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Chiroptical Detection Of Condensed Nickel(II)-Z-DNA In The Presence Of The B-DNA Via Porphyrin Exciton Coupled Circular Dichroism

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Abstract

Here, we report a highly sensitive and specific chiroptical detection method of condensed left-handed ZDNA in the presence of canonical right-handed B-DNA. The selective formation of a left-handed cytosine-guanine oligonucleotide (CG ODN) in the presence of a right-handed adenine-thymine oligonucleotide (AT ODN) was induced by millimolar concentrations of NiCl₂ and confirmed by electronic circular dichroism. The nickel(II) induced B-to-Z DNA transition of the CG ODN was accompanied by the concurrent condensation of the Ni(II)-Z-DNA as confirmed by resonance light scattering, transmission spectroscopy, and centrifugation. The selective condensation of the CG ODN allowed its separation from the AT ODN using centrifugation. No structural changes were observed for the AT ODN upon addition of Ni(II). Anionic nickel(II) *meso*-tetra(4-sulfonatophenyl) porphyrin (NiTPPS) spectroscopically detected the left-handed Z-DNA in the Z-DNA/B-DNA mixture via a strong exciton coupled circular dichroism (ECCD) signal induced in the porphyrin Soret band absorption region. The bisignate ECCD signal originates from the assembly of achiral porphyrins into helical arrays by intermolecular interactions with the condensed Z-DNA scaffold. No induced CD signal was observed for the Ni(II)-B-DNA-NiTPPS complex. Hence, an unambiguous spectroscopic recognition of Ni(II) induced condensed Z-DNA in the presence of B-DNA is possible. The sensitivity of this chiroptical method was as low as 5% of the Z-DNA (4.4 μmol base pair concentration) in the presence of 95% of B-DNA (80 μmol). Thus, NiTPPS is a highly sensitive probe for applications in bio-sensing via the CD signal amplification.

Keywords

Z-DNA detection; anionic nickel porphyrin; circular dichroism; DNA condensation; aggregation

INTRODUCTION

Intermolecular recognition in nucleic acids is among the most important phenomena of living systems. DNA can adopt various conformations that are very different from the canonical right-handed B-form double-helix. DNA polymorphism is believed to play a significant role in various biological processes including in genetic instability and

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SUPPORTING INFORMATION Additional circular dichroism and resonance light scattering spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

evolution.^{1–3} Left-handed Z-DNA is a minor DNA structure within the B-DNA and stabilized *in vivo* by negative supercoiling, mutations, or by Z-DNA binding proteins.^{4–12} The existence of proteins that bind Z-DNA with high affinity and specificity suggests that Z-DNA plays an important role *in vivo*.^{13–15} The Z-DNA binding domain in proteins (called Za) was first identified in the RNA-specific adenosine deaminase gene (ADAR1).^{9, 12} There is evidence that Z-DNA forms near a transcription start site in human cells resulting in the up-regulation of transcription of the factor-1 gene (CSF-1).¹⁶ Recently, however, inhibition of transcription was reported by the formation of a short Z-DNA sequence near a transcription site involving T7 RNA polymerase.¹⁷ Further biological significance of Z-DNA *in vivo* is demonstrated by the fact that Za in protein E3L (*vaccinia* virus) acts as a transcription modulator of several apoptosis-related genes.¹⁵ *In vitro*, Z-DNA can be stabilized by multivalent cations like $\text{Co}(\text{NH}_3)_6^{3+}$, spermine⁴⁺, spermidine³⁺,¹⁸ and very high salt environment.¹⁹ DNA mutations such as the 5-methylcytosine, 5-bromocytosine, 8-methylguanine, and 8-bromoguanine can also stabilize the left-handed Z-DNA form *in vitro*.^{20–22}

Circular dichroism (CD) is the technique commonly used to study DNA transitions as well as to identify different DNA structures.²³ However, studying one particular DNA form can be hampered due to the CD spectral overlap with other forms of DNA and chiral biomolecules that absorb light at similar wavelengths. It has been shown that the ECCD signal of porphyrin can be of higher sensitivity than the signal of the classical electronic CD (e.g. exciton chirality method),²⁴ and the porphyrin's absorption in the visible region is advantageous because this wavelength does not overlap with the UV absorbing chromophores of the DNA. DNA recognition using small molecular probes is a fast and convenient way for detecting different DNA conformations and both chiral and achiral molecular probes have been explored.²⁵ Specifically, Norden and Tjerneld were the first to report the preference of Δ enantiomer *tris*(dipyridyl)Fe(II) to bind to right-handed B-form DNA.²⁶ The Barton group has shown that chiral transition metal complexes recognize specific nucleic acid binding sites.^{27–29} Tanaka *et al.* reported *P*-helicenes selectively bind to Z-DNA, albeit accompanied by the undesired B-to-Z transition.³⁰ Furthermore, covalently linked probes were used to monitor the B-to-Z DNA transition.^{31–39} Metalloporphyrins are one of the most studied DNA binding agents due to their structural, photophysical and spectroscopic properties. Most studies focused on cationic porphyrins^{40–44} whereas negatively charged porphyrins have attracted, so far, much less attention.^{45–50} We have previously shown the spectroscopic detection of left-handed Z-DNA using achiral anionic or cationic metalloporphyrin probes. An ECCD signal was observed in the porphyrin absorption region (~400 nm) upon binding of a zinc or nickel porphyrin to Z-DNA, while no exciton coupled CD signal was observed in the presence of B-DNA.^{45, 51} The observed CD signal originated from an preferential interaction of the achiral porphyrins to the Z-DNA form.⁵²

Since Z-DNA is of higher energy than B-DNA, only a small fraction of DNA is in the left-handed conformation *in vivo*.^{2, 53, 54} Recent single-molecule studies using fluorescence assays showed that ZDNA is dynamically formed and only then stabilized by Z-DNA binding proteins through efficient trapping of the Z-conformation.⁵⁵ No induction of B-to-Z-DNA transition by Z-DNA binding proteins have been observed. Thus, it is important to explore probes that are able to selectively and sensitively detect native Z-DNA in the presence of B-DNA without altering the B-Z DNA equilibrium. To the best of our knowledge, detection of synthetically unmodified Z-DNA in the presence of B-DNA has not been reported yet. Here, we describe the selective formation and condensation of the Z-form of a cytosineguanine oligodeoxynucleotide (CG ODN) *in the presence* of the B-form adenine-thymine oligodeoxynucleotide (AT ODNs) and its spectroscopic detection using an induced CD signal of the anionic nickel(II) *meso*-tetra(4-sulfonatophenyl) porphyrin,

NiTPPS (Chart 1). A strong bisignate ECD signal is observed in the porphyrin Soret band region in the presence of any mixture of CG ZDNA and AT B-DNA whereas **NiTPPS** is spectroscopically silent in the presence of B-DNA only.

EXPERIMENTAL SECTION

Materials

Anionic nickel(II) porphyrin **NiTPPS** was purchased from Frontier Scientific, sodium cacodylate from Sigma-Aldrich, and HPLC purified ODNs from AlphaDNA. Water was obtained from Milli-Q system with a resistivity of 18.2 M $\Omega \cdot \text{cm}$. DNA samples were dissolved in sodium cacodylate buffer (1 mM, pH 7.0) and annealed at 80 °C for 20 min, then cooled at 1 °C/min and kept at 4 °C. The concentration of DNA stock solutions was quantified by UV-vis spectroscopy. Concentration of ODNs is per base pair. **NiTPPS** stock solution ($c = 0.83 \text{ mM}$) was prepared in sodium cacodylate buffer (1 mM, pH 7.0) and the concentration was determined by UV-vis spectroscopy using extinction coefficient ($\epsilon = 2.7 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ at 409 nm).

Circular Dichroism Spectroscopy

CD spectra were recorded at 20 °C using a Jasco J-815 spectropolarimeter equipped with a single position Peltier temperature control system. Scanning speed 50 nm/min, data pitch 0.5 nm, D.I.T. 2 sec, bandwidth 1 nm. A quartz cuvette with a 1 cm path length was used for all CD experiments. Each CD spectrum was an average of at least three scans.

UV-vis Absorption Spectroscopy

UV-vis absorption spectra were collected at 20 °C using a Jasco V-600 UV-vis double beam spectrophotometer equipped with a single position Peltier temperature control system. A quartz cuvette with a 1 cm path length was used for all UV-vis experiments.

Resonance Light Scattering

Resonance light scattering data were recorded at 20 °C using a Varian Cary Eclipse fluorescence spectrophotometer equipped with a Peltier temperature control system in a synchronous scan mode. Excitation slit 2.5 nm, emission slit 2.5 nm, scan rate 600 nm/min.

Sample Preparation

Method A1) Add buffer, CG 48mer, and 100 mM NiCl_2 into a vial A. 2) Add CG 48mer, vortex for 5s, wait for 5 min, then measure CD. 3) Add AT 48mer. Vortex for 5 s, wait 5 min, then measure CD. 4) Add 50 mM NiCl_2 , vortex for 5 s, wait 5 min, then measure CD. 5) Add **NiTPPS**, vortex the mixture for 5 s, wait 5 min, and then measure the CD. Method B1: 1) Add buffer, CG 48mer, and 100 mM MCl_2 into a vial A. 2) Add buffer and AT 48mer into a vial B. 3) Add the content of vial B to the vial A, vortex it for 5 s, place in the CD instrument, wait 5 min, and then measure the CD. 4) Add **NiTPPS**, vortex the mixture for 5 s, wait 5 min and then measure the CD. Method B2: 1) Add buffer and CG 48mer into a vial A. 2) Put buffer, AT 48mer, and 100 mM MCl_2 into a vial B. 3) Add the content of vial B to the vial A, vortex it for 5 s, place in the CD instrument, wait 5 min, and then measure the CD. 4) Add **NiTPPS**, vortex the mixture for 5 s, wait 5 min and then measure the CD.

RESULTS AND DISCUSSION

Selective induction and condensation of Z-DNA in the presence of B-DNA

We selected the 48mer 5'-(dGdC)₂₄ (CG Z-DNA) and the 48mer 5'-(dAdT)₂₄ (AT B-DNA) ODNs for our initial experiments (Chart 1). NiCl₂ was used as a Z-DNA inducer. Detection of Z-DNA in the presence of B-DNA requires these two DNA forms to coexist experimentally (Figure 1). It is thus imperative to choose a Z-DNA inducer allowing for such a selective scenario. Nickel(II) has been shown to induce left-handed Z-form of alternating CG polynucleotides and oligonucleotides whereas no B-Z DNA transition was observed for AT oligonucleotides.⁵⁶ The different behavior of AT and CG ODNs comes from a strong and specific binding of Ni(II) to the nitrogen N7 of guanine in the major groove resulting in the Z-DNA formation.^{57, 58} On the other hand, the interaction of Ni(II) with AT DNA bases is nonspecific without the B-to-Z DNA conformational change. However the formation of Z-DNA in the presence of B-DNA has not been reported previously.

No more than 2 mM concentration of NiCl₂ was necessary to invert the helicity of the 48mer 5'-(dGdC)₂₄ from the B to the Z conformation.⁵⁰ The appearance of the negative CD band at 290 nm, the positive CD band at 280 nm, and increase in magnitude of the negative CD band at 205 nm was observed and confirmed the inversion.^{23, 59–61} Furthermore, UV-Vis absorption spectroscopy showed a decreased transmittance suggesting the formation of aggregates. Nickel induced DNA condensation has been previously reported for polymeric DNA sequences such as the poly(dG-dC)₂ (~800 base pairs).⁵⁶ Supporting evidence for the condensation of the CG 48mer 5'-(dGdC)₂₄ came from the resonance light scattering (RLS) experiments. RLS has proved to be a very useful technique for studying electronically coupled arrays of chromophores where an enhanced signal of several order of magnitudes can be observed at the absorption bands of the aggregates.⁶² RLS data confirmed condensation upon addition of NiCl₂ to the CG 48mer 5'-(dGdC)₂₄ and a strong RLS signal was observed (Figure 2a, blue line). An analysis of the dependence of the CD and RLS signals on the Ni(II) concentration has revealed that the B–Z DNA transition is accompanied by the spontaneous and concomitant Z-DNA condensation (Figure 2b). The AT 48mer 5'-(dAdT)₂₄ remained in B-conformation upon NiCl₂ addition and no condensation was observed (almost no RLS signal, Figure 2a, green line). Hence NiCl₂ should not only allow the Z-form of the 48mer 5'-(dGdC)₂₄ to coexist in solution with the B-form of 48mer 5'-(dAdT)₂₄ but also to selectively condense the CG ODN in the presence of the AT ODN.

The 48mer 5'-(dAdT)₂₄ (80 μM) was added to the 48mer 5'-(dGdC)₂₄ (80 μM) in cacodylate buffer (1 mM, pH=7.0) followed by a stepwise addition of NiCl₂ (Method A). The appearance of the negative CD band at 290 nm and disappearance of negative band at 250 nm indicated the formation of Z-DNA (Figure 3, black curve). The coexistence of B-DNA and Z-DNA was confirmed by subtraction of the BDNA and Z-DNA CD signals, respectively, from the CD signal of the Z+B-DNA mixture. The subtraction of the Z-DNA CD signal from the Z+B-DNA CD signal gave the CD profile characteristic of right-handed B-DNA (positive CD band at 265 nm, negative CD band at 250 nm, Figure 3, red curve) while the subtraction of B-DNA CD signal gave a spectrum characteristic of Z-DNA (negative CD band at 290 nm, positive CD band at 275 nm, Figure 3, green curve). RLS experiments showed that the presence of AT 48mer does not significantly affect the condensation of the CG 48mer by NiCl₂. Figure 2 shows that similar RLS signals were observed for the CG 48mer and the 1:1 mixture of CG 48mer and AT 48mer (Figure 2, blue and red curves).

In order to test the participation of AT 48mer in the CG 48mer condensation along with possible CG+AT cross-condensation, we conducted simple centrifugation experiments. A

1:1 mixture of the CG 48mer and AT 48mer (80 μ M each) with 50 mM NiCl_2 (all together 2 mL) was centrifuged at 14500 rpm for 30 min in an Eppendorf MiniSpin plus centrifuge. The sample was then carefully divided into two parts: top and bottom half, 1.0 mL each. CD spectra of the upper half showed the presence of the B-form DNA with almost identical profile as the neat AT48mer in the presence of NiCl_2 (Figure 4a). The identity of the AT48mer was confirmed by a reverse phase HPLC. The CD spectrum did not show a Z-DNA CD signal demonstrating that no Z-DNA was present in the upper layer (Figure 4a, black curve). The re suspended bottom part of the solution showed the CD profile characteristic of Z-DNA with a negative CD band at ~ 295 nm. The UV-vis absorption spectra of the upper layer indicated that more than 90% of non-condensed B-form of the AT48mer stayed in the supernatant and did not precipitate (Figure 4b). The CD and UV-vis data thus suggested that NiCl_2 induced a selective condensation of the Z-form of the CG ODN in the presence of the B-form AT ODN. Only a small amount of cross-condensation was observed by a small decrease of the absorption signal ($\Delta A \sim 10\%$). Notably, a simple centrifugation allowed an efficient separation of the cytosine-guanine ODN from thymine-adenine ODN.

Detection of Z-DNA in the presence of B-DNA

Once the experimental conditions for the coexistence of B and Z-DNA were established, we investigated the ability of **NiTPPS** to spectroscopically detect CG Z-DNA in the presence of AT B-DNA. It appeared that **NiTPPS** is very efficient in detecting Z-DNA in the presence of B-DNA: a strong bisignate ECCD in the Soret absorption region with the positive Cotton effect at 415 nm and the negative Cotton effect at 392 nm was observed upon titration of **NiTPPS** to the Z+B-DNA mixture (80 μ M + 80 μ M) induced by 50 mM NiCl_2 (Figure 5). The ECCD signal increased steadily with increasing concentration of the **NiTPPS** from 2 μ M to 14 μ M (inset, Figure 5). As we have reported previously, the ECCD signal originates from the electronic dipole-dipole interaction between the porphyrins that are arranged helically on the DNA scaffold.^{52, 63} No changes were observed in the DNA region of the CD spectra (<300 nm) upon addition of **NiTPPS** indicating that the neither Z-DNA, B-DNA, or the equilibrium are disturbed.

No ECCD signal was observed when **NiTPPS** was titrated to the 5'-(dAdT)₂₄ (Figure S1) under identical experimental conditions (50 mM NiCl_2 , 1 mM cacodylate buffer, pH=7.0). Similarly, no ECCD signal was observed for the B+B-DNA mixture prepared of 48mer 5'-(dAdT)₂₄ (80 μ M) and the 48mer 5'-(dGdC)₂₄ (80 μ M) in the absence of NiCl_2 or in the presence of 50 mM MgCl_2 . Resonance light scattering experiments showed aggregation of **NiTPPS** upon its titration to the solutions of (CG 48mer + 50 mM NiCl_2), (AT 48mer + 50 mM NiCl_2), (CG 48mer + AT 48mer + 50 mM NiCl_2), or 50 mM NiCl_2 (Figure 6). The level of aggregation of **NiTPPS** on DNA is similar for CG and AT ODNs, as judged by the ΔRLS (Figure 6, inset). Although **NiTPPS** aggregation occurred in the presence of all DNA sequences, clearly, under our experimental condition, the B-forms of 48mer 5'-(dGdC)₂₄ and 5'-(dAdT)₂₄ do not provide the scaffold for the helical porphyrin assembly necessary for the ECCD signal to occur. No aggregation was observed for **NiTPPS** in cacodylate buffer in the absence of NiCl_2 .

UV-Vis absorption titration experiments of **NiTPPS** (from 2 μ M to 14 μ M) to the Z+B-DNA mixture as well as the AT B-DNA and CG Z-DNA alone (80 μ M each with 50 mM NiCl_2) were performed to further evaluate the interaction between the **NiTPPS** and ODNs (Figure 7). Addition of 2 μ M of **NiTPPS** to the B+Z DNA mixture consisted of the Z-form of the 48mer 5'-(dGdC)₂₄ and B-form of 48mer 5'-(dAdT)₂₄ (80 μ M + 80 μ M + 50 mM NiCl_2 , method A) led to a blue shift of the Soret band (from 402.6 nm to 401.6 nm, $\Delta\lambda = 1.0$ nm, Inset Figure 7, red line) in comparison to the absorption characteristics of the **NiTPPS** in 50 mM NiCl_2 in the absence of a DNA. Mixing the **NiTPPS** (2 μ M) with the B-form of

48mer 5'-(dAdT)₂₄ (80 μ M + 50 mM) gave a small bathochromic shift of the Soret band (from 402.6 nm to 403 nm, $\Delta\lambda$ = 0.4 nm, Inset Figure 7). Addition of 2 μ M of **NiTPPS** to the 48mer 5'-(dGdC)₂₄ (80 μ M + 50 mM NiCl₂) caused the hypsochromic shift of the Soret band (from 402.6 nm to 399.6 nm, $\Delta\lambda$ = 3.0 nm, Inset Figure 7). Minor changes of UV-vis spectra and small hypochromicity suggest the outside DNA groove binding of porphyrin.^{41, 64} The blue shift of **NiTPPS** absorption band observed for CG ODN suggests a cofacial porphyrin arrangement.^{65, 66}

The sensitivity and selectivity of **NiTPPS** as a chiroptical Z-DNA sensor were evaluated in the presence of B-DNA. The total concentration of the DNA was decreased while keeping the ratio of B-DNA to Z-DNA constant (1:1). The 48mer 5'-(dAdT)₂₄ (4.4 μ M) was added to the 48mer 5'-(dGdC)₂₄ (4.4 μ M) in cacodylate buffer (1 mM, pH=7.0) followed by addition of NiCl₂ (50 mM, method A). The coexistence of the Z-DNA and B-DNA was confirmed by CD by subtraction of the respective chiroptical signals. At these lower concentrations, unlike the 80 μ M Z-DNA + 80 μ M B-DNA mixture, the CD signal of the Z-DNA in the classic diagnostic window was significantly less pronounced with only a small negative CD band at 290 nm and did not allow to conclusively confirm the presence of the Z-form (Figures S2). However, in the visible region at 400 nm, **NiTPPS** selectively detected the Z-form in the Z+B DNA mixture as judged by the presence of the bisignate ECCD signal (Figures S3). The detection of Z-DNA in the presence of a shorter B-DNA sequence, using AT 24mer instead of AT 48mer, was also explored. The titration of **NiTPPS** to the solution consisting of 20 μ M CG 48mer, 20 μ M AT 24mer and 50 mM NiCl₂ gave rise to a strong bisignate ECCD, clearly showing of the presence of Z-DNA (Figures S4).

In order to study the selectivity of **NiTPPS** in Z-DNA detection in the presence of B-DNA, we decreased the content of Z-DNA from 50% to 10% while keeping the concentration of the B-DNA at 80 μ M. The 48mer 5'-(dAdT)₂₄ (80 μ M) was added to the 48mer 5'-(dGdC)₂₄ (8.9 μ M) in cacodylate buffer (1 mM, pH=7.0) followed by addition of NiCl₂ (50 mM, method A). The presence of both forms of DNA was confirmed by the CD and subtraction method as described above (Figure S5). Again, the addition of **NiTPPS** gave rise to a bisignate ECCD signal confirming the presence of Z-DNA (Figure S6). Upon further decrease of the Z-DNA concentration to 5% (4.4 μ M Z-DNA + 80 μ M B-DNA + 50 mM NiCl₂), the CD spectrum was too weak to unambiguously confirm the presence of Z-DNA (Figure 8a, red curve). Thus, the limit of Z-DNA detection by CD spectroscopy has been reached for the UV region. However, the addition of **NiTPPS** gave a clear-cut ECCD signal of the Z-DNA in the porphyrin Soret band region (Figure 8b). **NiTPPS** was able to successfully detect 5% Z-DNA in the mixture of 95% of AT B-DNA confirming its high selectivity and sensitivity for the left-handed DNA form.

In all experiments described so far, NiCl₂ was added to the mixture of CG+AT ODNs (method A). Thus, the formation and condensation of Z-form of CG ODN always happened in the presence of AT ODN. The presence of AT ODN during the B-to-Z DNA transition can, however, influence the structure of the Z-DNA and/or the structure of Z-DNA **NiTPPS** aggregates. Although the B- and Z-DNA are in equilibrium, once the condensed Z-DNA is formed, the B-Z DNA equilibrium may be hindered. To test whether the order of addition and the presence of AT ODN influenced the Z-DNA recognition by **NiTPPS** two additional set of experiments were performed. In the method B1, NiCl₂ was added to the 48mer 5'-(dGdC)₂₄ ([NiCl₂] = 50 mM), no NiCl₂ was added to the AT 48mer and only then CG and AT ODNs were mixed together. In the method B2, NiCl₂ was added to the AT 48mer ([NiCl₂] = 50 mM) and no NiCl₂ was added to the 48mer 5'-(dGdC)₂₄, before the ODNs were mixed together. Upon ODNs mixing, the final Ni²⁺ concentration in both cases was 50 mM. In all experiments, the presence of the Z-DNA form was confirmed by CD and subsequent subtractions as described above. Addition of **NiTPPS** to the mixtures of 48mer

5'-(dGdC)₂₄ and AT 48mer prepared by Methods B1 or B2 gave almost identical results as those obtained with Method A, *i.e.* strong bisignate CD signal with the positive CD band at 415 nm and the negative CD band at 390 nm (Figures S7, S8). These results confirmed that the order in which NiCl₂ was added to DNA had no effect on the formation or detection of Z-DNA in the presence of B-DNA.

CONCLUSION

For the first time, the selective formation, condensation, and sensitive spectroscopic detection of guanine-cytosine Z-DNA by Ni(II) in the presence of excess of the adenine-thymine B-DNA is reported. NiCl₂ selectively induced and condensed cytosine-guanine Z-DNA in the presence of adenine-thymine DNA. The selective condensation of the Z-form of the guanine-cytosine ODN allowed its separation from the adenine-thymine ODN using simple centrifugation. Almost no cross-condensation was observed as evidenced by UV-vis and CD spectroscopy. The achiral chiroptical porphyrin probe **NiTPPS** selectively detected the condensed Z-form of 48mer ODN 5'-(dGdC)₂₄ in the presence of the B-form of the 48mer ODN 5'-(dAdT)₂₄ and the 48mer ODN 5'-(dAdT)₂₄ at low Z-DNA to B-DNA ratio (5:95) as well as low Z-DNA concentrations (5 μM base pair concentration). The Z-DNA sensing was achieved via the induced ECCD signal of NiTPPS observed in the Soret band absorption region (~400 nm) avoiding overlap with the CD signal of the DNA and other chiral naturally occurring chromophores. The induced CD signal originated from the interaction between helically oriented **NiTPPS** units bound to the Z-DNA backbone. The Z-DNA detection in the presence of the AT B-DNA is far more sensitive using the **NiTPPS** chiroptical probe than the DNA spectral region (<300 nm) of the electronic CD spectrum. The **NiTPPS** interaction with the neat AT B-DNAs did not give rise to an ECCD signal (**NiTPPS** is chiroptically silent in the presence of the AT B-DNAs).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Chart 1.
Structure of nickel(II) *meso*-tetra(4-sulfonatophenyl) porphyrin, **NiTPPS**, and DNA sequences used in this study.

Figure 1. Schematic representation of the selective formation, condensation and detection of the left-handed Z-DNA in the presence of the right-handed B-DNA using **NiTPPS** chiroptical probe. Chiral **NiTPPS** assemblies formed on the Z-DNA give rise to the exciton coupled CD signal.

Figure 2.

a) RLS spectra of the CG 48mer 5'-(dGdC)₂₄ (blue curve), AT 48mer 5'-(dAdT)₂₄ (green curve), and the mixture of the CG 48mer + AT 48mer (red curve) upon addition of 50 mM NiCl_2 . b) Normalized intensity of the CD signal at 250 nm (black circles) and RLS signal at 490 nm (blue squares) of the CG 48mer 5'-(dGdC)₂₄ as a function of NiCl_2 concentration. Condition: $[5'-(\text{dGdC})_{24}] = 80 \mu\text{M}$, $[5'-(\text{dAdT})_{24}] = 80 \mu\text{M}$, Na-cacodylate buffer (1 mM, pH = 7.0).

Figure 3.

a) CD spectra of the mixture of the CG 48mer and the AT 48mer in the absence (blue curve, B+B DNA) and presence (black curve, Z+B DNA) of 50 mM NiCl_2 . b) CD spectra of Z-form of the 48mer 5'-(dGdC)₂₄ induced in the presence of B-form of the 48mer 5'-(dAdT)₂₄ with 50 mM NiCl_2 obtained by subtraction of B-DNA CD signal (green curve). Condition: [5'-(dGdC)₂₄] = 80 μM , [5'-(dAdT)₂₄] = 80 μM , 50 mM NiCl_2 , Na-cacodylate buffer (1 mM, pH = 7.0).

Figure 4.

CD spectra (a) and UV-vis absorption spectra (b) of the upper half of the solution obtained upon centrifugation (black curve) and a freshly prepared solution of 48mer 5'-(dAdT)₂₄ in the presence of 50 mM NiCl₂ (green curve). Condition: [5'-(dGdC)₂₄] = 80 μ M, [5'-(dAdT)₂₄] = 80 μ M, 50 mM NiCl₂, Na-cacodylate buffer (1 mM, pH = 7.0), centrifugation: 14500 rpm, 30 min.

Figure 5. CD spectra of the mixture of Z-form of the 48mer 5'-(dGdC)₂₄ and B-form of 48mer 5'-(dAdT)₂₄ titrated with **NiTPPS**. Inset: intensity of the ECD as a function of **NiTPPS** concentration. Condition: [5'-(dGdC)₂₄] = 80 μM, [5'-(dAdT)₂₄] = 80 μM, 50 mM NiCl₂, Na-cacodylate buffer (1 mM, pH = 7.0).

Figure 6.

RLS spectra of the **NiTPPS** added to a) the B-form AT 48mer 5'-(dAdT)₂₄, b) the Z-form CG 48mer 5'-(dGdC)₂₄, and c) the 1:1 Z+B DNA mixture in the presence of 50 mM NiCl₂. Inset: increased in intensity of the RLS at 490 nm as a function of **NiTPPS** concentration. Condition: [5'-(dGdC)₂₄] = 80 μM, [5'-(dAdT)₂₄] = 80 μM, Na-cacodylate buffer (1 mM, pH = 7.0).

Figure 7.

UV-vis absorption spectra of **NiTPPS** added to the Z-form of the 48mer 5'-(dGdC)₂₄ (blue curve), to the B-form of 48mer 5'-(dAdT)₂₄ (green curve), to the B+Z DNA mixture (1:1, red curve) consisted of CG 48mer and AT 48mer. Inset: Soret band absorption wavelength as a function of **NiTPPS** concentration. Condition: [5'-(dGdC)₂₄] = 80 μM, [5'-(dAdT)₂₄] = 80 μM, 50 mM NiCl₂, Nacacodylate buffer (1 mM, pH = 7.0), method A.

Figure 8.

a) CD spectra of the 5:95 mixture of Z-form 48mer 5'-(dGdC)₂₄ and B-form 48mer 5'-(dAdT)₂₄ (black curve) and the CD spectrum of the Z-form obtained by subtraction from the CD spectrum of Z+B DNA mixture (red curve). b) ECCD observed upon titration of **NiTPPS** into the 5:95 mixture of Z-form 48mer 5'-(dGdC)₂₄ and B-form of 48mer 5'-(dAdT)₂₄. Condition: [5'-(dGdC)₂₄] = 4.4 μM, [5'-(dAdT)₂₄] = 80 μM, 50 mM NiCl₂, Na-cacodylate buffer (1 mM, pH = 7.0).