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5′-Porphyrin–Oligonucleotide Conjugates: Neutral Porphyrin–DNA Interactions†

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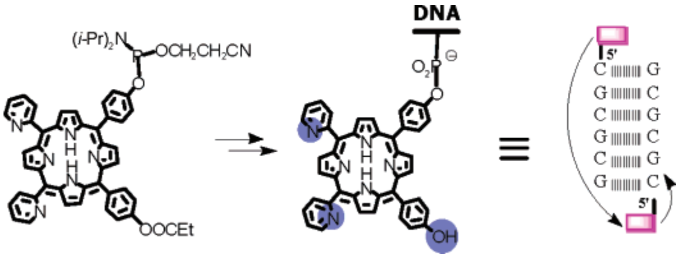
ABSTRACT



Incorporation of hydrophilic tetraarylporphyrin phosphoramidites into the 5′-termimus of the DNA as well as noncharged porphyrin–DNA interactions have been studied. Porphyrin-modified oligonucleotides show lower melting temperatures than their unmodified analogues. Single-stranded DNA interacts more strongly with porphyrin and causes more intense chiral disturbance in the porphyrin environment than the corresponding double strand.

Modified synthetic oligodeoxynucleotides[1] (ODNs) play important roles in molecular biology, biotechnology, and molecular medicine.[2] Of particular interest are ODNs carrying covalently attached porphyrins since these moieties are versatile and powerful molecular probes due to their unique electronic structure. Their planar macrocyclic ring can coordinate a variety of metals and can be easily chemically functionalized in order to tune their solubility, molecular recognition and aggregation.[3] In the past decade, several porphyrin−DNA conjugates have been synthesized and their biomedical,[4] oxidative cleavage,[5] and nuclease activity[6] extensively studied.

Previous studies on porphyrin−DNA interactions focused on charged water-soluble porphyrins.[7] In the case of cationic

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[†] Dedicated to Professor Koji Nakanishi on the occasion of his 80th birthday.

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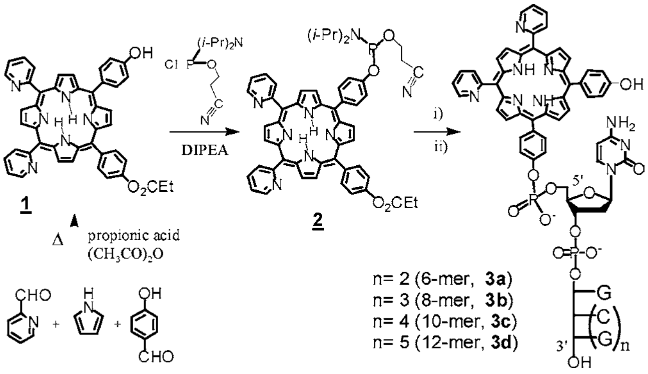
porphyrins, the binding modes (intercalation, outside groove binding, and outside binding with self-stacking) are driven by electrostatic attractions between the negatively charged DNA backbone and the positively charged porphyrins. Noncharged porphyrins have attracted, so far, much less attention, and only a few examples of porphyrin−ODN conjugates have been reported. It has been shown that a neutral porphyrin incorporated at C-1′ stabilizes the ds structure[8] while the porphyrins embedded in the DNA sequence destabilized the duplex.[9] In the case of 3′-modified self-complementary ODNs,[10] the porphyrin−porphyrin through-space interaction occurs over a large distance and leads to a CD exciton couplet.[11] To our knowledge, no prior studies focused on neutral porphyrin−DNA interactions.

To better understand the nonelectrostatic interactions of the porphyrin with the nucleobases and their effect on ODN stability, we decided to synthesize the oligonucleotides with a hydrophilic, neutral porphyrin attached to the DNA backbone via a natural phosphate linker at the 5′-position, which has not been reported. A short but flexible linker is expected to allow for an intimate contact of the porphyrin with the neighboring DNA. The selected porphyrin **1** contains two pyridyl moieties and a phenolic group, which enhance its water solubility relative to commonly used tetraphenylporphyrins prone to stacking in aqueous solution. On the other hand, the noncharged character of porphyrin **1** forces nonelectrostatic interactions with the hydrophobic nucleobases. Herein we report the synthesis of a neutral, hydrophilic, porphyrin phosphoramidite and its conjugation to ODNs to study the porphyrin−DNA interactions by T_m, UV, CD, and fluorescence spectroscopy.

Porphyrin **1** was synthesized by a modified Adler−Longo method[12] (Scheme 1, regioisomer not determined, see the Supporting Information). 2-Pyridinecarboxaldehyde and pyrrole were added to the mixture of 4-hydroxybenzaldehyde and acetic anhydride in propionic acid at 110 °C.[13] The presence of a small amount of acetic anhydride allowed for the in situ monoesterification of the *p*-phenol function by propionic acid. This modification further simplified the isolation of the desired monoprotected porphyrin **1** from the complex mixture by flash chromatography (see the Supporting Information). This protecting group was then removed, without an additional synthetic step, during the DNA cleavage from solid support.

Porphyrin **1** was *O*-phosphitylated with 2-cyanoethyl *N*,*N*-diisopropylchloro phosphoramidite providing the corresponding phosphoramidite **2** in 85−90% yield. Compound **2** was purified by flash chromatography and used immediately in order to avoid an over-oxidation to the unreactive phosphate.

Scheme 1. Synthesis of Porphyrin−Oligonucleotides **3**[a]



[a] Key: (i) 5′-CG(CG)$_n$-3′-solid support, $n = 2-5$, activation reagent (tetrazole/MeCN); (ii) oxidation reagent (I_2), then concd NH_4OH.

The porphyrin phosphoramidite **2** was reacted with the 5′-hydroxy group of the solid supported ODN sequences under standard phosphoramidite conditions (Scheme 1). The attractive feature of this approach is that it provides high coupling yields for bulky porphyrins (see the Supporting Information for more details). The intermediates were oxidized, deprotected, and cleaved from the resin by addition of concentrated aqueous ammonia solution. The crude porphyrin−ODNs **3a−d** were purified by reverse phase HPLC and their structures confirmed by MALDI-TOF mass spectroscopy.

The hydroxyphenylbispyridylporphyrin **1** absorbs at 414.5 nm (Soret band) in MeOH. The water-soluble porphyrin−ODN conjugates **3a−d** display characteristic spectroscopic profiles of porphyrin−ODNs with two absorption maxima at 255 and 423 nm (Figure SI-6). The shorter-wavelength absorption band occurs in a region where porphyrin absorbs only weakly, and thus, it represents the absorption of the nucleobases. The intensity of this band increases and the band maximum shifts to longer wavelengths as the ODN length increases (Table 1). We believe that the blue shift in the absorbance maximum of modified ODNs in comparison with corresponding unmodified ODNs (cf. Table 1) is the result of electronic interactions of the porphyrin chromophore with adjacent nucleobases. Interestingly, this effect diminishes as the number of C:G base pairs increases. The Soret band of porphyrin−ODNs is red shifted by ca. 8 nm, thus proving the interaction of the porphyrin macrocycle with the nucleobases. Since the length of the ODNs, e.g., **3a** and **3d** (Table 1), has no influence on Soret band absorption maximum, this suggests that porphyrin interacts only with neighboring nucleobases.

The steady-state fluorescence of porphyrin **1** and conjugates **3a−d** shows the expected large Stokes shifts with emission bands at ca. 649 nm (Figure SI-8). Remarkably, unlike many fluorophores such as pyrene,[14] the fluorescence properties of the porphyrin are not strongly affected by the

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Table 1. Characteristics of Synthesized Porphyrin−ODN Sequences^a

	porphyrin−ODN	T_m (°C)	$\Delta T_m{}^b$ (°C)	λ_{abs} P−ODN ($\Delta\lambda^c$) (nm)	λ_{abs} Soret band (nm)	$\lambda_{em}{}^d$ (nm)	mass calcd	MALDI-TOF
3a	**Pp**−5′-$(CG)_3$-3′	33.7	−5.7	250.6 (−4.2)	422.4	648	2496.5	2497.3
3b	**Pp**−5′-$(CG)_4$-3′	51.5	−5.6	252.8 (−2.6)	422.0	649	3120.6	3116.4
3c	**Pp**−5′-$(CG)_5$-3′	62.0	−5.1	255.2 (−0.8)	423.4	649	3738.7	3740.4
3d	**Pp**−5′-$(CG)_6$-3′	n.d.	n.d.	255.6 (−0.5)	421.7	649	4344.7	4360.9
4	**Pp**−5′-$(CT)_4$-3′			267.2 (−0.8)	426.4	653	3012.5	3014.1

^{a} All measurements done in the K-phosphate buffer (pH = 7.0, 50 mM, $c_{ODN} \approx 10^{-5}$ M). ^{b} Calculated by subtracting the unmodified sequence T_m from the porphyrin-modified sequence T_m. ^{c} Difference between the modified and unmodified ODNs. ^{d} Absorbance of solution <0.2.

presence of nucleobases. The ODN length also does not influence the fluorescence properties of the porphyrin moiety.

Thermal denaturation experiments were carried out to study the influence of the porphyrin moiety conjugated at the 5′-end on the stability of self-complementary duplexes. ODNs **3a−c** did show a slight decrease in the melting temperature relative to the unmodified sequences (Table 1). The slope of the melting curve with incorporated porphyrin was less steep than the unmodified duplex (Figure SI-14) indicative of lower cooperativity among the nucleobases due to interference by the porphyrin macrocycle.

On the other hand, the absorption spectrum of porphyrin is only marginally affected by heating. The Soret band displays only a small blue shift (2 nm, from 422 to 420 nm) and a small hypochromicity (∼10%) by increasing the temperature from 0 °C to 80 °C.

CD studies of duplexes **3a−d** containing porphyrin-phosphate in the 5′-position revealed, below 300 nm, spectral features of B-DNA similar to the unmodified (porphyrin-free) sequences with a positive CD band at 280 nm and negative band at 255 nm (Figure 1). Thus, the porphyrin incorporation does not cause any helicity changes. The change of the CD spectra of the duplexes containing 5′-porphyrin phosphate with the temperature (Figure 2) clearly showed the transition from the double-helix to the single strand associated with the decrease of the negative CD band at 255 nm.

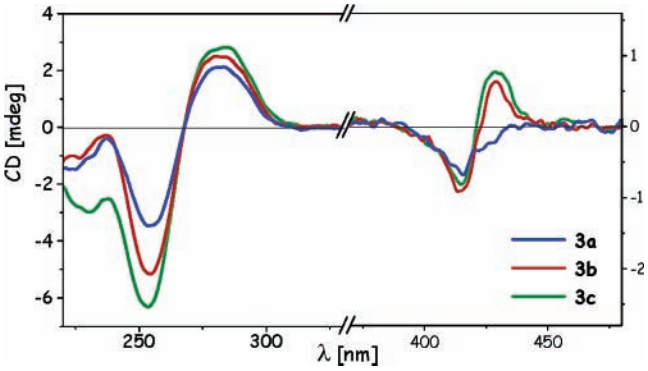

Figure 1. CD spectra of porphyrin−ODNs **3a−c** ($c_{ODN} \approx 10^{-5}$ M) in K-phosphate buffer (pH = 7.0, 50 mM) at 0 °C.

In addition, a characteristic CD can be observed in the porphyrin Soret band region. A bisignate CD curve (positive band at 435 nm and negative band at 405 nm) can be observed in duplexes **3b** and **3c**, while ODNs **3a** and **3d** (**3d** data not shown) exhibit negative CD bands at low temperatures (Figure 1). The bisignate curve is evidence for long-range electronic communication between the two porphyrin chromophores resulting, in the case of **3b** in a positive CD exciton couplet. It is known that such an interaction can occur only in the case of conformational rigidity around the porphyrin−ODN linkage, as well as favorable interchromophoric distance and twist.[15] The reason the porphyrin−ODN conjugates **3a** and **3d** show a negative CD bands in the porphyrin region is under study.

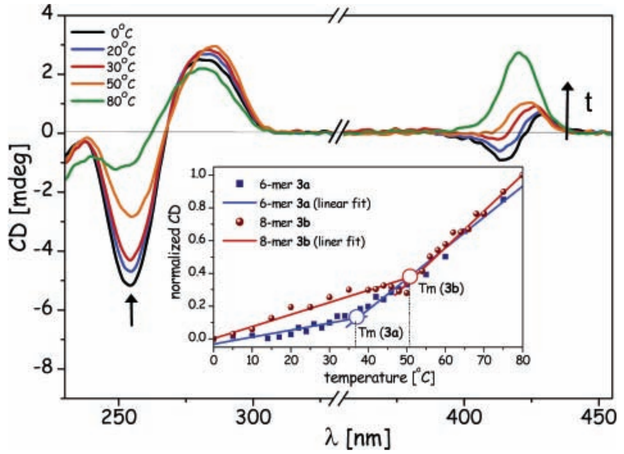

Figure 2. CD spectra of porphyrin−ODN **3b** ($c_{ODN} \approx 10^{-5}$M) in K-phosphate buffer (pH = 7.0, 50 mM) at different temperatures. Inset: The change of CD signals in the Soret region with temperature and their linear fits for 6-mer **3a** and 8-mer **3b**.

It is noteworthy that the melting of the duplexes **3a−d** caused significant changes in the shape and intensities of the CD in the Soret band region. The bisignate curve clearly observed at 0 °C for **3b** and **3c** slowly disappeared, giving

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rise to a positive band (425 nm) with increasing temperature. In the case of **3a** and **3d** with increase of the temperature the small negative CD bands also convert into strong positive bands (Figure SI-9). In previous studies on cationic porphyrins, induced positive CD bands in the Soret region have been associated with external DNA binding.[7] At temperatures higher than T_m, when the single strand prevails, the positive CD band is the only feature that can be observed in the Soret band region of porphyrin−ODNs **3a**−**d**.

The analysis of these data suggest that in buffer solution at low temperatures where ODN exists as a double strand, the porphyrins are not rotating freely, but more likely are in close contact with the DNA strands. This double-strand environment provides low conformational freedom of the phosphate linker. On the other hand, at higher temperature, the melting of the duplex results in a highly flexible single strand which has a higher degree of freedom for the phosphate linker. This interaction is clearly distinguishable by CD signals at 0 °C and at T_m, respectively.

The plot of the CD data in the inset of Figure 2 helps to distinguish the porphyrin interactions between single and double strand DNA. The intensity of the CD signal in the porphyrin region increases linearly with increasing temperature (decreasing amounts of double helix). This result reveals a more intense interaction of the porphyrin moiety with single strand than double strand DNA. Interestingly, the slope of the curve changes dramatically (3−4-fold increase) around the melting temperature. In summary, the more rigid helical structure (at 0 °C) gives rise to a bisignate curve or to the small negative CD band while a less rigid structure or single strand ($> T_m$) gives rise to the positive CD band.

The changes in the Soret band absorption and/or fluorescence spectra by heating the conjugate solutions **3a**−**d** from 0 °C to 80 °C suggest closer $\pi-\pi$ contact with the single strand than double strand DNA (Figure SI-7 and SI-15).

To clarify the differences in the interaction of the porphyrin chromophore with single and double strand DNA we synthesized a non self-complementary porphyrin-ODN 5′-CTCTCTCT-3′ **4** with the porphyrin in the 5′ position. Second, we prepared the double-strand sequence **5** formed from the conjugate **4** and its complementary porphyrin-free sequence, 5′-GAGAGAGA-3′. The single strand sequence **4** showed the expected positive CD signal at 0 °C as well at 80 °C. On the other hand, the duplex **5** containing only one porphyrin exhibited negligible CD at 0 °C (mostly double strand) and a positive CD band at 80 °C (single strand, Figure 3). These results strongly support the view that porphyrin-porphyrin through-space interactions are the origin of the split CD bands in case of **3b** and **3c**, as well as in the CD spectra previously reported.[11]

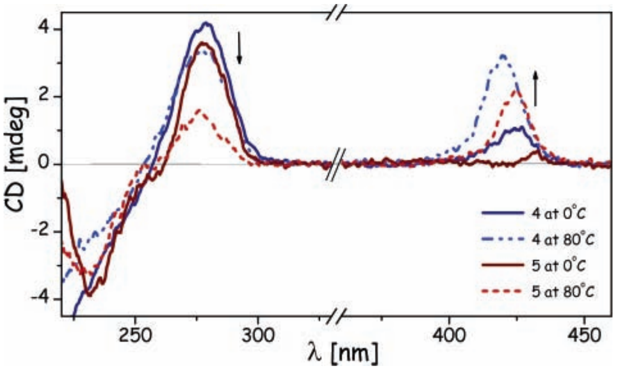

Figure 3. CD spectra of porphyrin−ODNs **4** and **5** ($c_{ODN} \approx 10^{-5}$ M) in K-phosphate buffer (pH = 7.0, 50 mM) at 0 and 80 °C.

In conclusion, we have shown that the neutral, hydrophilic, porphyrin labeled ODNs form stable B-form DNA duplexes in aqueous solutions with only slightly decreased Tms compared to their unmodified analogues. The CD data indicate that the porphyrins are not rotating freely in the buffer solution, and are in contact with double as well as single-stranded DNA. Most likely these are not close contacts since the fluorescence and the absorption data do not support either porphyrin intercalation or close $\pi-\pi$ interaction with neighboring nucleobases. We have also shown that single-stranded DNA interacts stronger with porphyrin and causes more intense chiral disturbance in porphyrin environment than the corresponding double strand. We confirmed that the bisignate CD curve originates from porphyrin-porphyrin through-space interactions and not from porphyrin-DNA interactions and can be used for DNA conformational studies. The moderate intensity of bisignate CD curve underscores the need for further optimization of the porphyrin linker. Our finding that the fluorescence properties of this neutral hydrophilic porphyrin are not strongly affected by the attachment on the ODNs reveals its potential use as a molecular probe for DNA labeling.

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Supporting Information Available: Experimental procedures and characterization (^{1}H and ^{31}P NMR, MS, HPLC, absorption, CD and fluorescence spectra) of compounds **1**, **2**, **3a**−**d**, **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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