# Interaction of Cu(II)-*meso*-tetrakis(*n*-*N*-methylpyridiniumyl)porphyrin (*n* = 2,3,4) with Native and Synthetic Polynucleotides Probed by Polarized Spectroscopy

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The interactions of Cu(II)-*meso*-Tetrakis(*n*-*N*-methylpyridiniumyl)porphyrin (*n* = 2,3,4), respectively referred to as *o*-, *m*- and *p*-CuTMPyP, and DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$] were investigated by circular and linear dichroism (CD and LD). In the *o*-CuTMPyP case, in which the rotation of the pyridinium ring is prevented, the shape of the CD spectrum when associated to DNA and poly[d(A-T)$_2$] resembles and is characterized by a positive band at a low drug to DNA concentration ratio (*R* ratio) and is bisignate at a high *R* ratio. The former CD spectrum shape has been attributed to porphyrin that is bound monomerically outside of DNA while the latter can be attributed to those that are stacked. When *o*-CuTMPyP is bound to poly[d(G-C)$_2$], the excitonic CD appeared at a relatively high *R* ratio. In contrast, a characteristic negative CD band in the Soret region was apparent for both *m*- and *p*-CuTMPyP when bound to DNA and poly[d(G-C)$_2$] at the low *R* ratios, indicating that the porphyrin molecule intercalates. However, the DNA is bent near the intercalation site and the plane of the porphyrin molecule tilts relative to the DNA helix axis, as judged by the magnitude of the reduced LD. Various stacking patterns were identified by the shape of the CD spectrum for *m*- and *p*-CuTMPyP when bound to poly[d(A-T)$_2$]. Three species for the former complex and two for the latter complex were found which may reflect the extent of the stacking.

**Key Words :** Cu(II)TMPyP, DNA, Linear dichroism, Porphyrin, Polynucleotide

## Introduction

Cationic porphyrins interact with DNA. The pattern of interaction depends on the nature of DNA and the structure of porphyrin, especially the central metal ions. In general, free-base porphyrin and that with the central metal that forms a planer square complex intercalate between the GC base pair while they bind externally along DNA at the AT base pairs. In the Cu(II)-*meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin (*p*-CuTMPyP, Figure 1) case, it intercalated between the GC base pairs while exhibiting outside binding at the AT sequences.[1,2] At the AT site, the formation of exciplex with the thymine C=O group that is located at the minor groove was reported.[3,4] The structure of periphery substituents on the porphyrin ring also affects the binding mode. It has been reported that the rotation ability of the pyridinium ring by introducing a methyl group at the position 2 of pyridinium ring, *i.e.*, *meso*-tetrakis(2-*N*-methylpyridiniumyl)porphyrin (*o*-TMPyP) alters the binding properties to a large extent.[5-7] While *p*-TMPyP and *meso*-tetrakis(3-*N*-methylpyridiniumyl)porphyrin (*m*-TMPyP) intercalated between GC base pairs, *o*-TMPyP, that cannot be a square planer structure due to hindrance of the rotation of the pyridinium ring, exhibited outside binding. Upon binding to poly[d(A-T)$_2$], *p*-TMPyP showed the groove binding at a low [porphyrin]/[DNA] ratio and they stacked at a high ratio. In contrast, various stacking modes of porphyrin were observed for *m*-TMPyP. The stacking patterns included moderate and extensive stacking,[8-11] which have been known since the outset of the porphyrin-DNA interaction study, and formation of extensive assemblies[12-14] as have been reported by other investigators.

Since the effects of the rotability of the pyridinium ring and of Cu as a central metal on the porphyrin's binding mode to DNA have been established, it is worth while to investigate the effect of the combined two factors on the formation of the porphyrin-DNA complex. In this work, we investigated the interactions of *p*-, *m*- and *o*-CuTMPyP (Figure 1) with calf thymus DNA, poly[d(G-C)$_2$] and

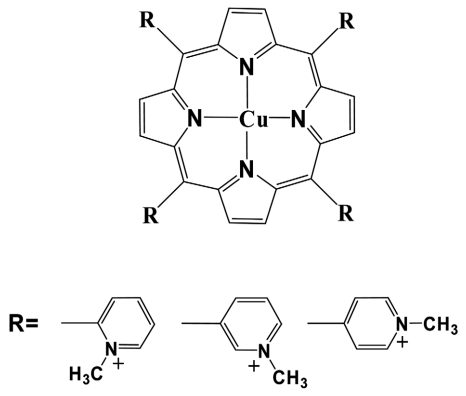


**Figure 1**. Chemical structures of Cu(II)*meso*-tetrakis(*n*-*N*-methylpyrimidiumyl)porphyrin referred to as *o*-, *m*- and *p*-CuTMPyP in the text, respectively, for *n* = 2, 3 and 4.

poly[d(A-T)$_2$] by polarized spectroscopy, including circular and linear dichroism (CD and LD). There was a special focus on the geometry of porphyrin in the complex formed with polynucleotide.

## Experimental Section

**Materials.** Porphyrins were purchased from MidCentury (Chicago, IL) and other chemicals from Sigma (Seoul, Korea), and used without further purification. The extinction coefficients were measured at the Soret maxim and were $\varepsilon_{416nm} = 2.19 \times 10^5$ cm$^{-1}$M$^{-1}$, $\varepsilon_{418nm} = 2.46 \times 10^5$ cm$^{-1}$M$^{-1}$, and $\varepsilon_{424nm} = 1.46 \times 10^5$ cm$^{-1}$M$^{-1}$ in 5 mM cacodylate buffer pH 7.0 for *o*-, *m*- and *p*-CuTMPyP, respectively. This buffer was used throughout this study. It was not known for what reason the extinction coefficient of *p*-CuTMPyP is particularly low. Calf thymus DNA (DNA) and polynucleotide were purchased from Amersham Biosciences (Piscataway, NJ) and they were further purified by the method described elsewhere.[5] The concentrations of polynucleotide were measured spectrophotometrically using the extinction coefficients of $\varepsilon_{262nm} = 6{,}600$ cm$^{-1}$M$^{-1}$, $\varepsilon_{254nm} = 8{,}400$ cm$^{-1}$M$^{-1}$, and $\varepsilon_{258nm} = 6{,}700$ cm$^{-1}$M$^{-1}$, respectively, thus indicating the concentrations of nucleo-bases. In this study, the concentration of the polynucleotides were fixed at 50 $\mu$M and that of the porphyrins were varied to give a desired [porphyrin]/[DNA] ratio (*R* ratio). Since the stacking of porphyrin itself in aqueous solution[15] affects the binding mode, porphyrin at a fixed concentration of stock solution (100 $\mu$M) was always added last.

**Measurements.** The absorption spectra of the porphyrin-DNA complex were measured on a Jasco V550 spectrophotometer, and CD on a J810 spectropolarimeter (Tokyo, Japan), displaying the CD in millidegree ellipticity. The path length of the sample for both absorption and CD measurements was 1.0 cm. Although CuTMPyP complex is an achiral molecule, the CD is induced by the interaction between the transition dipoles of porphyrin and those of chirally arranged DNA bases. In the porphyrin-DNA complex, a negative CD signal in the Soret absorption region is a diagnostic for porphyrin intercalation while a positive CD band is an indication for those bound monomerically at the groove.[16-18] A bisignate CD spectrum with the various magnitudes and signs reflects various types of porphyrin stacking along the DNA stem.[8-11,14,19]

Reduced linear dichroism (LD$^r$), defined by the ratio of measured LD to isotropic absorption spectrum, $A_{iso}$, at a given wavelength, $\lambda$, is related to the angle, $\alpha$, between the electric transition dipole of DNA-bound drugs and the local DNA helix axis.[20-22]

$$LD^r = \frac{LD(\lambda)}{A_{iso}(\lambda)} = \frac{3S}{2}(3\cos^2\alpha - 1)$$

Where $S$ is the orientation factor such that $S = 1$ for DNA perfectly aligned to the flow direction and $S = 0$ for random orientation. An effective angle, $\alpha$, can be calculated by assuming the angle of 86° between the DNA bases and the DNA helix axis at 260 nm.[23] In the porphyrin case a large absorption band in the Soret region makes it easy to obtain the LD$^r$ spectrum. However, since the $B_x$ and $B_y$ transitions are degenerated in the region, the true tilt may be obtained by replacing $\cos^2\alpha$ in the equation by $(1/2)\cos^2\beta$.[24] The LD spectrum on flow-oriented DNA and the porphyrin-DNA complex was measured in a flow Couvette cell with an inner rotating cylinder of Wada type on a Jasco 715 spectropolarimeter (Tokyo, Japan) by reported methods.[20-22]

## Results

The CD and LD spectra in this study were measured for the porphyrin-DNA complexes at a fixed polynucleotide concentration of 50 $\mu$M in base. The porphyrin concentration was varied to give the desired *R* ratios. The *R* ratios in this study were 0.02, 0.04, 0.08, 0.12, 0.16, 0.20 and 0.24. The resulting spectra for only selective *R* ratio are shown for reasons of clarity.

**CD spectra of the *o*-CuTMPyP bound to various DNAs.** The CD spectrum of *o*-CuTMPyP complexed with DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$] is depicted in Figure 2a, 2b, and 2c, respectively. When associated with DNA, *o*-

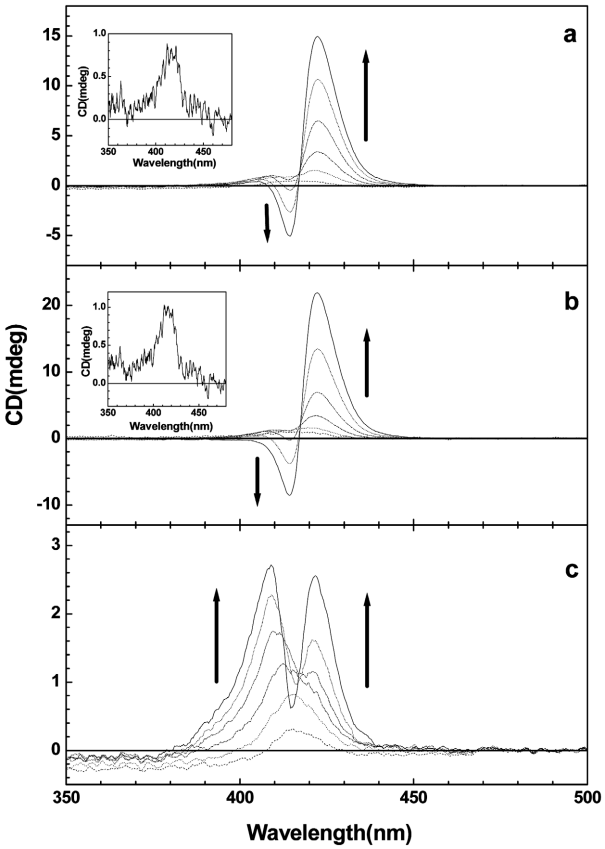


**Figure 2**. CD spectrum of the *o*-CuTMPyP when bound to DNA (panel a), poly[d(A-T)$_2$] (panel b) and poly[d(G-C)$_2$] (panel c). [Polynucleotide] = 50 $\mu$M in base, [*o*-CuTMPyP] = 2, 4, 6, 8, 10 and 12 $\mu$M to the arrow direction. CD spectrum of the *o*-CuTMPyP-DNA and -poly[d(A-T)$_2$] at a very low *R* ratio is inserted in panel a and b, respectively. The shapes of the CD spectra at the *R* ratio below 0.08 are essentially the same. See text.

CuTMPyP exhibited a positive CD signal with its maximum around 417 nm at the *R* ratio of 0.04 (Figure 2a, insertion), suggesting that the porphyrin binds monomerically near the minor groove. The shapes of the positive CD band were retained and the intensity was proportional to the *R* ratios between *R* = 0.02-0.08, indicating that the binding modes at these low *R* ratios are essentially identical and homogeneous. However, at an *R* ratio above 0.08, a minimum at *ca*. 414 nm and a maximum at 422 nm start to appear. At the higher *R* ratios, a bisignate CD spectrum was clearly observed (Figure 2a), suggesting that porphyrin stacking is the dominant binding mode. When *o*-CuTMPyP is associated with poly[d(A-T)$_2$], the shape of the CD spectrum was essentially the same as that bound to native DNA (Figure 2b). The positive maximum at *ca*. 419 nm was apparent at the low *R* ratios while the location of the negative minimum and the positive maximum of the bisignate CD at the high *R* ratios is the same as the *o*-CuTMPyP-DNA complex. The intensity of the bisignate CD of the *o*-CuTMPyP-poly[d(A-T)$_2$] complex is somewhat larger compared to that complexed with DNA. An isodichroic point at 417 nm for *o*-CuTMPyP complexed both with DNA and poly[d(A-T)$_2$] was found above the *R* ratio of 0.12, suggesting that the majority of the bound porphyrin was stacked. The amount of monomerically-bound porphyrin may be negligibly small at the high *R* ratios. The *R* ratio-dependent CD behavior of the *o*-CuTMPyP-poly[d(G-C)$_2$] complex (Figure 2c) is somewhat different with that of the *o*-CuTMPyP-DNA and -poly[d(A-T)$_2$] complexes. The positive CD signal with its maximum at *ca*. 416 nm, apparent at the low *R* ratio, is markedly symmetric. The positive CD signal was visible up to the *R* ratio of 0.16. At this *R* ratio, a minimum at *ca*. 415 nm started to appear, suggesting that porphyrin had begun to stack. However, the appearance of the bisignate CD is far less intense than those of the *o*-CuTMPyP-DNA and -poly[d(A-T)$_2$] complex. The similar positive CD signal for TMPyP complexed with DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$] at the R ratio of 0.05 were reported.[5]

**CD spectra of the *m*- and *p*-CuTMPyP bound to various DNAs.** Appearance of the *R* ratio dependent CD spectrum of *m*-CuTMPyP-DNA and *p*-CuTMPyP-DNA complexes resembles each other (Figure 3a and 4a). At the low *R* ratios, *R* = 0.02-0.08, the CD signals were negative with their maximums at *ca*. 427 nm and 425 nm, respectively, for *m*- and *p*-CuTMPyP (Figures 3a and 3b). The width of the *p*-CuTMPyP complex was wider. Similarly negative CDs were reported for both the *m*- and *p*-TMPyP free-base porphyrin complexed with DNA at a low *R* ratio.[5] As the *R* ratio increased, a bisignate CD signal appeared with its positive maximum at *ca*. 417 nm and negative

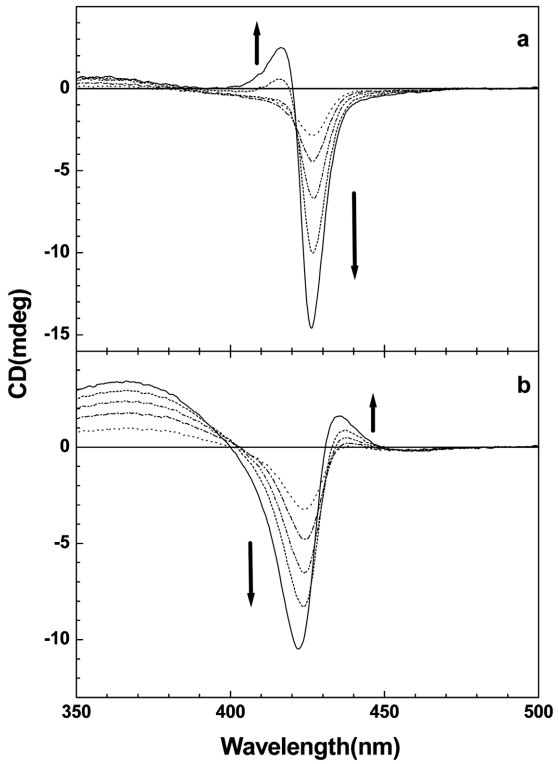


**Figure 3**. CD spectrum of *m*-CuTMPyP-DNA (panel a) and -poly[d(G-C)$_2$] complex (panel b). The concentrations of polynucleotide and porphyrin are the same as in Figure 2. The concentrations of *m*-CuTMPyP are 4, 6, 8, 10 and 12 $\mu$M to arrow direction. The shape of the CD spectra at *R* = 0.02 and 0.04, corresponding to [*m*-CuTMPyP] = 1 and 2 $\mu$M, are essentially the same as that at *R* = 0.4 therefore, are not shown.

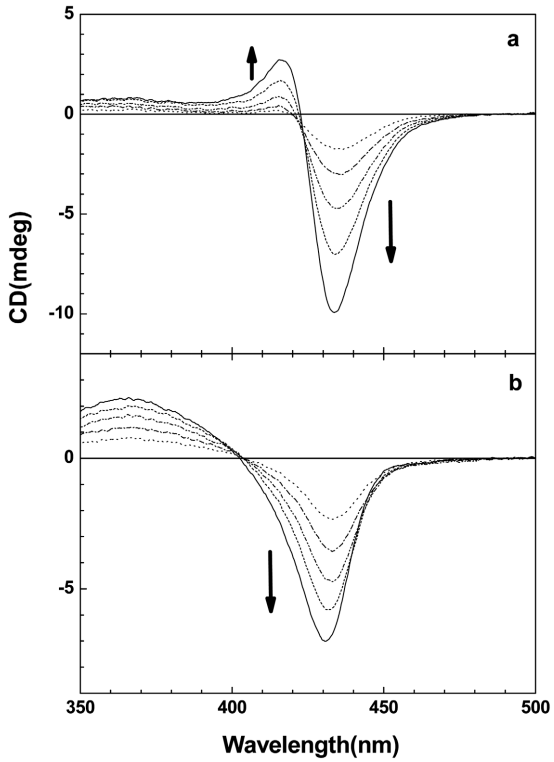


**Figure 4**. CD spectrum of *p*-CuTMPyP-DNA (panel a) and -poly[d(G-C)$_2$] complex (panel b). The concentrations of polynucleotide and porphyrin are the same as in Figure 3. The concentration of *p*-CuTMPyP increases to arrow direction. The shape of the CD spectra at *R* = 0.02, 0.04 and 0.08, corresponding to [*p*-CuTMPyP] = 1, 2, and 4 $\mu$M, are essentially the same.

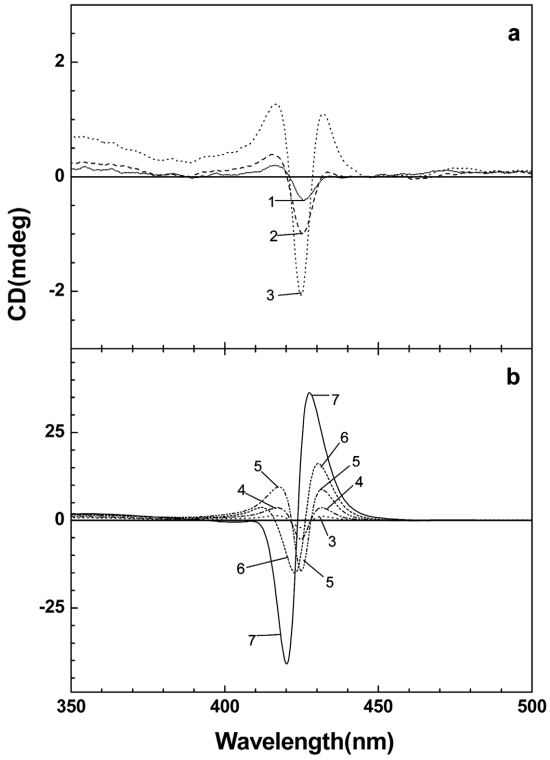


**Figure 5**. CD spectrum of *m*-CuTMPyP bound to poly[d(A-T)$_2$]. [Polynucleotide] = 50 $\mu$M, and [*m*-CuTMPyP] = 1, 2, 4, 6, 8, 10, 12 $\mu$M which are marked, respectively, with the number 1 through 7.

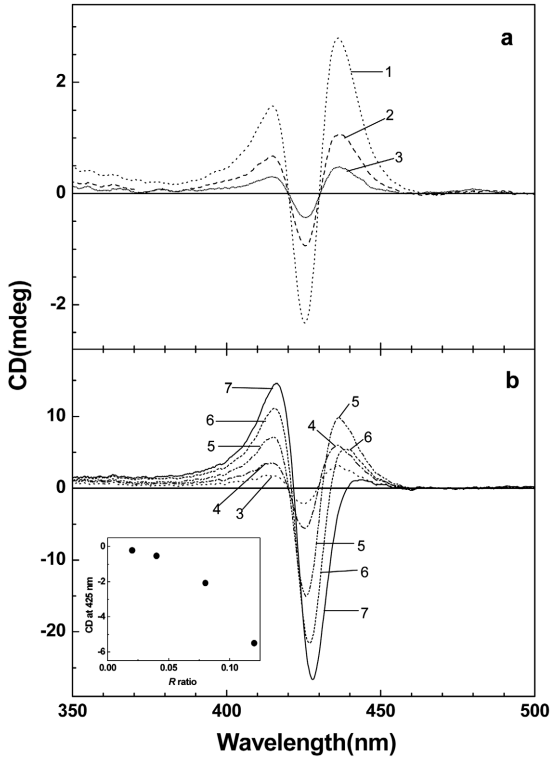


**Figure 6**. CD spectrum of *p*-CuTMPyP-poly[d(A-T)$_2$] complex. The concentrations and the curve assignment are the same as in Figure 5.

minimum at *ca*. 426 nm for the *m*-CuTMPyP-DNA complex (Figure 3). Those for the *p*-complex appeared at *ca.* 417 nm and *ca*. 433 nm (Figure 4). When complexed with poly[d(G-C)$_2$], a negative CD band was observed for both *m*- and *p*-CuTMPyP at the low binding ratios (R = 0.1, 0.2,0.4 and 0.8), with its minimum at *ca.* 424 nm and 433 nm, respectively (Figures 3b and 4b), suggesting that both porphyrins intercalate. As the *R* ratio increased, a positive band at the long wavelength region start to appear for the *m*-CuTMPyP-poly[d(G-C)$_2$] complex. This positive contribution in the long wavelength region of the Soret band was also noticed for the *p*-CuTMPyP-poly[d(G-C)$_2$] complex, but its extent was negligibly small.

The CD spectral characteristics of the *m*- and *p*-CuTMPyP-poly[d(A-T)$_2$] complexes are interesting because an abnormal behavior in the stacking of the free-base *m*-TMPyP along poly[d(A-T)$_2$] has been reported.[5-7] CD spectra of the *m*-CuTMPyP bound to poly[d(A-T)$_2$] are depicted in Figure 5. A bisignate CD spectrum with its positive maximum at *ca.* 415 nm and negative maximum at *ca.* 426 nm was apparent at the lowest mixing ratio (*R* = 0.02). A similar bisignate CD signal was observed for the free-base TMPyP-poly[d(A-T)$_2$] complex under similar condition.[5-7] However, at an *R* ratio as low as 0.04, a positive band at the long wavelength edge of the Soret band started to build up. At intermediate *R* ratios (*R* = 0.08-0.16), a complicated CD feature with its positive maximum at ca. 416 nm and *ca*. 432 nm, and a negative minimum at *ca*. 424 nm were apparent, suggesting that the pattern of the stacking of the porphyrin molecule is heterogeneous. A further increase in the *R* ratio resulted in a new CD spectrum with its negative minimum at *ca.* 420 nm and positive maximum at *ca.* 428 nm. In spite of some red-shift, the shape of the CD spectrum resembles that of the free-base TMPyP-poly[d(A-T)$_2$] complex at a high mixing ratio. The complicated CD spectra at the intermediate *R* range could not be explained by any combination of the CD spectra at the extremely low and high *R* ratios. In the *p*-CuTMPyP-poly[d(A-T)$_2$] complex case, a bisignate CD that was observed for the *m*-TMPyP case did not appeared. From the lowest *R* ratio, CD spectrum consisting of the positive-negative-positive band was apparent (Figure 6a). The shape of this CD spectrum remained up to the *R* ratio of 0.12 with two isodichroic points at ca. 420 nm and 430 nm. However, the intensity of the CD signal was not directly proportional to the porphyrin concentration. When the intensity of the CD at 425 nm was plotted with respect to the *R* ratio, a downward curve was apparent (Figure 6b, insertion), suggesting that the formation of this type of stacking is cooperative. As the *R* ratio further increased, a bisignate CD spectrum with its positive maximum at *ca.* 416 nm and a negative minimum at *ca.* 428 nm appeared (Figure 6b). It is interesting to note that the appearance of the CD spectrum of the *p*-CuTMPyP-poly[d(A-T)$_2$] complex at a high *R* ratio is symmetric to that of *m*-CuTMPyP-poly[d(A-T)$_2$] complex: the order of the negative and positive band appeared to be opposite.

**LD$^r$ spectra of the CuTMPyP-polynucleotide complexes.**

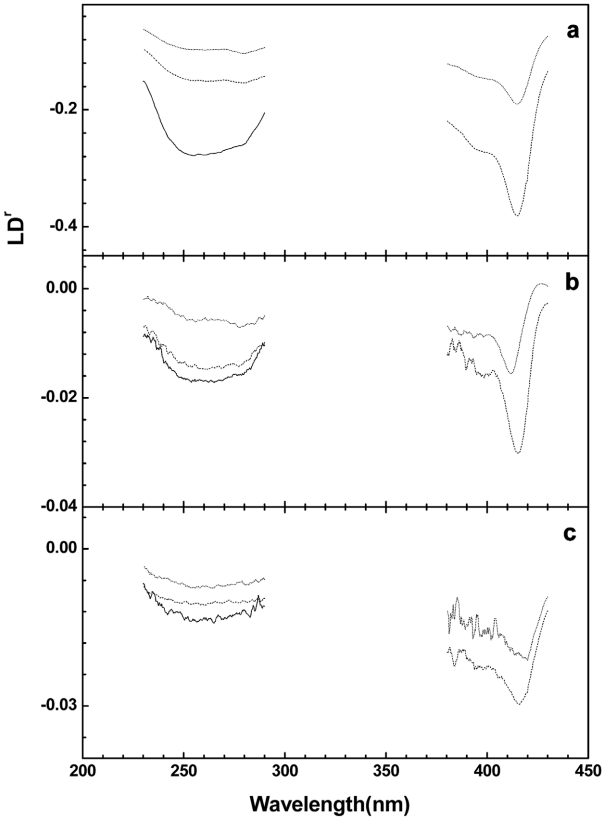


**Figure 7**. LD$^r$ spectrum of the *m*-CuTMPyP-DNA (panel a), -poly[d(A-T)$_2$] (panel b) and -poly[d(G-C)$_2$] (panel c) complex at the *R* ratio of 0.08 (dashed curve) and 0.24 (dotted curve). That of the corresponding porphyrin-free polynucleotide is shown for comparison as the solid curves. [polynucleotide] = 50 $\mu$M.

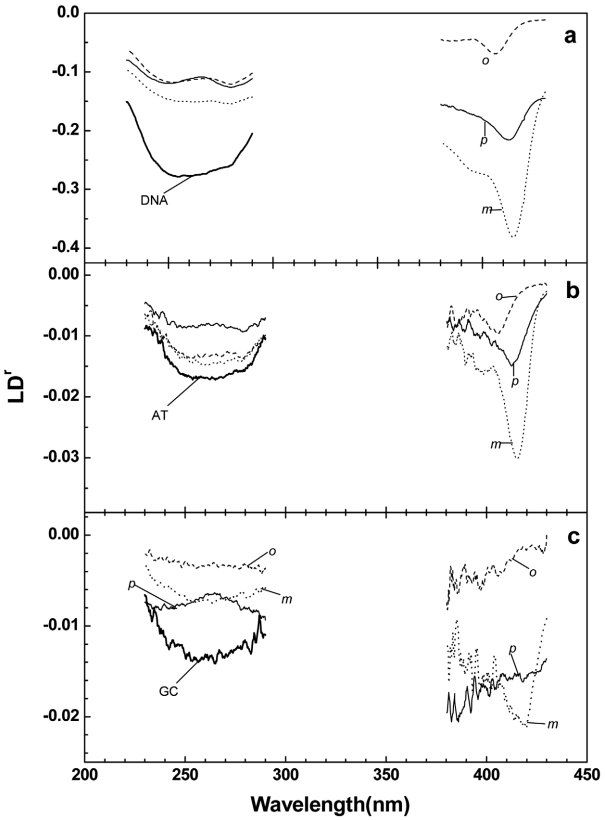


**Figure 8**. LD$^r$ spectrum of *o*-, *m*- and *p*-CuTMPyP complexed with DNA (panel a), poly[d(A-T)$_2$] (panel b) and poly[d(G-C)$_2$] (panel c) at R = 0.08. [polynucleotide] = 50 $\mu$M.

The LD$^r$ spectrum usually provides very detailed information about the angles between the drug's electric transitions and the DNA helix axis, thereby indicating the binding geometry of the drug relative to DNA.[20-22] However, in contrast with CD spectrum, the LD$^r$ spectrum was less sensitive for the *R* ratio in the case of CuTMPyP for all polynucleotide examined in this study. Figure 7a, 7b and 7c show the LD$^r$ spectrum of the *m*-CuTMPyP-poly[d(A-T)$_2$], -poly[d(G-C)$_2$] and -DNA complex at the low and high *R* ratios, as an example. At a low *R* ratio, *m*-CuTMPyP exhibited a negative CD band when complexed with DNA and poly[d(G-C)$_2$] while an excitonic, bisignate CD for poly[d(A-T)$_2$] was apparent. The former two complexes produced bisignate CD at a high R ratio. The appearance of the excitonic CD of the *m*-CuTMPyP-poly[d(A-T)$_2$] complex was even more complicated at a high *R* ratio. In spite of the *R* ratio dependent complicated CD spectrum, the appearance of the LD$^r$ spectrum was insensitive to the *R* ratio. Therefore, only the LD$^r$ spectra of *o*-, *m*- and *p*-CuTMPyP complexed with DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$] at a low *R* ratio are shown for reasons of clarity in Figure 8. The LD$^r$ of the porphyrin-free DNA is negative near 260 nm and is relatively wavelength independent as it is expected from our LD facility.[20-24] Upon binding of any of CuTMPyP, a significant decrease in the LD$^r$ magnitude was pronounced (Figure 8a), indicating the large decrease in the orientability of DNA. Since the chemical structures of DNA bases, which absorb the radiation in this wavelength region did not change, the large decrease in LD$^r$ magnitude indicates that the DNA is bent or partially denatured upon porphyrin binding. In the current case, bending or kinking of the DNA at the porphyrin binding site is more likely. In the Soret absorption region, the LD$^r$ magnitude of both *p*- and *m*-CuTMPyP is larger than that in the DNA absorption region, which has been often observed for drugs that intercalated.[25] The angle, a, cannot be calculated in this case. Strong wavelength-dependency in the Soret region was found for both complexes, suggesting that the $B_x$ and $B_y$ transition of the porphyrin relative to the DNA helix axis is tilted. In contrast, the LD$^r$ magnitude of the *o*-CuTMPyP-DNA complex is smaller in the Soret region compared to that in the DNA absorption region. The angles of the electric transitions relative to the DNA helix axis were calculated as 64° and 60° from the maximum and minimum value of the LD$^r$. Since the degeneracy in this region is at least partially removed, the calculation of the b angle[24] is not applicable in this case.

Binding of various CuTMPyP to poly[d(A-T)$_2$] also resulted in a decrease of LD$^r$ magnitude in the DNA absorption region, although the extent of decrease is less pronounced (Figure 8b). The shape of the LD$^r$ spectrum in the Soret absorption region is similar with that of the DNA complexes, although a larger tilt in the *o*-CuTMPyP case is

noticed. The angle of 65° and 57° were obtained from the maximum and minimum $LD^r$ for the *o*-CuTMPyP-poly[d(A-T)$_2$] case. For both DNA and poly[d(A-T)$_2$], the wavelength-dependency, hence tilt angle of the porphyrin plane with respect to DNA helix axis is largest for *m*-CuTMPyP. When associated with poly[d(G-C)$_2$], the $LD^r$ in the Soret region appeared to be remarkably independent on the wavelength for the *o*- and *p*-CuTMPyP case. However, the relative magnitude of the $LD^r$ in the DNA absorption region and Soret region is comparable in the *o*-CuTMPyP-poly[d(G-C)$_2$] complex while that in the Soret region is significantly larger for the *p*-CuTMPyP-poly[d(G-C)$_2$] complex. In the *m*-TMPyP-poly[d(A-T)$_2$] case, the $LD^r$ in the Soret region was wavelength dependent, similar with those discussed earlier.

## Discussion

**Known binding mode of *o*-, *m*-, and *p*-TMPyP and related Cu-porphyrins.** The binding mode of *o*-, *m*-, *p*-TMPyP and *p*-CuTMPyP to various DNAs was reported.[2,26,27] Summarizing the reported results may provide a good clue for analyzing the spectral observations presented in this study. *O*-TMPyP, in which rotation of the periphery pyridinium ring is prevented, exhibited a positive CD signal in the Soret region, similarly for all polynucleotides, namely DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$]. For all polynucleotides, *o*-TMPyP was concluded to bind at the major groove. On the other hand, *p*-TMPyP intercalates between the base pairs of both DNA and poly[d(G-C)$_2$] at a low *R* ratio, which was represented by a negative CD band in the Soret absorption region. In the poly[d(A-T)$_2$] case, *p*-TMPyP exhibited a monomeric outside binding that is represented by a positive CD signal at a low *R* ratio, while the stacking of porphyrin, producing a bisignate CD signal, appeared as the *R* ratio increased. When *m*-TMPyP was associated with DNA and poly[d(G-C)$_2$], the binding property was similar to that of *p*-TMPyP. The *m*-TMPyP-poly[d(A-T)$_2$] complex, however, exhibited an abnormal behavior. At a low *R* ratio, a bisignate CD spectrum in the Soret region was apparent, indicating that porphyrin was moderately or extensively stacked.[10,17,28] At a high *R* ratio, a bisignate CD with its intensity far higher than that in the low R ratio was observed. In the CD spectrum, the order of the negative and positive CD signal was opposite to that at a low R ratio. This species was assigned to the porphyrin that formed an extensive assembly.[6,7]

Binding of *p*-CuTMPyP and CuTAP, in which pyridinium ring is replaced by bulky 4-N,N',N''-trimethylanilinium to native DNA was reported. In CuTAP, the *t*-butyl groups provide steric hindrance preventing the intercalation of this porphyrin between the DNA bases similarly with the *o*-CuTMPyP. The CD spectrum of the *p*-CuTMPyP-DNA complex at $R = 0.08$ was characterized by a negative CD band in the Soret region, indicating that *p*-CuTMPyP intercalates. In contrast, CuTAP formed an external complex with DNA with some aggregation at a high drug load. The CD spectrum of the externally bound CuTAP was characterized by two positive maximum and one positive minimum between them.

**Binding mode of o-CuTMPyP to DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$].** The CD spectrum of the *o*-CuTMPyP-DNA and *o*-CuTMPyP-poly[d(A-T)$_2$] complex at low *R* ratios is characterized by a positive band in the Soret region, which is accompanied by an $LD^r$ spectrum with a decreased magnitude in the DNA absorption region. The possibility of porphyrin intercalation can be ruled out by the shape of the CD spectrum as well as the angle of 65°-55° between the porphyrin's molecular plane and the DNA helix axis. Since the rotation of the pyridinium ring is prevented for *o*-CuTMPyP, thereby porphyrin cannot be planar and intercalative binding is not expected. The positive CD signal in the Soret region upon porphyrin binding to the AT oligonucleotide was assigned to porphyrins that locate near the minor groove.[17,18] Apparent bisignate CD for both DNA and poly[d(A-T)$_2$] complexes at a high drug to DNA ratio indicates that the stacking of porphyrin molecule is significant. However, the stacking of porphyrin by themselves did not alter the binding geometry as judged by similar $LD^r$ spectrum at the low and high *R* ratios. Similar CD and $LD^r$ spectrum was reported for *o*-TMPyP-DNA and poly[d(A-T)$_2$] complex, suggesting that in the *o*-TMPyP case, the presence of $Cu^{2+}$ ion did not affect the binding mode. From the CD and LD data presented in this study, the location of the stacking, *i.e.*, minor *vs* major groove, cannot be determined unambiguously at this stage. In the *o*-CuTMPyP-poly[d(G-C)$_2$] complex case, a similar positive CD was observed. However, the appearance of the stacking requires a much higher drug to DNA load. The magnitude of $LD^r$ spectrum in the Soret region is similar to that in the DNA region, suggesting that the *o*-CuTMPyP is more parallel to the DNA base pairs when bound to poly[d(G-C)$_2$] than to the DNA and poly[d(A-T)$_2$] complex. Considering the fact that the spectral properties of *o*-CuTMPyP bound to DNA resemble poly[d(A-T)$_2$] more, this porphyrin may prefer to bind to the AT sequence than to the GC sequence.

**Binding mode of *m*- and *p*-CuTMPyP to DNA and poly[d(G-C)$_2$].** Both *m*- and *p*-CuTMPyP produced a negative CD band upon binding to DNA and poly[d(G-C)$_2$] at the low R ratios. A negative CD band for the *p*-CuTMPyP has been reported and was not affected by the concentration of salt.[1,2] The negative CD in the Soret region has been believed to be a diagnostic for porphyrin intercalation. The magnitude of $LD^r$ in the Soret region is larger than that in the DNA absorption region, supporting the intercalative binding mode of both *m*- and *p*-CuTMPyP. However, a deviation from the classic intercalation mode was also observed in the $LD^r$ spectrum. For a classic intercalator, such as ethidium[25] or 9-aminoacridine,[29] not only the larger or comparable $LD^r$ magnitude in the drug's absorption region relative to DNA absorption region but also the wavelength-independent $LD^r$ in the drug's absorption region are expected, reflecting that all in-plane $\pi^* \leftarrow \pi$ transitions of the drug are parallel to the DNA bases. The $LD^r$ magnitude in the DNA absorption region have a tendency to increase upon drug intercalation in

comparison with drug-free DNA.[21,22] These $LD^r$ criteria of intercalation is not applicable for *m*- and *p*-CuTMPyP complexed with DNA and poly[d(G-C)$_2$]. For all complexes, a significant decrease in $LD^r$ magnitude was observed. Although the magnitude of $LD^r$ in the Soret region is larger, a very strong wavelength dependence was found except in the *p*-CuTMPyP-poly[d(G-C)$_2$] complex. The wavelength dependency hence, the tilt of the porphyrin was always more pronounced for *m*-CuTMPyP than for its *p*- isomer. All this $LD^r$ analysis indicates that both DNA and porphyrin strongly tilt near the porphyrin intercalation site. In the absence of the central $Cu^{2+}$ metal ion, both *m*- and *p*-TMPyP exhibited similar CD and $LD^r$ behavior at a low *R* ratio, indicating that central $Cu^{2+}$ ion does not affect the binding mode of this class. As the *R* ratio increases, stacking of both *m*- and *p*-CuTMPyP was pronounced. The stacking did not significantly affect the $LD^r$ spectrum hence, the binding geometry. It is noteworthy that the order of positive and negative CD bands appeared to be opposite between DNA and poly[d(G-C)$_2$]. We do not have any explanation for these observations at the current stage. It is also noteworthy that the appearance of the CD and $LD^r$ spectrum of *m*- and *p*-CuTMPyP is similar when bound to DNA and poly[d(G-C)$_2$] and is significantly different when bound to poly[d(A-T)$_2$]. This result suggests that both *m*- and *p*-CuTMPyP prefer to bind at the GC site than AT site, which is in contrast with *o*-CuTMPyP.

**Stacking of *m*- and *p*-CuTMPyP along the poly[d(A-T)$_2$] stem.** The CD spectrum of both *m*- and *p*-CuTMPyP in the presence of poly[d(A-T)$_2$] appeared to be considerably complicated. In the *m*-CuTMPyP-poly[d(A-T)$_2$] complex case, at least three CD species were identified: a bisignate at the lowest *R* ratio, and that consists of two positive and one negative CD band at intermediate *R* ratios, and bisignate CD spectrum with very high intensity showing the order of positive and negative bands opposite to that observed at the lowest *R* ratio. The shapes of the CD spectrum as well as the intensities at the lowest and the highest *R* ratio resemble those of the free-base *m*-TMPyP-poly[d(A-T)$_2$] complex. These species were assigned to a moderately stacked porphyrin and formation of extensive porphyrin array, respectively.[5-7] The CD spectrum of intermediate *R* ratios could not be explained by the combination of those observed at the lowest and highest R ratios, suggesting that the nature of porphyrin in stacking the intermediate *R* range is different from that of the two former types although their binding geometries as elucidated by $LD^r$ resemble each other. Both the strong wavelength dependency in the Soret band and the decrease in the DNA absorption region indicated that poly[d(A-T)$_2$] is bent near the porphyrin stacking site. In the *p*-CuTMPyP-poly[d(A-T)$_2$] case, the bisignate CD signal observed for the *m*-CuTMPyP-poly[d(A-T)$_2$] at an extremely low drug to DNA ratio did not appear. When the central $Cu^{2+}$ was absent, a positive CD signal corresponding to *p*-TMPyP that was bound monomerically at the minor groove did not appear, suggesting that the presence of $Cu^{2+}$ ion may have enhanced the stacking in this case. Appearance of CD spectrum due to this stacking is cooperative. Although the order of the positive and negative CD band is opposite to that of the *m*-CuTMPyP-poly[d(A-T)$_2$] complex, the strong bisignate CD spectrum observed for the *p*-CuTMPyP-poly[d(A-T)$_2$] complex at a high R ratio may be assigned to a extension of the porphyrin stacking or porphyrin assembly.

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