Binding Mode of *meso*-Tetrakis(*N*-methylpyridinium-4-yl)porphyrin to Poly[d(I−C)$_2$]: Effect of Amino Group at the Minor Groove of Poly[d(G−C)$_2$] on the Porphyrin−DNA Interaction

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The spectral properties of *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (TMPyP) complexed with poly[d(I−C)$_2$] were compared with that bound to poly[d(G−C)$_2$] and poly[d(A−T)$_2$]. At low TMPyP/DNA base ratios, the TMPyP-poly[d(I−C)$_2$] complex exhibits a similar NaCl dependent absorption and circular dichroism spectrum to that of the TMPyP-poly[d(A−T)$_2$] complex. The negative CD band in the Soret absorption region of these complexes disappears as the NaCl concentration reaches around 10 mM. At a NaCl concentration higher than 10 mM, the resulting positive CD band is pronounced. In contrast, the change in negative CD band of the TMPyP-poly[d(G−C)$_2$] complex, which is believed to be intercalated, is small. The molecular plane of porphyrin in the TMPyP-poly[d(I−C)$_2$] complex for both species associated with negative and positive CD is perpendicular relative to the polynucleotide helix axis. Therefore, it is indicative that TMPyP (partially) intercalates between the base pairs of poly[d(I−C)$_2$] at a very low mixing ratio and NaCl concentration and exhibits an outside binding mode as the NaCl concentration increases. It then follows that the amine group of guanine that protrudes in to the minor groove either helps stabilize the intercalated TMPyP or serves to destabilize the minor groove binding.

Introduction

Interaction between DNA and porphyrin has been widely studied for its potential biological application such as in photodynamic therapy and for its unique physicochemical property in the interaction with nucleic acids (see refs 1−3 for review). The most extensively studied DNA binding porphyrin is *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (referred to as TMPyP, Figure 1) and its metal complex. It is now generally accepted that free porphyrin bases including TMPyP intercalate into the GC rich region,[4,5] whereas various binding modes for the TMPyP complexed with AT rich DNA, including outside stacking, outside random binding, and groove binding, have been suggested. In general, groove binding seems to be a preferable binding mode for the TMPyP-AT rich DNA complexes at low porphyrin to DNA base ratios,[6−10] and as the ratio increases, the outside binding mode dominates, in which porphyrin molecules form a moderate or extensive stacked array along the DNA template.[11−14] The nature of the groove binding mode of porphyrin, i.e., either major or minor groove, remains to be clarified.

It has been well-known that unfused aromatic hydrocarbons bind preferentially in the minor groove of the AT-rich region of DNA.[15−17] The majority of well-known minor groove binding drugs possess common structural motifs that they adopt as crescent shapes that match the turn of the helical motif in the DNA stem. There are chemical moieties in the crescent to enable the hydrogen bonds to form. Unfused or partially fused aromatic parts exhibit a certain degree of rotation for drugs to fit into the narrow minor groove. When the AT sequence is replaced by GC, the minor groove binders exhibit full or partially intercalative binding.[18−20] Therefore, the preferentiality of the minor groove binders toward the minor groove of AT sequences is inhibited, at least in part, by the amine group of the guanine base that protrudes into the minor groove. This results in the steric hindrance for incoming drugs.

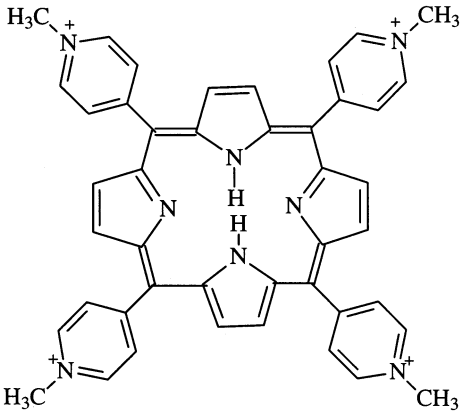

Figure 1. Molecular structures of *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin.

The side of the TMPyP shares a similar structural motif with conventional minor groove binders, which makes TMPyP a

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possible candidate of the minor groove binder. If TMPyP binds in the minor groove of AT rich DNA, the spectral properties of the TMPyP-poly[d(I−C)$_2$] complex are expected to be similar to those of the TMPyP-poly[d(A−T)$_2$] complex because the minor groove of the two polynucleotides is similar. Under this assumption, we compared the spectral properties of the TMPyP-poly[d(I−C)$_2$] complex to those of the TMPyP-poly[d(A−T)$_2$] and -poly[d(G−C)$_2$] complexes at the low [TMPyP]/[DNA base] ratio in this work.

Materials and Methods

Materials. Polynucleotides were purchased from Pharmacia and TMPyP from Mid Century (Chicago, IL) and directly dissolved in a 1 mM cacodylate buffer pH 7.0. This buffer was used throughout this work, except for the LD measurement, which was recorded in 5 mM cacodylate buffer. The concentrations of the TMPyP and poly[d(I−C)$_2$], poly[d(A−T)$_2$], and poly[d(G−C)$_2$] were determined spectrophotometrically using extinction coefficients of $\epsilon_{424\ nm} = 226\ 000\ cm^{-1}\ M^{-1}$, $\epsilon_{251nm} = 6900\ cm^{-1}\ M^{-1}$, $\epsilon_{262nm} = 6600\ cm^{-1}\ M^{-1}$, and $\epsilon_{254nm} = 8400\ cm^{-1}\ M^{-1}$, respectively. The mixing ratio, R, is defined by the ratio of the [TMPyP]/[DNA base]. Therefore, $R = 0.1$ indicates one porphyrin molecule per five DNA base pairs or 10 bases. Because the binding mode of porphyrin was reported to depend on the salt concentration and the order of the mixing,[21] measurements at various salt concentrations were also taken. Salt solution was always added to TMPyP−polynucleotide solution at the final step in order to obtain the desired salt concentration. In the following text, 0 mM NaCl indicates that 1 mM Na^+ is present in the solution that came from the counterion of the cacodylate molecule. 100 mM NaCl indicates 101 mM Na^+ (100 mM from NaCl and 1 mM from the buffering molecule). All measurements were performed right after mixing the salt at 20 °C.

Absorption, Circular, and Reduced Linear Dichroism. Absorption spectra were recorded on a Jasco V-550 spectrophotometer and circular dichroism (CD) spectra on a Jasco 715 spectropolarimeter, which displays the CD in milli degrees ellipticity. In the porphyrin case, a negative induced CD band in the Soret absorption region may be diagnostic of intercalation, whereas a positive feature indicates external groove binding.[22] CD spectra were averaged over an appropriate number of scans.

Reduced linear dichroism (LD^r), defined by the ratio of linear dichroism (LD) to the isotropic absorption spectrum, has been proved to be a powerful tool in measuring the angle between the electric transition moment of the DNA-bound drug and DNA helix axis.[23−26] A negative LD^r signal in the drug's absorption region with its magnitude comparable or larger than that in the DNA absorption region is generally accepted as an indication for a drug being intercalated, whereas a positive signal is typical for minor groove binding drugs. LD on flow-aligned polynucleotide was measured in a couette cell of Wada type[27] on a Jasco J500C spectropolarimeter as described by Nordén and coworkers.[25,26]

Results

Absorption Spectra. Self-assembly of porphyrin in aqueous solution is known to affect its binding property to DNA. For instance,[21] *t*-H_2P exhibits an intense Soret absorption band in aqueous solution, which shifts to the long wavelength upon increasing NaCl concentration up to 200 mM. This is accompanied by a decrease in the intensity of the Soret band. However, in the TMPyP case, the absorption spectrum in the presence of 1 mM Na^+ is identical with that in the presence of up to 200 mM NaCl (data not shown), indicating that the self-assembly of TMPyP is negligible and ensuring that the binding mode of porphyrin to polynucleotide would not be affected in our salt concentration. This observation is in contrast with the *t*-H_2P and *t*-CuP case,[21,28] which may be explained by number of positive charges on the porphyrins. *meso*-Tetrakis(4-*N*,*N*′,*N*″-trimethylanilinium)porphyrin forms aggregation at very high salt concentrations because of its four positive charges.[29]

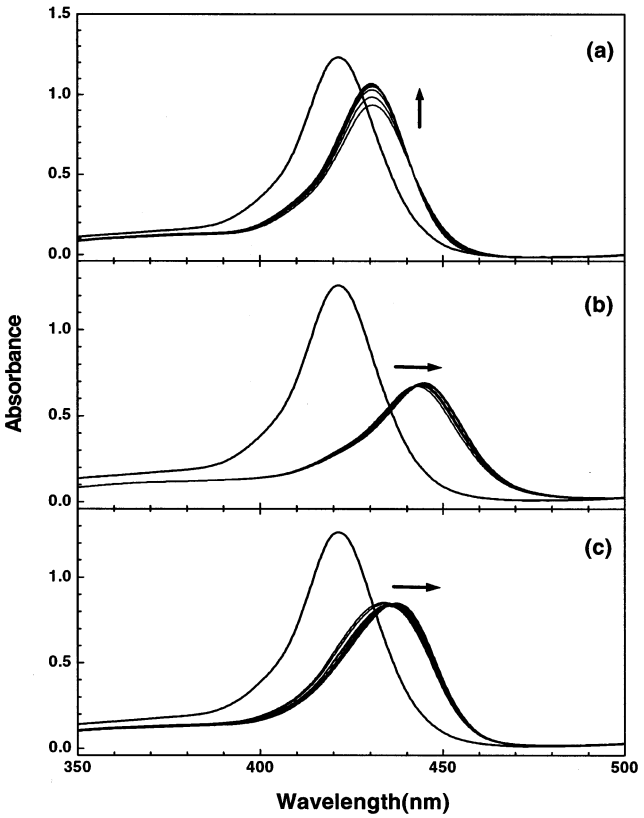

Figure 2. NaCl dependent absorption spectrum of TMPyP in the presence of poly[d(A−T)$_2$] (panel a), poly[d(G−C)$_2$] (panel b), and poly[d(I−C)$_2$] (panel c). $R = 0.05$. [polynucleotide] = 100 μM in nucleotide base. [NaCl] = 0, 1, 3, 10, 30, 50, 80, and 100 mM in direction of arrow. Absorption spectrum of polynucleotide-free TMPyP is shown as a thick solid curve.

Absorption spectra in the Soret band of TMPyP that complexed with poly[d(A−T)$_2$], poly[d(I−C)$_2$], and poly[d(G−C)$_2$] at an R ratio of 0.05 at various NaCl concentrations as well as that in the absence of polynucleotide are compared in Figure 2. When bound to poly[d(G−C)$_2$], TMPyP exhibited the largest change in absorption spectrum (Figure 3b; 0 mM NaCl): the red shift and hypochromism being 21 nm and 46.5%. The change was smallest in the TMPyP-poly[d(A−T)$_2$] complex case (8 nm red shift and 25.1 % hypochromism; Figure 3a; 0 mM NaCl). These observations are generally in agreement with previous reports.[7,8] In one of our reports, however, a far more pronounced red shift and hypochromism was observed for the TMPyP−poly[d(A−T)$_2$] complex[6] and that may have been an error. The changes in absorption spectrum of TMPyP upon binding to poly[d(I−C)$_2$] appeared to be intermediate (14 nm red shift compared that of the DNA free TMPyP at 422 nm and 34.2% hypochromism; Figure 3c; 0 mM NaCl). However, it should be noted that any combination of absorption spectra of the TMPyP−poly[d(A−T)$_2$] and TMPyP−poly[d(G−C)$_2$] complexes did not result in that of the TMPyP−poly[d(I−C)$_2$] complex (data not shown), indicating that the intermediate absorption spectrum of the latter complex is not a result of a

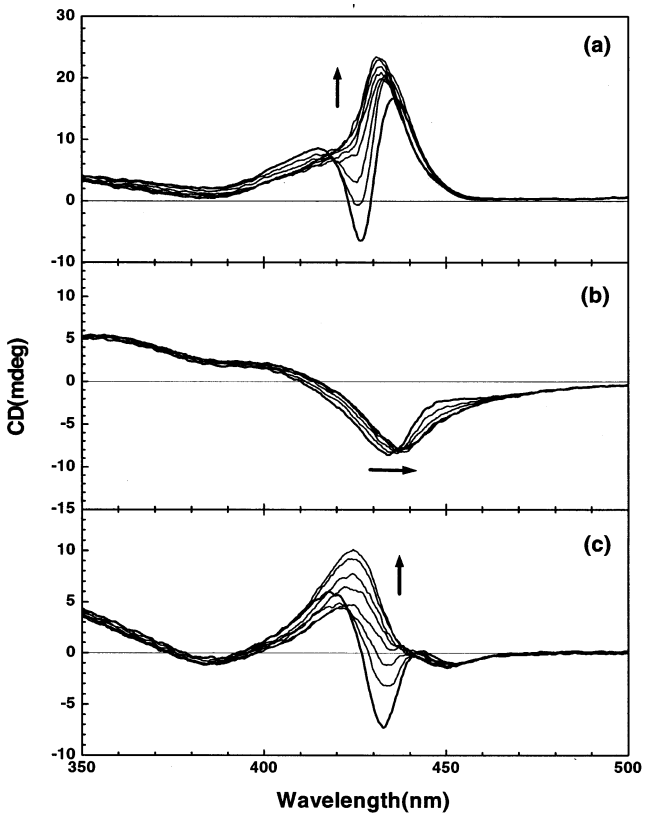

Figure 3. NaCl dependent CD spectrum of TMPyP in the presence of poly[d(A−T)$_2$] (a), poly[d(G−C)$_2$] (b), and poly[d(I−C)$_2$] (c). $R = 0.05$. [polynucleotide] = 200 μM in nucleotide base. [NaCl] = 0, 1, 3, 10, 30, 50, 80, and 100 mM in direction of arrow.

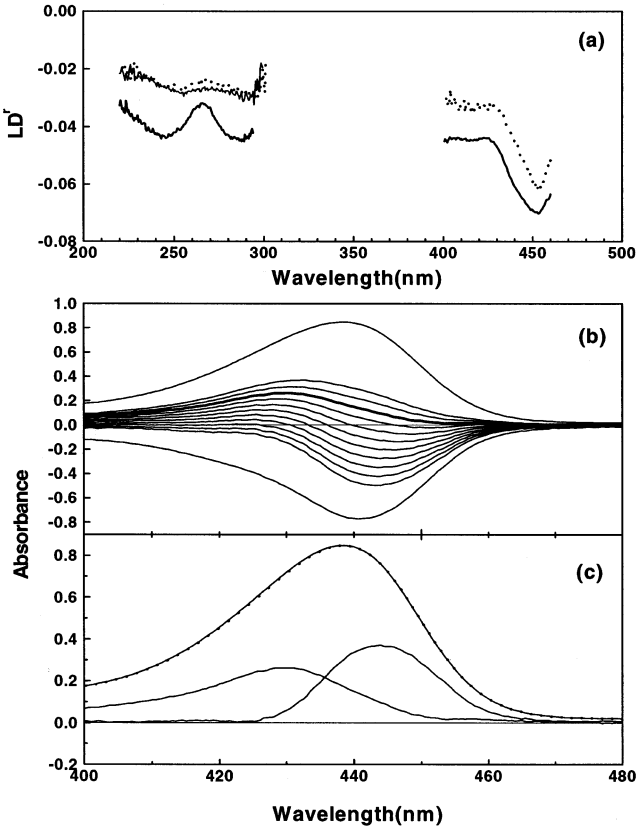

Figure 4. (a) Reduced linear dichroism spectrum of the TMPyP-poly[d(I−C)$_2$] complex at $R = 0.01$ (dotted curve) and 0.05 (solid curve) and that of porphyrin free poly[d(I−C)$_2$] (thin solid curve). The concentration of poly[d(I−C)$_2$] was 97.0 μM and that of NaCl 5 mM. (b) The different spectra $A(\lambda) - \kappa LD(\lambda)$ of TMPyP bound to poly[d(I−C)$_2$] at $R = 0.05$. Top and bottom curves are absorption and LD (multiplied by a factor of 13.5), respectively. The κ value varied between −10.5 and −25.5 with an increment of −1.5. The thick solid curve ($\kappa = -13.5$) was considered as the most representative profile of the short-wavelength transition. (c) Absorption spectrum of TMPyP bound to poly[d(I−C)$_2$] at $R = 0.05$, resolved into the contributions from two transitions. The dashed curve that is duplicated from the absorption spectrum represents the sum of $2.40T_1(\lambda) + 1.43T_2(\lambda)$. See text for explanation.

simple addition of the former two complexes. Very little R ratio dependent variation was noticed for all complexes at 0 mM NaCl (data not shown), indicating the binding mode is almost homogeneous at these low mixing ratios ($R < 0.05$).

As the NaCl concentration increases up to 100 mM, all three complexes exhibit a pronounced change in the Soret absorption band (Figure 2). An isosbestic point is also observed for all three complexes (441.6 nm for AT, 442.6 nm for GC, and 436.5 nm for IC), indicating that this change probably occurs between two states (two different binding modes).

CD Spectra. The CD spectra of TMPyP complexed with poly[d(A−T)$_2$], poly[d(G−C)$_2$], and poly[d(I−C)$_2$] at various NaCl concentration and at $R = 0.05$ are depicted in parts a−c in Figure 3, respectively. At 0 mM NaCl, both TMPyP−poly[d(A−T)$_2$] and TMPyP−poly[d(I−C)$_2$] complexes exhibit complicated CD spectrum in the Soret band (Figure 3 parts a and c), which consists, from short wavelength, positive (at 415 nm for AT and at 418 nm for IC), negative (427 nm for AT and 433 nm for IC), positive (at 436 nm for AT and at 444 nm for IC), and negative (at 452 nm for IC) bands. These complicated CD spectra are different from those observed in an excitonic CD spectrum which is originated from the aggregation (or stacking) of porphyrin at the outside of a DNA molecule.[7,8,11−14] As the salt concentration increases, the negative CD bands at 427 nm of the TMPyP−poly[d(A−T)$_2$] complex and at 433 nm of the TMPyP−poly[d(I−C)$_2$] complex start to disappear. A new positive CD band appears at 431 and 424 nm for AT and IC complexes, respectively. This change is very salt sensitive: disappearance of the negative band starts at 2 mM Na^+ for both complexes. In contrast, the negative CD band of the TMPyP−poly[d(G−C)$_2$] complex remains negative in the presence of 100 mM NaCl: the maximum shifts to red from 434 nm in the absence of NaCl to 438 nm at 100 mM NaCl with an isosbestic point at 437 nm.

The binding ratio dependent CD spectra of TMPyP complexed with poly[d(A−T)$_2$], poly[d(G−C)$_2$], and poly[d(I−C)$_2$] in the absence and presence of 100 mM NaCl were measured for $R = 0.01$, 0.02, 0.03, 0.04, and 0.05. In the absence of NaCl, the shape of CD spectra for all complexes at low binding ratio ($R = 0.01$) is essentially similar to that at $R = 0.05$ (data not shown), indicating that no significant alteration in the binding mode took place. However, a significant alteration in the CD band in the presence of 100 mM NaCl was observed for the TMPyP−poly[d(A−T)$_2$] complex. A similar CD spectrum was reported for the TMPyP−poly[d(A−T)$_2$] complex[6] and MnTMPyP−poly[d(A−T)$_2$] complex.[10]

Reduced Linear Dichroism. The LD^r spectrum of the flow oriented TMPyP−poly[d(I−C)$_2$] complex ($R = 0.01$, 0.02, 0.03, 0.04, and 0.05) at the NaCl concentration of 5 mM was measured. At a high NaCl concentration and high mixing ratio, the polynucleotide did not orient. Only those for porphyrin-free poly[d(I−C)$_2$] and $R = 0.01$ and 0.05 are shown in Figure 4a for clarity. It can be easily noticed that the overall magnitude of LD^r in the Soret band is larger than that in the DNA absorption region. A larger LD^r magnitude in the drug absorption

region than in the DNA region is usually attributed to an intercalative binding mode. However, a strong wavelength dependence on the LD^r magnitude observed for the TMPyP−poly[d(I−C)$_2$] complex indicates that TMPyP is not homogeneously intercalated. The wavelength-dependent LD^r may imply that the two transition moments in the Soret region are at different angles with respect to the DNA helix axis. Employing a similar approach as used by Kubista et al.[30] and Kim et al.,[31] we analyzed the LD^r spectrum as it was described by Michl and Thulstrup.[32] If the degeneracy of electric transition moments in the Soret band (x and y band) is removed upon binding to poly[d(I−C)$_2$] by having different angles with respect to the DNA helix axis, the absorption and LD spectra are the sum of contributions from the two transitions:

$$A(\lambda) = t_1T_1(\lambda) + t_2T_2(\lambda)$$

$$LD(\lambda) = t_3T_3(\lambda) + t_4T_4(\lambda)$$

where $T_1(\lambda)$ and $T_2(\lambda)$ are the normalized absorption spectral profiles of the two transitions and t_i's are the coefficients. Because the contributions of the two transition moments to absorption and LD spectrum are different, subtraction of the properly tuned LD spectrum by multiplying a weighting factor (κ) from the absorption spectrum results in an elimination of the contribution from one of the two transitions:

$$T_2(\lambda) = A(\lambda) - \kappa LD(\lambda)$$

The κ value can be determined by a stepwise reduction procedure. In Figure 4b, the top curve is the absorption spectrum and the bottom one is the LD spectrum multiplied by 15. The curves between show the $A(\lambda) - \kappa LD(\lambda)$spectrum with various κ values, which range from −10.5 (top) to −25.5 (bottom) with an increment of −1.5. We chose the one with $\kappa = -13.5$ as the curve best representing the short wavelength transition, $T_1(\lambda)$. By a similar calculation, $T_2(\lambda)$ was obtained, and those two transition moments are presented in Figure 4c. The LD^r values for each transition moment can be calculated by the expression[32]

$$LD_1{}^r = \frac{t_3T_1(\lambda)}{t_1T_1(\lambda)} = \frac{t_3}{t_1} \text{ and } LD_2{}^r = \frac{t_4}{t_2}$$

The LD^r values for short and long wavelength transition were calculated to be −0.0458 and −0.072, respectively. Both LD^r values are higher than that of the DNA absorption region, indicating that both transitions are almost perpendicular to the DNA helix axis. This approach is valid for the removed degeneracy of the x- and y-transition moments with different angles relative to the DNA helix axis. However, the two different binding modes of the TMPyP molecule bound poly[d(I−C)$_2$], with its two transition moments remaining to degenerate, would also exhibit a similar LD^r because the above approach only deals with two energy levels.

The positive LD^r contribution in the DNA absorption region at approximately 270 nm is also pronounced (Figure 4a). This positive LD^r suggests either a positive contribution from the transition moment of bound TMPyP or strong distortion of the polynucleotide bases. The positive LD^r signals in the DNA absorption region for other TMPyP−polynucleotide complexes were also reported.[6−8]

Discussion

In this work, we compared the absorption and induced CD spectrum of the TMPyP−poly[d(I−C)$_2$] complex with those complexed with poly[d(A−T)$_2$] and poly[d(G−C)$_2$]. Absorption spectra of TMPyP upon binding to all three polynucleotides depend on the salt concentration in the range of 1∼100 mM (Figure 2). The changes in absorption spectrum is accompanied by a well defined isosbestic point, indicating that this transition occurs between two states, unless two or more binding species exhibits exactly the same absorption spectrum, which is not likely in our case. Therefore, the changes in absorption spectrum should be understood via the different binding mode of TMPyP, which is induced by interaction between the TMPyP−polynucleotide complex and NaCl. The absorption spectrum recorded from the samples at various R ratios at the same NaCl concentration is similar when normalized to the concentration, indicating that the variation in the binding mode with the mixing ratio change is negligible at these low binding ratios(R = 0.01∼0.05).

A complex CD spectrum in the Soret band of TMPyP, which consists of two positive and two negative bands, is induced upon binding to poly[d(I−C)$_2$] in the absence of NaCl at $R = 0.05$ (Figure 3c). As the salt concentration increases, the most pronounced change in the CD spectrum is the disappearance of the negative band, which is usually attributed to porphyrin intercalation in the porphyrin−DNA system.[22] Salt-dependent change in the CD spectrum of the TMPyP−poly[d(I−C)$_2$] complex resembles that of the TMPyP−poly[d(A−T)$_2$] complex (Figure 3a). Together with LD^r result (see below), this negative CD band may be attributed to the intercalation binding mode. The positive CD band of the TMPyP−poly[d(I−C)$_2$] and TMPyP−poly[d(A−T)$_2$] complexes at high salt concentration may then be attributed to the outside binding. In the TMPyP−poly[d(I−C)$_2$] complex case, this species cannot be attributed to the groove binding mode because the molecular plane is perpendicular with respect to the DNA helix axis. In contrast, the negative CD band of the TMPyP−poly[d(G−C)$_2$] remains to be negative (Figure 3b), suggesting TMPyP is still intercalated at a high salt concentration (100 mM). Therefore, it is suggestive that the transition between the two intercalative states of the TMPyP−poly[d(G−C)$_2$] complex, observed from absorption and CD spectra, may be attributed to different intercalation sites, namely, CpG and GpC sites, although the CpG site has been known to be a particularly attractive site for intercalation.[33,34] Although there is a small R ratio dependence, overall shape and intensity of both the CD and absorption spectra are similar at an R ratio between 0.01 and 0.05, suggesting that the binding mode of porphyrin at these low binding ratios may be similar at both low and high salt concentration, except for the TMPyP−poly[d(A−T)$_2$] complex. In the TMPyP−poly[d(A−T)$_2$] complex case, a strong mixing ratio dependence in CD spectrum was apparent in the presence of 100 mM NaCl. The shape of CD spectrum resembles that already reported for TMPyP[6] and that of the MnTMPyP−poly[d(A−T)$_2$] complex[10] in similar condition and was attributed either to coexistence of the minor and major groove binding mode or to the binding at the surface of the groove.

The most direct evidence of TMPyP intercalation between the base pairs of poly[d(I−C)$_2$] at these low R ratios and low NaCl concentration is the LD^r result. Upon drug intercalation, the DNA helix is unwound and stiffened, resulting in an increase in orientability of DNA and hence, an increase in the LD^r magnitude in the DNA absorption region.[23,24] The magnitude of LD^r in the drug absorption region is comparable or often larger than that in the DNA absorption region, reflecting that the in-plane electric transition moment of the drug that is usually assigned to a $\pi^* \leftarrow \pi$ transition is perpendicular relative to the

DNA helix axis. A larger LD^r magnitude in the drug absorption region compared to that in the DNA absorption region reflects the tilt and/or bending of the DNA stem upon drug intercalation. These general features are what the LD^r spectrum of the TMPyP−poly[d(I−C)$_2$] complex appears to be. However, the wavelength dependence of the LD^r signal in the drug absorption region requires further explanation. In the Soret band, there are two in-plane perpendicular electric transition moments (*x* and *y* transition). If porphyrin is intercalated in a classic manner, i.e., the plane of the porphyrin molecule remains as a plane, both the in-plane electric transitions must be perpendicular to the DNA helix axis, resulting in a wavelength-independent (constant) LD^r. An observed strong wavelength-dependent LD^r therefore indicates that the plane of the porphyrin molecule is twisted or distorted in the intercalation pocket thereby resulting in a removal of energy degeneracy and wavelength-dependent LD^r in the Soret band. However, this description cannot explain the positive CD signal apparent at a relatively low NaCl concentration. The wavelength-dependent LD^r in the Soret absorption region can also be accounted for by a heterogeneous binding mode: coexistence of groove binding (or outside binding) modes and intercalation may result in a similar LD^r. Because the magnitude of both resolved LD^r signals in the Soret region are larger than that in the DNA absorption region, the molecular plane of TMPyP is perpendicular for both binding modes, in which one associated with negative CD and another positive. These results may omit the possibility of the groove binding of TMPyP in poly[d(I−C)$_2$], in which the edge of porphyrin is inserted along the groove thereby a positive LD^r is expected.

Removing the amine group from the guanine base which locates in the minor groove and provides steric hindrance for an incoming drug results in a similar binding mode for the minor groove binding drugs as was evident in the similar spectral properties of the DAPI(or Hoechst 33258)−poly[d(I−C)$_2$] and DAPI(or Hoechst 33258)−poly[d(A−T)$_2$] complexes. In the TMPyP case, the spectral properties and the salt dependent spectral change of the TMPyP−poly[d(I−C)$_2$] complex at a low binding ratio are very similar to that of the TMPyP−poly[d(A−T)$_2$] complex. However, close analysis of the CD and LD^r spectrum leads us to conclude that intercalative and outside binding modes of TMPyP coexist. This observation may raise a serious question of the minor groove binding mode of TMPyP at the AT rich region. It is also indicative that the amine group of guanine not only provides the steric hindrance for incoming classic minor groove binder but also take an important role in stabilizing the intercalated porphyrin.

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