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Circular Dichroism Spectroscopy of a Cationic Porphyrin Bound to DNA†

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Abstract: Recently, the porphyrin photosensitizer meso-tetra(4-N-methyl-pyridyl)-porphine was identified as a DNA-reactive agent demonstrating both electrostatic ar intercalative binding. A series of porphyrin derivatives were synthesized and studied to see if similar compounds manifested identical behavior. One derivative, meso-tetra(p-N-trimethylanilinium)porphine did not exhibit intercalation behavior l did show avid binding and novel circular dichroic features when bound to B-form DN At an ionic strength of 1.02, the binding constant was found to be on the order of 10^4 and higher at lower ionic strength. The large binding constants and induced optical activity suggest that at large porphyrin/DNA ratios the final porphyrin·DN complex may take the form of a suprahelical helix.

Introduction: The initial assignment of the photosensitizer (1,2) meso-tetra(4-N-methylpyridyl)porphine, T4MPyP, as an intercalating agent was made in consideratio of the physical properties of the porphyrin·DNA complex: stabilization of the heli against thermal denaturation, hypochroism, bathochromism, an induced optical activ in the visible region, altered ellipticity in the near ultra-violet region, an increased relative viscosity, and an unwinding of superhelical DNA (3,4). Subsequent the DNA binding characteristics of a large number of metallo- and nonmetallo-porphyrins has been studied. One derivative meso-tetra(p-N-trimethylanilinium)-porphi TMAP, was found unable to unwind superhelical DNA (5). Since unwinding of superhelical DNA is frequently diagnostic for intercalation the inability of TMAP to do so argues against this binding mode. However, this DNA-binding porphyrin manifests unique non-intercalative binding characteristics not observed in any of the other porphyrins studied to date. The results of our investigation (binding analysis an circular dichroism) of the interaction of TMAP with calf thymus DNA are presented this report.

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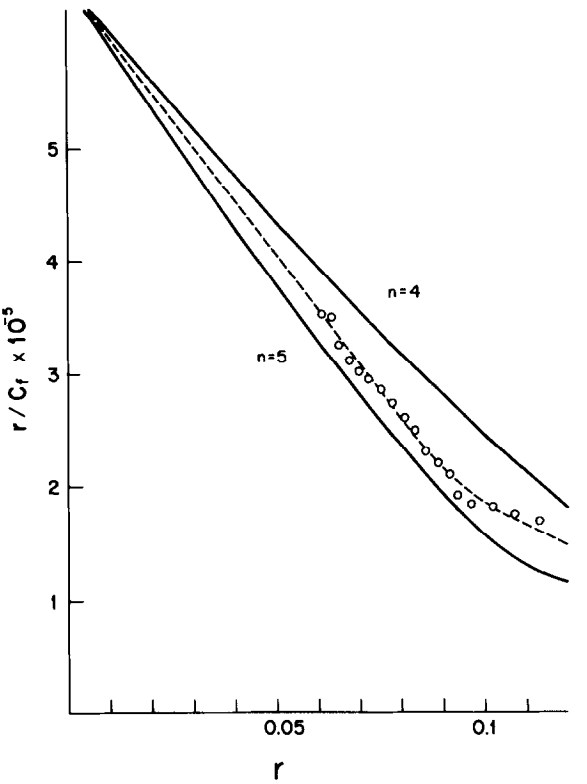

Figure 1. Binding isotherm measurements were carried out by titrating a solution containing calf thymus DNA (4.63 x 10^{-4} M (b.p.)) and TMAP (8.60 x 10^{-7} M) into a solution of TMAP (8.60 x 10^{-7} M) using 4 cm cells. The solutions were made up in BPES (.179 M NaCl) buffer. The decrease in absorbance was determined for each addition and the extinction coefficient for the porphrin·DNA complex was determined from the point at which a constant value for the apparent extinction coefficient was obtained. Nearest neighbor exclusion was determined from the data points shown, which were taken between the limits of 20 to 80% TMAP bound.

Materials and Methods: TMAP was prepared from meso-tetra(p-N,N-dimethylaminophenyl)-porphine dissolved in dimethylformamide and refluxed with methyl-p-toluene sulfonate for 10 minutes. The solvent was removed and the residue redissolved in water saturated with solid $NaClO_4$. The precipitate was recrystallized and dried. Calf thymus DNA was purchased from Worthington Biochemicals. All materials were dissolved in BPES buffer [2mM NaH_2PO_4, 6mM Na_2HPO_4, 1mM EDTA, 179mM NaCl, pH 6.8] with NaCl added or subtracted as described in the text. Binding isotherms were measured using a Beckman DB-G spectrophotometer and 4 cm cells. Circular dichroism studies employed a Jasco J41-C spectropolarimeter made available to us by Dr. Greyson Snyder, SUNY at Buffalo.

Results and Discussion: When solutions of TMAP are titrated with aliquots of calf thymus DNA the absorption maximum (414 nm) is reduced and shifted to longer wavelengths indicating that TMAP is binding to DNA. The binding isotherms calculated from the spectral changes are complex and display curvilinear plots of r/C_f vs. r, see figure 1. Scatchard analyses of these data inappropriately ignore the curva-

Table 1

Binding Affinities for TMAP*DNA Complexes

Buffer	K_{int}
BPES (without NaCl)	2.7×10^7
BPES (.05 M NaCl)	1.0×10^7
BPES (.10 M NaCl)	1.5×10^6
BPES (.179 M NaCl)	6.5×10^5
BPES (.30 M NaCl)	2.0×10^5
BPES (.50 M NaCl)	7.0×10^4
BPES (1.0 M NaCl)	4.2×10^4

*Calculated from data points of r/C_f vs. r between 20 and 80% bound TMAP.

ture at higher r and give values of K_{ap} which do not reflect an influence of ionic strength. A more detailed analysis using the neighbor exclusion model (6) accounts only for data in the mid-range of ionic strength, as shown in figure 1. At low ioni strength the binding isotherms are complicated due to the onset of an apparent positive cooperative effect at approximately 50% of dye bound (not shown). At higher ionic strength the range of r over which binding can be measured is too small (ca. 0.004 to 0.006 at 1 M Na^+) to provide an unequivocal fit of the experimental data to any theoretical model. An alternate procedure is to calculate the relative binding affinities or the intrinsic binding constant (K_{int}) from the intercept of $r/C_f = 0$ (7,8). K_{int} is independent of any particular model and is shown to decrease with ionic strength (Table 1), as would be expected for an electrostatically bound species.

The CD spectrum of the porphyrin and DNA complex was recorded as a function of ionic strength and [porphyrin]/[DNA] input ratio, R. The ultraviolet CD bears littl evidence of any interaction at small values of R, see figure 2. A decrease in the intensity of the positive peak at 275 nm and negative peak at 245 nm is seen at low ionic strength as R is increased. However, the overall contour of the spectrum is maintained. The effect is much less at 0.5 M NaCl (not shown). In contrast to thes results previous (3) and recent work (5) shows that binding of T4MPyP to DNA produce

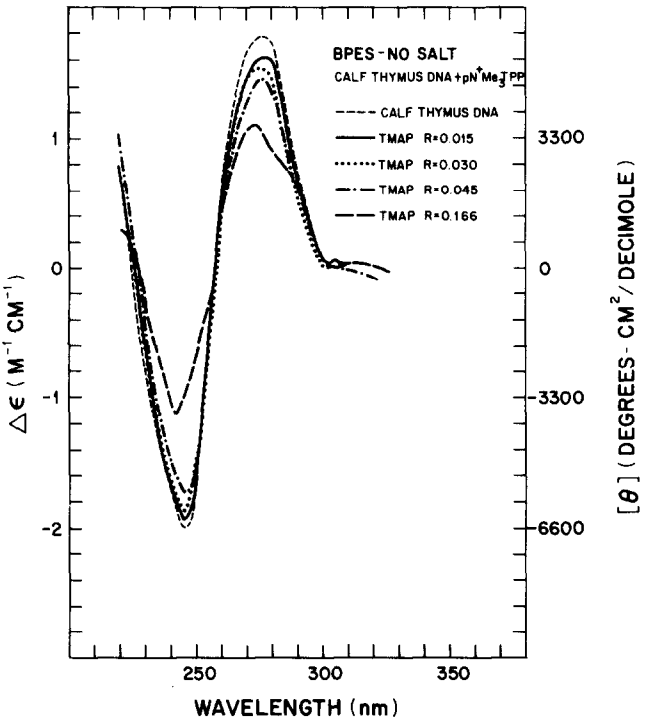

Figure 2. Ultra-violet CD spectra of TMAP·DNA in BPES buffer (without NaCl) as a function of R. ---- , calf thymus DNA; — R = 0.015; ···· R = 0.030; —·— R = 0.045; — — R = 0.166.

a much greater effect on the ultraviolet CD. Significant reduction and distortion of the 275 nm DNA peak occurs as R increases. These changes correlate with helix unwinding (7); however, unlike TMAP, T4MPyP has an absorption band in this region (ca. 265 nm) and it is not possible to determine the degree to which this contributes to the ellipticity.

The visible CD spectrum of TMAP·DNA is a sensitive measure of complexation, see figure 3. At low ionic strength and high R the spectrum is a conservative split pattern with a negative maximum centered at 422 nm and positive maximum centered at 412 nm. The zero point at 418 nm corresponds closely to the wavelength of maximum absorption for the free porphyrin. This pattern, which may be explained by exciton splitting (8-11), is unchanged when the amount of drug is varied between 1.0>R>0.1. When the amount of drug free in solution is high, R>1.0, the signal to noise ratio is very low and little useful information is available. If the DNA is present in a ten-fold or greater excess, 0.1>R>0.045, the conservative nature of the spectrum is lost

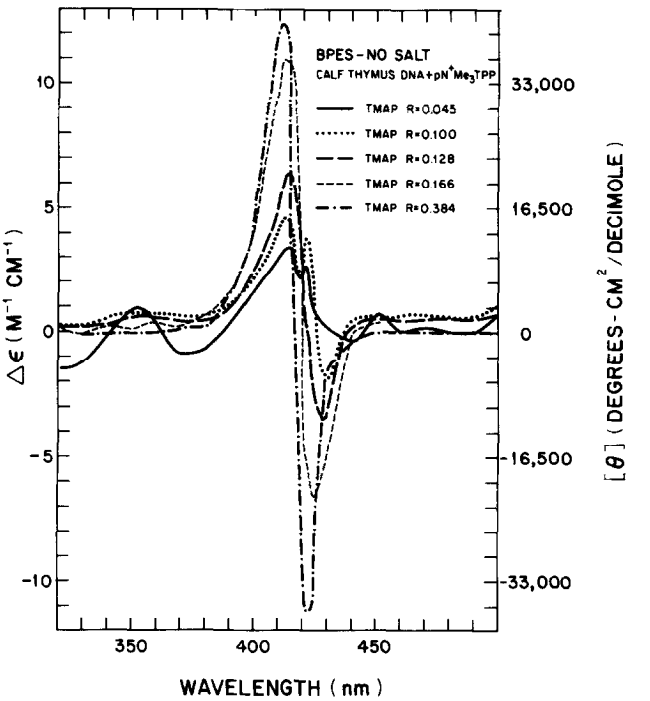

Figure 3. Induced visible CD spectra of TMAP·DNA in BPES buffer (without NaCl) as a function of R. —— R = 0.045; ···· R = 0.10; — — R = 0.128; - - - - R = 0.166; —·—R = 0.364.

and the positive peak appears to split into two peaks. For R≤0.03 the spectrum consists of a single positive peak of low intensity.

The visible CD spectrum measured in BPES/0.5 M NaCl shows no split peak and dichroic features are an order of magnitude less intense (not shown). The significant increase in noise under these conditions precluded the taking of reliable data.

The inability of TMAP to unwind supercoiled DNA indicates that it is not an intercalator. Therefore, the binding demonstrated by CD and binding isotherm measurements must be accounted for on the basis of an alternate interaction. Since the positively charged trimethylanilinium group is a powerful electrophile a charge-charge interaction with the backbone phosphate groups is expected to be very strong even in high ionic strength media. At small R this interaction would result in isolated TMAP molecules electrostatically tethered at the sites of negatively charged phosphate groups, see figure 4 (left). The CD spectrum shows a positive ellipticity under these conditions, as shown in figure 3 for R<0.045. The conservative negative positive CD spectrum is obtained when R lies between 0.1 and 1.0. This pattern has

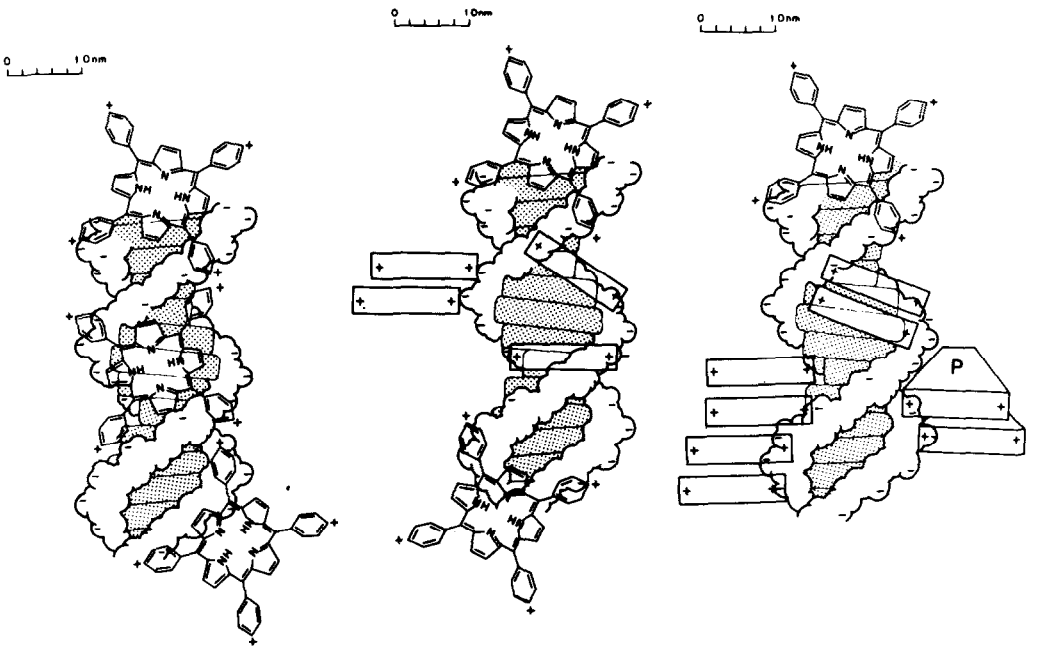

Figure 4. This schematic depicts porphyrin-DNA interactions in three ranges of R (porphyrin/DNA). Left: For 0.03>R>0 the binding is predominantly "face on", non-interacting i.e. the plane of the bound chromophore is oriented parallel to the helix axis. Middle: For 0.10>R>0.05 the binding is of two types: "face on" and "edge on". The "edge on" orientation, in which the plane of the bound chromophore is perpendicular to the helix axis, allows for porphyrin-porphyrin stacking. Right: For 1.00>R>0.10 a helical, stacked array of bound porphyrin molecules is established.

been observed in other systems in which the optical activity of dye-macromolecular complexes has been employed for the elucidation of conformation; in particular, to assess the helical content of these systems (12,13). For the porphyrin·DNA complex at low ionic strength and 0.1<R<1.0 we propose a model of a regularly stacked array of porphyrin molecules along the surface of the DNA, see figure 4 (right). In the limit of 1 porphyrin per base pair the stacked porphyrins would reproduce a right handed helix overlaying the polynucleotides' double helix. The DNA undergoes little structural alteration when accomodating the porphyrin in this fashion, as is shown by the ultra-violet CD. The induced ellipticity seen in the visible CD, is consonant with a helical porphyrin·DNA structure; particularly, as we have no evidence for self-association of this porphyrin in solution. Therefore, the porphyrin-porphyrin interaction and its representative CD would only be evident in the presence of a suitable polyanion which mediates the stacking in accord with the arrangement of its negative charges. Also, a threshold concentration of bound porphyrin would have to

be attained before stacking could be detected, and a very low concentration of drug would not exhibit the exciton splitting. At the intermediate range of R (0.10>R >0.045), a number of binding configurations appear to be possible as depicted in figure 4 (middle). Under these conditions the CD spectrum is characterized by a non-conservative split and the appearance of two closely spaced positive bands.

At higher ionic strengths the attractive force of the DNA phosphate backbone fo the porphyrin is screened by the increasing number of cations in solution. At any given value of R the binding is less, the amount of complex formed is less, and the observed CD spectrum is less intense. No data suggest there to be a superhelical structure of bound dye at higher ionic strengths.

As a final note, earlier results with T4MPyP·DNA indicated that its positive CD band (visible) increased under conditions favoring intercalation (low R and high ionic strength) and that its negative band decreased under conditions unfavorable fo electrostatic binding (3). A tentative association of negative band reflecting ele trostatic binding and positive band reflecting intercalative binding was made. Mor recently, preliminary CD studies of the interaction of T4MPyP with polynucleotides pointed to a possible base specificity as an additional factor in determining the sign of the ellipticity. The ellipticity is found to be predominantly positive for poly[d(A-T)]·[d(A-T)] and negative for poly[d(G-C)]·[d(G-C)]. However, TMAP shows evidence for strong DNA interactive behavior of a non-intercalative type and is characterized by CD spectra in which both positive and negative features are demonstrated. Hence, the sign of a given CD feature for a porphyrin·nucleic acid complex, and whether or not it displays splitting of its bands appears to be determined by the conformation of the nucleic acid and the mode of porphyrin·nucleic acid interaction.

In summary, we have found that TMAP demonstrates an extraordinarily high bind ing capacity to DNA that is non-intercalative. This may best be appreciated when t values in Table 1 are compared with the purely electrostatic binding of the hexacation ruthenium red to DNA for which K_{ap}= 2.5 x 10^4 M^{-1} in 0.165 M NaCl and is negligible for [NaCl] >0.2 M (14). At a comparable ionic strength (BPES buffer 0.1 M NaCl) TMAP has a binding constant more than an order of magnitude larger. At lo

a single CD band, not exciton split, is shown, while at high R a conservative feature is apparent. We suggest that this reflects a transition to a stacked state in which TMAP follows the helical structure of DNA. Judging from CD criteria alone, there can be no a priori assignment of base specificity in porphyrin·DNA complexes, nevertheless, carefully conceived CD experiments may substantiate an empirical designation of base specificity.

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