Supplementary Material (ESI) for Dalton Transactions

Electronic supplementary information

Molecular "light switch" for G-quadruplexes and i-motif of human telomeric DNA: $[Ru(phen)_2(dppz)]^{2+}$

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Table of contents

Fig. s1

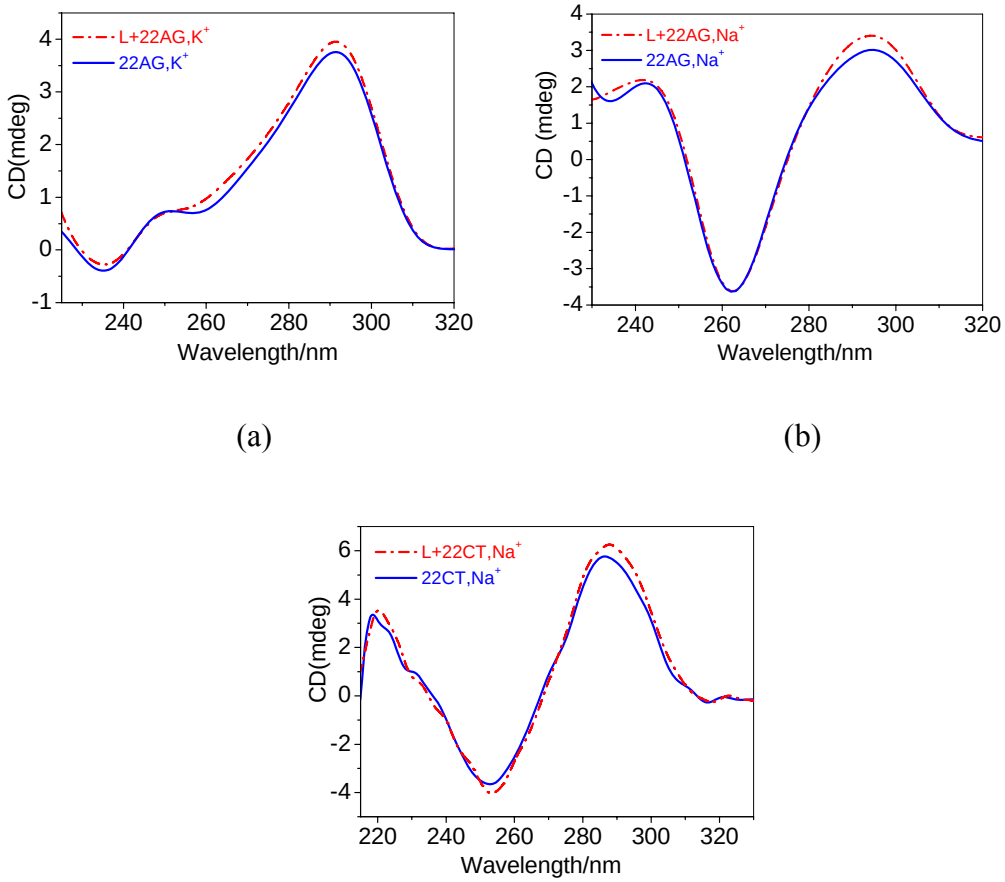

Fig. s1 CD spectra of human telomeric DNA in the absence (solid curves) and presence of $[Ru(phen)_2(dppz)]^{2+}$ (dash dot curves) pH 5.5, 25 °C.. [DNA] = 10μM, [Ru] = 50μM. (a) CD spectra of 22AG in K^+ buffer; (b) CD spectra of 22AG in Na^+ buffer; (c) CD spectra of 22CT in Na^+ buffer.

Table s1

Emission spectra, absorption spectra (λ_{max}/nm) and DNA binding constants K_b ($\times 10^5$ M^{-1}) of $[Ru(phen)_2(dppz)]^{2+}$.

Complex	Emission spectra				Absorption spectra				
	λ_{max}/free	λ_{max}/bound	I/I^0	$K_b/10^5M^{-1}$	λ_{max}/free	λ_{max}/bound	$\Delta\lambda$/nm	H(%)	$K_b/10^5M^{-1}$
Hybrid quadruplex		611	50	52	438	442	4	24.2	
					373	378	5	38.2	65
					263	265	2	37.7	
Antiparallel quadruplex		611	35	30	438	442	4	23.8	
					373	378	5	38.1	61
					263	265	2	36.9	
i-motif		611	11	4.7	438	439	1	13.9	
					373	375	2	20.5	6.7
					263	264	1	13.2	

Materials and methods

Materials: $[Ru(phen)_2(dppz)]^{2+}$ was prepared and characterized according to the literature.[1] Oligonucleotides 5′-AGGGTTAGGGTTAGGGTTAGGG-3′ (22AG) and its corresponding complementary strand 5′-CCCTAACCCTAACCCTAACCCT-3′ (22CT), were purchased from Sangon (Shanghai, China) and used without further purification. Concentrations of these oligonucleotides were determined by measuring the absorbance at 260 nm after melting. Single-strand extinction coefficients were calculated from mononucleotide data using a nearest-neighbor approximation.[2] The formations of intramolecular G-quadruplexes and i-motif were carried out as follows: the oligonucleotide samples, dissolved in different buffers, were heated to 90 °C for 5 min, gently cooled to room temperature, and then incubated at 4 °C overnight. Buffer

A: 100 mM NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, 1 mM Na_2EDTA, pH 5.5; Buffer B: 100 mM KCl, 10 mM KH_2PO_4/K_2HPO_4, 1 mM K_2EDTA, pH 5.5.

Circular dichroism measurements: Circular dichroism (CD) spectra were measured on a Jasco J-810 spectropolarimeter. The oligonucleotide samples were dissolved in the K^+ or Na^+ buffer. The corresponding samples of the DNA (22AG or 22CT) at a concentration of 10 μM were dissolved and placed in a quartz cuvette. During the titration, aliquot (1-10 μL) of $[Ru(phen)_2(dppz)]^{2+}$ solution was added to the cuvette, and the solutions were mixed by repeated inversion. After the solutions were mixed for ~5 minutes, CD spectra were recorded. The titration processes were repeated until there was almost no change, indicating binding saturation had been achieved. For each sample, at least four spectrum scans were accumulated over the wavelength range of 200-350 nm at the temperature 25 °C in a 1.0 cm path length cell at a scanning rate of 50 nm /min. The instrument was flushed continuously with pure evaporated nitrogen throughout the experiment. The scan of the buffer alone was subtracted from the average scan for each sample.

Emission spectra: Emission spectra were measured on a Shimadzu RF-5000 spectrofluorophotometer. The excitation wavelength was 460 nm, and the emission spectrum was collected from 500 to 800 nm. Excitation and emission slits were set at 10 and 5 nm, respectively. Luminescence titrations process was similar to CD titration experiment. Luminescence titrations: A 3000 μL of $[Ru(phen)_2(dppz)]^{2+}$ in a 1 cm path length quartz cuvette was loaded into the fluorimeter sample block, maintained at 25 °C. After 5 minutes to allow the cell to equilibrate, the first spectrum was recorded, and then 1-10 μL of DNA solution was then added to the sample cell, followed by thorough mixing. After 5 minutes, the spectrum was taken again. The titration processes were repeated until there was no change in the spectra for at least four titrations indicating binding saturation had been achieved.

The concentration of the bound compound was calculated using Eq. 1[3]

$$C_b = C_t[(F - F_0)/ (F_{max} - F_0)] \quad (1)$$

where C_t is the total compound concentration, F is the observed fluorescence emission intensity at given DNA concentration, F_0 is the intensity in the absence of DNA, and F_{max} is the fluorescence of the totally bound compound. Binding data were cast into the form of a Scatchard plot [4] of r/C_f versus r, where r is the binding ratio C_b /$[DNA]_t$ and C_f is the free complex concentration.

Absorption spectra titrations: Absorption spectra titrations were carried out at room temperature to determine the binding affinity between DNA and complex. Initially, 3000 μL solutions of the blank buffer and the ruthenium complex sample (20 μM) were placed in the reference and sample cuvettes (1 cm path length), respectively, and then first spectrum was recorded in the range of 200-600 nm. During the titration, aliquot (1-10 μL) of buffered DNA solution was added to each cuvette to eliminate the absorbance of DNA itself, and the solutions were mixed by repeated inversion. After the solutions were mixed for ~5 minutes, the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for four titrations at least, indicating binding saturation had been achieved. The changes in the metal complex concentration due to dilution at the end of each titration were negligible.

In order to compare quantitatively the binding strength of $[Ru(phen)_2(dppz)]^{2+}$ to each DNA, the intrinsic binding constants K_b with each DNA at 25 °C were obtained using the following eq. (2) [5]

$$\frac{\varepsilon_a - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{(b - (b^2 - 2K^2 C_t [DNA]/s)^{1/2})}{2KC_t} \quad (2a)$$

$$b = \frac{1 + KC_t + K[DNA]}{2s} \quad (2b)$$

where [DNA] is the concentration of DNA in base pair, ε_a, ε_f and ε_b are, the apparent extinction coefficient (A_{abs}/[M]), the extinction coefficient for free metal (M) complex and the extinction coefficient for the metal (M) complex in the fully bound form,

respectively. K is the equilibrium binding constant in M^{-1}, C_t is the total metal complex concentration, and s is the binding size.

Thermal DNA denaturation experiments: Thermal DNA denaturation experiments were carried out with a PerkinElmer Lambda 850 spectrophotometer equipped with a Peltier temperature-control programmer (± 0.1) °C. Melting curves were collected by UV absorbance as a function of temperature. Absorbance changes at either 295 nm (G-quadruplex) or 260 nm (i-motif) vs. temperature were collected at a heating rate of 1°C/min. The data were presented as $(A - A_0)/(A_f - A_0)$ versus temperature, where A_f, A_0, and A are the final, the initial, and the observed absorbance at 295 or 260 nm, respectively.

Quenching studies: Fluorescence quenching studies were carried out using the anionic quencher potassium ferrocyanide ($K_4Fe[CN)_6]$), monitoring the fluorescence intensity changes at 611 nm as a function of the quencher concentration. At least four measurements were taken and averaged. The data were plotted as I^o/I versus quencher concentration [Q] according to the Stern–Volmer equation, as described earlier.[6]

Fluorescence polarization anisotropy: The fluorescence polarization anisotropy experiment was performed as described earlier. [6] The samples were excited at 460 nm and the fluorescence signal was monitored at 611 nm through crossed polarizers.

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