Binding Mode of Cationic Porphyrin with CT-DNA: Importance of the Location and the Number of Positively Charged of Periphery Cationic Ions of Porphyrin

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ABSTRACT: The binding modes of o-, m-, and p-trans-BMPyP with DNA were studied using their spectroscopic properties. Also, the binding modes were compared based on the location and number of periphery cationic methylpyridine ions of the cationic porphyrins. The optical absorption spectra of the o-, m-, and p-trans-BMPyP when bound to DNA presented red shifts and hypochromicity compared to the optical absorption spectrum of DNA-free cationic porphyrins. m-trans-BMPyP–DNA presented the largest red shifts and hypochromicity. The results of the circular dichroism spectral analysis indicated positive and negative bisignate absorption bands in the Soret band of the porphyrins in the case of all concentration ratios of o- and p-trans-BMPyP–DNA, and two negative absorption bands were observed in m-trans-BMPyP–DNA. Compared to the size of the absorption band of the DNA optical absorption spectrum, the results of the reduced linear (LD') spectral analysis indicated mainly small sizes of Soret absorption bands (the absorption spectrum of porphyrins) and positive LD' values for o- and p-trans-BMPyP–DNA. In consideration of several of such spectroscopic properties, the binding of o- and p-trans-BMPyP with DNA can be said to be distant to insertion modes. Although the case of m-trans-BMPyP to DNA is an insertion mode, the m-trans-BMPyP molecular surface presented much tilt within the intercalation pocket. The results of comparing the binding modes of TMPyP having four periphery cationic methylpyridine ions of cationic porphyrin indicated that regardless of the number of periphery cationic methylpyridine ions of cationic porphyrin, in the case of the ortho-position, nonplanarity due to steric hindrance of the periphery cationic methylpyridine ions presented outside or groove-binding modes indicative of interaction with DNA phosphates. Unlike the ortho-position, the para-position presented different binding modes based on the number of periphery cationic methylpyridine ions. Only cationic porphyrins having four periphery cationic methylpyridine ions were inserted into the DNA. Lastly, regardless of the number of periphery cationic methylpyridine ions, all meta-positions were inserted into the DNA. This indicated that at the least the location and the number of periphery cationic methylpyridine ions of the porphyrins used in this experiment were important elements that determine insertion into DNA base pairs.

INTRODUCTION

Nucleic acid plays an important role in cellular processes including cellular separation (DNA replication) and protein synthesis (transcription and translation). During these processes, both healthy cells and cancerous cells are developed. Because of the immense strides made in the field of life sciences, many secrets of life have been uncovered. Yet there still exist several phenomena associated with life that are yet to be discovered. One of such phenomena includes the ability of a life-form to self-assemble. Life-forms, created through the process of self-assembly, have evolved numerous nanostructures that allow them to perform various functions. One of the most representative forms of self-assembly that occur in life-forms is DNA. Throughout the continued research to find treatments for cancer, DNA has both gained much attention as a storage substance of genetic information as well as a form of medication. Because of its high bioconformity, stability regarding its neighboring environment, and simple interaction between its four bases, DNA has been recognized as an excellent biomaterial that is both predictable and programmable. Ethidium bromide, acridine orange, methylene blue, Ru(II) complex, porphyrin, and small molecules are often used to understand the structure and properties of DNA through their interactions with drug–DNA and protein–DNA. The study of the interaction between porphyrins and DNA is continuously gaining interest. Porphyrins have been used to develop artificial receptors that are used to recognize molecules and to develop new chiral catalysts for asymmetric synthesis. They have also been used to explore biologically important response mechanisms such as photosynthesis and...
BMPyP binds with DNA. At maximum absorbency of the − their measurements were presented in each of the (A panels. Panel (A) presents the absorption spectra when concentration ratios (referred to as meta-, and para-positions (referred to as o-, m-, and p-trans-BMPyP, Scheme 1) bound with DNA and compared it with the binding mode of meso-tetakis(1-methylpyridinum-4-yl)-porphyrins (TMPyP) with four positive methylpyridine ions.

Scheme 1. Chemical Structures of trans-Bis(N-methylpyridinum-2-yl)diphenyl Porphyrin, trans-Bis(N-methylpyridinum-3-yl)diphenyl Porphyrin, and trans-Bis(N-methylpyridinum-4-yl)diphenyl Porphyrin, Respectively (Referred to as o-, m-, and p-trans-BMPyP in the Text)

| Entry | R  |
|-------|----|
| ortho | +N  |
| meta  | +N  |
| para  | +N  |

■ RESULTS

Absorption. In general, red shift and hypochromicity in absorption spectra are presented because of the interactions between DNA and small molecules. On the basis of the degree to which they change, binding modes can be predicted. Figure 1 presents the measured absorption spectra of various bindings between o-, m-, p-trans-BMPyP and DNA at various concentration ratios (R = 0.02, 0.04, 0.06, 0.08, and 0.1), and their measurements were presented in each of the (A–C) panels. Panel (A) presents the absorption spectra when o-trans-BMPyP binds with DNA. At maximum absorbency of the DNA-free o-trans-BMPyP and DNA binding at ~415 nm, an approximately 4 nm red shift to ~419 nm occurred. Absorbsivity was also found to gradually decrease as the concentration increased resulting in hypochromaticity. At the highest concentration ratio (R = 0.1), absorbsivity decreased by 39.71% compared to the DNA-free o-trans-BMPyP. Panel (B) presents the results of m-trans-BMPyP to DNA. The panel also presented red shifts and hypochromicity as was the case with o-trans-BMPyP–DNA. However, the results of panel (B) indicated greater hypochromicity (57.45%) and a larger red shift (~10 nm) of 10 nm from ~416 to ~426 nm compared to o-trans-BMPyP to DNA as the concentration ratios increased. Alongside the increases in the concentration ratios, small movements toward shorter wavelengths were observed at ~428 nm. Also, an isosbestic point was observed at ~422 nm. Although absorbsivity at ~428 nm decreased in small amounts as the concentration ratios increased, absorbsivity was found to increase in small amounts at the newly formed ~413 nm. This indicated that the type of binding of m-trans-BMPyP to DNA under the experiment conditions of this study may be different, that m-trans-BMPyP that did not bind may exist, or that this may be indicative of an interaction with m-trans-BMPyP after the final binding. Lastly, panel (C) presents absorbion regarding p-trans-BMPyP–DNA. Like other porphyrins, red shift (~4 nm) and hypochromicity (~42.92%) were observed. Similar to m-trans-BMPyP, on the basis of the concentration, the wavelengths of maximum absorbency indicated movement. However, no changes in the absorbency were presented. At lower concentration ratios (R = 0.02) of binding p-trans-BMPyP to DNA, red shift of approximately ~7 nm occurred.

Figure 1. Absorption spectra of (A) o-, (B) m-, and (C) p-trans-BMPyP complexed with DNA at various mixing ratios in the presence of DNA (black curve) and in the absence of DNA (red curve). [DNA] = 100 μM, R = 0.02, 0.04, 0.06, 0.08, and 0.1. The ratio is increasing with the direction of the arrow.

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from \( \sim 419 \) to \( \sim 426 \) nm, and as the concentration increased, wavelengths moved again to shorter wavelengths (\( \sim 422 \) nm). 

**Circular Dichroism Spectra.** Circular dichroism (CD) spectra are strong indicators used to easily distinguish the binding modes of porphyrins bound to DNA. Porphyrins are not chiral molecules. However, when bound to DNA, CD spectra induced by DNA can be observed in the Soret band of the porphyrin absorption spectrum, and through this CD spectrum, the binding modes of porphyrin bound to DNA can be distinguished.\(^{18-23}\) 

Figure 2 presents the CD spectra measured at various concentration ratios (\( R = 0.02, 0.04, 0.06, 0.08, \text{ and } 0.1 \)) of \( o-, m-, \text{ and } p-\text{trans}-\text{BMPyP} \) bound to DNA. As was the case with absorption, the results were presented through panels (A–C). Panel (A) presents the CD spectral analysis results regarding \( o-\text{trans}-\text{BMPyP} \) to DNA. When examining the DNA-induced porphyrin spectrum, the Soret spectrum, one positive peak was observed from the \( \sim 427 \) nm wavelength at low concentration ratios (0.02–0.04). On the other hand, as the concentration ratios increased, at wavelengths of \( \sim 413 \) and \( \sim 427 \) nm, a bisignate signal of a negative peak and a positive peak, respectively, was observed. The ratio of the size of the bisignate peak was not symmetrically the same, and at the highest concentration ratio (\( R = 0.1 \)), the positive peak was observed to be 2.86 greater in size than the negative peak. These results indicated that at low concentration ratios, a monomeric-binding mode was present in which binding occurs occasionally on the DNA grooves, and as the concentration ratios increase, outside stacking as a result of interactions with DNA phosphate groups or binding mode stacking along the DNA minor grooves was presented. Unlike this case, when \( m-\text{trans}-\text{BMPyP} \) binds to the DNA, negative peaks across the CD spectra at all concentration ratios were observed. Panel (B) indicates that at a low concentration ratio (\( R = 0.02 \)), one negative peak at \( \sim 440 \) nm and two negative peaks at \( \sim 419 \) and \( \sim 440 \) nm were observed, respectively, as the concentration increased, and their values were also found to increase gradually. However, at the maximum concentration ratio (\( R = 0.1 \)), compared to the absorption peak at \( \sim 440 \) nm, the absorption peak at \( \sim 419 \) nm was observed to be 2.30 times lower. Such a phenomenon can be considered to be the result of the following two explanations. First, as it is widely known, the result of insertion into the DNA base pairs results in observations of negative peaks, and the occurrence of another negative peak can be explained by the interactions between the porphyrins inserted into the base pairs.\(^{18-23}\) Second, CT-DNA incorporates a GC base of \( \sim 60\% \) and an AT base of \( \sim 40\% \). This indicates that it could be the result of binding affinities associated with GC or AT. The results of the CD spectral analysis of \( p-\text{trans}-\text{BMPyP} \) to DNA were presented in panel (C). Similar to \( o-\text{trans}-\text{BMPyP} \) to DNA, with the exception of a low concentration ratio (\( R = 0.02 \)), bisignate signals were observed and the positions shifted slightly to long wavelengths presenting a negative peak at \( \sim 418 \) nm and a positive peak at \( \sim 437 \) nm. However, unlike \( o-\text{trans}-\text{BMPyP} \) to DNA, the size of the bisignate signal was presented to be almost symmetrical. This indicated that it had a similar binding mode to \( o-\text{trans}-\text{BMPyP} \) to DNA or had better DNA stacking.

**Linear Dichroism and LD.** Figure 3 presents the linear dichroism (LD) spectra of \( o-, m-, \text{ and } p-\text{trans}-\text{BMPyP} \) bound with DNA at various concentration ratios. The measurements
were each presented, respectively, in panels (A–C). Although the changes in DNA absorption spectra as the concentration increased when binding \( o \)-, \( m \)-, and \( p \)-trans-BMPyP to DNA were similar, some differences were observed in the porphyrin absorption spectra between \( \sim 400 \) and \( \sim 500 \) nm. Compared to porphyrin-free DNA, all instances of \( o \)-, \( m \)-, and \( p \)-trans-BMPyP to DNA presented a tendency in which the DNA absorption spectra decreased as the concentration ratios increased. The reason behind such decrease can be attributed to an increase in positive contributions of porphyrins bound to DNA, a decrease in DNA orientation ability due to the porphyrin binding, or the result of both. Panel (A) presents the LD spectral analysis results regarding \( o \)-trans-BMPyP to DNA. Negative peaks of the porphyrin absorption spectra were observed at \( \sim 425 \) nm when the concentration ratio of \( o \)-trans-BMPyP to DNA was low \( (R = 0.02–0.04) \), and LD sizes indicated slight increases. Beginning at the concentration ratio of 0.06, bisignate peaks were formed. At the concentration ratio of 0.06 and \( \sim 410 \) nm, positive peaks began to form, LD sizes gradually increased with increase in the concentration ratio, and gradually increased even at negative absorption spectra \( (\sim 425 \) nm). However, as the concentration ratio increased \( (R = 0.08) \), the LD signal of the negative peaks shifted to long wavelengths at \( \sim 430 \) nm \( (R = 0.08) \) and \( \sim 436 \) nm \( (R = 0.1) \) while LD values decreased. Unlike this instance, the porphyrin absorption spectrum of \( m \)-trans-BMPyP to DNA did not present wavelength shifts based on different concentration ratios \( \text{[panel (B)]} \). At the lowest concentration ratio \( (R = 0.02) \), negative peaks were observed at \( \sim 435 \) nm, and with increase in the concentration ratio, bisignate signals were presented. Positive peaks formed at \( \sim 414 \) nm and negative peaks formed at \( \sim 435 \) nm, and their sizes presented gradual increases. LD sizes of the negative peaks were approximately 3.2 times larger than the positive peaks. Lastly, panel (C) presents the LD spectral analysis of \( p \)-trans-BMPyP to DNA. A bisignate signal was observed with a positive peak at \( \sim 420 \) nm and a negative peak at \( \sim 449 \) nm. Increase of positive peaks was clearly evident with increase in the concentration ratio while almost no changes were observed for the negative peaks. The LD sizes indicated the opposite results of the LD spectral analysis of \( m \)-trans-BMPyP to DNA in which large positive peaks were presented. When \( o \)-, \( m \)-, and \( p \)-trans-BMPyP bind to DNA, LD signal changes in the Soret absorption spectrum occur according to concentration ratios. At low concentration ratios, only one negative peak presented for all cases. Although sizes changed at different rates as concentration ratios increased, all cases presented bisignate signals. This was considered to be the result of interaction with porphyrins bound to DNA as concentration ratios increased or the result of the respective difference in interactions with AT and GC bases of DNA.

By dividing the measured LD spectra by the absorption spectra, LD' spectra can be found. Figure 4 presents the LD' spectra of \( o \)-, \( m \)-, and \( p \)-trans-BMPyP to DNA at various concentration ratios, and the results are presented in panels (A–C). In the DNA absorption spectra, the size of LD' decreased more with the binding of porphyrins than without the binding of porphyrins. This can be considered to be the result of the increase in DNA flexibility induced by the binding of porphyrins, the result of the bending of the DNA stem, or the result of both. LD' calculates the angles between the bound molecules with the DNA helix axis, when small molecules bind with DNA and through the use of such calculations, LD' is considered to be the most useful tool used to predict binding modes. In general, binding modes can be predicted by comparing the LD' sizes of the DNA spectra and drug spectra. Through this method, the interaction between DNA and drug can be understood. When examining the porphyrin absorption spectra, larger positive contributions and concentration ratios for \( o \)- and \( p \)-trans-BMPyP to DNA compared to \( m \)-trans-BMPyP to DNA result in decreases in LD' sizes and positive values. Positive LD' values were even observed at low concentration ratios in the case of \( p \)-trans-BMPyP to DNA. These results, as is the case with CD spectra, indicate outside-binding modes associated with binding along DNA phosphates or the binding of \( o \)- and \( p \)-trans-BMPyP with DNA grooves, resulting from the interaction between DNA and \( o \)- and \( p \)-trans-BMPyP DNA. Unlike this, in the case of \( m \)-trans-BMPyP to DNA, the LD' of the DNA absorption spectra and the Soret absorption spectra presented differences at low concentration ratios \( (R = 0.02 \) and 0.04). However, even with increases in concentration ratios, LD' values did not change at the Soret absorption spectrum at \( \sim 427 \) nm, and the higher the concentration ratios, compared to the LD' values of the DNA absorption spectra, the LD' values of the Soret absorption spectra were found to be smaller or similar. From the results of the LD' spectral analysis, at low concentration ratios, binding can be said to have occurred at the DNA grooves or
phosphates, whereas at high concentration ratios, the molecular surface of m-trans-BMPyP and the DNA helix axis were almost perpendicular, which was indicative of insertion into the DNA base pair. However, all concentration ratios of CD spectra indicated negative values at the Soret band, the m-trans-BMPyP absorption spectra. This indicated that even at low concentration ratios, m-trans-BMPyP was inserted into the DNA base pairs. Therefore, the reason behind the differences in LD' values at low concentration ratios was considered to be due to the binding of one of the porphyrin electric transition moments, Bx or By, at the DNA base surface in the horizontal direction, when the molecular surface of m-trans-BMPyP is inserted into the DNA base pair, whereas on the other hand, the other transition is tilted toward the DNA helix axis. That is, the molecular surface of m-trans-BMPyP was considered to have largely tilted within the intercalation pocket.

**DISCUSSION**

For the purpose of reaching conclusions regarding DNA-binding modes based on positions of periphery cationic methylpyridine ions of cationic porphyrins (o-, m-, and p-trans-BMPyP) and for the purpose of understanding the effects of binding modes based on the number of periphery cationic methylpyridine ions, the spectroscopic properties of DNA-binding modes of o-, m-, and p-TMPyP having four periphery cationic methylpyridine ions were studied and compared. All spectra were measured at a concentration ratio of 0.1 (Figures 5−7).

**Figure 5.** Absorption [panel (A)] and CD [panel (B)] spectra of o-trans-BMPyP (red curve) and o-TMPyP (blue curve) in the presence of DNA (solid curve) and in the absence of DNA (dashed curve). Panel (C) shows the LD' spectrum of o-trans-BMPyP−DNA (red curve) and o-TMPyP−DNA (blue curve) in the presence of DNA (solid curve) and in the absence of DNA (black dashed curve). [DNA] = 100 μM and [porphyrin] = 10 μM.

**Figure 6.** Absorption [panel (A)] and CD [panel (B)] spectra of p-trans-BMPyP (red curve) and p-TMPyP (blue curve) in the presence of DNA (solid curve) and in the absence of DNA (dashed curve). Panel (C) shows the LD' spectrum of p-trans-BMPyP−DNA (red curve) and p-TMPyP−DNA (blue curve) in the presence of DNA (solid curve) and in the absence of DNA (black dashed curve). [DNA] = 100 μM and [porphyrin] = 10 μM.

**Figure 6. Absorption [panel (A)] and CD [panel (B)] spectra of p-trans-BMPyP (red curve) and p-TMPyP (blue curve) in the presence of DNA (solid curve) and in the absence of DNA (dashed curve). Panel (C) shows the LD' spectrum of p-trans-BMPyP−DNA (red curve) and p-TMPyP−DNA (blue curve) in the presence of DNA (solid curve) and in the absence of DNA (black dashed curve). [DNA] = 100 μM and [porphyrin] = 10 μM.

**Binding Mode of o-trans-BMPyP to DNA.** In general, when porphyrins are inserted into DNA base pairs, large red shifts of the absorption spectra occur, negative CD spectra including hypochromicity are observed, and the porphyrin LD' sizes or values compared to the LD' sizes and values of the DNA absorption spectrum are larger or have larger values. However, when o-trans-BMPyP binds with DNA, unlike the insertion mode, small red shifts, hypochromicity, bisignate bands in the Soret absorption spectra, and largely reduced LD' of the Soret absorption spectra compared to the DNA absorption spectra can be observed. This results in the proposal of two possible binding modes due to the interaction between o-trans-BMPyP and DNA. This can be considered to be the result of groove binding between o-trans-BMPyP and DNA or outside binding between o-trans-BMPyP and DNA phosphate groups. Also, to compare the effects of the binding modes based on the number of periphery cationic methylpyridine ions of the cationic porphyrins, the results of absorption, CD, and LD' spectra analyses regarding the binding of DNA with o-TMPyP having four periphery cationic methylpyridine ions are presented in Figure 5.

As was the case with o-trans-BMPyP to DNA, the absorption spectra of o-TMPyP to DNA compared to o-trans-BMPyP to DNA indicated smaller changes, yet, red shifts and hypochromicity could be observed, and regarding the LD' spectra, the size of LD' in the Soret absorption spectra compared to the DNA absorption spectra was found to be especially smaller. However, significant differences were present in the CD analysis. Maintaining molecular planarity of
porphyrins is difficult for cationic porphyrins having periphery cationic methylpyridines at the ortho-position because of the difficulties in rotating due to steric hindrance. Because of the influence of such structural properties, o-trans-BMPyP and o-TMPyP are both difficult to insert into DNA base pairs and need to resort to groove binding or DNA outside binding through interactions with DNA phosphates. Such predicted binding modes show clear differences in binding modes based on the number of periphery cationic methylpyridine ions of the cationic porphyrins. Unlike the observed bisignate bands in o-trans-BMPyP to DNA having two periphery cationic methylpyridine ions in the Soret absorption spectra, a positive band can be observed, when o-TMPyP having four periphery cationic methylpyridine ions binds with DNA. In other words, o-TMPyP having four periphery cationic methylpyridine ions bind with the outside of DNA and o-trans-BMPyP having two ions bind through a moderate stacking binding mode by binding to grooves. These results indicate greater steric hindrance with larger numbers of periphery cationic methylpyridine ions at the ortho-position, resulting in porphyrin structures that are far from planarity. Such results are indicative of DNA outside binding because of the difficulties of groove binding.

**Binding Mode of p-trans-BMPyP to DNA.** Figure 6 presents the absorption, CD, and LD’ spectra, when p-trans-BMPyP and p-TMPyP bind with DNA. Both p-trans-BMPyP and p-TMPyP to DNA present large hypochromicity, where p-TMPyP to DNA presents a larger red shift compared to p-trans-BMPyP to DNA (Figure 6A). In the CD spectrum, both p-trans-BMPyP and p-TMPyP to DNA indicate bisignate bands in the Soret absorption spectrum. Although p-trans-BMPyP to DNA presents symmetrical similarities between the CD sizes of the negative and positive absorption bands, the size of the negative bands compared to the positive bands of p-TMPyP to DNA was found to be approximately 3 times larger (Figure 6B). Figure 6C presents the LD’ spectra of p-trans-BMPyP and p-TMPyP to DNA. An examination of the DNA absorption spectrum indicates a decrease in the p-trans-BMPyP to DNA LD’ size compared to when porphyrins are not present, whereas the LD’ size of p-TMPyP to DNA, on the other hand, was found to slightly increase. This indicated a change in the DNA length due to the binding of porphyrins. When comparing LD’ sizes of the Soret absorption spectra and the DNA absorption spectra in p-trans-BMPyP to DNA and p-TMPyP to DNA, compared to the LD’ of the DNA absorption spectrum of p-trans-BMPyP to DNA, the LD’ size of the Soret absorption spectrum decreased and presented a positive value. However, compared to p-TMPyP to DNA, the LD’ size of the Soret absorption spectrum was found to be almost the same as the DNA absorption spectrum. Although such results indicated that the p-TMPyP molecule bound to the DNA helix axis almost perpendicularly, that is, indicated a binding mode to the DNA base pair, this also indicated that p-trans-BMPyP was not inserted into the DNA base pairs but rather bound through DNA groove binding or DNA outside binding. Regardless of whether the periphery cationic methylpyridine ions of the cationic porphyrins were in the same position, there were differences in binding modes to DNA. This can be considered to be indicative of the fact that DNA binding modes are largely affected by the number of periphery cationic methylpyridine ions at the para-position of porphyrins.

**Binding Mode of m-trans-BMPyP to DNA.** Figure 7 presents the absorption, CD and LD’ spectra when m-trans-BMPyP and m-TMPyP bind with DNA. In the absorption spectrum, both the m-trans-BMPyP and m-TMPyP porphyrins indicated large red shifts and hypochromicity, when binding with DNA where larger red shifts were presented in the case of m-TMPyP to DNA. In the CD spectrum, negative bands were observed in the Soret absorption spectrum and the CD size value of m-TMPyP to DNA was approximately 2.5 times greater than m-trans-BMPyP to DNA. Such negative CD values in the Soret absorption spectrum are typical of binding modes, in which porphyrins have been inserted into DNA base pairs. In support of this fact, a study of the LD’ spectrum indicated the differences in DNA absorption spectrum LD’ sizes compared to when porphyrins do not exist as was the case with p-trans-BMPyP and p-TMPyP to DNA. This also indicated a change in the DNA length due to the binding of porphyrins. An examination of LD’ sizes of DNA absorption spectra and Soret absorption spectra of m-trans-BMPyP to DNA and m-TMPyP to DNA indicated similar values in the case of m-trans-BMPyP to DNA, whereas a notably larger negative value was present in the case of m-TMPyP to DNA. This, as was mentioned earlier, indicated that the porphyrin molecule was perpendicularly close to the DNA helix axis and that the porphyrin was inserted into the DNA base pair. On the other hand, the molecular surface of m-trans-BMPyP during the insertion into the DNA base pair was thought to have largely tilted in the intercalation pocket. On the basis of the number of periphery cationic methylpyridine ions, the para-position of cationic porphyrins indicated different binding modes to DNA, whereas in the meta-position, both porphyrins having two or...
**CONCLUSIONS**

On the basis of the observations reported, it is conclusive that m-trans-BMPyP exhibited an intercalative binding mode to native DNA, whereas the other two, that is, o- and p-isomers were not. In the latter two porphyrin cases, the porphyrin produced the spectral characteristics supporting either minor or major groove binding mode, in which one of the electric transition moments (either B<sub>x</sub> or B<sub>y</sub> transition) tilts the angle of the groove with respect to the local DNA helix axis (Figure 8).

**EXPERIMENTAL SECTION**

**Materials and Methods.** o-, m-, and p-trans-BMPyP and o-, m-, and p-TMPyP were purchased from Frontier Scientific, Inc. (Utah, USA) and used as received. DNA was purchased from Sigma-Aldrich and used without further purification. Buffer solution used was 5 mM cacodylate buffer and pH 7.0. The concentrations of the porphyrins were measured spectrophotometrically using the extinction coefficients of ε<sub>415nm</sub> = 233 960 cm<sup>-1</sup> M<sup>-1</sup>, ε<sub>418nm</sub> = 252 400 cm<sup>-1</sup> M<sup>-1</sup>, and ε<sub>419nm</sub> = 240 000 cm<sup>-1</sup> M<sup>-1</sup> for o-trans-BMPyP, m-trans-BMPyP, and p-trans-BMPyP and ε<sub>413nm</sub> = 239 000 cm<sup>-1</sup> M<sup>-1</sup>, ε<sub>417nm</sub> = 278 000 cm<sup>-1</sup> M<sup>-1</sup>, and ε<sub>421nm</sub> = 226 000 cm<sup>-1</sup> M<sup>-1</sup> for o-TMPyP, m-TMPyP, and p-TMPyP, respectively. The extinction coefficients for DNA was ε<sub>258nm</sub> = 6700 cm<sup>-1</sup> M<sup>-1</sup>. Absorption and CD spectra were recorded at room temperature, on a Cary 100 Bio spectropolarimeter (Australia) and on a JASCO J810 spectropolarimeter (Tokyo, Japan), respectively. The path length for all CD measurements was 1 cm. LD spectra were measured on J715 (Jasco, Tokyo, Japan) spectropolarimeters. A Wada-type inner-rotating flow cell was used to align the DNA sample for LD measurements, as described by Norden et al.<sup>19</sup>,<sup>27</sup> The division of measured LD by the isotropic absorption spectrum resulted in a dimensionless quantity LD<sup>'</sup>, which is related to the angle (α) of the transition moment of any DNA-bound drug with respect to the local DNA helix axis and the ability to orient the DNA–drug adduct through

\[ LD' = \frac{S}{(3 \cos^2 \alpha - 1)} \]

where S is the orientation factor, a measure of the sample’s ability to orient. The mixing ratio R, [porphyrin]/[DNA base], corresponded to the ratio of one porphyrin per polynucleotide bases, where R = 0.01 corresponded to one porphyrin per 100 DNA bases or phosphates.

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**Notes**

The authors declare no competing financial interest.

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