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A Naked-Eye On–Off–On Molecular "Light Switch" Based on a Reversible "Conformational Switch" of G-Quadruplex DNA

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Supporting Information

ABSTRACT: Herein, we report a new strategy for developing an on–off–on molecular "light switch" by utilizing the pH value to control the "conformational switch" of G-quadruplex DNA. A novel ruthenium(II) complex with an emission enhancement factor of 150 was synthesized and introduced to detect the switch by the naked eye. The "light switch" can be repeatedly cycled off and on through the addition of H^+ and OH^-, respectively. The conformational transitions of G-quadruplex DNA in K^+ solution at different pH values in the acidic region were evidenced by circular dichroism and fluorescence titrations. Computational calculations by applying density functional theory (DFT)/time-dependent DFT and molecular docking were also carried out to gain insight into the "light-switch" mechanism.

Polypyridyl-based transition-metal complexes that interact with DNA are excellent probes for the physical properties of DNA.[1] In particular, $[Ru(L)_2(dppz)]^{2+}$ (L = bpy (2,2′-bipyridine), phen (1,10-phenanthroline); dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), known as a DNA "light switch", has attracted intense interest.[2] This intriguing "light switch" has been widely used in applications such as molecular-scale logic gates,[3] DNA sensing,[4] and detection of mismatches.[5] The potential cause of the DNA light-switching properties is as follows: in a protic environment, hydrogen bonding with the phenazine (phz) N atoms of ruthenium(II) complexes lowers the energy of the metal-to-ligand charge-transfer (MLCT) dark state (DS) to below that of the bright state (BS) and quenches luminescence via nonradiative vibrational relaxation from the DS back to the ground state; in the bound form, the phz N atoms are shielded from the protic environment; thus, the lowest state is the BS and luminescence is observed.[6,7]

A G-rich telomeric DNA sequence can form a polymorphic quadruplex consisting of a four-stranded structure of stacked coplanar guanine-tetrads (G-quartets) stabilized by Hoogsteen hydrogen bonds.[8] The G-quadruplex architecture is sensitive to monocations, for example, the telomere sequence d[AGGG-$(TTAGGG)_3$] (22AG) was found to adopt an antiparallel G-quadruplex structure in the presence of Na^+ solution[9] but forms a hybrid-type mixed parallel/antiparallel-stranded G-quadruplex structure in K^+ solution.[10] These unusual DNA structures have been suggested to act as a negative regulator of telomere elongation by telomerase in vivo and are currently considered as a potential target for cancer therapy.[11] These potential roles of G-quadruplex DNA structures have sparked great interest in the design of molecules that can specifically recognize them.[12] Recently, our laboratory found that many ruthenium (Ru) complexes can also serve as a prominent molecular "light switch" for both G-quadruplex and i-motif DNA.[13] Nevertheless, most of them have low fluorescence enhancement and can hardly be observed by the naked eye. In the present work, we report a novel ruthenium(II) complex ($[Ru(phen)_2dppz\text{-}idzo]^{2+}$; dppz-idzo = dppz-imidazolone) as an excellent molecular "light switch" for G-quadruplex DNA (22AG), being superior to the well-known DNA molecular "light switches" of $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$ (Figure S1 in the Supporting Information, SI). The molecular structure is shown in Figure 1. For the first

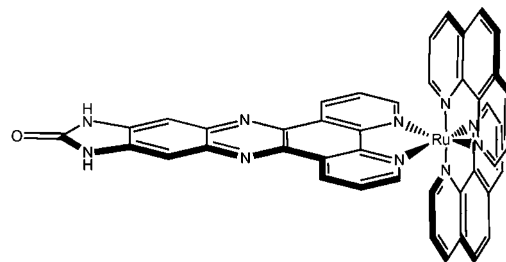

Figure 1. Molecular structure of $[Ru(phen)_2dppz\text{-}idzo]^{2+}$.

time, we introduced an imidazolone group to the main ligand of Ru complex and enhanced the fluorescence and selectivity to detect G-quadruplex DNA even with the naked eye. The synthesis and characterization of the compound are shown in the SI (Scheme S1).

The reversible "light switch" is very appealing in many applications. There are only a few ways to turn the DNA-based Ru complex "light switches" on and off repeatedly, such as temperature adjustment,[7] metal-ion-coupled ethylenediaminetetraacetic acid treatment,[14] and photoregulation.[15] In this research, we discovered that the conformation of G-quadruplex DNA can be regulated by adjusting the pH value in the acidic region, thereby accounting for the on–off–on "light-switch" effect.

Initially, luminescence titration of the Ru complex with G-quadruplex DNA (from 0.00 to 2.5 μM) in aqueous solution was performed, and the results are depicted in Figure 2a. Clearly, $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ is almost nonemissive in the absence of

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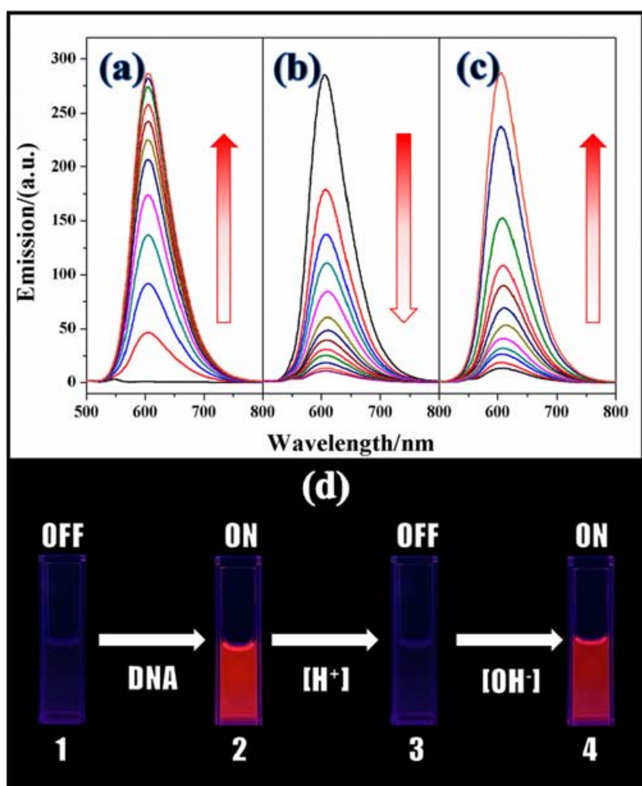

Figure 2. (a) Fluorescence titration of 2.5 μM $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ with 22AG DNA (0−2.5 μM). (b) H^+ titration within the pH range from 4.5 to 1.4. (c) OH^- titration within the pH range from 1.4 to 4.5. (d) Fluorescent distinction of the Ru complex by the naked eye under irradiation of UV light in the solutions containing (1) 5 μM Ru complex, pH 4.5, (2) 5 μM Ru complex and 2.5 μM DNA, pH 4.5, (3) 5 μM Ru complex and 2.5 μM DNA, pH adjusted to 1.4 by H^+, and (4) 5 μM Ru complex and 2.5 μM DNA, pH adjusted back to 4.5 by OH^-.

DNA, and a remarkable fluorescence enhancement (by a factor of 150; quantum yield φ = 0.088) can be observed after the addition of G-quadruplex DNA, resulting in an obvious "light-switch" effect. All measurements were carried out in 10 mM Tris-HCl buffer containing 100 mM KCl, pH 4.5.

Subsequently, fluorescent pH titrations were performed within the pH range from 4.5 to 1.4 via the addition of a HCl solution, and the pH effects on the fluorescence emission spectra of the Ru complex are shown in Figure 2b. In the acidic pH region below 1.4, the complex almost had no luminescence, indicating that the emission was switched "off". Interestingly, the emission spectra can be turned "on" through the successive addition of KOH, adjusting the pH range from 1.4 to 4.5 (Figure 2c). Figure S2 in the SI shows the changes in the relative emission intensity of $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ bound to G-quadruplex DNA as H^+ and OH^- were added successively, thus turning the DNA light switch on and off over a series of cycles. The pH effects on the color response of the Ru complex with DNA under UV light are shown in Figure 2d. We can clearly distinguish the "off" and "on" states by the naked eye. The absorption spectra upon pH titration of the complex on its own was also performed, and no notable change was observed (Figure S3), implying that the switch effect was due to changes of DNA.

Next, in order to elucidate the mechanism involved in the luminescent switch process, circular dichroism (CD) measurements were carried out and the conformational transitions of G-quadruplex DNA were evaluated. The CD spectra of 22AG upon pH titration are shown in Figure S4 in the SI. G-quadruplex DNA in K^+ buffer exhibited a major positive band around 295 nm and a smaller positive band at 246 nm, indicating that it favored a mixed-hybrid structure.[10,16] When the pH was adjusted to about 2.4 by the successive addition of H^+, the positive band at 295 nm increased and a negative band emerged gradually at 260 nm, suggesting that the antiparallel structure increased as the pH decreased.[17] Further decreases in the pH values from 2.4 to 1.4 resulted in the disappearance of the bands at both 260 and 295 nm, which means the G-quadruplex structure might be unfolded to protonize single-stranded forms in the acidic pH region below 1.4.[18] The conformation of DNA can be recovered by the successive addition of equimolar KOH. The changes of the CD signals and fluorescence emission as a function of the pH during titration are shown in Figure 3.

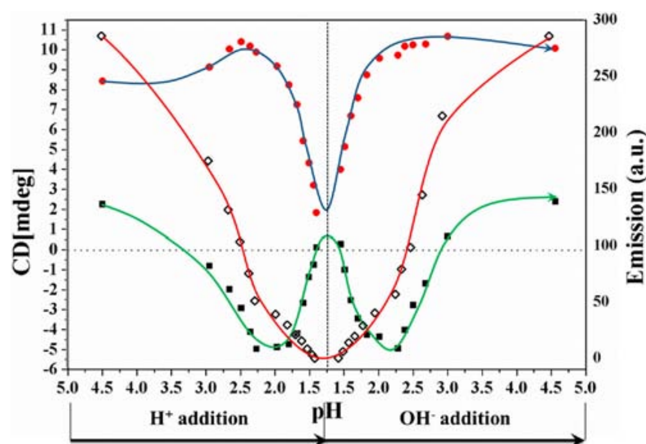

Figure 3. pH-dependent CD and fluorescence studies of the light-switch system (2.5 μM $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ in 2.5 μM 22AG) in 10 mM Tris-HCl buffer containing 100 mM KCl. The red spots (●) and black squares (■) represent the CD signals at 295 and 260 nm, respectively. The hollow diamonds (◇) represent the emission changes at 605 nm upon pH titration.

Combining the fluorescent and CD titrations, we speculate that the switch-off process consists of two steps: (1) over the pH range from 4.5 to 2.4, the conformation of the quadruplex transformed from a hybrid structure to an antiparallel structure,[17] which might account for the sharp decrease in the emission intensity; (2) in the pH range from 2.4 to 1.4, the antiparallel structure of DNA might dissociate to random-coil single-stranded DNA (ssDNA),[19−21] which gave rise to a slower decline in the emission intensity. The difference of the emission enhancement is closely correlated with the DNA-binding affinity. The quenching of fluorescence at pH 1.4 implied that $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ might no longer be bound to ssDNA. This was also evidenced by the UV-absorption spectrum (Figure S5 in the SI). During the switch-on process upon KOH titration, both the conformation and luminescence reversed in the same trend. Thus, the on−off−on "light-switch" effect was caused by the reversible two-step conformational transitions mediated by the pH values.

Fluorescence and CD titrations of duplex and antiparallel quadruplex DNA were also investigated (Figures S6−S9 in the SI) for comparison. As the results shown, different DNA structures exhibited different melting pH values. Thus, the fluorescence selectivity of the Ru complex between different DNA structures is closely related to the pH value (Figure S10 in

the SI): the selectivity toward G-quadruplex is much stronger at pH 2.5 than that at pH 4.5. That is interesting because it provides a possibility for the distinction of quadruplex and duplex DNA at low pH values (pH 2.5).

Finally, the structure of $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ was optimized on the basis of the density functional theory (DFT)-B3LYP method with the 6-31G* basis set for C, N, O, and H atoms and LanL2DZ for the Ru atom with *Gaussian03* (Figure S11 in the SI).[22] The distributions of molecular orbitals (MOs) were calculated, and some of the selected frontier orbitals are shown in the SI (Figures S12 and S13). The vertical singlet transition energies of the ruthnium(II) complex in water (Table S1 in the SI) and without water (Table S2 in the SI) were also calculated using time-dependent DFT (TD-DFT). The percentages of composition of the metal and ligands in each related MO are listed in Tables S3 and S4 in the SI. The results showed that the lowest MLCT states in the absence and presence of water are BS and DS, respectively. This is closely related to the "light-switch" effect of $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ in the presence of G-quadruplex DNA. The primitive calculated result was used for molecular modeling with AutoDock 4.2 Lamarckian Genetic Algorithm and Accelrys Discovery Studio 2.5,[23] which provided more information on the binding interactions between the Ru complex and G-quadruplex DNA (Figure S14 in the SI). The results revealed an "end-pasting"[12] binding model in which the Ru complex bound to human telomeric G-quadruplex at the 5′ terminus (Figure S15 in the SI). The planar dppz-idzo core participated in $\pi-\pi$ stacking with the guanine quartets on the end of the quadruplex, which may be responsible for the significant emission enhancement. In addition, the calculated binding energy of Ru-complex binding to hybrid (−13.0 kcal/mol) and antiparallel (−10.1 kcal/mol) G-quadruplexes explained the reason that the emission of $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ decreased as the content of the antiparallel DNA structure increased. This was also supported by the control experiment upon switching of quadruplex DNA from the antiparallel basket structure to the mixed-hybrid structure by starting with a Na^+-based buffer and then adding K^+ at the same pH value (Figure S16 in the SI).

In conclusion, a novel polypyridyl-based ruthenium(II) complex was synthesized and characterized. Fluorescence analysis showed that it had a strong emission enhancement in the presence of G-quadruplex DNA in K^+ solution. The combination of CD and luminescence titrations demonstrated that the dissociation and formation of G-quadruplex could be regulated successfully through the addition of H^+ and OH^- alternately, which leads to the "off" and "on" states in fluorescent emission, respectively. The on−off−on light switch could be observed clearly by the naked eye under UV light. This pH-controlled dual-switch (light and conformation switch) strategy may enable the development of convenient reversible molecular "light switches" as well as the visual detection of G-quadruplex DNA. The remarkable increase in the melting temperature of the G-quadruplex induced by $[Ru(phen)_2dppz\text{-}idzo]^{2+}$ also suggested the potential use of such a complex in therapeutic applications (Figure S17 in the SI). To our knowledge, this work presents the first example of a pH-controlled reversible visual light switch based on a conformational switch of G-quadruplex DNA.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis details of the Ru complex, fluorescence spectra, CD measurements, DFT/TD-DFT calculations, and molecular docking results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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