DNA Folding

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Ligand-Driven G-Quadruplex Conformational Switching By Using an Unusual Mode of Interaction**

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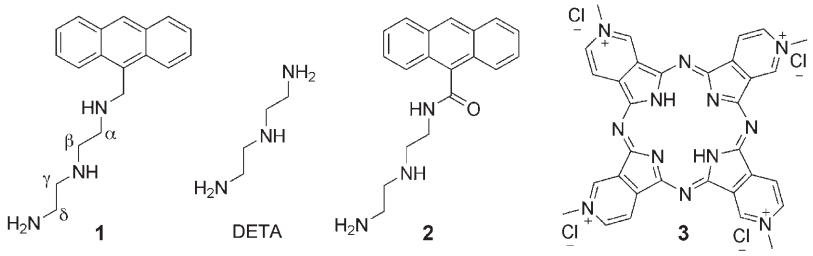
We report herein the successful design of the first molecule that induces the folding of the parallel human telomeric G-quadruplex from single-stranded DNA in the absence of added cation.[1] Ammonium ions stabilize quadruplex structures,[2] and K^+ ions have been shown to be separated by approximately 3.3 Å in the crystal structure of the human telomeric quadruplex.[3] Therefore, we synthesized **1** (Scheme 1) based on the hypothesis that the anthracene moiety would stack onto guanines, forming one of the external quartets and allowing the ammonium centers of **1**, which are separated by approximately 3.4 Å, to induce the DNA folding through hydrogen bonding and cation–dipole interactions, mimicking K^+ ions within the central quadruplex channel.[4]



Scheme 1. Ligands studied for G-quadruplex induction.

Upon the addition of **1** to a solution containing the human telomeric DNA d[TTAGGG]$_4$ (telo24) at room temperature and free of added cation, we observed positive and negative CD signals at 263 and 240 nm, respectively, within 30 s (Figure 1). This resultant CD spectrum is characteristic of a parallel quadruplex.[5] The human telomeric quadruplex is highly polymorphic and can exist as parallel,[3] antiparallel,[6a] and mixed-type parallel/antiparallel[6b] structures depending on strand orientation. However, ligand **1** appears to induce a single, parallel, quadruplex conformation. After addition of 10 equivalents of **1**, an induced CD signal at the absorbance of achiral **1** (253 nm) was observed as a result of **1** sensing the DNA chirality in the complex. In control experiments, we observed that the addition of 20 equivalents (200 μM) of diethylene triamine (DETA) to a solution free of added cation, but containing telo24, did not induce quadruplex structures as seen by CD spectroscopy. Furthermore, ligand **2**, in which an amide linkage reduces the protonation state and geometrical freedom of the molecule, also did not induce quadruplex formation under similar conditions (see the Supporting Information). These results suggest that the polyamine component of **1** is involved in a rather specific interaction with the DNA quadruplex.

Quadruplex-specific stabilization by cations has an order of preference of $K^+ > NH_4^+ > Na^+ > Li^+$ as a result of the relative free energies, hydration state, and ionic radius for optimal sphere coordination within the quadruplex cavity.[2] The more-general cation-induced stabilization owing to shielding of charge repulsion of the phosphate backbone is less sensitive to the nature of the monovalent cation. To explore the proposal that the polyamine of **1** is involved in a threading mode of interaction with the quadruplex, we studied the competition of **1** with specific monovalent cations. The presence of a large excess of K^+ ions

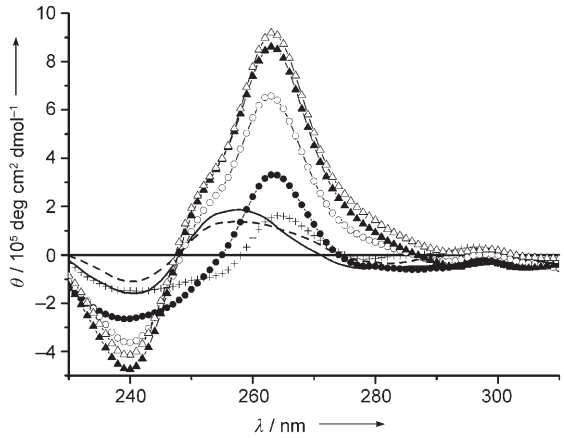


Figure 1. CD spectra of telo24 (10 μM) with 0 (——), 2 (-----), 3 (+), 5 (●), 10 (○), 12.5 (▲), and 15 equivalents (△) of **1** in Tris-HCl (10 mM, pH 7.4; Tris = tris(hydroxymethyl)aminomethane).

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should prevent the interaction of **1** with the DNA by saturating cation binding sites within the quadruplex core. The addition of **1** to a solution of telo24 preannealed with Li^+ induced changes in the DNA conformation, which are characteristic of a parallel conformer, within 2 min at room temperature with the emergence of signals at 263 and 240 nm (Figure 2). The addition of **1** to a solution of telo24

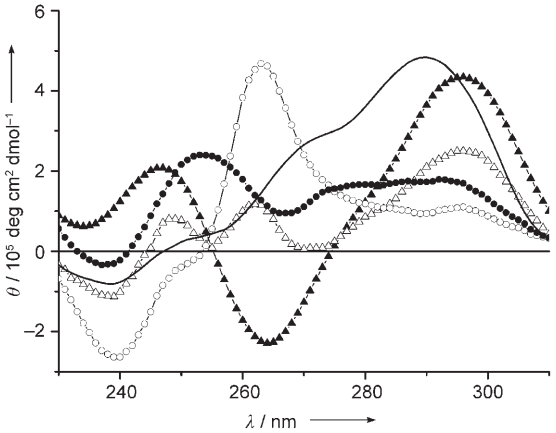

Figure 2. CD spectra of telo24 (10 μM) annealed with 100 mM Li^+ (●) followed by the addition of 20 equivalents of **1** (○); 100 mM Na^+ (▲) followed by the addition of 20 equivalents of **1** (△); 100 mM K^+ (——) in Tris-HCl (10 mM, pH 7.4).

preannealed with Na^+ led to a slow conformational switch of the DNA from the antiparallel to the parallel conformer. After 2 h incubation at room temperature, we observed that positive signals at 298 and 247 nm and a negative signal at 265 nm, characteristic of the antiparallel conformer,[6] decreased slowly while signals characteristic of the parallel conformer increased. The addition of **1** to a solution of telo24 preannealed with K^+ did not increase the signals characteristic of the parallel conformer even after two days at room temperature. Thus the apparent ability of **1** to bind and induce conformational changes in monovalent cation-complexed quadruplex DNA is in the order Li^+ (easier) > Na^+ > K^+ (difficult), reflecting the ease with which cations coordinated within the quadruplex core, rather than the phosphate backbone, can be displaced. These results are consistent with a mode of binding whereby the polyamine of **1** is threading through the quadruplex core, directly competing with and mimicking the central cations that stabilize the folded quadruplex.

To gain further insights into the interaction between **1** and the DNA, we carried out ^{1}H NMR spectroscopic studies in the absence of added cations (Figure 3). Upon the addition of 1 equivalent of **1** to telo24, we observed that all CH_2 signals of the ligand shifted downfield ($\approx$0.3 ppm) compared with the free ligand. The similarities in the $\Delta\delta$ observed are consistent with the presence of essentially identical hydrogen-bonding interactions occurring between the 12 guanine O6 lone pairs available from the DNA and the 8 methylene hydrogen atoms from the protonated ligand.[7] Upon increasing the concentration of **1** beyond 1 equivalent, we observed that a time-

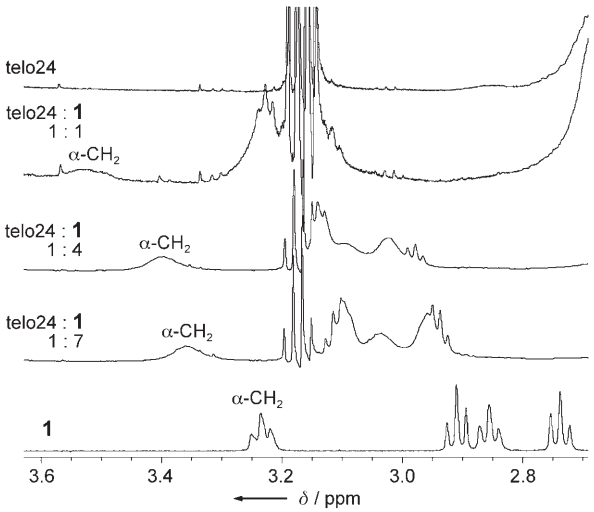

Figure 3. ^{1}H NMR spectra at 500 MHz of **1** (bottom), telo24 (top), and of telo24 with various mole equivalents of **1** (spectra in between). Conditions: 0.5 mM telo24 in D_2O/H_2O (1:9), pH 6.5–7.0, recorded at 298 K.

averaged set of the CH_2 signals between bound and free ligands progressively shifted upfield, indicating rapid exchange on the chemical-shift time scale. These observations are supportive of our threading model and highlight the dynamic character of the telomeric DNA.[8]

We recently reported that porphyrazine **3**[9] can induce and stabilizes the antiparallel quadruplex conformation in both the absence and presence of added cations. Given the ability of **1** and **3** to induce folding of the human telomeric DNA quadruplex preferentially into parallel or antiparallel structures, respectively, we reasoned that it may be possible to reversibly switch quadruplex conformations by sequential addition of both ligands. Figure 4a depicts a possible dynamic process of ligand-driven conformational switching as a result of two competing modes of interaction. The changes in the CD spectrum after the addition of 15 equivalents of **3** to a mixture of telo24 and **1** are illustrated in Figure 4b. We observed the disappearance of signals at 263 and 240 nm and the appearance of signals at 298 and 247 nm within 9 min of the addition of **3**, confirming a conformational switch from parallel to antiparallel structures. The opposite phenomenon was observed by the addition of **3** to telo24, followed by the addition of **1** (Figure 4c).[10] This demonstrates that the conformation of folded quadruplex DNA can be directed by one ligand, then subsequently switched by the addition of a second ligand.

A number of flat aromatic ligands have been reported that can stack onto the terminal G-tetrad of a prefolded quadruplex and thereby stabilize the structure.[11] Herein, we have described the first example of a molecule (**1**) capable of selectively inducing parallel quadruplex formation from unfolded DNA in the absence of added cations. Furthermore, this probably involves a combination of threading and stacking modes of interaction. This molecule was used along with a complementary ligand to controllably switch quadruplex conformations between the parallel and antiparallel

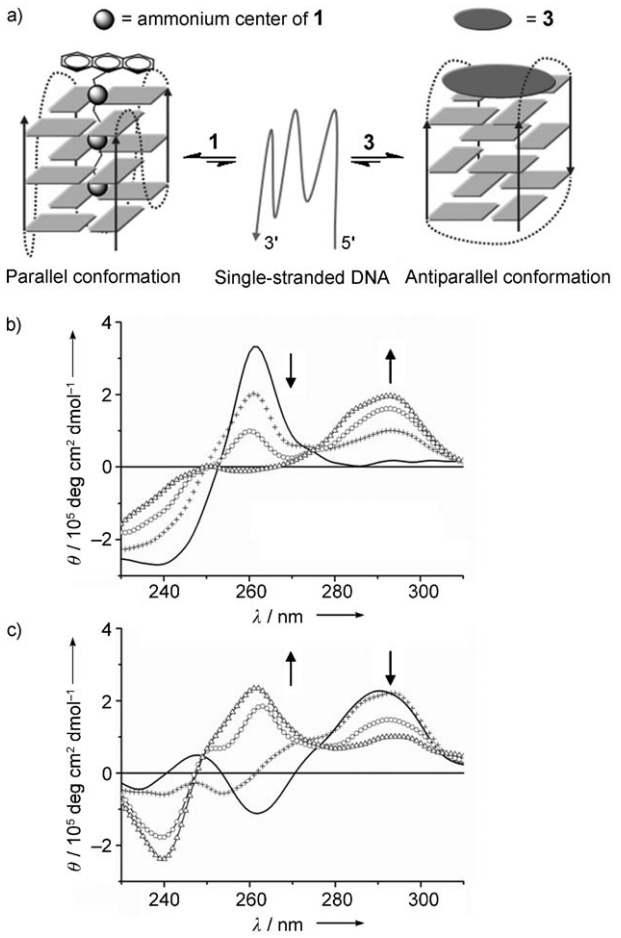

Figure 4. a) Schematic illustration of DNA conformational switching. b) CD spectra of telo24 (10 μM) with 5 equivalents of **1** (——), followed by the addition of 5 equivalents of **3** after 3 min (+), then after a further 3 min the addition of 5 equivalents of **3** (○), then the addition of 5 equivalents of **3** after a further 3 min (△) at RT. c) The above experiment repeated with an inverse ligand addition (**3** then **1**).

forms in either direction. Given the apparent prevalence of DNA quadruplexes in biology[12] and their application in nanoscience,[13] ligands such as **1** and **3** present the possibility of controlling biological or nanomolecular mechanisms by ligand-induced conformational switching.

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