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Classification of CD and absorption spectra in the Soret band of H_2TMPyP bound to various synthetic polynucleotides

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Abstract

The binding mode of porphyrins, namely *meso*-tetrakis(*N*-methyl pyridinium-4-yl)porphyrin (H_2TMPyP), was classified in this work by absorption and circular dichroism(CD) spectroscopy. The three binding modes of intercalation, minor groove binding and external stacking exhibit their own characteristic absorption and CD spectra. Intercalation occurs for this porphyrin when bound to GC-rich polynucleotides at a low mixing ratio, as expected. This binding mode produces hypochromism and a red shift in the absorption band and a negative CD band in the Soret absorption region. When it is complexed with AT-rich polynucleotides at a low mixing ratio, hypochromism and a red shift in the absorption band and a positive CD peak is apparent, and this species can easily be assigned to the minor groove-binding mode. For both AT- and GC-rich polynucleotides at a high binding ratio, an excitonic CD was apparent. The sign of excitonic CD depends on the order of the DNA bases; the CD spectra of H_2TMPyP complexed with non-alternating homopolymer (disregarding the nature of base pairs, i.e. AT or GC) are characterized by a positive band at short wavelengths followed by a negative band at long wavelengths. In contrast, those complexed with alternating polynucleotide were opposite to those of non-alternating homopolymers.

Keywords: Porphyrin; Circular dichroism; DNA; Synthetic polynucleotides

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1. Introduction

Interactions of water-soluble porphyrins and nucleic acids have been intensively studied since the pioneering work of Fiel and Pasternack and their co-workers [1,2]. The most extensively studied DNA binding porphyrin is *meso*-tetrakis(*N*-methylpyridium-4-yl)porphyrin (referred to as H_2TMPyP, Fig. 1) and its metal complex [3–12].

The binding mode of porphyrins is affected by the nature of the metal ion and the size and location of the substituent groups on the periphery of the porphyrin group [for review [13,14]]. In general, porphyrin free bases and square planar complexes such as those with Ni^{2+} and Cu^{2+} intercalate between the base pairs of DNA, while on those having axially bound ligands such as Mn^{3+}, Fe^{3+} and Co^{3+}, or those with bulky substituents on the periphery of the structure, intercalation is blocked and exhibit an 'outside binding' mode. Extensive NMR, equilibrium dialysis, flow linear dichroism, and viscometry measurements of oligonucleotides and DNA [13,14] have supported the original proposal [1] that porphyrins intercalate into GC-rich regions and that they bind in an outside manner at AT sites. The

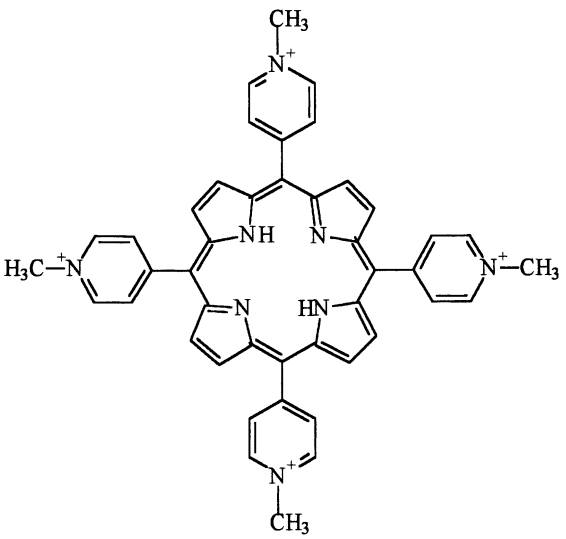

Fig. 1. Molecular structure of H_2TMPyP.

binding geometry of H_2TMPyP–DNA and ZnTMPyP–DNA complexes was determined by linear dichroism technique [15]. The molecular plane of H_2TMPyP, when complexed with DNA, is perpendicular relative to the DNA helix axis while that of ZnTMPyP lies at 62–67°. These structural features are consistent with the results of molecular modelling and energy minimization studies on dinucleotide [16] and oligonucleotide [17] complexes of H_2TMPyP.

More recently, the binding geometry of H_2TMPyP and CoTMPyP with various substituents on the periphery of porphyrin complexed with calf thymus DNA, poly[d(G–C)$_2$] and poly[d(A–T)$_2$] was investigated using polarized spectroscopy and fluorescence energy transfer techniques [18,19]. At a low porphyrin to DNA base ratio, H_2TMPyP was confirmed to intercalate into base pairs of DNA and poly[d(G–C)$_2$], while the angle between the molecular plane and DNA helix axis 40–50° for the H_2TMPyP–poly[d(A–T)$_2$] complex was consistent with groove binding geometry. In both cases, a strong energy transfer was observed from the excited state of DNA to bound porphyrin. Therefore, these porphyrins were concluded to be in close contact with DNA bases. CoTMPyP, on the other hand, did not exhibit any energy transfer and the angle was 45–50° for poly[d(A–T)$_2$] and 30–40° for poly[d(G–C)$_2$].

Circular dichroism studies were also reported for MnTMPyP [20] and *meso*-tetrakis[4-[(3-(trimethylammonio)propyl)-oxy]phenyl] porphyrin [21] complexed with various synthetic polynucleotides. At high porphyrin/DNA base ratios, these porphyrins exhibit an excitonic behaviour in the Soret band. At this binding ratio, an outside binding mode with self-stacking is important and is indistinguishable for porphyrins complexed with poly[d(A–T)$_2$] and poly[d(G–C)$_2$] [21]. These studies imply the importance of the base sequence in inducing the CD spectrum of the achiral porphyrin molecules in the porphyrin–DNA complex. However, a systematic and comparative CD and absorption pattern for metallo- and nonmetalloporphyrins bound to DNAs with a regular base sequence have not been reported. Thus, in

this work, we systematically investigated the CD spectrum of H_2TMPyP bound to poly[d(G–C)$_2$] and poly[d(A–T)$_2$], in which a guanine–cytosine base pair and an adenine–thymine base pair were regularly alternated, and to poly(dG)·poly(dC) and poly(dA)·poly(dT), in which the base pairs are not alternatively arranged. The arrangement of the base pairs of the former two polynucleotides and the latter two are anti-symmetric. The resulting CD spectra were then classified in terms of binding mode, namely intercalation and outside binding.

2. Materials and methods

2.1. Materials

Porphyrins were purchased from Midcentury (Chicago, IL) and used without further purification. All polynucleotides were purchased from Pharmacia and prepared as described elsewhere [18,19], except for poly(dG)·poly(dC) which is not soluble at intermediate pH. Poly(dG)·poly(dC) was dissolved at a high pH (above pH 10.0) and dialyzed five times with 5 mM of cacodylate buffer at pH 7.0 containing 100 mM NaCl and 1 mM EDTA, followed by the described preparation procedure [19]. The concentrations of porphyrin and polynucleotide were determined spectrophotometrically using extinction coefficients of $\varepsilon_{424\ nm} = 2.26 \times 10^5\ M^{-1}\ cm^{-1}$ for H_2TMPyP. Those for polynucleotides were $\varepsilon_{262\ nm} = 6600\ M^{-1}\ cm^{-1}$, $\varepsilon_{260\ nm} = 6000\ M^{-1}\ cm^{-1}$, $\varepsilon_{254\ nm} = 8400\ M^{-1}cm^{-1}$, and $\varepsilon_{253\ nm} = 7400\ M^{-1}\ cm^{-1}$, respectively for poly[d(A–T)$_2$], poly(dA)·poly(dT), poly[d(G–C)$_2$] and poly(dG)·poly(dC) in DNA bases. A 5-mM cacodylate buffer at pH 7.0 was used throughout this work and all measurements were performed at an ambient temperature. Throughout this work, the concentrations of porphyrins were fixed at 5 μM and that of polynucleotides were varied to achieve the desired [porphyrin]/[DNA base] ratio (R ratio). The different porphyrin–DNA solutions were prepared individually by mixing the porphyrin solution and the DNA solutions to obtain the final mixing ratio. The spectroscopic measurements were performed just after mixing. CD spectra were averaged over appropriate number of scans.

2.2. Absorption and circular dichroism spectra

The binding of certain ligands to DNA produces hypochromism, a broadening of the envelope, and a red shift of the ligand absorption band. These effects are particularly pronounced for intercalators; with groove binders, a large wavelength shift usually correlates with a ligand conformational change on binding or ligand–ligand interactions. All absorption spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer.

In general, DNA binding porphyrins do not possess a chiral center and are optically inactive. However, the CD spectrum in the drug absorption region, especially in the Soret band, is induced when it forms a complex with polynucleotides. Although the origin of induced CD of the achiral porphyrin–DNA complex is not clear, it is believed to be induced by the coupling of the transition moments of achiral drug and chirally arranged nucleobase transition or by excitonic interaction of the DNA-bound drug. The shape and magnitude of induced CD depends on the binding mode and location of the drug, and the nature of the nucleobases [18–21]. All CD spectra were measured on a Jasco J-715 spectropolarimeter.

3. Results

CD and absorption spectra were recorded for a fixed porphyrin concentration (5 μM). The different R ratios of 0.00, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1 and 2.1 were obtained by changing the polynucleotide concentration. Only the selective spectra are shown in this section for simplicity.

3.1. Absorption and CD spectra of H_2TMPyP complexed with poly[d(A–T)$_2$] and poly(dA)·poly(dT)

When H_2TMPyP is complexed with poly(dA)·

poly(dT), the CD spectrum at a low mixing ratio (below $R = 0.1$) is characterized by a strong positive band centered at approximately 428 nm (Fig. 2a). As the R ratio increases, a drastic change in the CD spectrum occurs between an R ratio of 0.1 and 0.3. Above an R ratio of 0.1, an excitonic CD with a positive band at approximately 415 nm and a negative band at approximately 435 nm are apparent. The intensity of these bands is largest at an R ratio of 0.3 and decreases upon a further increase in R ratio with an isosbestic point at 425 nm, suggesting that the porphyrin in this system (at a high R ratio) may be explained by only two states, namely excitonic and unbound species. For ease of comparison, a change in the CD intensity at 435 nm with respect to the R ratio is shown in Fig. 2a, insert. The change from monomeric CD to excitonic CD starts at R ratios as low as 0.1, which corresponds to one porphyrin molecule to five DNA base pairs. Above 0.3, a gradual de-

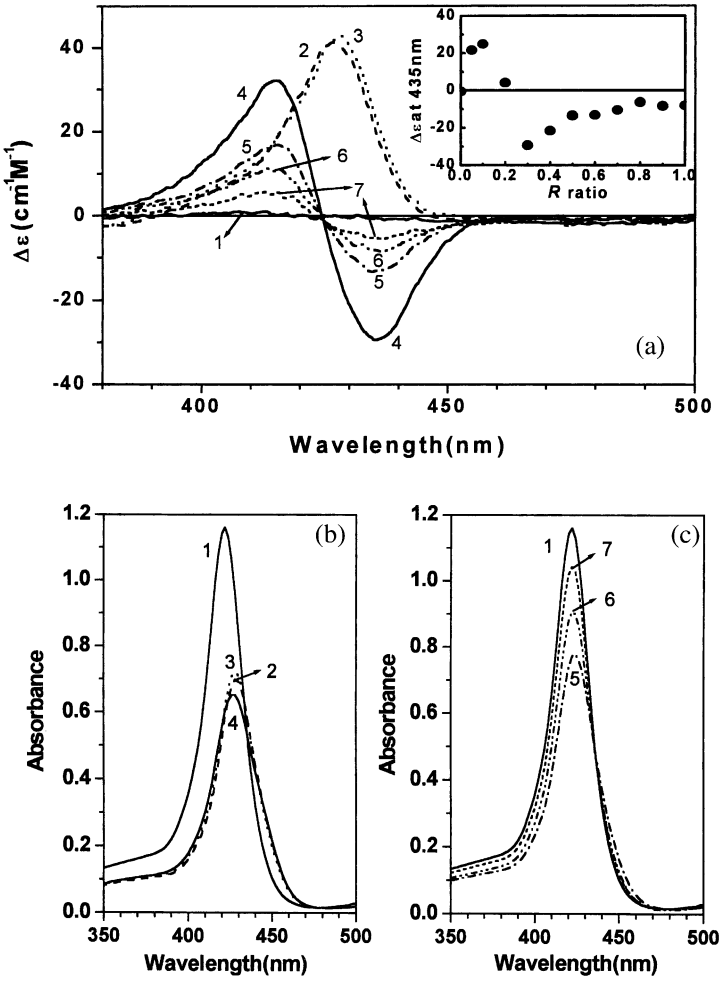

Fig. 2. CD (a) and absorption spectrum of H_2TMPyP at low (b) and high (c) R ratios in the presence of poly(dA)·poly(dT) and changes in CD intensity at 435 nm with respect to R ratio (insertion). [H_2TMPyP] = 5 μM, and the concentrations of polynucleotide were varied to achieve various R ratios. The spectra for only selective R ratios are shown for simplicity reasons (see text). Curve 1: $R = 0.00$; curve 2: $R = 0.05$; curve 3: $R = 0.1$; curve 4: $R = 0.4$; curve 5: $R = 0.6$; curve 6: $R = 1.0$; and curve 7: $R = 2.1$.

crease in CD intensity was observed and it reaches a plateau as the R ratio increases further. The absorption spectrum at a low R ratio, which corresponds to the monomeric positive CD, is characterized by a small red shift (~ 8 nm) and hypochromism ($\sim 38\%$) in the porphyrin's Soret band (Fig. 2b). As the R ratio increases, the shape of the absorption band becomes similar to that of the DNA-free porphyrin (Fig. 2c). An isosbestic point at 436 nm in the absorption spectrum suggests that a change in the absorption spectrum at high R ratios (above 0.3) occurs between two states. However, the absorbance never reaches the intensity of the DNA-free H_2TMPyP even at an extremely high R ratio ($R = 2.1$).

The CD spectrum of the H_2TMPyP–poly[d(A–T)$_2$] complex at various R ratios are depicted in Fig. 3a. In the presence of poly[d(A–T)$_2$], H_2TMPyP exhibits two positive bands centered at approximately 416 nm and approximately 435 nm at a low R ratio (below 0.1), where the band at

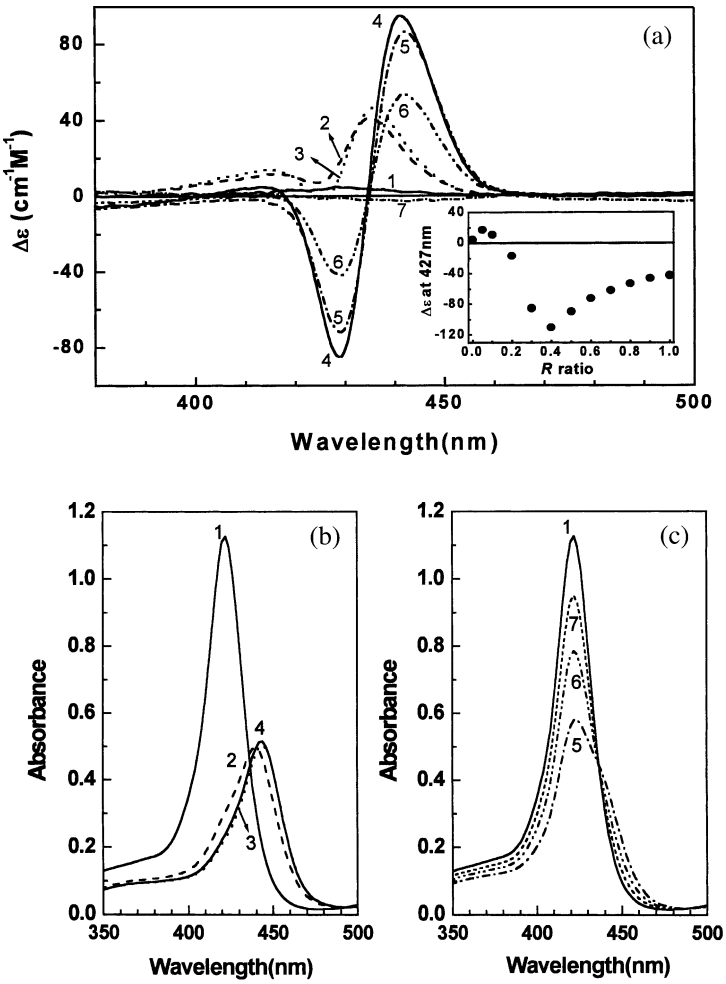

Fig. 3. CD (a) and absorption spectrum of H_2TMPyP at low (b) and high (c) R ratios in the presence of poly[d(A–T)$_2$] and changes in CD intensity at 427 nm with respect to R ratio (insertion). [H_2TMPyP] = 5 μM, and the concentrations of polynucleotide were varied. Curve 1: $R = 0.00$; curve 2: $R = 0.05$; curve 3: $R = 0.1$; curve 4: $R = 0.4$; curve 5: $R = 0.6$; curve 6: $R = 1.0$; and curve 7: $R = 2.1$.

longer wavelength is dominant. The shape of the CD spectrum at these R ratios is similar to that observed for the MnTMPyP–poly[d(A–T)$_2$] complex [20] at low R ratios. As the R ratio increases, a strong excitonic CD with a positive peak at approximately 442 nm and a negative peak at approximately 429 nm are apparent. For the same reason as in the poly(dA)·poly(dT) case, the CD intensity at 427 nm with respect to the R ratio is shown in Fig. 3a, insert. Changes in CD intensity with respect to the R ratio is similar to that in the poly(dA)·poly(dT) complex. Interestingly, the shape of the CD spectrum of the H_2TMPyP–poly[d(A–T)$_2$] complex looks antisymmetric compared to that of the H_2TMPyP–poly(dA)·poly(dT) complex. The hypochromism (54%) and red shift (22 nm) of porphyrin in the Soret band in the presence of poly[d(A–T)$_2$] at a low R ratio is larger than those of the poly(dA)·poly(dT) complex (Fig. 3b). Similar to the poly(dA)·poly(dT) case, the shape of the absorption spectrum of porphyrin starts to resemble that of polynucleotide-free porphyrin with further increases in R ratio (above $R = 0.4$, Fig. 3c). At a high R ratio, an isosbestic wavelength at 436 nm was observed. It is noteworthy that the absorption spectra of both H_2TMPyP–poly[d(A–T)$_2$] and H_2TMPyP–poly(dA)·poly(dT) complexes at a low R ratio lacks any isosbestic wavelength, suggesting that the porphyrin binding mode is heterogeneous even at low ratios.

3.2. Absorption and CD spectra of H_2TMPyP complexed with poly[d(G–C)$_2$] and poly(dG)·poly(dC)

Fig. 4a shows the CD spectra of porphyrin bound to poly(dG)·poly(dC), in which a guanine base occupies one strand and cytosine the other. At a low R ratio, the CD spectrum of H_2TMPyP–poly(dG)·poly(dC) is characterized by a negative band centered at approximately 440 nm. This CD spectrum remained the same until the R ratio reached 0.1. Then a heterogeneous, excitonic behavior starts to appear. Excitonic CD of this complex exhibits a positive band at approximately 430 nm and a negative band at approximately 448 nm with an isosbestic wavelength at 438 nm that starts to appear at an R ratio of 0.3–0.4. The lack of isosbestic wavelength between an R ratio of 0.1–0.3 is indictable for the heterogeneous binding of porphyrin. Overall, the magnitude of CD is comparable to that of the poly[d(A–T)$_2$] complex and larger than that of poly(dA)·poly(dT). The change in CD intensity at 449 nm with respect to the R ratio is shown in Fig. 4a. Here again, a drastic change from monomeric CD to excitonic CD occurs between an R ratio of 0.1 and 0.3. Above an R ratio of 0.4, the CD intensity gradually decreases. The change in the absorption spectrum is similar to that of the H_2TMPyP–poly[d(A–T)$_2$] with a maximum hypochromism of 58%, and a 20-nm red shift. An isosbestic wavelength at 438 nm was observed for an R ratio higher than 0.4. A further increase in R ratio results in an absorption spectrum that resembles that of DNA-free porphyrin.

Fig. 5a shows the CD spectrum of H_2TMPyP bound to poly[d(G–C)$_2$]. The complex exhibits a negative band centered near 437 nm at a low R ratio. As the R ratio was increased, an excitonic CD started to appear with its positive peak at approximately 446 nm and negative peak at approximately 429 nm. The shape and intensity of CD spectrum at a low R ratio is similar to that of the H_2TMPyP–poly(dG)·poly(dC) complex. As the R ratio increased, an excitonic CD with an opposite sign compared to poly(dG)·poly(dC) complex appeared. This point is similar to that of AT-rich polynucleotides, in which the alternating and non-alternating A–T polynucleotide induces the opposite CD signal in the Soret band (Fig. 2a and Fig. 3a). A change in CD intensity at 447 nm (Fig. 5a, insert) with increasing R ratio is similar to those of other complexes (drastic change from monomer to exciton between an R ratio of 0.1–0.3, followed by a gradual decrease). The absorption spectrum of the H_2TMPyP–poly[d(G–C)$_2$] complex is depicted in Fig. 5b,c. Again, a strong red shift (22 nm) and hypochromism (49%) was apparent at a low R ratio compared to its DNA-free porphyrin. However, as the mixing ratio increased above ~ 0.4, the absorption spectrum changed to that corresponding to the porphyrin–poly[d(A–T)$_2$]

complex at a high R ratio. At an R ratio of 0.3, a shoulder at the short wavelength starts to become apparent.

3.3. Dependence of CD in the porphyrin's Soret band on the DNA base pairs

Although it is already mentioned in the previous sections, it is worthy to classify the CD according to the nature of polynucleotides. At low R ratios, the CD spectrum of H_2TMPyP in the Soret band is positive when bound to A–T rich polynucleotides, while it is negative for a G–C pair: the intensity of those complexed with G–C polynucleotides is smaller than those bound to A–T. At high R ratios, a positive CD band at short wavelengths and a negative at long wave-

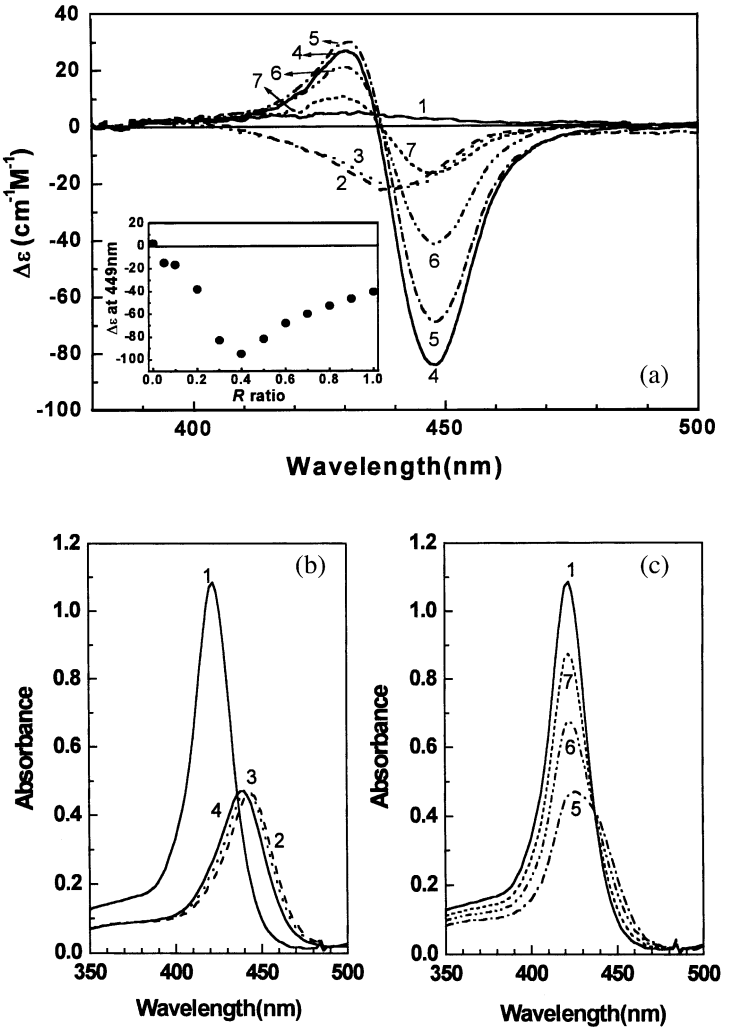

Fig. 4. CD (a) and absorption spectrum of H_2TMPyP at low (b) and high (c) R ratios in the presence of poly(dG)·poly(dC) and changes in CD intensity at 435 nm with respect to R ratio (insertion). [H_2TMPyP] = 5 μM, and the concentrations of polynucleotide were varied. Curve 1: $R = 0.00$; curve 2: $R = 0.05$; curve 3: $R = 0.1$; curve 4: $R = 0.4$; curve 5: $R = 0.6$; curve 6: $R = 1.0$; and curve 7: $R = 2.1$.

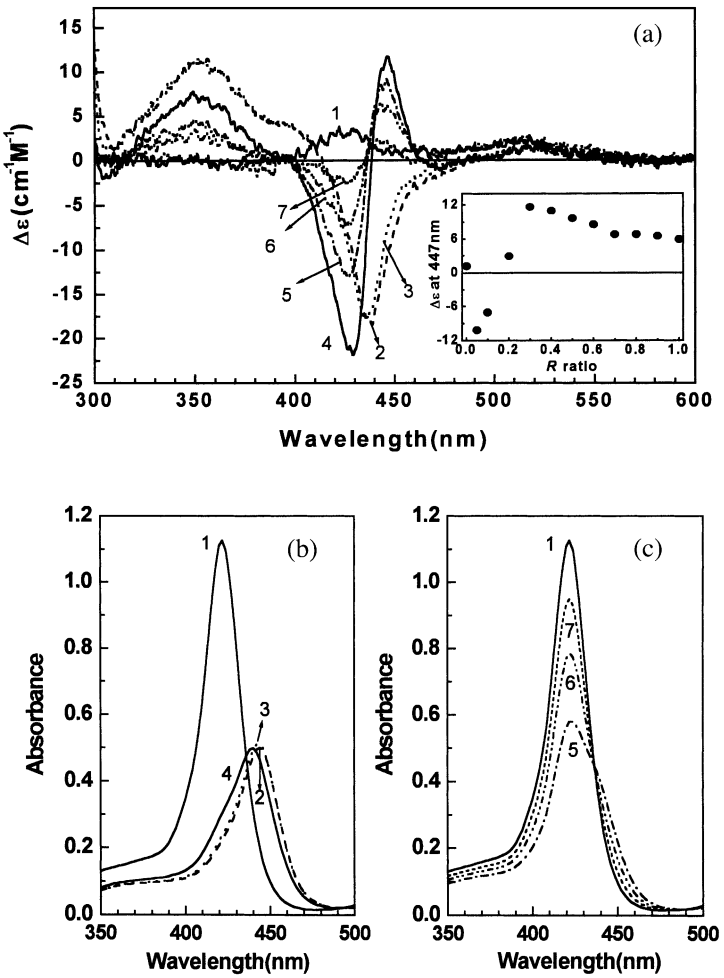

Fig. 5. CD (a) and absorption spectrum of H_2TMPyP at low (b) and high (c) R ratios in the presence of poly[d(G–C)$_2$] and changes in CD intensity at 435 nm with respect to R ratio (insert). [H_2TMPyP] = 5 μM, and the concentrations of polynucleotide were varied. Curve 1: $R = 0.00$; curve 2: $R = 0.05$; curve 3: $R = 0.1$; curve 4: $R = 0.4$; curve 5: $R = 0.6$; curve 6: $R = 1.0$; and curve 7: $R = 2.1$.

lengths are apparent for both AT and GC rich non-alternating polynucleotides, i.e. purine on one strand and pyrimidine on the other. In contrast, a negative CD band at short wavelengths and a positive CD band at long wavelengths was observed for alternating AT and GC pairs, where purine and pyrimidine were alternatively attached to one strand. Hence, the signs of excitonic CD depend on the arrangement of the DNA bases, not on the nature of them. These observations are summarized in Table 1.

4. Discussion

4.1. Binding mode and spectral properties at low R ratios (R < 0.1)

The absorption spectrum of H_2TMPyP when complexed with synthetic polynucleotide can be classified in two species. At a low binding ratio, the absorption spectrum of H_2TMPyP bound to poly[d(A–T)$_2$], poly[d(G–C)$_2$] and poly(dG)·poly(dC) exhibits strong hypochromism and a

Table 1
CD signal of porphyrin at the Soret absorption region in the presence of various polynucleotides

Polynucleotide	Low R ratio (below ~ 0.1)	High R ratio (at 0.4)
Poly(dA)·poly(dT)	+ (428 nm)	+ (415 nm) and − (435 nm)
Poly[d(A−T)$_2$]	+ and + (416 nm, 435 nm)	− (429 nm) and + (442 nm)
Poly(dG)·poly(dC)	− (440 nm)	+ (430 nm) and − (448 nm)
Poly[d(G−C)$_2$]	− (437 nm)	− (429 nm) and + (446 nm)

red-shift. The H_2TMPyP−poly(dA)·poly(dT) complex is an exception: red-shift and hypochromism of this complex is less pronounced. A positive (alternating or homo-AT polynucleotide) or negative (alternating or homo-GC polynucleotide) induced CD band in the Soret region accompanies this species. This observation agrees with the previous report for the H_2TMPyP complexed with poly[d(A−T)$_2$] and poly[d(G−C)$_2$] at an extremely low binding ratio [19]. The angle of 45–50° between in-plane transition moments of porphyrin and the polynucleotide helix were reported for the H_2TMPyP−poly[d(A−T)$_2$] complex in the same report. This angle is consistent with that of well known minor groove binding drugs such as 4′,6-diamidino-2-phenylindole [22,23] and Hoechst [24]. On the other hand, the molecular plane of porphyrin is almost parallel to the plane of the nucleo-base in the H_2TMPyP−poly[d(G−C)$_2$] complex, supporting an intercalation binding mode. In addition to the LD measurements, there is abundant evidence for these binding geometries, including NMR, equilibrium dialysis, viscometric titration and optical spectroscopy [13,14]. The sign of the CD signal, therefore, may be caused either by different binding geometries of porphyrin or a difference in the nature of the DNA bases. At this point, no evidence can be supplied to support either of the reasons.

The CD spectrum, which is similar to the H_2TMPyP−poly[d(A−T)$_2$] complex at low R ratios in this work was reported for MnTMPyP bound to polynucleotide [20]. When MnTMPyP was bound to poly[d(A−T)$_2$], two positive CD bands in the Soret absorption region were revealed in a wide R ratio range, while one positive CD band was apparent for the MnTMPyP−poly[d(G−C)$_2$] complex. The CD spectrum of the MnTMPyP−poly[d(I−C)$_2$] complex was similar to that of the MnTMPyP−poly[d(A−T)$_2$] complex. Since inosine is identical to guanine, except that it lacks the 2-amino group which prevents the minor groove binding of MnTMPyP, this porphyrin was concluded to bind to the major groove of poly[d(G−C)$_2$] and to the minor groove of poly[d(A−T)$_2$] and poly[d(I−C)$_2$]. This point was supported by the disappearance of one of two CD peaks observed for the MnTMPyP-poly[d(A−T)$_2$] complex in the presence of the minor groove binding drug berenil and distamycin; since the minor groove of poly[d(A−T)$_2$] is blocked, porphyrin has to bind to the major groove, resulting in one CD band [20].

4.2. Binding mode and spectral properties at an intermediate R ratio ($R \cong 0.4$)

The second absorbing porphyrin species, which is characterized by no shift in absorption maximum and smaller absorbance at a high R ratio was noted. An excitonic CD accompanied all of these complexes. A negative CD signal at short wavelengths was followed by a positive CD band at long wavelengths for the H_2TMPyP−poly[d(G−C)$_2$] and H_2TMPyP−poly[d(A−T)$_2$] complex. The sign of an exciton-like CD signal is opposite when H_2TMPyP is complexed with homopolynucleotides. It is, therefore, conclusive that the sign of the excitonic CD in the Soret band of H_2TMPyP is governed by the arrangement of DNA bases. A similar excitonic behavior for the TθOPP−poly[d(A−T)$_2$] and TθOPP−poly[d(G−C)$_2$] complex was reported (where TθOPP denotes *meso*-tetrakis[4-[(3-trimethylammonio)-propyl]-oxy]phenyl)porphyrin) [21]. In contrast to

this study, excitonic CD appeared even at a very low R ratio ($R = 0.01$). This discrepancy can be described by the inhibition of the minor groove binding or intercalation of TθOPP by long and bulky trimethylammonio side chains; H_2TMPyP, which does not have bulky side chain, is able to squeeze in to the minor groove or intercalation pocket until the population of porphyrin becomes large enough to form an exciton. Based on the excitonic CD observed for TθOPP–poly[d(A–T)$_2$] and TθOPP–poly[d(G–C)$_2$] complexes, extensively stacked and moderated stacked binding modes were proposed [21]. Although this binding mode of TθOPP seems to be generally accepted, this model can not explain how porphyrin, which is stacked outside of DNA, can recognize the alternating and non-alternating arrangement of DNA bases which was observed in this work.

4.3. Dependence of spectral properties on R ratio

A drastic change from monomeric to excitonic behavior is observed for all complexes between $R = 0.1$ and 0.4. In this range, monomeric and excitonic porphyrin, and DNA-unbound porphyrin may co-exist. An increase in excitonic porphyrin accompanies the disappearance of the monomeric porphyrin, which is assigned either to the minor groove binding mode (to AT-rich polynucleotide) or intercalation (to GC-rich polynucleotide). As the population of the porphyrin increases, stacking interaction between porphyrin becomes dominant and this interaction may be strong enough to change the binding mode of the porphyrin that is bound to the groove or intercalation. Above an R ratio of 0.4, the CD signal starts to collapse while the absorption spectrum remained at an equilibrium between the DNA-free and stacked porphyrin. The linear dichroism spectra of the complexes in these R ratios appear to collapse (data not shown), indicating that the ability of orientation of polynucleotide diminished. A complete loss of orientation upon an increasing porphyrin population is not surprising because the repulsion between the phosphate group would be reduced by the electrostatic interaction between positively charged porphyrin and a negatively charged phosphate group. The decrease in the intensity of excitonic CD may be related to this collapsing of DNA. Pasternack et al. observed a very strong exciton CD for H_2TMPyP and its Cu(II) complex when bound to DNA at a high ionic strength (0.10–0.17 M) and at a mixing ratio of 0.125 [25]. The appearance of exciton CD was attributed to the association of porphyrins in the solution prior to the binding to DNA. This observation does not agree with our results in the sense that strong exciton CD was observable even at a high ionic strength, at which DNA is possibly collapsed. However, the ionic strength and DNA are different for these two works and, hence, these results cannot be directly compared.

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