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Perylene Diimide G-Quadruplex DNA Binding Selectivity is Mediated by Ligand Aggregation

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Abstract—Two *N,N′*-disubstituted perylene diimide G-quadruplex DNA ligands, PIPER [*N,N′*-bis-(2-(1-piperidino)ethyl)-3,4,9,10-perylene tetracarboxylic acid diimide] and Tel01 [*N,N′*-bis-(3-(4-morpholino)-propyl)-3,4,9,10-perylene tetracarboxylic acid diimide] were studied. Visible absorbance, resonance light scattering, and fluorescence spectroscopy were used to characterize the pH-dependent aggregation of these ligands. The G-quadruplex DNA binding selectivity of these ligands as monitored by absorption spectroscopy is also pH-dependent. The ligands bind to both duplex and G-quadruplex DNA under low pH conditions, where the ligands are not aggregated. At higher pH, where the ligands are extensively aggregated, the apparent G-quadruplex DNA binding selectivity is high.

G-quadruplex DNA is a diverse family of DNA structures in which up to four separate DNA strands associate through specific hydrogen bonding interactions between four guanosine residues (G-tetrads).[1] A number of compounds have been reported to bind to G-quadruplex DNA.[2–5] Many of these G-quadruplex ligands have been shown to inhibit telomerase,[3–5,6a] the enzyme responsible for the de novo synthesis of telomeric DNA.[6] Because telomerase levels are high in the majority of tumor cells and largely absent in normal cells,[7] these telomerase inhibiting G-quadruplex ligands have been proposed as selective anti-cancer agents.[8] However, one obstacle to the use of these compounds is the high level of double-stranded DNA binding that they exhibit, which leads to non-specific cytotoxic effects.[4b,8,9] Understanding the factors involved in G-quadruplex binding selectivity is an essential step towards the design of G-quadruplex ligands for potential therapeutic use.

By employing the DOCK program to select molecular scaffolds with a high degree of shape complementarity to G-quadruplex DNA, we identified the *N,N′*-disubstituted 3,4,9,10-perylenetetracarboxylic acid diimides as promising G-quadruplex DNA ligands.[10] On the basis of 2-D NOESY NMR data, a structural model was derived for the interaction of one such diimide, *N,N′*-bis(2-(1-piperidino)ethyl)-3,4,9,10-perylenetetracarboxylic acid diimide (PIPER), with the intermolecular G-quadruplexes formed by oligonucleotides containing the human telomeric sequence d(TTAGGG).[10] The perylene diimide binds by stacking on the 3′- or 3′- and 5′-faces of the core $[d(GGG)]_4$ G-tetrad helix. These interactions stabilize the G-quadruplex form of the telomeric DNA, resulting in inhibition of human telomerase[10] and the G-quadruplex helicase activity of yeast Sgs1.[11] PIPER facilitates the formation of G-quadruplex DNA from single-stranded DNA[12] and certain double-stranded DNA oligonucleotides,[13] but no evidence for duplex DNA binding by PIPER was observed in ^{1}H NMR titrations.[10,12]

Here, we report our investigation of pH effects on the G-quadruplex DNA binding by PIPER and *N,N′*-bis-(3-(4-morpholino)-propyl)-3,4,9,10-perylenetetracarboxylic acid diimide (Tel01).[14] We find that the G-quadruplex DNA binding selectivity of these compounds is pH-dependent. By characterizing the aggregation state of these ligands as a function of pH, we find that under conditions where these ligands are extensively aggregated, they bind to G-quadruplex DNA preferentially over duplex DNA; however, under conditions in which the ligands are not extensively aggregated, the binding selectivity for G-quadruplex DNA is much less. These findings demonstrate a novel consequence of ligand aggregation, and highlight the importance of monitoring self-assembly of G-quadruplex ligands, which are extremely prone to aggregation.[15]

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In 40 mM phosphate buffer between pH 2 and 4, the absorption spectra of PIPER and Tel01 display maxima at 545 and 500 nm with a shoulder at 480 nm (Fig. 1), which is similar to the spectra reported for other perylene diimides under conditions where the compounds are not aggregated.[16] At higher pH, there is a marked hypochromism and a shift in maxima, resulting in a broad peak around 470 nm. These changes reflect a change in aggregation state of these perylene diimides.[17] From the point dipole model of Kasha and co-workers,[18] the appearance of a blue-shifted absorption band indicates that the transition dipoles of individual perylene diimide units in the aggregate are oriented in a parallel fashion. The pH dependence of these spectral changes differs depending upon the nature of the side chain: PIPER, with the more basic piperidino side chain, requires higher pH (>8–9) as compared to Tel01, with the less basic morpholino side chain, which is extensively aggregated at pH >7–8.[19]

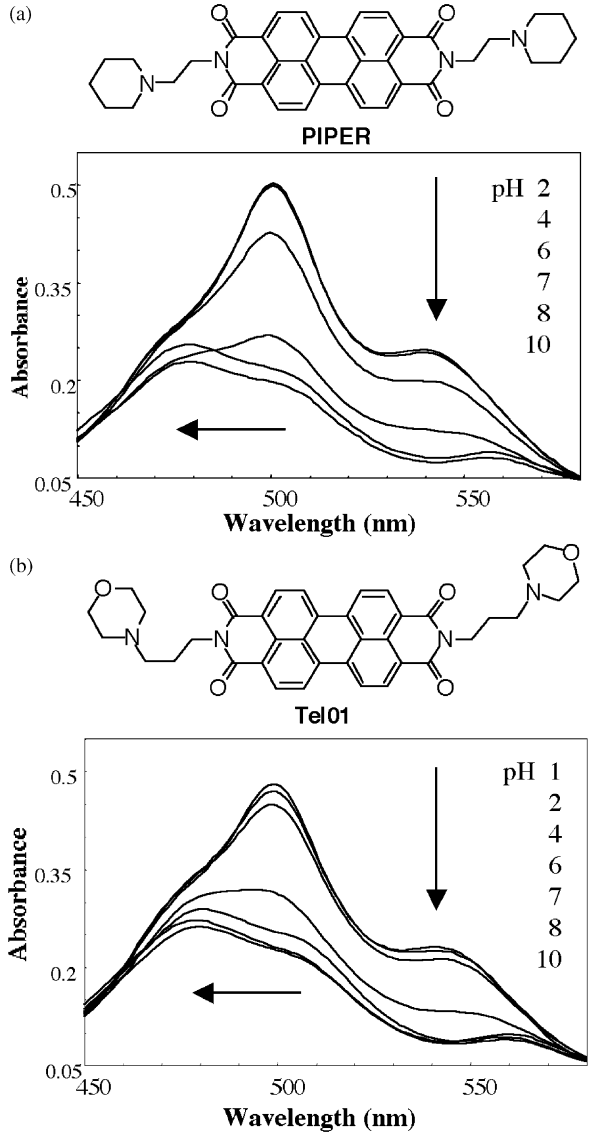

Figure 1. Chemical structure and absorption spectra of (a) PIPER and (b) Tel01 (20 μM) in potassium phosphate buffer (40 mM) at the indicated pH.

Further evidence for pH-dependent aggregation was obtained from resonance light scattering (RLS) experiments of more dilute ligand solutions in higher salt buffers that favor G-quadruplex DNA formation. For dyes in which aggregation promotes good electronic coupling between chromophores, a substantial enhancement in the scattering of light matching the absorption band of the aggregate is observed.[20] There is a pronounced RLS signal for solutions of Tel01 at pH 7 that is absent at pH 6 (Fig. 2a). The maximum of the RLS profile at 470 nm at pH 7 corresponds to the blue-shifted band in the absorption spectrum at this pH, confirming the assignment of this band to the aggregate. A much weaker RLS signal is observed for solutions of PIPER at pH 7 (Fig. 2b).

Non-aggregated perylene diimides are highly fluorescent in solution.[21] Solutions of PIPER fluoresce (Ex 520 nm/Em 550 nm) at pH <7.0 and solutions of Tel01 fluoresce only at pH <6.5 (data not shown). Failure to detect fluorescence from aqueous solutions of Tel01 at pH 7.0 and above or from PIPER solutions at pH 8.5 is also indicative of aggregation.[18]

We determined the UV–Vis absorption spectra of solutions of Tel01 and PIPER (20 μM) in pH 7.0 potassium phosphate buffer (170 mM K^+) alone or in the presence of excess single-stranded DNA [ssDNA: d(TTTTTTTT)], double-stranded DNA [dsDNA: $[d(CGCGCGATATCGCGCG)]_2$], intermolecular G-quadruplex DNA [G4-DNA: $[d(TAGGGTTA)]_4$], or

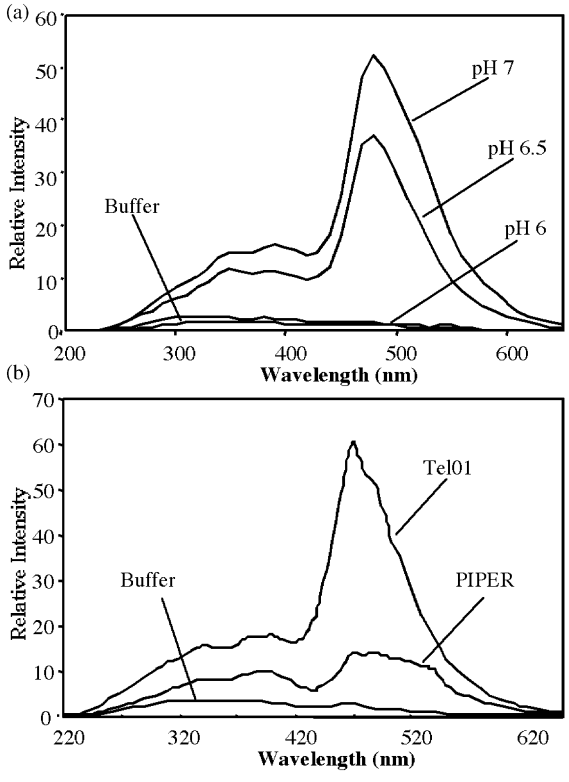

Figure 2. (a) Resonance light scattering (RLS) spectra of Tel01 (1 μM) in 170 mM potassium phosphate buffer at different pH. (b) RLS spectrum of Tel01 and PIPER (1 μM) in 170 mM potassium phosphate buffer, pH 7.

intramolecular G-quadruplex DNA [G4′-DNA: $[d(TTAGGG)_4]]$[22] (Fig. 3). In the presence of excess G4-DNA, G4′-DNA, or dsDNA, the absorption spectrum for PIPER shows a prominent peak at ca. 550 nm and a smaller peak at ca. 550 nm. In the presence of excess ss-DNA, the absorbance peak at 510 nm is larger than the peak at 550 nm. The absorption spectrum of Tel01 is only slightly shifted in the presence of excess ssDNA or dsDNA, but shows peaks at 510 nm and 550 nm in the presence of either G4-DNA or G4′-DNA.

We confirmed the nature of the G4-DNA binding by Tel01 through an NMR titration experiment in H_2O/D_2O using G4-DNA (data not shown). The results were similar to those reported for PIPER: addition of sub-stoichiometric amounts of Tel01 led to the appearance of two separate sets of resonances for the imino protons of the free DNA (10.9–11.6 ppm) and the Tel01–DNA complex (10.1–11.5 ppm). The upfield shift of the imino proton resonances indicates that Tel01 binds by stacking interactions with the terminal G-tetrads.

In contrast to these results obtained at pH 7.0, where the Tel01 selectivity for G-quadruplex DNA is estimated to be $>10^3$-fold, at lower pH, where Tel01 is not aggregated, Tel01 undergoes spectral changes in the presence of dsDNA that are similar to the spectral changes observed in the presence of G-quadruplex DNA (Fig. 4a). At pH 6.5, Tel01 demonstrates only a 10-fold preference for binding G4-DNA versus dsDNA.[23] Similarly, PIPER displays only about a 10-fold selectivity for binding G4-DNA versus ds-DNA under conditions where it is not aggregated (pH 7.0), and a much higher degree of selectivity under aggregating conditions (pH 8.5) (Fig. 4b).

Here we show that PIPER and Tel01 bind preferentially to G4-DNA but not dsDNA under conditions that favor ligand aggregation. Support for ligand aggregation, and not some other pH-mediated effect,[24] as the cause of G-quadruplex DNA binding selectivity is provided by experiments in which non-aggregated Tel01 is added to dsDNA at pH 7.0. When stock solutions of Tel01 in 0.1% TFA/H_2O are added to dsDNA in pH 7.0 phosphate buffer, the absorbance spectrum of the dsDNA–Tel01 complex is initially observed. Over the course of ca. 30 min, the spectrum reverts to that of the uncomplexed, aggregated Tel01 (Fig. 5). Under conditions where the stability constant for the perylene diimide aggregate is more favorable than the association constant for double-stranded DNA binding, little binding is observed. In contrast, when the stability constant for the aggregate is not as favorable, a slow duplex DNA binding interaction is observed. The dsDNA binding by perylene diimides likely involves intercalation interactions. Evidence for intercalation is provided

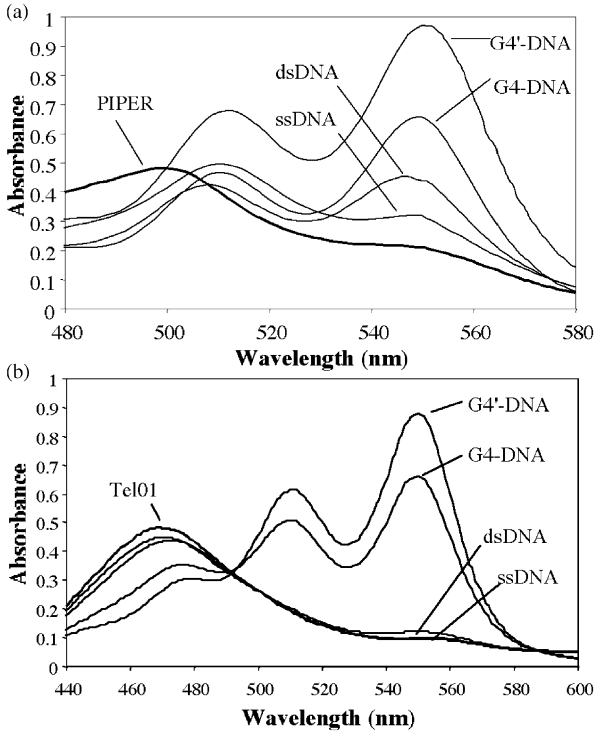

Figure 3. (a) Absorption spectra of PIPER (20 μM) in 70 mM potassium phosphate/100 mM KCl buffer (pH 7.0) alone and in the presence of $[d(TAGGGTTA)]_4$ (G4-DNA) (640 μM phosphate); $[d(TTAGGG)_4]$ (G4′-DNA) (480 μM phosphate); $[d(CGCGCGATATCGCGCG)]_2$ (dsDNA) (500 μM phosphate); and [(TTTTTTTT)] (ssDNA) (500 μM phosphate). (b) Absorption spectra of Tel01 (20 μM) in the same buffer as above alone or in the presence of G4-DNA (640 μM phosphate); G4′-DNA (480 μM phosphate); dsDNA (500 μM phosphate); and ssDNA (500 μM phosphate).

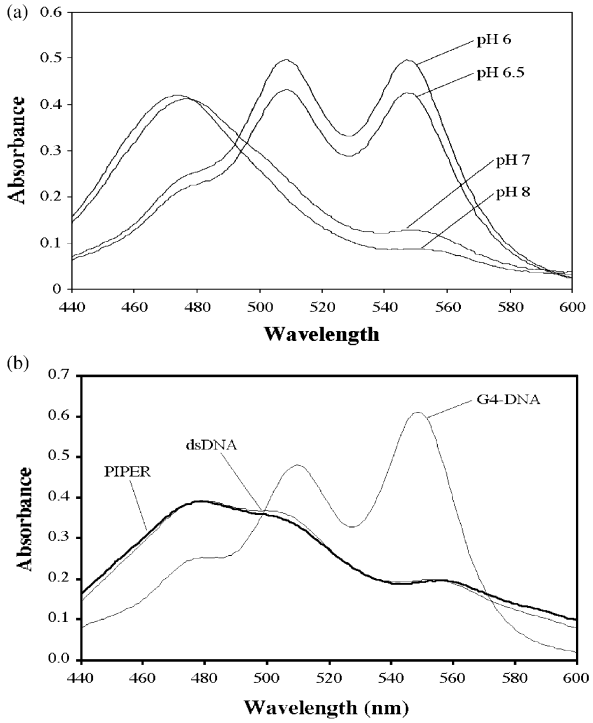

Figure 4. (a) Absorption spectrum of Tel01 (20 μM) in the presence of double-stranded $[d(CGCGCGATATCGCGCG)]_2$ (320 μM base-pairs) in 70 mM potassium phosphate buffer/100 mM KCl at the indicated pH. (b) Absorption spectra of PIPER (20 μM) in 70 mM potassium phosphate buffer/100 mM KCl, pH 8.5, alone or in the presence of $[d(TAGGGTTA)]_4$ (G4-DNA) (640 μM phosphate) or $[d(CGCGCGATATCGCGCG)]_2$ (dsDNA) (320 μM phosphate).

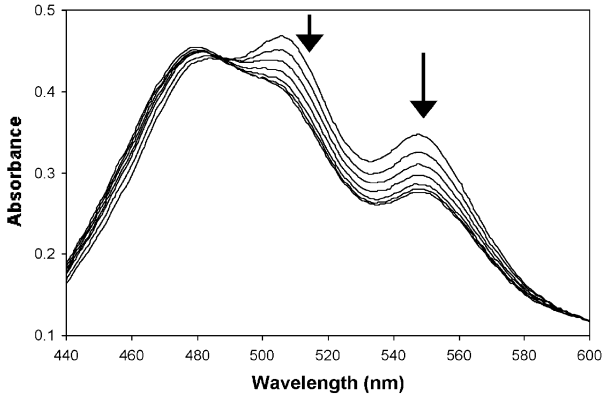

Figure 5. Time-dependent absorbance spectra of Tel01 in the presence of dsDNA in 70 mM potassium phosphate buffer/100 mM KCl, pH 7.0. Tel01 was added to the dsDNA solution as a 2 mM stock solution in 0.1% aq TFA, and the absorbance spectra were acquired over 30 min. The arrows indicate the decrease in absorption at 500 and 550 nm over time.

by unwinding experiments, in which relaxed closed-circular ϕX174 phage DNA in the presence of PIPER (30–180 μM) and topoisomerase I at pH 7.5 affords supercoiled DNA products (data not shown). The duplex DNA intercalation by these perylene diimides likely requires the ligand to be monomeric. In contrast, G-quadruplex DNA binding by perylene diimides occurs by end stacking interactions with G-tetrads. Because this stacking interaction requires only one free chromophore face, it is possible that G-quadruplex DNA binding may occur from either aggregated or monomeric ligand. While it is doubtful that the high degree of G-quadruplex DNA binding selectivity observed for aggregated perylene diimides would have relevance in the biological milieu, understanding the role of ligand aggregation in G-quadruplex DNA binding will allow the design of new ligands having the type of selectivity required for use as probes for G-quadruplex structures in biological systems, or as potential drugs.

Acknowledgements

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References and Notes

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22. The ds-, G4-, and G4′-DNA secondary structures were annealed and confirmed by non-denaturing gel electrophoresis.
23. Spectrophotometrically determined apparent association constants uncorrected for ligand aggregation for dsDNA are Tel01 (pH 6.5) 1.4×10^4 M^{-1} and PIPER (pH 7) 8.2×10^4 M^{-1} and for G4-DNA are Tel01 (pH 6.5) 3.6×10^5 M^{-1} and PIPER (pH 7) 4×10^6 M^{-1}.
24. The stability of the ds-DNA is expected to be relatively insensitive to pH in the range employed for these studies.