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Porphyrins as spectroscopic sensors for conformational studies of DNA*

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Abstract: Molecular systems containing two or more interacting porphyrins show remarkable spectroscopic features that allow for a very sensitive detection of conformational changes on the microscale level by different methods, such as fluorescence and electronic circular dichroism (ECD). Covalent porphyrin-DNA assemblies can provide a CD profile (exciton couplet) within the porphyrin Soret band region which is very diagnostic for DNA conformational changes. Additionally, covalently linked porphyrins have been shown to function as DNA molecular caps and to stabilize the non-self-complementary non-Watson–Crick guanine–adenine DNA sequence via their strong π–π stacking.

Keywords: porphyrins; circular dichroism; DNA conformation; DNA probe; B- to Z-DNA transition; oligonucleotides; DNA conjugates.

INTRODUCTION

DNA is polymorphic biopolymer and can adopt alternative conformations that can be very different from the classical B-form double-helix structure described by Watson and Crick over 50 years ago. Under physiological conditions, specific sequences of DNA can form non-B-DNA structures, such as left-handed Z-DNA, cruciform DNA, hairpins, and triplex and quadruplex DNA [1]. The biological roles associated with the different structural conformations of DNA are not well understood because of the short lifetime of appearance of each structure and the difficulty in creating a system to demonstrate the DNA local structure. However, this diversity of DNA structures is believed to be important for recognition by control proteins in various biological processes [2].

Circular dichroism (CD) in the region below 300 nm is the technique commonly used to study DNA transitions and to identify the different forms of DNA [3]. However, distinguishing one particular DNA form from another can be hampered due to the CD spectral overlap below 300 nm of other forms of DNA, and/or of other DNA-binding molecules [4]. In order to gain greater sensitivity in monitoring DNA transitions, we considered that porphyrins attached to opposite ends of duplex DNA oligomers might be useful as sensitive reporter groups for CD analysis. This was based on the assumption that DNA conformational changes owing to salt effects and DNA-binding drugs would be reflected by changes in the CD in the Soret region (400–425 nm) of the spectrum, sufficiently removed from the CD of the DNA or that of other small molecules in the 220–350 nm region. For example, with the minor-groove DNA binding small molecules such as netropsin and distamycin, the DNA conforma-

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tional changes are relatively slight and have mainly been analyzed by X-ray [5]. Solution data by CD have been difficult to analyze because the induced CD Cotton effects for netropsin and distamicin overlap with that of the DNA [6].

The CD exciton chirality method is based on the through-space electronic coupling of two or more chromophores in chiral substrates, giving rise to a bisignate CD curve [7]. This results in an exciton-split CD spectrum (a CD couplet) consisting of two Cotton effects of opposite signs flanking the absorption band. Porphyrins, in particular, owing to their unique electronic and geometric properties, are versatile and powerful CD reporter groups for structural studies by the exciton chirality method [8]. They have intense extinction coefficients ($\varepsilon \sim 400\,000$) in the visible region ($\lambda_{max} \sim 415$ nm) and a known direction of the effective transition moments [9]. In addition, their planar macrocyclic ring can coordinate a variety of metals and can be easily chemically functionalized in order to tune the solubility, molecular recognition, and aggregation. Porphyrins and metalloporphyrins covalently linked to peptides [10], oligosaccharides [11], glycol lipids [12], dimeric steroids, and the marine neurotoxins [13] have been employed for conformational and configurational studies by exciton chirality CD.

Not surprisingly, there are many reports on porphyrin–DNA interactions [14]. However, most of them have focused on the binding mode of free cationic porphyrin to the DNA. The CD sign in the porphyrin Soret region is diagnostic for the mode of porphyrin interaction with DNA. The first porphyrinyl-nucleosides were synthesized in the early 1990s having the 5'-ether linker [15]. Since then, many porphyrin-nucleosides and porphyrin-oligonucleotides have been reported in the literature [16]. Meunier et al. have prepared a series of cation manganese porphyrin-oligodeoxynucleotide (ODN) conjugates attached in the 2' and 5' positions using long aminoalkyl linkers. They have shown that porphyrin conjugates can selectively and efficiently oxidize and cleave DNA [17]. Richert et al. embedded the porphyrin into the DNA backbone, replacing one of the nucleotides [18]. The embedded porphyrin did not interact with the nucleic acid, but intercalated into the duplex structure. Kool et al. published 1'-substituted porphyrin oligonucleotides [19]. The duplex was found to be thermally and thermodynamically stabilized with porphyrin heavily intercalated into the duplex.

Only recently, porphyrins [20–24] and stilbenes [25] covalently attached to ODN have been used as chiroptical sensors studied by electronic CD. Development of new systems that are able to sense the transition between different DNA forms is of high importance. Therefore, much broader and comprehensive studies on the usefulness of DNA/porphyrin conjugates are obviously well justified. The goal of our research has been to explore the possibility of following different DNA transitions in real time via exciton and induced CD resulting from porphyrin–porphyrin and porphyrin–DNA interactions. In parallel, we have also studied the effect of a noncharged porphyrin attached to the DNA termini on the DNA structure and stability.

We attached the porphyrin to the 5'- or 3'-termini of DNA using amide or phosphate linkers. These short linkers allow for an intimate contact between the porphyrin and the neighboring DNA nucleotides. A short linker also provides good control over the position of the porphyrin on the DNA and prevents porphyrin intercalation. The noncharged character of selected porphyrins forces nonelectrostatic modes of interaction with adjacent hydrophobic nucleobases. Porphyrins attached to both ends of a double helix function as sensitive reporter groups through exciton-coupled CD and are able to detect geometrical changes in the backbone of the DNA sequence. Figure 1 summarizes the porphyrin phosphoramidites used for incorporation of the porphyrin moiety into the DNA.

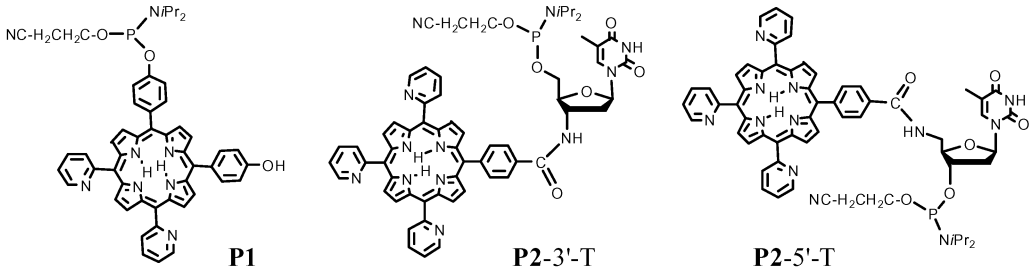

Fig. 1 Porphyrin phosphoramidites used to incorporate porphyrin chromophores into the ODNs.

5'-PHOSPHATE LINKER

The porphyrin was attached to the DNA backbone via a DNA natural phosphate linker at the 5'-position using standard phosphoramidite chemistry [22]. The selected porphyrin **P1** contains two pyridyl moieties and a phenolic group, which enhance the porphyrin water solubility relative to commonly used tetraphenyl-porphyrins.

Thermal denaturation experiments carried out on **P1**-5'-$(CG)_n$, n = 3–5, did show a slight decrease (~5 °C) in the melting temperature relative to the unmodified sequences. The slope of the melting curve with incorporated porphyrin was less steep than the unmodified duplex indicative of lower cooperativity among the nucleobases owing to interference by the porphyrin macrocycle. CD studies of self-complementary duplexes containing porphyrin-phosphate in the 5'-position revealed spectral features of B-DNA similar to the porphyrin-free sequences. A bisignate CD curve could be observed in the porphyrin Soret region in duplexes at low temperatures [22]. The melting of the duplexes caused significant changes in the shape and intensities of the CD in this Soret band region. The bisignate curve clearly observed at 0 °C slowly disappeared, giving rise to a positive band (425 nm) with increasing temperature (Fig. 2a).

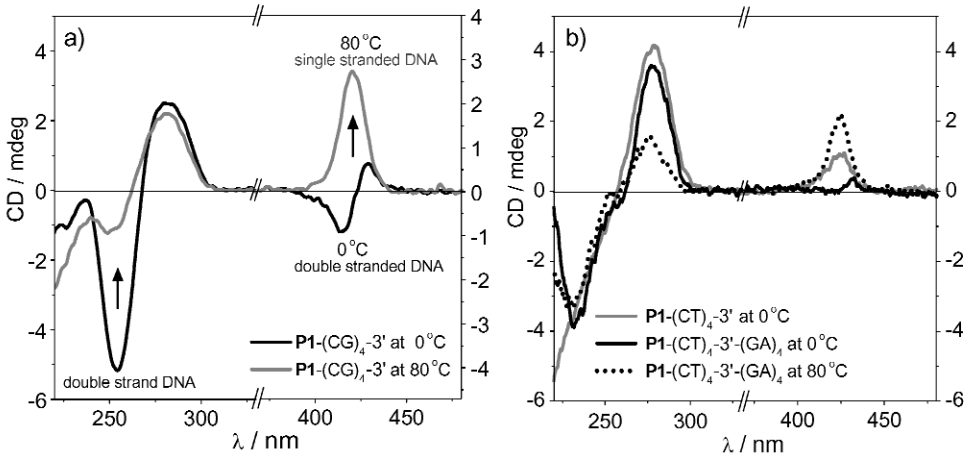

Fig. 2 (a) CD spectra of 8-mer **P1**-5'-phosphate-$(CG)_4$-3' at 0 and 80 °C; (b) CD spectra of 8-mer **P1**-5'-phosphate-$(CT)_4$-3' at 0 °C and 8-mer **P1**-5'-phosphate-$(CT)_4$-$(GA)_4$-3' at 0 and 80 °C.

In order to confirm that porphyrin–porphyrin through space interaction is the origin of the observed split CD bands, we prepared the double-stranded sequence formed from the non-self-complementary conjugate **P1**-5'-$(CT)_4$-3' and its complementary porphyrin-free sequence, 5'-$(GA)_4$-3'. Such a

duplex carries only one porphyrin unit, and porphyrin–porphyrin interaction can be excluded. The CD spectrum (Fig. 2b) showed only a negligible positive CD at 0 °C (mostly double strand) and the expected strong positive CD band for single strand at 80 °C. These results are evidence for dipole–dipole, long-range electronic interaction between the two porphyrin chromophores that give rise to exciton-coupled CD when ODN conjugates form a duplex as in the case of **P1**-5'-$(CG)_4$ at 0 °C.

DETECTION OF THE B- TO Z-DNA TRANSITION

Although the biological role of Z-DNA still remains to be clarified, it has recently been inferred by the discovery that certain classes of proteins bind to it tightly and specifically. It is likely that the B-form represents the predominant conformation of DNA in cells, and it is possible that left-handed DNA arises transiently during the course of cellular processes such as transcription [26]. Our goal was to explore the possibility of following the B- to Z-DNA transition in real time via porphyrin–porphyrin exciton couplet CD and to study the effect of a bulky porphyrin on the salt-induced B- to Z-DNA transition.

Alternating CG sequences are known to form stable Z-form structures at high salt concentration. Figure 3a shows how the Soret region of the CD spectrum of 8-mer **1P**-5'-$(CG)_4$ sensitively reflects the change from B- to Z-DNA [24]. The CD spectrum of **1P**-5'-$(CG)_4$ in the B-form shows a weak bisignate curve with a positive band at 427 nm and a negative band at 417 nm while the CD spectrum in the Z-form shows a strong bisignante curve with a positive band at 441 nm (red shift, Δ = 14 nm) and negative band at 407 nm (blue shift, Δ = 10 nm). The observed porphyrin CD bisignate curve can also be used to quantify the amounts of B- and Z-DNA during the sodium chloride-induced transition. We found that the change in intensity and position of the band at ca. 440 nm wavelength (positive band of the porphyrin bisignate CD curve) best reflects the B–Z transition of the studied ODN. Figure 3b compares the monitoring of the B–Z transition using 294 nm (conventional) and porphyrin 442 nm wavelength. The tetraarylporphyrin attached in the 5'-position also enhanced the high salt-induced B–Z transition. The porphyrin-modified 8-mer **1P**-$(CG)_4$ reached 50 % of the Z-DNA form (the midpoints of the B–Z transition) at 0.75 M NaCl (Fig. 3b, solid line). On the other hand, the porphyrin-free 8-mer 5'-$(CG)_4$ reached 50 % Z-DNA form at 2.6 M NaCl (Fig. 3b, dotted line).

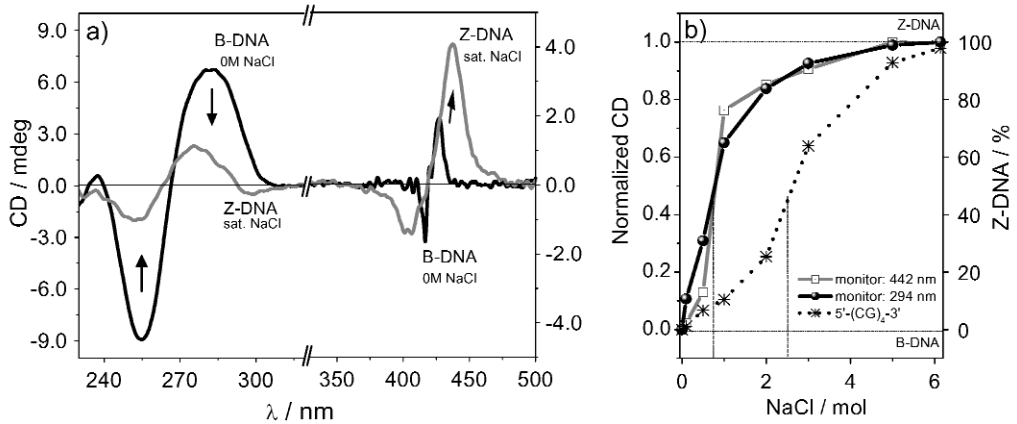

Fig. 3 (a) CD spectra of 8-mer **P1**-5'-phosphate-$(CG)_4$-3' at different salt concentrations; (b) B–Z transition of **P1**-5'-phosphate-$(CG)_4$-3' monitored at two different wavelengths, and the B–Z transition of the 5'-$(CG)_4$-3'.

Using 5'-porphyrin-modified ODN, we have shown that if the interporphyrinic twist and distance are favorable, the porphyrin chromophore can simultaneously serve as a real-time reporter of the

B–Z transition by the exciton couplet CD signal in the Soret band region. This signal allows the detection of the B- to Z-DNA transition in the spectral region free of undesired spectral overlaps.

NON-SELF-COMPLEMENTARY NON-WATSON–CRICK GUANINE–ADENINE DUPLEX

Noncovalent interactions between aromatic molecules are important factors in maintaining the structural integrity of all biopolymers. Far more important among them is the molecule of DNA. The termini of DNA duplexes are hot spots of low base-pairing fidelity. Small aromatic molecules are able to bind to the ODN termini and stabilize its structure. Such a mode of interaction is called "molecular capping", and it is believed to have high potential to help explore less stable and nonclassical DNA structures [27]. The capping can be favored by covalently linking the small molecule to the termini of the DNA oligonucleotides. We found out that covalently linked tetraarylporphyrin **P1** could arrange a non-self-complementary $d(GA)_4$ 8-mer into an antiparallel duplex with continuous noncanonical Watson–Crick guanine–adenine base pairs [23].

The CD of the non-self-complementary and unmodified sequence OH-$(GA)_4$-OH shows the expected weak CD spectrum with a small negative band at 285 nm and a small positive band at 250 nm, resulting from unorganized single-stranded structure [23]. The CD spectrum of porphyrin **1P**-$(GA)_4$ (Fig. 4a), however, shows a spectrum characteristic of duplex DNA with A-like features.

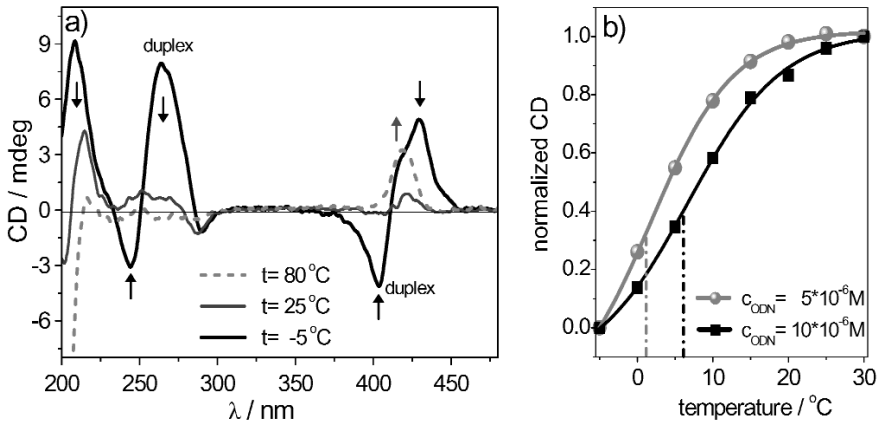

Fig. 4 (a) CD spectra of 8-mer sequence **P1**-5'-phosphate-$(GA)_4$-3' at different temperatures; (b) melting curve of **P1**-5'-phosphate-$(GA)_4$-3' at two different concentrations.

The CD spectrum of the porphyrin conjugate **1P**-$(GA)_4$ at –5 °C shows the ODN CD spectrum below 320 nm and a bisignate curve in the porphyrin Soret band absorption region. The exciton couplet curve (positive band at 425 nm and negative band at 407 nm) is evidence for the dipole–dipole through-space long-range electronic interaction between the two porphyrin chromophores. Upon increasing the temperature from –5 to 80 °C, the Soret band CD signal changes dramatically. The porphyrin bisignate curve disappears at temperatures higher than 30 °C, indicating a loss of ODN secondary structure due to strand separation (denaturation). A positive CD band can be observed at temperatures above 30 °C.

The bisignate CD signal in the porphyrin Soret band absorption region indicates porphyrin–porphyrin long-range interaction resulting from an antiparallel duplex structure. Porphyrin bisignate CD curve also excludes the existence of hairpin, organized single-strand or tetraplex as possible structures of porphyrin-GA sequence. As expected, the CD spectrum of porphyrin-free sequence 5'-$(GA)_4$ varied only slightly on heating from –5 to 80 °C. Figure 4b (solid line) illustrates the melting curve of porphyrin–ODN **1P**-$(GA)_4$ with the melting temperature (T_m) equal to approximately 7 °C. The melting

CD experiments run at half concentration (c = 5.10^{-6} M) still show stabilization of the secondary structure but with a lower T_m value equal to approximately 1 °C (Fig. 4b, gray line). The concentration dependency of T_m allows us to rule out organized single-strand and hairpin structures of **P1**-$(GA)_4$.

These results indicate that the tetraarylporphyrin **P1** can serve as a selective molecular cap for stabilization of a non-self-complementary non-Watson–Crick 5'-$(GA)_4$-3' sequence reminiscent of an A-form-like DNA anti-parallel helix. More importantly, the porphyrin not only acts as a specific DNA cap, but owing to its unique spectroscopic features, it also serves as an internal and highly sensitive CD reporter group of newly formed secondary structure. Thus, it is the example of CD spectroscopic discrimination between parallel and antiparallel homoduplexes (Fig. 5).

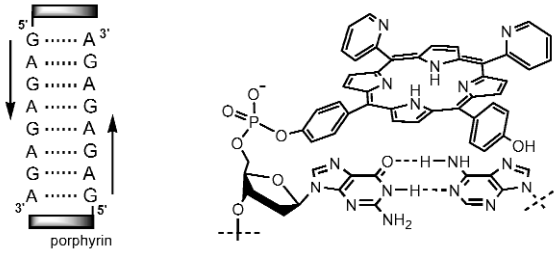

Fig. 5 Schematic representation of the antiparallel duplex **P1**-$(GA)_4$ with porphyrins serving as molecular caps (left), diagram demonstrating the overlap of the porphyrin **P1** with a guanine–adenine base pair (right).

3'-AMIDE LINKER

A short and more rigid amide bond was selected in order to restrict the conformational flexibility at the site of attachment between the porphyrin moiety and the nucleobase (Fig. 6) [28]. Since the porphyrin group blocks the 3'-position of the thymidine, the standard 3' → 5' synthesis could not be applied for the last coupling step. Thus, the porphyrin thymidine phosphoramidite **P2**-3'-T was attached through a 5'-to-5' coupling on a solid-supported 5'-GCGCGCA-3' 7-mer or 5'-GCGCA-3' 5-mer (both prepared by conventional 3' → 5' synthesis) [28].

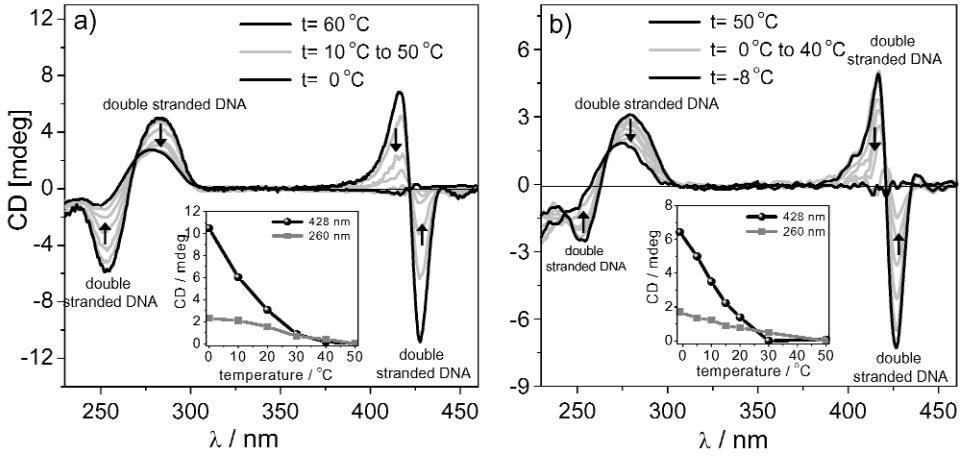

Fig. 6 (a) CD melting spectra of 8-mer **P2**-3'-T-5'-GCGCGCA-3' (b) CD spectrum of 6-mer **P2**-3'-T-5'-GCGCA-3'. Inlets: Difference in sensitivity in detection of structural changes detected at 260 and 428 nm.

The porphyrin in the 3' position does not appear to perturb the DNA duplex. Thermal denaturation experiments revealed that the stability of porphyrin-ODN duplexes was not significantly influenced by incorporation of the porphyrin. The CD spectra of the modified and unmodified sequences exhibited almost identical CD signals below 300 nm, typical of a right-handed B-DNA.

The annealed CD spectra of the 8-mer conjugate **P2**-3'-T-5'-GCGCGCA as well as 6-mer conjugate **P2**-3'-T-5'-GCGCA at 0 °C consist of two regions (Fig. 6): (i) the right-handed B–ds helix signal in the 220–320 nm region, and (ii) a split Soret band in the 380–440-nm region. An exciton-coupled CD signal in the Soret region is direct evidence of the long-range through space electronic interaction between two porphyrins [20]. Interestingly, unlike in the case of the porphyrin **P1** attached to ODN via the phosphate linker, the single-stranded ODNs with porphyrin **P2** attached in the 3'-position did not lead to any CD signal in the porphyrin Soret region.

The CD signals in the 220–320 nm region for 8-mer **P2**-3'-T-5'-GCGCGCA-3' and 6-mer **P2**-3'-T-5'-GCGCA-3' sequences vary only slightly upon increasing the temperature from 0 to 50 °C, as in the case with porphyrin-free ODNs. The CD couplets in the porphyrin region, however, disappear indicating a loss in helicity owing to strand separation (denaturation). The temperature-driven melting behavior of the porphyrin-ODNs shows that porphyrin attached in 3'-position properly report the double- to single-strand transition of ODNs. The detection sensitivity is enhanced in the porphyrin region of the CD spectrum. The inlets in Fig. 6 show the remarkable difference in the change of CD signal (mdeg) of the bands at 260 and 428 nm for **P2**-8-mer and **P2**-6-mer.

5'-AMIDE LINKER

We reasoned that attachment of a porphyrin directly to the 5' carbon of deoxythymidine via an amide linkage provides a way to prepare oligomers in which the attached porphyrin is in more intimate contact with the neighboring DNA nucleotides than if attached through a phosphate. Such an intimate linkage would result in greater sensitivity to conformational changes of the ODNs.

The annealed CD spectra of the 8-mer conjugate **P2**-5'-T-5'-$(GC)_3$A-3' at –5 °C consist of the right-handed B-DNA signal in the 220–320-nm region, and a split Soret band in the 400–440-nm region (Fig. 7). The temperature increase caused significant changes in the CD in the Soret band region. The bisignate curve disappeared, giving rise to a positive band (424 nm) at 60 °C. Although our studies with 5'-amide-linked porphyrins are at an early stage, 5'-amide-linked **P2**-5'-T-5'-$(CG)_3$A-3' indicates a greater sensitivity in the Soret region over that of the 5'-phosphate-linked **P1**-5'-$(CG)_4$-3'. Still to be determined is the effect of orientation of the electric transition moments of the 5' attached porphyrins on through-space exciton coupling as a function of oligomer length and structure.

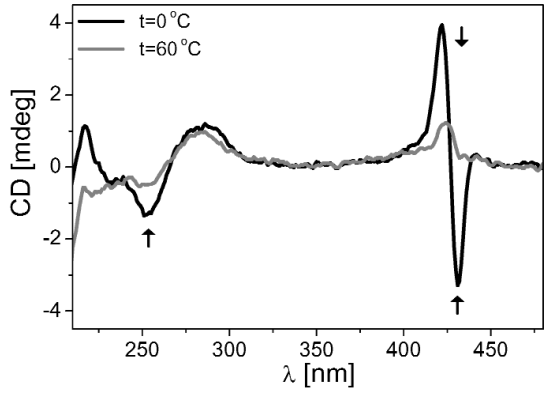

Fig. 7 CD spectrum of 8-mer **P2**-5'-T-5'-$(GC)_3$A-3' at two different temperatures. The arrows show increasing temperature.

CONCLUSION

Our studies demonstrate the application of porphyrins as reporter groups for oligonucleotide conformational studies. If the twist and distance are favorable, porphyrin chromophores covalently attached to the oligonucleotides give rise to an exciton-coupled CD. We confirmed that the bisignate and the monosignate CD curves originate from porphyrin–porphyrin through-space interactions and porphyrin–DNA interactions, respectively. Our CD data indicate that the porphyrins are in contact with double- as well as single-stranded DNA. The newly formed bisignate CD signal in the visible region originates from the chiral twist between the porphyrin chromophores and can be used for the detection of the DNA conformational changes, such as B- to Z-DNA or double- to single-strand DNA transition. The advantage of this signal is that it appears in a spectral region free of undesired spectral overlaps.

Our results also reveal the ability of porphyrins to serve as DNA "molecular caps". The tetraarylporphyrin can stabilize the non-self-complementary non-Watson–Crick guanine–adenine DNA sequence. Porphyrins here also act as the internal CD sensors of the newly formed secondary structure that allows the spectroscopic discrimination between parallel and antiparallel homoduplexes.

ACKNOWLEDGMENT

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