Porphyrins conjugated to DNA as CD reporters of the salt-induced B to Z-DNA transition†,‡

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The porphyrin chromophore incorporated at the 5′-position of an oligonucleotide allows the simultaneous detection of the B- to Z-DNA transition *via* the porphyrin Soret band circular dichroism exciton couplet signal around 420 nm and the oligonucleotide CD region below 300 nm, at micromolar concentrations.

DNA can adopt a variety of secondary structures that range from the canonical right-handed B-form to the left-handed Z conformation. Such polymorphism of the DNA structure is believed to play an important role in various biological processes.[1] While the specific biological function of Z-DNA is unknown, the recent discovery of classes of proteins that bind tightly and specifically to Z-DNA clearly signifies its importance.[2] Circular dichroism (CD) is the most commonly used and convenient spectroscopic technique for detecting DNA conformational changes.[3] However, determination of Z-DNA can be complicated by the presence of other forms of DNA, and/or of other biomolecules that make the CD region below 300 nm difficult to analyze. To overcome these problems, probes such as pyrenes and porphyrins have been introduced and analyzed using fluorescence or exciton circular dichroism.[4]

Our goal was to explore the possibility of following the B- to Z-DNA transition in real time *via* porphyrin–porphyrin exciton coupled circular dichroism and to study the effect of a bulky porphyrin on the salt-induced B- to Z-DNA transition. For this purpose we attached a non-charged hydrophilic tetraarylporphyrin to the 5′ end of self complementary oligodeoxynucleotide (ODN) **1** (Fig. 1) through solid support synthesis.[5] Alternating CG sequences are known to form stable Z-form structures at high salt concentrations. The porphyrin modified ODN **1P** was obtained in good yield using reverse-phase HPLC[6] and its structure was confirmed through MALDI-TOF mass spectroscopy. We have previously shown that neutral porphyrins attached in the 5′ position can interact through space and give rise to a bisignate CD curve.[5]

Fig. 2 shows the sodium chloride-dependent absorption spectrum of porphyrin–ODN **1P**. The UV spectrum of the porphyrin–ODN conjugate at 0.0 M sodium chloride consists of two bands, an ODN absorption band at 260 nm and the porphyrin Soret band at 420 nm. With increasing amounts of salt, strong hypochromicity

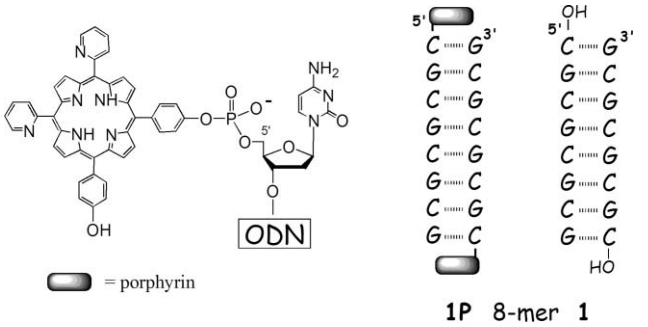

Fig. 1 Tetraarylporphyrin attached to the cytosine *via* phosphate linker and studied ODNs **1P** and **1**.

of both absorption bands is observed. This is apparently a result of increased shielding between the porphyrin and nucleotide π-electron systems in the Z-conformation.[7] The presence of one isosbestic point in the ODN absorption region (212 nm) shows that only two DNA forms (the B- and Z-forms) are in equilibrium. The isosbestic point in the porphyrin Soret band region (436 nm) indicates that the porphyrin absorption band also reflects this equilibrium. Strong hypochromicity was also observed for 8-mer **1** with increasing amounts of sodium chloride (see ESI†).

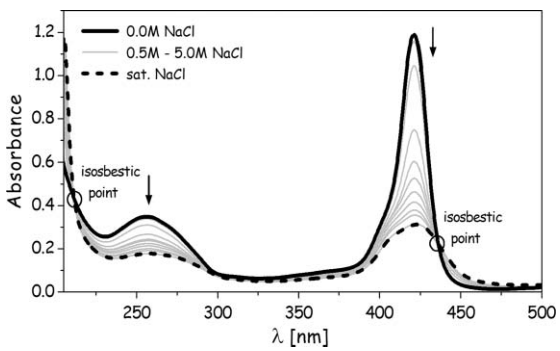

Fig. 2 Absorbance spectrum of ~5 μM porphyrin–ODN **1P** in 50 mM phosphate buffer, pH = 7.0 at different sodium chloride concentrations. The arrows show increasing amounts of sodium chloride.

It is noteworthy that more sensitive detection of conformational changes associated with the B- to Z-DNA transition in the same broad spectral region was achieved by circular dichroism spectroscopy. Below 300 nm increasing amounts of salt induce a dramatic change in the CD spectrum as a consequence of the conformational change from right-handed B-DNA to left-handed Z-DNA. In 0.0 M NaCl, the duplex **1P** exists in the B-DNA conformation, while in saturated NaCl it exists predominantly, but not completely, in the Z-DNA form (Fig. 3a).

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† Electronic supplementary information (ESI) available: HPLC purification profile of **1P**. Absorption and CD spectra of **1P**. NaCl + $NiCl_2$ UV and CD titration spectra. See DOI: 10.1039/b603409h

‡ All measurements were done at room temperature in 50 mM potassium phosphate buffer at pH = 7.0.

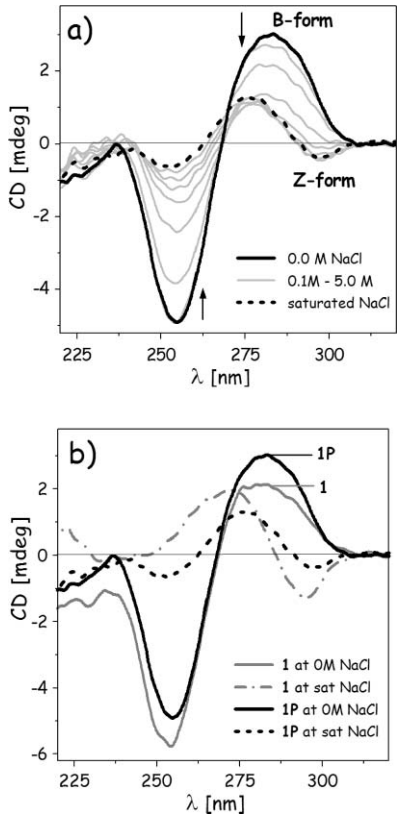

Fig. 3 a) CD spectra of ~10 μM porphyrin–ODN **1P** in 50 mM potassium phosphate buffer, pH = 7.0 at different sodium chloride concentrations. The arrows show increasing amount of sodium chloride. b) CD spectra of 10 μM each of porphyrin–ODN **1P** (black) and ODN **1** (grey) in 50 mM potassium phosphate buffer, pH = 7.0 at different sodium chloride concentrations.

In addition to the CD signal below 300 nm (ODN absorption), an exciton coupled CD can be observed in the porphyrin Soret band absorption region (around 420 nm) in **1P**. Fig. 4b shows how the Soret region of the CD spectrum of 8-mer **1P** sensitively reflects the change from B-DNA to Z-DNA. The CD spectrum

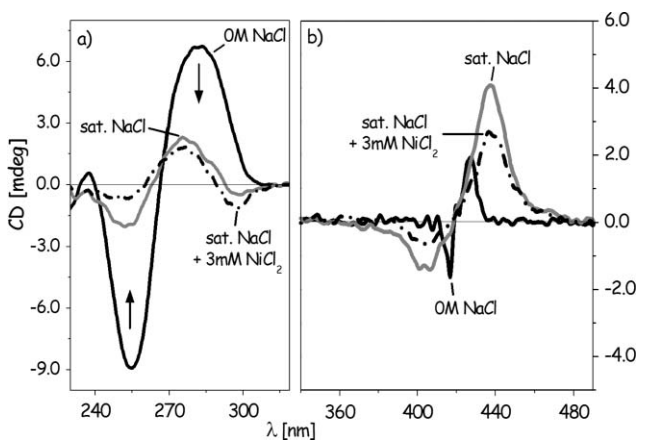

Fig. 4 CD spectra of ~10 μM porphyrin–ODN **1P** in 50 mM potassium phosphate buffer, pH = 7.0. Black line, with 0.0 M sodium chloride; grey line, with saturated sodium chloride; black dash-dotted line, with saturated sodium chloride and 3 mM nickel(II) chloride.

of **1P** in the B-form (black curve) shows a small bisignate curve with a positive band at 427 nm and a negative band at 417 nm while the CD spectrum of **1P** in the Z-form (grey curve) shows a strong bisignate curve with a positive band at 441 nm (red shift, $\Delta = 14$ nm) and negative band at 405 nm (blue shift, $\Delta = 12$ nm).

The bisignate CD curves are evidence for dipole–dipole, long-range electronic interaction between the two porphyrin chromophores.[8] It is known that such an interaction depends on interchromophoric distance and twist, as well as the conformational rigidity around the porphyrin–ODN linkage.[9] The origin of the difference in the porphyrin CD signal for B- and Z-DNA is based on the differences in their respective structures. The bases in left-handed Z-DNA helix alternate between the *anti*- and the unusual *syn*-conformation. This dinucleotide repetition causes the ODN strand to follow a zigzag path around the helix, while the base pairs are located nearly perpendicular to it.[10] A porphyrin attached in the 5′-position through a flexible but short phosphate linker helps to analyze, through a separate CD signal in the porphyrin Soret region, the unique chiral environment in the B- and Z-DNA.

The observed porphyrin CD signal can also be used to quantify the amounts of B and Z-DNA during the sodium chloride induced transition. We found that the CD signal at 442 nm wavelength (positive band of the porphyrin bisignate CD curve) best fits the B–Z transition of the studied ODN. Fig. 5 compares the monitoring of the B–Z transition using 294 nm (conventional) and porphyrin 442 nm wavelengths.

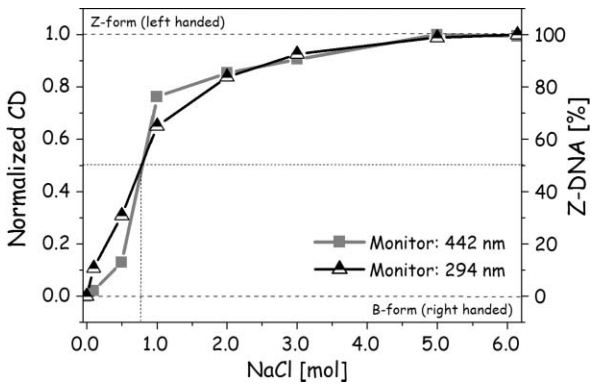

Fig. 5 The salt-induced B–Z transition of the porphyrin–ODN **1P** monitored at two different wavelengths, 294 nm (black line, triangles) and 442 nm (grey line, squares).

In order to evaluate the influence of a bulky porphyrin on the B–Z transition, we compared the sodium chloride titration curves for **1** and **1P**. The slope of the titration curve of ODN **1P** with incorporated porphyrin was steeper than the unmodified duplex **1** (Fig. 6). This result is indicative of higher cooperativity of the B–Z transition. The midpoints of the B–Z transition (the amount of NaCl needed to reach 50% Z-DNA conversion) suggest that the tetraarylporphyrin attached in the 5′-position of the ODN enhanced the salt-induced B- to Z-DNA transition. The porphyrin modified 8-mer **1P** reached 50% of the Z-DNA form at 0.75 M NaCl (Fig. 6, black curve, circles). The porphyrin-free 8-mer **1**, however, reached 50% Z-DNA form at 2.6 M NaCl (grey curve, triangles). The CD signal at 295 nm of the unmodified sequence **1** (grey curve) changed from +2.2 mdeg to −1.25 mdeg, whereas the CD signal of porphyrin–ODN **1P** (black curve) changed from

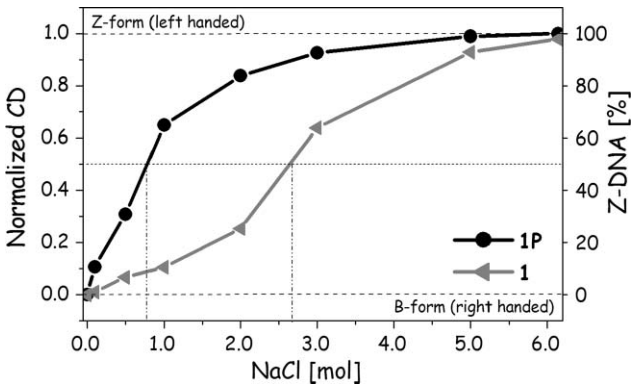

Fig. 6 The salt-induced B–Z transition of the porphyrin-ODNs **1** (grey line, triangles) and **1P** (black line, circles) monitored at 295 nm.

+3.0 mdeg to −0.5 mdeg, Fig. 3b. The weaker CD signal at 290 nm of **1P** suggests that the Z-form in the presence of porphyrin is stabilized with a smaller helical twist than in the Z-form of the corresponding porphyrin-free ODN **1**. The structure of **1P** requires more detailed study.

To further differentiate between the two observed exciton CD signals in the porphyrin Soret region at 0.0 M and 5.0 M sodium chloride and to confirm that the strong bisignate curve reports the Z-DNA form, we added a micromolar concentration of $NiCl_2$ to the NaCl saturated solution of **1P**. It is known that Ni(II) enhances the Z-DNA polymorph through an interaction with nitrogen N7 of guanine.[11] Fig. 4 shows the expected increase in intensity of the negative CD band at 295 nm as well as a decrease of the CD band at 255 nm upon addition of millimolar quantities of $NiCl_2$. It is important to note that the $NiCl_2$ addition also resulted in a similar effect on the unmodified sequence **1** (see ESI†). The porphyrin CD signal became slightly weaker (Fig. 4, black dash-dotted line) but did so without any absorption wavelength shift, thus proving that the bisignate curve observed under saturated NaCl solutions indeed belongs to the Z-conformer.

In conclusion, we have shown that if the twist and distance are favorable, the porphyrin chromophore can serve as a real time reporter of the B–Z transition *via* Soret band CD signal. The advantage of this signal is that it appears in a region that is clean and uncomplicated with other undesired spectral overlaps. We have also shown that a tetraarylporphyrin attached in the 5′-position of the studied ODN enhanced the high salt concentration cooperative B–Z transition and stabilized the Z-form with a smaller helical twist with respect to the unmodified sequence. Most notably, our end-capped approach does not significantly destabilize the B-DNA form as do the usual base-modified promoters of Z-DNA.[12] Future studies will explore the structure of the Z-form of **1P** and the effect of porphyrin on the B–Z transition of ODNs of different length and structure.

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