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The Aggregation and G-Quadruplex DNA Selectivity of Charged 3,4,9,10-perylenetetracarboxylic Acid Diimides

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Abstract—Two *N,N′*-disubstituted perylene diimide G-quadruplex DNA ligands, Tel11 (*N,N′*-bis-[3-(4-methyl-morpholin-4-yl)-propyl]-3,4,9,10-perylenetetracarboxylic acid diimide diiodide) and Tel12 (*N,N′*-bis-[(3-phosphono)-propyl]-3,4,9,10-perylene-tetracarboxylic acid diimide tetrapotassium salt) were synthesized and studied. Visible absorbance spectroscopy, resonance light scattering, and fluorescence spectroscopy were utilized to explore the aggregation state, affinity for various DNA structures, and G-quadruplex selectivity of these ligands. The water-soluble ligands exist in a monomer–dimer equilibrium with the cationic Tel11 exhibiting a greater affinity for various DNA structures than the anionic Tel12. Tel12 has greater selectivity for G-quadruplex DNA over double-stranded DNA than Tel11.

G-Quadruplex DNA is a diverse family of structures characterized by the association of up to four G-rich DNA sequences to form stacked G-tetrads.[1] A number of molecular scaffolds have been shown to interact with G-quadruplex DNA, including the porphyrins,[2] fluoroquinophenoxazines,[3] carbocyanine dyes,[4] and benzoindoloquinolones.[5] The 3,4,9,10-perylenetetracarboxylic acid diimides (PTCDI) are another series of demonstrated G-quadruplex DNA interactive agents with several interesting properties. PIPER, the *N,N′*-bis[2-(1-piperidino)-ethyl] PTCDI variant, has been shown to stack on the terminal faces of G-tetrads in G-quadruplex DNA,[6] promote the formation of G-quadruplex DNA from single-stranded[7] and double-stranded[8] DNA precursors, inhibit the ability of RecQ DNA helicase Sgs1p to unwind G-quadruplex DNA,[9] and inhibit telomerase,[6] the enzyme responsible for telomere maintenance. Recently, we reported on the ability of PTCDIs bearing basic sidearms, like PIPER, to selectively bind G-quadruplex DNA.[10,11] We concluded that the G-quadruplex selectivity of these ligands is based on the pH-dependent aggregation of the molecules in solution. As nearly all of the recently reported G-quadruplex interactive ligands are cationic in solution, we decided to investigate the effects of charge, both cationic and anionic, on the PTCDI scaffold. The incorporation of charge into these molecules should attenuate the aggregation of these molecules. Studying these ligands in the presence of DNA will further increase our understanding of the factors involved in G-quadruplex DNA affinity and selectivity.

Here, we report the synthesis and characterization of PTCDIs bearing cationic and anionic sidearms. We investigate the role that charge plays in PTCDI solubility and aggregation compared to the previously studied PTCDIs.[10,11] The thermodynamic and kinetic affinity and selectivity for G-quadruplex DNAs using visible absorbance and fluorescence spectroscopy are also detailed.

Tel11[12] is prepared by reaction of the previously described Tel01[10] with 20 equivalents of methyliodide in chlorobenzene at 140 °C for 90 min. Filtration of the warm reaction mixture and subsequent washing with methanol gives the final compound in quantitative yield (Fig. 1).[13] For Tel12, an aqueous solution of 15 equivalents of 3-aminopropylphosphonic acid and 30 equivalents of potassium hydroxide were added drop wise to a stirring solution of 3,4,9,10-perylene tetracarboxylic dianhydride in DMSO at 100 °C. The reaction proceeded for 3 h. Filtration of the warm reaction mixture and washing with methanol and DMSO gives the final product in 52% yield.[14]

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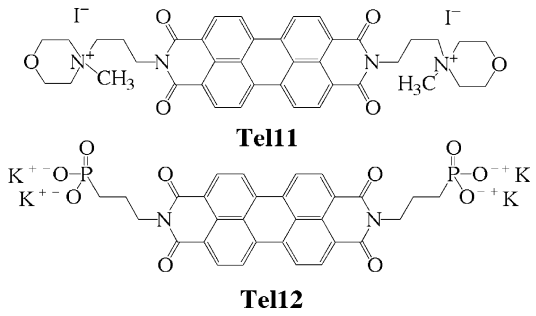

Figure 1. Chemical structures of Tel11 and Tel12.

Both Tel11 and Tel12 are water-soluble ligands. Investigations into the aggregation of Tel11 and Tel12 demonstrate that these charged PTCDIs have a significantly altered degree of self-association when compared to our previous PTCDIs.[10,11] In 70 mM potassium phosphate/100 mM potassium chloride/1 mM EDTA buffer at pH 7.0 (170 mM phosphate buffer), 20 μM solutions of Tel11 and Tel12 do not display a resonance light scattering (RLS) signal,[15] unlike previously reported PTCDI G-quadruplex DNA ligands (data not shown). This indicates that Tel11 and Tel12 do not undergo extensive aggregation, or, if aggregates are formed, they are not RLS active. Absorbance spectroscopy experiments under the same buffer conditions demonstrate isobestic behavior for both charged compounds as the concentration is decreased from 100 μM (Fig. 2). This isobestic behavior is indicative of a two-state monomer–dimer equilibrium.[16] Above 100 μM ligand, isobestic behavior is lost, most likely due to the formation of higher order aggregates (data not shown). The dimerization of these ligands is extensive in 170 mM phosphate buffer, and we were unable to obtain spectra corresponding to monomeric ligand even at 1 μM ligand concentration. However, the dimers can be dissociated in the presence of organic solvents or in low ionic strength buffers. Figure 3 shows the absorbance and fluorescence profiles of the monomeric ligands compared to the predominantly dimeric ligands in 170 mM phosphate buffer.

The absorbance spectra of both Tel11 and Tel12 dimers have a maximum at 500 nm with a shoulder at 550 nm. The monomer spectra, obtained in the presence of ethanol for Tel11 or a 50/50 mixture of ethanol and DMSO for Tel12, have peaks at 425, 450, and 535 nm. In high ionic strength buffer, Tel11's fluorescence is only 15% of that of the monomer while Tel12's fluorescence is only 4% that of the monomer. This decrease in fluorescent intensity has also been seen in the dimerization of di(glycyl) PTCDI.[16] It should be noted that the addition of charged sidearms is not the only method for limiting PTCDI aggregation, as PTCDIs containing neutral bulky peripheral groups also have limited aggregation as indicated by fluorescence spectroscopy.[17]

The absorbance and fluorescence spectra of these molecules in the presence of DNA allow us to gauge the impact of charge on the PTCDIs affinity and selectivity

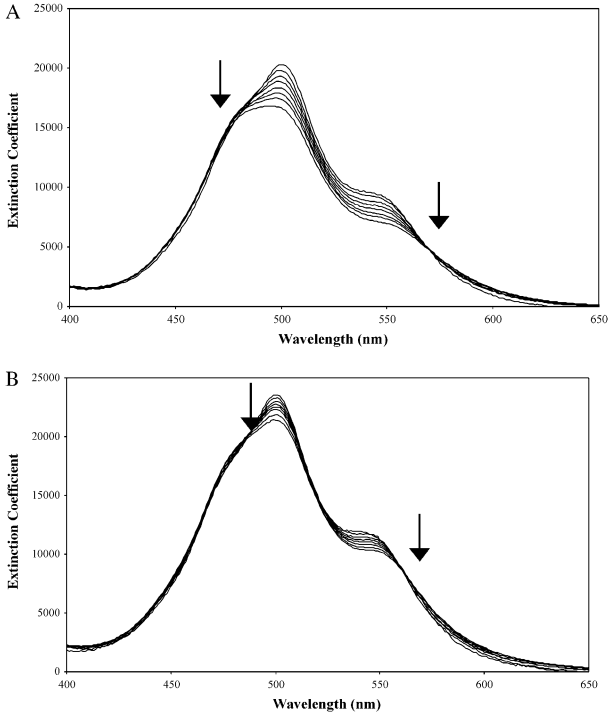

Figure 2. Visible absorbance spectra of serial dilutions of Tel11 (A) and Tel12 (B) in 170 mM phosphate buffer. Curves represent the extinction coefficient of solutions of 9.4, 12.5, 18.8, 25, 37.5, 50, 75, and 100 μM ligand, with the more dilute solutions demonstrating the higher extinction coefficient at 500 nm for both ligands. Arrows indicate isobestic points.

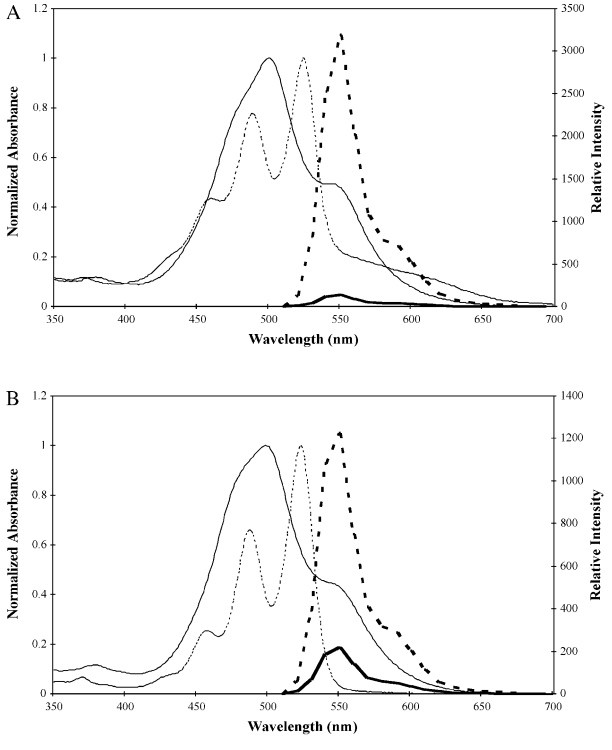

Figure 3. Normalized visible absorbance spectra (thin lines, left *y*-axis) and relative fluorescent spectra (bold lines, right *y*-axis) for solutions of Tel11 (A) and Tel12 (B) under conditions (170 mM phosphate buffer) that favor dimerization (solid lines) and conditions favoring the monomeric state (EtOH for absorbance and 1 mM HCl for fluorescence for Tel11 and EtOH/DMSO for absorbance and 1 mM NaOH for fluorescence for Tel12, dashed lines).

for various DNA structures. The absorbance spectra of Tel11 and Tel12 alone and with various DNA structures are shown in Figure 4. Tel11 or Tel12 (20 μM) were combined with 20 μM structure of intermolecular G4-DNA [d(TAGGGTTA)]$_4$, intramolecular G4′-DNA [d(TTAGGG)$_4$], double-stranded DNA [d(CGCGC GATATCGCGCG)]$_2$, or single-stranded DNA [d(T)$_8$] in 170 mM phosphate buffer. The absorbance spectrum was recorded until equilibrium was achieved. As with previously investigated PTCDI G-quadruplex DNA ligands, we observe a marked hyperchromicity at 550 nm accompanied by a more gradual bathochromic shift from 500 nm to 510 nm for Tel11 in the presence of intermolecular G-quadruplex DNA (G4-DNA), intramolecular G-quadruplex DNA (G4′-DNA), as well as double-stranded DNA (dsDNA). Very little hyperchromicity at 550 nm and a minimal bathochromic shift from 500 nm is observed in the presence of single-stranded DNA (ssDNA). These results indicate that Tel11 has a similar, strong interaction with G4-DNA, G4′-DNA, and dsDNA. For Tel12, hyperchromicity at 550 nm and a bathochromic shift from 500 to 510 nm is only observed in the presence of G4′DNA. Hyperchromicity at 550 nm is observed with G4-DNA, and only subtle changes in absorbance are observed for Tel12 with dsDNA or ssDNA. These results indicate that Tel12 interacts most strongly with G4′-DNA and also interacts with G4-DNA. In contrast to Tel11, however, Tel12 has little affinity for double-stranded DNA, making it a rather selective ligand. We note that Tel11′s absorbance spectra indicate rapid initial binding with no change after 5 min following the addition of the DNAs. In contrast, the absorbance spectrum of Tel12 undergoes gradual change after the addition of DNA, typically requiring 60 min to achieve equilibrium. Particularly in the case of binding to G4′-DNA, comparison of the absorbance spectra of the two ligands indicates that Tel11 achieves equilibrium over 1,000 times faster than Tel12 (data not shown). This difference in binding kinetics may be attributed to the charge–charge effects of the ligands with the polyanionic backbone of the DNA. The cationic ligand, Tel11, would have electrostatic attraction for the DNA in solution while the anionic Tel12′s electrostatic repulsion with the DNA would slow binding.

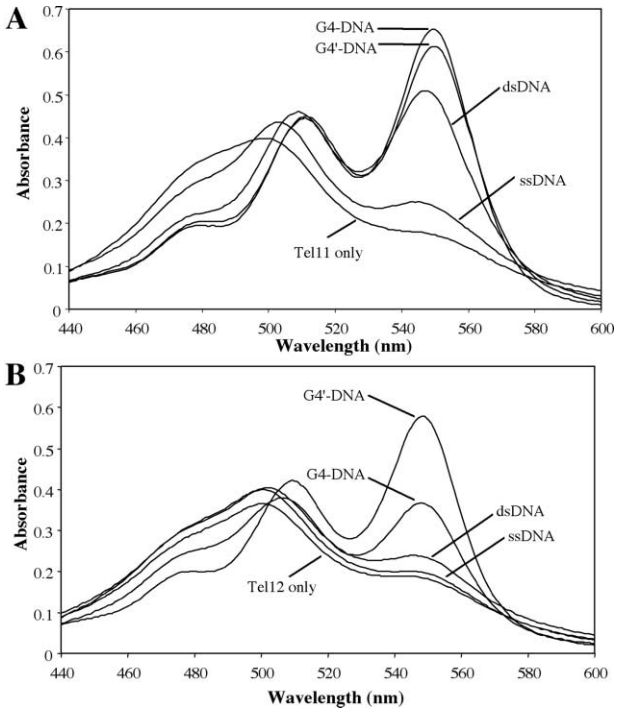

Figure 4. Absorption spectra of Tel11 (A) or Tel12 (B) in 170 mM phosphate buffer alone or in the presence of 1 equivalent of G4-DNA [d(TAGGGTTA)]$_4$, G4′-DNA [d(TTAGGG)$_4$], dsDNA [d(CGCGCGATATCGCGCG)]$_2$, or ssDNA [d(T)$_8$] structure.

PTCDI fluorescence is quenched upon ligand binding to DNA structures.[18] Fluorescence titration experiments of Tel11 and Tel12 with intermolecular G4-DNA, intramolecular G4′-DNA, and double-stranded DNA were carried out by adding stock solutions of each DNA structure to 1 μM solutions of each ligand in 170 mM phosphate buffer in quartz cuvettes treated with SigmaCote to minimize nonspecific interactions between the ligand and quartz. For Tel11, the fluorescence signal is almost completely quenched at very low ratios of DNA structure to ligand (Fig. 5), from which we conclude that several Tel11 molecules are binding to each DNA structure. Scatchard analysis of the data presented in Figure 5 for Tel11 demonstrates pronounced curvature indicative of positive cooperativity, with an apparent saturation binding of six Tel11 molecules per G4-DNA structure. Resonance light scattering experiments to determine if Tel11 ligands were aggregating on DNA, analogous to the aggregation on DNA templates that has been observed with carbocyanine dyes,[19] were inconclusive.[20] These data, however, do not exclude the possibility that Tel11 may aggregate on the DNA, as the aggregate simply may not be RLS active.

Table 1 provides a synopsis of the fluorescent titration experiments expressed as the ratio of the concentration of each DNA structure required to quench half of the ligands fluorescence. The fluorescence-quenching data in Table 1 demonstrate good agreement with the previously described absorbance binding experiments. While Tel11 has similar affinity for all three DNA structures, Tel12 is more G-quadruplex selective, requiring 83-fold more

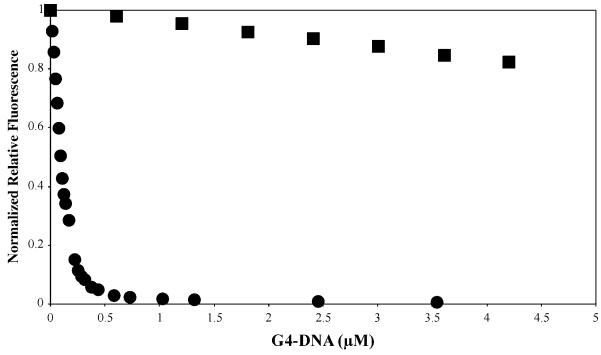

Figure 5. Normalized fluorescent titration data at an emission wavelength of 550 nm for 1 μM Tel11 (circles) or Tel12 (squares) with G4-DNA in 170 mM phosphate buffer. For each DNA concentration, fluorescence emission was determined after equilibrium had been reached (10–60 min).

Table 1. Ratio of DNA required to quench 50% of ligand fluorescence[a,b]

DNA structure	Tel11	Tel12
G4-DNA[c]	0.14±0.05	9.0±1.0
G4′-DNA[d]	0.12±0.06	2.8±0.2
dsDNA[e]	0.17±0.07	46.5±1.5

[a]Numbers are reported as the average of two separate experiments± standard deviation.
[b]DNA solutions were titrated into 1 μM solutions of ligand in 170 mM phosphate buffer and the fluorescence decrease (ex = 500 nm, em = 550 nm, measurement after 10–60 min equilibration) versus ratio of added DNA structure concentration to ligand concentration was plotted in order to determine the concentration of DNA required to decrease the initial fluorescence by 50%.
[c][d(TAGGGTTA)]$_4$.
[d][d(TTAGGG)$_4$].
[e][d(CGCGCGATATCGCGCG)]$_2$.

dsDNA base pairs than G4-DNA and 266-fold more dsDNA base pairs than G4′-DNA to quench half of the ligand's initial fluorescence. While Tel12 may be more selective for G-quadruplex DNA than Tel11, Tel11 interacts more readily with the all of the DNA structures, requiring between 23- and 274-fold less DNA than Tel12 to reach 50% fluorescence decrease.

We have reported here the novel synthesis and characterization of two water-soluble 3,4,9,10-perylenetetracarboxylic acid diimides. We find that they do not extensively aggregate, forming dimers in the absence of DNA in 170 mM phosphate buffer. This limited aggregation is quite different than the pH-dependent aggregation observed in previously investigated PTCDI G-quadruplex ligands. Aggregation, then, is not the determining factor for the selectivity differences observed here for G-quadruplex DNA over double-stranded DNA. Fluorescent titration experiments indicate these ligands may aggregate on DNA structures and that Tel11 binds with greater affinity, but little selectivity for G-quadruplex DNA over double-stranded DNA. Further structural investigations of Tel11 and Tel12 are required in order to elucidate the nature of their G-quadruplex DNA interactions, which may differ from the end-stacking binding mode of the PTCDIs PIPER[6] and Tel01.[11] Tel12 binds with lower affinity and kinetically slower to the DNA structures, but exhibits increased selectivity for G-quadruplex DNA over double-stranded DNA due to charge-charge repulsion. It is interesting to note that *N*-methyl mesoporphyrin IX (NMM), the most G-quadruplex DNA selective porphyrin identified to date, is also anionic in solution.[21] These investigations continue to shed light on the aggregation and forces governing G-quadruplex DNA selectivity of 3,4,9,10-perylenetetracarboxylic acid diimides.

Acknowledgements

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13. ^{1}H NMR (D_2O, 300 MHz, 25 °C) δ 8.26 (s, 4H), 7.98 (s, 4H), 4.5 (br, 12H), 4.06 (s, 4H), 3.94 (s, 4H), 3.83 (s, 4H), 3.56 (s, 6H), 2.63 (s, 4H). ESIMS (M-2) 337.5. EA calcd for $C_{40}H_{42}N_2O_6I_2{\cdot}2H_2O$: C 49.80, H 4.81, N 5.81; found C 49.52, H 4.46, N 5.62.
14. ^{1}H NMR (D_2O, 300 MHz, 25 °C) δ 7.08 (br, 8H), 3.75 (br, 4H), 1.62 (br, 8H). ^{31}P NMR (D_2O, 300 MHz, 25 °C, external reference: H_3PO_4) δ 25.40. ESIMS (M-1) 633.6. EA calc'd for $C_{30}H_{20}N_2O_{10}P_2K_4{\cdot}3H_2O$: C 42.85, H 3.12, N 3.33; found C 42.99, H 3.48, N 3.36.
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