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Perylene ligand wrapping G-quadruplex DNA for label-free fluorescence potassium recognition

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ABSTRACT

A perylene ligand, *N,N*-bis-(1-aminopropyl-3-propylimidazol salt)-3,4,9,10-perylene tetracarboxylic acid diimide ligand (PDI), which consisted of π-conjugated perylene moiety and hydrophilic side chains with positively charged imidazole rings, was used to wrap G-quadruplex for fluorescence turn-on K^+ recognition. Electrostatic attraction between PDI's positively charged imidazole rings and DNA's negatively charged phosphate backbones enabled PDI to accumulate on DNA. Upon trapping K^+, these G-rich DNA sequences transitioned to G-quadruplex. Subsequently, PDI ligands wrapped G-quadruplex, in which the flat aromatic core of PDI ligand interacted with G-quartet through π–π stacking and the side chains were positioned in grooves through electrostatic interactions. Consequently, the interaction mode change and conformational transition from PDI stacked G-sequence to PDI wrapped G-quadruplex led to PDI fluorescence enhancement, which was readily monitored as the detection signal. This strategy excluded the sequence tagging step and exhibited high selectivity and sensitivity towards K^+ ion with the linear detection range of 10–150 nM. Besides, PDI ligands may hold diagnostic and therapeutic application potentials to human telomere and cancer cells.

1. Introduction

Potassium ions (K^+) play an important role in biological systems, such as the maintenance of extracellular osmolarity, the regulation of other ions' concentration in living cell and reducing the risk of high blood pressure (He et al., 2005; Qin et al., 2010), etc. Various strategies have been adopted to K^+ recognition and detection (Kong et al., 2009; Li et al., 2009; Thibon and Pierre, 2009; Wu et al., 2003; Yuanboonlim et al., 2012). One important issue concerning K^+ is the ability to stabilize guanine (G)-rich oligonucleotide sequences to form G-quadruplex, composed of stacked G-quartets which are a noncanonical base pairing motif with four G-bases forming a cyclic and coplanar Hoogsteen hydrogen bonding array (Basu et al., 2000; Huppert and Balasubramanian, 2005; Read and Neidle, 2000; Smargiasso et al., 2008). The unique G-quadruplexes, including both parallel and anti-parallel alignments, are exceptionally stable and suitable for monovalent cations trapping. Cations are positioned between the G-quartets (Scheme 1a) and each is coordinated to eight different G-carbonyl groups that line the interior channel of the G-quadruplex (Basu et al., 2000). Depending on the free energies, hydration state and ionic radius, monovalent cations stabilize G-quadruplex in the order: $K^+ > NH_4^+ > Na^+ > Li^+$ for optimal sphere coordination within G-quadruplex cavity (Murat et al., 2011; Rodriguez et al., 2007). In this respect, conformational transition from G-rich sequences to G-quadruplex induced by K^+ exhibits its potential for K^+ assay. Based on fluorescence resonance energy transfer (FRET), G-rich sequences with dual tag or single tag (coupling to conjugated polymers) have been constructed (He et al., 2005; Lee et al., 2008; Lv et al., 2006; Nagatoishi et al., 2005; Ueyama et al., 2002). Using redox tag, an electrochemical platform is also fabricated (Wu et al., 2008). These strategies require tagging of the oligonucleotide with sensing element, which possess the drawbacks of time-consuming, expensive and contamination to the sample. In the present work, we would like to develop a label-free G-quadruplex K^+ recognition method.

G-qudaruplex ligands, which selectively bind to G-quadruplex motifs, have been shown to interfere with the telomere structure, telomere elongation/replication and proliferation of cancer cells, thus leading to the possibility of novel cancer therapeutics (De Cian and Mergny, 2007; Huppert, 2008). General features of ligands include planar aromatic system, containing delocalized π-electrons and favoring interactions with G-quartets, and

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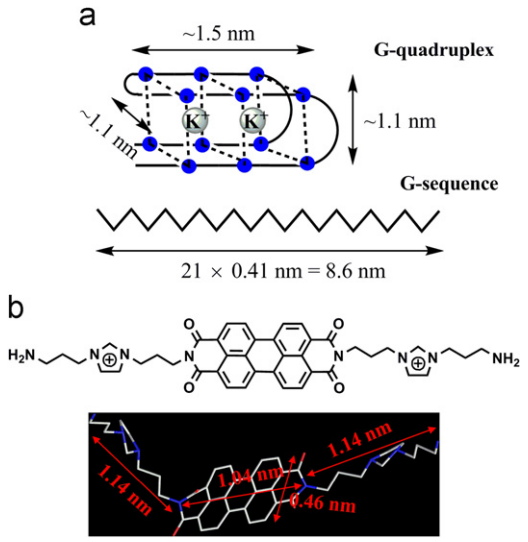

Scheme 1. (a) Schematic representation of the persistence length of G-quadruplex and random coil G-sequence and (b) Molecular structure and model of PDI ligand.

positively charged side chains, interacting with the grooves/loops or negatively charged phosphate backbones of G-quadruplex (Alzeer et al., 2009; Bugaut et al., 2008; Di Antonio et al., 2009; Murat et al., 2011; Tera et al., 2008). Among these ligands, perylene diimides fit the criteria well and have been widely studied as G-quadruplex binders and telomerase inhibitors (Dincalp et al., 2007; Franceschin et al., 2004; Han et al., 1999; Kern and Kerwin, 2002; Kern et al., 2002; Kerwin et al., 2002; Luigi et al., 2002; Rossetti et al., 2005; Tuntiwechapikul et al., 2001). Results illustrate that perylene diimides exhibit strong affinity and selectivity for G-quadruplex over duplex by mediating the biological milieu and the side chains, and show the therapeutic potential to human telomere. Perylene diimides are also known as excellent fluorescent dyes due to the chemical resistance, high photostability and fluorescence quantum yield (Abdalla et al., 2004). While researchers mainly focus on perylene ligands' ability to discriminate G-quadruplex and inhibit telomere, little has been reported concerning the ligand wrapped G-quadruplex for K^+ recognition based on the fluorescent signal.

Herein we report on the synthesis and characterization of *N*,*N*-bis-(1-aminopropyl-3-propylimidazol salt)-3,4,9,10-perylene tetracarboxylic acid diimide ligand (PDI, Scheme 1b), which consists of π-conjugated perylene moiety and hydrophilic side chains with positively charged imidazole rings, to wrap G-quadruplex for fluorescence turn-on K^+ recognition. Conformational transition from G-sequence to G-quadruplex upon trapping K^+ ions leads to PDI ligand wrapping, which causes PDI fluorescence enhancement and the signal is adopted for K^+ recognition. This strategy excludes the sequence tagging step. On the occasion of G-quadruplex formation, PDI ligands wrap it immediately, providing a thermodynamic driving force for the configuration conversion from G-sequence to G-quadruplex (Kern et al., 2002). In this respect, PDI ligand should match G-quadruplex size well, including the binding sites. As illustrated in Scheme 1b, the hydrophobic perylene moiety is ~1.04 nm and hydrophilic side chain is ~1.14 nm, which match the G-quartet plane (~1.1 nm) and G-quadruplex (to be ~1.5 nm including the loops) well (Scheme 1a), respectively. The flat aromatic core of PDI ligand interacts with G-quartet through π–π stacking and the side chains are positioned in grooves through electrostatic interactions. As demonstrated in the reference, the side chains' cationic charges and the locations may be crucial for binding with G-quadruplex (Bertrand et al., 2011). PDI side chains contain positively charged imidazole rings and terminal amine groups. While PIPER (Fedoroff et al., 1998), a widely studied G-quadruplex stabilizing ligand, possesses comparatively short side chains and lack hydrogen-bond donors in an equivalent position on the chromophore (Haider et al., 2003).

2. Experimental section

2.1. Materials

The 21-mer human telomeric sequence: 5′-GGGTTAGGGTTAGGGTTAGGG-3′ (G1) and thrombin binding aptamer: 5′-GGTTGGTGTGGTTGG-3′ (G2) were synthesized and purified with PAGE by Sangon Biotechnology Co., Ltd. (Shanghai, China). 3,4,9,10-Perylenetetracarboxylic dianhydride (PTCDA, 97%) was obtained from Sigma-Aldrich, USA. *N*-(3-Aminopropyl)-imidazole (98%) and 3-bromopropylamine hydrobromide (98%) were purchased from Alfa Aesar. Other reagents were of analytical grade and used as received. The DNA sequences were stored at 4 °C before use and diluted to required concentration in EtOH/H_2O mixture for use. All aqueous solutions were prepared with ultrapure water (> 18 MΩ) from a Milli-Q Plus system (Millipore).

2.2. PDI synthesis

The synthesis procedure and NMR characterizations of PDI ligand were illustrated in our previous work (Hu et al., 2012). Briefly, PTCDA (3.923 g, 10 mmol) and *N*-(3-aminopropyl)-imidazole (3.75 g, 30 mmol) were added to isobutanol (200 mL) and heated at 90 °C for 24 h with stirring under N_2 atmosphere. The crude product was filtered, washed with ultrapure water and ethanol. The obtained residue was treated with 150 mL 5% aqueous NaOH solution at 90 °C for 30 min to remove the unreacted PTCDA. The mixture was filtered, washed with ultrapure water and ethanol and dried under vacuum (5.40 g, 89%). ^{1}H NMR (CF_3COOD, 400 MHz, 25 °C), δ/ppm: 2.33–2.37 (t, 4H), 4.24–4.27 (t, 4H), 4.3–4.33 (t, 4H), 7.27 (s, 2H), 7.36 (s, 2H), 8.60–8.66 (m, 8H), 8.75 (s, 2H). ^{13}C NMR (CF_3COOD, 400 MHz, 25 °C), δ/ppm: 29.1, 38.7, 48.5, 111.1, 113.9, 119.6, 121.1, 122.7, 125.0, 133.6, 135.3, 136.6 and 166.4.

The above obtained *N*,*N*-bis-(1-propylimidazole)-3,4,9,10-perylene tetracarboxylic acid diimide (3.03 g, 10 mmol) and 3-bromopropylamine hydrobromide (0.438 g, 20 mmol) were added to ethanol (100 mL), refluxed for 24 h under N_2 atmosphere. After the mixture was cooled to room temperature, the precipitate was filtered and washed with ethanol. The resulting mixture was purified by re-crystallization from ethanol and dried under vacuum to give a red PDI product (3.2 g, 93%). ^{1}H NMR (DMSO, 400 MHz, 25 °C), δ/ppm: 1.23 (t, 4H), 2.26–2.30 (t, 4H), 4.11 (s, 4H), 4.33–4.37 (s, 4H), 7.75 (s, 2H), 7.86 (s, 2H), 8.34–8.36 (d, 4H), 8.61–8.63 (d, 4H), 9.19 (s, 2H). ^{13}C NMR (CF_3COOD, 400 MHz, 25 °C), δ/ppm: 29.21, 38.78, 48.59, 111.03, 113.85, 119.48, 120.84, 122.50, 122.74, 124.88, 126.19, 129.46, 133.24, 135.72, 135.79 and 165.91.

2.3. Measurements

UV–vis absorption spectra were recorded using a Hitachi U-3900 spectrophotometer. Fluorescence emission spectra were recorded using a Hitachi F-4600 fluorescence spectrophotometer with an excitation wavelength of 495 nm. Excitation and emission slit widths were both of 5 nm. Quartz cuvettes with 10 mm path length were used for UV–vis and emission measurements. Circular dichroism (CD) spectra were collected using a JASCO J-810 spectropolarimeter (Tokyo, Japan). The optical chamber with