Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 18, Issue Number 5, (2001)

Interactions of Meso-tetra-(4-N-oxyethylpyridyl) Porphyrin, Its 3-N Analog and Their Metallocomplexes with Duplex DNA

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Abstract

Interactions of meso-tetra-(4-N-oxyethylpyridyl) porphyrin (TOEPyP(4)), its 3-N analog (TOEPyP(3)) and their Co, Cu, Ni, Zn metallocomplexes with duplex DNA have been investigated by uv/visible absorbance and circular dichrosim spectroscopies. Results reveal the interactions of these complexes with duplex DNA are of two types. (1) External binding of duplex DNA by metalloporphyrins containing Zn and Co, and (2) Binding of duplex DNA both externally and internally (by intercalation) by porphyrins not containing metals, and metalloporphyrins containing Cu and Ni. Results indicate that (4N-oxyethylpyridyl) porphyrins intercalate more preferably in the structure of duplex DNA and have weaker external binding than 3N-porphyrins.

Introduction

For both chemical and clinical reasons the study of porphyrins and modified porphyrins and their interactions with duplex DNA is an important area of research. Porphyrins comprise an important class of compounds whose chemical and photochemical properties are widely exploited in both medical and biological applications. Due to their ability to accumulate in malignant cells, porphyrins have found utility as diagnostic agents for determination of tumor design (4,5). This peculiarity of porphyrins as well as their photosensitivity have enabled their use in successful photodynamic treatments of tumors (1,2). Some porphyrins have also been shown to exhibit anti-viral activity (3).

Many of the important biological and medically relevant properties of porphyrins are dependent on the type of peripheral substituents. For example, we previously reported synthesis of water-soluble meso-tetra (4-N-oxyethylpyridyl) porphyrin (TOEPyP(4)), its 3-N analog (TOEPyP(3)) and their metallocomplexes with Co, Cu, Ni, Zn, which had been shown to have both antibacterial and antifungal activities (6-8). Although the anti tumor and viral activities of modified porphyrins have been established, the mechanisms of their interactions with biological macromolecules, in particular with duplex DNA, have not been thoroughly characterized. Interactions of meso-tetra (4-N-methylpyridyl) porphyrin (TMPyP) with DNA have been studied previously (9-21) and mechanisms of their complex formation with DNA have been determined (9,10,17). These studies have established, that the positions of the 2N -, 3N -, and 4N - methyl-group on the pyridylic ring influences interactions of porphyrin with duplex DNA (11,17). In addition the type of the central metal in metallocomplexes of TMPyP porphyrins is known to strongly influence mechanisms of binding duplex DNA (11,14,17-21). Investigations of the interactions of TOEPyP(4) and its Cu, Ni and Mn derivatives with duplex DNA were also reported (22).

In this paper uv/visible and circular dichroism (CD) spectroscopies were applied to investigate the interactions of TOEPyP(4), TOEPyP(3) and their Co, Cu, Ni, Zn metallocomplexes with duplex DNA. These investigations indicate the predominate DNA binding modes exhibited by the modified porphyrins, and how DNA binding depends on the chemical structures of the porphyrins and their metallocomplexes.

Materials and Methods

DNA Molecules:
Ultra-pure DNA from Calf Thymus served as the duplex DNA samples for these studies (protein<0.1%, RNA<0.2%, M.w.>30 MDa). The properties of this DNA described in (23). Poly(dA-dT)$_2$ was purchased from Sigma Chemical Company.

Porphyrin Compounds:
Structures of meso-tetra-(4-N-oxyethylpyridyl) porphyrin (TOEPyP(4)), its 3-N analog (TOEPyP(3)) and their metallocomplexes with Co, Cu, Ni, Zn are shown in Figure 1. These compounds were synthesized as described in (24).

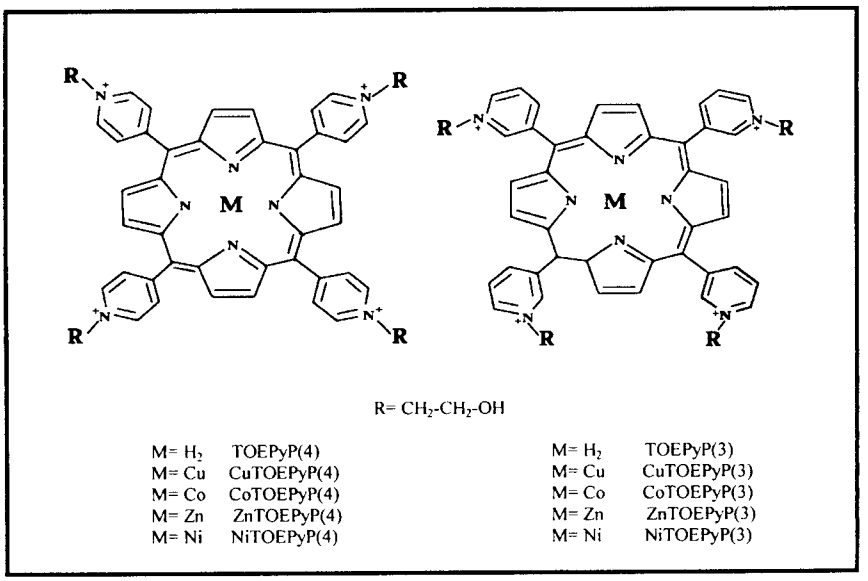

Figure 1: Structure of porphyrins.

Ultraviolet melting curves of porphyrin/DNA complexes were recorded on a Lambda-20-Bio (single beam) spectrophotometer. For all experiments the heating rate was 0.5°C/min while absorbance at 260 nm was recorded. Absorbance spectra were recorded on a Specord UV-VIS spectrophotometer. Circular dichroism spectra were recorded on a Roussel Jouan-II dichrograph.

Concentrations of porphyrin solutions ranged from 10^{-6} to 10^{-4} M. When in the presence of duplex DNA at ratios of porphyrin compounds to nucleotide base pair concentrations, r, ranged from $0.002 < r < 0.8$, where $r = C_{porf} / C_{\text{base pair of DNA}}$. All solutions were buffered in 0.1 BPS, pH = 6.85, ionic strength [Na^+] = 0.02. (1BPS = 6 mM Na_2HPO_4 + 2 mM NaH_2PO_4+185 mM NaCl).

Results and Discussion

The Absorption Spectra of Porphyrin/DNA Complexes

For our spectroscopic investigations the highly intense Soret peak absorbance near 420 nm was used to monitor interactions of TOEPyP(4) and TOEPyP(3) and two of their metallocomplexes with duplex DNA. The absorbance spectra collected from 375 to 485 nm at increasing DNA concentrations for these DNA/porphyrin complexes are displayed in Figure 2. These spectra reveal for all four complexes, albeit to differing extents, that as DNA concentration increases in solutions con-

taining a constant concentration of porphyrins, a hypochromic and bathochromic shift of the absorbance at 420 nm (shown by the vertical lines on the spectra in Figure 2) occurs. Similar spectral changes are also observed for the other varieties of DNA-porphyrin complexes examined. The spectroscopic data obtained for the porphyrin-DNA complexes that were examined are summarized in Table I.

As clearly seen in Figure 2 and summarized in Table I, when DNA is added in increasing concentrations to a porphyrin solution, the porphyrin spectra are affected in a titratable manner. However, the strength of the effect depends on the type of porphyrin and its interactions with duplex DNA.

Table I

Position of the Soret band for pure porphyrins ($\lambda_{max,0}$); maximal values of bathochromic and hypochromic effects for this band caused by titration with DNA. The maximum concentration of DNA is 5×10^{-4} M of nucleotide pairs. Porphyrin concentration is given in Figure 1.

No	Type of Porphyrins	$\lambda_{max,0}$ of Soret band of pure porphyrin	Bathochromic shift of DNA $\Delta\lambda_{max}=\lambda_{max,C}-\lambda_{max,0}$, nm	Maximal hypochromic effect of Soret band, %
1.	TOEPyP(4)	426	12	46
2.	TOEPyP(3)	420	9	38
3.	CoTOEPyP(4)	438	2	24
4.	CuTOEPyP(4)	429	5	34
5.	NiTOEPyP(4)	424 (shoulder at 444 nm)	11 (main peak)	23
6.	ZnTOEPyP(4)	440	4	24
7.	CuTOEPyP(3)	422	5	45
8.	CoTOEPyP(3)	434	2	6
9.	ZnTOEPyP(3)	430	6	18
10.	NiTOEPyP(3)	413 (shoulder at 434 nm)	15 (main peak)	37

A hypochromic effect on the Soret band when DNA is added to TOEPyP solutions has been reported (9). The authors proposed an intercalation mechanism to explanation their observations. Additional studies of TOEPyP porphyrins and their metal derivatives with duplex DNA have suggest that these compounds interact with DNA by essentially three different modes (9-17,20,25-27). These compounds bind duplex DNA by (1) intercalation, where porphyrins are inserted between adjacent nucleotide base pairs such that the pyridylic groups reside in the grooves, two in each groove (28,29); (2) Ordered external (surface and groove) interactions, where planar porphyrins stack on top of each other along the duplex stabilized by

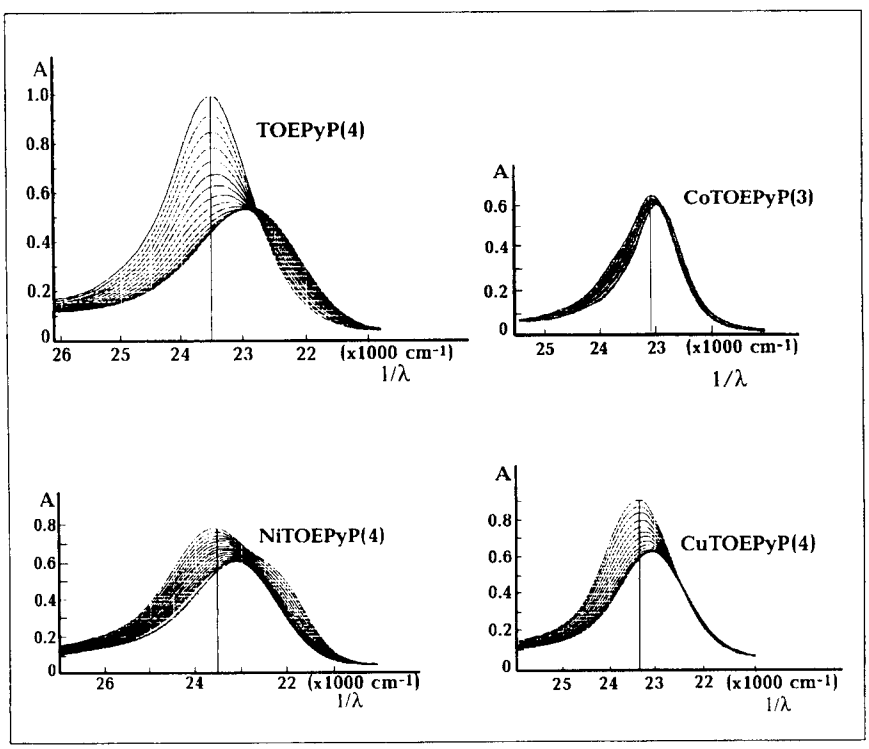

Figure 2: The absorption spectra of some porphyrin-DNA complexes.The position of the maximum of absorption spectra of pure porphyrin is indicated by vertical line. DNA was added to the porphyrin solution by small portions from concentrated ($1.1\cdot10^{-4}$ M of base pairs) DNA solution. During experiment the concentration of DNA was increased ~ 15-20 times from minimum concentration 5×10^{-7}. The concentration of porphyrin is 5×10^{-6} for TOEPyP(4) and CuTOEPyP(4); 8×10^{-6} for CoTOEPyP(3); 10^{-5} for NiTOEPyP(4).

interactions in the minor groove of DNA and negatively charged phosphate groups. Both (1) and (2) rely on stacking interactions, either between nucleotide bases and porphyrins, or porphyrin-porphyrin stacking. Either case results in a hypochromic effect around 420 nm. (3) The third type of binding mode is assumed to occur through less ordered interactions between porphyrins and duplex DNA when the planar porphyrin molecules are located in the major groove, with the plane of the porphyrin ring parallel to the DNA helix axis, in a so-called "face-on" orientation (9-11,20). This third type of binding does not result in hypochromic effects when DNA is added to porphyrin solutions. The absence of a clear isobestic point on the absorption spectra of the porphyrin/DNA complexes in Figure 2, except TOEPyP(4), suggests these porphyrins may participate in more than one type of binding.

Circular Dichroism Spectra

It is expected that the interplanar interactions of porphyrins with duplex DNA can induce conformational changes of the DNA. Valuable information regarding conformational changes in DNA induced by interactions with ligands can be obtained from measurement of the CD-spectra of the ligand/DNA complexes. It has was shown that the formation of complexes of TMPyP(4) with duplex DNA could be monitored by changes in two prominent regions of the CD spectra (9,10) These are, the region from 220 to 310 nm, corresponding to the CD band of native duplex DNA and, the Soret region from 400 to 470 nm which corresponds to the porphyrins. DNA does not have a measurable CD in this region of the spectrum.

Presumably, the CD band observed for porphyrin/DNA complexes in the visible region, the so-called induced CD, corresponds to packing of porphyrins on the DNA. This CD signal of the porphyrins is DNA dependent because porphyrins,

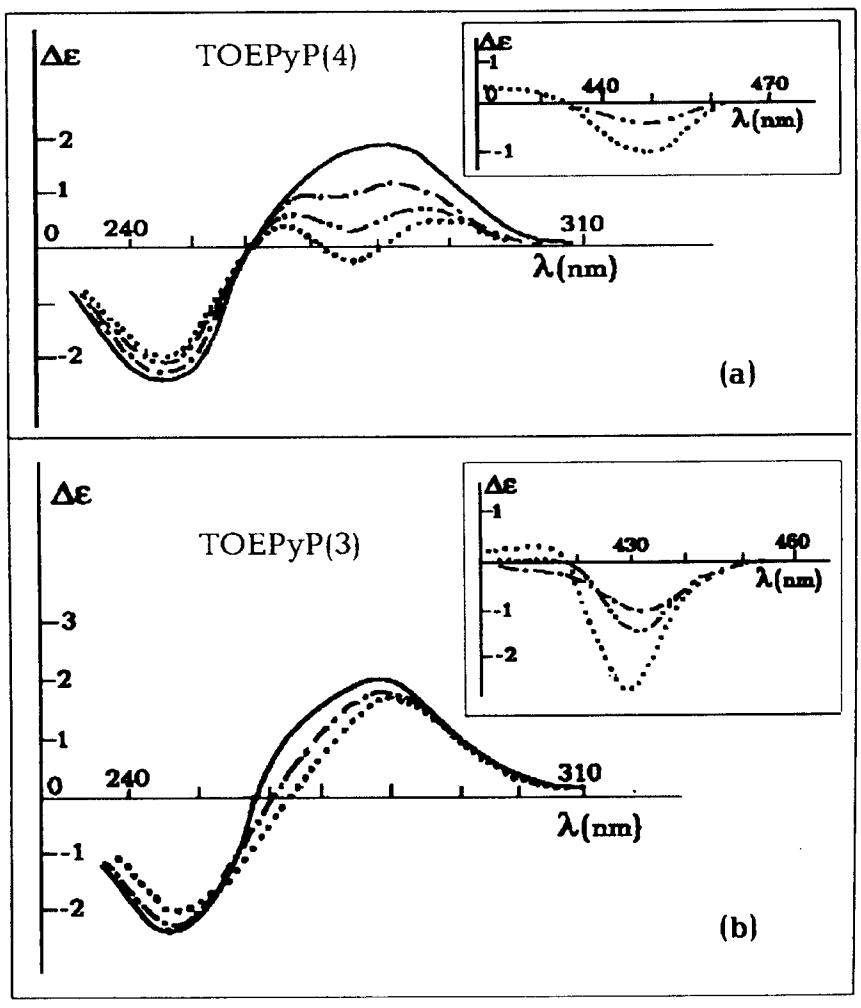

Figure 3: Circular dichroism spectra of DNA complexes with TOEPyP(4) (a) and TOEPyP(3) (b) at various relative porphyrin concentrations: (—) - r = 0; (-•-) - r = 0.077; (- •• -) - r = 0.15; (••••) - r = 0.77.

being planar, symmetrical complexes do not possess natural optical activity and in fact have zero dichroism in the entire spectral region of their electronic transitions.

Binding of TMPyP and duplex DNA was monitored by CD (9,10). The noted negative value of the induced CD was taken as indication of the intercalation of the porphyrin, while a positive value corresponds to the external binding mode (9,10). Thus, the sign of the induced CD in the visible region of the spectrum can serve as an indicator of the type of porphyrin-DNA binding.

The UV-region of the CD spectra for porphyrin/DNA complexes is also quite informative. The CD spectra of complexes of TOEPyP(4) and TOEPyP(3) with duplex DNA from 230 to 310 nm are shown in Figure 3. The visible spectra are shown in the insets. As seen in Figure 3a, increasing concentrations of TOEPyP(4) results in splitting of the positive CD band at 276 nm, $\Delta\varepsilon_{276}$, into two bands. In contrast, as seen in Figure 3b, addition of TOEPyP(3) induces only a slight decrease in intensity of the 276 nm band. The negative band at 248 nm is only slightly affected for either porphyrin/DNA complex. These observations suggest the locations of peripheral substituents on the pyridylic ring of the porphyrins determine the conformations of the porphyrin/DNA complexes. As observed for the complexes in the visible region of the CD spectra (insets of Figure 3), addition of these porphyrins induces a negative CD from 420 to 440 nm, for both complexes, consistent with an intercalation binding mode for these compounds.

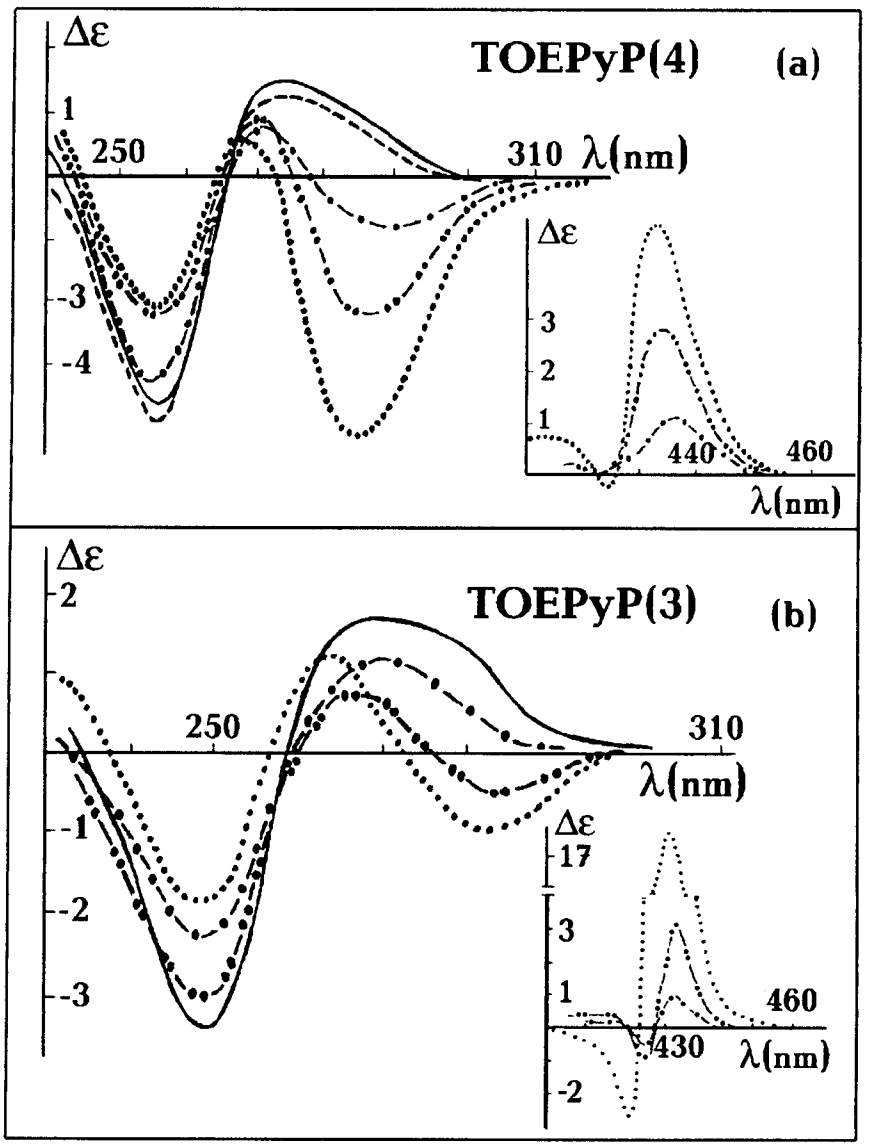

Figure 4: Circular dichroism spectra of Poly(dA-dT)$_2$ complexes with TOEPyP(4) (a) and TOEPyP(3) (b) at various relative porphyrin concentrations: (—) - r = 0; (- - -) - r = 0.013; (- • -) - r = 0.066; (- •• -) - r = 0.13; (•••) - r = 0.66.

Published results on studies of TMPyP/DNA complexes showed that an intercalation binding mode is preferred for GC-rich regions of duplex DNA. For AT-rich regions of duplex DNA porphyrin binding occurs by means of external groove binding (11,30,31). To determine whether our porphyrins can also participate in external binding with duplex DNA, the CD spectra of their complexes with poly(dA-dT)$_2$ were recorded in the visible and uv regions of the spectra. The spectra of these complexes in the visible region of the spectra are shown in Figure 4.

This DNA polymer is stearically unable to accommodate intercalation by porphyrins, and external binding is preferred. This is clearly demonstrated by the changes in the CD spectra in Figure 4a for the complex of TOEPyP(4) and poly(dA-dT)$_2$. There is a positive band at 435 nm, but with increased porphyrin concentrations, a negative band at 425 nm emerges. Meanwhile, in the same region of the spectrum, the CD of the TOEPyP(3)/poly(dA-dT)$_2$ complexes are nearly conservative with a negative band at 425 nm and positive band at 435 nm) at low porphyrin concentration. With increased porphyrin concentration the positive band increases several times more than the negative one. In the UV region of the CD spectra (insets of Figure 4), dramatic changes are also observed with increased concentrations of porphyrins. The positive band from 310-260 nm inverts in sign from positive to negative ellipticity.

Comparison of these changes in the CD spectrum of the porphyrin/DNA with those shown in Figure 3 it is concluded that the characteristic splitting of the CD at 276 nm is an indication of external binding of porphyrins with AT-rich regions of Calf thymus DNA. As shown in several studies (13,17,30,31), at high ionic strengths, porphyrins can form rather extended self-assembled complexes (aggregates) in the

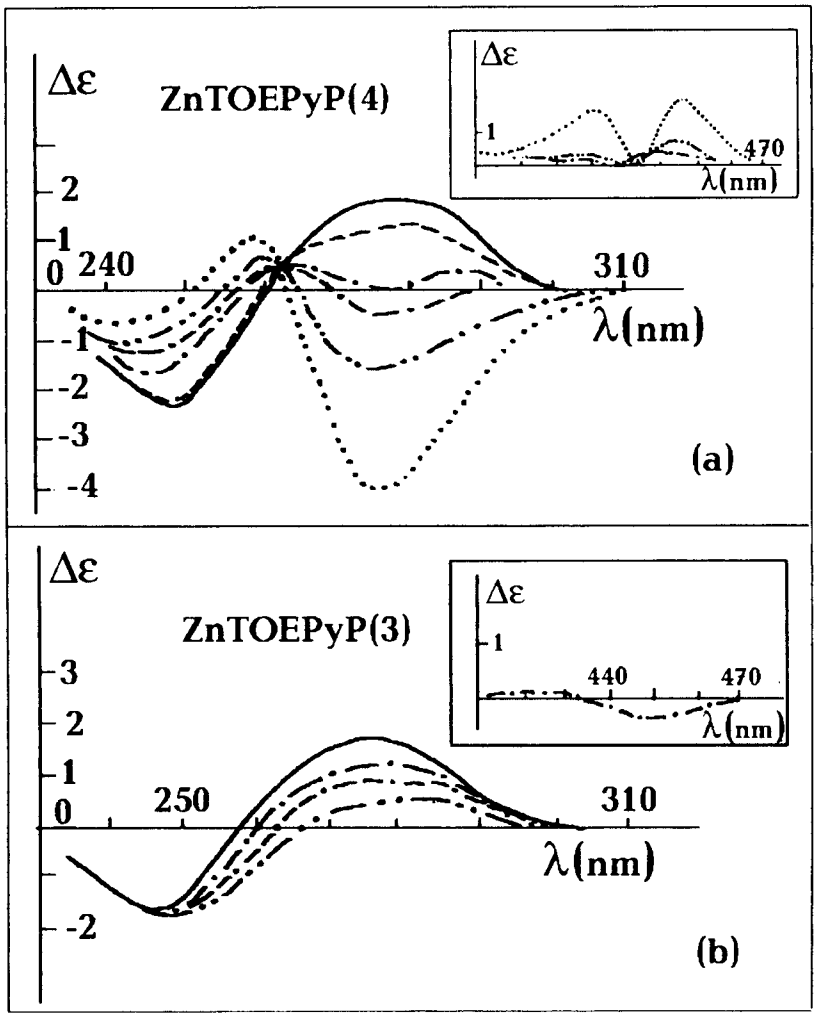

Figure 5: Circular dichroism spectra of DNA complexes with ZnTOEPyP(4) (a) and ZnTOEPyP(3) (b) at various relative porphyrin concentrations:
(—) - r = 0; (- - -) - r = 0.03; (- • -) - r = 0.077;
(• - - •) - r = 0.11; (- •• -) - r = 0.15; (•••) - r = 0.77.

minor groove of poly(dA-dT)$_2$. These complexes then exhibit strong bands in the visible region of CD-spectra. On the basis of these data we conclude, that the porphyrins TOEPyP(4) and TOEPyP(3) can interact with duplex DNA both by intercalation and external groove binding .

Comparison of the CD-spectra of the complexes of TOEPyP(4) and TOEPyP(3) with Calf thymus DNA (Figure 3) and poly(dA-dT)$_2$ (Figure 4), reveals that the 4N-porphyrins preferably intercalate into duplex DNA and exhibit less external binding than the 3N-porphyrins.

The CD spectra of metalloporphyrin -DNA complexes strongly depends on the type of the central metal and the type of porphyrin (TOEPyP(4) or TOEPyP(3)). The CD in the uv region of the spectra of complexes of Calf thymus DNA with ZnTOEPyP(4) and ZnTOEPyP(3) are shown in Figure 5. The spectra of the CuTOEPyP(4) and CuTOEPyP(3) complexes are shown in Figure 6.

From the UV region of the CD spectra for the metalloporphyrins that contain the same metal, it is possible to interpret, on the basis of what was observed for non-metalloporphyrin, changes of the CD spectra. As shown in Figure 5 the DNA porphyrin complex containing ZnTOEPyP(4) results in inversion of the positive band at 276 nm. This same type of CD spectra change is characteristic of left-handed Z-

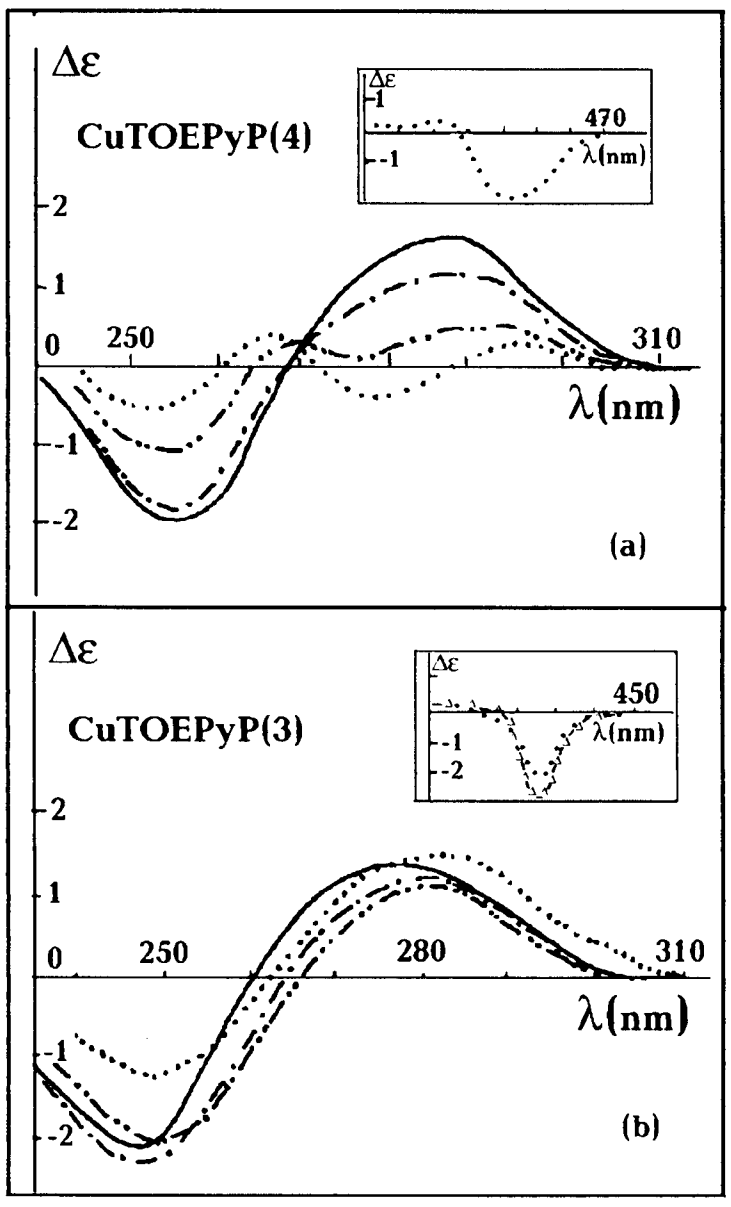

Figure 6: Circular dichroism spectra of DNA complexes with CuTOEPyP(4) (a) and CuTOEPyP(3) (b) at various relative porphyrin concentrations: (—) - r = 0; (- • -) - r = 0.027; (- •• -) - r = 0.08; (••••) - r = 0.77; (-Δ-) - r = 1.35.

DNA and aggregated ψ- forms of DNA (32-35). An isoelliptic point is observed. It is surmised, that due to external packing of porphyrins on duplex DNA, rather extended porphyrin structures result which have a negative CD from 260-310 nm, together with the DNA positive band in this region leads to splitting of the positive CD band at 274 nm. This interpretation is also consistent with results of flow dichroism measurements of duplex DNA in the presence of TMPyP(4) and its metallocomplexes. These hydrodynamic measurements suggested when porphyrins are involved external groove binding the planes of the porphyrin are not parallel or perpendicular to the helix axis, but tilted at an angle. For TMPyP(4) this angle is 55° (27), for ZnTMPyP(4) – 65° (25), and for CoTMPyP(4) – 45° (26).

The sign of the induced CD for these complexes leads to the conclusion that ZnTOEPyP(4) and ZnTOEPyP(3) (Figure 5) bind DNA preferably in an external mode while CuTOEPyP(4) and CuTOEPyP(3) (Figure 6) bind preferably by an intercalation mechanism. These observations suggest that metalloporphyrins with Co are related to the metalloporphyrins with Zn, because in the visible regions of the spectrum they display a positive CD.

Thus, the Zn containing TOEPyP interact with DNA by external groove binding, and the Cu-containing TOEPyP interact by an intercalation mode. This raises the question of whether the Cu-containing TOEPyP porphyrins also bind to duplex DNA in an external mode. To address issue, the CD spectra of CuTOEPyP(3)/poly(dA-dT)$_2$ complexes (Figure 7) were measured. The strong responses of the CD spectra in the visible region with increased concentrations of CuTOEPyP(3) are consistent with the proposition that these porphyrins interact with AT-pairs in an external binding mode.

On the basis of this CD spectral analysis of these porphyrin/DNA complexes with Calf thymus DNA and poly(dA-dT)$_2$ it appears porphyrins containing Zn and Co interact with DNA only by external binding, and the nonmetalloporphyrins and Cu and Ni metalloporphyrins interact both by intercalation binding and by external binding.

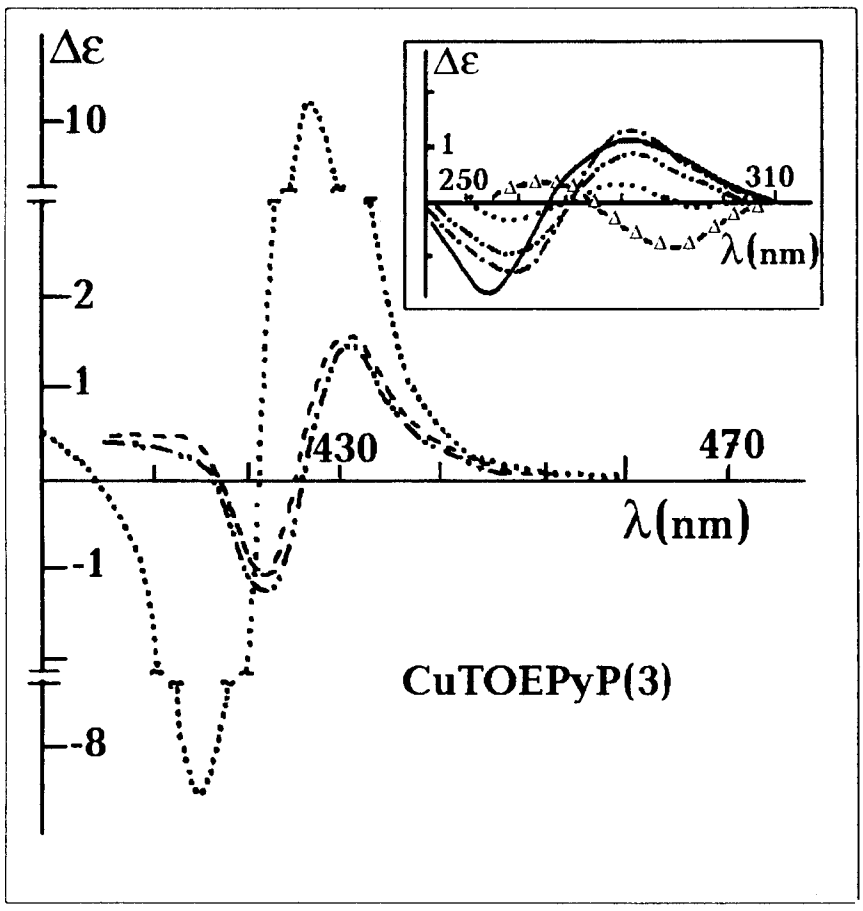

Figure 7: Circular dichroism spectra of Poly(dA-dT)$_2$ complexes with CuTOEPyP(3) at various relative porphyrin concentrations:
(—) - r = 0; (- • -) - r = 0.022; (- •• -) - r = 0.11; (- - -) - r = 0.22; (•••) - r = 1.1; (-Δ-) - r = 3.0.

Results of melting curve measurements for the variety of porphyrin/DNA complexes monitored at λ=260 nm are presented in Table II. There effects of melting of Calf thymus DNA in the presence of various concentrations of TOEPyP(4), ZnTOEPyP(4) and CuTOEPyP(4) are summarized.

Table II

The melting temperature (T_m), melting interval (ΔT) and hypochromic effect (ΔH) (λ=260 nm) for Calf Thymus DNA for various concentrations of some porphyrins. $C_{DNA}=5\times10^{-5}$ M of nucleotide pairs.

C_{porf}/C_{DNA} (r)	TOEPyP(4)			ZnTOEPyP(4)			CuTOEPyP(4)		
	T_m,°C	ΔT,°C	ΔH,%	T_m,°C	ΔT,°C	ΔH,%	T_m,°C	ΔT,°C	ΔH,%
0	71.8	9.8	37	71.8	9.8	37	71.8	9.8	37
0.001	72.8	9.3	38	-	-	-	-	-	-
0.002	70.6	9.6	39	71.4	9.9	37	71.9	9.6	37
0.01	71.6	9.4	36	-	-	-	-	-	-
0.02	-	-	-	72.8	11.5	37.5	72.5	10.2	37
0.05	73.3	9.5	34	-	-	-	-	-	-
0.1	75.6	11.8	33	80.6	13.6	39	80.9	12.2	28
>0.3	aggregation								

Table II shows that at porphyrin-DNA concentration ratios, r, in the range $0.001 < r < 0.01$ melting curve parameters relatively insensitive to the presence of porphyrin. At these low porphyrin concentration ratios external binding is probably more preferable. The hypochromicity of the complexes is essentially unchanged over the concentration range examined. For TOEPyP(4) the hyperchromicity change is only 1-2 %. At greater porphyrin:DNA ratios, $0.02 < r < 0.3$, both the Tm and ΔT values increase with increasing concentration of porphyrins. At concentration ratios, $r > 0.3$, aggregation effects are observed which hinder acquisition of the melting curves. (The most significant changes in the CD spectra of the porphyrin/DNA complexes were also observed at these concentration ratios).

Values of T_m, ΔT and the hyperchromicity change (ΔH) the porphyrin/DNA complexes at a relative ratio, r = 0.1 are given in Table III.

Table III shows that at porphyrin concentration ratios, r = 0.1, the melting temperature and melting curve widths increase for all porphyrins. However, the magnitude

Table III

Parameters of the melting curves of Calf Thymus DNA in the presence of various porphyrins (r = 0.1).

No	DNA/Porphyrin Complexes	T_m, °C	ΔT, °C	ΔH, %
1.	DNA without porphyrins	71.8	9.8	37
2.	DNA+ TOEPyP(4)	75.8	11.8	33
3.	DNA+ ZnTOEPyP(4)	80.6	13.6	39
4.	DNA+ CoTOEPyP(4)	78.6	14.3	35
5.	DNA+ CuTOEPyP(4)	80.9	12.2	28
6.	DNA+ NiTOEPyP(4)	82.9	13.1	25
7.	DNA+ TOEPyP(3)	75.0	14.9	32
8.	DNA+ ZnTOEPyP(3)	77.2	10.5	40
9.	DNA+ CoTOEPyP(3)	77.8	11.8	36
10.	DNA+ CuTOEPyP(3)	72.6	10.4	25
11.	DNA+ NiTOEPyP(3)	73.0	12.0	30

of the increases depends on the type of metal and positions (3N or 4N) of peripheral substituents on the pyridylic ring. As shown in Table 3, T_m and ΔT show greater changes at increased concentrations of TOEPyP(4) and its metallocomplexes, than TOEPyP(3). For nonmetalloporphyrins TOEPyP(4) and TOEPyP(3) the hypochromic effect (ΔH) at 260 nm decreases 4-5 % compared to DNA alone. The hypochromic effect is specific to the type of the central metal. The hypochromicity changes of the metalloporphyrin complexes are of two types. For the Zn and Co

metallocomplexes and both types of nonmetalloporphyrins, the hyperchromicity changes only slightly (in case of Co) or increases only slightly (in case of Zn). Alternatively, for the Cu and Ni metallocomplexes the hyperchromicity of melting is significantly reduced. For Cu, ΔH is 28 % and 25 %; for Ni, the decreases of ΔH are 25 % and 30 %, for TOEPyP(4) and TOEPyP(3), respectively.

Increases in T_m for TOEPyP(4) and its metallocomplexes CuTOEPyP(4) and NiTOEPyP(4) complexes with duplex DNA in 0.1 M Na^+ were noted previously (9,11). In this relatively high ionic strength, the melting temperature of DNA/porphyrin complexes is above 90° and it is technically difficult record the entire melting transitions. Consequently, it is not possible to accurately determine melting curve parameters under these conditions. Due to Coulombic screening of phosphates at high ionic strengths, many subtleties of the thermodynamic behavior of the DNA/porphyrin complexes are not detectable. In contrast, our experiments at relatively lower ionic strength (20 mM Na^+) allow acquisition of the entire melting curve and thereby accurate acquisition of melting curve parameters. The increased melting temperature (T_m) and melting interval (ΔT), observed under these conditions, are most likely connected with higher binding constant of porphyrins with helical DNA and the additional energy necessary for disruption of both internally (intercalated) and externally stacked porphyrins.

Decrease in the hypochromicity of duplex DNA in the presence of some porphyrins suggests weakening of interplanar stacking interactions between base pairs. This can occur if the plane of the porphyrin rings intercalate between base pairs and thereby disrupt stacking interactions between adjacent base pairs. If so, then the metalloporphyrins containing Cu and Ni must bind mainly by intercalation.

Alternatively, external binding would not be expected to disrupt significantly stacking of duplex DNA base pairs, and consequently the hypochromicity for melting complexes bound in this mode does not decrease. This is the case for complex of duplex DNA with porphyrins containing Zn and Co.

Thus, these investigations of porphyrin/DNA complexes by absorbance and CD spectroscopy have shown their preferable binding modes are determined by the type of central metal. It is known that the stearic chemistry of metalloporphyrins depends on the coordination number of transition metal atom (14). The coordination number for Cu is four. In the structure of metalloporphyrins it is located in the plane of the porphyrin ring and does not form any axial ligand. The atoms of Ni, having coordination number four may be related to this group. Such metalloporphyrins easily intercalate between base pairs of DNA.

The coordination number for Zn and Co is five. The porphyrins with these metals have one axial ligand, out of the plane of the porphyrin ring. As a result, these metalloporphyrins are stearically occluded from intercalating between base pairs of duplex DNA and therefore only participate in external binding.

Conclusions

In summary, comparison of the results obtained by CD and spectrophotometry reveal that: The porphyrins interact with Calf thymus DNA by two possible mechanisms depending on the porphyrin compound. (1) Metalloporphyrins, containing Zn and Co, bind duplex DNA only by an externally stacked mode, and (2) Nonmetalloporphyrins and metalloporphyrins, containing Cu and Ni, bind duplex DNA by both external stacked and internal (intercalation) modes. Replacement of a methyl-group in the pyridilic ring of TMPyP to the oxyethyl-group, does not change the mechanisms of their binding to duplex DNA.

The position of peripheral substituents on the pyridylic ring influences the interactions of porphyrins with DNA. That is (4N-oxyethylpyridyl) porphyrins bind preferably by intercalation and show worse external binding than 3N-porphyrins.

Acknowledgments

The investigations described in this publication were carried out under grants CRDF # AB1-963 / NFSAT # ACB-014-98, CRDF # AB2-2006 and ISTC #A-301d.

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Date Received: December 14, 2000

Communicated by the Editor Valery I. Ivanov