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

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Experimental CD procedure:

CD spectra were recorded on a JASCO J-710 circular dichroism spectropolarimeter using a 10-mm path length quartz cuvette. Scans were performed at controlled temperature (20°C) over a wavelength range of 210-380nm (only 240-330 is shown), with response time of 0.5s, 1nm pitch, 1nm bandwidth and a scanning speed of 200nm/min. Blank spectra of sample containing buffer were subtracted from collected data. The CD spectra represent an average of three scans and are zero-corrected at 330nm.

Oligonucleotides (22AG (5'-AG3(T2AG3)3-3')) or TBA (5'-G2T2G2TGTG2T2G2-3')) were purchased from Eurogentec (Belgium) as RP-HPLC purified oligonucleotides and were used without further purification. Quadruplexes were annealed by heating the oligonucleotide at 90°C for 5min in sodium cacodylate buffer (10mM, pH 7.4) with 100mM of KCl (22AG K⁺, TBA) or NaCl (22AG Na⁺), and cooled directly to 4°C to favor the intramolecular folding by kinetic trapping. Concentrations are evaluated by UV measurements (after thermal denaturation for annealed 22AG (5min at 90°C)) before use.

22AG and TBA were used at 3µM in sodium cacodylate buffer (10mM, pH 7.4) with 100mM of KCl or NaCl where appropriate. 360A and MMQ₁₆ were used as 0.5mM solution in DMSO; CuSO₄ and Na₂.EDTA were used as 2mM solution in water.

For studying the modification of the quadruplex-structure induced by 360A or MMQ₁₆:

A solution of annealed 22AG (3µM) in 10mM sodium cacodylate buffer with 100mM of KCl (pH 7.4, 20°C) is added of increasing amounts (from 0 to 3equiv., with 0.5equiv. step) of 360A or MMQ₁₆ (500µM in DMSO); each addition is followed by a 2min equilibration time, after which the CD spectrum is recorded.

For studying the effect of sequential addition of Cu(II) ions and EDTA on quadruplex-structure stabilized by 360A or MMQ₁₆:
A 3µM solution of 360A- or MMQ16-stabilized 22AG quadruplex (obtained as indicated above) in 10mM sodium cacodylate buffer with 100mM of KCl (pH 7.4, 20°C) is added of 30equiv. of CuSO₄ (2mM in water); the CD spectrum is recorded after 10min equilibration time. To renaturate the quadruplex signal, 30equiv. of Na₂EDTA (2mM in water) is added; the CD spectrum is recorded after 20min equilibration time.

Fig. S1. UV-Vis titration of 360A and MMQ16 upon sequential addition of CuSO₄ (from 0 to 2equiv., 0.1equiv. pitch) in 10mM sodium cacodylate buffer (pH 7.4), 100mM KCl (20°C). Upper panel: dose-response curves of UV-Vis signal at 274 (blue curve) or 343nm (red curve) for 360A and MMQ16 respectively as a function of the number of equivalents of Cu(II) salts added. Lower panels: related UV-vis spectra.

Fig. S2. Thermal denaturation of 22AG (3µM, in 20mM lithium cacodylate, 100mM KCl, pH 7.2) in absence (black curve) or in presence of various concentrations of Cu(II) ions (3, 30 and 60µM, red, orange and green curves respectively) followed by the DNA absorbance at 295nm: while the typical denaturation sigmoid-shaped curve of quadruplex-DNA is monitored with 0 and 3µM of Cu(II) (black and red curves), no transitions are observed with higher copper concentrations, meaning that in these cases, the quadruplex-DNA has been already unfolded by the action of Cu(II) ions. Similar results have been obtained with less quadruplex-stabilizing initial cationic conditions (90mM lithium cacodylate + 10mM KCl and 99mM lithium cacodylate + 1mM KCl, not
shown), thus demonstrating that the loss of transitions with high copper loadings is only due to the Cu-
destabilization of quadruplex-DNA (and not to the formation of complexes of higher stability).

Fig. S3. 360A-stabilized 22AG quadruplex denaturation as a function of Cu(II) ion concentration, followed by
CD spectroscopy. 360A (9µM) is added on 22AG (3µM) in 10mM sodium cacodylate buffer (pH 7.4), 100mM
KCl (20°C); after 5min of equilibration, the 360A-stabilized quadruplex (black curve) is added of 30µM
(10equiv., ~0.4 atom/nucleotide, A), 60µM (20equiv., ~0.9 atom/nucleotide, B) or 90µM (30equiv., ~1.4
atom/nucleotide, C) of CuSO4, CD spectra (green curve) were recorded after 10min equilibration time.

Fig. S4. Denaturation of various quadruplex-DNA by Cu(II) ions. To a solution of 22AG K+ (A), 22AG Na+ (B)
or TBA (C), as 3µM solution in 10mM sodium cacodylate buffer (pH 7.4), 100mM KCl (A,C) or 100mM NaCl
(B) was added of 90µM of CuSO4 (30equiv., ~1.4 atom/nucleotide); CD spectra were recorded after 10 (A, B) or
60min (C) equilibration time.

**FRET-melting experiments:**

a) **Principles:**

FRET-melting is based on the use of a quadruplex-forming oligonucleotide (F21T) doubly-labeled with a pair of
FRET donor and acceptor (usually FAM: 6-carboxyfluorescein and Tamra: 6-carboxy-tetramethylrhodamine)
that allows to monitor the melting of the quadruplex via a FRET (fluorescence resonance energy transfer) effect.
Semi-quantitative evaluation of ligand binding affinity is obtained by measuring of the increase in melting
temperature induced by the ligand (ΔT_{1/2}). The quadruplex- over duplex-DNA selectivity is established via
competitive FRET-melting which is carried out in presence of competitive duplex-DNA.

b) Protocol:

Labelled oligonucleotide is purchased from Eurogentec (Belgium); after an initial dilution at 100µM concentration in purified water, further dilutions are carried out in the relevant buffer.

FRET assay is performed as a high-throughput screen in a 96-well format, with F21T (FAM-G3[T2AG3]3-Tamra, with FAM: 6-carboxyfluorescein and Tamra: 6-carboxy-tetramethylrhodamine). Fluorescence melting curves were determined with a Stratagene Mx3000P real-time PCR machine, using a total reaction volume of 25µL, with 0.2µM of tagged oligonucleotide in a buffer containing 10mM lithium cacodylate pH 7.2 and 100mM NaCl. After a first equilibration step at 25°C during 5 minutes, a stepwise increase of 1°C every minute for 71 cycles to reach 95°C was performed and measurements were made after each “cycle” with excitation at 492nm and detection at 516nm. The melting of the G-quadruplex was monitored alone or in the presence of various concentrations of compounds and/or double-stranded competitor ds26 (5’-CAATCGGATCGAATTCGATCCGATTG-3’).

Final analysis of the data was carried out using Excel and Kaleida graph software. Emission of FAM was normalized between 0 and 1, and T1/2 was defined as the temperature for which the normalized emission is 0.5. ΔT1/2 values are mean of 2 to 4 experiments ± standard deviation.

c) Results:

![Normalized FAM fluorescence vs Temperature](image)

**Fig. S5.** FRET-melting curves obtained with F21T (0.2µM) alone (black circles) and with 1µM of 360A (right) or MMQ16 (left) in absence (white squares) or presence (3 black triangles) or 10µM (black crosses) of competitive duplex-DNA ds26.

G4-FID experiments:

a) Principles:

Fluorescent Intercalator Displacement (G4-FID) assay is based on the displacement by ligands of the fluorescent probe thiazole orange (TO) from quadruplex- and duplex-DNA matrices. This test allows a convenient ranking of putative ligands as a function of their quadruplex-DNA affinities and their quadruplex- over duplex-DNA selectivities. This assay is based on the use of quadruplex-forming oligonucleotide 22AG (that mimics the human-telomeric sequence (5’-AG3(T2AG3)3-3’)) or TBA (thrombin binding aptamer, (5’-G3T3G3TGTG3T3G2-3’)). The quadruplex- vs duplex-selectivity is estimated by comparison of the results obtained with a 17-bp duplex (ds17, 5’-CCAGTTCTGTAACCC-3’/5’-GGTACTACGAAGTGG-3’)).

b) **Protocol:**

A temperature of 20 °C is kept constant with thermostated cell holders. Each experiment is performed in a 3-mL cell, in 10 mM sodium cacodylate buffer pH 7.3 with 100 mM KCl or 100 mM NaCl depending on the experiments, in a total volume of 3 mL. The G4-FID assay is designed as follows: 0.25µM pre-folded DNA target is mixed with thiazole orange (0.50 µM). Each ligand addition step (from 0.5 to 10 equivalents) is followed by a 3-min equilibration period after which the fluorescence spectrum is recorded. The percentage of displacement is calculated from the fluorescence area (FA, 510-750nm, \(\lambda_{ex} = 501\text{nm}\)), using: percentage of displacement = 100-\([\text{FA}/\text{FA}_0] \times 100\], FA₀ being the fluorescence of TO bound to DNA without added ligand. The percentage of displacement is then plotted as a function of the concentration of added ligand.

c) **Results:**

![Graphs showing G4-FID results obtained with 360A (right) and MMQ 16 (left) with quadruplex- (22AG (blue line) and TBA (black line)) or duplex-DNA (ds17, red line).](Fig_S6)