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Intercalative and nonintercalative binding of large cationic porphyrin ligands to polynucleotides

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ABSTRACT

The interactions of two positional isomers and one analogue of meso-tetra(4-N-methylpyridyl)porphine, with the synthetic polynucleotides poly[d(A-T)]·poly[d(A-T)] and poly[d(G-C)]· poly[d(G-C)] have been investigated by circular dichroism. All four porphyrins were found to bind to the polynucleotides as shown by the induction of circular dichroism in their Soret bands. Furthermore, the sign of the induced ellipticity reflects selective occupation of binding sites by the porphyrin ligands. The conformational lability of poly[d(A-T)]·poly[d(A-T)] was found to be appreciable as micromolar amounts of meso-substituted 4-N-methylpyridyl, 3-N-methylpyridyl, and p-N-trimethylanilinium porphines induced a CD spectrum similar but not identical to that of DNA in the Z-form, i.e. a negative band at 280 nm and a positive band at 259 nm. The effect of porphyrin binding to poly[d(G-C)]·poly[d(G-C)] was less pronounced and dissimilar to that seen in the AT polymer.

INTRODUCTION

Extensive studies using synthetic polynucleotides have shown that double stranded DNAs composed of alternating purine-pyrimidine sequences are capable of extreme reorganization in structure as dictated by solvent conditions (1-10). Furthermore, recent crystallographic and solution studies suggest that the conformational states available to copolymers of AT and GC base pairs may be quite different (11-14). This implies that distinct structural families, A-type, B-type, Z-type, or any others may or may not be expressed for a given degree of hydration and linear array of base pairs in any polynucleotide, natural or synthetic, whose sequence is characterized as alternating purine-pyrimidine (15). Ligand binding to DNAs of different compositions is one means by which this phenomenon of conformational lability has been demonstrated (16-19). In the present work, we have employed the following DNA interactive ligands as probes to identify and characterize ligand-induced al-

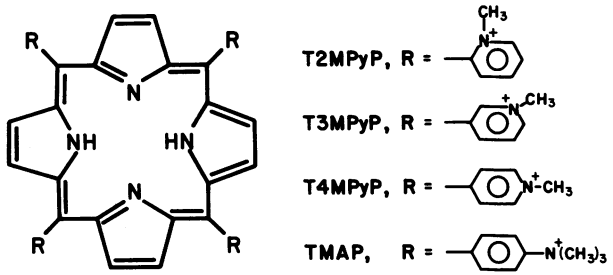

Figure 1. Meso-substituted porphines used in this study: (a) meso-tetra(4-N-methylpyridyl)porphine, (b) meso-tetra(3-N-methylpyridyl)porphine, (c) meso-tetra(2-N-methylpyridyl)porphine, (d) meso-tetra(p-trimethylanilinium)-porphine.

teration in polynucleotide structure: meso-tetra(4-N-methylpyridyl)porphine (T4MPyP), meso-tetra(3-N-methylpyridyl)porphine (T3MPyP), meso-tetra(2-N-methylpyridyl)porphine (T2MPyP), and meso-tetra(para-N-trimethylanilinium)porphine (TMAP), see figure 1. T4MPyP unwinds covalently closed circular DNA and is a known intercalating agent (20,21). T3MPyP also unwinds supercoiled DNA and is presumed to bind to DNA by intercalation (22). TMAP does not unwind supercoiled DNA (22) and binds by self-stacking along the external surface of DNA (23). T2MPyP, a second positional isomer of T4MPyP, is also a nonintercalating porphyrin as characterized by its inability to unwind supercoiled DNA (22). Although this porphyrin binds to the external surface of DNA it probably does not self-stack, as does TMAP, since this would result in superposition of its charged groups.

Preliminary studies have shown differential effects between the binding of T4MPyP to AT versus GC containing copolymers and indicate that alterations in the polynucleotide structure occur consequent to binding (24). These effects were monitored through the use of circular dichroism spectroscopy. In this work we exploit the multiple binding modes of all four different porphyrins in order to investigate these effects in detail and to examine the polymorphism of poly[d(A-T)] · poly[d(A-T)] and poly[d(G-C)]· poly[d(G-C)]. The qualitative features of these differential effects are used to interpret results obtained previously for porphyrins complexed with calf thymus DNA (22).

EXPERIMENTAL

Materials. Poly[d(A-T)]·poly[d(A-T)] and poly[d(G-C)]·poly[d(G-C)] were purchased from P-L Biochemicals. The lyophilized material, 100 A_{260} units, was dissolved in 1 ml of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 6.8 (BPES/no salt) to form a stock solution. CD measurements were conducted on solutions containing 0.150 ml of stock polynucleotides mixed with drug and buffer to a final volume of 1.50 mls. The buffer used for dilution is BPES/0.5 M NaCl, i.e. 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, 0.5 M NaCl, pH 6.8. Meso-tetra(4-N-methylpyridyl)porphine was purchased from Strem Chemicals. Meso-tetra(3-N-methylpyridyl)porphine, meso-tetra(2-Nmethylpyridyl)porphine and meso-tetra(para-N-trimethylanilinium)porphine were provided by Dr. N. Datta-Gupta.

Spectroscopy. Circular dichroism experiments were conducted on a Jasco-J41C spectropolarimeter at a 2 nm spectral bandwidth. Absorption spectroscopy measurements were performed with a Beckman model DB-G recording spectrophotometer.

RESULTS

The visible CD spectra of T2MPyP, T3MPyP, T4MPyP and TMAP complexed with poly[d(G-C)]·poly[d(G-C)] are shown in figure 2, parts a,b,c,d. The ellipticity seen for T3MPyP and T4MPyP, 2b and 2c, is predominantly negative in character, centered at 436 nm in the case of T4MPyP and 427 nm for T3MPyP. The intensity of the negative trough is sensitive to the concentration of porphyrin with the greatest ellipticity occuring at the porphyrin to DNA (phosphate) ratio, R, of 0.235. At values of R greater than 0.235 the negative ellipticity decreases markedly for T3MPyP while a positive peak appears at 418 nm for T4MPyP. The spectrum for T4MPyP·poly[d(G-C)] at this ratio resembles that of T4MPyP·DNA (calf thymus) (20), an indication of structural similarity in the environment of the bound porphyrin in each case.

In contrast the spectrum of T2MPyP, figure 2a, complexed with poly[d(G-C)]·poly[d(G-C)] presents a positive ellipticity. At low values of R there is a single peak centered at 410 nm. Increasing the porphyrin/DNA ratio causes the peak to split into two maxima located at 406 nm and 418 nm. The inability of this porphyrin to unwind DNA, as previously noted, suggests that the induced CD

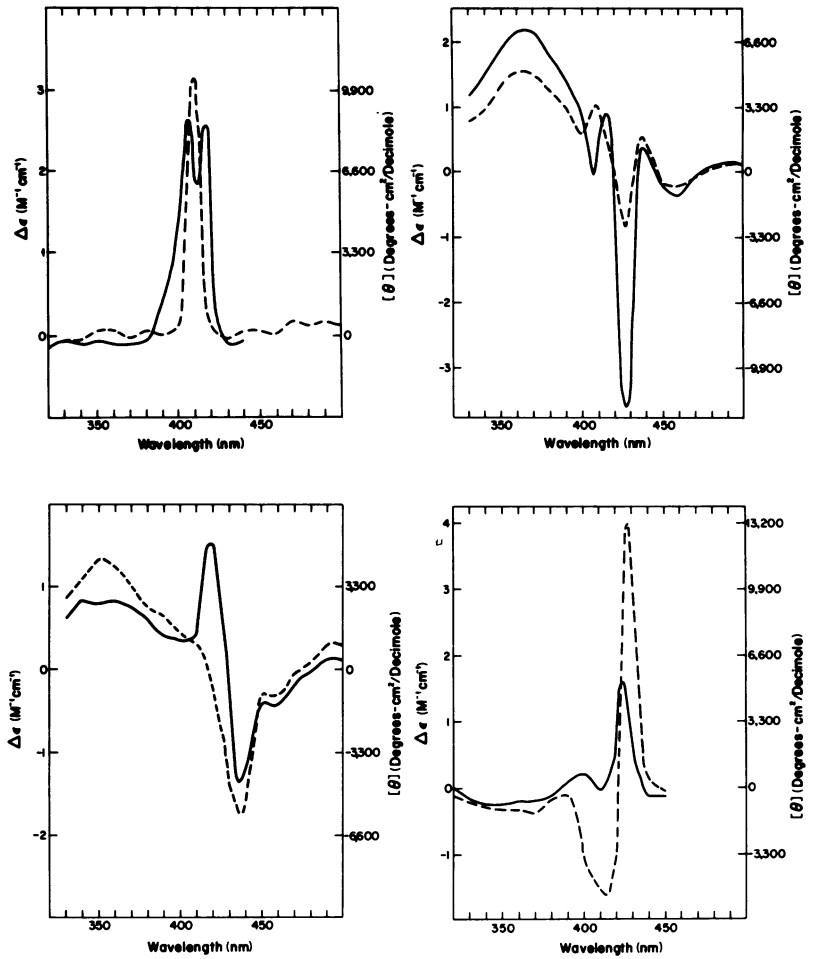

Figure 2. Visible CD spectra of poly[d(G-C)]·poly[d(G-C)] in BPES/0.5 M NaCl buffer with (a) T2MPyP, (b) T3MPyP, (c) T4MPyP, (d) TMAP. Input ratio of [porphryin]/[DNA], R, is 0.235 (- - -) and 0.354 (——). Also, (d) TMAP and poly[d(G-C)]·poly[d(G-C)] in BPES/no salt buffer with input ratios of 0.100 (——) and 0.235 (----).

spectrum is characteristic of the porphyrin externally bound to the polynucleotide. The anilinium derivative, TMAP, which has been shown to bind externally to DNA (23), does not manifest measurable circular dichroism when bound to poly[d(G-C)]·poly[d(G-C)] in BPES/0.5 M NaCl buffer. However, it has a positive ellipticity when bound to calf thymus DNA in this buffer (23) and when bound to

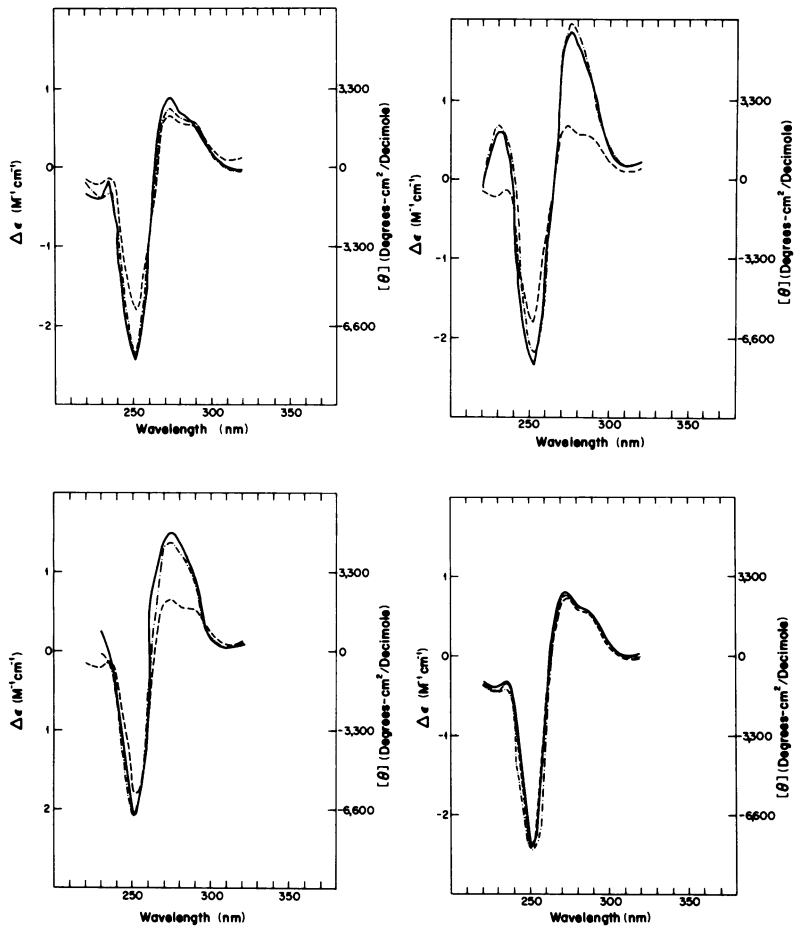

Figure 3. Ultraviolet CD spectra of poly[d(G-C)]·poly[d(G-C)] in BPES/0.5 M NaCl buffer with (a) T2MPyP, (b) T3MPyP, (c) T4MPyP, (d) TMAP. Input ratio of [porphyrin]/[DNA], R, is 0.235 (-·—·) and 0.354 (——). Polynucleotide control is (----).

poly[d(G-C)]·poly[d(G-C)] at lower ionic strength, as in figure 2d.

The ultraviolet CD spectra for T2MPyP, T3MPyP, T4MPyP, and TMAP with poly[d(G-C)]·poly[d(G-C)] are shown in figure 3, parts a,b,c,d. Each porphyrin derivative causes an increase in the intensity of the polynucleotide's characteristic dichroic features, i.e. there is an increase in the broad positive band at 275 nm and

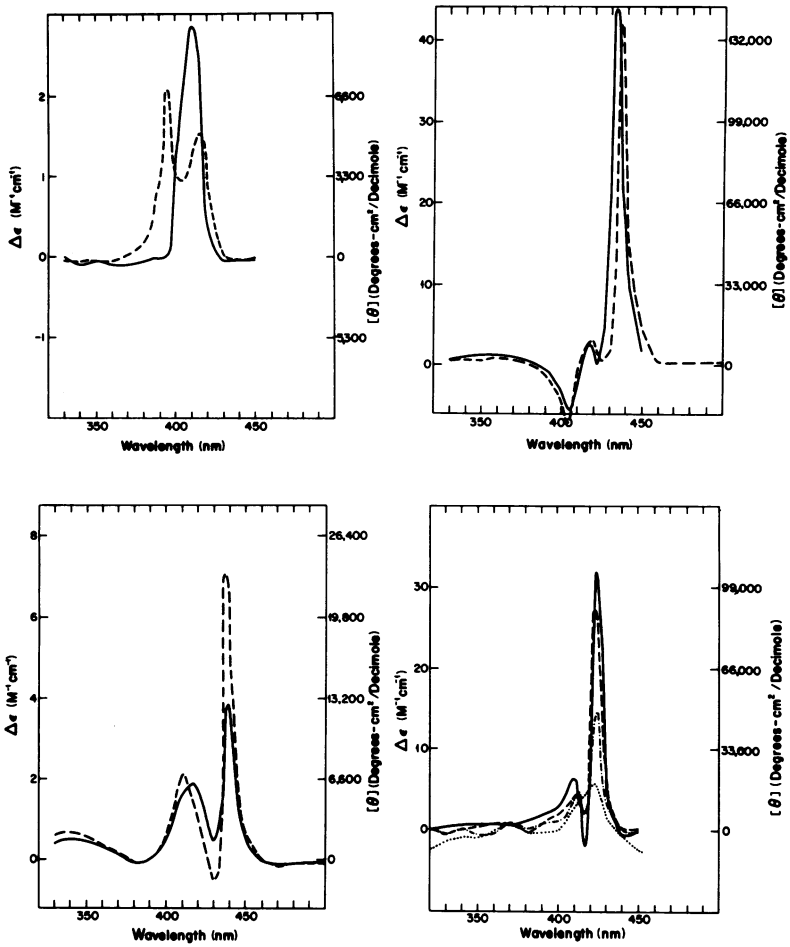

Figure 4. Visible CD spectra of poly[d(A-T)]·poly[d(A-T)] in BPES/0.5 M NaCl with (a) T2MPyP, (b) T3MPyP, (c) T4MPyP. Input ratio of [porphyrin]/[DNA], R, is 0.235 (——) and 0.354 (– – –). Also, (d) TMAP with input ratios of 0.015 (· · · ·), 0.030 (–·–·), 0.045 (– – –), 0.100 (——).

in the negative band at 250 nm. These effects are most pronounced for T4MPyP and T3MPyP, the intercalating porphyrins, while T2MPyP and TMAP, the non-intercalating porphyrins, demonstrate little effect. In the case of T4MPyP and T3MPyP, the CD spectra point to a final porphyrin·poly[d(G-C)] conformation that is altered from that of the native B-form. Similar optical anisotropy has been observed for poly[d(G-C)]·poly[d(G-C)] under dehydrating conditions, e.g.,

78-84% trifluoroethanol/water or 80% ethanol/ water (25-27).

In figure 4, parts a,b,c,d the ellipticity induced in the Soret bands of porphyrins bound to poly[d(A-T)]·poly[d(A-T)] is shown. The visible CD can be generally described as possessing positive ellipticity. The T2MPyP·poly[d(A-T)] spectrum is very similar to that of T2MPyP·poly[d(G-C)]: a positive peak at 411 nm at $R \leq 0.235$ and a split pattern, 395 nm and 415 nm, for $R > 0.235$. Conversely, complexes of poly[d(A-T)]·poly[d(A-T)] and T3MPyP, T4MPyP, and TMAP display distinctive circular dichroic features, not evident in other DNAs. There is a signature peak located approximately 14 nm to the red of the maximum visible absorption band. This positive ellipticity (in the visible) is very intense and can be as much as two orders of magnitude greater than bands observed in poly[d(G-C)]·poly[d(G-C)] at comparable ionic strength. The rotational strength of the peak increases with increasing R up to a value of 0.235 and decreases for larger values. Proceeding to shorter wavelengths from the signature peak there occurs a negative-positive-negative group of bands that is much less intense.

The ultraviolet CD spectra of poly[d(A-T)]·poly[d(A-T)] bound T3MPyP, T4MPyP, TMAP are also quite distinctive and represent the most interesting aspect of this investigation, see figure 5 b,c,d. Here the longest wavelength feature is negative and occurs between 275 nm and 280 nm. This same feature has been observed for poly[d(A-T)]·poly[d(A-T)] in concentrated solutions of CsF, CsCl, $CaCl_2$, and NaCl (28,29). Also, poly[d(I-C)]·poly[d(I-C)] manifests a similar spectrum under low ionic strength conditions (7). Finally, there are a number of circumstances in which poly[d(G-C)]·poly[d(G-C)] or a modified GC copolymer has been shown to express a negative band as the longest wavelength feature in its ultraviolet CD (1-6, 16-19). T2MPyP does not induce this same radical alteration in the ultraviolet CD when it binds to poly[d(A-T)] poly[d(A-T)], see figure 5a, except under conditions of low salt (24).

The remainder of the ultraviolet circular dichroism spectrum is positive for T3MPyP, and positive-negative for T4MPyP and TMAP. The pattern seen in these three cases has extrema at 258-261 nm and at 245 nm. The shorter wavelength components of these spectra re-

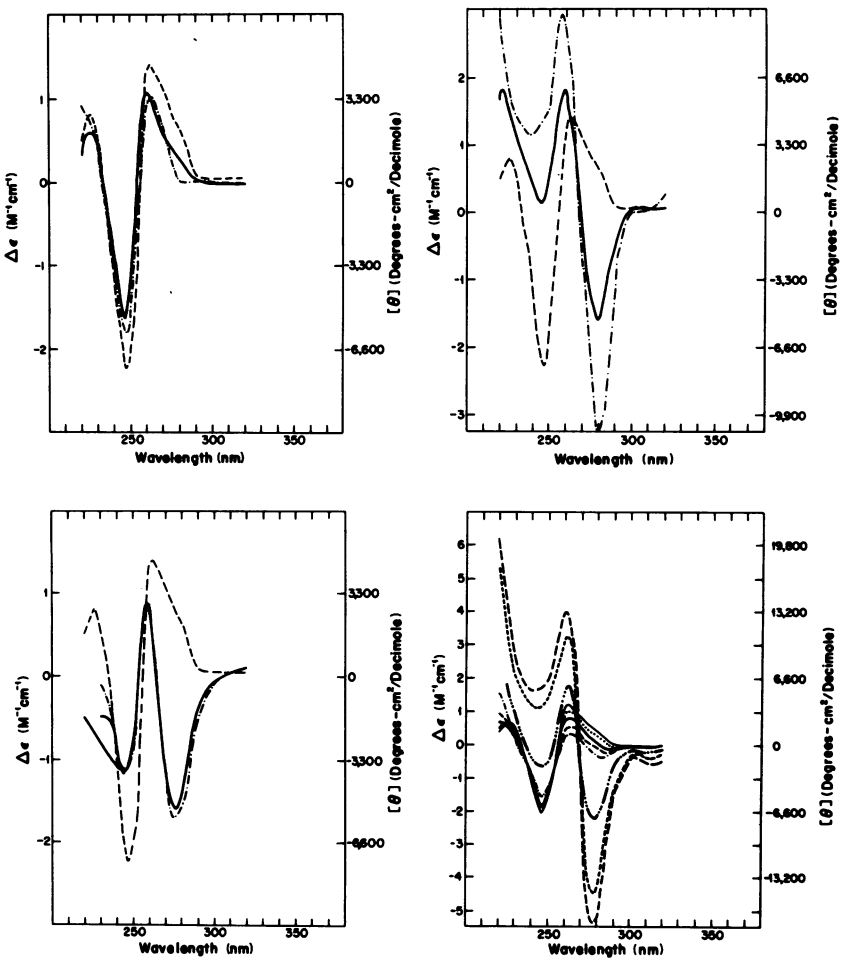

Figure 5. Ultraviolet CD of poly[(d(A-T)]·poly[d(A-T)] in BPES/ 0.5 M NaCl buffer with (a) T2MPyP, (b) T3MPyP, (c) T4MPyP. Input ratio of [porphyrin]/[DNA], R, is 0.235 (——) and 0.354 (— · —). Polynucleotide control is (----). (d) TMAP with input ratios of 0.015 (· · · ·), 0.030 (▬▬), 0.045 (— — —), 0.100 (— · — · —), 0.235 (— · · — · · —), 0.354 (----), 0.710 (— —). Polynucleotide control is (——).

semble features seen in other deoxyribose polynucleotides with alternating purine-pyrimidine sequences, such as the above mentioned poly[d(G-C)] poly[d(G-C)] and poly[d(I-C)] poly[d(I-C)].

DISCUSSION

T2MPyP and T3MPyP are positional isomers of T4MPyP and differ only in the location of the N-methyl groups, see figure 1. In the case of T2MPyP the proximity of the N-methyl to the central tetrapyrrole ring imposes steric constraints, thereby limiting the rotational freedom of the pyridyl group. Also, that the positive N-methyl is situated out of the plane of the porphyrin ring precludes stacked structures. In contrast T3MPyP appears, on the basis of its ability to unwind supercoiled DNA (22), to bind by intercalation. In this porphyrin the N-methyl restricts rotational freedom to a lesser degree; however, the N-methyl located in the 3-position may still affect the molecule's ability to stack with itself or with nitrogen bases. In TMAP, a second nonintercalator, the substituted nitrogen is located remote from the tetrapyrrole ring in an "equatorial" orientation such that rotation of the anilinium group is not hindered by interaction with the porphyrin ring. Since electrostatic and stacking interactions are the key determinants of the final conformation of the complex, molecules with different binding potential serve as keen probes by which to assess the importance and extent of these binding modes.

In these experiments the nonintercalating isomer T2MPyP serves as a restricted control. In the case of calf thymus DNA, this isomer is always bound externally and always demonstrates positive circular dichroic features. The single peak is centered at 410 nm for both poly[d(G-C)]·poly[d(G-C)] and poly[d(A-T)]·poly[d(A-T)] and is of moderate intensity. What we learn in such a limited situation is that monomeric binding of the ligand yields positive ellipticity with a band profile similar to that observed in absorption spectroscopy.

Several distinctive CD features are perceived from the binding of the two intercalating porphyrins T4MPyP and T3MPyP. First, the association with alternating and homo GC sequences produces a CD spectrum that is principally negative at lower values of R (24). This resembles the results found for calf thymus DNA measured at low R (20) and suggests that some structural homology exists in the bound states. Second, at higher values of R, R=0.354, the visible CD of T4MPyP approaches a conservative spectrum while that of T3MPyP does not. Conservative spectra are frequently observed for

chromophore-chromophore interactions of the exciton type (30-32). This same conservative spectrum is observed for TMAP bound to DNA at higher R (23) and reflects extensive dye stacking upon the surface of the polymer. It is probable, therefore, that the nature of the bound states of T4MPyP and T3MPyP (intercalation, outside bound, stacked outside bound, or others) varies as a function of input ratio, R, and ionic strength. This is corroborated by the results obtained in the ultraviolet CD. At any given input ratio the ultraviolet CD spectra of poly[d(G-C)]·poly[d(G-C)] complexed with T4MPyP or T3MPyP are comparable to corresponding spectra for calf thymus DNA at small R (22). Therefore, extrapolation of the characteristic features of the ultraviolet-visible CD spectra found for T4MPyP and T3MPyP bound to these polymers indicates that these porphyrins are bound by intercalation to GC base pairs. However, it is emphasized that this selectivity is only apparent through a small aperture of solution conditions dictated by R and ionic strength.

For poly[d(A-T)]·poly[d(A-T)] the visible CD shows the induction of positive ellipticity on binding of T4MPyP and, predictably, T3MPyP as shown in figure 4. Interesting characteristic features are also noted in the ultraviolet CD that are not apparent in the GC analogue. These probably result because the AT copolymer is thermodynamically more labile than the GC counterpart, in that the T_m is substantially lower and there can exist various hairpin and clover leaf excrescences (33). Hence the structure of this AT polymer appears to be more readily altered by the steric/electronic forces produced through ligand interaction than is its GC analogue, see also NMR evidence concerning AT base pair lability (34, 35). Binding of the porphyrin ligands with the AT helix results in a non-cooperative relaxation of polymer twist for $R \leq 0.100$, as is shown by changes in the positive ellipticity at 275 nm. For $R > 0.100$ a cooperative transition to some other, non-B like, conformation is observed that is remarkably similar to that described as X-DNA by Vorlíčková et al (29). Surprisingly, the structure of poly[d(A-T)]·poly[d(A-T)] is so inherently adaptable that both TMAP, as shown here, and T2MPyP at low salt (not shown), derivatives not capable of unwinding supercoiled DNA, can induce this transition. This distinctive spectral feature of negative

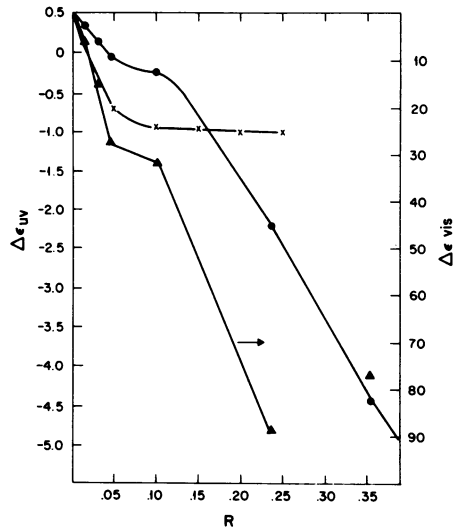

Figure 6. Change in ellipticity as a function of input ratio, R, [porphyrin]/[DNA base pairs]. T4MPyP at 277 nm (-x-x-), TMAP at 278 nm (-●-●-), left legend; TMAP at 424 nm, (-▲-▲-) right legend. Note that the ellipticity in the visible for TMAP•poly[d(A-T)] decreases for R>0.235.

ellipticity at 275 nm seen in poly[d(A-T)]·poly[d(A-T)] is also induced in calf thymus DNA at large R by T4MPyP and its metallo-derivatives: Fe(III), Ni(II), Mn(III), Co(III) (22). We suggest that this binding results in a significant change in the conformation of AT base pairs, although it is not clear that the binding is intercalative.

In figure 6 the CD changes observed upon the binding of porphyrins to poly[d(A-T)]·poly[d(A-T)] are summarized. Although both T4MPyP and TMAP induce an inversion in the ultraviolet CD there appears to be a difference in the concentration dependence between the two. The negative ellipticity at 277-278 nm attains a maximum value for T4MPyP at R=0.100 and varies little at higher R. For TMAP the ellipticity continues increasing to R=0.710. We have considered the possibility that these tetracationic porphyrins are capable of evoking tertiary structure and that the quantitative differences shown in figure 6 are a reflection of these processes, i.e. binding versus condensation. For reasons already stated in the accompanying paper we feel that condensation, particularly for

the intercalator T4MPyP, is unlikely. Also, evidence from ^{31}P NMR studies using the T4MPyP·poly[d(A-T)] complex, R=0.470, shows the two phosporous resonances of poly[d(A-T)]·poly[d(A-T)] separated by 2.4224 ppm (-2.4783 ppm and -4.9007 ppm) (36). (These NMR results are being presented more completely in a separate publication). These data indicate that a profound change in the phosphodiester backbone has taken place that is similar but not necessarily identical to that observed for the Z-form of poly[d(G-C)]·poly[d(G-C)]. Nevertheless, the prospect of condensation will be tested more rigorously.

Our results to date are consistent with a model in which complexation to GC base pairs occurs at low R and low ionic strength. Binding of porphyrin to GC base pairs induces a distortion in the standard B-form DNA so that the conformation of the complex resembles (caveat: by CD criteria) the A-form of DNA. Binding to AT base pairs occurs at higher R and higher ionic strength such that structural alterations closely associated with an inverted, or X-type helix are induced (29,37).

In summary, we would like to outline the experimental parameters which we have found to be important determinants of porphyrin binding to DNA. First, the molecular conformation of the porphyrin ligand, that is the location and orientation of the positively charged substituents, alters the intercalation profile of a meso-substituted porphine and determines whether a given derivative can be stably incorporated within the DNA helix. Second, the extensibility of the DNA molecule, that is the extent of motion between base pairs (same strand) and among hydrogen bonded base pairs, governs the size of the intercalation site and the degree to which the polymer may be favorably deformed when complexed with ligands. Third, the solution conditions of input ratio and ionic strength promote sequence selective binding of porphyrin to DNAs.

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