Cleavage of Telomeric G-Quadruplex DNA with Perylene-EDTA·Fe(II)†

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ABSTRACT: Interest in the development of compounds that bind G-quadruplex DNA selectively has been sparked by the discovery that these compounds can inhibit the activity of telomerase. Compounds that bind to this unique structure, as well as compounds that recognize it and cleave it upon binding, have potential uses as anticancer agents and as probes for the presence of G-quadruplex structures in chromatin. Here we report on the G-quadruplex-specific cleavage properties of a perylene derivative, perylene-EDTA·Fe(II). This water-soluble complex cleaves G-quadruplex DNA selectively in the presence of dithiothreitol. We characterize the cleavage reaction with respect to perylene-EDTA·Fe(II) concentration, DNA structural type, cation type (Na^+ vs K^+), cation concentration, and pH. Visible spectroscopy confirms the selectivity of the perylene-EDTA·metal complex for G-quadruplex DNA. NMR titration of a parallel G-quadruplex with perylene-EDTA (without metal) indicates that the compound binds to G-quadruplex DNA by stacking externally on the 3′ G-tetrad.

With a few exceptions, telomeres consist of sequence repeats that are known to terminate in single stranded G-rich overhangs (*1*). The DNA sequences that make up these single-stranded overhangs are known to form G-quadruplex structures readily under physiologically relevant conditions, and it is thought that the formation of G-quadruplexes by these overhang regions may prevent their extension by telomerase (*2*−*6*). Thus, because of the critical role that telomerase is thought to play in the survival of cancer cells (*7*), there has been increased interest recently in the development of compounds that inhibit telomerase by virtue of their interaction with telomeric G-quadruplex DNA (*2*−*6*, and references therein).

Although the existence of G-quadruplex structures in vivo has not been unequivocally proven, there is indirect evidence that such structures may in fact exist (*4*, *5*, and references therein). Thus, various classes of compounds have been developed to target G-quadruplex structures, including porphyrin derivatives, anthraquinone derivatives, cyanine dyes, perylene derivatives, and most recently ethidium and dibenzophenanthroline derivatives (*4*, *8*, *9*). These compounds have potential therapeutic applications as telomerase inhibitors by virtue of their ability to stabilize G-quadruplex structures formed by telomeric G-rich DNA.

Although the anticancer therapeutic value of telomere interactive agents, particularly those that target G-quadruplex DNA, remains to be determined, there have been some promising preclinical results (*10*, *11*). This prompted us to undertake the development of compounds that not only bind to G-quadruplex DNA but also cleave this unique structure selectively upon binding. Presumably such compounds would cleave telomeric G-rich overhangs selectively (assuming that these overhangs form G-quadruplex structures in vivo) and would hopefully be less cytotoxic. Cleavage of the G-rich overhangs would also abolish the action of telomerase, since this enzyme is incapable of extending blunt-ended telomeric DNA. To this end, we have employed the perylene core chemical structure, which has proven to be an excellent G-quadruplex binding motif (*4*, *5*, *12*−*15*) and modified it with an EDTA·Fe(II) complex, which is known to lead to cleavage of DNA through hydroxyl radical generation in the presence of a reducing agent (*16*−*19*). Here we show that the resulting, water-soluble, perylene-EDTA·Fe(II) selectively cleaves G-quadruplex DNA in the presence of dithiothreitol (DTT) in a concentration-dependent manner. We characterize G-quadruplex cleavage with respect to cation concentration, cation type (Na^+ versus K^+), and pH. Visible spectra confirm the selectivity of the related perylene-EDTA-Ni·(II) complex for G-quadruplex DNA. NMR titration data indicate that perylene-EDTA binds to parallel G-quadruplex DNA in a manner similar to that established for the related molecule PIPER (*13*).

MATERIALS AND METHODS

DNA Preparation. All DNA sequences used in this study (Table 1) were synthesized on an Expedite 8909 Nucleic Acid Synthesis System (Perseptive Biosystem) and purified by denaturing polyacrylamide gel electrophoresis (PAGE). The 5′-end-labeled DNA strands were labeled with [γ-^{32}P]-ATP (Amersham) using T4 polynucleotide kinase (New

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[1] Abbreviations: EDTA, ethylenediaminetetraaccetic acid; DTT, dithiothreitol; ATP, adenosine triphosphate; PIPER, *N*,*N*′-bis[2-(1-piperidino)-ethyl]3,4,9,10-perylenetetracarboxylic diimide; PAGE, polyacrylamide gel electrophoresis; RT, room temperature; DMS, dimethyl sulfate; UV, ultraviolet; cpm, counts per minute; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; PEDTA, perylene-EDTA.

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Table 1: DNA Sequences Used in This Work

name	sequence
T2AG3	5′-GCT GCG TCC AAC TAT GTA TAC **TTA GGG TTA GGG TTA GGG TTA GGG** TTA GCG GCA CGC AAT TGC TAT AGT GAG -TCG TAT TA −3′
T2G4	5′-GCT GCG TCC AAC TAT GTA TAC **TTG GGG TTG GGG TTGGGG TTG GGG** TTA GCG GCA GCG AAT TGC TAT AGT GAG TCG TAT TA-3′
C3TA2	5′-TAA TAC GAC TCA CTA TAG CAA TTG CGT GCC GCT AA**CCCT AAC CCT AAC CCT AAC CCT AA**G TAT ACA TAG TTGGAC GCA GC-3′
C4A2	5′- TAA TAC GAC TCA CTA TAG CAA TTG CGT GCC GCT AA**C CCC AAC CCC AAC CCC AAC CCC AA**G TAT ACA TAG TTG GAC GCA GC-3′
T6	5′ -TAA TAC GAC TCA CTA TAG CAA TTG CGT GCC GCT **TTTTTT** GTA TAC ATA GTT GGA CGC AGC-3′
G3	5′ -TTA GGG TTA GGG TTA GGG TTA GGG- 3′
G4	5′ -TTG GGG TTG GGG TTG GGG TTG GGG- 3′
A4	5′ -TTA AAA TTA AAA TTA AAA TTA AAA- 3′
D12	5′-CGC GAA TTC GCG-3′
NMR1	5′-TAG GGT TA-3′

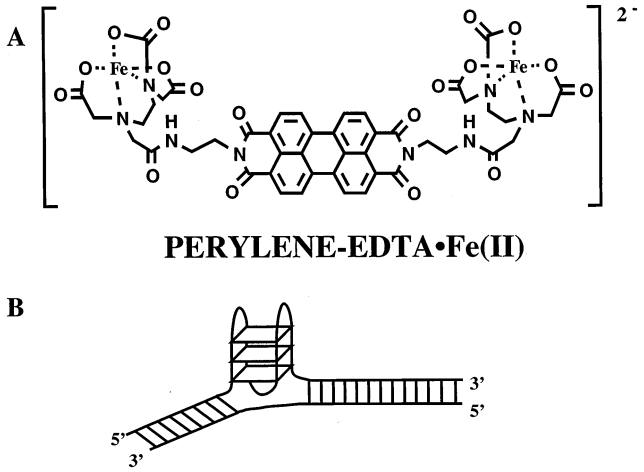


FIGURE 1: (A) Chemical structure of perylene-EDTA·Fe(II). (B) The quadruplex-duplex substrate used to assay cleavage by perylene-EDTA·Fe(II). The top strand consists of either the sequence T2G4 (Table 1) or the sequence T2AG3. The bottom strand is the sequence T6 in Table 1 and contains a single-stranded region of six T residues bridging the G-quadruplex region in the top strand.

England Biolabs). For the cleavage experiments, the quadruplex-duplex DNA substrates (Figure 1B) were prepared as follows. First, the G-rich strands (T2AG3 and T2G4 sequences, Table 1) were denatured by heating to 95 °C for 5 min in a buffer consisting of 10 mM Tris HCl (pH 8.0) and 100 mM KCl. The samples were then allowed to cool slowly to room temperature (RT), then kept at 4 °C overnight to allow time for complete formation of the G-quadruplex. The complementary strand (T6 sequence, Table 1) was then added, and the mixture was incubated at 55 °C for 1 h and then cooled slowly to RT. The duplex DNA substrates were prepared by denaturing the complementary strands at 95 °C for 5 min in a buffer consisting of 10 mM Tris HCl (pH 8.0) and 100 mM KCl. The samples were then allowed to cool to RT overnight. All DNA substrates were then purified by 8% nondenaturing PAGE and subsequently stored at 4 °C in a buffer consisting of 10 mM Tris HCl (pH 8) and 100 mM KCl. In all cases, the ratio amount of end-labeled strand to its nonlabeled counterpart was 1:3.

Dimethyl Sulfate (DMS) Footprinting. The 800 μL reaction mixture consisted of 80 000 cpm of the quadruplex-duplex DNA substrate (~800 fmol), 40 μg of calf thymus DNA in 10 mM Tris HCl (pH 8.0) and either 10 mM KCl or 100 mM KCl. After adding 1 μL of neat DMS (Sigma), the mixture was split into four tubes. The reactions were stopped by adding 50 μL of stop buffer (1.0 M mercaptoethanol, 1.5 M sodium acetate, pH 5.2, and 10 μg of tRNA) at the indicated times. The samples were then subjected to ethanol precipitation and piperidine heat treatment, before the cleavage products were resolved by 8% denaturing PAGE. The control set was prepared in the same manner with an extra denaturation step in a 10 mM Tris HCl buffer (pH 8.0) without KCl. The DNA substrate was denatured at 95 °C for 5 min and then cooled immediately to 0 °C prior to adding DMS.

Preparation of perylene-EDTA·Fe(II) and EDTA·Fe(II). Fresh stock solutions of 1 mM perylene-EDTA·Fe(II) and 10 mM EDTA·Fe(II) were prepared by mixing equimolar amounts of perylene-EDTA or EDTA (sodium salt) with ammonium iron(II) sulfate hexahydrate in purified water. The stock solutions were then adjusted to the desired concentrations prior to use.

Cleavage Reactions. For the perylene-EDTA·Fe(II) concentration-dependent experiments (Figures 2 and 3), the 20 μL reaction mixtures consisted of DNA substrate (30 000 cpm, ~300 fmol) plus the indicated amount of perylene-EDTA·Fe(II) in a buffer consisting of 10 mM Tris-HCl (pH 8.0) and 100 mM KCl. With the exception of the control sample lane 8 (Figures 2 and 3), the samples were incubated at RT for 4 h before adding 2 μL of 10 mM dithiothreitol (DTT). The cleavage reactions were then allowed to proceed for 2 h before adding 100 μL of stop buffer [0.1 M thiourea, 0.3 M sodium acetate (pH 5.2), and 10 μg of tRNA]. The samples were then subjected to ethanol precipitation before the cleavage products were resolved by 8% denaturing PAGE.

For the cation concentration-dependent experiments, the DNA substrates were washed with a 50-fold volume of 100 mM Tris HCl (pH 7.4) twice, using Microcon 10 spin column (Millipore), before mixing in the cleavage reaction mixture. The reaction mixture consisted of DNA substrate (30 000 cpm), 10 μM perylene-EDTA·Fe(II), 10 mM Tris HCl (pH 7.4), and the indicated salt concentrations. The cleavage procedures were the same as above.

For the pH-dependent experiments, the reaction mixtures consisted of DNA substrate (30 000 cpm), 10 μM perylene-EDTA·Fe(II), 100 mM KCl, and 10 mM of the indicated buffer. Buffers used in these experiments were sodium acetate pH 5.2, sodium phosphate pH 6.0, and Tris HCl pH 7.0, 8.0, and 9.0.

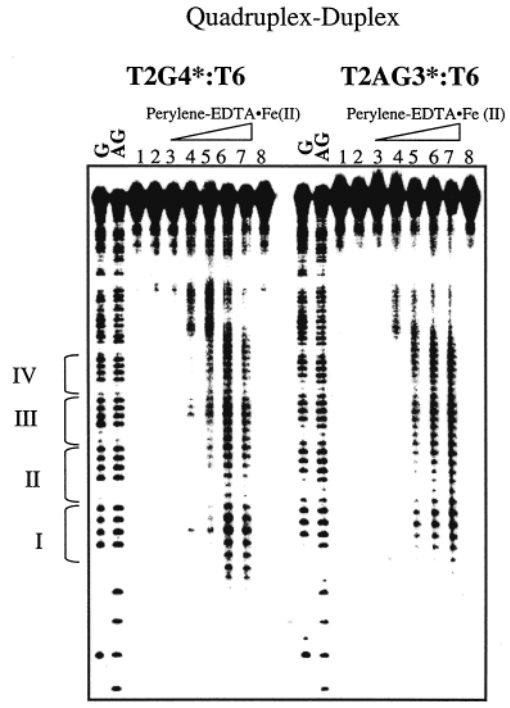


FIGURE 2: Phosphorimage of the cleavage products from the reaction of perylene-EDTA·Fe(II) with the quadruplex-duplex substrate in Figure 1B, where the quadruplex region consists of either four repeats of T_2G_4 or four repeats of T_2AG_3. Only the results for the labeled G-rich strands are shown. Lane 1: Control experiment in which the DNA underwent the same treatment as the DNA with perylene-EDTA·Fe(II). Lane 2: DNA in the presence of 100 mM EDTA·Fe(II). Lanes 3−7: DNA with perylene-EDTA·Fe(II) added at concentrations of 100 nM, 500 nM, 1 μM, 4 μM, and 16 μM, respectively. Lane 8: DNA with 16 μM perylene-EDTA·Fe(II) but without added DTT. The G and AG lanes are Maxam−Gilbert sequencing lanes. Brackets I−V denote the four G-rich repeat sequences.

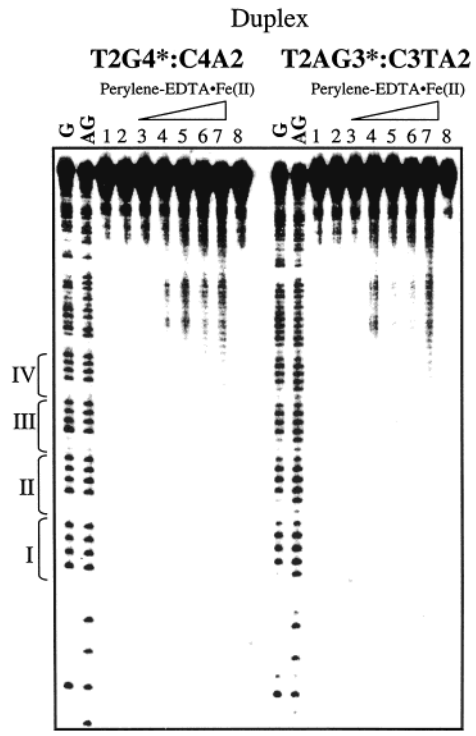


FIGURE 3: Phosphorimage of the products from the reaction of perylene-EDTA·Fe(II) with a duplex formed from the G-rich T2G4 sequence and its complementary C4A2 sequence and a duplex formed from the G-rich T2AG3 sequence and its complementary sequence C3TA2 (see Table 1). Only the results for the labeled G-rich strands are shown. Lane 1: Control experiment in which the DNA underwent the same treatment as the DNA with perylene-EDTA·Fe(II). Lane 2: DNA in the presence of 100 μM EDTA-Fe(II). Lanes 3−7: DNA with perylene-EDTA·Fe(II) added at concentrations of 100 nM, 500 nM, 1 μM, 4 μM, and 16 μM, respectively. Lane 8: DNA with 16 μM perylene-EDTA·Fe(II) but without added DTT. Cleavage reaction conditions were otherwise identical to those used for the experiments in Figure 2. The G and AG lanes are Maxam−Gilbert sequencing lanes. Brackets I−V denote the repeat sequences in the G-rich strand.

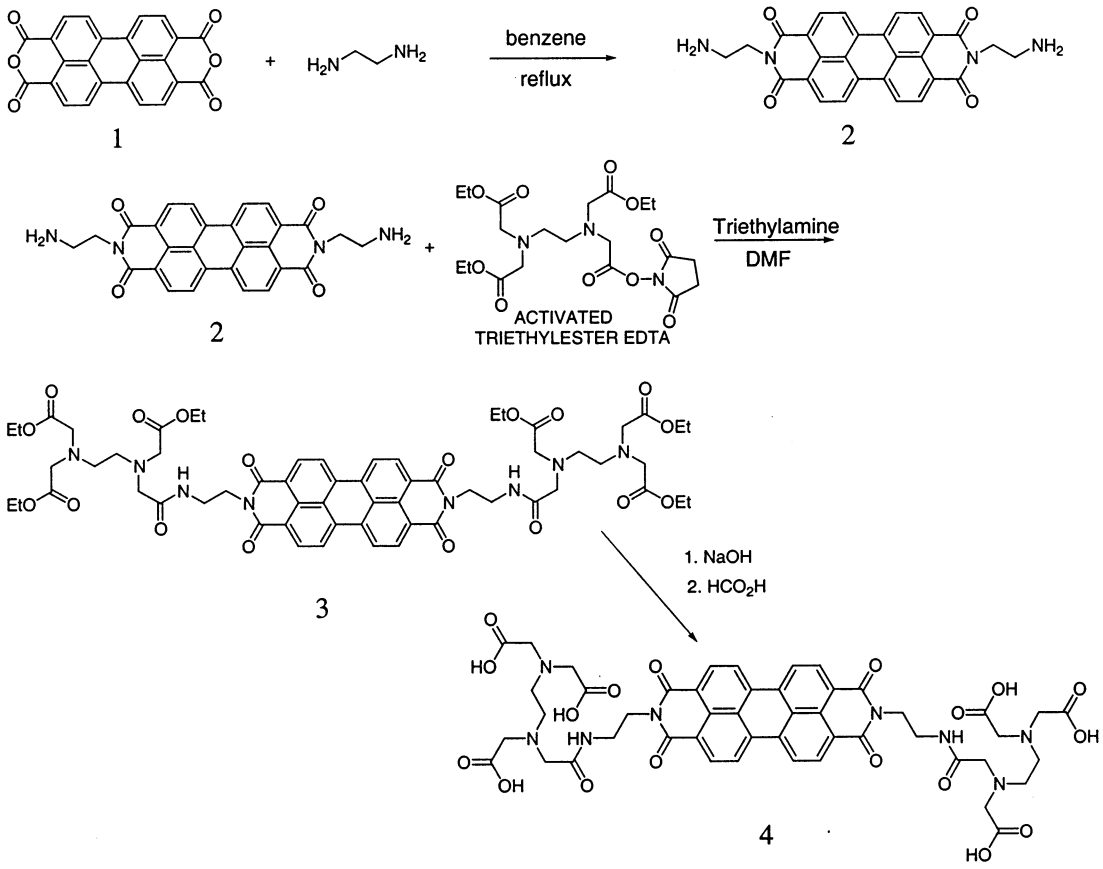
Visible Spectra. The concentrations of all oligonucleotides were estimated by measuring the UV absorbance at 260 nm. All the oligonucleotides were adjusted to the same concentration (except for the dodecamer duplex in which the concentration was doubled) in 10 mM Tris HCl (pH 8.0) and 100 mM KCl buffer. All the DNA solutions were heated at 95 °C for 5 min, then allowed to cool slowly to RT, and kept at 4 °C overnight. The DNA substrates were then incubated with perylene-EDTA or perylene-EDTA·Ni(II) for at least 12 h, and then the spectra were recorded on a Cary 100 spectrophotometer in the region 400−620 nm. Each sample contained 4 μM of DNA and 8 μM of perylene-EDTA or perylene-EDTA·Ni(II) in a buffer consisting of 10 mM Tris HCl (pH 8.0) and 100 mM KCl.

NMR Spectra. Imino proton spectra were acquired on a Varian UNITYplus 500 MHz spectrometer. The HPLC purified DNA (NMR1 sequence, Table 1) was dissolved in 600 μL of a pH 8.0 buffer consisting of 150 mM KCl, 50 mM KH_2PO_4, and 1 mM EDTA. The sample was then stored at 4 °C overnight to allow for complete quadruplex formation (final concentration was 1 mM in quadruplex). The sample was then lyophilized to dryness and redissolved in 600 μL of a 10%D_2O/90%H_2O mixture. A 40 mM stock solution of perylene-EDTA in 10 mM Tris HCl (pH 9.0) was then used to titrate the DNA directly in the NMR tube. The spectra were acquired at 7 °C and 64 scans were acquired for each spectrum with a relaxation delay of 2 s. Water suppression was achieved through the use of a standard 1-1 echo pulse sequence with maximum excitation centered at 12.0 ppm. The imino protons of $[d(TAGGGTTA)]_4$ were assigned as described previously (*13*).

RESULTS

Synthesis. The synthesis and analytical characterization of perylene-EDTA (Scheme 1) has been described in detail elsewhere (*20*). Briefly, commercially available 3,4,9,10-perylenetetra-carboxylic dianhydride (**1**) is allowed to react with excess ethylenediamine by refluxing in benzene to afford *N*,*N*′-bis[diethylamine]-3,4,9,10-perylenetetra-carboxylic diimide (**2**) (*21*). An excess of activated triethyl ester EDTA, obtained following published procedures (*16*−*19*), is then allowed to react with (**2**) by sonicating in anhydrous DMF in the presence of a trace amount of triethylamine under an inert atmosphere, to afford the hexa-ethyl ester of perylene-EDTA (**3**). The water-soluble sodium salt of perylene-EDTA is obtained from (**3**) by sonication of (**3**) in an aqueous solution containing 6 equiv of NaOH for 30 min. Purification of perylene-EDTA (**4**) is readily achieved by precipitation of an aqueous solution of the sodium salt of (**3**) with formic acid.

Scheme 1



Cleavage of G-Quadruplex DNA by Perylene-EDTA·Fe(II). To assay for cleavage of G-quadruplex DNA by perylene-EDTA·Fe(II), we designed the rather unusual substrate shown in Figure 1B. This substrate consists of an intramolecular G-quadruplex structure [formed by four repeats of either the *Tetrahymena* telomeric repeat sequence T_2G_4 or the human telomeric repeat sequence T_2AG_3 (sequences T2G4 and T2AG3, respectively, see Table 1)] flanked by random sequence duplex regions on both ends. The opposite strand is only complementary to the G-rich DNA in the regions that remain single-stranded after formation of the G-quadruplex. The quadruplex is bridged in the opposite strand with six T residues (sequence T6 in Table 1). In constructing this substrate, the G-quadruplex in the top strand was first preformed in 100 mM K^+ buffer, and its structure was confirmed by DMS footprinting, as described in the Experimental Section. The top (G-rich) strand was then annealed to the bottom strand, and then the identity of the duplex-quadruplex substrate (including the presence of the intramolecular G-quadruplex in the top strand) was reconfirmed by nondenaturing PAGE and DMS footprinting (Supporting Information), prior to its use in the cleavage reactions. The advantage of this type of substrate is that it allows for simultaneous evaluation of duplex and G-quadruplex cleavage by perylene-EDTA·Fe(II) within the same substrate. Figure 2 shows the concentration-dependent cleavage pattern observed for the top strand of the two G-rich substrates when they are incubated in the presence of perylene-EDTA·Fe(II) and DTT. These data show clear evidence of cleavage of the DNA residues involved in G-quadruplex formation, as well as cleavage of residues immediately adjacent to the G-quadruplex region. DNA degradation due to the radiolabeling process precluded a more detailed evaluation of cleavage of the top strand in the 3′-duplex region. However, with the exception of the DNA residues immediately adjacent to the 5′-region of the G-quadruplex, there was no noticeable cleavage of the 5′-duplex region. Thus, perylene-EDTA·Fe(II) shows remarkable selectivity for G-quadruplex over duplex structure, regardless of the G-rich repeat sequence used.

To further assess the selective recognition and cleavage of DNA structure by perylene-EDTA·Fe(II), we tested for cleavage of duplex DNA substrates. Two duplexes consisting of either the T2G4 repeat sequence and its complementary strand (C4A2) or the T2AG3 repeat sequence and its complementary strand (C3TA2) [see Table 1] were tested. In both cases, the two strands were carefully annealed in 100 mM KCl buffer and purified by nondenaturing PAGE to ensure the purity of the duplex. Figure 3 shows the perylene-EDTA·Fe(II) concentration-dependent cleavage patterns for the top (G-rich) strand when these two duplexes are incubated in the presence of perylene-EDTA·Fe(II) and DTT. Clearly, with the exception of negligible cleavage near the 3′ end of the DNA substrate, perylene-EDTA·Fe(II) does not lead to cleavage of this substrate. Thus, the data in Figures 2 and 3 show that perylene-EDTA·Fe(II) is selective for G-quadruplex DNA over duplex DNA.

Effect of Cation Type and Cation Concentration on the Cleavage Reactions. The stability of G-quadruplex DNA is known to be largely dependent on the cation type and cation concentration. Potassium is known to be more effective than sodium at stabilizing the G-quadruplex structure, and the

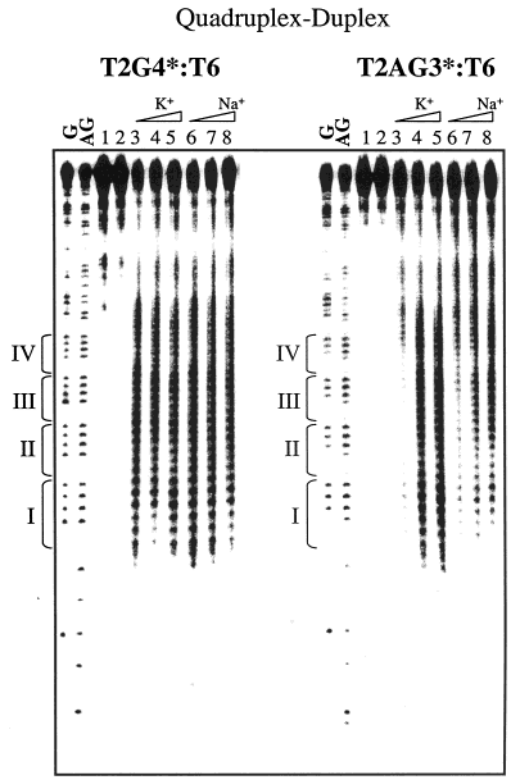


FIGURE 4: Phosphorimage of the salt-dependent cleavage products from the reaction of perylene-EDTA·Fe(II) with the quadruplex-duplex substrate in Figure 1B, where the quadruplex region consists of either four repeats of T_2G_4 or four repeats of T_2AG_3. Only the results for the labeled G-rich strands are shown. Lane 1: Control experiment in which the DNA underwent the same treatment as the DNA with perylene-EDTA·Fe(II). Lane 2: DNA in the presence of 10 μM perylene-EDTA·Fe(II) but without added DTT. Lanes 3−5: DNA with 10 μM perylene-EDTA·Fe(II), and 0.0 mM KCl, 10 mM KCl, and 100 mM KCl, respectively. Lanes 6−8: DNA with 10 μM perylene-EDTA·Fe(II), 0.0 mM NaCl, 10 mM NaCl, and 100 mM NaCl, respectively. The G and AG lanes are Maxam−Gilbert sequencing lanes. Brackets I−V denote the four G-rich repeat sequences. The reactions were run in 10 mM Tris pH 7.4 buffer.

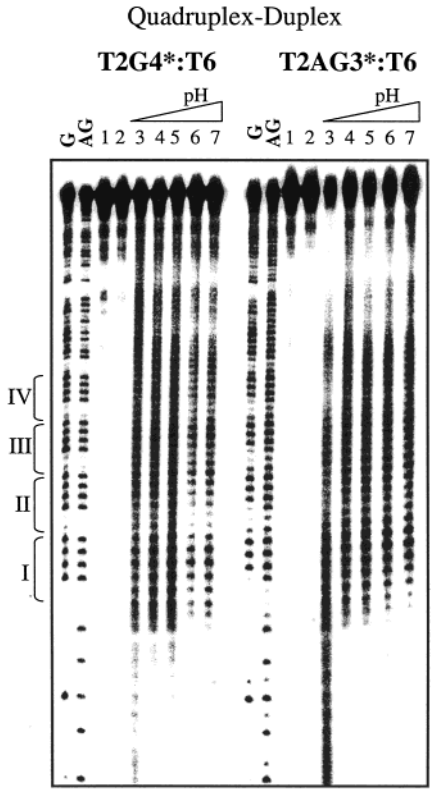


FIGURE 5: Phosphorimage of the pH-dependent cleavage products from the reaction of perylene-EDTA·Fe(II) with the quadruplex-duplex substrate in Figure 1B where the quadruplex region consists of either four repeats of T_2G_4 or four repeats of T_2AG_3. Only the results for the labeled G-rich strands are shown. Lane 1: Control experiment run at pH 7.0 in which the DNA underwent the same treatment as the DNA with perylene-EDTA·Fe(II). Lane 2: DNA in the presence of 10 μM perylene EDTA-Fe(II) at pH 7.0 but without added DTT. Lanes 3−7: Cleavage reactions run at pH values of 5.2, 6.0, 7.0, 8.0, and 9.0, respectively, in the presence of 10 μM perylene-EDTA·Fe(II), DTT, and 100 mM KCl. The G and AG lanes are Maxam−Gilbert sequencing lanes. Brackets I−V denote the four G-rich repeat sequences.

G-quadruplex structure is typically more stable at higher cation concentrations (*1*, *4*). We examined the effects of cation type and cation concentrations on the cleavage of G-quadruplex by perylene-EDTA·Fe(II) using the same substrate in Figure 1B. Figure 4 shows the cleavage pattern observed when this preformed substrate, where the G-quadruplex region consists of either four repeats of T_2G_4 or four repeats of T_2AG_3 [T2G4 and T2AG3 sequences in Table 1, respectively], is incubated with 10 μM perylene-EDTA·Fe(II) in the presence of DTT in buffers containing K^+ or Na^+ at concentrations of 0, 10, and 100 mM. For the T2G4 sequence, there is rather uniform cleavage of the G-quadruplex region over the entire cation concentration ranges in the presence of both K^+ and Na^+, indicating that once preformed, the T2G4 quadruplex is very stable. However, in the case of the T2AG3 sequence cleavage of the G-quadruplex region is more pronounced at higher concentrations of Na^+ and K^+. Cleavage of the preformed G-quadruplex region in the T2AG3:T6 substrate is reduced in the absence of either cation, and higher amounts of Na^+ (versus K^+) are needed to induce significant cleavage of the G-quadruplex region. This provides indirect evidence that the preformed intramolecular quadruplex structure from T_2G_4 repeats is much more stable than that from T_2AG_3 repeats, even after removal of excess cation (compare lanes 3 and 6 for T2G4:T6 with lanes 3 and 6 for T2AG3:T6 in Figure 4), and that perylene-EDTA·Fe (II) is very selective in cleaving G-quadruplex over duplex DNA.

pH-Dependent Cleavage of G-Quadruplex DNA by Perylene-EDTA·Fe(II). Figure 5 shows the cleavage pattern observed when the DNA substrate in Figure 1, containing the G-quadruplex structure formed from four repeats of either T_2G_4 or T_2AG_3, is incubated with 10 μM perylene-EDTA·Fe(II) in the presence of DTT at various pH values. The cleavage reactions were carried out at increasing pH values of 5.2, 6.0, 7.0, 8.0, and 9.0 and a constant K^+ concentration of 100 mM. The effect of pH is similar for both G-quadruplex structures in that decreasing pH values lead to progressively more generalized cleavage of the top strand in the DNA substrate. At pH 5.2, the G-rich region of the top strand and the region involved in duplex formation are both significantly cleaved. This lack of structure selectivity at lower pH is likely due to the ionization state of the EDTA moiety in the molecule. Thus, at pH 5.2, the EDTA moiety is less ionized and it is thus less effective at binding Fe(II). This leads to generalized cleavage of the DNA substrate in the presence of DTT due to free Fe(II) in solution. On the

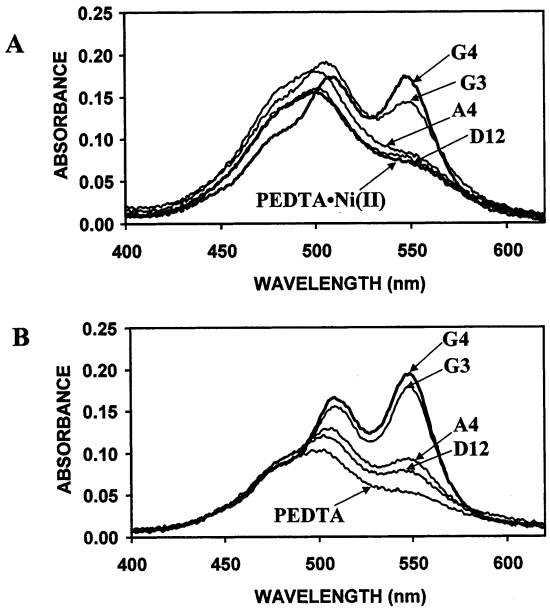


FIGURE 6: Visible spectra of (A) perylene-EDTA·Ni(II) [PEDTA·Ni(II)] and (B) perylene-EDTA (PEDTA) in the absence and presence of the G3 and G4 sequences (both of which form intramolecular G-quadruplex structures), single-stranded DNA (A4 sequence), and the dodecamer duplex d[(CGCGAATTCGCG)$_2$] (from the D12 sequence). The G3, G4, A4, and D12 sequences are shown in Table 1. In both cases, the molar ratios of DNA to perylene-EDTA·Ni(II) and DNA to perylene-EDTA are 1:2.

other hand, increasing pH values lead to stronger binding of Fe(II) by the EDTA moiety and thus more specific cleavage of the G-quadruplex region.

Visible Spectroscopy Studies of Structure Recognition and Binding by Perylene-EDTA and Perylene-EDTA·Ni(II). The cleavage data in Figures 2 and 3 provide evidence that perylene-EDTA·Fe(II) is selective for G-quadruplex over duplex DNA. Also, the data in Figure 4 for the T2AG3:T6 substrate indicate that as the G-quadruplex region becomes unstable and single-stranded at lower salt concentrations, it is no longer recognized by perylene-EDTA·Fe(II). Additional evidence that perylene-EDTA·Fe(II) does not recognize and cleave single-stranded DNA is the lack of significant cleavage of the T-rich single stranded region opposite the G-quadruplex in this type of substrate (*20*). We further investigated the selectivity of the perylene-EDTA·metal complex for G-quadruplex versus duplex and single-stranded DNA by visible spectroscopy using Ni(II) as the metal ion. Although DTT was not included in the buffer used for the spectroscopic measurements, we used Ni(II) rather than Fe(II) to avoid the possibility of DNA cleavage during the measurements. Figure 6A shows the visible spectra of perylene-EDTA·Ni(II) alone and that of perylene-EDTA·Ni(II) in the presence of single stranded and duplex DNA (A4, and D12, respectively; see Table 1). The spectra are not much different, indicating that there is little interaction between perylene-EDTA·Ni(II) and either single stranded or duplex DNA. However, the spectra of perylene-EDTA·Ni(II) alone is significantly different from the spectra in the presence of preformed G-quadruplexes from either the G4 or G3 sequences (Table 1). Thus, there is a slight increase in the peak absorbance at 500 nm that is accompanied by a slight bathochromic shift of this peak in the presence of either the G4 or the G3 sequences. In addition, there is an approximately 2-fold increase in the peak absorbance at 550 nm in the presence of G-quadruplex DNA versus a negligible increase in the presence of either single stranded or duplex DNA. On the other hand, the same type of measurements for perylene-EDTA in the absence of metal (Figure 6B) show that there is a significant difference in the spectra of perylene-EDTA alone and that of perylene-EDTA in the presence of single-stranded and duplex DNA. Thus, there is a slight bathochromic shift and a significant increase in the intensity of the peak absorbance at 500 nm for the perylene-EDTA in the presence of both single-stranded and duplex DNA. Furthermore, there is a ~1.5- and a ~1.8-fold increase in the intensity of the peak at 550 nm in the presence of duplex and single stranded DNA, respectively. However, spectral changes in the presence of intramolecular G-quadruplex DNA are dramatically more significant. Thus, there is a significant bathochromic shift, and a significant increase in the intensity of the peak absorbance at 500 nm in the presence of G-quadruplex DNA. This is accompanied by a 3.4- to 3.7-fold increase in the peak absorbance at 550 nm. Thus, while perylene-EDTA without metal retains some selectivity, the data indicate that the perylene-EDTA·metal complex is more selective toward intramolecular G-quadruplex DNA. However, it is noteworthy that the spectral changes for perylene-EDTA (Figure 6B) in the presence of single stranded and duplex DNA occur only after incubation at room temperature for several hours, whereas the spectral changes in the presence of intramolecular G-quadruplex occur almost immediately.

DISCUSSION

A nuclease that recognizes G-quadruplex and cleaves DNA residues adjacent to this structure was discovered recently (*22*, *23*). Some porphyrins can cleave G-quadruplex DNA (*24*−*25*), but they are also known to cleave duplex DNA. Thus, to our knowledge, there are no other synthetic cleaving reagents specific for G-quadruplex DNA reported to date. Perylene-EDTA·Fe(II) is thus the first compound of its kind that selectively binds to G-quadruplex DNA and cleaves it. We show here that this compound is selective for G-quadruplex DNA versus single stranded and duplex DNA, and that cleavage is more pronounced with increased stability of the G-quadruplex. Increasing pH leads to more localized cleavage of the G-quadruplex region in the substrate used; an effect likely due to more effective localization of Fe(II) by perylene-EDTA. Visible spectra confirm that the perylene-EDTA·metal complex is significantly more selective for G-quadruplex than perylene-EDTA alone.

The precise mode of binding of perylene-EDTA·Fe(II) [or perylene-EDTA·Ni(II)] to the intramolecular G-quadruplex DNA substrates studied here is not yet known. Presumably, perylene-EDTA·Fe(II) binds by stacking externally to the G-tetrads in the G-quadruplex motif, as shown earlier for the related G-quadruplex ligand PIPER (*13*). The complexity of the NMR spectra of the intramolecular G-quadruplex DNA, coupled with severe line broadening upon titration with PIPER, has precluded a detailed structural study of a PIPER: intramolecular G-quadruplex complex. We have encountered a similar problem in the case of the perylene-EDTA: intramolecular G-quadruplex complex. Nevertheless, partial titration of the parallel G-quadruplex [d(TAGGGTTA)]$_4$ with

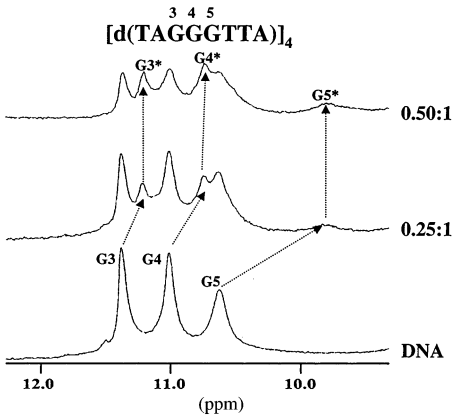


FIGURE 7: Imino proton region of the 500 MHz ^{1}H NMR spectra of the parallel G-quadruplex formed from the NMR1 sequence in Table 1. The spectra were acquired at 7 °C and show the titration of the quadruplex with perylene-EDTA in the absence of metal. G3, G4, and G5 are the original imino proton resonances, which gradually decrease in intensity as the concentration of perylene-EDTA is increased. The G3*, G4*, and G5* resonances correspond to the ligand-quadruplex complex. Only partial titration to a 0.50:1 ratio of ligand to quadruplex is shown.

perylene-EDTA (Figure 7) indicates a similar mode of binding to G-quadruplex as that observed for PIPER (*13*). Thus, titration of this parallel G-quadruplex leads to the appearance of new peaks corresponding to the perylene-EDTA:G-quadruplex DNA complex. Titration leads to severe line-broadening of the 3′ guanine involved in tetrad formation coupled with a significant upfield shift (G5* in Figure 7), indicating that perylene-EDTA stacks externally to the G-quadruplex 3′-end. Thus, it is likely that the perylene-EDTA·Fe(II) complex binds to intramolecular G-quadruplex DNA in a similar mode as reported for PIPER (*13*), thus leading to localized G-quadruplex cleavage through the generation of hydroxyl radicals in the presence of DTT.

In summary, perylene-EDTA·Fe(II) is remarkably selective toward G-quadruplex DNA, and it thus serves as a starting point for the design of more efficient G-quadruplex-specific cleaving reagents. Such cleaving reagents may prove useful as telomere-interactive anticancer agents, and as probes for the existence of G-quadruplex structures in chromatin.

SUPPORTING INFORMATION AVAILABLE

Nondenaturing PAGE showing the purification of the annealed duplex-quadruplex substrates T2G4:T6 and T2AG3:T6, and the duplex substrates T2G4:C4A2 and T2AG3:C3TA2. DMS footprinting (in 10 mM KCl and 100 mM KCl) of the purified T2G4:T6 and T2AG3:T6 duplex-quadruplex substrates used for the cleavage reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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