THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

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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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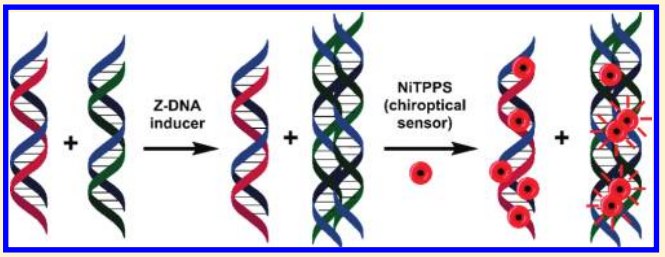
Supporting Information

ABSTRACT: Here, we report a highly sensitive and specific chiroptical detection method of condensed left-handed Z-DNA 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 $NiCl_2$ 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% B-DNA (80 μmol). Thus, NiTPPS is a highly sensitive probe for applications in biosensing via the CD signal amplification.



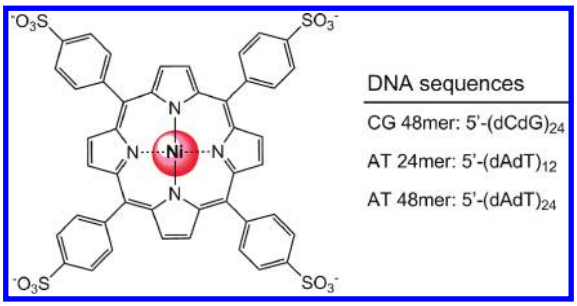
INTRODUCTION

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 thought to play a significant role in various biological processes, including in genetic instability and evolution.[1−3] Left-handed Z-DNA is a minor DNA structure within the B-DNA and is stabilized in vivo by negative supercoiling, by 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 Zα) 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 the transcription of the factor-1 gene (CSF-1).[16] However, the inhibition of transcription by the formation of a short Z-DNA sequence near a transcription site involving T7 RNA polymerase was reported.[17] Further biological significance of Z-DNA in vivo is demonstrated by the fact that Zα 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 such as $Co(NH_3)_6^{3+}$, spermine^{4+}, and spermidine^{3+} [18] and a very high salt environment.[19] DNA mutations such as 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 as a result of 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 the Δ enantiomer

Received: May 21, 2011
Revised: July 13, 2011
Published: July 20, 2011

Chart 1. Structure of Nickel(II) *meso*-Tetra (4-sulfonatophenyl) Porphyrin (NiTPPS) and the DNA Sequences Used in This Study



tris(dipyridyl)Fe(II) to bind to the 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 that *P*-helicenes selectively bind to Z-DNA, albeit accompanied by the undesired B- to Z-DNA 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 because of 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 a chiral arrangement of the achiral porphyrins on the Z-DNA.[52]

Because 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] Single-molecule studies using fluorescence assays showed that Z-DNA is dynamically formed and is only then stabilized by Z-DNA binding proteins through the efficient trapping of the Z-conformation.[55] No inductions of the 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, the 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 cytosine−guanine 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 ECCD signal is observed in the porphyrin Soret band region in the presence of any mixture of CG Z-DNA 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 a Milli-Q system with a resistivity of 18.2 MΩ·cm. DNA samples were dissolved in a sodium cacodylate buffer (1 mM, pH 7.0), annealed at 80 °C for 20 min, cooled at 1 °C/min, and kept at 4 °C. The concentration of the DNA stock solutions was quantified by UV−vis spectroscopy. The concentration of ODNs is per base pair. The NiTPPS stock solution (c = 0.83 mM) was prepared in a sodium cacodylate buffer (1 mM, pH 7.0), and the concentration was determined by UV−vis spectroscopy using the extinction coefficient $\varepsilon = 2.7 \times 10^5\ M^{-1}\ cm^{-1}$ 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. Conditions were as follows: scanning speed 50 nm/min, data pitch 0.5 nm, DIT 2 s, and 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. Conditions were as follows: excitation slit 2.5 nm, emission slit 2.5 nm, scan rate 600 nm/min.

Sample Preparation. Method A: (1) Add the buffer to a vial. (2) Add the CG 48mer, vortex for 5 s, wait 5 min, and then measure the CD. (3) Add the AT 48mer, vortex for 5 s, wait 5 min, and then measure the CD. (4) Add 50 mM $NiCl_2$, vortex for 5 s, wait 5 min, and then measure the CD. (5) Add NiTPPS, vortex the mixture for 5 s, wait 5 min, and then measure the CD.

Method B1: (1) Add the buffer, the CG 48mer, and 100 mM $NiCl_2$ into vial A. (2) Add the buffer and the AT 48mer into vial B. (3) Add the contents of vial B to vial A, vortex 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 the buffer and the CG 48mer into vial A. (2) Add the buffer, the AT 48mer, and 100 mM $NiCl_2$ into vial B. (3) Add the contents of vial B to vial A, vortex 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). $NiCl_2$ 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 the left-handed Z-form of alternating CG polynucleotides and oligonucleotides whereas no B- to 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

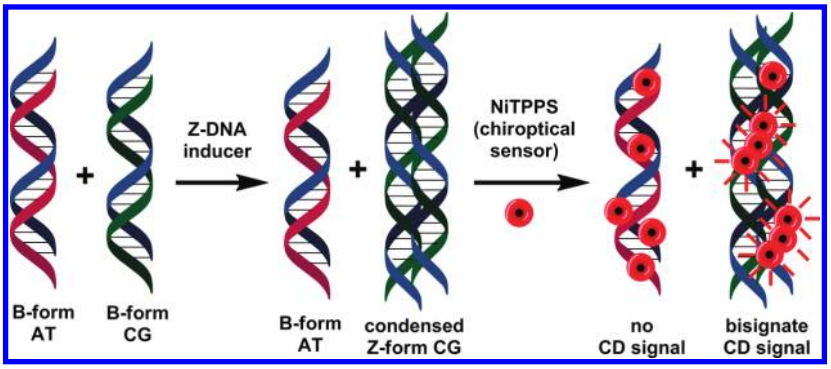


Figure 1. Schematic representation of the selective formation, condensation, and detection of the left-handed Z-DNA in the presence of the right-handed B-DNA using the NiTPPS chiroptical probe. The chiral NiTPPS assemblies formed on the Z-DNA give rise to the exciton coupled CD signal.

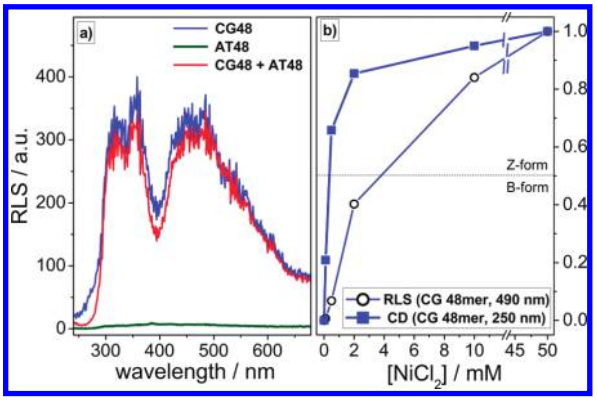


Figure 2. (a) RLS spectra of the CG 48mer 5′-$(dGdC)_{24}$ (blue curve), the AT 48mer 5′-$(dAdT)_{24}$ (green curve), and the mixture of the CG 48mer and the AT 48mer (red curve) upon addition of 50 mM $NiCl_2$. (b) Normalized intensity of the CD signal at 250 nm (black circles) and the RLS signal at 490 nm (blue squares) of the CG 48mer 5′-$(dGdC)_{24}$ as a function of $NiCl_2$ concentration. Conditions: [5′-$(dGdC)_{24}$] = 80 μM; [5′-$(dAdT)_{24}$] = 80 μM; [Na-cacodylate buffer] =1 mM, pH = 7.0.

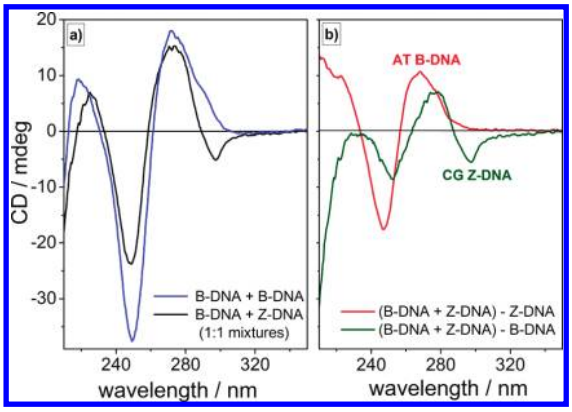


Figure 3. (a) CD spectra of the mixture of the CG 48mer and the AT 48mer in the absence (blue curve, B + B-DNA) and presence (black curve, Z + B-DNA) of 50 mM $NiCl_2$. (b) CD spectra of the Z-form of 48mer 5′-$(dGdC)_{24}$ induced in the presence of the B-form of 48mer 5′-$(dAdT)_{24}$ with 50 mM $NiCl_2$ obtained by subtraction of the B-DNA CD signal (green curve). Conditions: [5′-$(dGdC)_{24}$] = 80 μM; [5′-$(dAdT)_{24}$] = 80 μM; 50 mM $NiCl_2$; [Na-cacodylate buffer] = 1 mM, pH = 7.0.

Z-DNA in the presence of B-DNA has not been reported previously.

No more than a 2 mM concentration of $NiCl_2$ 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 the increase in the 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 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 proven to be a very useful technique for studying electronically coupled arrays of chromophores where an enhanced signal of several orders of magnitude can be observed at the absorption bands of the aggregates.[62] The RLS data confirmed condensation upon addition of $NiCl_2$ 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- to Z-DNA transition is accompanied by the spontaneous and concomitant Z-DNA condensation (Figure 2b). The AT 48mer 5′-$(dAdT)_{24}$ remained in the B-conformation upon $NiCl_2$ addition, and no condensation was observed (almost no RLS signal, Figure 2a, green line). Hence, $NiCl_2$ should not only allow the Z-form of 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 $NiCl_2$ (method A). The appearance of the negative CD band at 290 nm and disappearance of the 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 the subtraction of the B-DNA 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 and 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 and positive CD band at 275 nm, Figure 3, green curve). The RLS experiments showed that the

dx.doi.org/10.1021/jp2047213 |J. Phys. Chem. B 2011, 115, 10182–10188

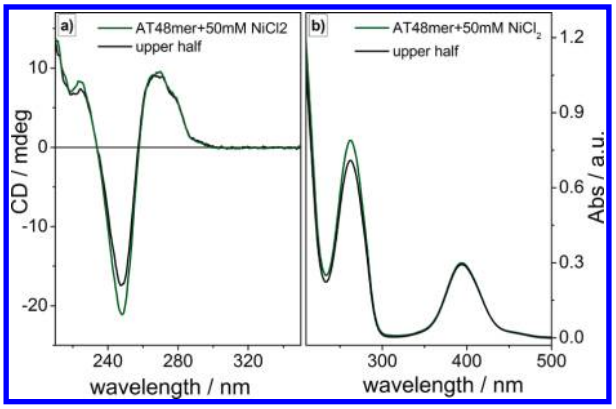


Figure 4. CD spectra (a) and UV−vis absorption spectra (b) of the upper half of the solution obtained upon centrifugation (black curve) and a freshly prepared solution of the 48mer 5′-$(dAdT)_{24}$ in the presence of 50 mM $NiCl_2$ (green curve). Conditions: [5′-$(dGdC)_{24}$] = 80 μM; [5′-$(dAdT)_{24}$] = 80 μM; 50 mM $NiCl_2$; [Na-cacodylate buffer] = 1 mM, pH = 7.0. Centrifugation: 14 500 rpm, 30 min.

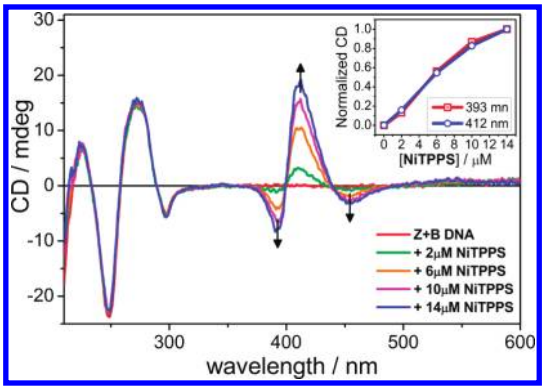


Figure 5. CD spectra of the mixture of the Z-form of 48mer 5′-$(dGdC)_{24}$ and the B-form of 48mer 5′-$(dAdT)_{24}$ titrated with NiTPPS. Inset: intensity of the ECCD as a function of NiTPPS concentration. Conditions: [5′-$(dGdC)_{24}$] = 80 μM; [5′-$(dAdT)_{24}$] = 80 μM; 50 mM $NiCl_2$; [Na-cacodylate buffer] = 1 mM, pH = 7.0.

presence of the AT 48mer does not significantly affect the condensation of the CG 48mer by $NiCl_2$. Figure 2 shows that similar RLS signals were observed for the CG 48mer and the 1:1 mixture of the CG 48mer and the AT 48mer (Figure 2, blue and red curves).

To test the participation of the 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 the AT 48mer (80 μM each) with 50 mM $NiCl_2$ (all together 2 mL) was centrifuged at 14 500 rpm for 30 min in an Eppendorf MiniSpin Plus centrifuge. The sample was then carefully divided into two parts: the top and bottom halves, 1.0 mL each. The CD spectra of the upper half showed the presence of the B-DNA with an almost identical profile to that of the neat AT 48mer in the presence of $NiCl_2$ (Figure 4a). The identity of the AT 48mer was confirmed by reverse phase HPLC. The CD spectrum did not show any Z-DNA CD signals, demonstrating that no Z-DNA was present in the upper layer (Figure 4a, black curve). The resuspended bottom part of the solution showed the CD profile characteristic of Z-DNA with a

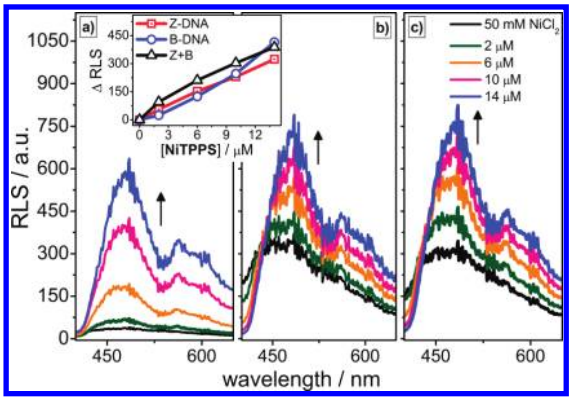


Figure 6. RLS spectra of NiTPPS added to (a) the B-form AT 48mer 5′-$(dAdT)_{24}$, (b) the Z-form CG 48mer 5′-$(dGdC)_{24}$, and (c) the 1:1 Z + B-DNA mixture in the presence of 50 mM $NiCl_2$. Inset: increase in intensity of the RLS at 490 nm as a function of the NiTPPS concentration. Conditions: [5′-$(dGdC)_{24}$] = 80 μM; [5′-$(dAdT)_{24}$] = 80 μM; [Na-cacodylate buffer] = 1 mM, pH = 7.0.

negative CD band at ~295 nm. The UV−vis absorption spectra of the upper layer indicated that more than 90% of the non-condensed B-form of AT 48mer 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 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 appears 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 μM + 80 μM) induced by 50 mM $NiCl_2$ (Figure 5). The ECCD signal increased steadily with increasing concentration of NiTPPS from 2 μM to 14 μM (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 neither Z-DNA, B-DNA, nor the equilibrium are disturbed.

No ECCD signal was observed when NiTPPS was titrated to the 5′-$(dAdT)_{24}$ (Figure S1 in the Supporting Information) 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 of the 48mer 5′-$(dAdT)_{24}$ (80 μM) and the 48mer 5′-$(dGdC)_{24}$ (80 μM) in the absence of $NiCl_2$ or in the presence of 50 mM $MgCl_2$. Resonance light scattering experiments showed aggregation of the NiTPPS upon its titration to the solutions of CG 48mer + 50 mM $NiCl_2$, AT 48mer + 50 mM $NiCl_2$, and 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 the NiTPPS aggregation

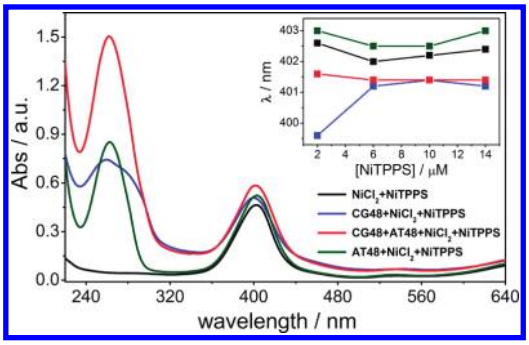


Figure 7. UV−vis absorption spectra of NiTPPS added to the Z-form of 48mer 5′-$(dGdC)_{24}$ (blue curve), to the B-form of 48mer 5′-$(dAdT)_{24}$ (green curve), and to the B + Z-DNA mixture (1:1, red curve) consisting of the CG 48mer and the AT 48mer. Inset: Soret band absorption wavelength as a function of the NiTPPS concentration. Conditions: [5′-$(dGdC)_{24}$] = 80 μM; [5′-$(dAdT)_{24}$] = 80 μM; 50 mM $NiCl_2$; [Na-cacodylate buffer] = 1 mM, pH = 7.0; method A.

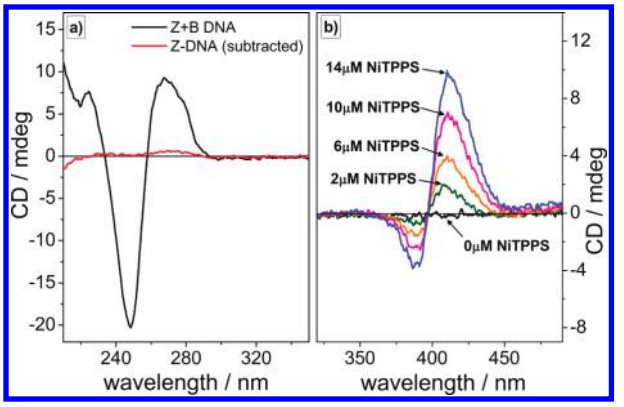


Figure 8. (a) CD spectra of the 5:95 mixture of the Z-form 48mer 5′-$(dGdC)_{24}$ and the B-form 48mer 5′-$(dAdT)_{24}$ (black curve) and the CD spectrum of the Z-form obtained by subtraction from the CD spectrum of the Z + B-DNA mixture (red curve). (b) ECCD observed upon titration of NiTPPS into the 5:95 mixture of the Z-form 48mer 5′-$(dGdC)_{24}$ and the B-form of 48mer 5′-$(dAdT)_{24}$. Conditions: [5′-(dGdC)24] = 4.4 μM; [5′-$(dAdT)_{24}$] = 80 μM; 50 mM $NiCl_2$; [Na-cacodylate buffer] = 1 mM, pH = 7.0.

occurred in the presence of all DNA sequences, clearly, under our experimental condition, the B-forms of the 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 to 14 μM) to the Z + B-DNA mixture and to the AT B-DNA and CG Z-DNA alone (80 μM each with 50 mM $NiCl_2$) were performed to further evaluate the interaction between the NiTPPS and the ODNs (Figure 7). The addition of 2 μM NiTPPS to the B + Z-DNA mixture consisting of the Z-form of 48mer 5′-$(dGdC)_{24}$ and the B-form of 48mer 5′-$(dAdT)_{24}$ (80 μM + 80 μM + 50 mM $NiCl_2$, method A) led to a blue shift of the Soret band (from 402.6 to 401.6 nm, $\Delta\lambda$ = 1.0 nm; see Figure 7 inset, red line) in comparison to the absorption characteristics of NiTPPS in 50 mM $NiCl_2$ in the absence of DNA. Mixing NiTPPS (2 μM) with the B-form of 48mer 5′-$(dAdT)_{24}$ (80 μM + 50 mM) gave a small bathochromic shift of the Soret band (from 402.6 to 403 nm, $\Delta\lambda$ = 0.4 nm; see Figure 7 inset). The addition of 2 μM NiTPPS to the 48mer 5′-$(dGdC)_{24}$ (80 μM + 50 mM $NiCl_2$) caused the hypsochromic shift of the Soret band (from 402.6 to 399.6 nm, $\Delta\lambda$ = 3.0 nm; see Figure 7 inset). Minor changes of the UV−vis spectra and small hypochromicity suggest the outside DNA groove binding of porphyrin.[41,64] The blue shift of the NiTPPS absorption band observed for the CG ODN suggests a cofacial porphyrin arrangement.[65,66]

The sensitivity and selectivity of the NiTPPS as a chiroptical Z-DNA sensor were evaluated in the presence of the 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 μM) was added to the 48mer 5′-$(dGdC)_{24}$ (4.4 μM) in cacodylate buffer (1 mM, pH = 7.0) followed by the addition of $NiCl_2$ (50 mM, method A). The coexistence of the Z-DNA and B-DNA was confirmed by CD followed by subtraction of the respective chiroptical signals. At these lower concentrations, unlike the case of the 80 μM Z-DNA + 80 μM 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 it did not allow for the conclusive confirmation of the presence of the Z-form (Figure S2 of the Supporting Information). However, in the visible region at 400 nm, the NiTPPS selectively detected the Z-form in the Z + B-DNA mixture, as judged by the presence of the bisignate ECCD signal (Figure S3 in the Supporting Information). The detection of Z-DNA in the presence of a shorter B-DNA sequence, using the AT 24mer instead of the AT 48mer, was also explored. The titration of the NiTPPS to the solution consisting of 20 μM CG 48mer, 20 μM AT 24mer, and 50 mM $NiCl_2$ gave rise to a strong bisignate ECCD, clearly showing of the presence of Z-DNA (Figure S4 in the Supporting Information).

To study the selectivity of the NiTPPS in the Z-DNA detection in the presence of B-DNA, we decreased the amount of the Z-DNA from 50 to 10% while keeping the concentration of the B-DNA at 80 μM. The 48mer 5′-$(dAdT)_{24}$ (80 μM) was added to the 48mer 5′-$(dGdC)_{24}$ (8.9 μM) in cacodylate buffer (1 mM, pH = 7.0) followed by addition of $NiCl_2$ (50 mM, method A). The presence of both forms of DNA was confirmed by the CD subtraction method as described above (Figure S5 in the Supporting Information). Again, the addition of NiTPPS gave rise to a bisignate ECCD signal confirming the presence of Z-DNA (Figure S6 in the Supporting Information). Upon further decreasing the Z-DNA concentration to 5% (4.4 μM Z-DNA + 80 μM B-DNA + 50 mM $NiCl_2$), 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 the NiTPPS gave a clear-cut ECCD signal of the Z-DNA in the porphyrin Soret band region (Figure 8b). The NiTPPS was able to successfully detect 5% Z-DNA in the mixture of 95% AT B-DNA, confirming its high selectivity and sensitivity for the left-handed DNA form.

In all experiments described so far, $NiCl_2$ was added to the mixture of CG + AT ODNs (method A). Thus, the formation and condensation of the 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 the Z-DNA NiTPPS aggregates. Although the B- and Z-DNA are in equilibrium, once the condensed Z-DNA is formed, the B- to Z-DNA equilibrium may be hindered. To test whether the order of addition and the

presence of the AT ODN influenced the Z-DNA recognition by the NiTPPS, two additional sets of experiments were performed. In method B1, $NiCl_2$ was added to the 48mer 5′-$(dGdC)_{24}$ ($[NiCl_2]$ = 50 mM), no $NiCl_2$ was added to the AT 48mer, and only then were the CG and AT ODNs mixed together. In method B2, $NiCl_2$ was added to the AT 48mer ($[NiCl_2]$ = 50 mM), and no $NiCl_2$ was added to the 48mer 5′-$(dGdC)_{24}$ before the ODNs were mixed together. Upon the ODNs mixing, the final Ni^{2+} concentration in both cases was 50 mM. In all experiments, the presence of the Z-DNA form was confirmed by the CD and subsequent subtractions as described above. The addition of the NiTPPS to the mixtures of the 48mer 5′-$(dGdC)_{24}$ and the AT 48mer prepared by method B1 or B2 gave results almost identical to those obtained with method A, that is, a strong bisignate CD signal with the positive CD band at 415 nm and the negative CD band at 390 nm (Figures S7 and S8 in the Supporting Information). These results confirmed that the order in which $NiCl_2$ was added to DNA had no effect on the formation or detection of the Z-DNA in the presence of the 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 adenine−thymine B-DNA are reported. $NiCl_2$ 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 48mer ODN 5′-$(dAdT)_{24}$ and the 48mer ODN 5′-$(dAdT)_{24}$ at a low Z-DNA to B-DNA ratio (5:95) as well as at low Z-DNA concentrations (5 μM base pair concentration). The Z-DNA sensing was achieved via the induced ECCD signal of the 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 the 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 it is in 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 (the NiTPPS is chiroptically silent in the presence of the AT B-DNAs).

ASSOCIATED CONTENT

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.

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ACKNOWLEDGMENT

This research was supported in part by the University of Wyoming Start-Up Fund (M.B); the NIH, Grant Nos. P20 RR016474 (M.B.) and P20 RR016469 (A.E.H) from the INBRE Programs of the National Center for Research Resources; and the NSF, Grant Nos. CHE-0747949 (A.E.H) and NSF-EPSCoR EPS-1004094 (A.E.H.). G.S. thanks the University of Wyoming School of Energy Resources Graduate Assistantship.

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