Chiroptical Probes

A Cationic Zinc Porphyrin as a Chiroptical Probe for Z-DNA**

Milan Balaz, Massimo De Napoli, Andrea E. Holmes, Angela Mammana, Koji Nakanishi, Nina Berova, and Roberto Purrello**

DNA is a polymorphic polymer that can adopt a variety of secondary structures ranging from the canonical right-handed B form to the left-handed Z conformation.[1] The latter conformation is less common than the right-handed B-DNA because under physiological conditions Z-DNA has a higher energy.[2–4] Although the biological role of Z-DNA still remains to be clarified, it has recently been inferred that certain classes of proteins bind to it both tightly and specifically.[5–8]

The different handedness of the two DNA forms is reported in the spectral region below 300 nm of circular dichroism (CD) spectra. B-DNA is characterized by a complex positive band centered at 274 nm and a negative Cotton effect at 253 nm, while Z-DNA exhibits a negative Cotton effect at 293 nm and a positive Cotton effect at 263 nm. Determination and sensing of Z-DNA based on these spectral differences, however, can be hampered by the concomitant presence of the two forms of DNA and/or other biomolecules such as proteins that make the region below 300 nm in the CD spectra difficult to analyze. To solve this problem, it is desirable to design chiroptical probes that discriminate between the B and Z structures and absorb above 300 nm, a region that is free from interferences, to provide characteristic induced circular dichroism (ICD) signals.

Recognition of B- and Z-DNA has been achieved by using the Δ or Λ enantiomers, respectively, of chiral complexes of transition metals (Fe, Ru, and Rh).[9,10] Recently, it has been reported that the enantiomeric pairs of daunorubicin or helicene display structural selectivity in binding to B- or Z-DNA.[11] However, none of these systems can be used as chiroptical probes because recognition of the Z or B forms is not reflected in unique spectroscopic features, such as new CD bands, which unequivocally show the detection event.

[*] Dr. M. Balaz, Dr. A. E. Holmes, Prof. K. Nakanishi, Prof. N. Berova
Department of Chemistry
Columbia University
3000 Broadway, NY, 10027 (USA)
Fax: (+1) 212-932-1289
E-mail: ndb1@columbia.edu

M. De Napoli, A. Mammana, Prof. R. Purrello
Dipartimento di Scienze Chimiche
Università di Catania
Viale Andrea Doria 6, 95125 Catania (Italy)
Fax: (+39) 095-580-138
E-mail: rpurrello@dipchi.unict.it

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Porphyrins and their metallo-derivatives are very attractive molecules because of their unique spectroscopic and geometric properties. They have been covalently linked to specific chiral sequences, and their CD spectral properties have been used to resolve complicated stereochemical issues[12–15] as well as monitor polynucleotide dynamics.[16] Also, the noncovalent interactions of cationic or anionic water-soluble achiral porphyrins with chiral templates have been exploited to build supramolecular species to detect and/or amplify the handedness of the matrix.[17,18] Achiral cationic porphyrins are indeed good candidates as noncovalent probes for right- and left-handed DNA.[19] Up to now, cationic porphyrins and metalloporphyrins have been used as reporters of different sequences of DNA bases,[20–22] but have never been employed for recognizing the handedness of a DNA helix.

Circular dichroism spectroscopy has been very useful for distinguishing the three main DNA-binding modes of porphyrins: intercalation (negative CD), outside groove binding (positive CD), and outside stacking (bisignate CD).[20,21] However, few studies have been performed on the interactions of cationic porphyrins with Z-DNA because the intercalation of porphyrins reverts the helix back to the B form.[23] The latter data suggest that pentacoordinated cationic metalloporphyrins, such as the zinc(II) derivative of *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (ZnT4, **1**; Figure 1), should be an ideal chiroptical probe for Z-DNA because the axially coordinated water molecule hinders intercalation[24–28] and prevents the Z-to-B transition.

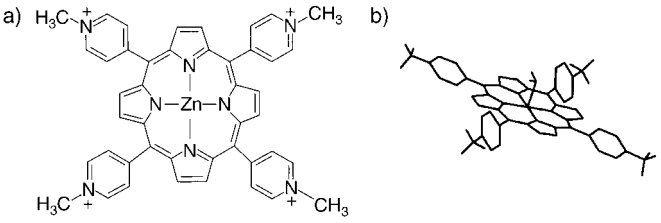

Figure 1. a) Chemical structure of zinc(II) porphyrin **1** and b) its 3D structure which shows the axially coordinated water molecule.

In our study we used poly(dG-dC)$_2$ (poly(deoxyguanylic-deoxycytidylic) acid; average base length 960), as this alternating purine–pyrimidine sequence favors the Z conformation. The B-to-Z transition can be induced in vitro by molar concentrations of NaCl, millimolar concentrations of multivalent cations such as $Co(NH_3)_6^{3+}$, or micromolar concentrations of protonated spermine.[29,30] Among these, spermine seems to be the most appropriate choice as this tetraamine, which is fully protonated at pH 7, is ubiquitous in cells and other biological systems.[31]

With B-DNA (Figure 2, blue line) only a small ICD signal is detectable in the visible region, while with Z-DNA (Figure 2, red line) a very intense bisignate curve, with $\Delta\varepsilon = -154\,M^{-1}cm^{-1}$ at $\lambda = 449$ nm and $\Delta\varepsilon = +87\,M^{-1}cm^{-1}$ at $\lambda = 433$ nm, is observed even at concentrations as low as 1 μM of **1**. The ICD spectra remain remarkably different at concentrations of **1** ranging from 1 to 8 μM (see Supporting Information) which thus shows that ZnT4 (**1**) can be highly diagnostic of the different DNA structures. The region below 300 nm in the CD spectra suggests changes in the Z-DNA helix upon interactions with **1** which become more apparent with increasing concentrations of porphyrin.

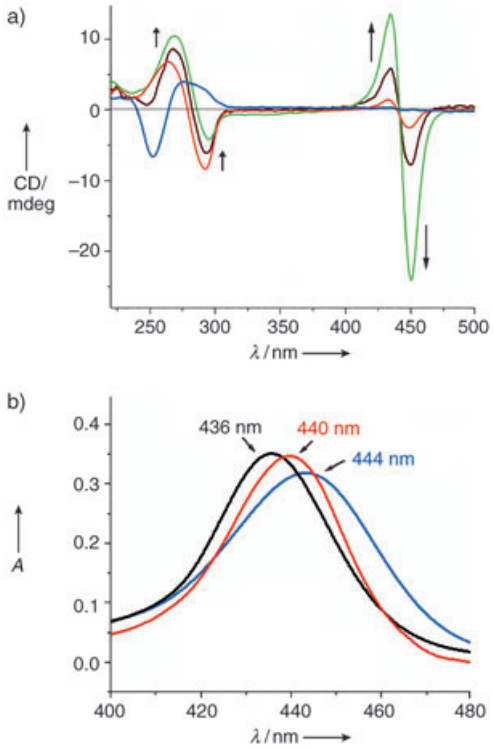

Figure 2. a) Circular dichroism (CD) spectra of Z-DNA (50 μM) in the presence of **1** (1 μM (red), 4 μM (brown), and 8 μM (green)), and B-DNA (50 μM) in the presence of **1** (1 μM; blue). b) Variation of the Soret band in the UV/Vis spectra of unbound **1** (black), **1**+Z-DNA (red), and **1**+B-DNA (blue).

So far,[21,32] observation of a ICD bisignate signal has been related to the aggregation of porphyrins. However, absorption data of ZnT4 (**1**) in the presence of Z-DNA (Figure 2b) does not support porphyrin aggregation, which should lead to quite extensive hypochromicity and broadening of the Soret band. None of these changes are observed upon binding of **1** to Z-DNA. To understand the origin of the bisignate signal we also performed fluorescence measurements, as porphyrin aggregation usually leads to severe quenching of the fluorescent emission. While the fluorescence of ZnT4 (**1**) is quenched upon complexation with B-DNA, it is only marginally affected in the presence of the Z form (Figure 3). In particular, the emission of **1** at $\lambda = 633$ nm is blue-shifted to 622 nm and quenched slightly upon binding to Z-DNA, while the intensity of the band at 670 nm increases by about 10%. This behavior clearly indicates the absence of close π–π interactions. The increase in fluorescence of **1** upon binding to Z-DNA suggests that the porphyrins lose some vibrational freedom as a result of a tighter interaction with Z-DNA than that found with B-DNA.

While the presence of the bisignate signal implies electronic communication between the porphyrin chromo-

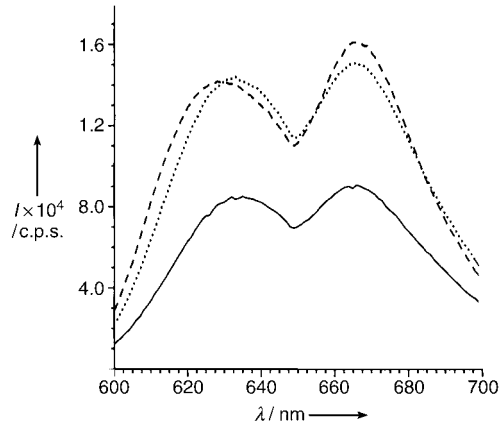

Figure 3. Fluorescence spectra (*I*/counts per second) of **1** unbound (••••), in the presence of Z-DNA (50 μM; -----), and in the presence of B-DNA (50 μM; ——).

phores (Figure 2), this is not inferred from either the absorption or fluorescence spectra (Figure 2b and Figure 3, respectively).

To better understand the binding modes, resonance light scattering (RLS) measurements were performed.[33] One advantage of RLS compared with other scattering techniques is that it provides information on the coupling between the transition dipole moments of interacting chromophores. RLS data provided a clear difference in the interactions of **1** with B- and Z-DNA (Figure 4). The RLS spectrum for the system

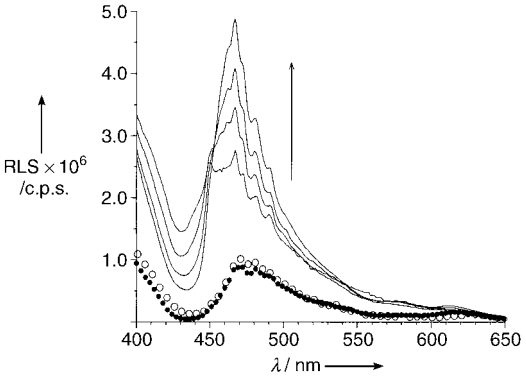

Figure 4. Resonance light scattering (RLS/counts per second) spectra of unbound **1** (2 μM; ○), **1** (2–8 μM) in the presence of B-DNA (50 μM; ●), and **1** (2, 4, 6, and 8 μM) in the presence of Z-DNA (50 μM; ——).

1·B-DNA is superimposable on that of free ZnT4 (**1**) at the same concentration, and its intensity did not change upon changing the concentration of **1**. In contrast, the intensity of the RLS spectrum of **1** in the presence of Z-DNA (**1**·Z-DNA) was higher than that of unbound **1** at the same concentration and increases with increasing concentrations of **1** (Figure 4). Therefore, in agreement with ICD data, RLS indicates that the porphyrins ZnT4 (**1**) are electronically coupled on Z-DNA but not on B-DNA.

Thus the CD, absorption, fluorescence, and RLS spectra show a profound difference in the binding modes of **1** with the two forms of DNA, B and Z . ICD and RLS data reveal that the porphyrin probes are dispersed disorderly on B-DNA and, therefore, not involved in interporphyrin coupling. In contrast, the structure of Z-DNA induces an ordered distribution of **1** which allows electronic coupling between porphyrin molecules that are not in close π–π contact (according to fluorescence measurements).

The different modes of interporphyrin coupling observed with B- or Z-DNA suggests that interaction of ZnT4 (**1**) with Z-DNA involves an additional binding site. Moreover, it is quite unlikely that only the different geometry of the phosphate backbone of the two DNA forms might cause such a different distribution of the porphyrin molecules. Inspection of the structure of Z-DNA reveals that the alternate *anti* and *syn* conformation of nucleobases leads to exposure of guanine atoms N7 and C8, which are both shielded in the B form.[1,3] In Z-DNA, nitrogen atom N7 is available for coordination with transition-metal ions, as shown previously by IR,[34,35] and therefore can provide a site for axial coordination with the central zinc atom of **1**. This hypothesis is corroborated by recent fluorescence studies which reported that the interactions of a cationic zinc(II) porphyrin with polynucleotides caused a blue shift in the emission of the porphyrin despite the red shift in the absorption Soret band,[28,36] similar to what is observed here in the emission of **1**·Z-DNA. The "anomalous" shift of the emission maximum of the porphyrin was interpreted in that case as arising from a change in the binding with respect to the fifth ligand, that is, upon changing from a molecule of water to DNA. The change in the fifth ligand around the Zn(II) ion may also be the case here for **1**·Z-DNA, with substitution of the axial water molecule in ZnT4 (**1**) by guanine N7 of Z-DNA.

Compelling evidence for the occurrence of this binding event came from a competition experiment. Figure 5 shows the decrease in ICD in the Soret region of **1**·Z-DNA and the partial restoration of the original CD features of unbound Z-DNA upon addition of micromolar concentrations of Ni^{II} ions to a solution of the DNA–porphyrin complex. This behavior can be explained by the complexation of Ni^{II} ions to site N7 in Z-DNA and the simultaneous release of porphyrins **1**, together with the strains related to their binding as shown

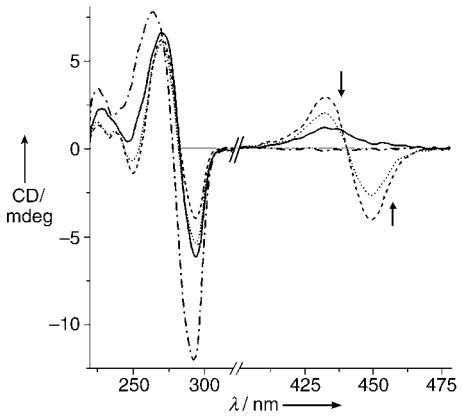

Figure 5. CD spectra of Z-DNA (–•–•), complex **1**·Z-DNA (-----), and **1**·Z-DNA after the addition of $NiCl_2$ (5 μM (••••) and 46 μM (——)).

by the decrease of the ICD of **1** and the partial restoration of the CD spectrum of Z-DNA in Figure 5. A possible model of the **1**·Z-DNA complex is shown schematically in Figure 6b.

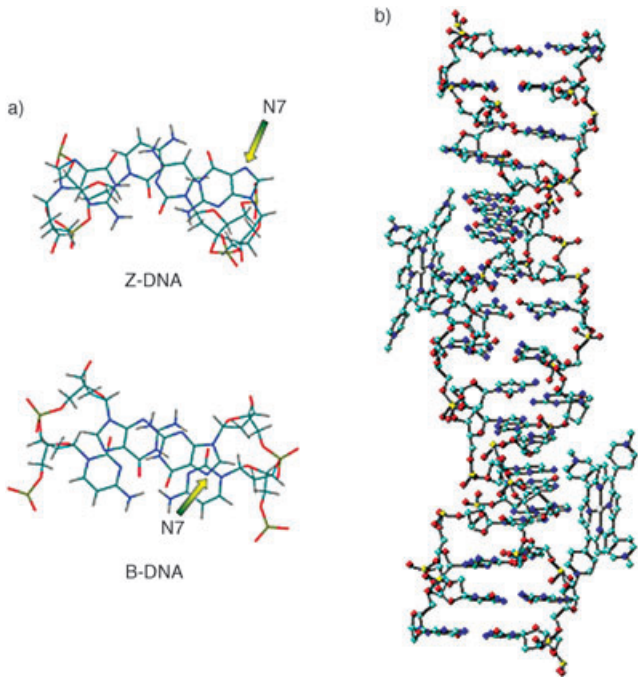

Figure 6. a) Stacking diagram demonstrating the overlap of successive bases (CpG = cytidine-phosphate–guanosine) along the helices of Z- and B-DNA (the arrows point to guanine N7 to highlight their different environments in the two forms), and b) schematic of the proposed structure of the complex **1**·Z-DNA.

In conclusion we have shown that cationic zinc porphyrin **1** is a promising chiroptical probe, which operates at micromolar concentrations, for the structure of left-handed Z-DNA. By application of different spectroscopic techniques, we have proposed the possible origin for a clear-cut discrimination between B- and Z-DNA in the presence of zinc porphyrin **1**. We have clarified the interaction of **1** with left-handed Z-DNA which in turn has led us to propose a structure for the complex **1**·Z-DNA. Studies aimed to better understand the interaction between Z-DNA and porphyrin molecules as well as the design of new porphyrin derivatives with improved discrimination abilities are underway.

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