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# The influence of pH on the G-quadruplex binding selectivity of perylene derivatives

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**Abstract**—Three new perylene derivatives with branched ionizable side chains were synthesized, and their G-quadruplex binding specificities were compared by spectroscopic and electrophoretic analysis with two well-studied G-quadruplex ligands: PIPER and $TmPyP_4$. The value of pH and consequent charge formation and self-aggregation of these perylene derivatives influences not only the type of G-quadruplex formation, but also the G-quadruplex binding selectivity.
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G-rich DNA sequences can adopt a special class of DNA structure called a G-quadruplex, which comprises a stack of G-tetrads, the planar association of four guanines in a cyclic Hoogsteen hydrogen bond. G-quadruplex ligands are proposed to be selective anticancer agents by acting as telomerase inhibitors[1] and/or transcriptional repressors of c-*MYC* oncogene.[2] However, one problem that faces many G-quadruplex ligands is non-specific cytotoxicity, which is believed to arise from their interaction with duplex DNA.[1b,3] Ideal G-quadruplex ligands, therefore, should bind selectively to their target and have little interaction with duplex DNA. Studies regarding G-quadruplex binding selectivity are essential to the development of G-quadruplex ligands for therapeutic use.

Among several classes of G-quadruplex ligands, perylene is one of the most widely studied.[4] The perylene derivatives, including the prototype, PIPER, have been well characterized with regard to their G-quadruplex-bound structure,[4a] G-quadruplex induction,[4b,c,h,i] G-quadruplex binding selectivity,[4d–i] and telomerase inhibition.[4h,i] The binding specificity of perylene toward G-quadruplex DNA is exemplified by the cleavage experiment of perylene-EDTA·Fe(II). This complex, upon initiating hydroxyl radical production, cleaved preferentially at the G-quadruplex region, with little effect on the duplex region of the same DNA molecule.[4d] To develop this class of molecule into useful therapeutic agents, studies of G-quadruplex binding selectivity are essential.

Here, we report the synthesis and characterization of three new perylene derivatives (see Fig. 1): P-GLU, P-HIS, and P-TRIS,[5] and the influence of pH on the solubility and G-quadruplex binding selectivity of these molecules in comparison with the two well-studied G-quadruplex ligands: PIPER and $TmPyP_4$. The four perylene derivatives possess side chains that are ionizable with systematically varying charges as a function of pH (see Fig. 2).[6] Over the entire range of buffers used in this study (pH 5–9), P-GLU is negatively charged, while PIPER and P-TRIS are positively charged. P-HIS, however, is neutral at lower pH ($\sim$5) but is negatively charged at higher pH. Therefore, the effect of neutral and negatively charged P-HIS toward G-quadruplex selectivity can be compared directly without considering structural differences. The solubility profiles of these perylene derivatives are shown in Table 1.

We first determined the binding specificity of the perylene derivatives with various preformed DNA structures by spectrophotometry. Each perylene derivative (40 μM) was incubated with each preformed DNA

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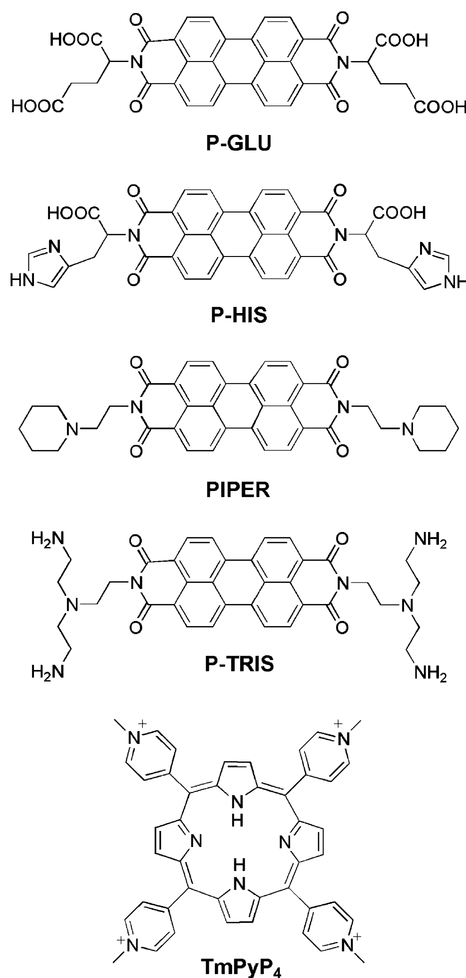


**Figure 1.** Structures of perylene derivatives and $TmPyP_4$.

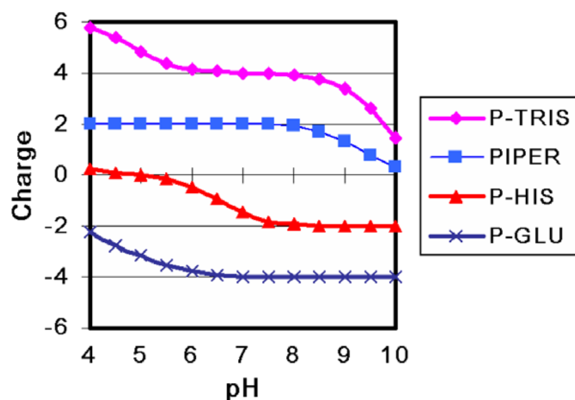


**Figure 2.** Calculated charges of perylene derivatives as a function of pH.

**Table 1.** Solubility profiles of perylene derivatives

| pH | 5* | 6** | 7** | 8** | 9*** |
|---|---|---|---|---|---|
| P-GLU | + | + | + | + | + |
| P-HIS | – | – | ± | + | + |
| PIPER | + | + | – | – | – |
| P-TRIS | + | – | – | – | + |

Solubility was recorded after dispersion of the compound (50 μM) in the designated buffer for seven days. Symbols: (+) soluble, (±) partially soluble, (–) precipitate, (*) potassium acetate buffer, (**) potassium phosphate buffer, and (***) Tris–HCl buffer.

structure[7] (20 μM): single-stranded DNA (ss-DNA, 24A4), double-stranded DNA (ds-DNA, 12D), G3-quadruplex DNA (G3-DNA, 24G3), or G4-quadruplex DNA (G4-DNA, 24G4), in a designated buffer containing 100 mM KCl for 18 h, and absorption spectra between 400 and 600 nm were recorded (Fig. 3). The sequences of all oligonucleotides used in this study are shown in Table 2.

The spectra of P-GLU with the various DNA substrates show no significant changes from the spectrum of P-GLU alone, indicating that P-GLU fails to interact with any of the DNA structures. Considering that P-GLU is negatively charged and dissolves well at all buffers used, it is plausible that the branched negatively charged side chains of P-GLU prevent the interaction with DNA via electrostatic repulsion with the phosphate backbone of DNA.

The spectra of P-HIS with the various DNA substrates show pH-dependent and substrate-dependent interactions. At pH 6, spectra of P-HIS with ss-DNA, ds-DNA, or P-HIS alone are almost flat across all wavelengths due to aggregation, indicating that P-HIS fails to interact with these DNA substrates. In contrast, the spectra of P-HIS with G-quadruplex DNAs (both G3-DNA and G4-DNA) show a significant increase in absorption intensity, especially at 550 nm, indicating strong interaction of P-HIS with these DNA substrates. When the pH is increased, the specific interaction of P-HIS with the G-quadruplex DNAs diminishes, which is reflected by the small differences in spectral intensity. The absorption intensity of P-HIS alone increases with pH, consistent with the enhanced solubility of this compound at higher pH values.

Considering the charge of the P-HIS side chains, the two carboxylic groups are deprotonated at pH 6, and some of the imidazole nitrogens are protonated. The average charge of P-HIS at pH 6 is −0.45.[6] When the pH of the buffer increases, P-HIS becomes more negatively charged (pH 7 = −1.49 and pH 8 = −1.93),[6] and the specific interactions of P-HIS with the G-quadruplex DNAs are less, which is again reflected by the small differences in spectral intensity.

The spectra of PIPER with the various DNA substrates also show pH-dependent and substrate-dependent interactions. The binding preference follows the order G4-DNA ≅ G3-DNA > ds-DNA > ss-DNA. When the pH is increased, self-aggregation of PIPER increases, as illustrated by the general decline in absorption intensity. The G-quadruplex selectivity also increases with increasing pH, as illustrated by the increasing differences in spectral intensity for the various DNA substrates.

The spectra of P-TRIS with the various DNA substrates show patterns similar to those of PIPER. Although P-TRIS aggregates at all pH values used, it appears that the aggregation of P-TRIS is enhanced at higher pH. The binding preference follows the order G4-DNA > G3-DNA > ds-DNA > ss-DNA. Compared to PIPER, the G-quadruplex binding selectivity is less,

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