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Synthesis and Circular Dichroism of Tetraarylporphyrin−Oligonucleotide Conjugates

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Circular dichroism (CD) spectra in the 200−300 nm region are widely used to investigate DNA conformations and their changes. However, subtle conformational changes caused by DNA−drug binding, intercalation, etc. are difficult to analyze, particularly when the drug UV bands overlap with those of DNA.[1] We describe the results of using covalently attached porphyrins to the termini of an oligonucleotide in order to cope with such cases. This will allow clear-cut detection of conformational variations in the visible region.

Long-range exciton coupling between stilbene chromophores separated by (A−T) sequences has been reported.[2] However, porphyrins with far more intense extinction coefficients (ϵ = 400 000−550 000) in the visible region (λ_{max} 415 nm), adjustable hydrophilicities, and a known direction of the effective transition moments[3] should reflect subtle DNA conformational changes in the visible 420 nm region at even larger interchromophoric distance.[4] CD couplets in the Soret region have been detected in the 10 Å range for porphyrin derivatives of 1,*n*-glycol lipids,[5] and at larger distances (13−50 Å) in the case of steroids, the marine neurotoxin brevetoxin B (BTX-B),[6] as well as in various peptides.[7]

Although, exciton-coupled CD resulting from true-space chiral interactions of covalently attached porphyrins to peptides,[7,8] cellulose strand,[9] polyisocyanides,[10] and self-assembling guanosines[11] has been employed for conformational studies; with respect to porphyrins incorporated into DNA sequences, so far only the fluorescence[12] and the DNA cleaving properties[13] have been investigated.

We have prepared the trispyridylphenyl porphyrin (TPyrPhP) conjugate of self-complementary DNA sequences, 3′-T-5′-GCGCG-CA-3′ (**1a**); 5′-ACGCGCGT-3′ (**1b**); and 3′-T-5′ACGCGCG-3′ (**1c**), carrying a TPyrPhP residue at the 3′ end (Figure 1) of the different single-stranded (ss) sequences. The porphyrin probe has been attached to the nucleotide 3′-amino group via the more rigid amide bond rather than to the more flexible 3′ or 5′ hydroxyl group.

We anticipate the porphyrins attached to both ends of the double-stranded (ds) helical backbone will serve as sensitive CD sensors for detecting subtle DNA conformational changes.

Tetraarylporphyrin oligonucleotides **1** (ODN) were prepared according to Scheme 1.[14] Trispyridylphenylporphyrin **2** was attached to 3′-amino-5′-dimethoxytrityl thymidine **3**[15] via EDC coupling. The dimethoxytrityl (DMT) protecting group was removed with *p*-TsOH, and the nucleoside was phosphitylated with 2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite following a standard protocol[16] to yield 5′-phosphoramidites **4**. Since the porphyrin group blocks the 3′ position of the thymidine, the standard 3′ → 5′ synthesis could not be applied. Thus, we pursued two different synthetic routes to prepare the desired oligonucleotides. (a) For oligo **1b**, using reverse 5′-phosphoramidites,[17] we synthesized the ODN in the 5′ → 3′ direction via oligonucleotide automated solid-phase synthesis, in which trispyridylporphyrin thymidine phosphoramidite **4** was attached to the 3′ end of a growing 7-mer chain 5′-ACGCGCG-3′ on solid-supported CPG resin. (b) For oligos **1a** and **1c**, the trispyridylphenylporphyrin thymidine phosphoramidite **4** was attached through a 5′-to-5′ coupling on a solid-supported 5′-GCGCGCA-3′ 7-mer (commercially available) and prepared by conventional 3′ → 5′ synthesis.[18] After cleavage from the solid support and deprotection, the crude porphyrin−oligonucleotides were purified by semipreparative reverse-phase HPLC[19] and characterized by MALDI-TOF MS (see Supporting Information, Figures SI-1,2).

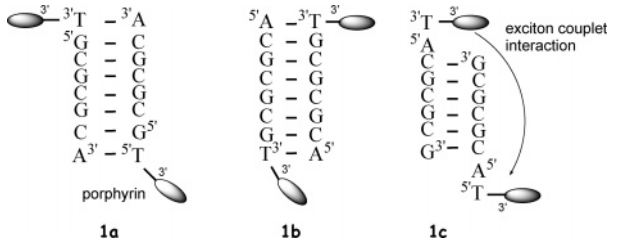
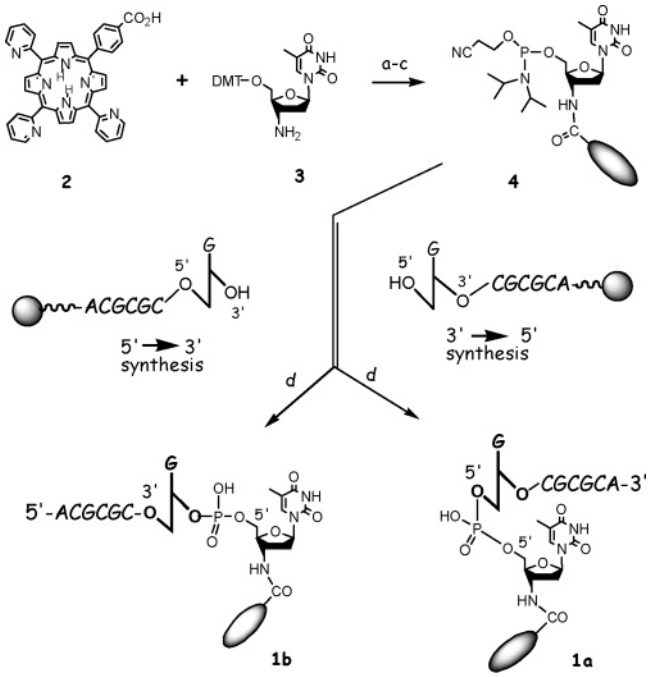


Figure 1. Double-stranded (ds) oligonucleotides with the TPyrPhP attached in 3′ position.

Scheme 1 [a]



[a] (a) EDC, DMAP, CH_2Cl_2; (b) *p*-TsOH, CH_2Cl_2, MeOH; (c) 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite, DIPEA, CH_2Cl_2; (d) oxidation, cleavage from resin, deprotection (shaded balls represent resin).

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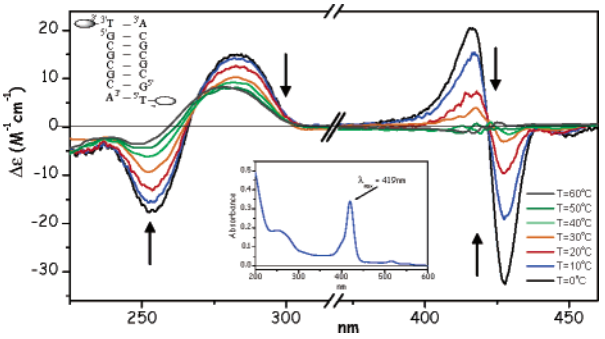

Figure 2. Variable temperature CD data of double-stranded trispyridylphenyl porphyrin ODN **1a** (50 mM phosphate buffer, pH = 7.0).

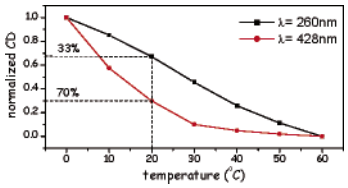

Figure 3. Difference in sensitivity in detection of structural changes of porphyrin−oligonucleotide **4a** detected at 260 and 428 nm.

While the B form of the 8-mer is ca. 27 Å long (3.4 Å per base), the interchromophoric distance in the ds porphyrin conjugate should be at least 10 Å larger.[6] Figure 2 shows the variable temperature (denaturation−renaturation) CD data for **1a**. The CD of the ds conjugate **1a** at 0 °C consists of two regions: (a) the B-ds helix signal in the 225−320 nm ("285 band"), and (b) a coupled Soret band in the 375−470 nm region ("430 band") that represents a long-range interaction between the porphyrins in this 8-mer.

Upon increasing the temperature from 0 to 60 °C, the CD signal in the 200−300 nm region varies only slightly as in the case with porphyrin-free ODN (Figure SI-7). On the other hand, the CD couplet disappears in the porphyrin region, indicating a loss in helicity due to strand separation (denaturation). Thus, the single-stranded ODN does not lead to any CD signal in the porphyrin region. (Figure SI-3) The porphyrin does not appear to perturb the DNA duplex. The CD spectra of the modified and unmodified sequences exhibited the same CD profile below 300 nm, typical of a right-handed B-DNA.

Figure 3 reveals remarkable differences in CD sensitivity between the 225−320 nm and the 375−470 nm regions upon changing the temperature. From 0 to 20 °C, the CD intensity in the typical DNA UV region changes only by 33% compared to the 70% change in the porphyrin region.

As expected, the porphyrin Soret band region thus reflects changes more sensitively in ODN helicity accompanying the ds to ss transition. The possibility that the induced CD[20] arises from intermolecular porphyrin−porphyrin interactions was excluded by repeated melting experiments. Thus, contrary to induced CD originating from a random porphyrin arrangement, the CD amplitudes were reproducible and consistent in each thermal cycle. This fact was further confirmed by the absence of porphyrin Soret CD bands upon mixing 3′-tetraarylporphyrin thymidines and/or tetraarylporphyrin methylesters with the corresponding ODN in different ratio and concentrations (Figure SI-11). The CD couplet clearly originates from electronic interactions between the two porphyins, and not from intercalation or stacking.

To further test this approach, we also examined the following variations: (i) the same sequences in **1a** but derivatized with tetraphenyl porphyrin instead of TPyrPhP; (ii) the 8-mer **1b**; and (iii) the 8-mer sequence **1c**, where the porphyrins are separated by 10 bases. Despite these variations in the sequences, increasing the number of residues, different linkages between the nucleotides, and different tetraarylporphryins, the CD profiles were qualitatively similar, showing negative couplets in the Soret region (Figures SI-8−10). According to this approach, it is expected that the amplitudes and the sign of the porphyrin CD couplets will vary with the change in sequences and number of bases, hence, the distance and mutual orientation of porphyrins.[3,4] Yet, the net difference CD in the Soret band region may still serve as a probe for conformational changes.

In conclusion, these studies show the possibility of using porphyrin exciton-coupled CD for ODN conformational studies. In particular, the exciton-coupled CD signal in the visible region originating from the chiral twist between the porphyrin chromophores is more sensitive than the conventional 200−300 nm region for investigating ODN 3D structural changes. Studies are ongoing to employ this approach utilizing the porphyrin Soret region for the detection of ligand binding, photomodification, as well as B−Z transitions induced in the ODN sequence.

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Supporting Information Available: MALDI-TOF MS spectra of compound **1a**, CD and UV−vis spectra at different temperatures, and melting curves for the native ODN sequences and ODN−porphyrin conjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Mahadevan, S.; Palaniandavar, M. *Inorg. Chem.* **1998**, *37*, 3927−3934. (b) Lee, E. J.; Yeo, J. A.; Cho, C. B.; Lee, G. J.; Han, S. W.; Kim, S. K. *Eur. J. Biochem.* **2000**, *267*, 6018−6024. (c) Parkinson, A.; Hawken, M.; Hall, M.; Sanders, K. J.; Rodger, A. *Phys. Chem. Chem. Phys.* **2000**, *2*, 5469−5478.
(2) (a) Lewis, F. D.; Liu, X.; Wu, Y.; Zuo, X. *J. Am. Chem. Soc.* **2003**, *125*, 12729−12731. (b) Lewis, F. D.; Yangsheng, W.; Zhang, L.; Zuo, X.; Hayes, R. T.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 8206−8215.
(3) Pescitelli, G.; Gabriel, S.; Wang, Y.; Fleischhauer, J.; Woody, R. W.; Berova, N. *J. Am. Chem. Soc.* **2003**, *125*, 7613−7628.
(4) Huang, X.; Nakanishi, K.; Berova, N. *Chirality* **2000**, *12*, 237−255.
(5) MacMillan, J. B.; Molinski, T. F. *J. Am. Chem. Soc.* **2004**, *126*, 9944−9945.
(6) (a) Matile, S.; Berova, N.; Nakanishi, K. *Chem. Biol.* **1996**, *3*, 379−392. (b) Matile, S.; Berova, N.; Nakanishi, K.; Fleischhauer, J.; Woody, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 5198−5206.
(7) (a) Oancea, S.; Formaggio, F.; Campestrini, S.; Broxterman, Q. B.; Kaptein, B.; Toniolo, C. *Biopolymers* **2003**, *72*, 105−115. (b) Formaggio, F.; Peggion, C.; Crisma, M.; Kaptein, B.; Broxterman, Q. B.; Mazaleyrat, J. P.; Wakselman, M.; Toniolo, C. *Chirality* **2004**, *16*, 388−397.
(8) (a) Mihara, H.; Haruta, Y.; Sakamoto, S.; Nishino, N.; Aoyagi, H. *Chem. Lett.* **1996**, 1−2. (b) Arai, T.; Inudo, M.; Ishimatsu, T.; Sasaki, T.; Kato, T.; Nishino, N. *Chem. Lett.* **2001**, *12*, 1240−1241.
(9) Redl, F. X.; Lutz, M.; Daub, J. *Chem.—Eur. J.* **2001**, *7*, 5350−5358.
(10) Takei, F.; Hayashi, H.; Onitsuka, K.; Kobayashi, N.; Takahashi, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 4092−4094.
(11) Masiero, S.; Gottarelli, G.; Pieraccini, S. *Chem. Commun.* **2000**, 1995−1996.
(12) (a) Berlin, K.; Jain, R. K.; Simon, M. D.; Richert, C. *J. Org. Chem.* **1998**, *63*, 1527−1535. (b) Morales-Rojas, H.; Kool, E. *Org. Lett.* **2002**, *4*, 4377−4380.
(13) Mestre, B.; Jakobs, A.; Pratviel, G.; Meunier, B. *Biochemistry* **1996**, *35*, 9140−9149.
(14) Balaz, M.; Holmes, A. E.; Benedetti, M.; Proni, G.; Berova, N. *Bioorg. Med. Chem.* **2005**, in press.
(15) (a) Chen, J. K.; Schultz, R. G.; Lloyd, D. H.; Gryaznov, S. M. *Nucleic Acids Res.* **1995**, *23*, 2661−2668. (b) Zuckerman, R.; Corey, D.; Schultz, P. *Nucleic Acids Res.* **1987**, *15*, 5305−5321. (c) Glinski, R.; Kahn, M.; Kalamas, R. *J. Org. Chem.* **1973**, *38*, 4299−4305.
(16) (a) Dubey, I.; Pratviel, G.; Meunier, B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3088−3095. (b) Haraguchi, K.; Delaney, M. O.; Wiederholt, C. J.; Sambandam, A.; Hantosi, Z.; Greenberg, M. M. *J. Am. Chem. Soc.* **2002**, *124*, 3263−3269.
(17) Purchased from ChemGenes, Boston, MA.
(18) Purchased from Alpha DNA, Montreal, Canada.
(19) Fountain, K. J.; Gilar, M.; Gebler, J. *Commun. Mass Spectrosc.* **2003**, *17*, 646−653.
(20) Pasternack, R. F. *Chirality* **2003**, *15*, 329−332.

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