THE DNA–PORPHYRIN INTERACTIONS STUDIED BY VIBRATIONAL AND ELECTRONIC CIRCULAR DICHROISM SPECTROSCOPY

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The interactions of three different porphyrins, without axial ligands – 5,10,15,20-tetrakis-(1-methylpyridinium-4-yl)porphyrin-Cu(II) tetrachloride (Cu(II)TMPyP), with axial ligands – 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin-Fe(III) pentachloride (Fe(III)TMPyP), and with bulky meso substituents – 5,10,15,20-tetrakis(*N*,*N*,*N*-trimethylanilinium-4-yl)-porphyrin tetrachloride (TMAP), with calf thymus DNA were studied by combination of vibrational circular dichroism (VCD) and electronic circular dichroism (ECD) spectroscopy, and by IR and UV-VIS absorption spectroscopy. It has been shown that Cu(II)TMPyP prefers the intercalative binding mode with DNA in the GC-rich regions and the intercalative sites are saturated at the *c*(DNA)/*c*(Cu(II)TMPyP) ratio ~3:1, where *c*(DNA) and *c*(Cu(II)TMPyP) are total molar concentrations of nucleic acid in base pairs and porphyrin, respectively. Fe(III)TMPyP does not intercalate between the GC base pairs but binds to DNA in the minor groove. At higher *c*(DNA)/*c*(TMAP) ratios, TMAP interacts with DNA in the minor groove, but at lower ratios in the major groove and by the external binding mode accompanied by self-stacking of porphyrins along the phosphate backbone. VCD spectroscopy reliably discriminates the binding modes and specifies the conformational changes of the DNA matrices. It has been also shown that VCD spectroscopy is an effective tool for the conformational studies of DNA–porphyrin complexes. New spectroscopic "markers" in VCD spectra have been found for the specific DNA–porphyrin interactions.

Keywords: Vibrational circular dichroism spectroscopy; Electronic circular dichroism spectroscopy; DNA–porphyrin interactions; CD; VCD; Porphyrins; Intercalation.

Complexes of DNA or polynucleotides with water-soluble cationic porphyrins and their metal derivatives[1,2] have been widely studied by different spectroscopic methods involving UV-VIS absorption, circular dichroism (CD), NMR, Raman scattering, or by X-ray crystallography[3–14]. Three major binding modes of porphyrins with DNA were identified: (i) intercalation

between the GC base pairs, (ii) external minor or major groove binding, and (iii) external binding to the sugar-phosphate backbone.

Characterization of the cationic porphyrins–DNA interactions using electronic circular dichroism (ECD) spectroscopy is summarized in Pasternack's review[2]. There are two spectral regions where the porphyrin–DNA interactions are observed: 230–300 nm, where the π–π* transitions of the nitrogen bases occur and, therefore, the conformational changes of DNA can be observed, and 300–500 nm (Soret spectral region), where electronic absorption and the induced CD signal of the porphyrin part of complex are observed. The intercalative binding mode is characterized by a large red shift (≥15 nm) and substantial hypochromicity (≥30%) in the Soret maximum and by negative induced CD band in the Soret spectral region. On the other hand, the external binding mode is characterized by a small red shift (≤8 nm) and limited hypochromicity (≤10%) or even hyperchromicity of the Soret band and by positive induced CD band in the Soret spectral region[11,13].

Vibrational circular dichroism (VCD)[15–20] is an analog of electronic circular dichroism in the infrared spectral region. VCD spectroscopy has been used to determine the absolute configuration of small or medium-size molecules[15,16,21,22], in semiempirical studies of proteins and peptides[23,24], and DNA and oligonucleotides[25–30]. As for DNA, the characteristic absorption bands and VCD signals assigned to C=O and C=N stretching vibrations of DNA nitrogen bases are observed at 1750–1550 cm^{-1}. In this spectral region, the π–π and ionic interactions of porphyrins with DNA are expected to be demonstrated.

Cu(II) and Fe(III) derivatives of 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetrachloride (Cu(II)TMPyP and Fe(III)TMPyP) and 5,10,15,20-tetrakis(*N*,*N*,*N*-trimethylanilinium-4-yl)porphyrin tetrachloride (TMAP) were chosen for this study because they interact with DNA in different manner.

Cu(II)TMPyP, a porphyrin with non-axial ligands, is capable of intercalation favored in the GC-rich regions of DNA[12]. The spectral "marker" of this type of interaction is a negative induced ECD signal in the Soret spectral region[12]. Due to the clashing between the DNA backbone and the porphyrin pyridine groups, the interior of the complex is destacked and cytosine is flipped out of the helical stacking[8].

Fe(III)TMPyP is a porphyrin with axial ligands, which does not intercalate into DNA due to steric demands imposed by the axial ligands and tends to form complexes with DNA by electrostatic forces[4], by the so-called external minor groove binding mostly in AT regions of DNA. The spectral "marker"

of this mode, which was proved by measurements of Fe(III)TMPyP–poly(dA-dT)$_2$ complexes, is a positive ECD signal in the Soret spectral region[11], but the equilibrium is complicated by several porphyrin species that co-exist in the solution[31,32].

Due to the steric hindrance, TMAP possesses the minor groove binding mode to DNA[5] and external interactions accompanied by self-stacking of TMAP along the sugar-phosphate backbone[12]. This type of interaction provides the bisigate conservative ECD signal. TMAP affects DNA structure due to strong interactions of trimethyl groups and deoxyribose leading to a small increase in the major groove width[5].

The aim of this work has been the determination of the VCD response to the different binding modes that were identified using ECD and other spectroscopic techniques. VCD spectroscopy as a key technique used in this work combines the intrinsic sensitivity of circular dichroism techniques to the spatial structure with the assignment of individual signals to the characteristic vibrations. This new approach in testing DNA–porphyrin interaction enables to specify which characteristic vibrations of DNA are influenced by porphyrin interaction and which binding mode causes variations in the DNA spatial structure. The new VCD "markers" for the specific type of interaction enable to follow how the *c*(DNA)/*c*(porphyrin) ratios influence the binding modes.

EXPERIMENTAL

The sodium salt of calf thymus DNA, highly polymerized, type I, and sodium cacodylate trihydrate were purchased from Sigma. 5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)-porphyrin-Fe(III) pentachloride was obtained from Porphyrin Systems GbR, Germany, and 5,10,15,20-tetrakis(*N*,*N*,*N*-trimethylanilinium-4-yl)porphyrin tetrachloride from Frontier Scientific. 5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)porphyrin-Cu(II) tetrachloride was prepared from 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetratosylate salt (Porphyrin Systems GbR, Germany) according to published procedures[33]. The structure of all the porphyrins used is given in Scheme 1.

All the solutions were measured in a cacodylate buffer (0.02 mol l^{-1}, pH 7) and 10^{-1} M NaCl in D_2O or H_2O. The highly polymerized DNA was sonicated to decrease its viscosity. The final average DNA length was ~800 base pairs, as determined by gel electrophoresis.

The total molar concentration of DNA used for all IR and VCD measurements was 20 mg per ml (30 mmol l^{-1} per base pair). The ratios *c*(DNA)/*c*(porphyrin) were in the range 2:1–10:1, where *c*(porphyrin) and *c*(DNA) are the total molar concentrations of porphyrin and DNA per base pair, respectively. The lowest *c*(DNA)/*c*(porphyrin) ratios used were limited by the viscosity of the complexes, as the addition of porphyrins to the DNA solution increases its viscosity. The VCD spectra were measured in the 1750–1550 cm^{-1} spectral region in a demountable cell consisting of CaF_2 windows separated with a 50 μm Teflon spacer. The DNA–porphyrin solution was thoroughly mixed for ca. 45 min. The IR and VCD spectra were col-

lected with a Bruker IFS66 FTIR VCD spectrometer at 8 cm^{-1} spectral resolution, as described previously[34]. The total number of scans was 33 120 (180-min scan time).

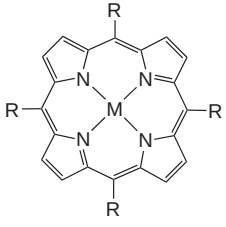
The total molar concentration of DNA used for all absorption and ECD measurements was 100 μmol l^{-1} in the UV range. Total molar concentrations of Cu(II) and Fe(III) derivatives of TMPyP and TMAP were 15 and 8 μmol l^{-1} in the visible spectral range, respectively. The absorption and ECD spectra of aqueous solutions were measured with a JASCO J-810 spectrophotometer in the 230–500 nm spectral region, using a 1-cm quartz cuvette.

RESULTS AND DISCUSSION

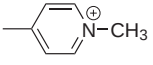
UV-VIS Absorption and ECD Study

Figure 1 shows the UV-VIS absorption and ECD spectra of the DNA–Cu(II)TMPyP (A), Fe(III)TMPyP (B), and TMAP (C) complexes at different ratios, demonstrating clearly the different binding modes achieved for different porphyrins used.

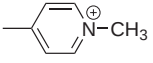
Significant hypochromicity and red shifts of 3 and 6 nm in the Soret band for the 2:1 and 3:1–10:1 ratios, respectively, for DNA–Cu(II)TMPyP complexes versus neat Cu(II)TMPyP prove that interaction took place[13]. Neat Cu(II)TMPyP provides a nonspecific noisy ECD signal in the Soret region. The significant negative ECD band at 426(–) nm, characteristic for the intercalation binding mode[13] observed for the DNA–Cu(II)TMPyP complexes, reaches its maximum intensity for the 3:1 ratio. This observation suggests that the intercalation sites are saturated at this ratio. This hypothesis is also supported by the UV-ECD spectrum measured at the 3:1 ratio (Fig. 1, part A), which resembles the spectrum of poly(dA-dT)$_2$ (refs[35,36]), indicating that mainly the GC part of DNA was substantially disturbed.



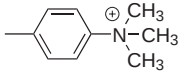
Cu(II)TMPyP: M = Cu

R = 

Fe(III)TMPyP: M = Fe

R = 

TMAP: M = 2 H

R = 

SCHEME 1
Cationic parts of Cu(II)TMPyP, Fe(III)TMPyP, and TMAP

The lower intensity of the ECD band at the 2:1 ratio can be explained by Cu(II)TMPyP–Cu(II)TMPyP self-interaction that prevents intercalation, or by an interaction between Cu(II)TMPyP and DNA different from intercalation. Except for negative ECD band that is characteristic of intercalation, the weak positive features indicate that an external minor groove binding occurred[2].

The UV spectral changes of DNA after complexation with Cu(II)TMPyP are documented in the inset to Fig. 1, part A. The UV absorption band increased its intensity going from neat DNA to the 2:1 ratio, suggesting a distortion of DNA caused by a higher loading of Cu(II)TMPyP, which is also demonstrated in UV-ECD by the positive band splitting and the decrease of the entire ECD spectrum intensity.

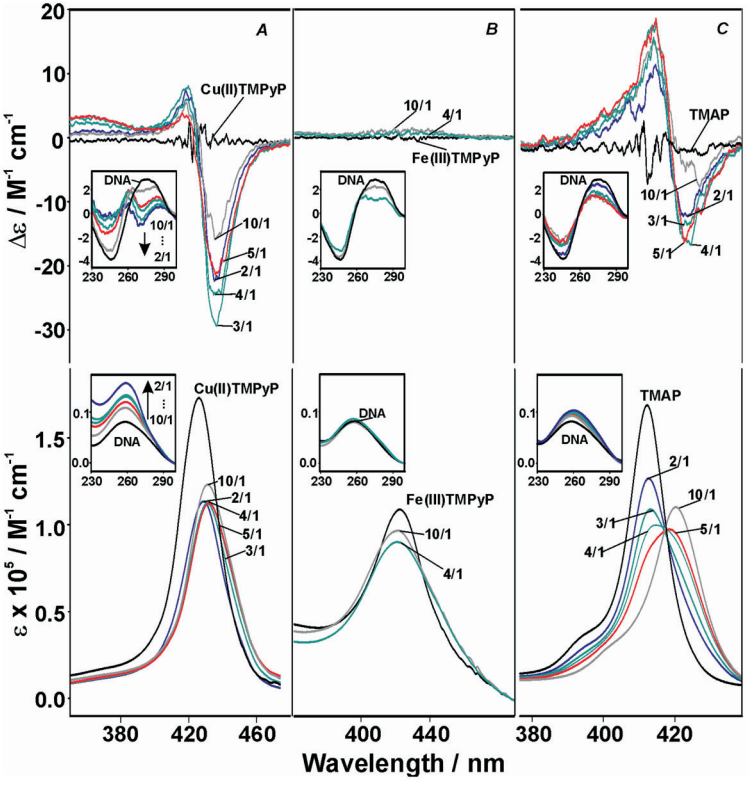


FIG. 1

Visible absorption (bottom) and ECD (top) spectra of DNA–Cu(II)TMPyP (A), DNA–Fe(III)TMPyP (B), and DNA–TMAP (C) complexes in the Soret spectral region at different *c*(DNA)/*c*(porphyrin) concentration ratios. UV spectra given as insets

Figure 1, part B shows the electronic absorption and ECD spectra of the DNA–Fe(III)TMPyP complexes in the visible spectral region. The DNA–Fe(III)TMPyP complexes are characterized by hypochromicity and a small blue shift of ~2 nm typical of derivatives of porphyrin with axial ligands[11,13]. Neat Fe(III)TMPyP, as well as its DNA complexes, provided only a weak nonspecific signal in the Soret spectral region. These spectral observations are typical of the external minor groove binding mode of porphyrin[2,13]. Although a splitting of the positive band in the UV spectral region occurred in the ECD spectrum of the DNA–Fe(III)TMPyP complex at 4:1, the two new bands were not as separated as for the DNA–TMPyP (ref.[28]) or DNA–Cu(II)TMPyP complexes at the same ratio (cf. insets in Fig. 1, parts A and B).

The *c*(DNA)/*c*(TMAP) ratio plays a significant role in the binding mode of TMAP to DNA (Fig. 1, part C). The gradual hypochromicity and red shift are observed after the addition of DNA. The decrease of the shoulder at 394 nm that is characteristic of stacking of porphyrins, is also observed on the DNA addition. For the 2:1–5:1 ratios, the ECD spectra exhibit a conservative couplet. The observation of this couplet is caused by exciton coupling, which suggests stacking interactions of porphyrins along the phosphate backbone[12]. In contrast to a distinct ECD signal, the absorption bands were not distinctly red-shifted for 2:1–4:1 ratios. At a higher value of the DNA loading, i.e. at 10:1 ratio, the absorption spectrum is 8 nm red-shifted compared to neat TMAP. In this case, the conservative ECD couplet is replaced by strong positive and weak negative ECD bands in the Soret region. This phenomenon is probably caused by the external minor groove binding of TMAP. It is notable that the decay of the minor groove binding at lower DNA loading is probably due to a stacking interaction of porphyrins along the DNA backbone which competes with minor grove binding in this case.

In the UV spectral region, neither electronic absorption nor ECD spectra of DNA–TMAP complexes significantly changed their shape or band position, and only minor variations of intensity were observed (Fig. 1, part C, inset). From this fact it follows that a high concentration of TMAP accompanied by stacking interaction of porphyrins disturbs the DNA structure less than external minor groove binding or intercalation (cf. insets in Fig. 1, parts A, B and C).

The absorption and ECD spectral changes in the UV region observed for all the studied complexes suggest that the DNA structure was affected by addition of porphyrins. A detailed study of these structural variations caused by the DNA–porphyrin interactions was performed using VCD.

IR Absorption and VCD Spectral Study

Figure 2 shows the IR (bottom) and VCD (top) spectra of DNA–Cu(II)TMPyP (A), DNA–Fe(III)TMPyP (B), and DNA–TMAP (C) complexes at different ratios of the components. IR and VCD spectra of neat DNA, common to all complexes, are shown in Fig. 2, part B only. The assignment of the IR and VCD bands[25,37] is summarized in Table I.

The IR and VCD spectra of the DNA–Cu(II)TMPyP complexes are shown in Fig. 2, part A. The intensity of the IR band at 1642–1646 cm^{-1} increased in parallel to the increasing concentration of the porphyrin. This band corresponds to the pyridinium C=N stretching vibrations[38], its increase reflecting only the increasing concentration of Cu(II)TMPyP. It does not provide the VCD signal and is not essential for a conformational study. The other absorption bands are specific to definite DNA vibrations. The gradual rela-

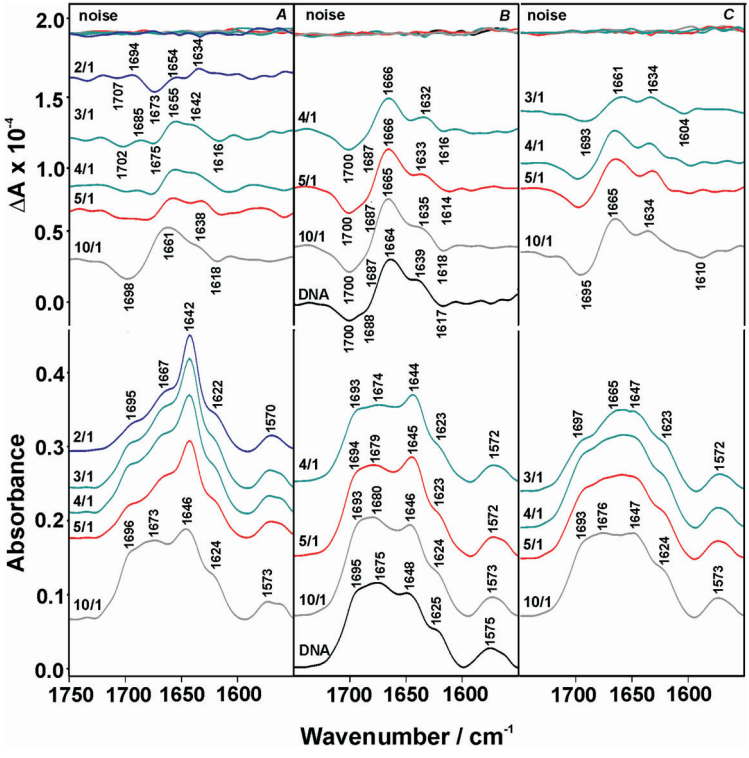


FIG. 2
IR (bottom) and VCD (top) spectra of DNA–Cu(II)TMPyP (A), DNA–Fe(III)TMPyP (B), and DNA–TMAP (C) complexes at different *c*(DNA)/*c*(porphyrin) concentration ratios

tive decrease in the intensity of the absorption band at 1695–1696 cm^{-1}, assigned to the C2=O thymine stretching vibrations, and the shift of the band from 1675 to 1667 cm^{-1}, corresponding to C6=O guanine coupled with C2=O cytosine and C4=O thymine vibrational stretching, with the increase of the porphyrin load, suggest that DNA was influenced by the addition of Cu(II)TMPyP, particularly in the GC region. The IR absorption spectra for the 5:1–2:1 ratios, i.e. at a higher porphyrin load, look similar to the IR spectrum of the B-form of poly(dA-dT)$_2$ (ref.[29]).

More pronounced changes were observed in the VCD spectra. In the VCD spectrum of the DNA–Cu(II)TMPyP complex at the 10:1 ratio, the couplet 1688(–)/1663(+) cm^{-1}, assigned to C6=O guanine and C2=O cytosine, is slightly disturbed compared to the spectrum of neat DNA shown in Fig. 2, part B. Distinct changes occurred in the spectra for 5:1–3:1 ratios. The VCD couplet, originally at 1688(–)/1664(+) cm^{-1}, which represents GC base pairs, almost disappeared. The band pattern between 1690 and 1700(–) cm^{-1} became less intense. We have interpreted the similar phenomenon previously[27] as an increase of the positive VCD signal at 1690(+) cm^{-1}, which is characteristic of interacalation between GC base pairs. On the contrary, the

TABLE I
IR and VCD spectral bands of DNA and DNA–porphyrin complexes

IR, cm^{-1}	VCD, cm^{-1}	Assignment of vibration stretchings of DNA[a]
1695	1700(–)	C2=O thymine
1675	1688(–)/1664(+)	C6=O guanine coupled with C2=O cytosine and C4=O thymine
1648	1639(+)	C2=O cytosine and C4=O thymine coupled with C6=O guanine
1625	1617(–)	C=C ring of adenine
1575		C=N ring of adenine and guanine

IR, cm^{-1}	VCD, cm^{-1}	Binding mode of the DNA–porphyrin complexes
	1690(+)	GC region, intercalative binding mode[b]
	1639(+) shifted to 1632(+) intensity decrease	AT region, minor groove binding
1665	1661(+) intensity decrease	external major groove binding

[a] Refs[25,35]. [b] Ref.[27]

VCD bands at 1639(+) and 1617(–) cm^{-1}, which represent AT base pairs, did not change their intensity and position. In addition, the VCD spectrum for the 3:1 ratio is close to that of the B-form of poly(dA-dT)$_2$ (ref.[29]), suggesting that all intercalative sites are saturated at this ratio. From these facts it follows that Cu(II)TMPyP interacts preferentially with GC base pairs. The minor groove binding to the AT reach regions, suggested from the existence of the positive ECD signal in the Soret region, does not disturb the DNA part of the complex.

For the 2:1 ratio, the VCD spectrum, especially the pattern characteristic of the vibrations in the CG region, is highly disturbed, suggesting that the DNA structure is highly disordered due to the presence of Cu(II)TMPyP at high concentrations. These results are consistent with the results of the ECD study where the UV-ECD characteristic of the DNA is highly disturbed (Fig. 1, part A, inset).

Figure 2, part B, shows the IR and VCD spectra of neat DNA and the DNA–Fe(III)TMPyP complexes at different ratios. The variations of IR absorption spectra in the presence of Fe(III)TMPyP are quite limited, as no significant changes were observed except for the pyridinium stretching vibration at ~1646 cm^{-1} reflecting the increasing concentration of Fe(III)TMPyP. Contrary to the spectrum of the copper derivative, distinct changes were not observed in VCD. The VCD band at 1700(–) cm^{-1} and the couplet at 1687(–)/1664(+) cm^{-1}, characteristic of GC base pairs, did not change significantly its magnitude or position. Hence, the interaction of Fe(III)TMPyP did not affect the GC base pairs. A more distinct change was observed for the band at 1639(+) cm^{-1}. The intensity of this band decreased with the increasing concentration of Fe(III)TMPyP and shifted from 1639(+) to 1632(+) cm^{-1}. At the 4:1 ratio, the band intensity decreased to the half of the original value for neat DNA, suggesting that the AT base pairs were affected by the interaction with Fe(III)TMPyP binding to DNA in the minor groove. The presence of the band at 1632(+) cm^{-1}, characteristic of AT base-pair stacking, suggests that the interacting sites are not saturated at the 4:1 ratio. Some parts of the AT regions of DNA remained unchanged since the VCD spectrum differs from that of the B-form of poly(dG-dC)$_2$ (ref.[39]). The DNA–Fe(III)TMPyP solution became very viscous and DNA aggregated at high Fe(III)TMPyP loads. Therefore, we were not able to measure spectra for lower ratios.

Figure 2, part C, shows the IR and VCD spectra of the DNA–TMAP complexes at different ratios. The intensity of the IR absorption band at ~1695 cm^{-1} decreased with increasing concentration of TMAP. The band at ~1675 cm^{-1} did not change up to the 4:1 ratio. It shifted to 1665 cm^{-1} with-

out any change of its intensity for the 3:1 ratio. We interpret these observations as a consequence of conformational changes of GC base pairs. The same phenomenon was observed in the spectra of DNA–Cu(II)TMPyP complexes (Fig. 2, part A). Because of the pyridinium absence in TMAP, the absorption spectra of DNA–TMAP complexes are not distorted by the absorption peak that grows in parallel with the porphyrin load. The IR spectrum for the 3:1 ratio is similar to that of the B-form of poly(dA-dT)$_2$ (ref.[29]), suggesting that the GC base pairs are affected by the addition of TMAP.

In the VCD spectra, the couplet at 1688(–)/1664(+) cm^{-1} does not change its intensity for the 10:1–4:1 ratios, indicating that the GC base pairs are not significantly affected by TMAP. The decrease in the intensity and the band shift from 1639(+) to 1634(+) cm^{-1} confirm that the external minor groove binding took place (cf. the Fe(III)TMPyP–DNA study above). The shape of the spectra at the 10:1–4:1 ratios is very similar to that of the VCD spectra of the DNA–Fe(III)TMPyP complexes (cf. Fig. 2, part B). Therefore, we suggest that the same DNA regions participate in the binding mode of the DNA–TMAP complexes up to the 4:1 ratio. The VCD spectrum of the DNA–TMAP complex at the 3:1 ratio differs from those for other ratios. The intensity of the couplet at ~1688(–)/1663(+) cm^{-1} decreased so that the intensity of the band at 1661(+) cm^{-1} became similar to that of the band at 1634(+) cm^{-1}. From this fact it follows that the GC base pairs were further affected by TMAP at the 3:1 ratio. Since the positive band at ~1690(+) cm^{-1}, which represents intercalation[27], did not appear and only minor perturbation of the ECD spectrum of the DNA part of the complex was observed (Fig. 2, part C), we suggest that intercalation does not take place in this case due to the bulky trimethyl substituents. Rather than the other binding modes that employ the GC part of DNA, external major groove binding is possible.

CONCLUSIONS

Three types of DNA–porphyrin interactions were observed in ECD and characterized by VCD spectra. Although ECD spectra are treated generally as a reliable "marker" for different binding modes of porphyrin with DNA, VCD spectra obtained for different porphyrin–DNA systems possess additional information about specific groups of atoms involved in the interactions (Table I).

Cu(II)TMPyP with DNA provides an intercalative binding mode that corresponds to a negative band at 426(–) nm in ECD spectra and a new positive band at 1690(+) cm^{-1} in the VCD spectra. The binding of Cu(II)TMPyP

affects substantially the DNA structure in the GC base-pair regions at higher Cu(II)TMPyP loadings. Intercalative sites are saturated at the 3:1 ratio, which is demonstrated by the fact that the VCD and ECD spectra resemble those of poly(dA-dT)$_2$ (refs[29,35,36]).

Fe(III)TMPyP with DNA provides an external minor groove binding mode that is revealed by a positive band in the ECD spectra and the shift of the positive VCD band from 1639(+) to 1632(+) cm^{-1} accompanied by intensity decrease.

ECD spectra show that TMAP with DNA provides a minor groove binding mode at higher *c*(DNA)/*c*(TMAP) ratios and external binding accompanied by self-stacking of TMAP along the phosphate backbone at smaller *c*(DNA)/*c*(TMAP) ratios, which are manifested in ECD as a positive band and as a couplet with both positive and negative bands of similar intensities, respectively. In the VCD spectra, the minor groove binding is demonstrated by the shift of the band from ~1639(+) to 1634(+) cm^{-1}, and the external major groove binding as a less intense positive band at 1661(+) cm^{-1}.

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