G-Quadruplex DNA

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A Metal-Mediated Conformational Switch Controls G-Quadruplex Binding Affinity**

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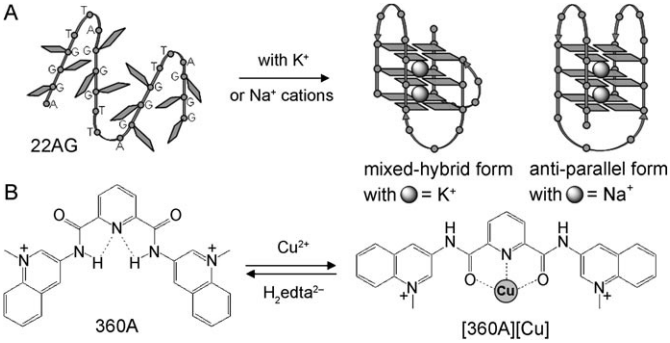
Since the seminal report in 1997 on small molecules designed to interact with G-quadruplex DNA,[1] an impressive number of candidates have been evaluated for binding to this particular DNA structure.[2] The ligands are structurally diverse, combining flat aromatic cores that π-stack on external tetrads and cationic centers that bind primarily through electrostatic interactions with DNA.[2] In the overwhelming majority of cases, the ligand is a structurally frozen aromatic molecule. We chose to investigate whether a flexible and externally controllable molecular conformation would lead to a modulation of the quadruplex–ligand interaction. This approach would allow the association process to be governed through external signals, with the potential for reversible and tunable quadruplex affinity that could lead to quadruplex-based nanodevices.[3]

Herein, we describe the first example of a metal-mediated conformational switch that controls the G-quadruplex binding affinity of a structurally flexible ligand. This unprecedented multipartner system relies on the use of a quadruplex DNA that mimics the human telomeric sequence (22AG, Scheme 1 A), a G-quadruplex ligand that can adopt two well-defined and controllable conformations (360A, Scheme 1 B),[4] and switch-off (a Cu^{II} salt, $CuSO_4$) and switch-on agents (Na_2H_2edta).[5]

The bisquinolinium 360A is among the highest-affinity and most selective G-quadruplex ligands reported to date.[4] A tritiated derivative of 360A was used recently to gain insight into the prevalence of G-quadruplex DNA in cells.[6] The central pyridodicarboxamide motif of 360A has a unique duality that can be used to control the geometry of the molecule (Scheme 1 B): the presence of two internal H bonds leads to a molecular V shape (Scheme 1 B, left) that is crucial for quadruplex affinity,[7] whereas the pyridodicarboxamide unit is also a tridentate center able to chelate a metal,[8] which forces the molecule to adopt a linear conformation that does not bind G-quadruplex DNA (Scheme 1 B, right).[7] Thus, we hypothesized that the quadruplex affinity of 360A could be cycled by alternating the addition of a metallic ion, to force the ligand to adopt its inactive conformation, with the addition of a poison for this ion, to free the form of 360A with affinity for the quadruplex (Scheme 1 B).



Scheme 1. A) 22AG ($d[AG_3(T_2AG_3)_3]$) in the unfolded form (left) and possible folded structures (right). B) Structure of 360A (with iodide as counterion) in the V shape (left) and chelated to copper in the linear conformation (right). edta = ethylenediaminetetraacetate.

We thus decided to use Cu^{II} ions, as they are promptly chelated by a pyridodicarboxamide unit (see the Supporting Information).[8] However, high concentrations of Cu^{II} ions (that is, more than about 0.7 molar equivalents per nucleotide) are known to induce a duplex-to-coil transition of DNA.[9] This denaturation originates in a specific interaction of copper with nucleobases (particularly with the N7 of the guanine residue), which skews the normal hydrogen-bond pairing between the nucleotides, thus destabilizing base–base interactions.[9] Consequently, G-rich DNA strands such as 22AG should be highly sensitive to the presence of Cu^{II} ions.

To determine the influence of ligands and ions on the structure of 22AG, we used circular dichroism (CD) spectroscopy. On the basis of extensive comparison between CD spectra, NMR spectra, and/or X-ray crystallographic data, it has been shown that the various topologies of quadruplex DNA are associated with specific CD signatures.[10] A quadruplex with a "parallel" structure (all its glycosidic angles in the *anti* conformation) is generally characterized by ellipticity (Θ) maxima at 264 (positive) and 240 nm (negative).[10] In contrast, the "antiparallel" structure (a combination of *syn* and *anti* angles, Scheme 1 A, right) has maxima at 295 (positive) and 264 nm (negative), and the "mixed-hybrid" structure (a different combination of the *syn* and *anti*

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angles, Scheme 1 A, center) has two positive maxima at 295 and 268 nm.[10] The characteristic spectra originate in the guanine glycosidic torsion angles[11] or in the interplay between the polarity of the neighboring quartets.[12] Great caution must be exercised in CD interpretation; assignments used herein are based on recent bibliographic consensus.[10] This said, CD offers a reliable means for following the structural conversion of the quadruplex to the unfolded conformation.

The CD spectrum of the quadruplex structure of 22AG in K^+-rich conditions (Figure 1, black curves) corresponds to a mixed-hybrid type like the one shown in Scheme 1 A, center (a positive maximum at 295 nm and a shoulder at 264 nm). The spectrum was dramatically altered by the addition of Cu^{II} ions. Indeed, the addition of 30 equiv of $CuSO_4$ led to the total disappearance of the signal at 295 nm (Figure 1 A, dark blue curve), strongly suggesting that the 22AG quadruplex was denatured. This was confirmed by the appearance of a shoulder near 260 nm, which probably corresponds to the signal of the random-coil 22AG (characterized by a positive peak at 257 nm).[13]

This excess of Cu^{II} ions (ca. 1.4 molar equivalents per nucleotide) was selected because it resulted in a rapid and complete denaturation of 22AG, which was not obtained with lower copper concentrations (see the Supporting Information). This denaturation, which can also be followed by UV-melting studies (see the Supporting Information), was not dependent on the buffer cation (Na^+ versus K^+) nor was it sequence-dependent (thrombin-binding aptamer (TBA), another intramolecular quadruplex,[14] is also unfolded in the presence of Cu^{II} ions; see the Supporting Information). Interestingly, the addition of H_2edta^{2-} restored the signal at 295 nm (Figure 1 A, red curve) with an efficiency of about 88 %. Three consecutive cycles of Cu^{II}/H_2edta^{2-} addition led to a 32 % decrease of the intensity of the 295 nm signal (Figure 2, black squares). We thus demonstrated that Cu^{II} effectively unfolds quadruplex DNA, a process that can be reversed with H_2edta^{2-}.

Comparable studies were then performed in the presence of 360A (Figure 1 B). As shown in the Supporting Information, 360A chelated copper with a high efficiency. As a control, experiments were performed with another known quadruplex ligand, MMQ_{16} (Figure 1 C).[15] This N-methylated quinacridine was selected because it does not interact with copper, as a result of the electronic poorness of its intracyclic nitrogen atoms and the lack of an *ortho*-chelating system (see the Supporting Information). As seen in Figure 1 B and C, the presence of either 360A or MMQ_{16} led to an increase of the 295 (positive) and 265 nm (negative) signals, as previously reported for strong quadruplex binders.[7,13,16] These observations imply that in the presence of either ligand, the antiparallel structure is stabilized (Scheme 1 A, right).

We then analyzed the effect of sequential Cu^{II}/H_2edta^{2-} additions on the ligand-stabilized structures of 22AG. As seen in Figure 1 D, the presence of 360A did not impede unfolding of the quadruplex upon addition of Cu^{II} ions, as indicated by a decrease of the positive signal at 295 nm and the appearance of a positive signal at 257 nm. The process was reversed by

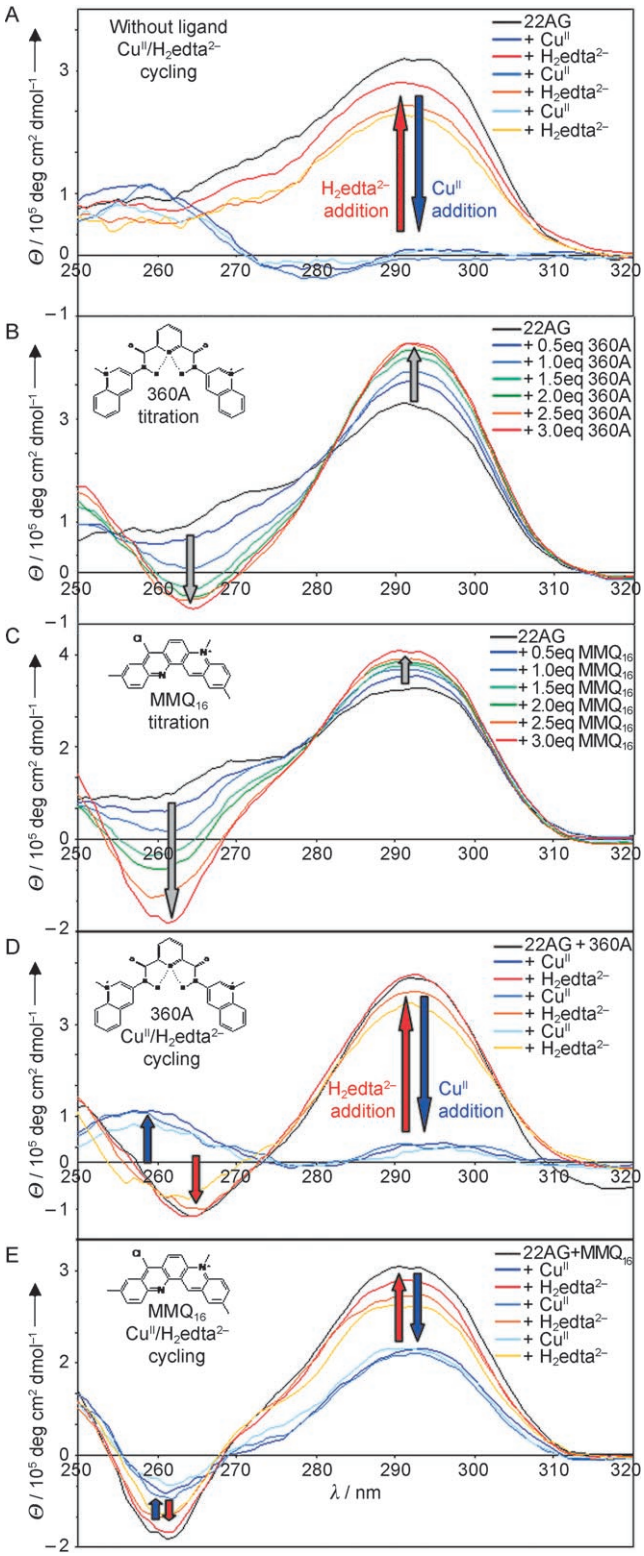


Figure 1. CD studies of 22AG quadruplex (3 μM in 10 mM sodium cacodylate buffer, 100 mM KCl, 20 °C) upon A) sequential Cu^{II}/H_2edta^{2-} additions (30 equiv), B) addition of 360A (0 to 3 equiv), and C) addition of MMQ_{16} (0 to 3 equiv). CD profiles of D) 360A-stabilized 22AG (3:1 equiv) upon sequential Cu^{II}/H_2edta^{2-} additions (30 equiv) and E) MMQ_{16}-stabilized 22AG (3:1 equiv) upon sequential Cu^{II}/H_2edta^{2-} additions (30 equiv).

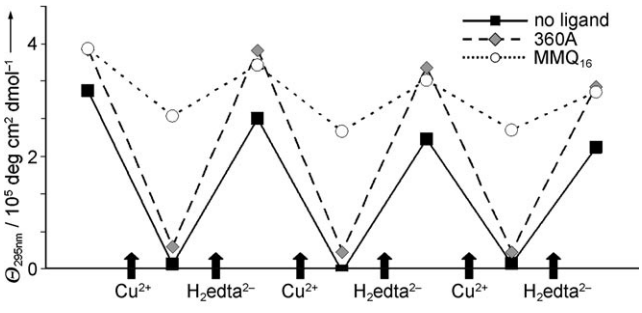


Figure 2. Cycling of the Cu-mediated structural conversion of 22AG monitored by the CD intensity at 295 nm, without ligand, with 360A, or with MMQ_{16}.

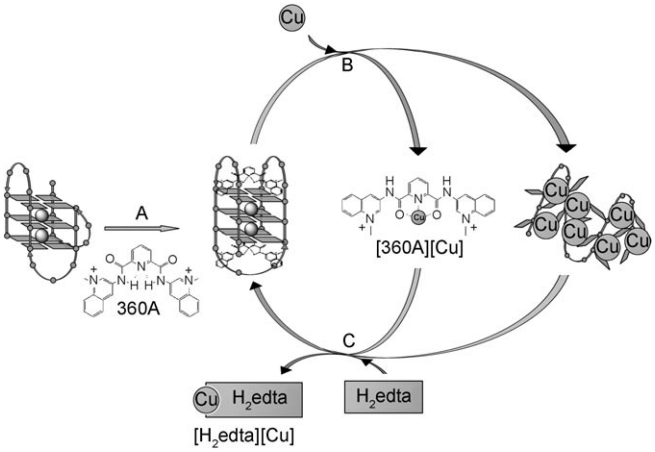
addition of H_2edta^{2-} with an efficiency of about 94% (Figure 2, gray diamonds). The better efficiency of the process as compared to cycling in the absence of ligand (Figure 2, black squares) may originate in a chaperone activity of 360A.[4b]

The situation is dramatically different in the presence of MMQ_{16} (Figure 1E): the intensity of the signals was somewhat altered, but the overall shape of the CD signal (positive signal at 295 nm and negative at 265 nm) was unchanged in the presence of Cu^{II} ions. This observation strongly suggests that the presence of MMQ_{16} inhibits the structural reorganization of 22AG observed upon copper addition in the absence of MMQ_{16}. The cycling has a cost in terms of signal intensity (with an efficiency of about 93%; Figure 2, white circles), thus indicating that cycle waste (herein, the $[H_2edta][Cu]$ complex, 90 equiv after the third run) somewhat interfered with the system.

As demonstrated by the example of MMQ_{16} (Figure 1E), a tight-binding quadruplex ligand impeded the Cu-mediated unfolding of the 22AG quadruplex. However, 360A, which is known to have a higher affinity for the quadruplex than MMQ_{16} ($\Delta T_{1/2} = 21$ versus 16 °C, respectively; see the Supporting Information),[4a,15,17] did not inhibit Cu^{II}-mediated denaturation (Figure 1D). This observation can only be explained by a copper-related modification of the 360A quadruplex affinity, an alteration that must originate from a specific and reversible Cu^{II} interaction with 360A. Copper controls the quadruplex affinity of 360A through a structural reorganization of the pyridodicarboxamide unit.

A schematic representation of the proposed cycle is depicted in Scheme 2: A) the addition of 360A causes the quadruplex structure of 22AG to shift from a mixed-hybrid type to the antiparallel conformation; B) the addition of Cu^{II} ions traps 360A in a complex that has no affinity for quadruplex DNA and concomitantly denatures the quadruplex by favoring the helix-to-coil transition of 22AG; C) the addition of Na_2H_2edta traps the Cu^{II} ions in a complex with H_2edta^{2-}, therefore releasing free 360A that stabilizes the folded structure of 22AG. The efficiency of the cycle relies on a dual, synergistic action of copper on both the DNA structure and the ligand.

The described study demonstrates that the affinity of a ligand for G-quadruplex DNA can be controlled by external signals, provided the structure of the ligand is appropriate.



Scheme 2. Cycling of 360A quadruplex affinity. A) 360A-mediated structural modification of the 22AG quadruplex. B) Poisoning of 360A upon addition of Cu^{II} salts and the resulting denaturation of the 22AG quadruplex. C) Regeneration of free 360A upon removal of Cu^{II} ions.

Our quadruplex-templated molecular machine is based on 360A, a well-characterized, high-affinity G-quadruplex ligand that adopts two different conformations depending on its free or Cu-complexed state. Only the free conformation has affinity for the quadruplex. Thus, the reversibility of this metal-mediated structural transition allowed the cycling of 360A quadruplex affinity. The system presented herein is fueled by alternate additions of small inorganic ($CuSO_4$) and organic (Na_2H_2edta) compounds and generates $[H_2edta][Cu]$ complex as waste. The waste interferes with the system in a very limited manner, thus ensuring a high cycling efficiency.

DNA-based nanodevices and nanostructures are becoming increasingly popular and very complex objects can now be assembled with DNA strands. Most reported conformational changes rely on the use of DNA single strands as fuels or varying pH. The copper-mediated approach we have described herein is different and attractive as it is fast, reversible, and differentially modulated by the presence of quadruplex ligands (copper-sensitive or not). Finally, this study clearly demonstrates that internal hydrogen-bond-based molecular organization is a valuable parameter for controlling the affinity of a quadruplex ligand, as recently confirmed by an elegant use of a helically folded oligoamide as G-quadruplex ligand.[18]

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