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Promoting the Formation and Stabilization of G-Quadruplex by Dinuclear Ru^{II} Complex $Ru_2(obip)L_4$

Shuo Shi,[†,‡] Jie Liu,[*,†] Tianming Yao,[‡] Xiaoting Geng,[‡] Lingfeng Jiang,[‡] Qingyuan Yang,[†] Lin Cheng,[†] and Liangnian Ji[*,†,‡]

Key Laboratory of Bioinorganic and Synthetic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, People's Republic of China, and Department of Chemistry, Tongji University, Shanghai 200092, People's Republic of China

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The remarkable ability of a new dinuclear Ru^{II} complex $Ru_2(obip)L_4$ [obip = 2-(2-pyridyl)imidazo[4,5-*f*][1,10]-phenanthroline; L = 2,2′-bipyridine] to promote the formation and stabilization of the human telomeric repeat AG3(T2AG3)3 quadruplex was reported. The experimental results indicated that $Ru_2(obip)L_4$ could induce the formation of an antiparallel G-quadruplex structure in the absence of metal cations. It could induce positive T_m shifts of +9.4 and +5.8 °C in Na^+ and K^+ buffers, respectively, in which an increase in the melting temperature of the quadruplex indicated a stabilizing effect. Binding stoichiometry with the quadruplex was investigated through a luminescence-based Job plot. The major inflection point for $Ru_2(obip)L_4$ at $x = 0.48$ was observed. The data were consistent with a 1:1 [quadruplex]/[complex] binding mode, which was suggestive of a specific $Ru_2(obip)L_4$−quadruplex interaction with a single guanine tetrad.

Guanine-rich regions abound in the human genome; they have the propensity to form tetraplex structures known as G-quadruplexes.[1,2] Such structures are made up of G-quartet subunits, where four coplanar guanines (G) are linked together by Hoogsteen hydrogen bonds, as shown in Figure 1a. These G-quadruplexes have recently received great attention because they can inhibit the telomere maintenance provided by telomerase activity, thus affecting the lifespan of the cells of a number of cancer types.[3−5] The best-studied example is the human telomeric repeat AG3(T2AG3)3 quadruplex, which leads to inhibition of telomere extension by the enzyme telomerase,[6] whose activity is up-regulated in cancer cells. In particular, quadruplexes from any one sequence are often capable of conformational plurality (Figure 1b); for example, the NMR structure of AG3(T2AG3)3 in the presence of Na^+ was an antiparallel basket quadruplex,[7] but the X-ray structure for the same sequence in the presence of K^+ revealed a parallel propeller quadruplex.[8] A recent paper indicated that it favored a mixed parallel/antiparallel structure in the presence of a K^+ solution based on circular dichroism (CD).[9,10]

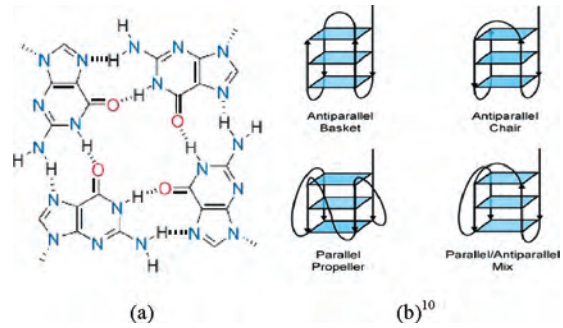

Figure 1. (a) Structure of a G-quartet with a cyclic array of four guanines linked by Hoogsteen hydrogen bonds. (b) Four different strand orientations for a G-quadruplex.

Recently, a number of promising small molecules have been devised to selectively promote the formation and/or stabilization of G-quadruplex structures.[11] It has been shown that these compounds that interact with quadruplexes can also act as inhibitors of the enzyme telomerase,[12] whose function is to protect tumor cells against telomere loss during replication. Ru^{II} complexes have prominent DNA binding properties. In particular, $[Ru(bpy)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), known as a "DNA light switch",

* To whom correspondence should be addressed. E-mail: cesliuj@mail.sysu.edu.cn (J.L.), cesjln@mail.sysu.edu.cn (L.N.J.).
† Sun Yat-Sen University.
‡ Tongji University.

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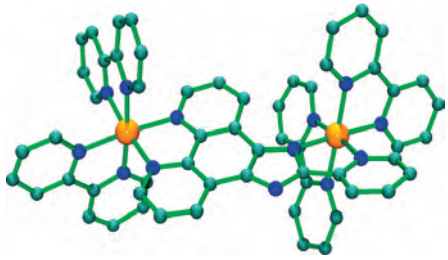

Figure 2. ORTEP drawing of of $Ru_2(obip)L_4$.

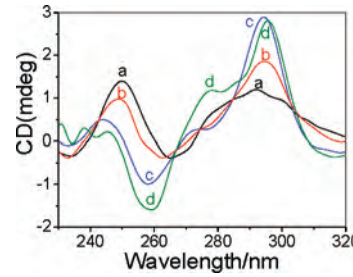

Figure 3. CD spectra of the human telomeric AG3(T2AG3)3 (20 μM) in H_2O: (a) without $Ru_2(obip)L_4$; (b) with 1 μM $Ru_2(obip)L_4$; (c) with 10 μM $Ru_2(obip)L_4$; (d) with 50 μM $Ru_2(obip)L_4$.

and its derivatives have attracted particular attention. They can intercalate between the duplex DNA base pairs and stabilize the DNA. Some of them have been investigated as nucleic acid probes, synthetic restriction enzymes, anticancer drugs, and DNA footprinting agents, etc.[13] However, the direct interaction between quadruplex and Ru^{II} complexes is rare. Herein, we report the remarkable ability of the novel dinuclear complex (Figure 2) to promote the formation and stabilization of G-quadruplex DNA. Synthetic procedures for $Ru_2(obip)L_4$ are given in the Supporting Information. The crystal structure of $Ru_2(obip)L_4$ was determined by single-crystal X-ray diffraction analyses, and the structure was solved by direct methods and refined using full-matrix least-squares/difference Fourier techniques using *SHELXTL*.[14]

$Ru_2(obip)L_4$-induced formation of the human telomeric intramolecular G-quadruplex structure in the absence of K^+ or Na^+ was monitored using CD spectroscopy. Figure 3 shows the CD spectra for the titration of AG3(T2AG3)3 with increasing amounts of $Ru_2(obip)L_4$. Without any metal cations, the CD spectra of the human telomeric AG3(T2AG3)3 at room temperature exhibited a negative band centered at 235 nm, a major positive band at 252 nm, and a rather broad positive signal around 290 nm (Figure 3a). These results suggested that telomeric AG3(T2AG3)3 might coexist as a single strand and two types of quadruplex DNA structures: parallel and antiparallel.[15] However, upon the addition of $Ru_2(obip)L_4$ (1 μM; Figure 3b) to the AG3(T2AG3)3 oligonucleotide, a dramatic change in the CD spectrum was observed. The maximum at 252 nm was gradually suppressed and shifted to 245 nm, while the band centered at about 290 nm increased dramatically along with an increase of the concentration of $Ru_2(obip)L_4$. At the same time, a major negative band at about 260 nm started to appear. As the $Ru_2(obip)L_4$ concentration increased to 10 μM (Figure 3c), the CD spectrum of this new DNA conformation was virtually identical with the CD spectra of antiparallel G-quadruplexes described in previous studies, where the major positive band was usually observed around 290 nm with a negative band at 265 nm and a smaller positive band at 246 nm.[16] A significant change was observed in the CD spectra when excess $Ru_2(obip)L_4$ (50 μM; Figure 2d) was added to the preformed antiparallel basket-type G-quadruplex in the presence of $Ru_2(obip)L_4$; the strong shoulder at 272 nm as the spectral characteristic of a parallel G-quadruplex appeared. It seemed that the structure was a G-quadruplex that contained mixed parallel/antiparallel components or that there was a mixture of G-quadruplex structures, but the increased intensity of the negative band at 260 nm disagreed with this assumption.The CD signal at 272 nm may be induced through the strong absorbance of $Ru_2(obip)L_4$ at about 280 nm (Figure S1 in the Supporting Information). So, AG3(T2AG3)3 still adopted an antiparallel intramolecular G-quadruplex structure at the concentration of 50 μM $Ru_2(obip)L_4$. However, upon the addition of $Ru_2(obip)L_4$ to AG3(T2AG3)3 in 100 mM NaCl or KCl buffer, the CD spectrum exhibited a maxima−minima pattern similar to, but not identical with, the spectrum in Na^+ or K^+ without the addition of $Ru_2(obip)L_4$ (Figure S2 in the Supporting Information), which implied that the conformation of G-quadruplex was stabilized by Na^+ or K^+ and $Ru_2(obip)L_4$ could not change the conformation of the G-quadruplex at high ionic strength.

The addition of a G-quadruplex to 100 mM NaCl-buffered solutions of $Ru_2(obip)L_4$ resulted in an emission enhance-

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(14) Crystal data for $Ru_2(obip)L_4$: $C_{59}H_{45}F_{24}N_{13.50}OP_4Ru_2$, $M_r = 1741.10$, triclinic, space group $P\bar{1}$, $a = 13.232(5)$ Å, $b = 16.521(6)$ Å, $c = 16.640(6)$ Å, $\alpha = 80.433(6)°$, $\beta = 88.1732(7)°$, $\gamma = 69.784(6)°$, $V = 3365(2)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.719$ g cm^{-3}, $\mu = 0.663$ mm^{-1}, $T = 293$ K, 13 099 reflections collected, 6845 unique ($R_{int} = 0.0538$), R1 = 0.0691, wR2 = 0.1780 [$I > 2\sigma(I)$].

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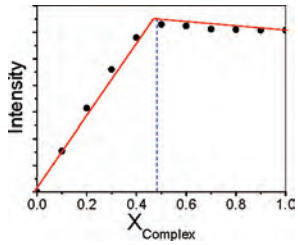

Figure 4. Job plot using luminescence data for $Ru_2(obip)L_4$ with a final G-quadruplex at 10 μM using 100 mM NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, 1 mM Na_2EDTA, pH = 7.0, and x = mole fraction of complex added to DNA.

ment, about 1.5 times larger than that observed without a G-quadruplex, which was obviously larger than that observed for duplex DNA (Figure S3 in the Supporting Information). At the same time, the interaction between the complex and DNA depends on the ionic strength. The increment of emission intensities in H_2O was larger than that in a 100 mM NaCl buffer (Figure S4 in the Supporting Information). On the basis of the luminescence emission enhancement observed for the binding of $Ru_2(obip)L_4$ with a G-quadruplex and duplex DNA, an intrinsic binding constant was obtained according to the Scatchard equation. The values of the binding constant were 5.4×10^5 and 4.1×10^4 for quadruplex and duplex DNA, respectively. These observations indicated that the interaction between $Ru_2(obip)L_4$ and DNA was strong and $Ru_2(obip)L_4$ interacted preferentially with G-quadruplexes over duplex DNA.

Binding stoichiometry with a quadruplex was then investigated through the luminescence-based Job plot (Figure 4). One major inflection point for $Ru_2(obip)L_4$ at $x = 0.48$ was observed. The data were consistent with a 1:1 [quadruplex]/[complex] binding mode, which was suggestive of a specific Ru−quadruplex interaction with a single guanine tetrad.

To gain insight into the interaction between $Ru_2(obip)L_4$ and G-quadruplex, we examined the ability of $Ru_2(obip)L_4$ to stabilize G-quadruplex DNA by thermal denaturation profiles. As expected, the melting profiles of AG3(T2AG3)3 in the absence of K^+ or Na^+ showed almost no transition (not shown), suggesting that it did not form a stable G-quadruplex structure. As for the dissociation of a G-quadruplex, the T_m of the monovalent ion K^+induced an intramolecular G-quartet structure of AG3(T2AG3)3 sequence that was more stable than its Na^+ counterpart. As the ratio of $Ru_2(obip)L_4$ to AG-22 equaled 1, the transition temperature of the G-quadruplex increased from 55.2 to 64.6 °C in a Na^+ buffer and increased from 65.1 to 70.9 °C in a K^+ buffer, in which an increase in the melting temperature of the quadruplex indicated a stabilizing effect (Figure 5). The results indicated that $Ru_2(obip)L_4$ strongly stabilized the G-quadruplex in both Na^+ and K^+ buffers. It was reported that the telomerase inhibition activity of drugs was strongly

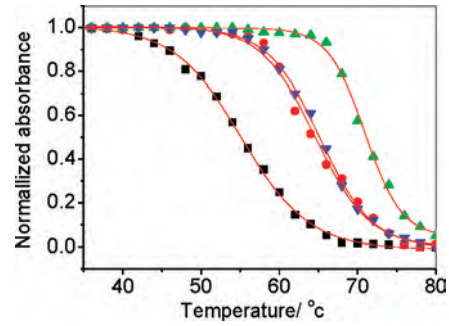

Figure 5. Normalized UV melting curves for a 10 μM G-quadruplex (■), a 10 μM G-quadruplex + 10 μM $Ru_2(obip)L_4$ (●) in a buffer of 100 mM NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, 1 mM Na_2EDTA; 10 μM G-quadruplex (▼), 10 μM G-quadruplex + 10 uM $Ru_2(obip)L_4$ (▲) in a buffer of 100 mM KCl; 10 mM K_2HPO_4/K_2HPO_4, 1 mM K_2EDTA (pH 7.0), and 1 mM K_2EDTA. Melting of the antiparallel G-quadruplex was assessed by UV absorbance at 295 nm.

related to the stabilization of the quadruplex structure. Therefore, $Ru_2(obip)L_4$ might be a promising candidate for potent anticancer drugs.

Small molecules can potentially bind to a quadruplex by externally stacking below the quartets, intercalating between the quartets, or nonspecifically binding to some random location on the DNA strand.[10] Intercalators will often exhibit lower intensity CD spectra compared with groove binders because of the fact that a groove binder contacts a larger part of the DNA and twists to follow the groove.[17] Given the CD results, the structure of $Ru_2(obip)L_4$, and the lateral loop conformations delineated in a recent X-ray structure of the G-quadruplex,[7,8] intercalation of $Ru_2(obip)L_4$ in between successive G-tetrads of the conformation would seem to be an unlikely binding mode. In contrast, either $Ru_2(obip)L_4$ in nonspecifically bound complexes simply attaches itself to the sugar–phosphate backbone on the DNA strand, presumably via electrostatic means, or the binding of $Ru_2(obip)L_4$ to G-quadruplex takes place via external stacking or both. The details of the binding modes and an evaluation of biological activity are in progress.

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Supporting Information Available: Synthetic procedures for $Ru_2(obip)L_4$; details for circular dichroism (CD) spectra, emission spectra, absorption spectra, and thermal denaturation profiles; X-ray crystallographic files in CIF format for $Ru_2(obip)L_4$. This material is available free of charge via the Internet at http://pubs.acs.org.

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