Notes

B-DNA Binding with Cobalt(III) and Vanadyl(2+) Derivatives of Tetracationic 5,10,15,20-Tetrakis(4-*N*-methylpyridyl)porphine: Combined CD, Optical, and Electronic MCD Spectra

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Introduction

Electronic MCD spectroscopy is used for the first time to probe the binding interaction between porphyrins **CoP(4)** and **VOP(4)** and duplex B-DNA.[1] Since MCD selection rules[2] are significantly different from those of optical and natural CD absorption spectroscopies, one can often extract from MCD new complementing electronic and sometimes geometric structural information. The three methods do have in common the need for a nonvanishing electric dipole transition moment (edtm), $\langle a|\mu_e|j\rangle$, for electronic excitation $|a\rangle \rightarrow |j\rangle$. Here we compare optical, CD, and MCD band heights, respectively: $\epsilon_{max} = 102.34(\nu_0/\Gamma)D_{aj}$, $\Delta\epsilon_0 = 4.57(\lambda_0^{CD}/\Gamma^{CD})R_{aj}$, and $[\theta]_M = -21.349[f_A A_{aj} + f_B(B_{aj} + C_{aj}/kT)]$, where $D_{aj} = |\langle a|\mu_e|j\rangle|^2$ is the electric dipole strength (eds) in Debye[2], $R_{aj} = \mathrm{Im}\langle a|\mu_m|j\rangle\cdot\langle j|\mu_e|a\rangle$ is the rotational strength in μ_B·Debye, Γ (cm^{-1}) and Γ^{CD} (nm) are the optical and CD full widths at half-height, $f_{A,B}$ are band shape functions,[2,3] ν_0 (cm^{-1}) and λ_0^{CD} (nm) are positions of band extrema, μ_e is the electric dipole operator, μ_m is the magnetic dipole operator, and the usual MCD quantum parameters[2,3] (Faraday parameters) are A_{aj}, B_{aj}, and C_{aj} for the most general molecule. The Soret band, or B_0, of closed-shell **CoP(4)** can have only A_{aj} and B_{aj} parameters (giving rise to A- and B-terms, or bands) for Soret[3] transition $^1A_1 \rightarrow {}^1E_u$ of $1a_{1u}^2\ 3a_{2u}^2\ 4e_g$ MO origin.[4] However, open-shell **VOP(4)** ($^2\Gamma \rightarrow {}^2E$) may, in principle, also have a second-order C-term, but its intensity is expected to be negligible. Upon binding with CT DNA, the observed genuine Soret MCD (+) A-term[5] of these tetragonal, free **MP-(4)**s degenerates to a (+) or (−) pseudo-A-term, due to the absence of any axially symmetric CT DNA sites at which **MP-(4)** binds. Therefore, each of the Soret B_0's excited low-symmetry, nondegenerate daughter states ($^{1,2}B_{0x}$, $^{1,2}B_{0y}$), derived from the tetragonal (D_{4h}), free **MP(4)**'s degenerate $^{1,2}E$ parent excited state, will have only an MCD B-term, but the sum of the two ($^{1,2}B_{0x}+{}^{1,2}B_{0y}$) will be a (+) or (−) pseudo-A-term (Figure 1).[5]

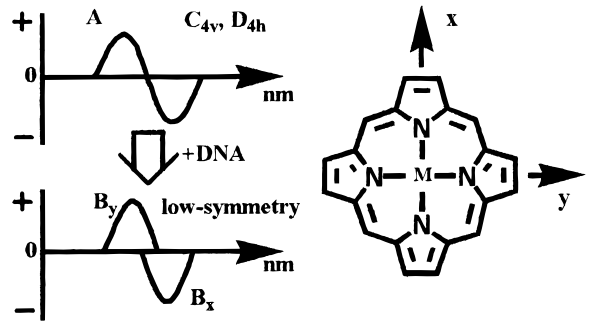

Figure 1. Reduction of the genuine MCD (+) A-term of a tetragonal **MP(4)** into the (+) pseudo-A-term (sum of MCD B_x- and B_y-terms) of outside-bound **MP(4)**/CT DNA; *x* is the direction in which the π-electron system of bound **MP(4)** is most perturbed.

The MCD spectra of these two cationic "drugs", **CoP(4)** and **VOP(4)**, were studied together because (i) in the unbound state they are similarly "thick" in the direction normal to their porphyrin planes due to axially ligating one (**VOP(4)**) or two (**CoP(4)**) water molecules, (ii) the "in-plane" porphine ligand is the same for each, and (iii) each free **MP(4)** has a large MCD (+) A-term, whose features are potentially interesting to follow upon binding. For optimum information extraction our interpretations and conclusions rely on the composite of optical, CD, and MCD spectral data measured for these free and DNA-bound species (Figure 2), as well as literature FLD^r data and CD sector rules.

Experimental Section

The cobalt(III) and vanadyl(2+) derivatives of 5,10,15,20-tetrakis-(4-*N*-methylpyridyl)porphine, **CoP(4)** and **VOP(4)**, were purchased as the chloride salts from Midcentury Chemicals (Posen, IL). Each metalloporphyrin was subsequently purified under nitrogen by gradient elution adsorption chromatography using Sephadex LH-20 (Pharmacia Biotech) as the adsorbing stationary phase and H_2O/H_2SO_4 (pH ∼ 2−3) as the eluting mobile phase. Fractions containing **CoP(4)** or **VOP(4)** were collected, combined (when having the same optical spectra), neutralized with $Ba(OH)_2$, subsequently centrifuged and filtered, and finally lyophilized on a LABCONCO Lyophilizer Freeze-Dryer 8 for the recovery of the purified metalloporphyrin solid. A stock solution of each porphyrin was prepared in buffer (pH ∼ 7.0, $\mu = 0.20$ M), and the concentration of metalloporphyrin was determined spectrophotometrically using Beer's Law and $\epsilon_{436} = 1.68 \times 10^5\ cm^{-1}\ M^{-1}$ and $\epsilon_{440} = 2.47 \times 10^5\ cm^{-1}\ M^{-1}$ for the Soret bands of **CoP(4)** and **VOP(4)**, respectively.

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(1) Abbreviations and symbols: MCD, electronic magnetic circular dichroism; CD, electronic natural circular dichroism; FLD^r, reduced flow linear dichroism; RR, resonance Raman; **MP(4)** [**M** = Co^{III} and VO^{2+}, **P(4)** = 5, 10, 15, 20-tetrakis(4-*N*-methylpyridyl)porphine], metalloporphyrins employed in this study; MO, molecular orbital; HOMO, highest energy occupied MO; LUMO, lowest energy unoccupied MO; ϵ_{max}, molar absorptivity ($cm^{-1}\ M^{-1}$); $\Delta\epsilon_0$, differential molar absorptivity ($\epsilon_l - \epsilon_r$) using left- and right-circularly polarized light ($cm^{-1}\ M^{-1}$); $[\theta]_M$, MCD molar ellipticity per Gauss of externally applied longitudinal magnetic field; μ_B, Bohr magneton; *k*, Boltzmann constant; *T*, sample temperature (K).

(2) (a) Buckingham, A. D.; Stephens, P. J. *Annu. Rev. Phys. Chem.* **1966**, *17*, 399−432. (b) Schatz, P. N.; McCaffery, A. J. *Quart. Rev.* **1969**, *23*, 552−584.

(3) (a) Stephens, P. J.; Suetaka, W.; Schatz, P. N. *J. Chem. Phys.* **1966**, *44*, 4592−4602. (b) Gale, R.; McCaffery, A. J.; Rowe, M. D. *J. Chem. Soc., Dalton Trans.* **1972**, 596−604. (c) Barth, G.; Linder, R. E.; Bunnenberg, E.; Djerassi, C. *Ann. N. Y. Acad. Sci.* **1973**, *206*, 223−246.

(4) Gouterman, M. *J. Mol. Spectrosc.* **1961**, *6*, 138−163.

(5) A genuine MCD A-term or pseudo-A-term is positive (+) when its high-energy lobe has (+) ellipticity. Also, the pseudo-A-term (composite of two B-terms of opposite sign) is observed because Soret band splitting is always small (<500 cm^{-1}).

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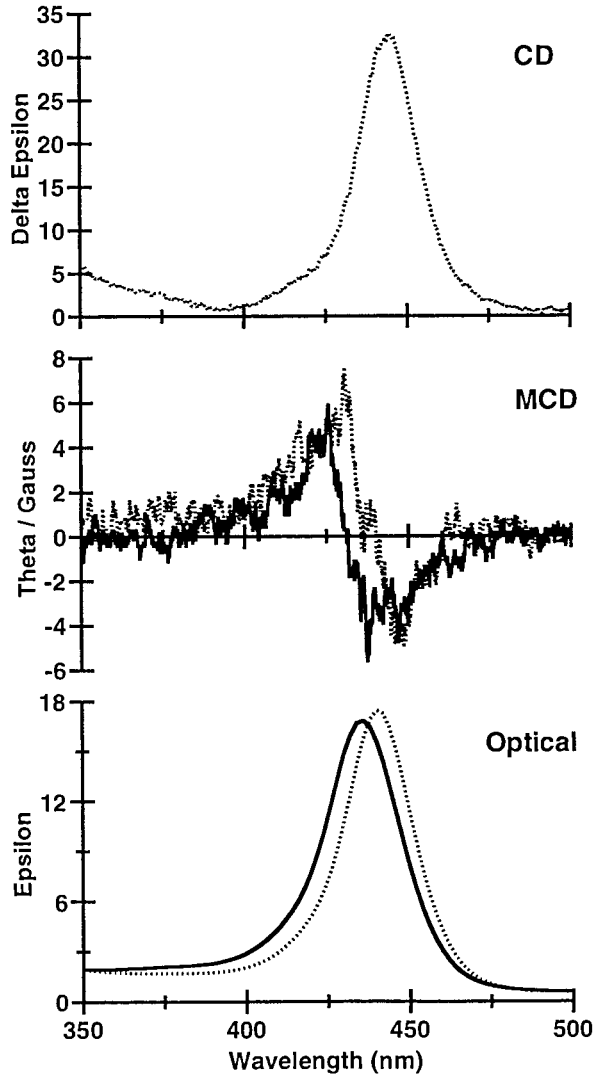

Figure 2. Room-temperature Soret spectra of unbound **CoP(4)** (solid trace, $r_0 = 0.0$) and **CoP(4)**/CT DNA (dotted trace, $r_0 = 0.030$, [base pair] = 1.5 mM) in sodium phosphate buffer ($\mu = 0.20$ M, pH ~ 7.0). Spectral units: Delta Epsilon in cm^{-1} M^{-1}; Theta in deg cm^2 $dmol^{-1}$; and Epsilon in units of 10^4 cm^{-1} M^{-1}. The net MCD spectra are shown.

Sonicated calf thymus DNA (CT DNA), phenol-extracted and lyophilized, was purchased from Pharmacia Biotech and used without further purification. The average length of the DNA fragments was determined by agarose gel electrophoresis to be ca. 1500 base pairs. A stock solution of the nucleic acid was prepared in phosphate buffer (pH ~ 7.0, μ ~ 0.20 M), and the molar base-pair concentration, or [base pair], of the solution was determined spectrophotometrically using $\epsilon_{260} = 1.31 \times 10^4$ cm^{-1} M^{-1} for CT DNA.[6]

Porphyrin−nucleic acid working solutions were prepared by mixing appropriate aliquots of the fresh metalloporphyrin and nucleic acid stock solutions so as to obtain $r_0 = 0.030$ and [base pair] = 1.5 mM, where r_0 = [**MP(4)**]/[base pair]. Room-temperature MCD, CD, or optical absorption measurements were made immediately following the preparation of each combined solution.

All porphyrin and DNA solutions were prepared in a sodium phosphate buffer solution (pH ~ 7.0) containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and sufficient NaCl to give a final ionic strength of μ ~ 0.20 M. Unless otherwise stated, all chemicals were reagent grade and purchased from Fisher Scientific.

Optical spectra were measured on a Hewlett-Packard (HP) 8452A diode-array spectrophotometer at a spectral band-pass of 2 nm, giving 2 nm spectral resolution. Wavelength calibration was verified using an HP holmium oxide−glass standard.

Induced CD spectra were obtained on a carefully calibrated JASCO J-600 scanning spectropolarimeter. Wavelength calibration was carried out using a Nd glass standard (JASCO), and the intensity ($\Delta\epsilon$) calibration was done using an aqueous 0.060% (w/v) solution of *l*-10-ammonium camphorsulfonate (Aldrich). CD spectra were recorded for each metalloporphyrin−nucleic acid solution with the following instrument parameter settings: 2.0 nm bandwidth, 1.0 s time constant, 0.2 nm step resolution, and 25 nm/min scan speed. Signal-to-noise ratios ranging from 7:1 to 55:1 were achieved by storing the average of five spectra, after having performed careful cyclic alignments of monochromator mirrors M_1 and M_0.

MCD spectra were measured on a JASCO J-500 scanning spectropolarimeter, additionally equipped with a JASCO water-cooled electromagnet and magnetic shielding of the detector. Instrument calibration of wavelength and intensity ($\Delta\epsilon$) was ascertained by using 1 mM aqueous solutions of $K_3[Fe(CN)_6]$ and (+)-$[Coen_3]Cl_3$. Measurements were made in an applied longitudinal magnetic field of H_0 = 1.1 T and with the following instrument parameters: 2.0 nm bandpass, 2.0 s time constant, 0.1 nm step resolution, and 50 nm/min scan speed. Total MCD spectra, [CD+MCD], were recorded for free and DNA-bound porphyrin solutions. Each MCD recording was preceded by a CD recording ($H_0 = 0.0$ T) of the sample in order to obtain the pure, net MCD spectrum, i.e., net MCD = [CD + MCD] − [CD].

Results and Discussion

Upon binding of **CoP(4)** to CT DNA, the single optical B_0 band shifts[7] ($\Delta\lambda = 4$ nm, $H = -4\%$) are small (Figure 2), which is the case for nearly all outside binding porphyrins, and they agree well with earlier Soret data.[7] The two changes, H and $\Delta\lambda$, taken together indicate a lesser perturbation of the relevant pπ MOs ($1a_{1u}^2$ $3a_{2u}^2$ $4e_g$) of **CoP(4)** than that experienced by intercalating porphyrin and metalloporphyrin species.[7] These relative changes and perturbations (exterior versus intercalation modes) also parallel, at least qualitatively, the binding constants (K_B) characteristic of these binding modes.[7b,8]

The B_0 MCD spectrum (Figure 2) of free **CoP(4)** has a (+) A-term, a sign also observed for other free tetragonal[3] metalloporphyrins (e.g., D_{4h} and C_{4v}) whose in-plane polarized transition $^1A_1 \rightarrow {}^1E_u$ derives from the primarily porphine-localized configuration $1a_{1u}^2$ $3a_{2u}^2$ $4e_g$. The directly measurable MCD/optical parameter ratio, $\Delta[\theta]_{MCD}^{p-t}/\epsilon_{max}$, is used, since it is proportional to the excited-state angular momentum, $\langle L_j \rangle$.[9] For **CoP(4)** this ratio is ~0.44 deg cm^2 $dmol^{-1}$ $Tesla^{-1}/(cm^{-1}$ $M^{-1})$, and it is virtually unchanged upon binding (Figure 2), so that $\langle L_j \rangle$ remains nearly unquenched upon binding. In turn, this strongly implies that at least this pπ set of parent MOs $1a_{1u}^2$ $3a_{2u}^2$ $4e_g$ is not strongly deviating from the original tetragonality and π-electron distribution, which is consistent with exterior binding usually causing smaller optical changes ($\Delta\lambda$ and H) than intercalation.[7] It can also be noted that the MCD crossover position and pseudo-A-term extrema are likewise red-shifted (~6 nm) only little, similar to the optical shift, which is an additional new indicator of a weaker perturbation upon outside binding.

The third significant MCD feature is that the pseudo-A-term of **CoP(4)**/CT DNA, in which **CoP(4)** must have lower than

(6) Wells, R. D.; Larson, J. E.; Grant, R. C.; Shortle, B. E.; Cantor, C. R. *J. Mol. Biol.* **1970**, *54*, 465−497.

(7) (a) $\Delta\lambda$ is the DNA-induced wavelength shift of the Soret maximum, $\Delta\lambda = \lambda^0$(bound) − λ^0(free). H is the percent hypochromicity (decrease in ϵ_{max}) of the Soret maximium, defined as $H = [(\epsilon_{free} - \epsilon_{bound})/\epsilon_{free}] \times 100$; therefore, (−) H values represent percent *hyper*chromicities. Typically, only small $\Delta\lambda$ and H Soret shifts accompany outside binding, i.e., $\Delta\lambda \leq 8$ nm and $H \leq 10\%$; intercalation, however, usually leads to much larger Soret shifts, i.e., $\Delta\lambda \geq 15$ nm and $H \geq 35\%$. (b) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983,** *22*, 2406−2414.

axial symmetry, has the same (+) sign as the genuine A-term of the tetragonal free **CoP(4)** (Figures 1 and 2). This sign retention informs that the LUMO (doubly degenerate $4e_g$) energy splitting (ΔLUMO), into two singly degenerate MOs, upon binding is less than the HOMO $|1a_{1u} - 3a_{2u}|$ energy separation (ΔHOMO).[10] This is further verification that the low-symmetry external binding sites on CT DNA are at least not severely perturbing the Soret band's involved pπ MOs $1a_{1u}^2\ 3a_{2u}^2\ 4e_g$. A sign-reversed (−) pseudo-A-term would be observed for a strong perturbation effect in cases that lead to ΔLUMO > ΔHOMO.[10]

Our MCD (+) pseudo-A-term of **CoP(4)**/CT DNA also allows us to draw still another conclusion. It was shown[11] for strongly perturbed porphyrins that the least perturbed molecular direction, labeled by Weiss as the *y*-axis (passing through two opposite pyrrole N's), defines edtm μ_{ey} and has a (+) MCD B-term, or B_y-term. On the other hand, the most perturbed molecular direction (*x*), orthogonal to *y* and defining edtm μ_{ex}, was found[11] to have the (−) MCD B-term (B_x) lobe of this lower symmetry pseudo-A-term (two B-terms) of the porphyrin whose axiality is destroyed (Figure 1). By direct analogy, for **CoP-(4)**/CT DNA we assign our higher energy (+) MCD lobe (∼428 nm) of the pseudo-A-term to Soret band (B_0) component B_{0y}, and its associated edtm is μ_{ey}; it follows then that this is the least perturbed direction within **CoP(4)** of **CoP(4)**/CT DNA. In contrast, the lower energy (−) MCD component (∼447 nm) of this pseudo-A-term is assigned to B_{0x} (and its edtm is μ_{ex}), which is thereby associated with the most perturbed direction in the bound **CoP(4)**.

It is of advantage to next examine the CD and reported[12] FLD^r data and relate them to our MCD conclusions. First, the large amplitude of the (+) CD band ($\lambda_0^{CD} = 444$ nm, $\Delta\epsilon_0 = +32\ cm^{-1}\ M^{-1}$) is definitive for groove binding, which is in contrast to much weaker CD bands observed in cases of weak "nonspecific" outside binding (wnob), e.g., $\Delta\epsilon \sim 1.5\ cm^{-1}\ M^{-1}$ in **PdP(2)**/poly(G-C)$_2$.[13] Second, the (+) CD sign would tentatively be assignable to edge-on minor groove binding (AT-rich segments[14]) with one drug edtm, μ_e^D (either μ_{ex}^D or μ_{ey}^D of the porphyrin's orthogonal edtm pair), having orientation angles $\alpha/\beta/\beta' = \sim 45°/0°/90°$.[15] [This binding mode assignment is based on the nondegenerate μ_e^D-μ_e^{DNA} coupling model (1988) and its CD sector rules[15b] for random-sequence DNAs, which have repeatedly been shown to correctly predict DNA-induced CD signs.] These angles would place μ_e^D ($\alpha/\beta/\beta' = \sim 45°/0°/90°$), with associated (+) CD, along the minor groove and tangent to the helix cylinder with tilt angle ∼45°relative to the helix axis. On the other hand, a (−) CD band is predicted[15b] (but not observed) for the second daughter transition, i.e., for the *most* perturbed edtm μ_{ex}^D (ca. perpendicular to the helix axis (*z*) and $\alpha/\beta/\beta' = \sim 90°/0°/0°$), which is orthogonal to the tangential μ_e^D. This is the *most* perturbed direction because it includes two adjacent *N*-methylpyridinium rings of **CoP(4)**, and their in-between pyrrole, penetrating deepest into the minor groove. In other words, the observed (+) CD band can, in principle, be associated with the *y*-component, μ_{ey}^D ($\alpha/\beta/\beta' = \sim 45°/0°/90°$ along the least perturbed direction *y)*, and therefore our (+) MCD lobe would then be the higher energy MCD B_{0y}-term, as concluded above from MCD alone. Of course, the nearby second edtm, μ_{ex}^D ($\alpha/\beta/\beta' = \sim 90°/0°/0°$), with its lower energy (−) MCD (B_{0x}) must be considered as well, i.e., the CD sector rules[15b] for random DNA sequences predict a (−) CD band that is *not* observed. This 1988 model does not predict magnitudes. However, the same group showed later by a matrix method[16] CD (mmCD) sector model that magnitudes can actually be estimated well. When we use its results,[16] we find that there would be two (+) CD bands of different intensities for these two B_0-band's edtm orientations if **CoP(4)** were bound edge-on in AT-rich minor groove sequences and centered at 5′TA3′ sites, i.e., μ_{ex}^D and μ_{ey}^D are calculated to have weak and strong (+) CD intensities. These would account well for our observed single (+) CD band, since Soret bands split so little (<500 cm^{-1}). However, insight taken from FLD^r measurements[12] across the Soret band of **CoP(4)**/CT DNA gives two very similar values of tilt angle α (48°, 41°), whereas the presently considered minor groove binding orientation described above requires $\alpha \sim 45°$ and $\sim 90°$! Therefore, this suggests a more likely outside binding mode as follows, since the fit of all experimental and predicted data is significantly better.

The FLD^r spectrum[12] of **CoP(4)**/CT DNA was taken to have two main polarization values across the B_0 optical band envelope, so the presence of two Soret band edtms was suggested, but which have only slightly different orientations with respect to the DNA helix axis (z). Tilt angles (α) of 48° ($\alpha_{old} = 62°$) and 41° ($\alpha_{old} = 58°$) were extractable for the blue and red portions of B_0, respectively. From our above MCD conclusions we can associate the higher energy (+) MCD lobe (B_{0y}, μ_{ey}^D) with the blue FLD^r component ($\alpha = 48°$) and the lower energy (−) MCD lobe (B_{0x}, μ_{ex}^D) with the red FLD^r component ($\alpha = 41°$). Consequently, a face-on[17] major groove binding mode (Figure 3) fits all the data definitely best. For

(8) (a) In general, porphyrin/DNA binding constants are typically ca. 1 order of magnitude larger for intercalating systems ($K_B \sim 10^6\ M^{-1}$) than for outside binding systems ($K_B \sim 10^5\ M^{-1}$). (b) Kelly, J. M.; Murphy, M. J.; McConnell, D. J.; OhUigin, C. *Nucleic Acids Res.* **1985**, *13*, 167−184. (c) Strickland, J. A.; Marzilli, L. G.; Gay, K. M.; Wilson, W. D. *Biochemistry* **1988**, *27*, 8870−8878. (d) Sari, M. A.; Battioni, J. P.; Mansuy, D.; LePecq, J. B. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 643−649.

(9) (a) For A-terms and pseudo-A-terms, $\Delta[\theta]_M^{p-t}$ is the apparent peak-to-trough A-term amplitude: $\Delta[\theta]_M^{p-t} = 32.44[(2\nu_0 + \Delta\nu)/\Delta\nu^2]A_{aj}$ and $\Delta[\theta]_M^{p-t}/\epsilon_{max} = 0.317p(A_{aj}/D_{aj})$, where $\Delta\nu$ is the peak-to-trough energy (cm^{-1}) separation, $A_{aj} = (1/2)\Sigma\langle j|\mu_m|j\rangle\cdot Im\langle a|\mu_e|j\rangle\langle j|\mu_e|a\rangle$, $p = \Gamma(2\nu_0 + \Delta\nu)/(\Delta\nu^2\nu_0)$, and D_{aj} is as defined in the text. For present transitions, $(A_{aj}/D_{aj}) = c_1\langle^1E|\mu_m|^1E\rangle = c_1(2\mu_B)^{1/2}\langle^1E_x|L_z|^1E_y\rangle$, where $c_1 = -i2^{-3/2}$, $\mu_m = -\mu_B L$, and μ_B is the Bohr magneton. (b) Trexler, J. W.; Fuentes, M.; Ober, G. E.; Schreiner, A. F.; Knopp, J. A. *J. Coord. Chem.* **1994**, *32*, 11−25. (c) Schreiner, A. F.; Amer, S.; Duncan, W. M.; Ober, G.; Dahlgren, R. M.; Zink, J. *J. Am. Chem. Soc.* **1980**, *102*, 6871−6872.

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(13) Barnes, N. R.; Schreiner, A. F. *Biospectroscopy* **1998**, in press.

(14) (a) Such a proposal is reasonable, since (i) the vast majority of exterior binding drugs are known to bind in the minor groove of AT-rich sequences, (ii) the C_2-NH_2 group of guanine sterically hinders GC minor groove binding, (iii) the negative electrostatic groove potential of the AT minor groove is much larger than that of the GC minor groove, (iv) the highly flexible AT minor groove can adopt a variety of conformations to snugly fit around the drug. (b) *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M., Gait, M. J., Eds.; Oxford University: Oxford, 1996; Chapter 2. (c) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapters 9, 16−17. (d) Lavery, R.; Pullman, B. *J. Biomol. Struct. Dyn.* **1985**, *2*, 1021−1031.

(15) (a) In this model α is the drug's edtm angle with respect to the DNA helix axis (*z*), *β* is the angle from the glycoside-bonds' bisector axis (*x*) to the line connecting a base pair's μ_e^{DNA} and the drug's μ_e^D, and *β*′ is the angle from this +*x* axis to the projection of edtm μ_e^D in the *xy*-plane; *x*, *y*, and *z* axes define a right-hand Cartesian coordinate system. (b) Kubista, M.; Akerman, B.; Nordén, B. *J. Phys. Chem.* **1988**, *92*, 2352−2356.

(16) (a) Lyng, R.; Rodger, A.; Nordén, B. *Biopolymers* **1992**, *32*, 1201−1214. (b) Lyng, R.; Rodger, A.; Nordén, B. *Biopolymers* **1991**, *31*, 1709−1720.

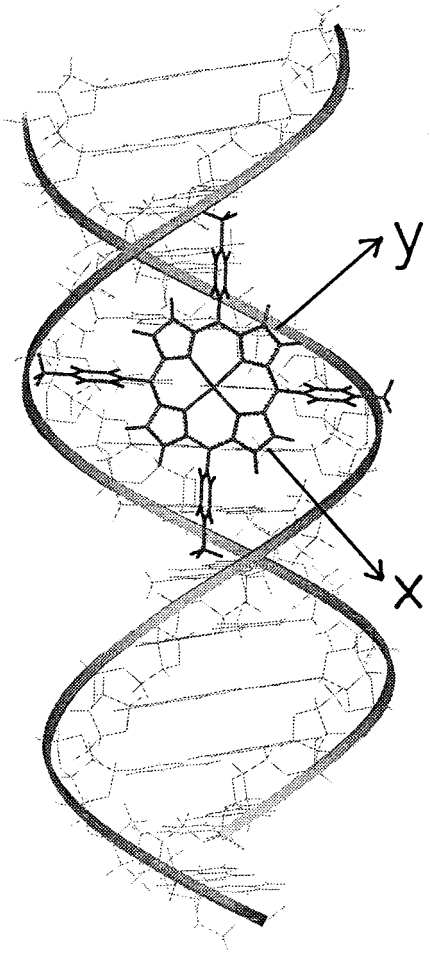

Figure 3. Proposed face-on orientation of **MP(4)** in the major groove of CT DNA; *x* and *y* are, respectively, the most and least perturbed molecular directions of the externally bound **MP(4)** (see Figure 1 caption).

example, it accounts for observing only one single (+) CD band, because **CoP(4)**'s orthogonal edtm pair, μ_{ex}^{D} and μ_{ey}^{D} with $\alpha/\beta/\beta' = 40-50°/180°/90°$ and $40-50°/180°/270°$, respectively, each has (+) CD by sector rules[15b] for heterobase DNA sequences. Moreover, mm-CD sector rules[16] can be used to predict weak (+) and strong (+) CD bands for μ_{ex}^{D} and μ_{ey}^{D}, respectively, for **CoP(4)** binding face-on in the major groove of AT- or GC-rich sequences and specifically at 5′AT3′ or 5′CG3′ sites. For such an orientation (Figure 3) μ_{ey}^{D} (ca. parallel to groove direction) is the least perturbed porphyrin direction and has expected (+) MCD,[11] as observed at ~430 nm (MCD B_{0y}-term); μ_{ex}^{D} (ca. perpendicular to groove direction) is the most perturbed direction with (−) MCD, as observed at ~447 nm (MCD B_{0x}-term). What mitigates strongly against associating the two FLD^r polarizations with two different binding modes (minor *plus* major groove binding) is that there is only one observed (+) CD band (Figure 2), whereas such dual-mode binding in analogous **ZnP(4)**/CT DNA[7b,18] and **MnP(4)**/CT DNA[19] produces two well separated (+) CD bands with nearly equal sizes.

In summary the combined data of these five MCD/CD/optical spectra, the two FLD^r α values of 41° and 48°, and successful sector rule predictions can all be correlated with a *single* binding mode in the major groove, i.e., by positioning the **CoP(4)** face-on in the major groove so as to orient μ_{ey}^{D} (least perturbed) at $\alpha/\beta/\beta' = \sim 41°/180°/270°$ and its ca. orthogonal partner, μ_{ex}^{D} (most perturbed), at $\alpha/\beta/\beta' = \sim 49°/180°/90°$. For these two edtms (i) the mm-CD sector rule model predicts (+) CD for each of μ_{ey}^{D} (strong) and μ_{ex}^{D} (weak) (consistent with one band of (+) CD intensity being observed), (ii) an MCD pseudo-A-term is still expected (± for B_{0y}/B_{0x}), (iii) seeing only one (+) CD band when two (+) CD bands are predicted is consistent with the fact that porphyrin B_0 band splitting[20] is always small ($<500\ cm^{-1}$), and (iv) the requirement of two values of similar sizes of angle α for this binding mode is in agreement with the FLD^r experiment of the Soret band. Also, that face-on major groove binding is suggested to occur at GC- or AT-rich regions of DNA is consistent with RR[18c] interpretations for **CoP(4)**/CT DNA as well as earlier CD[19] results reported for somewhat analogous **MnP(4)**/B-DNA systems.

The **VOP(4)** and **VOP(4)**/CT DNA spectra are not shown because they are so very similar to those of the analogous cobalt(III) systems just described. The combined MCD, CD, and optical Soret spectra of $^2\Gamma \rightarrow {}^2E$ parentage show that the (i) small optical ($\Delta\lambda = 4$ nm, $H = 5\%$)[21] and MCD band shifts upon binding, (ii) (+) MCD A-term of free **VOP(4)**, (iii) dominant single (+) CD band ($\lambda_0^{CD} = 444$ nm, $\Delta\epsilon_0 = +45.2\ cm^{-1}\ M^{-1}$),[21] and (iv) (+) MCD pseudo-A-term of **VOP(4)**/CT DNA very strongly resemble the spectra of **CoP(4)** and **CoP(4)**/CT DNA above, so that similar assignments and conclusions are suggested.

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