to (i) produce small viscometric changes in DNAs with greater than 40% GC content[1,8] but significant changes in the SRV of CP DNA (78% AT),[1] (ii) give large negative ^{red}D results in FD experiments,[1] (iii) broaden ^{31}P NMR signals of DNA but not induce downfield shoulders.[1,4] (iv) produce no upfield signals or shifts in imino ^{1}H NMR signals of DNA and oligonucleotides but, instead, eliminate the signals,[1,4] and (v) be unable to unwind CCS DNA[8].

All of the group III porphyrins give results characteristic of outside binding at both AT and GC sites: (i) they do not cause any significant increase in the SRV for any of the linear or superhelical DNA samples,[1,3,8] (ii) they exhibit ^{red}D values that are much lower than the DNA complexes of group I porphyrins,[1] (iii) they do not induce any significant downfield peaks in ^{31}P NMR spectra of DNA,[1,3] (iv) they cause some broadening of DNA imino proton NMR signals but the effects are much less than for group I and group II porphyrins,[1] and (v) they have been shown to nick CCS DNA.[12,16,25]

Since the metalloporphyrins studied here exhibit effects characteristic of either group I or group III, we have organized the presentation of the results of each type of physical measurement along these lines. Group I porphyrins, i.e. PdTMpyP(4) and NiTMpyP(4), have loosely held axial ligands or none at all and can achieve a temporarily planar structure; the position of the *N*-methyl group in the para position allows rotation of the pyridyl ring.[8]

Group III porphyrins, i.e. CoTMpyP(4), SnTMpyP(4), FeTMpyP(4), ZnTMpyP(4), and NiTMpyP(2), cannot achieve a planar structure because they have either tightly bound axial ligands or a 2-N-methyl pyridyl group that prevents the pyridyl moiety from rotating into the porphyrin plane. CoTMpyP(4)[41] and SnTMpyP(4)[32] are six coordinate. FeTMpyP(4)[42] exists as a six-coordinate species or as a μ-oxo dimer, whereas NiTMpyP(4)[43] can exist as either a six-coordinate or four-coordinate structure. ZnTMpyP(4)[44] is five coordinate, with the Zn atom slightly out of the plane of the porphyrin ring and a water molecule occupying the axial position.

Viscometric Titrations. Group I Porphyrins. As seen in Figure 2, NiTMpyP(4) and PdTMpyP(4) produced large increases, to ca. 2, in the SRV of ML DNA, a GC-rich DNA. Similar increases in the SRV were also observed when titrations were performed with poly[d(G-C)$_2$] (Figure 2).

AT-rich DNAs exhibited little or no increase in the SRV at low R when titrated with NiTMpyP(4) or PdTMpyP(4), as shown in Figure 3. When poly[d(A)·d(T)] was titrated with NiTMpyP(4) and PdTMpyP(4) (Figure 3), the SRV decreased until at

(33) Pasternack, R. F.; Huber, P. R.; Boyd, P.; Engasser, L.; Francesconi, L.; Gibbs, E.; Fasella, P.; Ventura, C. G.; Hinds, L. C. *J. Am. Chem. Soc.* **1972**, *194*, 4511.
(34) Hambright, P.; Gore, T.; Burton, M. *Inorg. Chem.* **1976**, *15*, 2314.
(35) Krishnamurthy, M. *Indian J. Chem., Sect. B* **1977**, *15B*, 964.
(36) Sharp, P. A.; Sagden, B.; Sawbrook, J. *Biochemistry* **1973**, *12*, 3055.
(37) Davidson, M. W.; Griggs, B. G; Lopp, I. G.; Wilson, W. D. *Biochem. Biophys. Acta* **1977**, *479*, 378.
(38) Banville, D. L.; Marzilli, L. G.; Wilson, W. D. *Inorg. Chem.* **1985**, *24*, 2481.
(39) Redfield, A. G.; Kunz, S. D.; Ralph, E. K. *J. Magn. Reson.* **1975**, *19*, 114.
(40) Redfield, A. G.; Kunz, S. D. *NMR and Biochemistry*; Opella, S. J., Lu, P., Eds., Marcel Dekker: New York, 1979; p 225.
(41) Rohrbach, D. F.; Deutsch, E.; Heineman, W. R.; Pasternack, R. F. *Inorg. Chem.* **1977**, *16*, 2650.
(42) Pastnernack, R. F.; Lee, H.; Malek, P.; Spencer, C. *J. Inorg. Nucl. Chem.* **1977**, *39*, 1865.
(43) Pasternack, R. F.; Sutin, N.; Turner, D. H. *J. Am. Chem. Soc.* **1976**, *98*, 1908.
(44) Hoard, J. L. *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: Amsterdam, 1979; p 318.

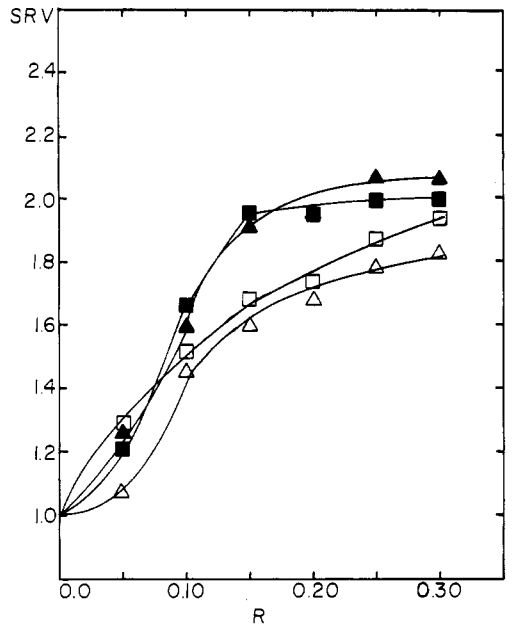

Figure 2. Plots of the solution-reduced viscosity ratio (SRV) vs the molar concentration ratio (R) of the porphyrin to DNA-P in PIPES 10 buffer at 31.00 °C: ML DNA with NiTMpyP(4) (■) and PdTMpyP(4) (▲); and of poly[d(G-C)$_2$] titrated with NiTMpyP(4) (□) and PdTMpyP(4) (△).

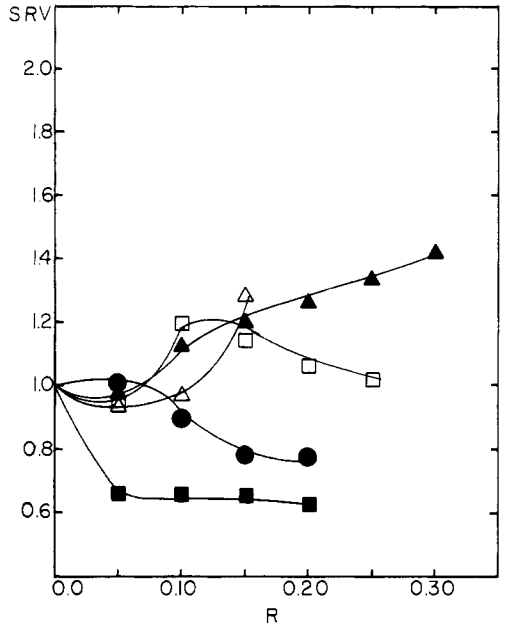

Figure 3. Plots of the SRV vs R in PIPES 10 buffer at 31.00 °C: poly[d(A)·d(T)] with NiTMpyP(4) (●) and PdTMpyP(4) (■); poly[d-(A-T)$_2$] with NiTMpyP(4) (□) and PdTMpyP(4) (△); CP DNA with PdTMpyP(4) (▲).

R = 0.20, a visible precipitate was noted. Precipitation also was observed at R = 0.25 and 0.15 when poly[d(A-T)$_2$] was titrated with NiTMpyP(4) and PdTMpyP(4), respectively (Figure 3). Titrations of CP DNA with NiTMpyP(4) gave scattered results. Precipitation was visible at and above R = 0.15. Before precipitation, the SRV values were scattered outside experimental error. For PdTMpyP(4), results with CP DNA were quite different from that for NiTMpyP(4) (Figure 3). By R = 0.30, the SRV had increased to ca. 1.4.

CT DNA was titrated with NiTMpyP(4) and with PdTMpyP(4) as shown in Figure 4. At low R, the results were similar. At higher R values, PdTMpyP(4) produced the larger increase in the SRV of CT DNA, to ca. 2, while NiTMpyP(4) produced an SRV of ca. 1.6. When poly[d(A-C)(G-T)] was titrated with the group I porphyrins, quite dramatic differences

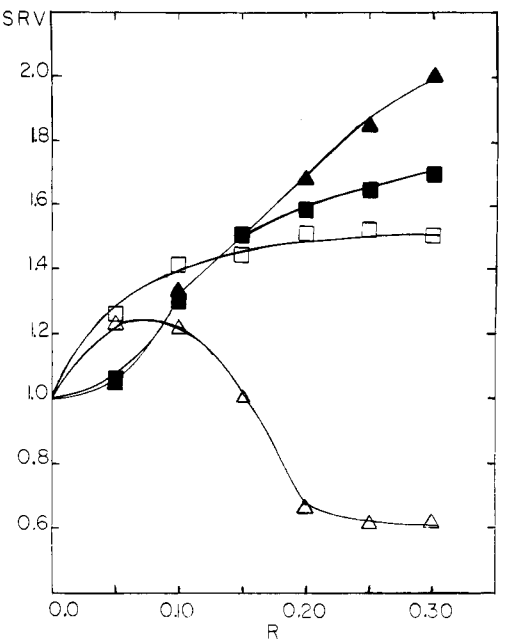

Figure 4. Plots of the SRV vs R in PIPES 10 buffer at 31.00 °C: poly[d(A-C)(G-T)] with NiTMpyP(4) (□) and PdTMpyP(4) (△); CT DNA with NiTMpyP(4) (■) and PdTMpyP(4) (▲).

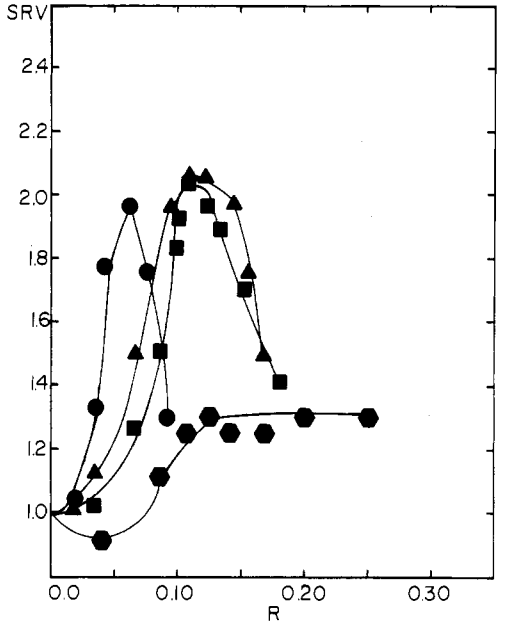

Figure 5. Plots of the SRV of CCS DNA in PIPES 10 buffer at 31.00 °C vs R with NiTMpyP(4) (■), PdTMpyP(4) (▲), ethidium bromide (●), and ZnTMpyP(4) (●).

were noted at high R as shown in Figure 4. Although a large decrease in the SRV was observed when poly[d(A-C)(G-T)] was titrated with PdTMpyP(4), no precipitate was noted. At low R, the effects on SRV were similar for the two porphyrins.

A summary of the group I viscosity results for linear DNAs at low R follows: NiTMpyP(4) and PdTMpyP(4) caused (a) large increases in the SRV of GC-rich DNAs, (b) moderate increases in the SRV of poly[d(A-C)(G-T)], and (c) very small increases in the SRV and, at higher R, often precipitation in AT-rich DNAs, e.g. CP DNA, poly[d(A-T)$_2$] and poly[d(A)]·d(T)]. Viscometric results with linear DNA similar to those obtained particularly with PdTMpyP(4) have been reported for TMpyP(4) by Banville et al.[1]

CCS DNA was titrated with both NiTMpyP(4) and PdTMpyP(4) (Figure 5). In the titration with NiTMpyP(4), an increase and then a decrease in the SRV similar to changes observed with ethidium bromide were found but with a maximum

Table I. Effects of Group III Porphyrins on Solution-Reduced Viscosity of CT DNA[a]

	SRV					
porphyrin	R = 0.04	R = 0.08	R = 0.12	R = 0.16	R = 0.20	R = 0.27
SnTMpyP(4)	1.03	1.05	1.06	1.05	1.06	
ZnTMPyP(4)	0.98	0.94	0.92	0.90	0.88	0.88
CoTMpyP(4)	0.90	0.90	0.86	0.82	0.79	
FeTMpyP(4)	1.03	1.06	1.08	1.01	0.98	0.87
NiTMpyP(2)	1.10	1.11	1.12	1.15	1.11	

[a] PIPES 10, 30.03 °C.

SRV of ca. 2.0 at $R = 0.12$. The dependence of the SRV on added PdTMpyP(4) was similar when titrations were conducted in the dark. An increase in the SRV to ca. 2 at $R = 0.11$ was noted, followed by a decrease to ca. 1.5 at $R = 0.17$. An aliquot was removed and later examined by gel electrophoresis. PdTMpyP(4) titrations of CCS DNA were also conducted in the light. An increase in the SRV to ca. 2 at $R = 0.11$ was noted but a decrease in the SRV upon the addition of PdTMpyP(4) to $R = 0.25$ was never observed. An aliquot was taken from the $R = 0.25$ solution and examined by gel electrophoresis. It was observed that the lanes of the electrophoresis gel that contained the nonirradiated PdTMpyP(4)–CCS DNA solutions showed the mobility expected of CCS DNA. The lanes containing the irradiated solution produced a large smear. This result is consistent with extensive nicking. No photo effect on SRV was observed in titrations performed with CCS DNA and NiTMpyP(4). Even after exposure of an $R = 0.12$ solution to light for 3–4 h, no effect of light was observed.

The apparent unwinding angle of NiTMpyP(4) with CCS DNA was determined according to the methods described by Jones et al.[45] Four titrations were carried out at various CCS DNA concentrations ((3.87–12.09) × 10^{15} M P). The porphyrin concentration that produced the maximum viscosity was determined and plotted against the DNA concentration at the same point of each titration. The slope of the resulting straight line was 0.075, with a correlation coefficient of 0.995. The unwinding angle of NiTMpyP(4) was calculated to be ca. 13°, about half that of ethidium bromide.[46] It should be noted that this is a relative value because of the strong possibility of outside binding of the porphyrins to the DNA.

Group III Porphyrins. Viscometric titrations were carried out with the Group III porphyrins with CT DNA (Table I). None produced significant increases in the SRV (SRV < 1.5). A titration was performed with ZnTMpyP(4) and CCS DNA (Figure 5). Initially, the SRV decreased to 0.86 at $R = 0.04$ and then increased to a maximum SRV of 1.25 at R = 0.13. From that point on, the SRV stayed the same, within experimental error, until the end of the titration.

CCS DNA was titrated with SnTMpyP(4). It was noted that at $R = 0.01$, the SRV was 1.06 but after remaining in fluorescent light for 0.5 h, the SRV increased to 1.34. After a total of 2 and 4 h, the SRV had increased to 1.57 and 1.64, respectively. Examination of the viscometric solution with gel electrophoresis revealed a smear, indicative of nicking.

Flow Dichroism. Group I Porphyrins. The interactions of the Group I porphyrins with CT DNA were studied by FD (Table II). As shown in Figure 6, both NiTMpyP(4) and PdTMpyP(4) exhibited a negative ^{red}D in PIPES 10 both at 260 nm and in the Soret region, where the major absorbing species are the DNA and the porphyrin, respectively. It is important to note that two different samples of CT DNA were used. The initial ^{red}D of the DNA (260 nm) used for PdTMpyP(4) was −0.133. The initial ^{red}D of the DNA used for NiTMpyP(4) and the group III porphyrins (vide infra) was −0.075. After addition of the porphyrin, the ^{red}D values of the DNA for PdTMpyP(4) and NiTMpyP(4)

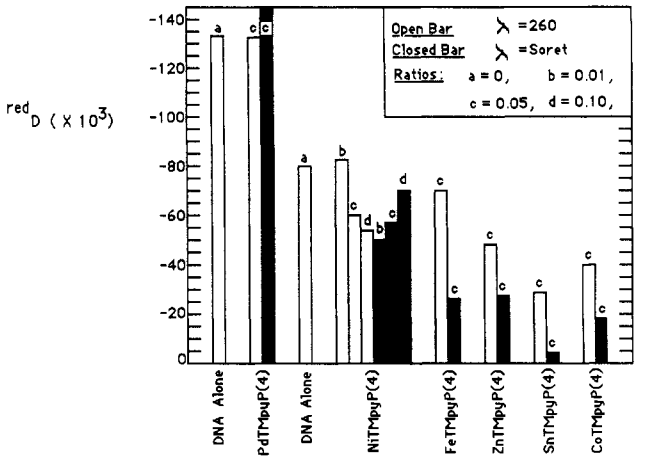

Figure 6. Bar graph of ^{red}D values obtained from FD studies performed with unsonicated CT DNA in PIPES 10 buffer at room temperature with the group I and group III metalloporphyrins.

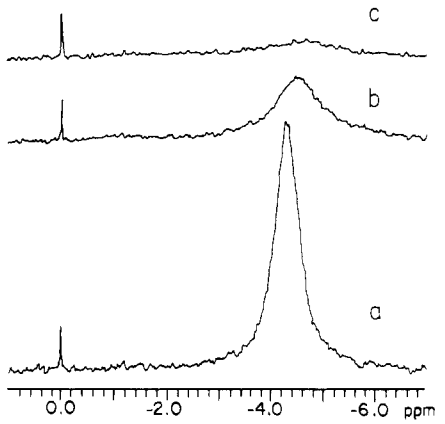

Figure 7. 81-MHz ^{31}P NMR spectra ($T = 22$ °C) of SS DNA (0.04 M P) before and after the addition of NiTMpyP(4) at (a) R = 0.0, (b) $R = 0.10$, and (c) $R = 0.20$.

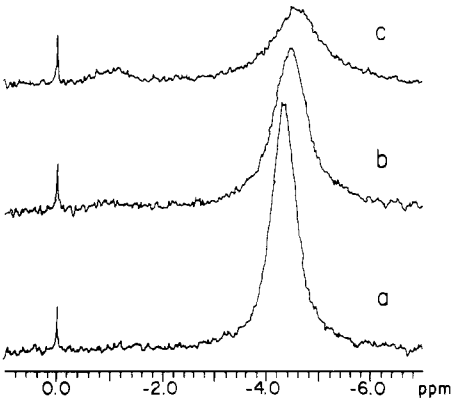

Figure 8. 81-MHz ^{31}P NMR spectra (T = 22 °C) of SS DNA (0.04 M P) before and after the addition of PdTMpyP(4) at (a) $R = 0.0$, (b) $R = 0.10$, and (c) $R = 0.20$.

were −0.134 and −0.060, respectively, at $R = 0.05$. The Soret ^{red}D values for PdTMpyP(4) and NiTMpyP(4) were −0.145 and −0.057, respectively.

Group III Porphyrins. The interaction of the group III porphyrins with CT DNA was studied by FD (Table II). As shown in Figure 6, none of the group III porphyrins produced a very large ^{red}D value, either in the Soret region or at 260 nm.

^{31}P NMR. The ^{31}P NMR spectrum of SS DNA in PIPES 10 consists of a single broad signal at ca. −4.33 ppm from TMP with a full line width at half-height (HHW) of 46 Hz at 81 MHz. A list of the chemical shift and HHW effects of the group I and group III porphyrins on SS DNA is given in Table III. When

(45) Jones, R. L.; Lanier, A. C.; Keel, R. A.; Wilson, W. D. *Biochem. Biophys. Res. Commun.* **1980**, *8*, 1613.
(46) Wang, J. C. *J. Mol. Biol.* **1974**, *89*, 783.

Table II. Flow Dichroism Results with Calf Thymus DNA[a]

porphyrin	ratio	init abs	λ, nm	$A_{\parallel}$	$A_{\perp}$	D	^{red}D
NiTMpyP(4)[b]	0.01	1.348	260	−0.0788	+0.033	−0.112	−0.083
		0.144	428	−0.0052	+0.0021	−0.0073	−0.051
	0.05	1.66	260	−0.070	+0.029	−0.099	−0.060
		0.863	431	−0.035	+0.015	−0.049	−0.057
	0.10	1.77	260	−0.067	+0.029	−0.096	−0.054
		1.62	431	−0.078	+0.033	−0.114	−0.070
PdTMpyP(4)[c]	0.05	0.873	260	−0.066	+0.051	−0.117	−0.134
		0.599	437	−0.062	+0.025	−0.087	−0.145
FeTMpyP(4)[b]	0.05	1.54	260	−0.076	+0.032	−0.108	−0.070
		0.480	428	−0.0093	+0.004	−0.013	−0.027
ZnTMpyP(4)[b]	0.05	1.62	260	−0.054	+0.024	−0.078	−0.048
		1.63	439	−0.024	+0.012	−0.036	−0.022
SnTMpyP(4)[b]	0.05	1.61	260	−0.033	+0.013	−0.046	−0.029
		2.76	424	−0.0056	+0.0030	−0.0086	−0.003
CoTMpyP(4)[b]	0.05	1.61	260	−0.045	+0.019	−0.064	−0.040
		1.06	427	−0.013	+0.0064	−0.019	−0.018

[a] Flow rate = 17.7 mL/min; PIPES 10 buffer. [b] Calf thymus 7.5 mM phosphate; dichroism (D) at 260 nm = −0.0984; reduced dichroism (^{red}D) = −0.0795. [c] Calf thymus 4.26 mM phosphate; dichroism (D) at 260 nm = −0.0937; reduced dichroism (^{red}D) = −0.133.

Table III. Effects of Metalloporphyrins on the ^{31}P NMR Spectrum of Salmon Sperm DNA

porphyrin or DNA	porphyrin/ DNA-P	chem shift,[a] ppm	HHW, Hz	chem shift diff,[b] ppm
SS DNA		−4.33	46	0
SnTMpyP(4)	0.10	−4.34	63	+0.01
	0.20	−4.39	80	+0.05
NiTMpyP(2)	0.10	−4.04	77	−0.29
	0.20	−3.85	128	−0.48
FeTMpyP(4)	0.10	−4.17	72	−0.16
	0.20	−4.07	167	−0.26
ZnTMpyP(4)	0.10	−4.37	96	+0.04
	0.20	−4.54	105	+0.21
CoTMpyP(4)	0.10	−4.27	87	−0.06
	0.20	−4.28	137	−0.05
NiTMpyP(4)	0.10	−4.56	169	+0.23
	0.20	−4.71		+0.48
PdTMpyP(4)	0.10	−4.45	58	+0.12
	0.20	−4.51	102	+0.18

[a] Relative to TMP, [DNA] = 0.04 M in P. [b] Key: +, upfield; −, downfield.

SS DNA was treated with NiTMpyP(4), an upfield shift was noted as shown in Figure 7. This shift change is about twice that reported for TMpyP(4).[1,3] An increase in the HHW of the SS DNA in the presence of NiTMpyP(4) was also observed. Note the emergence of only a weak downfield signal at ca. −1.0 ppm. In spectra taken with SS DNA and PdTMpyP(4) (Figure 8), small upfield shifts of the main signal (less than 0.25 ppm) were noted, as well as an increase in the HHW and the now clearer emergence of a weak downfield signal at ca. −0.9 ppm. For both PdTMpyP(4) and NiTMpyP(4), there was a decrease in the intensity of the main body of peaks at ca. −4.3 ppm with increasing R. Results similar to NiTMpyP(4) have been observed for TMpyP(4).[1] For PdTMpyP(4), a decrease in either DNA concentration (to 0.015 M P) or temperature (to 5 °C) did not affect the intensity decrease of the main body of peaks located at ca. −4.3 ppm. With the group III porphyrins, either small upfield or downfield shifts of the signal with increases in the HHW were observed.

^{1}H NMR. The ^{1}H NMR spectrum of the imino region of SS DNA consists of two broad overlapping signals at ca. 13.6 and 12.7 ppm from TSP that have been assigned to the AT and GC imino protons, respectively.[47] Previously, it had been reported that addition of TMpyP(4), TMpyP(3), or TMAP caused an 80–90% decrease in the total imino signal area.[1]

Group I Porphyrins. SS DNA was treated with the group I porphyrins at different ratios, and the ^{1}H NMR spectra were taken. When NiTMpyP(4) was added, a large decrease in the imino signal area was observed (Figure 9). By R = 0.05, the area loss was about 100%. In the titration with PdTMpyP(4),

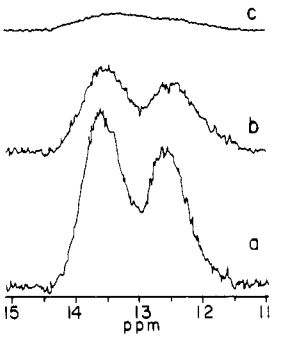

Figure 9. 361-MHz ^{1}H NMR spectra (T = 22 °C) of SS DNA (0.04 M P) before and after the addition of NiTMpyP(4) at (a) R = 0.0, (b) R = 0.02, and (c) R = 0.05.

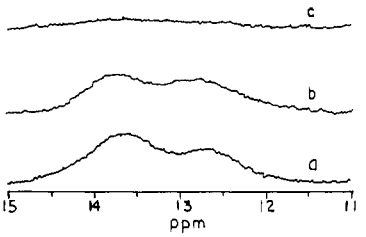

Figure 10. 361-MHz ^{1}H NMR spectra (T = 22 °C) of SS DNA (0.04 M P) before and after the addition of PdTMpyP(4) at (a) R = 0.0, (b) R = 0.05, and (c) R = 0.10.

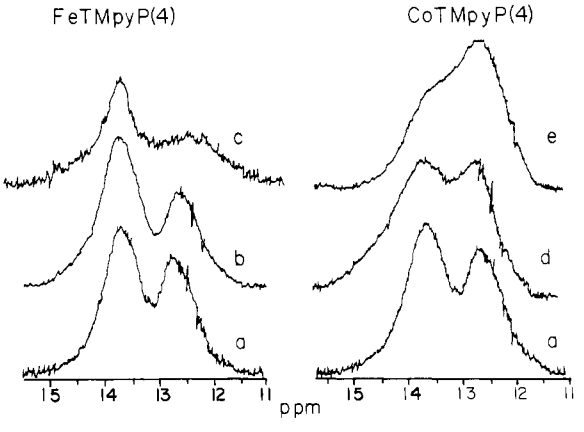

Figure 11. 361-MHz ^{1}H NMR spectra (T = 22 °C) of SS DNA (0.04 M P): before and after the addition of FeTMpyP(4) at (a) R = 0.0, (b) R = 0.05, and (c) R = 0.10; before and after the addition of CoTMpyP(4) in the presence of SS DNA at (a) R = 0.0, (d) R = 0.05, and (e) R = 0.10.

(47) Early, T. A.; Kearns, D. R.; Hillen, W.; Wells, R. D. *Nucleic Acids Res.* **1980**, *8*, 5795 and references therein.

ca. 100% area loss of the imino signals was observed by $R = 0.10$ as shown in Figure 10.

Group III Porphyrins. Of the group III porphyrins, SnTMpyP(4), ZnTMpyP(4), and NiTMpyP(2) had very little effect on the total area of the imino signals with less than a 10% area loss. It is interesting to note that FeTMpyP(4) reduced the GC imino proton signal area by ca. 50% at $R = 0.20$. In contrast, CoTMpyP(4) selectively broadened the AT imino proton signal of SS DNA (Figure 11).

Discussion

This section will be divided as follows. First, we will discuss evidence that indicates two broad classes of binding, namely, intercalative binding at "GC" regions and outside binding (groove binding, partial intercalation, and/or outside self-stacking) primarily at "AT" regions but also conceivably at "GC" or mixed AT/GC regions. Second, we will discuss some of the problems in applying information from oligonucleotides and synthetic polymers to the explanation of the behavior of native DNA. We will highlight studies that have played a major role in identifying base selectivity. Third, we will consider our results for group I metalloporphyrins in the light of the foregoing. Fourth, we will discuss previous studies with the group III porphyrins and analyze our new results. Finally, we will evaluate an alternative explanation for the relationship between binding mode and porphyrin structure in the light of known information.

Binding Modes and Selectivity. Binding Modes. The early work of Fiel and co-workers provided evidence for three types of binding of TMpyP(4) and other porphyrins, particularly TMAP, to DNA.[7,8,10] Some spectral changes (UV–vis, CD) and viscosity results were interpreted as arising from intercalative binding of TMpyP(4).[5-8,11] These data were also analyzed as indicating a second outside binding role for TMpyP(4).[1] Work with TMAP confirmed outside binding and, furthermore, suggested strongly that TMAP at high R could self-stack outside along the helix.[10] Subsequently, studies from both Pasternack's and Fiel's laboratories indicated that some metalloporphyrins have properties similar to TMpyP(4) and thus can also act as intercalators.

In a recent ^{1}H NMR and ^{31}P NMR study with tetradecadeoxyribonucleotides, Marzilli et al.[4] showed that TMpyP(4) bound in a selective, symmetric manner to the 5′-CG-3′ sequences, giving pronounced spectral changes consistent with intercalation and with TMpyP(4) in slow exchange on the NMR time scale. Such pronounced spectral changes were not observed for 5′-GC-3′ or 5′-GG-3′ sequences or for sequences involving AT base pairs. Similar spectral changes were observed with poly[d(G-C)$_2$].[1,4] Viscosity changes with this polynucleotide are also consistent with intercalative binding.[1]

GC vs AT Selectivity. Indications that intercalalative binding was "GC" specific came from nearly simultaneous reports on CD and UV–vis measurements on TMpyP(4) and NiTMpyP(4).[6,7,9] For example, CT DNA and poly[d(G-C)$_2$] produced a 17-nm red shift and hypochromicities of 38% and 46%, respectively, for the Soret band of NiTMpyP(4). When NiTMpyP(4) was titrated with poly[d(A-T)$_2$], a small red shift and a 20% hypochromicity were observed.

Pasternack et al. have explained these UV–vis and other CD and kinetic results as indicating a fundamental difference in binding of these porphyrins between GC and AT base pairs in DNA, with intercalation occurring at the GC base pairs and partial intercalation or outside binding occurring at AT base pairs. It has also been suggested that, in the presence of excess DNA, NiTMpyP(4) prefers to populate GC base pairs but that TMpyP(4) interacts significantly with both AT and GC base pairs; i.e., NiTMpyP(4) is more selective than TMpyP(4) for "GC" sites.[9] With TMpyP(4), the binding constant to poly[d(A-T)$_2$] was twice that of poly[d(G-C)$_2$].[13]

Binding to Native DNAs. Since the original general model of Fiel (intercalation, outside binding, and outside self-stacking) did not include sequence or base pair selectivity, the factors requiring further consideration were delineated.[1] In particular, in native DNAs, mixed-binding modes and their relative influence on DNA properties needed to be considered. The large viscosity changes in DNA were difficult to rationalize in the light of the relatively small percentage of the 5′-CG-3′ sequence in DNA. In particular, could intercalative or other binding modes at other DNA sequences be increasing the DNA viscosity? In contrast to results obtained with poly[d(G-C)$_2$] by ^{1}H and ^{31}P NMR, studies with native DNAs did not reveal clear evidence for a binding mode similar to that identified with oligonucleotides having 5′-CG-3′ sequences or with poly[d(G-C)$_2$]. Furthermore, signal loss was evident, suggesting DNA aggregation at NMR concentrations. For example, self-stacked porphyrin molecules could interact with two DNA duplexes or with the DNA folded back on itself. FD studies did suggest that there was a high degree of alignment with TMpyP(4) and TMAP along the DNA.[1] In light of Pasternack's results,[9,13] it appeared to us that greater insight into the binding mode could be gained by studying NiTMpyP(4) and other metalloporphyrins since more selective GC binding could lead to clearer evidence consistent with intercalative binding in native DNAs.

Group I Porphyrins. Increase in viscosity of native DNA solutions has usually been considered a necessary but insufficient criterion for intercalation. The highly charged porphyrin cations are relatively unique species, and it is not clear whether they could increase viscosity without intercalating or whether they would necessarily increase the viscosity even if intercalated.

The clearest evidence for intercalation is found for poly[d(G-C)$_2$] with TMpyP(4) where the NMR shift effects are consistent with intercalative binding and are very similar to those obtained with oligonucleotides containing the 5′-CG-3′ sequence.[1,4] Both NiTMpyP(4) and PdTMpyP(4) strongly increase the SRV of poly[d(G-C)$_2$] (Figure 2). The increase to ca. 1.8 at R of ca. 0.25 is very close to that found with TMpyP(4).[1] Such similar results suggest a nearly identical binding mode for these three porphyrin species.

It is interesting that, in titrations of CT DNA with NiTMpyP(4) and PdTMpyP(4) (Figure 4), the SRV increases are comparable to those found for poly[d(G-C)$_2$]. In the TMpyP(4) study,[1] the SRV increases for CT DNA were less than those for poly[d(G-C)$_2$]. In view of the relatively small number of 5′-CG-3′ sites in CT DNA, it is puzzling that such large increases were observed with the metalloporphyrins. We believe that these SRV changes with all group I porphyrins are sufficiently large to support the view that sites other than 5′-CG-3′ are involved. The large effects of NiTMpyP(4) and PdTMpyP(4) suggest that, perhaps, these species are less selective than TMpyP(4) for 5′-CG-3′ sites. This possibility is also suggested by the studies with ML DNA (ca. 14.3% 5′-CG-3′ sites)[18] where the group I metalloporphyrins have a larger viscometric effect than that for poly[d(G-C)$_2$] (Figure 2). The TMpyP(4) effects are comparable for these two DNAs, again suggesting greater selectivity of TMpyP(4); i.e., it binds to fewer sites than the group I metalloporphyrins. For AT-rich polymers (Figure 3), the group I metalloporphyrins had relatively minor effects on the SRV except that PdTMpyP(4) increased the SRV of CP DNA at high R. Furthermore, NiTMpyP(4) increased the SRV of poly[d(A-C)(G-T)] at high R (Figure 4). In contrast, the SRV of PdTMpyP(4) and TMpyP(4)[1] decreased at high R. If this effect is indicative of intercalation, then intercalation must occur at sites other than 5′-CG-3′. As indicated above, however, it could be that viscosity changes may not necessarily imply intercalation or that a given type of intercalation would always increase the SRV by a particular amount. Nevertheless, the results are unusual.

A stricter viscometric criterion for intercalation involves CCS DNA. All known intercalators unwind CCS DNA.[45] Both NiTMpyP(4) and PdTMpyP(4) have this ability. The results of Fiel et al.[8] with pBR 322 DNA and NiTMpyP(4) agree with our observations.

Intercalation requires alignment of the planes of the intercalator with the bases of DNA. The DNA is aligned by flowing the DNA solution through a narrow tube, leading to differences in absorbance for light polarized parallel and perpendicular to the flow. The ^{red}D observed for PdTMpyP(4) and NiTMpyP(4) are quite

large (Figure 6), and for NiTMpyP(4), the effect is observed up to $R = 0.1$, well beyond the percentage of 5′-CG-3′ sequences in CT DNA. These results are also consistent with intercalation and imply additional sites of interaction. Furthermore, at low R, the ^{red}D effect with NiTMpyP(4) is comparable to that of TMpyP(4), but at $R = 0.1$, the NiTMpyP(4) ^{red}D is larger. Again, this result suggests that NiTMpyP(4) can bind intercalatively at sites unavailable to TMpyP(4).

Our ^{31}P NMR studies of SS DNA with PdTMpyP(4) and NiTMpyP(4) are comparable to those with TMpyP(4)[1] in that the ^{31}P signal decreases due to aggregation. We had hoped that greater selectivity by the Group I metalloporphyrins might lead to clearly identifiable downfield ^{31}P signals. In the case of the Pd compound, some area at ca. −1.0 ppm is observed and perhaps also with NiTMpyP(4). However, the area is quite small, consistent either with limited selective intercalation or with intercalation that is quite different than that for 5′-CG-3′ sites.

In the ^1H NMR experiments, at high DNA concentration, the ^1H NMR signals decreased, eventually to the base line, without any evidence of upfield-shifted imino signals characteristic of intercalation.[4] Again these results are similar to those found with TMpyP(4).[1] Therefore, no evidence for greater selectivity was observed, but outside self-stacking for the group I metalloporphyrins was noted.

The PdTMpyP(4) species has no axial ligands and, like TMpyP(4), can intercalate without change in the solvated species. On the other hand, NiTMpyP(4) has axial ligation. If the loss in the ^1H NMR and ^{31}P NMR signal intensity is due to outside self-stacking as we suggest, the NiTMpyP(4) would need to lose its axial water molecules since no porphyrin species with axial ligands (i.e. group III) induce as extensive a loss in signal intensity. Overall, PdTMpyP(4) appears to be more similar in its properties to TMpyP(4) than to NiTMpyP(4). The effect of NiTMpyP(4) on poly[d(A-C)(G-T)] could be the result of coordination via some axial site, although, at this time, we have no evidence for such an interaction.

We conclude that, on the basis of our experiments, we cannot support the suggestion by Pasternack et al.[9] that NiTMpyP(4) is more selective than TMpyP(4) for "GC" over "AT" base pairs. Clearly, the situation is more complex. Research currently in progress with oligonucleotides should help to clarify the nature of the binding interaction.

Group III Porphyrins. On the basis of spectrophotometric and kinetic data[5,7,9,13], it has been suggested that the group III porphyrins interact with AT base pairs more extensively than with GC base pairs. Since no large spectral changes are observed, binding of the group III porphyrins with GC base pairs is primarily electrostatic, whereas porphyrin–base overlap may be involved in binding of the group III porphyrins to AT sites.[9] Pasternack et al.[9] have stated that the type of helical distortions that would need to occur for overlap of the porphyrin with GC base pairs would be energetically unfavorable due to the stability of the helix. AT-rich DNA, however, could form external complexes with the group III porphyrins that involve a larger degree of drug–base overlap.[9] Footprinting experiments with group III porphyrins of Mn reveal AT selectivity.[25] It was suggested that the 2-amino group in guanine, which extends into the minor groove, could sterically disfavor outside binding, an interpretation used to explain the AT selectivity of netropsin.[48] Theoretical calculations on the electrostatic potential of DNA have shown that the minor groove of AT sequences possesses a high negative potential.[49,50] This potential would greatly favor the binding of the cationic porphyrins to AT regions.

Viscometric titrations have been performed on DNA with the group III porphyrins.[1,3,5] None of the group III porphyrins produced significant changes over a wide range of porphyrin concentration. We have observed similar results, thus suggesting that group III porphyrin binding to DNA does not produce a helical extension.

When CCS DNA was titrated with ZnTMpyP(4), no significant viscosity increase and thus no unwinding was observed (Figure 5). ZnTMpyP(4), with its one axial water ligand, has an approximate 0.5-nm thickness.[9,44] The ZnTMpyP(4) cation would not be able to intercalate. Fiel et al[5] have observed viscosity increases of CCS DNA with some group III porphyrins at 4–20 times the concentrations of the group I porphyrins. This result was interpreted as an unwinding of the CCS DNA. We do not think, however, that the viscosity increases of CCS DNA produced by the group III porphyrins at higher concentrations are indicative of intercalation (see below).

Our FD results with group III porphyrins show a small ^{red}D when compared to the results for the group I porphyrins (Figure 6). Intercalation requires the insertion of the drug between the base pairs of DNA. This would suggest a highly ordered system. Since the small ^{red}D values with the group III porphyrins are consistent with little alignment of the porphyrin plane with respect to the DNA axis, intercalation by the group III porphyrins seems unlikely.

CoTMpyP(4) selectively broadened the AT imino signals. Rohrbach et al.[41] have shown that the diamagnetic Co^{III}TMpyP(4) species can be reduced easily to the paramagnetic Co^{II}TMpyP(4) species. When reduction occurs, the absorbance maximum exhibits a blue shift from 433 to 429 nm. We observed such a shift. Therefore we propose that Co^{III}TMpyP(4) is reduced at least partly to Co^{II}TMpyP(4). If this reduced, paramagnetic form has an ability to recognize AT sites, then a broadening of the AT imino signal is explained. However, the preferential broadening may be due to greater efficiency of line broadening of the thymine N(3)H than of the guanine N(1)H signal. In footprinting experiments with CoTMpyP(4), a specificity for cleavage at AT-rich sites has been noted.[25] This result suggests preferential AT binding. Viscosity, FD, spectral,[9] and kinetic[13] data provide strong evidence that the loss of the AT signal area is not due to intercalation. FeTMpyP(4) broadened the GC imino signal. Since FeTMpyP(4) can exist in different forms,[42] it is possible that the broadening is caused by one of these paramagnetic FeTMpyP(4) species recognizing GC regions of the DNA and relaxing the imino proton. The selective broadening could be due to a greater efficiency for relaxation of the guanine N(1)H. Again, it is unlikely that FeTMpyP(4) binds by intercalation based on FD, viscosity, spectral,[9] and kinetic[13] data.

It has been shown that some of the group III metalloporphyrins nick DNA.[11,25] Our results suggest that SnTMpyP(4) can nick DNA. According to Richoux et al.,[51] the strongly electronegative Sn(IV) can stabilize a radical on the pyridyl ring of SnTMpyP(4). Aeration of the photolyzed solution restores the original porphyrin. The oxidation and reduction of the porphyrin ring via a radical mechanism is accompanied by spectral changes. We observed color changes of the solution that depend on the presence of light. It has been shown that both TMpyP(4) and ZnTMpyP(4) nick pBR 322 CCS DNA in the presence of light and the nicking mechanism involves the production of singlet oxygen.[12] Therefore we propose that the SnTMpyP(4) nicking of CCS DNA involves a redox process, possibly on the porphyrin ring. Nicking would also explain the unusually low ^{red}D value obtained in our FD study (Figure 6). Such nicking does not require intercalation.

In summary, none of our results with the group III porphyrins are consistent with intercalation. However, our results do not exclude partial intercalation or some base–porphyrin overlap.

Kinetic vs Thermodynamic Explanations for Differences in Binding. Comparative effects of changing the porphyrin species or the DNA on the resulting binding have been explained by several factors, which can be divided crudely into kinetic/steric and thermodynamic/steric effects. For example, the apparent selectivity of outside binding for AT or GC regions is explained

(48) Kopa, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.
(49) Lavery, R.; Pullman, B. *J. Biomol. Struct. Dyn.* **1982**, *2*, 1032.
(50) Weiner, P. K.; Langeridge, R.; Blaney, J. M.; Schaefer, R.; Kollman, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3754.
(51) Richoux, M. C.; Neta, P.; Harriman, A.; Baral, S.; Hambright, P. *J. Phys. Chem.* **1986**, *90*, 2462.

by thermodynamic arguments: the greater flexibility of AT vs GC regions, the steric effects of the guanine amino group, the differences in potential in the GC and AT regions, etc. Alternatively, the apparent intercalative binding of TMpyP(4) and group I metalloporphyrins contrasted with the absence of such binding by group III porphyrins is explained by either a thermodynamic/steric argument (the presence of axial ligands) or a kinetic/steric argument (inability to achieve a planar structure for TMpyP(2), etc).

As stated above, our major objectives here involved evaluating the effects of metalloporphyrins on DNA properties to identify areas where fruitful studies might be conducted with oligonucleotides. However, the apparent differences in effects of group I porphyrins (TMpyP(4), NiTMpyP(4), and PdTMpyP(4)) when combined with the selectivity of TMpyP(4) for 5'-CG-3' over 5'-GC-3' sites have led us to wonder if all effects are not best explained by primarily a thermodynamic/steric argument. The highly charged porphyrins appear to be binding by many competitive modes. If such subtle differences between the group I porphyrins and between sequences lead to differences in binding, then perhaps major changes (TMpyP(4) to TMpyP(2), *N*-methylpyridiniumyl to trimethylanililiniumyl) could make intercalative binding unfavorable. (Again, we repeat that we do not claim that intercalative binding has been established beyond question). For example, steric effects of the *N*-methyl groups of TMpyP(2) could easily prevent intercalation from being thermodynamically possible. Likewise, intercalated TMAP, with its positive groups ca. 1.5 Å further out from the porphyrin center than TMpyP(4), might not be able to form as favorable interactions with the phosphate groups of DNA as does intercalated TMpyP(4).

We believe that this thermodynamic/steric model explains the interaction of complex molecules, like the porphyrins, with DNA and offers an alternative to the "temporarily planar" kinetic explanation for additional reasons. First, complex molecules such as nogalamycin[52,53] and naphthalenediimides,[54,55] which have bulky groups on opposite sides of the planar ring system and which cannot become planar, bind to DNA by intercalation. Second, model building studies indicated that porphyrins, even with the pyridyl groups in the plane of the porphyrin ring, still require significant disruption of the double helix to allow intercalation.

It is clear that a complete understanding of the factors influencing binding of these intriguing porphyrin species must await further information on binding modes and additional kinetic measurements. In particular, studies with oligonucleotides may allow us to differentiate between the models. Oligonucleotides undergo large amplitude dynamic motions, including complete strand separation. These motions would easily allow TMpyP(2) to intercalate if only a kinetic barrier prevented insertion of the porphyrin.

Acknowledgment. This work was supported by NIH Grant GM 29222 to L.G.M. and NSF Grant DBM 8603566 to W.D.W. The Col E_1 CCS DNA was a gift from Dr. Robert L. Jones. The 360-MHz NMR spectrometer was purchased with partial support from the NSF through a departmental grant to Emory University.

(52) Arora, S. K. *J. Am. Chem. Soc.* **1983**, *105*, 1328.
(53) Collier, D. A.; Neidles, S.; Brown, J. R. *Biochem. Pharmacol.* **1984**, *33*, 2877.
(54) Yen, S. F.; Gabbay, E. J.; Wilson, W. D. *Biochemistry* **1982**, *21*, 2070.
(55) Hopkins, H. P.; Stevenson, K. A.; Wilson, W. D. *J. Solution Chem.* **1986**, *15*, 563.

Notes

Contribution from the Dipartimento di Chimica Inorganica, Metallorganica ed Analitica, Università di Padova, Padova, Italy, Istituto di Chimica e Tecnologia dei Radioelementi del CNR, Padova, Italy, and Dipartimento di Chimica Inorganica e Struttura Molecolare, Università di Messina, Messina, Italy

UV Photoelectron Spectra and DV-Xα Calculations on Rh_2^{4+} Formamidinate Complexes

Gian Andrea Rizzi,[1a] Maurizio Casarin,[1b] Eugenio Tondello,[1a] Pasquale Piraino,[1c] and Gaetano Granozzi*[1a]

Received March 2, 1987

Complexes containing the Rh_2^{4+} core have been extensively investigated from both the synthetic[2] and spectroscopic[2] points of view, and their possible applications have been outlined.[3] Most of these studies have been directed toward complexes containing four carboxylate groups as bridging ligands.

Several theoretical studies (using both MS-Xα and minimal basis-set ab initio methods) on the nature of the Rh-Rh bond in carboxylates have been reported.[4] They are all in agreement with the existence of a single Rh-Rh bond, but different electronic configurations for the tetraformate $Rh_2[O_2CH]_4$ complex (**1**) have been obtained according to the different theoretical approaches (MS-Xα,[4a] $\sigma^2\pi^4\delta^2\pi^{*4}\delta^{*2}$; ab initio,[4b] $\pi^4\delta^2\pi^{*4}\delta^{*2}\sigma^2$). However, no experimental determination of one-electron levels by gas-phase photoelectron (PE) spectroscopy has been reported so far because of the thermal decomposition of the carboxylate dimers in the ionization chamber. To our knowledge, the unique PE literature data regarding Rh_2^{4+} complexes refer to the complex $Rh_2(mhp)_4$ (mhp = 2-oxo-6-methylpyridinate), where a $\sigma^2\pi^4\delta^2\pi^{*4}\delta^{*2}$ configuration was proposed in order to nicely fit the experimental data.[5]

Recently, complexes containing formamidinate ligands have been prepared and structurally characterized[6] and the attempt to record their PE spectra has been successful. In this contribution we report the UV excited PE spectra of $Rh_2[HC(NR)_2]_2[O_2CCF_3]_2$ (**2**) and $Rh_2[HC(NR)_2]_4$ (**3**) (R = *p*-tolyl) coupled with the results of *first-principle* discrete variational (DV) Xα quantum-mechanical calculations[7] carried out on the $Rh_2[HC(NH)_2]_2[O_2CH]_2$ (**2a**) and $Rh_2[HC(NH)_2]_4$ (**3a**) models.

Experimental Section

Synthesis. The samples of $Rh_2[HC(NR)_2]_2[O_2CCF_3]_2(H_2O)_2$ (**2c**) and $Rh_2[HC(NR)_2]_4$ (**3**) (R = *p*-tolyl) were synthesized according to the published procedures.[6] After crystallization, their purity was checked by IR and NMR measurements.

(1) (a) University of Padova. (b) CNR of Padova. (c) University of Messina.
(2) (a) Boyar, E. B.; Robinson, S. D. *Coord. Chem. Rev.* **1983**, *50*, 109. (b) Felthouse, T. R. *Prog. Inorg. Chem.* **1982**, *29*, 73.
(3) (a) Hughes, R. G.; Bear, J. L.; Kimball, A. P. *Proc. Am. Assoc. Cancer Res.* **1972**, *13*, 120. (b) Howard, R. A.; Kimball, A. P.; Bear, J. L. *Cancer Res.* **1979**, *39*, 2568.
(4) (a) Norman, J. G., Jr.; Kolari, H. J. *J. Am. Chem. Soc.* **1979**, *101*, 5256. (b) Nakatsuji, H.; Ushio, T.; Kanda, K.; Onishi, Y.; Kawamura, T.; Yonezawa, T. *Chem. Phys. Lett.* **1981**, *79*, 299.
(5) Berry, M.; Garner, C. D.; Hillier, I. H.; McDowell, A. A.; Clegg, W. *J. Chem. Soc., Chem. Commun.* **1980**, 494.
(6) (a) Piraino, P.; Bruno, G.; Tresoldi, G.; Lo Schiavo, S.; Zanello, P. *Inorg. Chem.* **1987**, *26*, 91. (b) Piraino, P.; Bruno, G.; Tresoldi, G.; Lo Schiavo, S.; Zanello, P. *Inorg. Chem.*, in press.
(7) (a) Averill, F. W.; Ellis, D. E. *J. Chem. Phys.* **1973**, *59*, 6411. (b) Rosen, A.; Ellis, D. E.; Adachi, H.; Averill, F. W. *J. Chem. Phys.* **1976**, *65*, 3629 and references therein. (c) Trogler, W. C.; Ellis, D. E.; Berkowitz, J. *J. Am. Chem. Soc.* **1979**, *101*, 5896.

0020-1669/87/1326-3406$01.50/0