$Os(phen)_2(dppz)^{2+}$: A Red-Emitting DNA Probe

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Received August 18, 1994

Polypyridyl complexes of ruthenium(II) provide sensitive, luminescent probes for double helical DNA in solution.[1,2] The mixed-ligand complexes $Ru(phen)_2dppz^{2+}$ (phen = 1,10-phenanthroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) and $Ru(bpy)_2dppz^{2+}$ (bpy = 2,2′-dipyridyl) have been shown to be molecular "light switches" for DNA.[3] No detectable emission is observed in aqueous solution due to quenching by hydrogen bonding between water and the phenazine nitrogens of the dppz ligand.[3,4] Binding to DNA, however, protects the phenazine nitrogens from water through preferential intercalation of the dppz ligand and leads to intense photoluminescence. This light-switch effect has been shown to be sensitive to perturbations in the ligand architecture.[5] On the basis of analogies between ruthenium and osmium polypyridyls,[6] we anticipated that substitution of osmium(II) would not significantly alter the light-switch characteristic but would yield a lower energy emitting species with fast decay kinetics. As part of our ongoing effort to identify novel DNA diagnostics, we report here the preparation and characterization of $Os(phen)_2dppz^{2+}$ as the first osmium-containing DNA light switch.[7] This complex represents one of a new class of red-emitting DNA probes, active on a fast time scale.

Figure 1 shows the steady-state emission profile of $Os(phen)_2dppz^{2+}$ [8] in buffered aqueous solution both in the presence and absence of double-stranded DNA. No luminescence is detected with irradiation (λ_{ex} = 480 nm) of an aqueous solution of $Os(phen)_2dppz^{2+}$. Upon addition of DNA, however, significant long-wavelength emission (λ_{max} = 738 nm) is observed, demonstrating the light-switch behavior of $Os(phen)_2dppz^{2+}$. On the basis of this steady state signal of the complex bound to DNA, we estimate a quantum yield for emission, $\Phi_{em} \sim 0.0001$.[9] While this Φ_{em} is low compared to other luminescent probes of DNA, $Os(phen)_2dppz^{2+}$ is unique because of its broad emission profile at long wavelengths (700−800 nm).[10]

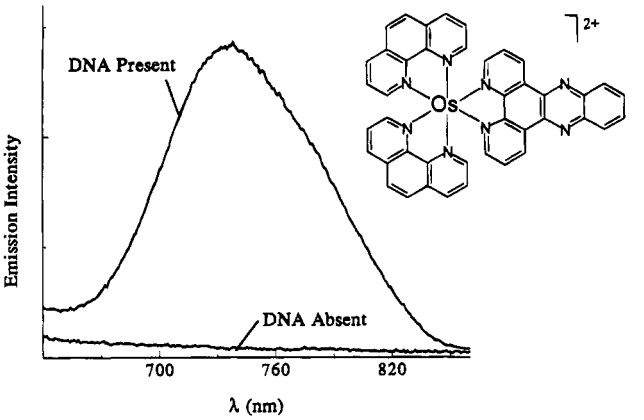

Figure 1. Steady-state emission spectrum of $Os(phen)_2dppz^{2+}$ (50 μM Os, 5 mM Tris, 50 mM NaCl, pH 8.5, air-equilibrated) in the absence and presence of sonicated calf-thymus DNA (1000 μM nucleotide). Excitation was at 480 nm, and the emission maximum occurs at 738 nm (standardized to $Ru(bpy)_3^{2+}$ emission maximum at 610 nm). Steady-state luminescence was measured using an SLM 8000 spectrofluorimeter, and peak integrals were computed using the SLM software package. $Os(phen)_2dppz^{2+}$ is also schematically illustrated.

Visible absorption spectroscopy reveals intense hypochromism (38% at 372 nm) and a red shift (7 nm) in the dppz $\pi-\pi^*$ transition with the complex fully bound to DNA. These spectroscopic features are equivalent to those observed[11] with $Ru(phen)_2dppz^{2+}$ and suggest that the osmium complex binds by intercalation in a manner that parallels $Ru(phen)_2dppz^{2+}$.[12]

The excited state lifetimes of racemic $Os(phen)_2dppz^{2+}$ in acetonitrile and with sonicated calf-thymus DNA have been measured by time-correlated single-photon counting.[13] Parts A and B of Figure 2 show the luminescence decay in acetonitrile and with DNA, respectively. In acetonitrile, the excited state decay is strictly mono-exponential (τ = 3.4 ns) as illustrated by the linear semi-log plot extending over 5 radiative lifetimes. By contrast, a multi-exponential decay is observed when the complex intercalates into DNA. The data shown here are best described by a tri-exponential fit. Bi-exponential decays in

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(8) The complex was prepared by coordination of dppz to $Os(phen)_2Cl_2$[6a] in ethylene glycol at 170 °C over 2 h and then isolated as the hexafluorophosphate salt. Following alumina chromatography (acetonitrile eluent), trace impurities were rigorously excluded using reverse phase HPLC. Data for $[Os(phen)_2dppz](PF_6)_2{\cdot}2H_2O$ are as follows. ^{1}H-NMR (acetonitrile-d_3): δ 9.45 (d, 2H), 8.53 (dd, 2H), 8.46 (dd, 4H), 8.31 (s, 4H), 8.19 (dd, 2H), 8.17 (dd, 2H), 8.08 (d, 2H), 7.97 (d, 2H), 7.72 (dd, 2H), 7.64 (dd, 2H), 7.62 (dd, 2H). FABMS (*m/z*): $[MH^{2+} + PF_6^-]^+$, 979; M^+, 834. Anal. Calcd for $C_{42}H_{30}N_8F_{12}O_2P_2Os$: C, 43.53; H, 2.61; N, 9.67. Found: C, 43.52; H, 2.32; N, 9.76.

(9) The quantum yield was determined by comparison to $Ru(bpy)_3^{2+}$. Since a significant fraction of the decay occurs within the response time of the instrument, and the signal was not corrected for photomultiplier tube insensitivity at long wavelengths, this yield is a lower limit.
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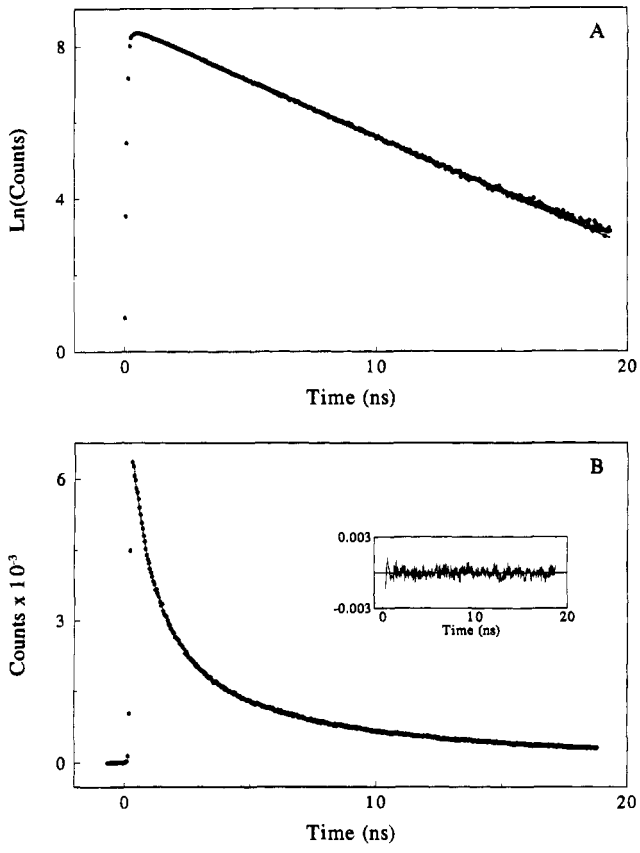

Figure 2. (A) Semi-log plot of the luminescence decay profile of Os(phen)$_2$dppz^{2+} in aerated acetonitrile (50 μM Os), with a computer-generated fit of the emission overlayed. The radiative lifetime for the mono-exponential decay is 3.4 ns. (B) Luminescence decay profile of Os(phen)$_2$dppz^{2+} in sonicated calf-thymus DNA (50 μM Os, 1000 μM nucleotide, 5 mM Tris, 50 mM NaCl, pH 8.5). A computer-generated tri-exponential fit of the decay overlays the data following the initial rise. The inset shows the weighted residuals for the fit. The radiative lifetimes and their respective contributions to the decay (taken as the ratio of the pre-exponential factor to the sum of such factors) are as follows: τ_1 = 0.76 ns (49 %); τ_2 = 2.4 ns (33%); τ_3 = 11 ns (18%); a small linear offset is typically included in the fit, contributing less than 1% of the decay.

emission have been observed[11] for both Λ- and Δ-Ru(phen)$_2$dppz^{2+} bound to DNA, and the increased quantum yield for emission by the Δ-isomer leads to an apparent bi-exponential decay for *rac*-Ru(phen)$_2$dppz^{2+}.[3,4] The longest component of the decay by Os(phen)$_2$dppz^{2+} with DNA reflects the long-lived component of emission by the Δ-isomer, while the remaining two decay components may correspond to the shorter lived component of Δ-isomer emission combined with both components of emission by the Λ-isomer.[14] The short lifetimes observed for Os(phen)$_2$dppz^{2+} with DNA compared to ruthenium(II) are understandable because of the high spin−orbit coupling constant for osmium[15,16] and allow a probe for DNA on a fast time scale. Indeed Os(phen)$_2$dppz^{2+} may serve as a general probe of water accessibility in picosecond experiments.

The remarkable luminescent characteristics of Ru(phen)$_2$dppz^{2+} and Ru(bpy)$_2$dppz^{2+} with DNA and their utility as diagnostic probes may therefore be extended to include Os(phen)$_2$dppz^{2+}. Important for applications in biosensor technology, these osmium polypyridyl complexes are unique in their capability to probe events occurring on time scales below 10 ns while emitting at wavelengths in the red.

Acknowledgment. We are grateful to the NIH (Grant GM49216) and to the NSF (a predoctoral fellowship to R.E.H.) for their financial support. We also thank J. Winkler, C. Kenyon, and the Beckman Institute Laser Resource Center for expert assistance and facilities.

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(13) A synchronously pumped dye laser containing rhodamine-6G dye (Exciton) provided 590 nm excitation light. Emission from the sample was collected with a fused silica lens at ~*f*/4 and directed through a monochromator typically set at 770 nm, with slits open to 1.75 mm, into a multichannel plate/photomultiplier tube (MCP/PMT) equipped with an S1 photocathode. Data were collected over 20 ns, and 50 ns time windows in reverse mode, with the start pulse generated at the MCP/PMT. Photon-counting apparatus was standard, and data deconvolution was accomplished using a nonlinear least squares fitting routine.

(14) Preliminary measurement of Δ-Os(phen)$_2$dppz^{2+} emission decay in DNA at the DNA:Os ratio of 10:1 reveals a bi-exponential decay with τ_1 = 2.0 ns (55%) and τ_2 = 11 ns (45%).

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(16) Nonetheless it appears that the ratio of emission quantum yields for Os(phen)$_2$dppz^{2+} versus Os(bpy)$_3^{2+}$ is lower than for their ruthenium counterpart.