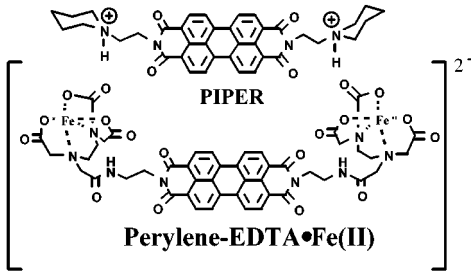
Design and Synthesis of the G-Quadruplex-Specific Cleaving Reagent Perylene-EDTA·Iron(II)

Wirote Tuntiwechapikul, Jeong Tae Lee, and Miguel Salazar*

Division of Medicinal Chemistry
College of Pharmacy and Institute for
Cellular and Molecular Biology
The University of Texas at Austin
Austin, Texas 78712

Received February 7, 2001
Revised Manuscript Received April 25, 2001

The structural uniqueness of G-quadruplex DNA makes it an ideal target for drug design. Various small molecules have been found to bind to G-quadruplex DNA. These include porphyrins, diamidoanthraquinones, carbocyanine dyes, and perylene diimides, among others.[1] The perylene diimide, *N*,*N*′-bis[2-(1-piperidino)-ethyl]3,4,9,10-perylenetetracarboxylic diimide (PIPER) has been reported to be selective for G-quadruplex structures with little affinity for either single- or double-stranded DNA.[1] Herein we report on the synthesis and characterization of a PIPER derivative, perylene-EDTA·iron(II) that selectively cleaves G-quadruplex DNA in the presence of dithiothreitol (DTT).



EDTA·Fe(II) has been tethered to a variety of small molecules, peptides, and nucleic acids to effect cleavage of single- and double-stranded DNA in the presence of dioxygen and a reducing agent such as DTT.[2] A similar approach has been taken to effect cleavage of triple-stranded DNA.[3] Bleomycin−nickel(III) has been found to cleave loop residues in the intramolecular G-quadruplex structure formed by $d(T_2G_4)_4$,[4] and certain porphyrins are known to effect cleavage of G-quadruplex DNA when irradiated with UV light.[5] However, both porphyrins and bleomycin also bind to duplex DNA. Thus, although a nuclease specific for G-quadruplex DNA has been found,[6] no G-quadruplex-specific cleaving reagent has been reported to date.

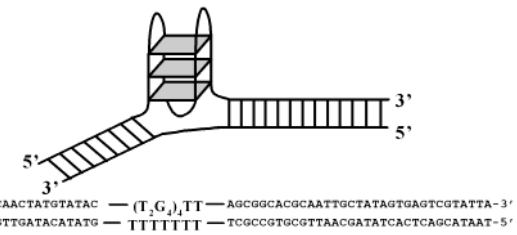


Figure 1. Diagram of the duplex−quadruplex substrate used to assay cleavage of G-quadruplex DNA by perylene-EDTA·Fe(II).

Among the G-quadruplex binding molecules that have been reported, PIPER appears to be one of the most selective. This molecule is known to bind by stacking on the 3′-G tetrad of parallel stranded G-quadruplex DNA and positioning the side chains in opposing grooves.[7] Indirect evidence indicates that this molecule can also bind to intramolecular G-quadruplex structures,[1] although the precise mode of binding to this type of quadruplex structure motif is not known. PIPER has also been shown to facilitate the formation of G-quadruplex structures from single-stranded DNA.[8] Furthermore, as a result of its binding to G-quadruplex structures, PIPER has been found to inhibit the extension of G-rich telomeric primers by telomerase.[1,7] Due to its strong binding to G-quadruplex DNA, it was thus natural to select this molecule as a starting point in the design of a G-quadruplex specific cleaving reagent.

The synthesis of perylene-EDTA was carried out by coupling triethylester EDTA to *N*,*N*′-bis[diethylamine]-3,4,9,10-perylene-tetracarboxylic diimide, both of which were obtained following published procedures.[2,9] Cleavage of the triethylester was then carried out with NaOH (see Supporting Information). Figure 1 shows the DNA substrate used for assaying cleavage of G-quadruplex DNA by perylene-EDTA·Fe(II). The G-quadruplex region consists of four repeats of the *Tetrahymena* telomeric repeat sequence T_2G_4, flanked by two random-sequence duplex regions consisting of 21 base pairs and 33 base pairs, respectively. Seven T residues in the bottom strand were used to bridge the G-quadruplex section in the top strand. The G-quadruplex in the top strand was preformed in 100 mM K^+ buffer prior to annealing to the bottom strand. The duplex−quadruplex substrate was then purified by nondenaturing PAGE. DMS methylation protection analysis (Supporting Information) of the top strand hybridized to the bottom strand in 100 mM K^+ confirmed that the four T_2G_4 repeats form a stable G-quadruplex structure in which only three guanines in each of the four repeats are involved in G-tetrad formation.

Figure 2 shows the cleavage pattern observed for the top strand when the duplex−quadruplex substrate in Figure 1 is incubated in the presence of perylene-EDTA·Fe(II) and DTT. Two prominent cleavage sites within the G-quadruplex region centered on G4 and G16 were observed. Cleavage around G4 includes some cleavage of the two T residues of the first T_2G_4 repeat, while cleavage around G16 includes some cleavage of the two T residues in the third T_2G_4 repeat. Relatively minor cleavage of the second and fourth sets of guanine repeats was observed and only at high concentrations of perylene-EDTA·Fe(II). Degradation products resulting from the radiolabeling process precluded a detailed analysis of the 3′-end of the top strand. However, except for cleavage centered at the first A immediately adjacent to the last T_2G_4 repeat, no other significant cleavage was observed. Also,

* To whom correspondence should be addressed. E-mail:m-salazar@mail.utexas.edu. Fax: 512-232-2606.

(1) (a) Kerwin, S. M. *Curr. Pharm. Design* **2000**, *6*, 441−471 and references therein. (b) Hurley, L. H.; Wheelhouse, R. T.; Sun, D.; Kerwin, S. M.; Salazar, M.; Fedoroff, O. Y.; Han, F. X.; Han, H.; Izbicka, E.; Von Hoff, D. D. *Pharmacol. Ther.* **2000**, *85*, 141−158 and references therein.

(2) (a) Dervan, P. B. *Science* **1986**, 232, 464−471. (b) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645−650. (c) Sluka, J. P.; Griffin, J. H., Mack, D. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 6369−6374. (d) Wade, W. S.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 8783−8794.

(3) Zain, R.; Marchand, C.; Sun, J−s.; Nguyen, C. H.; Bisagni, E.; Garestier, T.; Hélène, C. *Chem. Biol.* **1999**, *6*, 771−777.

(4) Guan, L. L.; Kuwahara, J.; Sugiura, Y. *Biochemistry* **1993**, *32*, 6161−6145.

(5) (a) Wheelhouse, R. T.; Sun, D.; Han, H.; Han, F. X.; Hurley, L. H. *J. Am. Chem. Soc.* **1998**, *120*, 3261−3262. (b) Han, F. X.; Wheelhouse, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* **1999**, *121*, 3561−3570.

(6) (a) Liu, Z.; Frantz, J. D.; Gilbert, W.; Tye, B.-K. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3157−3161. (b) Liu, Z.; Gilbert, W. *Cell* **1994**, *77*, 1083−1092.

(7) Fedoroff, O. Y.; Salazar, M.; Han, H.; Chemeris, V.; Kerwin, S. M.; Hurley, L. H. *Biochemistry* **1998**, *37*, 12367−12374.

(8) Han, H.; Cliff, C. L.; Hurley, L. H. *Biochemistry* **1999**, *38*, 6981−6986.

(9) Lukáč, I.; Langhals, H. *Chem. Ber.* **1983**, *116*, 3524−3528.

10.1021/ja0156439 CCC: $20.00
Published on Web 05/19/2001

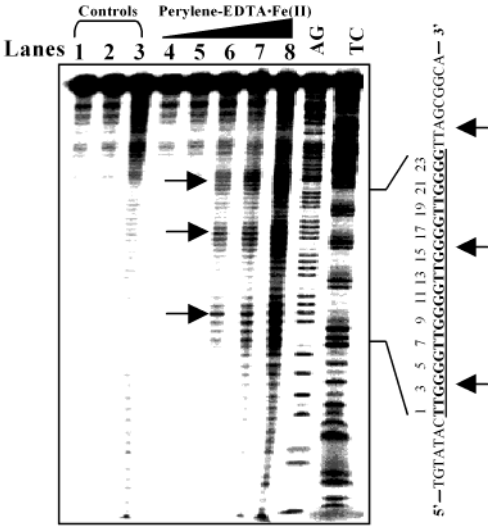


Figure 2. Autoradiograph of the cleavage pattern observed for the top strand in the duplex−quadruplex substrate shown in Figure 1. Lane 1: 5′-^{32}P-labeled DNA only (some degradation products from the ^{32}P-labeling process are evident). Lane 2: Mock experiment in which the labeled DNA underwent the same treatment as the DNA with perylene-EDTA·Fe(II). Lane 3: DNA in the presence of 1 mM EDTA·Fe(II). Lanes 4−8: DNA with perylene-EDTA·Fe(II) added at concentrations of 0.01, 0.1, 1.0, 10, and 100 μM, respectively. The arrows point to the cleavage sites in the substrate. The AG and TC lanes are Maxam−Gilbert sequencing lanes.

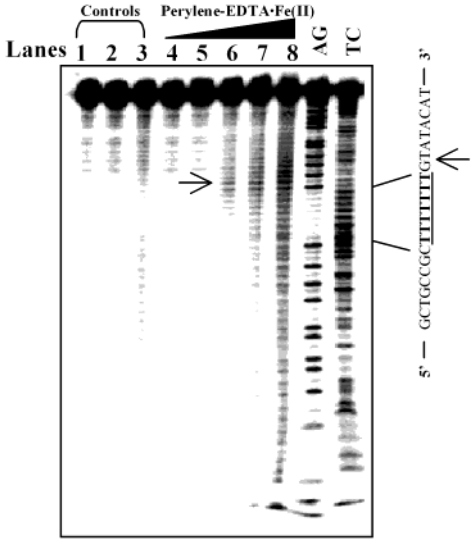


Figure 3. Autoradiograph of the cleavage pattern observed for the bottom strand in the substrate shown in Figure 1. The experimental conditions for each lane are the same as in Figure 2. The arrow points to a minor cleavage site at the 3′-end of the T_7 segment.

the top strand within the 21 base pair double-stranded region remains essentially intact. Figure 3 shows the cleavage pattern observed for the bottom strand under the same conditions. Only minor cleavage of the bottom strand centered on the first T following the T_7 bridge was observed. No noticeable cleavage of the bottom strand within either of the two double-stranded regions was observed.

Overall, the results show that perylene-EDTA·Fe(II) can bind to G-quadruplex DNA and cleave it. The combined data from

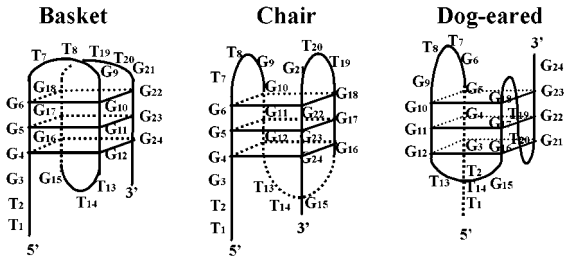


Figure 4. G-quadruplex structures proposed for the *Tetrahymena* telomeric repeat sequence.

Figures 1 and 2 show some cleavage of the double-stranded regions but only in the immediate vicinity of the G-quadruplex, indicating that this molecule is selective for G-quadruplex DNA. There are some similarities in the cleavage pattern in Figure 2 and that observed in the photocleavage of intramolecular G-quadruplex (formed from single-stranded DNA containing T_2AG_3 repeats) with the cationic porphyrin derivative TMPY4.[5] Here we also observe cleavage around the 5′- and 3′-ends of the G-quadruplex, and around the 5′-G of the third G-rich repeat. However, the cleavage pattern differs from that observed with TYPYP4 in that there is lack of significant cleavage around the 3′-Gs of the second and last G-rich repeats (G12 and G24, respectively, in the present substrate). It is likely that lack of cleavage around G12 and G24 may be a result of steric hindrance that may prevent either of the EDTA moieties from approaching these two residues in this rather unusual substrate

Different folding patterns for $d(T_2G_4)_4$ have been proposed[10] (Figure 4). The different folding patterns could arise due to differences in the microenvironment and the manner in which the DNA is annealed. Although the mode of G-quadruplex binding by perylene-EDTA·Fe(II) and the conformations of the EDTA moieties is not yet clear, the cleavage pattern suggests that the G-rich region in the present substrate assumes a conformation closer to that of the basket or chair forms and that perylene-EDTA·Fe(II) binds in the vicinity of the outer G-tetrad formed by G4, G12, G16, and G24. A "dog-eared" G-quadruplex topology is less likely since cleavage is observed at both 5′- and 3′-ends of the G-rich region. However, additional data will be needed to unambiguously determine the overall conformation of the G-quadruplex DNA/perylene-EDTA·Fe(II) complex.

In summary, although its mode of binding is not precisely known, perylene-EDTA·Fe(II) is the first DNA-cleaving reagent of its kind with a strong affinity and selectivity for G-quadruplex DNA. It should prove a useful tool for analyzing G-quadruplex structures and as a prototype for the design of G-quadruplex-specific molecules that can be used to probe chromatin structure.

Acknowledgment. This work was supported by Grants from the National Institutes of Health (CA77000) and the Robert A. Welch Foundation (F-1409) and graduate fellowships from the William Orr Dingwall Foundation. We thank Dr. Laurence H. Hurley, Dr. Sean M. Kerwin, Dr. Qingping Zeng, and Dr. T. Randall Lee for helpful discussions.

Supporting Information Available: Detailed procedures for the synthesis of the perylene-EDTA; experimental details of the DMS methylation protection and cleavage reactions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org

JA0156439

(10) Williamson, J. R.; Raghuraman, M. K.; Cech, T. R. *Cell* **1989**, *59*, 871−880; Wang, Y.; Patel, D. J. *Structure* **1994**, *2*, 1141−1156; Han, H.; Hurley, L. H.; Salazar, M. *Nucleic Acids Res.***1999**, *27*, 537−542.