Luminescence Retrieval of $[Ru(bpy)_2(HNOIP)]^{2+}$: A Novel Molecular "Light Switch" for DNA

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A novel molecular "light switch", $[Ru(bpy)_2(HNOIP)]^{2+}$ (HNOIP = 2-(2-hydroxy-5-nitrophenyl)imidazo[4,5-*f*][1,10]phenanthroline), for DNA was developed. In aqueous solution the suppressed luminescence can be revived by the presence of Poly[dA]•Poly[dT], Poly[dG]•Poly[dC] and calf thymus DNA.

The development of new molecular sensory devices has initiated vigorous interests in the syntheses of various responsive molecules whose light emission can be switched by metal ions, organic molecules, protons or redox potentials.[1] However, the emission of transition metal complexes switched by polyanionic double strand DNA is rare.[2] Complexes $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-a:2'3'-c]phenazine), the only and the most intensively investigated molecular "light switches" for DNA, exhibit a negligible background emission in water but luminesce brightly in the presence of double strand DNA with high binding affinity ($k_a \sim 10^6$ M^{-1}).[2a] Polypyridyl complexes of ruthenium(II) can provide sensitive, luminescent probes for double strand DNA in solution. Nevertheless, the background luminescence of the free complexes in aqueous solution and their relatively weak binding constants appeared insufficient for their broad application as general nonradioactive nucleic acid probes, which stimulated us to develop new systems as DNA probes.

As demonstrated in our previous studies, the derivatives of the complexes of $[Ru(bpy)_2(PIP)]^{2+}$ (PIP = 2-phenylimidazo[4,5-*f*][1,10]phenanthroline) can bind with calf thymus DNA intercalatively with relatively high binding affinity, but their strong background luminescence in the free form give way to molecular "light switch" for DNA.[3] Considering the introduction of nitro group may frequently quench its luminescence in aqueous solution, we design a novel complex $[Ru(bpy)_2(HNOIP)]^{2+}$ (HNOIP = 2-(2-hydroxy-5-nitrophenyl)imidazo[4,5-*f*][1,10]phenanthroline),[4] whose luminescence is suppressed in aqueous solution. Surprisingly, its luminescence can be revived by the addition of double strand DNA and it shows the characteristics of a molecular "light switch" for DNA.

$[Ru(bpy)_2(HNOIP)]^{2+}$ was prepared by coordination of HNOIP to $Ru(bpy)_2Cl_2$ in ethylene glycol at 120 °C for 3 h under an argon atmosphere. Following alumina chromatography, the hexafluorophosphate salt was isolated. $[Ru(bpy)_2(HNOIP)]^{2+}$ shows negligible luminescence in aqueous solution at ambient temperature, but luminesces in organic solvents such as CH_2Cl_2, DMF and CH_3CN (Table 1). The most interesting features gathered from our data is that the polarity of the medium correlates well with its luminescence intensities. The results likely suggest that the more polarity of the solvent, the smaller relative intensities are observed. This phenomenon has also been found with the dppz ruthenium(II) complexes in the similar conditions.[5]

Figure 1 shows the steady-state emission spectra of $[Ru(bpy)_2(HNOIP)]^{2+}$ in buffer solution in the absence and presence of different B-form double strand DNA. No detecterable luminescence is observed for the complex in buffer solution. Upon addition of DNA, however, luminescence is apparent, displaying the light-switch behavior of $[Ru(bpy)_2(HNOIP)]^{2+}$. The maximum wavelength for emission shifts from 607 nm for GC sequences to 615 nm for AT sequences. Results with mixed-sequence calf thymus DNA are intermediate (Table 1). Data from the emission titration were employed to determine the binding constant of $[Ru(bpy)_2(HNOIP)]^{2+}$ with CT-DNA according the McGhee - Von Hippel equation.[6] The best fit was obtained with

Table 1. Emission characteristics of $Ru(bpy)_2(HNOIP)^{2+}$ in various solutions at 298 K

Solvent	DNA	$I^{rel\ a}$ (steady-state)	λ_{em} nm
H_2O		0.0	
5 mM Tris / 50 mM NaCl, pH 7.0		0.0	
CH_2Cl_2		1.0	612
DMF		0.67	621
CH_3CN		0.54	613
MeOH		0.15	613
5 mM Tris / 50 mM NaCl, pH 7.0	Poly[dA]•Poly[dT]	0.68	615
5 mM Tris / 50 mM NaCl, pH 7.0	Poly[dG]•Poly[dC]	0.53	607
5 mM Tris / 50 mM NaCl, pH 7.0	CT-DNA	0.56	613

[a]The values are given for the maximum intensity found relative to that found in CH_2Cl_2 at 612 nm. In the presence of DNA, the values are given as [DNA] / [Ru] = 40.

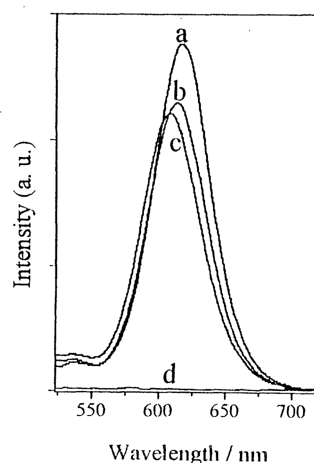

Figure 1. Emission spectra of $Ru(bpy)_2(HNOIP)^{2+}$ (10 μmol dm^{-3}) in 5 mmol dm^{-3} Tris-HCl, 50 mmol dm^{-3} NaCl buffer (pH 7.0) at 298 K in the presence of (100 μmol dm^{-3}) (a) Poly[dA]• Poly[dT], (b) calf thymus DNA, (c) Poly[dG]•Poly[dC] and (d) absence of DNA.

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the site-covering size parameter, n, equal to *ca.* 3 base pairs and an intrinsic binding constant of $K = 6.2 \times 10^5$ M^{-1}. The luminescence retrieval of $[Ru(bpy)_2(HNOIP)]^{2+}$ in the presence of double strand DNA may be understood by comparing the structures of itself with $[Ru(bpy)_2(HPIP)]^{2+}$, (HPIP = 2-(2-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline),which shows intense luminescence in aqueous solution. The results strongly suggest that the introduction of a nitro group to the HNOIP ligand is responsible for the absence of its luminescence. Consequently, by addition of double strand DNA, $[Ru(bpy)_2(HNOIP)]^{2+}$ may intercalate its ligand HNOIP into the DNA base pairs, shielding the ligand esp. the nitro-group from solvent water and switching on its luminescence. Viscosity experiments (data not shown) also confirm that the complex binds with CT-DNA intercalatively.

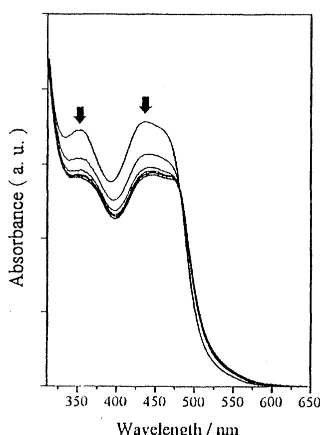

Figure 2. Absorption spectra of $Ru(bpy)_2(HNOIP)^{2+}$ (10 μmol dm^{-3}) in 5 mmol dm^{-3} Tris-HCl, 50 mmol dm^{-3} NaCl buffer (pH 7.0) in the presence of 0.0, 7.0, 14.0, 30.0, 80.0 and 113.0 μmol dm^{-3} calf thymus DNA.

The electronic absorption titration of $[Ru(bpy)_2(HNOIP)]^{2+}$ with CT-DNA reveals intense hypochromism and bathochromism in both the ligand π-π^* transition (λ_{max}, 350 nm) and the metal–to–ligand charge transfer (MLCT) transition in the visible region (Figure 2). Upon addition of DNA, the MLCT band at 434 nm exhibits a decrease with a maximal value of 23.1% and the concomitant shift of the peak position to 442 nm. At the same time, the HNOIP π-π^* transition band at 350 nm displays hypochromism as 20.6% and a red shift of *ca.* 4 nm. An isobestic point at 472 nm was also observed. These spectroscopic features are comparable to those observed with ruthenium(II) complexes which bind with DNA by intercalation.[7]

In conclusion, a novel molecular "light switch" for DNA was developed. We found the complex $[Ru(bpy)_2(HNOIP)]^{2+}$ to be a highly sensitive lüminscent sensor for double strand DNA. In aqueous solution, the suppressed emission is revived when $[Ru(bpy)_2(HNOIP)]^{2+}$ has intercalated in the DNA base pairs. Therefore, $[Ru(bpy)_2(HNOIP)]^{2+}$ may be served as a true molecular "light switch" for DNA and the potential application to nonradioactive nucleic acid probe is optimistically prospected.

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References and Notes

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4 Data for HNOIP: Found: C, 63.8; H, 3.3; N, 19.5%. Calcd for $C_{19}H_{11}N_5O_3$: C, 63.9; H, 3.1; N, 19.6%; ^1H NMR (ppm, DMSO-d_6) 14.02 (s, 1H), 9.15 (s, 1H), 9.05 (d, 2H, J = 4.2), 8.89 (d, 2H, J = 8.1), 8.20 (d, 1H, J = 9.1), 7.83 (q, 2H), 7.17 (d, 1H, J = 9.2 Hz); FAB-MS : 358, $[M+1]^+$. Data for $[Ru(bpy)_2(HNOIP)][PF_6]_2 \cdot H_2O$: Found: C, 43.5; H, 2.4; N, 11.7%. Calcd for $C_{39}H_{29}F_{12}N_9O_4P_2Ru$: C, 43.4; H, 2.7; N, 11.7%; ^1H NMR (ppm, DMSO-d_6) 9.15 (s, 1H), 9.08 (d, 2H, J = 8.4), 8.87 (d, 2H, J = 7.8), 8.83 (d, 2H, J = 8.4), 8.21 (t, 2H), 8.09 (t, 2H), 8.05 (d, 1H, J = 9.0), 7.91 (d, 2H, J = 4.8), 7.86 (d, 2H, J = 5.4), 7.81 (m, 2H), 7.61 (d, 2H, J = 6.0), 7.59 (t, 2H), 7.36 (t, 2H), 6.91 (d, 1H, J = 9.0 Hz); UV-VIS (H_2O) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 242 (30900), 254 (29900), 286 (62300), 350 (17500), 434 (18600); FAB-MS: 915 ($[M+PF_6]^+$), 770 (M^{2+}).

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