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SELF-ASSEMBLY OF PORPHYRINS ON NUCLEIC ACID TEMPLATES

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Summary: The cis and trans isomers of dicationic bis(4-N-methylpyridyl)diphenylporphine show a much greater tendency to aggregate than similar tetracationic porphyrins. Upon binding to nucleic acids these aggregating dicationic porphyrins form long-range structures on the polymer template giving intense circular dichroism signals whose profile reports the helical sense of the DNA.

Introduction: A variety of techniques both experimental and theoretical have been employed to probe the nature of the reactions of synthetic, cationic porphyrins with nucleic acids (1-19). These studies, which have emphasized the tetracationic, meso-tetrakis(N-methylpyridyl) family of metallo and nonmetallo porphyrins (see figure 1), have shown these substances to have considerable versatility in how they bind to DNA. Based upon results from our laboratories, we suggested that at low levels of drug load, the sign of the induced CD spectrum of porphyrins bound to nucleic acids can be used as a signature for the binding mode: a positive induced CD band in the Soret region is indicative of external groove binding and a negative induced CD band is present upon intercalation (1). Theoretical calculations have recently been presented which indicate that such correlations between binding mode and/or geometry and the sign of the CD are not unreasonable (20,21). Important relationships have been revealed between the binding mode and the nature of the inserted metal (1,2) as well as the position of the N-methyl group on the

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Abbreviations: tetrakis(X-N-methylpyridyl)porphine, H_2TMpyP-X where X indicates the position of the N-methyl group on the pyridine ring; bis(4-N-methylpyridyl)-diphenylporphine, $H_2(Ph)_2(4\text{-N-Mpy})_2P$; circular dichroism, CD.

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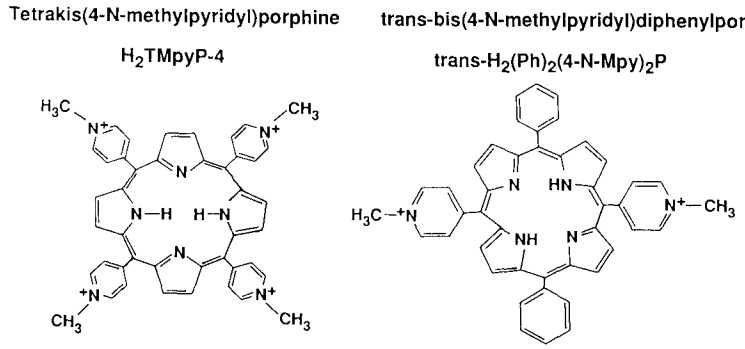

Figure 1. Structure of Porphyrins

pyridyl ring (1,2,11,12). In addition, the non-metallo derivatives, H_2TMpyP-4 and H_2TMpyP-3 show a dependence of binding mode to DNA on the ionic strength of the medium (3).

It was recently reported that the trans isomer of the dicationic bis(4-N-methylpyridyl)-diphenylporphine (see figure 1) is capable of intercalating into DNA (22,23). However, the results to be reported in this communication with cis and trans $H_2(Ph_2)(4\text{-}Mpy)_2P$ indicate that neither of these porphyrins is a particularly avid intercalator; rather they prefer to bind externally to form long-range stacked structures on the exterior of nucleic acids. This stacked drug structure leads to an unusually intense circular dichroism spectral signature whose detailed profile appears to report the helical sense of the DNA matrix.

Methods and Materials: The porphyrins were obtained from Midcentury as the chloride salt. Their concentrations were determined by absorbance using ε=1.4 x $10^5 M^{-1}cm^{-1}$ for the cis isomer and 2.4 x $10^5 M^{-1}cm^{-1}$ for the trans isomer. Calf thymus DNA was obtained from Sigma and poly(dG-dC)$_2$ and poly(dA-dT)$_2$ were from Pharmacia. These nucleic acids were purified as previously described (1). ΨDNA was prepared as described by Maestre, et al. (24). The concentration of nucleic acids were obtained by absorbance using literature values for ε and are reported in molar basepairs (1). All solutions were 0.8mM in phosphate at pH6.8 and thermostated at 25°C. Absorbance data were obtained on a Varian 2200 and CD spectra on an Aviv 60DS or Jasco 600. Scattering intensities were measured using a Perkin Elmer 650-10s spectrofluorimeter.

Results and Discussion: Experiments were conducted in the absence of DNA in which solutions containing the cis or trans $H_2(Ph)_2(4\text{-}Mpy)_2P$ were titrated with sodium chloride. As shown in figure 2 for the cis isomer, there is a 26nm bathochromic shift and an approximate 50% hypochromicity of the Soret maximum upon increasing the concentration of salt. An isosbestic point at 434 nm is observed. Similar results were obtained for the trans isomer; although for this latter porphyrin the changes in the Soret maximum occurred at lower salt concentration than required for the cis isomer. Solutions left undisturbed for periods of 15 to 30 minutes show further spectral changes in the Soret region, with eventual loss of the isosbestic point. This time dependent effect is more pronounced for the trans isomer and for solutions at high ionic strength. The observed absorbance changes in the absence of DNA which have not been previously reported, are suggestive of aggregation, with the salt serving to screen the charge of the dications to allow close approach of two or more porphyrin units. Similar absorbance spectral changes

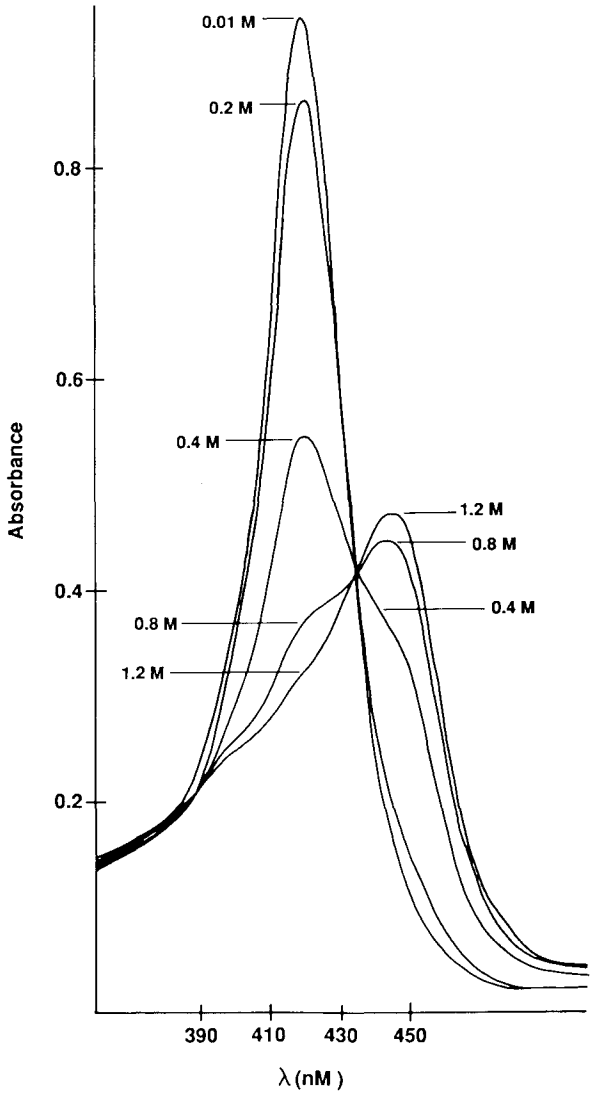

Figure 2. Titration of cis-bis(4-N-methylpyridyl)diphenylporphine with sodium chloride.

All solutions contain 5.3 x 10^{-6} M porphyrin buffered at pH 6.8. Shown on the figure are the concentrations of NaCl. Spectra were run immediately after preparation of solutions.

have been obtained for a number of porphyrins with negatively charged groups attached to the porphine core and have been ascribed to the formation of dimers (25,26). For the cis and trans $H_2(Ph)_2(4\text{-}Mpy)_2P$, the presence of the isosbestic point suggests that at least initially only one aggregated species is formed. With time, and especially at high salt concentration, higher order aggregates form; the trans isomer having the greater tendency to aggregate. Although there have been reports indicating that tetrakis(4-N-methylpyridyl)porphine exists in solution as a dimer, even at low porphyrin concentration and ionic strength conditions (27-29), the bulk of the

experimental evidence weighs heavily against this stacking model (25,30). The titration results of the cis and trans diphenyl porphyrins as a function of salt concentration provide additional evidence for a nonaggregation model for tetrakis(4-N-methylpyridyl)porphine: up to salt concentrations as high as 2M, small (1 or 2 nm) spectral shifts with almost no changes in absorbance intensity occur for the tetracationic porphyrin (25). Thus the dicationic porphyrins appear to have a much larger tendency to aggregate than does the tetracationic H_2TMpyP-4.

Similar salt titrations were conducted in which a given porphyrin isomer was first preincubated with either poly(dG-dC)$_2$ or poly(dA-dT)$_2$ at ratios of [polymer]/[porphyrin] of 37 and 47, respectively. The results were monitored by visible absorbance and circular dichroism spectroscopy (see figure 3a and 3b). For trans-$H_2Ph_2(4\text{-}Mpy)_2P$ with poly(dG-dC)$_2$ at 0.01M NaCl, the Soret absorbance band broadened and decreased in intensity with its maximum 19 nm red shifted relative to the free porphyrin. The CD in the Soret region of the porphyrin chromophore (the nucleic acid does not absorb in this wavelength region) appeared as a small, broad negative band with two extrema at approximately 438 and 448nm. At this low ionic strength condition, the trans porphyrin in the absence of nucleic acid has an absorbance band at 418 nm and a weak, negative CD band with its maximum centered at 419 nm. Based upon results obtained with H_2TMpyP-4 and its metallo derivatives, the large red shift and hypochromicity of the Soret absorbance maximum and the negative CD band in the Soret region would indicate that at these conditions trans-bis(4-N- methylpyridyl)diphenylporphine intercalates into poly(dG-dC)$_2$, as previously reported(22,23). When the NaCl concentration was increased to 0.087M a broad Soret band was initially observed having a maximum at 420nm (near the position of free porphyrin) but with a shoulder at longer wavelengths. After 15 minutes in this higher NaCl medium, the Soret band shifted to the red with a shape and position similar to what was observed at lower salt but with reduced intensity. However, CD measurements show that this high salt complex is very different from the one formed at μ=.01M; at the higher salt concentration after a 15 minute incubation period, the CD in the Soret region is dramatically different in shape and intensity from the negative CD band obtained earlier: a conservative exciton-type CD spectrum is observed having a positive band centered at 439nm and a negative band at 463nm. Even more dramatic is the fact that both these bands have intensities more than two orders of magnitude larger than observed at low salt. The intensity of the CD signal for the high salt solution (0.087M NaCl) required about 30 to 40 minutes to reach its final value. With addition of more NaCl (up to 0.22M), the intensity of the CD signal increased further. From 0.22M to 0.50M salt, the CD intensity in the Soret region remained unchanged. At still higher NaCl concentrations the CD intensity begins to decrease most probably due to a lowered binding affinity at very high salt (3). Since the trans porphyrin at these higher ionic strength conditions has no CD in the absence of the nucleic acid polymer, the CD spectra observed must be due to porphyrin bound to poly(dG-dC)$_2$ and not due to porphyrin aggregates free in solution. Although conservative type induced CD have been obtained for other porphyrins with nucleic acids (31), their appearance requires much higher levels of drug load and none have shown nearly the intensity observed here. The conservative nature of the CD spectrum in the Soret region for the trans porphyrin•poly-(dG-dC) $_2$ complex at high salt suggests there is a strong interaction occurring between bound porphyrins (32), while the intensity of the CD suggests a long range organization of porphyrin

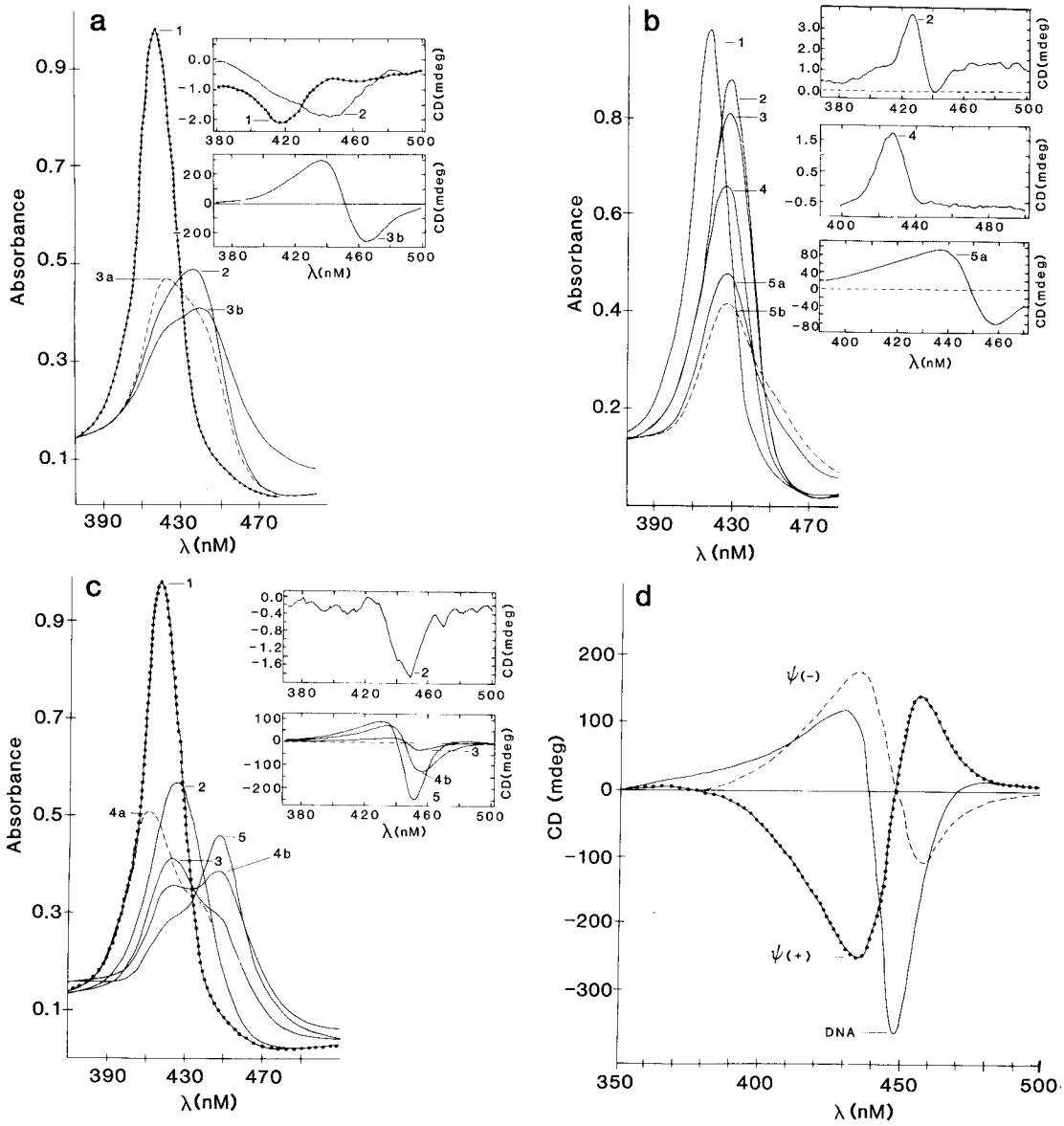

Figure 3. Absorbance and CD spectra of trans- (4-N-methylpyridyl)diphenylporphine upon Reaction with Nucleic Acids

(3a) [poly(dG-dC)$_2$] = 1.86 x 10^{-4} M; [trans-P] = 5.07 x 10^{-6} M;
(1) (. . . . .) no poly(dG-dC)$_2$, [NaCl] = 0.01 M; (2) [NaCl] = 0.01 M
(3a) [NaCl] = 0.087 M, spectrum run immediately after solution preparation (3b) same as (3a) except 15 minutes after mixing.

(3b) [poly(dA-dT)$_2$] = 2.34 x 10^{-4} M; [trans-P] = 5.07 x 10^{-6} M;
(1) (. . . . .) no poly(dA-dT)$_2$, [NaCl] = 0.01 M;
(2) [NaCl] = 0.01 M; (3) [NaCl] = 0.087 M NaCl;
(4) [NaCl] = 0.34 M NaCl; (5a) [NaCl] = 0.49 M;
(5b) same as (5b) except 15 minutes after mixing.

molecules (24). We propose that this long range order involves a helical arrangement of the porphyrin molecules on the nucleic acid polymer which results in a helical alignment of porphyrin transition dipole moments. When light interacts with this helical structure a resonance phenomenon between the interacting porphyrin units results, leading to large CD signals for the porphyrin chromophore. No changes in the CD of the poly(dG-dC)$_2$ (350-220nm region) were observed, suggesting there is no major change in the conformation of the nucleic acid upon formation of the high salt complex.

For trans-$H_2(Ph)_2(4\text{-}Mpy)_2P$ with poly(dA-dT)$_2$ at 0.010M NaCl and at large excess of polymer, there is an 8nm red shift and 10% hypochromericity of the Soret maximum. These are smaller changes than observed with poly(dG-dC) $_2$. A small positive induced CD band in the Soret region appears at 428nm. These spectral patterns are analogous to those obtained for H_2TMpyP-4 with this polymer and are signatures for external binding to poly(dA-dT)$_2$; this polymer is unable to accommodate the bulky porphyrin molecules as an intercalated complex, preferring to form external "groove" bound complexes (1,2). No changes in the porphyrin CD were observed up to 0.34M NaCl. At 0.34M salt concentration, there was a time dependent broadening and hypochromicity of the Soret absorbance band with the concomitant appearance of a conservative CD in the Soret region, several orders of magnitude larger than the CD observed at low ionic strength. No changes in the CD of the poly(dA-dT)$_2$ were observed at these high salt concentrations. These results parallel those obtained with the trans porphyrin and poly(dG-dC)$_2$ except that the initial blue shift of the Soret maximum when additional salt is added is not seen with the AT polymer and higher ionic strength conditions and longer times (several hours) are required to attain equilibrium. These results suggest that the trans porphyrin also forms a long-range helical structure with the AT polymer but that this process is thermodynamically less favorable (requires higher salt) and kinetically less facile than with the GC polymer. The initial formation of stable external complexes with poly(dA-dT)$_2$ in which the porphyrin molecule is buried deep in a groove, may inhibit formation of the long range helical porphyrin structure, especially if this structure involves self stacking of porphyrin molecules on the surface of the nucleic acid. Poly(dG-dC)$_2$ is not a good substrate for porphyrin groove binding. (1,2)

At 0.01M salt and large excess of polymer, absorbance and CD spectra obtained for the cis-diphenyl isomer with the synthetic nucleic acids are qualitatively similar to results obtained with the trans porphyrin. Thus, the cis isomer, at these conditions also appears to intercalate into poly(dG-dC)$_2$ and bind externally to poly(dA-dT)$_2$. However, unlike the trans isomer, when the salt concentration was increased as high as 0.34M, the only spectral change observed was a small decrease in the Soret absorbance. The large conservative CD was never observed with the cis

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(3c) [NaCl] = 0.1 M; [trans-P] = 5.07 x 10^{-6} M;
(1) (. . . . .) no calf thymus DNA; (2) [DNA] = 1.14 x 10^{-4} M;
(3) [DNA] = 2.92 x 10^{-5} M; (4a,b) [DNA] = 1.75 x 10^{-5} M;
(4b) after 15 incubation period; (5) [DNA] = 2.92 x 10^{-6} M.

(3d) [NaCl] = 0.10 M; [trans-P] = 9 x 10^{-6} M; [DNA] = 5 x 10^{-5} M;
(. . . .) $\Psi_{(+)}$; (- - - -) $\Psi_{(-)}$ (____) "naked" DNA.

porphyrin. Apparently, the inherently smaller tendency of the cis porphyrin to aggregrate reduces the driving force for formation of a stacked structure on the surface of nucleic acid stuctures. Higher salt and/or drug loads may be required to foster formation of the long range structure of the cis porphyrin on these synthetic polymers .

Qualitatively, spectral and kinetic results obtained with calf thymus DNA for the trans isomer are similar to those obtained in salt titrations (at high excess of base pairs to drug) with poly-$(dG\text{-}dC)_2$. For the cis isomer with calf thymus, unlike results obtained with the synthetic homopolymers, salt titrations produced conservative type CD in the Soret region of the porphyrin at high salt (approximately 0.5M NaCl), but with intensities only five to ten times larger than signals at lower salt conditons. No changes in the CD of the DNA were observed at any salt concentration with either isomer. Titration experiments were also conducted at a constant [NaCl]=0.05M but varying the total concentration of calf thymus DNA. As can be seen in figure 3c for the trans isomer, similar absorbance and CD spectral changes were observed with decreasing DNA concentration as were observed as a function of increasing salt. Qualitatively similar results were obtained for the cis-diphenyl porphyrin. The formation of the proposed long-range porphyrin structure with its intense CD signal therefore appears also to be facilitated when the number of DNA binding sites is reduced.

An alternative explanation for the observed spectral and kinetic effects, particularly the appearance of the intense CD of the porphyrin chromophore, involves aggregation of the DNA facilitated by binding of the cis and trans diphenyl porphyrins. A number of intercalating drug molecules are known to cause the condensation of DNA at high binding ratios (33,34) and large induced CD spectra in the Soret region have been observed upon intercalation of porphyrins into polypeptide-induced aggregated DNA, Ψ_{DNA} (24). Condensed or aggregated DNA such as Ψ_{DNA} can be detected by their increased scattering properties. To test this alternate hypothesis, we conducted scattering experiments on solutions of aggregated Ψ_{DNA}; nonaggregated, "naked" DNA and "naked" DNA with trans $H_2(Ph)_2(4\text{-}N\text{-}Mpy)_2P$. Scattering intensity was monitored from 280-350nm. "Naked" DNA and the two solutions of "naked" DNA with trans $H_2(Ph)_2(4\text{-}N\text{-}Mpy)_2P$ both at high and low ionic strength conditions where the small CD and the large conservative CD for the bound porphyrin chromophore are observed, respectively all had approximately the same intensity of scattering; whereas the Ψ_{DNA} had significantly larger scattering signals. This suggests that the trans-dicationic porphyrin is not causing extensive aggregation of DNA.

Ψ_{DNA} not only scatters light but is also characterized by CD signals which are different in shape and intensity from nonaggregated B-DNA, even though the individual DNA helices are considered to remain B-form (35); $\Psi_{(+)}$ has a large positive CD in the UV region and $\Psi_{(-)}$ a negative CD in this region. This inversion of the CD of the DNA is believed to reflect a switch in this handedness of the superhelicity of the aggregated structure (24). We do not observe any changes in the CD of the nucleic acid at any conditions upon interaction with the cis or trans diphenyl porphyrins. Although this does rule out DNA aggregation (other DNA aggregates do not have unusual CD (35,36)), it is consistent with a nonaggregating model.

When tetracationic porphyrins intercalate into Ψ_{DNA} their CD mimic the handedness of the superhelix; with $\Psi_{(+)}$ the CD of porphyrin intercalators are large and all positive and with

$\Psi_{(-)}$ large and all negative induced CD are observed(24). Nonaggregating porphyrins which are not capable of intercalating into DNA display only small induced CD bands with Ψ type DNA. When trans-$H_2(Ph)_2(4\text{-}Mpy)_2P$ is bound to $\Psi_{(-)}$ a large but conservative CD is obtained for the porphyrin chromophore, similar to what was observed with "naked" DNA (see figure 3d). With $\Psi_{(+)}$ a large conservative CD is also observed but in this case the CD spectrum is inverted (mirror image). The conservative, exciton nature for the CD of bound trans-$H_2(Ph)_2(4\text{-}Mpy)_2P$ to DNA aggregates suggests that porphyrin-porphyrin interactions are occurring. The sign pattern of the porphyrin CD for these complexes may well be related to how the bound monomers are oriented with respect to one another on the DNA aggregate. This orientation is apparently dictated by the helical structure of the polymer matrix (at least for aggregated DNA) to which the porphyrin molecules are bound. Thus trans-$H_2(Ph)_2(Mpy\text{-}4)_2P$, like the previously studied intercalating porphyrins, appears to be reporting the superhelical sense of the DNA aggregate to which it is bound.

We are continuing studies to further characterize the nature and mechanism of formation of these long-range porphyrin structures on nucleic acids, the physical basis for the intense CD and to determine whether the CD spectral pattern of the porphyrin chromophore when bound to nonaggregated DNA is also a signature for the handedness of the individual helices.

Acknowledgments

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