

Redox-activated luminescence and light-induced nuclease activity of a new mixed-ligand ruthenium(II) complex

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Abstract. The new metallointercalator 1, a mixed-ligand ruthenium(II) complex incorporating a quinone-fused dipyridophenazine ligand, effects an efficient cleavage of the supercoiled plasmid DNA upon activation with visible light. In addition, the quinone/hydroquinone redox couple 1/2 represents a molecular light-switching device displaying interconversion between non-luminescent state 1 and luminescent state 2.

Keywords. Metallointercalator; DNA-binding and photocleavage; redox-switching of luminescence.

1. Introduction

Currently, a great deal of attention is being paid to DNA interactions of mixed-ligand ruthenium(II) complexes containing 1,10-phenanthroline (phen) and modified phen ligands, with the latter being specially designed to augment intercalative interaction by the complexes (Murphy and Barton 1993; Holmlin and Barton 1995; Jacquet *et al* 1995; Liu *et al* 1995; Schoch *et al* 1996). Such complexes can also be employed in research related to molecule-based electronic devices and solar energy conversion processes (Balzani 1987; Harriman and Zissel 1996; Constable *et al* 1996). During our recent studies (Arounagui and Maiya 1996) on the DNA interactions of nickel(II) and cobalt(III) complexes containing dipyrido[3,2-*a*:2',3'-*c*]-phenazine (dppz, a modified phen ligand), it occurred to us that further derivatization of this ligand with appropriate functional group/s might not only accentuate DNA-binding and photocleavage efficiencies of the ensuing complexes but also serve to explore other interesting functional aspects associated therein. Towards this end, we have now synthesized a novel mixed-ligand complex 1, containing a quinone-fused di-pyridophenazine ligand (10,11-[1,4-naphthalene-dione]dipyrido[3,2-*a*:2',3'-*c*] phenazine, Aqphen). The quinone moiety has been specifically chosen here owing to its known intercalating ability and rich redox chemistry (Bhattacharya and Mandal 1996; Breslin and Schuster 1996). Indeed, as will be demonstrated in this communication, complex 1 is not only an avid binder of CT-DNA but is also an efficient photocleaving agent of supercoiled pBR 322 DNA. In addition, results of combined coulometry and fluorescence studies reveal that the redox (quinone/hydroquinone) couple 1/2 unites an electroactive component with a light emitting centre and that it

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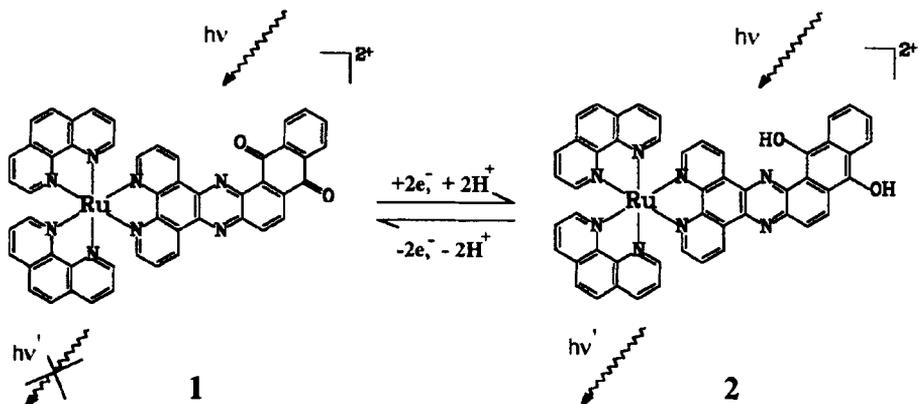


Figure 1. Structures of redox-related ruthenium complexes 1 and 2.

can, in principle, be used in redox-activated optical signal generation and allied applications (figure 1).

It should be noted here that, while this manuscript was being prepared, synthesis and characterization of Aqphen and a rhenium complex containing it ($\text{Re}(\text{Aqphen})(\text{CO})_3\text{Cl}$) have been reported by Lopez et al (1996). However, the focal theme of their paper was entirely different from that envisaged in the present study.

2. Results and discussion

2.1 Synthesis and characterization

Refluxing an ethanol solution (250 ml) containing readily accessible 1,10-phenanthroline-5,6-dione (0.21 g, 1 mmol) and 1,2-diamino anthraquinone (0.23 g, 1 mmol) for 5 h and evaporation of the solvent gave a greenish-brown residue which was taken up in 100 ml CHCl_3 and warmed to $\approx 50^\circ\text{C}$. The solution was cooled and filtered, and a yellow precipitate obtained upon addition of diethyl ether to the filtrate. It was vacuum dried, after washing thoroughly with diethyl ether, to give the pure sample of Aqphen in $\approx 70\%$ yield. Complex 1·(PF_6)₂ was prepared by condensing $\text{Ru}(\text{phen})_2\text{Cl}_2$ and Aqphen in refluxing diethylene glycol. Addition of NH_4PF_6 precipitated crude 1·(PF_6)₂, which was purified by recrystallization (acetone–diethyl ether, yield $> 80\%$). The PF_6 salt was converted to the water-soluble chloride salt 1· Cl_2 , by the standard procedure using tetrabutylammonium chloride (TBACl). Both Aqphen and 1 were characterized by elemental analysis, FAB-MS, IR, UV-Vis, NMR and cyclic voltammetric methods.[†]

[†]Selected data for Aqphen, and 1

Aqphen: Analysis: Found: C, 73.02; H, 3.16; N, 11.75%; Calcd. for $\text{C}_{26}\text{H}_{12}\text{N}_4\text{O}_2$: C, 73.73; H, 2.98; N, 12.59%; IR (KBr pellet): 1670, 1585, 1462 cm^{-1} . UV-Vis (CH_2Cl_2) λ_{nm} (log ϵ): 410 (4.13), 394 (4.13), 281 (4.58), 259(4.62). ^1H NMR (200 MHz, CDCl_3 , 298 K): δ 9.85 (d, 1H), 9.65(d, 1H), 9.35 (d, 2H), 8.71 (dd, 2H), 8.32 (q, 2H), 7.88(m, 4H).

1. (PF_6)₂: Analysis: Found: C, 50.86; H, 2.37; N, 9.22%; calcd. for $\text{C}_{50}\text{H}_{32}\text{N}_8\text{O}_4\text{P}_2\text{F}_{12}\text{Ru}$: C, 50.06, H, 2.49; N, 9.34%; IR (KBr pellet): 1670, 1589, 1427, 837 cm^{-1} UV-Vis (CH_3CN) λ_{nm} (log ϵ): 438(4.29), 388(4.32), 302(4.83), 278(4.92), 263(5.09). ^{13}C NMR(200 MHz, $\text{CD}_3\text{CN}/10\%$ D_2O , 298 K): δ 181 (ArC=O)

Complex $\underline{1}(\text{PF}_6)_2$ showed its characteristic MLCT band at 438 nm and bands due to the intra-ligand transitions at 388 (Aqphen) and 263 (phen) nm in the UV-Vis spectrum. The well-defined cyclic voltammetric responses seen at +1.36 and -0.37 V for the complex can be ascribed to $\text{Ru}^{\text{III}}/\text{Ru}^{\text{II}}$ and Aqphen/Aqphen $^-$ couples respectively. The complex was found to be weakly luminescent ($\phi < 10^{-4}$) in many organic solvents, presumably due to an intramolecular photoinduced electron transfer (PET) quenching of its MLCT state by the appended quinone fragment. The spectral and redox characteristics of $\underline{1}\cdot\text{Cl}_2$ were found to be essentially similar to those of $\underline{1}(\text{PF}_6)_2$. In aqueous buffered or aqueous CH_3CN (4–5% H_2O) solutions, this chloride salt was found to be totally non-fluorescent.

2.2 DNA-binding and photocleavage

DNA binding by $\underline{1}\cdot\text{Cl}_2$ was monitored by absorption titration (MLCT band, buffer A = 5 mM tris, pH 7.1, 50 mM NaCl), thermal denaturation (monitoring A_{260} of DNA, buffer B = 1 mM phosphate, pH 7.0, 2 mM NaCl) and differential pulse voltammetric (quinone reduction peak, buffer A) methods. In the presence of increasing amounts of calf-thymus (CT) DNA, the complex showed bathochromic shifts (maximum: 4 ± 1 nm) and hypsochromism (maximum: $25 \pm 3\%$) in the UV-Vis spectrum, diminution of peak current (maximum: $40 \pm 5\%$) and cathodic shifts of the peak potential (maximum: 35 ± 5 mV) in differential pulse voltammograms and increase in the DNA melting temperature ($4 \pm 1^\circ\text{C}$ at $[\text{DNA}]_{\text{phosphate}}/[\underline{1}] = 25$) in thermal denaturation experiments. These observations are reminiscent of those reported earlier for various metallointercalators including the metal-dppz complexes and suggest that the complex $\underline{1}\cdot\text{Cl}_2$ binds strongly to DNA by an intercalative mode (Gupta *et al* 1992; Murphy and Barton 1993; Holmlin and Barton 1995; Arounaguiiri and Maiya 1996). It was not possible to evaluate the accurate binding constant (K_b) for DNA intercalation by $\underline{1}$ by the absorption titration method because the complex was found to bind too strongly (stoichiometric binding) even at micromolar concentrations of DNA as was the case with the previously studied dppz complexes (Hiort *et al* 1993; Arounaguiiri and Maiya 1996).

In agarose gel electrophoresis experiments, control runs suggested that untreated plasmid pBR 322 DNA (supercoiled form, 100 μM in nucleotides) does not show any perceptible cleavage in the dark and even upon irradiation by 440 ± 5 nm light for 2 h in the sample chamber of a Jasco model 777 spectrofluorimeter (slit width 5/5 nm). Similarly, DNA nicking was not observed for the plasmid treated with $\underline{1}\cdot\text{Cl}_2$ (10 μM) in the dark run. On the other hand, irradiation of DNA in the presence of the complex for 25 min caused complete conversion of the supercoiled form (form I), generating relaxed circular DNA (form II) under similar experimental conditions.

2.3 Redox-switching of luminescence

Exhaustive coulometric reduction of $\underline{1}$ in deaerated, aqueous (4–5% H_2O) CH_3CN at -0.5 V (0.1 mol dm^{-3} TBAPF $_6$, Pt working electrode; $E_{1/2}(\text{red})\underline{1} = -0.27$ V) generated $\underline{2}$ as identified by its UV-Vis, NMR and IR spectra.[†] The same electrolytic

[†] Selected data for $\underline{2}$

$\underline{2}(\text{PF}_6)_2$: UV-Vis($\text{CH}_3\text{CN}/10\%\text{H}_2\text{O}$) λ_{nm} (log ϵ): 442(4.28), 348(4.22), 300 (*sh*, 4.74), 263(5.06); ^{13}C NMR (200 MHz, $\text{CD}_3\text{CN}/10\%\text{D}_2\text{O}$, 298 K): δ 153 ($\underline{\text{ArC}}\text{-OH}$).

solution containing the hydroquinone complex could be reoxidized to obtain 1 by exhaustive coulometry at +1.1 V ($E_{1/2}(\text{ox } \underline{2}) = +0.92 \text{ V}$) and the redox-cycle was repeated thrice with < 10% loss of the material. Whereas, as stated earlier, the quinone form 1 was found to be almost non-luminescent, the electrochemically (or chemically, by $\text{Na}_2\text{S}_2\text{O}_4$) generated hydroquinone form 2 showed the MLCT luminescence at 601 nm ($\phi = 0.018 \pm 0.03$). Thus the couple 1/2, which combines an electroactive component with a light-emitting centre (figure 1), represents a redox activated luminescence on/off switching device with its function being similar to that exhibited by $[\text{Ru}(\text{bpy})_2(\text{bpy-BQ})]^{2+}$ -a covalently-linked, Ru(II) complex-benzoquinone system reported previously (Goulle *et al* 1993).

3. Conclusion

In summary, the new ruthenium(II) complex 1 endowed with a novel, quinone-fused, dipyrrophenazine ligand exhibits three interesting properties: (i) it strongly binds to DNA, (ii) it efficiently photocleaves DNA, and (iii) the redox couple 1/2 represents an 'electro-photoswitch'. These characteristics testify to the utility of this complex as a photonuclease and also as a molecule-based photodevice. Detailed studies investigating these aspects of complexes 1 and 2 are currently in progress.

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