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Intercalative and nonintercalative binding of large cationic porphyrin ligands to calf thymus DNA

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ABSTRACT

The large meso-substituted porphine, meso-tetra(4-N-methylpyridyl)porphine has been identified as a DNA-interactive ligand with a capacity for intercalation (1,2). Subsequently, the 2-N-methyl, 3-N-methyl and N-trimethylanilinium analogues of this porphyrin intercalator have been obtained for physico-chemical analyses (absorption spectroscopy, viscometry, circular dichroism, unwinding of supercoiled DNA). In this paper we discuss the factors affecting the character of porphyrin binding (intercalative, as is the case for the 4-N-methyl and 3-N-methyl porphines, versus non-intercalative, as is the case for the 2-N-methyl and N-trimethylanilinium porphines) and the impact that porphyr ins' binding has upon the structure of DNA. The molecular conformation of the porphyrin ligand varies slightly within this series so that the ability of a given porphyrin to intercalate may be correlated with the arrangement of charg ed groups, the planarity of the porphine ring and the effective width of the individual molecules. The results from these studies indicate that sequence selective binding occurs within a small aperture of solution conditions.

INTRODUCTION

It is generally accepted that the inherent biological properties of nucleic acids depend on the sequence of their constituent purine and pyrimidine base pairs. More recently, this dictum has been enlarged to include the concept of DNA as a polymorphic, dynamic biopolymer. A right-handed, B-form, double helix is not the only conformation available to DNA in solution. A-DNA (3), C-DNA (4), D-DNA (5), X-DNA (6), Z-DNA (7) or alternating B-form DNA (8) all serve to illustrate the point that base sequence affects the conformation adopted for any given set of solvent conditions. Furthermore, the DNA molecule cannot be simply conceived of as a rigid rod, whatever helical sense it may possess. Rather, transient uncoupling of base pairs occurs as do other temporary structural deformations (9-14).

DNA interactive ligands have been utilized by a number of workers to probe the extent to which and locations at which conformational changes occur within native biopolymers (15,16). We have employed a series of porphyrin derivatives: meso-tetra(4-N-methylpyridyl)porphine, T4MPyP; meso-tetra(3-N-methyl-

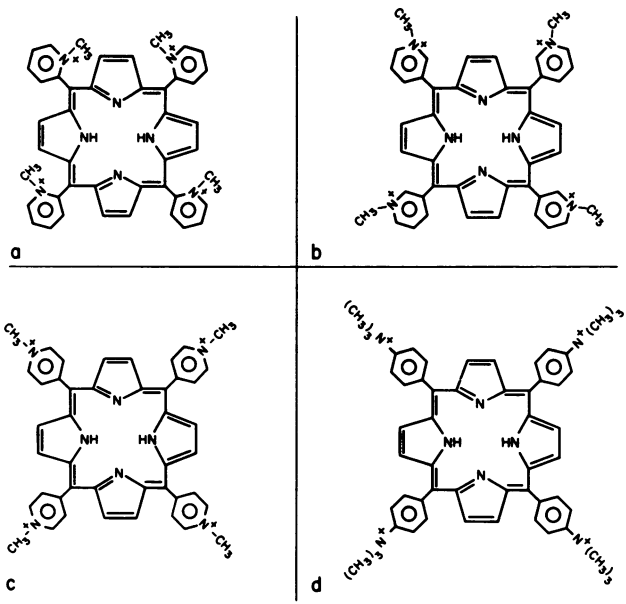

Figure 1: DNA interactive porphyrins (a) meso-tetra(2-N-methylpyridyl)-porphine, T2MPyP, (b) meso-tetra(3-N-methylpyridyl)porphine, T3MPyP, (c) meso-tetra(4-N-methylpyridyl)porphine, T4MPyP, (d) meso-tetra(para-N-trimethylanilinium)porphine, TMAP.

pyridyl)porphine, T3MPyP; meso-tetra(2-N-methylpyridyl)porphine, T2MPyP; meso-tetra(para-N-trimethylanilinium)porphine, TMAP; see figure 1, to study the protean nature of DNA. Quite surprisingly, T4MPyP has demonstrated an intercalative binding to DNA (1,2), this despite the presence of the four N-methylpyridyl groups at the periphery of the tetrapyrrole ring. The apparently restrictive bulkiness of T4MPyP is emphasized in a comparison of the models of ethidium bromide and proflavin, as shown in figure 2; however, it is important to note that the largest planar dimension of the porphine ring is comparable in length to that of both intercalators.

Intercalation has been verified for T4MPyP binding to DNA by hypochromism and bathochromism in the Soret absorption band, increased T_m, induced ellipticity in the visible region and altered ellipticity in the ultraviolet region, and more definitively by its ability to unwind covalently closed circular DNA and to increase the relative viscosity of linear DNA (1,2).

Although T2MPyP, T3MPyP, and TMAP are similar to T4MPyP and each is tetracationic, the location of their positive charges varies from a position close to the porphine ring in T2MPyP to an extreme peripheral position for TMAP, see

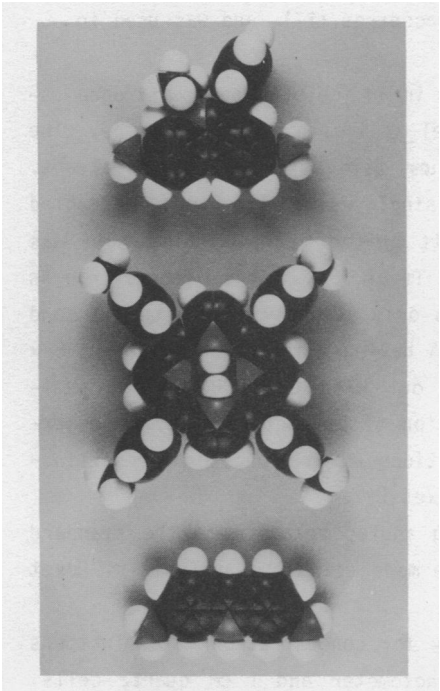

Figure 2: CPK models comparing T4MPyP (middle) with proflavin (top) and ethidium bromide (bottom).

figure 1. This variation also determines the axial dimension, "thickness", of the porphyrin so that the TMAP and T2MPyP are nearly equal and somewhat greater than T3MPyP which is greater than T4MPyP. These differences provide an indirect assessment of the limiting degree of the fluctuations allowed in DNA i.e., the helix's capacity for deformation is measured first by its ability or inability to accomodate a particular porphine ligand in an intercalation mode and second by the conformational properties of the resulting complexes.

EXPERIMENTAL PROCEDURES

Solutions. All materials were dissolved in a biphosphate buffer, BPES, which contains 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, at pH 6.8 with either no added NaCl, (BPES/no salt) or NaCl added to 0.5 M, (BPES/0.5 M NaCl).

Porphyrins. Meso-tetra(4-N-methylpyridyl)porphine was purchased from Strem chemicals. Meso-tetra(3-N-methylpyridyl)porphine, meso-tetra(2-N-methylpyridyl)porphine, and meso-tetra(para-N-trimethylanilinium)porphine were provided by Dr. N. Datta Gupta.

Nucleic Acids. Calf thymus DNA was purchased from Worthington Biochemicals and was dissolved in the appropriate buffer following published procedures (1)

PM-2 DNA was isolated following published procedures (17), and was used in the unwinding experiments.

Viscosity. Calf thymus DNA was sonicated in an ice bath under nitrogen using a Heat System-Ultrasonics sonifier, Model W185 with a Bronson probe. The DNA was fractionated on a Sepharose 6B column using 1 M NaCl as an eluent. Fractions were pooled from the maximum of a single asymmetric band. The pooled fractions were dialyzed against BPES/no salt buffer. The final sample was found to have a sharp melting profile with a Tm of 76°C. This material ran as a single band on agarose gel electrophoresis only slightly slower than a band from the Hind III digest of lambda phage DNA having a molecular weight of 8 x 10^4. Viscosity measurements were carried out with Cannon-Ubbelohde semi-micro dilution viscometers. The concentration of bound porphyrin was determined from parallel spectrophotometric titrations using pre-determined extinction coefficients for the fully bound complexes (1).

Viscosimetric determinations of unwinding angles relative to the standard value of 26° for ethidium bromide (18) were made using the analysis of Revet et al. (19).

Instrumentation. Absorption spectroscopy for concentration determinations was conducted with a Beckman DB-G spectrophotometer and 1 cm quartz cells. Circular dichroism spectroscopy was performed on a Jasco J41-C spectropolarimeter and 1 cm cells maintained at 25 $\pm$ 1°C.

RESULTS

In figure 3, the visible absorption spectra for T2MPyP, T3MPyP, T4MPyP, and TMAP are shown. There is an intense absorption band at 400-450 nm, the Soret band, for which the molar extinction coefficient is on the order of 10^5 ($M^{-1}cm^{-1}$), see Table 1. The extraordinarily large extinction coefficients for the characteristic Soret band of these derivatives allows spectrophotometric detection of porphyrin-DNA interactions to very low porphyrin concentrations.

Titrating solutions of porphyrin with calf thymus DNA causes a reduction in the intensity of the Soret band and a shift of maximum absorption to longer wavelength as in figure 3. While the absorption band of the free porphyrin remains hypochromic throughout the titration, the extinction of the red shifted peak increases as the titration proceeds and additional complex is formed. These spectral changes indicate that porphyrin-DNA association or DNA induced porphyrin-porphyrin associations occur in each case. It should be mentioned that in the absence of DNA no aggregation between free ligands

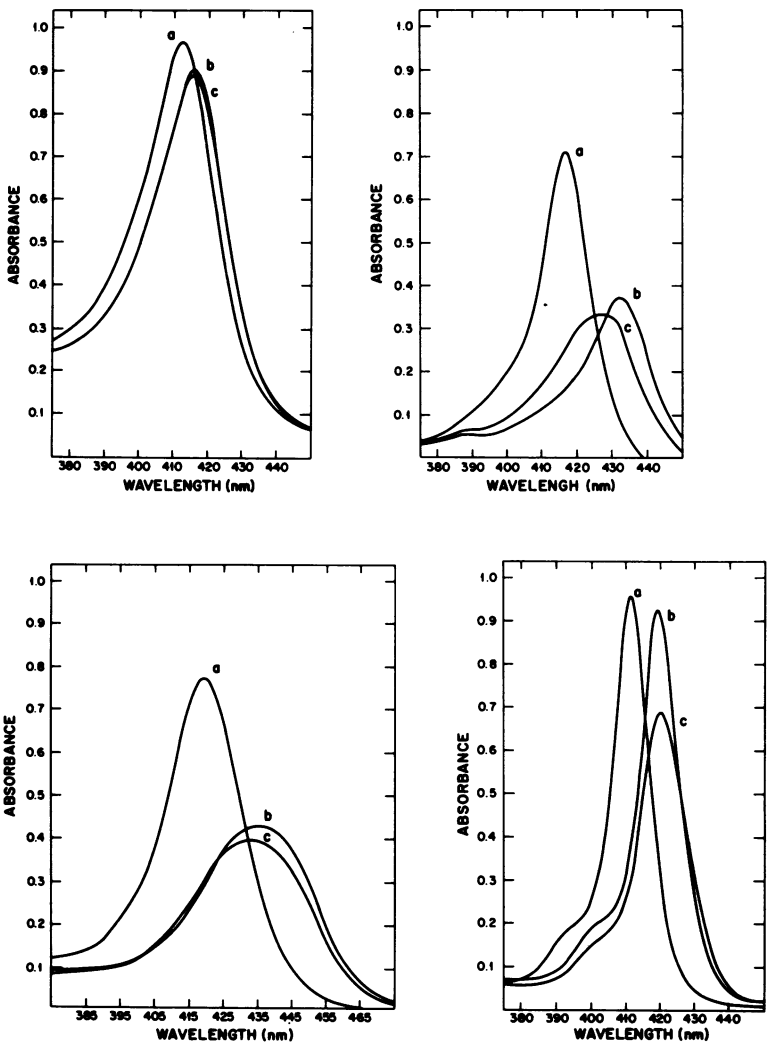

Figure 3. Visible absorption spectra of four porphyrins, free and in the presence of DNA. The input ratio of porphyrin to DNA base pairs, R, is (a) free dye, R=0; (b) R=0.10; (c) R=0.40. Upper left, T2MPyP; upper right, T3MPyP; lower left, T4MPyP; lower right, TMAP.

has been observed for these porphyrins up to a concentration of 10^{-4} M.

To fully characterize the mode and extent of porphyrin binding to DNA the spectroscopic titration data must be analyzed as binding isotherms using appropriate models. In all of the cases studied here the isotherm plots show

TABLE 1
Molar Extinction Coefficients for DNA Interactive Porphyrins

Porphyrin	Buffer	λ_{max} (nm)	ε_{max} ($M^{-1}cm^{1}$)
T2MPyP	BPES/no salt	416 260	2.15×10^5 3.63×10^4
	BPES/0.5 M NaCl	416	2.71×10^5
T3MPyP	BPES/no salt	419 260	2.54×10^5 3.18×10^4
	BPES/0.5 M NaCl	419	3.14×10^5
T4MPyP	BPES/no salt	424 260	2.06×10^5 2.86×10^4
	BPES/0.5 M NaCl	424	2.15×10^5
TMAP	BPES/no salt	414	2.31×10^5
	BPES/0.5 M NaCl	414	2.84×10^5

a very steep linear region at low r (ratio of bound porphyrin/base pairs) coupled with a pronounced curvature at higher values of r (not shown). The binding appears to be very complex in the case of all four porphyrin derivatives and has not been resolved; however, preliminary determinations indicate that in BPES/no salt binding constants on the order of at least 10^7 may be obtained (see also, 20).

A direct approach to establish the mode by which a particular drug may bind to DNA is to determine its ability to unwind covalently closed circular DNA. A negative result is indicative of a non-intercalative mode (21-23). As a test for intercalation, however, this assay is somewhat equivocal as there are at least two instances in which non-intercalating drugs have been shown to unwind supercoiled DNA (24,25). Nevertheless, the ability to unwind supercoiled DNA is a characteristic property of intercalators and when accompanied by supporting data serves as primary evidence for intercalative binding.

Both T4MPyP and T3MPyP unwind covalently closed circular DNA. Results for T4MPyP using an electrophoresis unwinding assay have been reported (2). T3MPyP has also been shown to unwind supercoiled DNA, but T2MPyP and TMAP do not un-

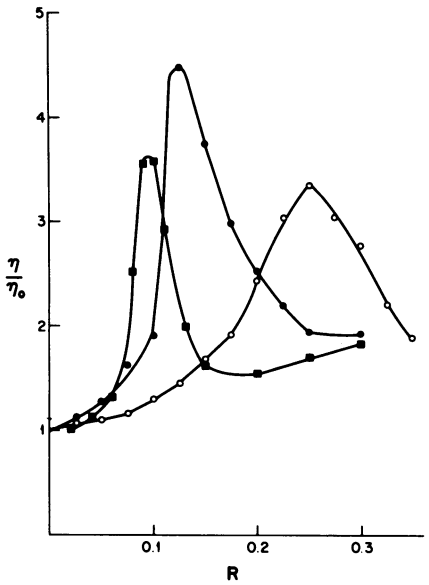

Figure 4. Viscosimetric unwinding assay of PM-2 DNA in BPES/no salt expressed as a plot of relative viscosity versus the input ratio of ligand to DNA base pairs. Ethidium bromide, -■-; T4MPyP, -●-; T3MPyP, -O-.

wind supercoiled DNA even at concentrations 100 times that required for T3MPyP and T4MPyP (26).

In addition to the electrophoresis assay, unwinding of supercoiled DNA can be easily detected by viscometry. A typical example is shown in figure 4. This measurement can be used to determine the unwinding angle for a given intercalator relative to ethidium bromide, whose unwinding angle has been established as 26° (18,19). The unwinding angles measured for T3MPyP and T4MPyP are shown in Table 2.

Besides their ability to unwind supercoiled DNA, intercalating drugs can also be identified by their characteristic ability to increase the length of sonicated DNA (27). The relative increase in length, L/L_0, can be related to a corresponding increase in relative viscosity with the following equation:

$$L/L_0 = (\eta/\eta_0)^{1/3}$$

where L is the length of the DNA molecule in the presence of bound ligands, L_0 is the length of free DNA, and η/η_0 is the corresponding relative viscosity. As shown in figure 5, the plot of L/L_0 vs. r, the moles of bound porphyrin per mole of DNA base pairs, three of the four porphyrins tested register an increase in the length of DNA. T3MPyP and T4MPyP demonstrate a rapid increase in the relative length to a maximum of between 20% and 30% at a binding ratio of approximately one porphyrin to four base pairs. TMAP does not affect an increase in relative length until r>0.250, at which point a

Table 2
Unwinding Angles for DNA Interactive Porphyrins

Ligand	Unwinding Angle
Ethidium bromide	26[19]
T2MPyP	0
T3MPyP	10
T4MPyP	19
TMAP	0

gradual increase is seen. T2MPyP produces a very slight decrease through the entire range of r.

Based on the evidence obtained from the viscosity measurements of linear and supercoiled DNA, and our previous experience with T4MPyP (1,2) and TMAP (20), we conclude that both T3MPyP and T4MPyP bind by intercalation, whereas, T2MPyP and TMAP do not. Circular dichroism spectroscopy has been employed as a means of obtaining additional insight into the nature and consequences of porphyrins binding to DNA. The circular dichroism induced in the Soret band of optically inactive porphyrin molecules occurs as a consequence of interactions between the porphyrin and the asymmetric binding sites of the helical polymer. Figure 6, parts a,b,c,d shows the visible CD for complexes of T2MPyP, T3MPyP, T4MPyP and TMAP with calf thymus DNA at low salt conditions, BPES/no salt. The circular dichroic features of T4MPyP and T3MPyP bound to calf thymus DNA are very similar, and are characterized by a predominating negative band at 429-435 nm which increases in intensity as the concentration of porphyrin in solution increases. However, at R (input mole ratio of porphyrin to DNA base pairs) values greater than 0.354 this trend reverses and the peak decreases as additional porphyrin is introduced. At slightly shorter wavelengths, 408-417 nm, the spectrum displays positive ellipticity. This peak shows a similar relationship of $\Delta\varepsilon$ vs. R, i.e. increasing until a ratio of 0.354 and decreasing thereafter. At all values of R, for both T4MPyP and T3MPyP, the negative ellipticity is greater than the positive ellipticity. The crossover point, at which $\Delta\varepsilon = 0$, corresponds almost exactly to the Soret absorption for these porphyrins, T3MPyP and T4MPyP. The predominance of a negative band in the visible CD of both T3MPyP and T4MPyP at small R and low

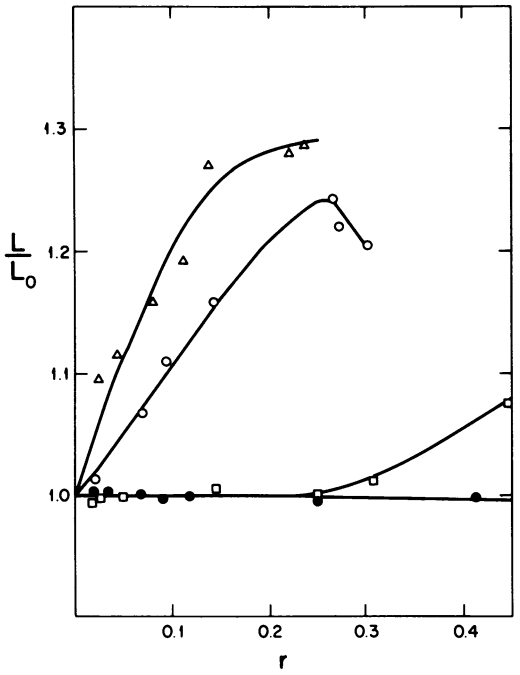

Figure 5: Plot of the relative increase in length of calf thymus DNA L/L_0, versus the ratio of porphyrin bound to DNA base pairs (r). T2MPyP, -●-; T3MPyP, -Δ-; T4MPyP, -o-, TMAP, -[].

salt suggests that it is reflective of the intercalation binding mode. Since it is an exclusive characteristic for the visible CD spectra of the complexes of T3MPyP and T4MPyP with poly[d(G-C)]·poly[d(G-C)], it also suggests that GC selectivity may occur in the binding of these porphyrins at small R (28). We have also noted this negative feature to occur in the visible CD spectrum of the complex of DNA and the nickel and copper derivative of T4MPyP, but not in the iron, cobalt, manganese or zinc analogues. We have also determined that the nickel derivative unwinds supercoiled DNA over a concentration range similar to T4MPyP, and that it increases the viscosity of linear DNA (26).

The visible CD of TMAP complexed with calf thymus DNA manifests a pattern of negative (422-430 nm) followed by positive (412-414 nm) ellipticity as do the complexes of T3MPyP and T4MPyP, see figure 6; however, there are several important differences. First, the intensity of the negative and positive peaks is nearly equal so that the spectrum is conservative in nature for $1.0 \geq R \geq 0.1$. Second, the positive peak splits into two peaks, 414 nm and 421 nm, at lower values of R, $0.1 \geq R \geq 0.05$, something not seen for either T3MPyP or T4MPyP. Finally, the negative peak is lost entirely for $R \leq 0.05$ as the spectrum decomposes into two smaller and finally one positive peak at these

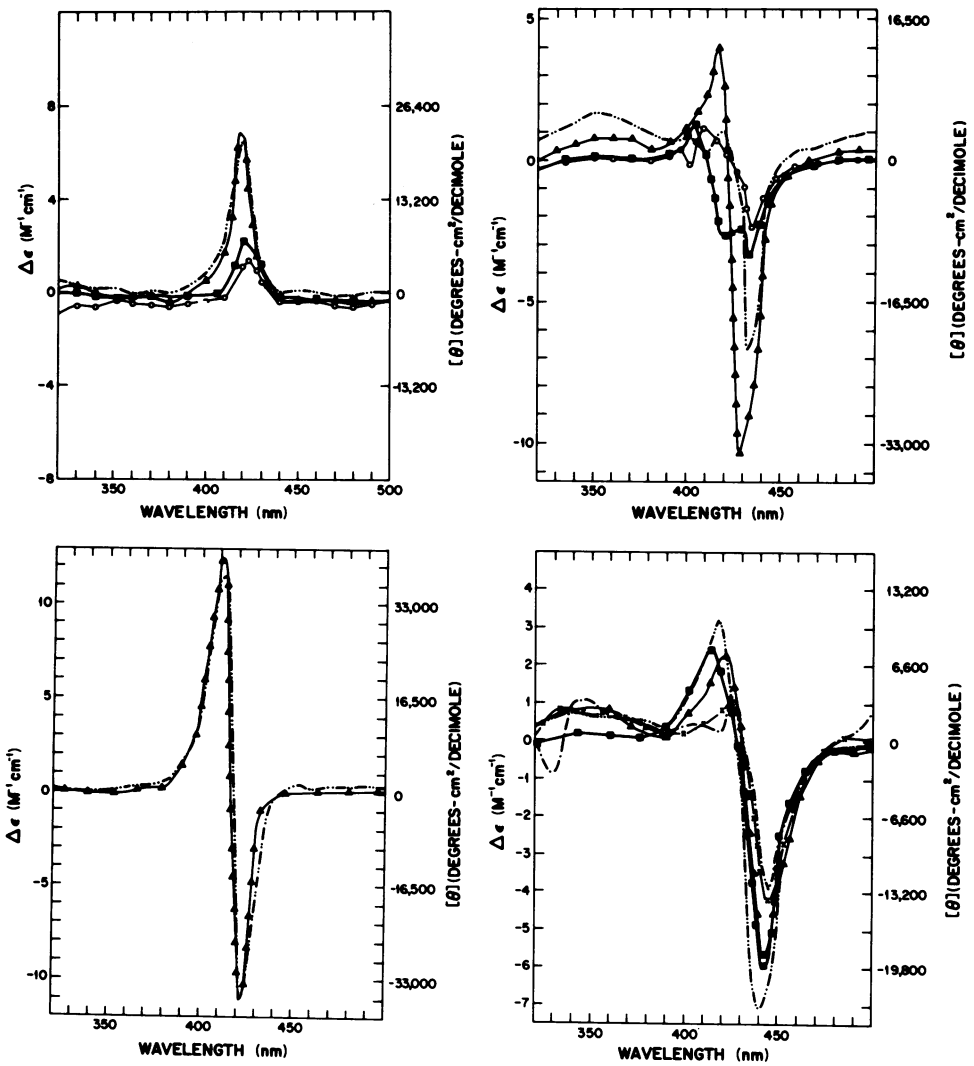

Figure 6. Ellipticity induced in the Soret absorption band of porphyrins bound to DNA in BPES/no salt buffer as a function of the input ratio of porphyrin/DNA base pairs, R. For (a) T2MPyP, (b) T3MPyP. R=0.235 (---), R=0.354 (-▲-▲-), R=0.710 (-■-■-), and R=1.00 (-0-0-); (c) TMAP, R=0.235 (---), R=0.354 (-▲-▲-); (d) T4MPyP, R=0.045 (-·-·-), R=0.100 (-x-x-), R=0.235 (---), R=0.354 (-▲-▲-), R=0.710 (-■-■-).

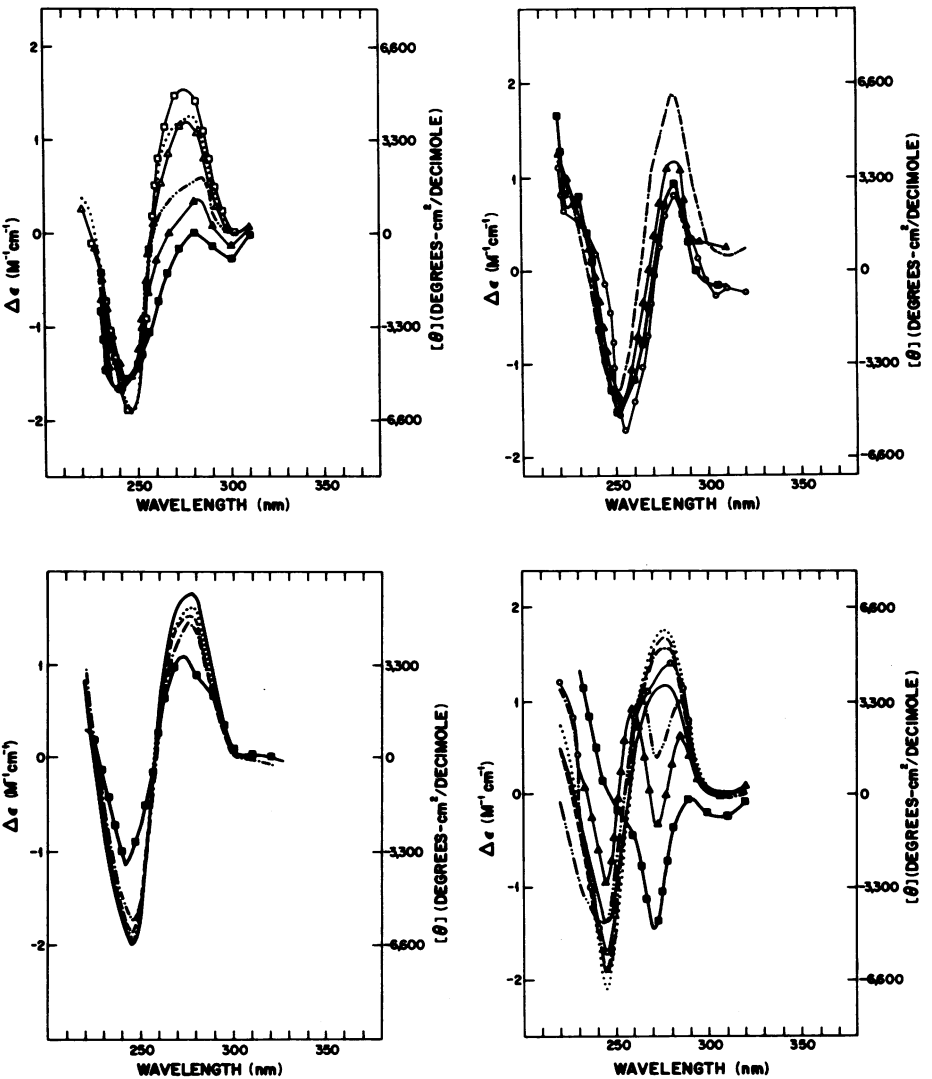

Figure 7. Ellipticity in the ultraviolet for calf thymus DNA titrated with porphyrins in BPES/no salt buffer as a function of the input ratio of porphyrin/DNA base pairs, R. For (a) T2MPyP, R=0.013 (·····), R=0.064 (-[]-[]-), R=0.127 (-Δ-Δ-), R=0.235 (-·-·-), R=0.354 (-▲-▲-), R=0.710 (-■-■-); (b) T3MPyP, R=0.235 (-·-·-), R=0.354 (-▲-▲-), R=0.710 (-■-■-), R=1.00 (-0-0-); (c) TMAP, R=0.015 (·····), R=0.030 (---), R=0.045 (-·-·-), R=0.166 (-■-■-), DNA control (——); (d) T4MPyP, R=0.015 (·····), R=0.030 (- -), R=0.045 (-·-·-), R=0.100 (-0-0-), R=0.235 (-·-·-), R=0.354 (-▲-▲-), R=0.710 (-■-■-), DNA control (——).

values.

Although T2MPyP is similarly constrained in its interactions with DNA, its circular dichroism spectrum is quite different from that of TMAP. At all values of R there is a single positive peak observed in the wavelength region 418-423 nm for T2MPyP and calf thymus DNA. The intensity of the peak attains a maximum value at R=0.354 and decreases at the larger values of R. We have found no conditions at which a negative CD band develops for T2MPyP.

At higher ionic strengths, e.g. BPES/0.5 M NaCl, the spectra obtained for T4MPyP and T3MPyP are qualitatively the same as those seen at the lower salt conditions (not shown). The features which characterize the bound states of these porphyrins develop at higher drug/DNA ratios indicating that binding is reduced at higher salt. The effect of increasing the ionic strength is more pronounced for TMAP and T2MPyP, the non-intercalating derivatives. In these cases the CD spectrum seen at low salt is not induced in the higher ionic strength buffers.

The well-known CD spectrum of calf thymus DNA is a conservative pattern with a positive ellipticity at approximately 275 nm and negative ellipticity at approximately 245 nm. The major effect of DNA complexation with T2MPyP, T3MPyP, and TMAP is to alter the intensity of these characteristic dichroic features, see figure 7 parts a,b,c. Increasing amounts of T2MPyP and T3MPyP lead to a decrease in the positive band, and a slight change in the ellipticity of the negative band, whereas, addition of TMAP to calf thymus DNA causes uniform attenuation of both bands. The ultraviolet CD displayed in the case of T4MPyP, see figure 7d, is notably different from the other porphyrins. At low values of R the spectrum shows an increase in ellipticity in the DNA extrema. The positive Cotton effect that is characteristic of double stranded B-form DNA is maintained when the concentration of base pairs is at a tenfold or greater excess. However, for $R \geq 0.100$ the positive CD at 275 nm is resolved into two peaks, one at 285 nm and other at 260 nm, which are separated by a trough. This declivity enlarges as the input ratio of porphyrin increases so that at R=0.710 the positive peaks are no longer distinct features. We believe that the effect of T4MPyP at $R \geq 0.100$ is of particular significance in that it signals a change in the conformation of DNA.

DISCUSSION

The appearance of hypochromicity and a bathochromic shift in the Soret band of each porphyrin subsequent to the addition of DNA is definitive evidence of the interaction of these components. It remains to establish the nature of

this interaction. In the case of T2MPyP there appears to be little diversity in its bound state. It neither unwinds supercoiled DNA nor increases the length of short linear fragments of calf thymus DNA, see figure 5. Although a detailed structural interpretation of the binding cannot be provided by CD data, it can be said that the development of the induced ellipticity as a function of R reflects the orientation of T2MPyP in the electrostatic field of the double helix. Moreover, this "outside" binding is characterized by a single positive ellipticity centered on the Soret absorption. This pattern is also observed for TMAP at small R, T4MPyP at 1 M salt (1) and for all four porphyrins under all conditions tested with poly[d(A-T)]·poly[d(A-T)] (37). It has also been noted for the iron, cobalt , manganese and zinc derivatives of T4MPyP (26). Although all of these analogues have been shown to unwind supercoiled DNA, unlike the nickel derivative of T4MPyP or T4MPyP itself, they require very large concentrations to do so and probably have unwinding angles even smaller than T3MPyP (26). We believe that the appearance of a single positive band in the visible CD indicates nonintercalative or outside binding. Furthermore, we suggest that this may also represent selective occupation of AT binding sites (28).

The ultraviolet CD spectra of T2MPyP·DNA complexes show a reduction in the intensity of the band at 275 nm. A reduction in positive ellipticity at 275 nm has been reported for DNA dissolved in non-aqueous solvents or in concentrated solutions of alkali metal salts (29,30). Also, a similar decrease has been related to the degree to which DNA is unwound due to ligand interaction (31-33). Since T2MPyP is unable to increase the length of DNA, as measured by viscosity, and is unable to unwind supercoiled DNA, as measured by gel electrophoresis (data not shown), the observed CD changes must arise from an indirect perturbation of DNA confromation, such as that caused by dehydration rather than a direct perturbation, such as that caused by intercalation.

The binding characteristics of TMAP appear to be somewhat novel as already noted (20). It is incapable of unwinding supercoiled DNA at large values of R, but it induces an increase in the apparent length of linear DNA. This result is consistent with our earlier determination that this derivative, although not an intercalator, can self-stack along the surface of DNA. Unlike T2MPyP, the charge distribution in TMAP is at the periphery and generally in the plane of the porphine ring. At low R, the visible CD spectrum of TMAP is that of DNA-bound, but non-interacting porphyrin chromophores, i.e. a single positive peak centered at the wavelength of maximum absorption, as in the case of T2MPyP. The singly bound porphyrin molecules may be oriented in a face-on

or edge-on fashion inasmuch as the quaternary anilinium nitrogen is located in the para position. At higher R, chromophore interactions occur as is reflected by the split in the visible CD peak and the appearance of a negative trough. A conservative split pattern develops within the porphyrin/DNA range of 1.0 to 0.1 (input ratio, R), a spectrum consonant with exciton interaction between bound dye. Interestingly, at a ratio, r, of one bound TMAP for every four base pairs, the viscosity data indicate the beginning of a gradual increase. We believe, however, that this results from a reduction in the flexibility of DNA by the stacking of outside-bound porphyrin, rather than an increase in its length as shown by the intercalation effects of T3MPyP and T4MPyP.

In contrast to the exclusive outside-binding characteristics of T2MPyP and TMAP, T3MPyP and T4MPyP display the binding characteristics of intercalation and outside binding. Both porphyrins are capable of unwinding DNA, as previously shown for T4MPyP (2) and demonstrated in this work for T3MPyP, see figure 4. The unwinding angle of 19° calculated from these data for T4MPyP is less than the standard value of 26° for ethidium bromide (18), but larger than the value of 17° for proflavin (23). Considering the large meso-substituents on the porphyrin ring, one may have anticipated a somewhat larger unwinding angle for T4MPyP (see figure 1). However, it is important to consider that in the porphyrin·DNA intercalation complex, only the planar porphine ring is stacked between the base pairs and the bulky N-methylpyridyl groups are projected away from the helix axis into the helix grooves. Therefore, although the N-methylpyridyl groups may restrict the insertion of T4MPyP into an intercalative binding-site and may require "breathing modes" to initiate the process, these groups are not restrictive to the final stacking process. This has been demonstrated using simple model studies and is consistent with our preliminary ^{1}H NMR results for complexes of T4MPyP with dinucleotides (34) and a hexanucleotide (35). In both instances ring current induced shifts appear for the pyrrole protons of the porphyrin ring, whereas, the protons of the pyridyl rings are only slightly affected. In addition to this we believe that T4MPyP has a smaller unwinding angle than ethidium bromide because not all of the bound porphyrin is intercalated, but that a fraction is bound externally in a site selective manner (28). That this fraction increases with increasing R and increasing ionic strength is reflected in the decrease of the unwinding angle with increasing ionic strength (26).

The unwinding angle of 10° found for T3MPyP is even lower than the apparent unwinding angles of 12° found for the anthracycline drugs (36) and brings

into question whether T3MPyP actually intercalates. Again, as with T4MPyP it is possible that only a fraction of the bound T3MPyP is actually intercalated. As noted previously, the ability to unwind supercoiled DNA does not provide a positive identification of an intercalator. The steroidal diamines, for example, unwind supercoiled DNA with extraordinarily small unwinding angles (24). These ligands do not affect the intrinsic viscosity of the relaxed form of PM-2 DNA and are not considered to be intercalators (24). That the unwinding of PM-2 DNA by T3MPyP and T4MPyP represents intercalative binding is corroborated by the increase in viscosity corresponding to an increase in length of low molecular weight linear fragments of calf thymus DNA obtained on titration with these porphyrins.

The CD spectra of T3MPyP and T4MPyP·DNA complexes are less tractable than those previously discussed because so many tessellae of information are found superimposed one on the other. During the course of a titration the asymmetric environment of the bound porphyrin will change as a result of the unwinding/intercalation process. The close correspondence between the visible CD of T3MPyP and that of T4MPyP indicates that the asymmetric binding site of the porphine ring is similar, if not identical, for both complexes.

T3MPyP complexed with DNA gives an ultraviolet CD spectrum only slightly different from that of the free nucleic acid. T4MPyP, on the other hand, induces conspicuous alterations in the ellipticity of calf thymus DNA: the negative peak at 245 nm is reduced at high values of R and the positive peak at 275 nm is split into a distinct positive-negative-positive feature. If these spectra actually monitor changes in the DNA, one must conclude that the final conformation adopted by the DNA in a porphyrin·DNA complex is ultimately determined by small differences in the structure of the ligand employed. Although T4MPyP and T3MPyP do possess their several, characteristic molecular conformations, both porphyrins are similar enough in their axial dimensions that they may exploit the transient opening of DNA in order to be accommodated within its helix. However, the orientation that the pyridyl groups adopt once the central porphine ring is intercalated will differ for these porphyrins, thus providing a degree of discrimination regarding the final structure of their respective intercalation complexes.

The important question remains as to whether the drastic changes induced in the ultraviolet CD spectra of DNA on addition of T4MPyP are actually reflecting alterations in the conformation of DNA. Since the free base pyridine absorbs in the ultraviolet at 250 nm, 256 nm, and 263 nm and all of the pyridine containing porphyrins possess electronic transitions in this region,

it must be established that the features observed in the ultraviolet CD actually express polymer asymmetry and not induced asymmetry in the porphyrin chromophore. First, the features of the CD spectra closely parallel those obtained through the use of high ionic strength media (30,37) or solvent conditions in which no absorbing ligand was present (38,39). Second, results from viscosity, unwinding experiments and visible CD indicate that T3MPyP binds to DNA in a manner similar to T4MPyP. It also has similar ultraviolet absorption characteristics but it does not induce a similar pattern in the ultraviolet CD. Third, other work performed with synthetic polynucleotides indicates that all four porphyrins induce a striking inversion in the ultraviolet CD of poly[d(A-T)]·poly[d(A-T)] (28). This is particularly important because TMAP has a negligible extinction in the ultraviolet, therefore the inversion must reflect the conformation of the polynucleotide. The significance of this result is that it represents the first demonstration of an intercalator-induced inversion of the ultraviolet CD of DNA; however, it is not clear as to which characteristic of the binding of T4MPyP leads to the inversion. This effect is not apparent for poly[d(G-C)]·poly[d(G-C)] suggesting, along with the reported findings of Early et al. (40), that the inclusion of AT base pairs results in the formation of a more labile helix. We conclude that the helix structure of calf thymus DNA is permissive for the insertion of even very large, bulky ligands. Yen and co-workers (41) have come to much the same conclusion studying aromatic imide binding to DNA.

One final alternative explanation for the observed CD spectra, that of porphyrin mediated DNA condensation, has been suggested. The porphyrins with their charge of +4 could feasibly act as condensing agents, particularly at the higher values of R employed. If a tertiary structure is enforced through porphyrin binding the CD spectra should demonstrate certain diagnostic features: (i) very intense ellipticity, either positive or negative, in the wavelength region 320-250 nm; (ii) extraneous CD bands, or tails appended to known CD bands that extend into non-absorbing regions; (iii) broadening or flattening of otherwise sharp CD bands; (iv) time dependent spectral shifts (42). Since the CD spectra we have recorded do not manifest these characteristics we feel that the CD is primarily measuring a change in the secondary structure of DNA. However, the possibility does exist that a condensed form of DNA occurs as a metastable intermediate, as has recently been shown to be the case for poly[d(G-C)]·poly[d(G-C)] in sodium acetate buffer (43). In addition, there is no reason to exclude contemporaneous states of intercalated dye and condensed DNA. Widom and Baldwin (44) have

shown that the DNA interactive properties of polyvalent cations, namely condensation and intercalation, need not be considered as mutually exclusive effects. Freeze-etch electron microscopy is now being used to definitively resolve this question.

We believe that it is appropriate at this point to present a working hypothesis to describe the binding of porphyrins to DNA, based upon our accumulated results (1,2,20,26,28,34,35). This initial model is proposed for the porphyrins studied in this report although it is equally applicable to several metalloporphyrin analogues of T4MPyP as already indicated (45).

At small R and low ionic strength, T4MPyP and T3MPyP bind by intercalation and possibly with some degree of GC selectivity. This is shown by the sum of the evidence derived from unwinding experiments and viscosity measurements of linear DNA along with the negative character of the visible CD band of both porphyrins. As R increases the GC selectivity is diminished as indicated by the appearance of a positive band to give a split CD spectrum. The positive band is characteristic of AT binding as demonstrated by the results of our measurements with polynucleotides (28). The difference demonstrated in the binding characteristics of T4MPyP and T3MPyP is largely one of degree. The smaller unwinding angle found for T3MPyP indicates that at a given input ratio, a smaller fraction of this ligand is bound by intercalation. It is apparent that the binding of these porphyrins is strongly dependent on ionic strength, but varies within the series. Recently we have found that the unwinding angles of T4MPyP and T3MPyP decrease with increasing ionic strength (26). We interpret this to mean that with increasing ionic strength the fraction of porphyrin bound by intercalation is decreasing concurrently with a shift to greater occupation of AT sites. Consistent with this is our result showing that T4MPyP can bind to DNA at 1 M Na^+ and shows a single positive band in its CD spectrum (1). In addition T4MPyP can bind to poly[d(A-T)]·poly[d(A-T)] but not to poly[d(G-C)]·poly[d(G-C)] up to at least 4.0 M Na^+ with a characteristic positive CD spectrum (34). It has proven useful to identify DNA-interactive ligands as being either intercalating such as ethidium bromide or outside binding, such as netropsin. Many of the outside-bound drugs appear to exercise AT-site selective association (46). A unique characteristic shared by T3MPyP and T4MPyP is that they demonstrated both binding modes and that the relative occupation of these "sites" depends on R and ionic strength.

T2MPyP and TMAP only demonstrate outside binding, but based on their CD features it can be argued that at small R there is a degree of AT specificity.

Binding of T2MPyP appears to fall rapidly with increasing ionic strength, whereas, TMAP binding can be demonstrated up to at least 1 M Na^+ (20). We believe this is due to the ability of TMAP to stack along the surface of DNA and that this process is favored by increasing ionic strength. That there is some specificity for AT is suggested by the finding that TMAP binds very poorly to poly[d(G-C)]·poly[d(G-C)] even at low salt concentrations (26,28). As already noted, the charge distribution for T2MPyP would be likely to prevent stacking and it is therefore unable to attain that state and consequently bind at high salt. One question that we have not addressed is the nature of the AT-bound porphyrin. Although we have described this as outside binding, there remains the possibility that some type of partial intercalation may be involved (47).

We realize that the validity of this working hypothesis depends to a large degree on our intrepretation that the sign of the visible CD indicates base pair selectivity. Recently, we have received information that provides verification of our results. Pasternack et al. (48) have performed kinetic experiments and, paralleling our earlier efforts, CD spectroscopy to examine the DNA interactive nature of certain metalloporphyrin derivatives of T4MPyP. Their findings indicate, as we have suggested (45), that binding selectivity for either AT or GC rich regions in DNA is a property of some tetrapyrrole ligands.

The problems inherent with the interpretation of CD and the pitfalls associated with over interpretation of these spectroscopic data are well-known and suggest a prudent approach. We believe, however, our analysis to be generally correct and that it can serve as a useful basis for the design of additional experiments.

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