Molecular "Light Switch" for DNA: Ru(bpy)$_2$(dppz)$_2$$^{2+}$

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Considerable research has focused on the development of nonradioactive probes for nucleic acids.$^1$ On the basis of the well-characterized photophysical properties of ruthenium polypyridyls,$^2$ we earlier developed Ru(phen)$_2^{2+}$ (phen = 1,10-phenanthroline) and its derivatives as spectrophotometric probes for DNA structure.$^{3,4}$ Extensive photophysical studies$^4$ indicate that Ru(bpy)$_2^{2+}$ bound to double-helical DNA displays an increase in luminescence owing to intercalation; emission from the metal-to-ligand charge transfer (MLCT) excited state decays as a biexponential with one lifetime of 2 ns attributed to the intercalative form and a second lifetime of 0.6 ns (indistinguishable from the free species) assigned to the surface-bound form.$^5$ This enhancement in luminescence, coupled to shape- and symmetry-selective binding, has been useful in probing nucleic acid conformations and in the design of new phototherapeutics.$^6,7$ However, the background luminescence of the free form, the relatively weak binding, and the extent of enhancement on binding appeared insufficient for the broader application of the ruthenium complexes as general nonradioactive nucleic acid probes. Here we report the application of a novel transition-metal complex as a true molecular "light switch" for DNA. This probe is Ru(bipy)$_2$(dppz)$_2$$^{2+}$ (bpy = 2,2'-bipyridine, dppz = dipyrido[3,2-a:2',3'-c]phenazine),$^8$ which shows no photoluminescence in aqueous solution at ambient temperatures, but displays intense photoluminescence in the presence of double-helical DNA, to which the complex binds avidly.$^9$

Ru(bpy)$_2$(dppz)$_2$$^{2+}$ appeared to be a prime candidate for application as a spectrophotometric probe for nucleic acids owing to its shape$^{10}$ and the sensitivity of its excited-state properties to environment.$^9$ While the complex does not luminesce in aqueous solution, it shows appreciable solvatochromic luminescence in ethanol ($\lambda_{max} = 482$ nm, $\lambda_{em} = 610$ nm), acetonitrile ($\lambda_{max} = 482$ nm, $\lambda_{em} = 615$ nm) and 2-propanol ($\lambda_{max} = 482$ nm, $\lambda_{em} = 622$ nm) (Table 1). Electrochemical and photophysical measurements of Ru(bpy)$_2$(dppz)$_2$$^{2+}$ in its ground and excited states show that the charge transfer is directed from the metal center to the phenazine ring, and the major nonradiative deactivation pathway for the complex likely involves the protonation of the phenazine nitrogen atoms in the excited state.$^9$ Not surprisingly owing to the extended planar structure of the dppz ligand$^{10}$ and in contrast to Ru(bpy)$_2^{2+}$, Ru(bpy)$_2$(dppz)$_2$$^{2+}$ binds to double-helical DNA by intercalation; topoisomerase assays$^1$ indicate an unwinding angle of 30° ± 1° per ruthenium bound. Given the preferential charge transfer onto the intercalating dppz ligand, the luminescence of the bound complex therefore provides a sensitive reporter of its helical environment.

![Diagram](image.png)

Table 1. Luminescence of Ru(bpy)$_2$(dppz)$_2$$^{2+}$ in the Absence and Presence of Polynucleotides

<table>
<thead>
<tr>
<th>Solvent</th>
<th>DNA$^a$</th>
<th>$\lambda_{em, d}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$r, ns$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>30 mM NaCl/5 mM</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Tris, pH 7.0</td>
<td>2-propanol</td>
<td>1.0</td>
<td>622</td>
<td>210</td>
</tr>
<tr>
<td>methanol</td>
<td>50 mM NaCl/5 mM</td>
<td>0.23</td>
<td>610</td>
<td>30</td>
</tr>
<tr>
<td>Tris, pH 7.0</td>
<td>poly[d(GC)]</td>
<td>0.61</td>
<td>632</td>
<td>75, 259</td>
</tr>
<tr>
<td>Tris, pH 7.0</td>
<td>poly(d(AU))</td>
<td>0.72</td>
<td>628</td>
<td>75, 250</td>
</tr>
<tr>
<td>Tris, pH 7.0</td>
<td>poly[r(AU)]</td>
<td>0.09</td>
<td>650</td>
<td></td>
</tr>
<tr>
<td>Tris, pH 7.0</td>
<td>poly[r(AU)]</td>
<td>0.61</td>
<td>632</td>
<td></td>
</tr>
</tbody>
</table>

*All measurements were conducted at 25 °C with Ru(bpy)$_2$(dppz)$_2$$^{2+}$ at 10 μM. $^a$The concentration of DNA used was 100 μM nucleotides. $^b$The luminescence spectra were measured by using an SLM 8000C spectrofluorimeter with excitation at 482 nm. $^c$Values for the maximum intensity found relative to that found in 2-propanol at 622 nm. $^d$Wavelength with maximum emission (uncorrected for photomultiplier tube response) upon excitation at 482 nm. $^e$Emission lifetimes were determined by deconvolution of biexponential decay traces in single photon counting experiments as described previously.$^2$ $^f$Values given have an estimated uncertainty of 10%.

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(6) More recent $^h$NMR experiments further support these assignments. See: Rehmann, J. P.; Barton, J. K. Biochemistry 1990, 29, 1701, 1710.


(10) The equilibrium binding constant of Ru(bpy)$_2$(dppz)$_2$$^{2+}$ to calf thymus DNA in 50 mM NaCl/5 mM Tris, pH 7.0, at ambient temperatures is $>10^7$ M$^{-1}$ based upon absorption titrations and equilibrium dialysis experiments (A. M. Pyle, data in our laboratory). The complex binds comparably to AT-rich and GC-rich DNA.


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Preparations\textsuperscript{5,12} of Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} (10 \(\mu\)M) in buffered aqueous solutions show no detectable luminescence. In the presence of 100 \(\mu\)M calf thymus DNA, however, luminescence, with emission centered at 632 nm and comparable in intensity to that found in 2-propanol (where \(\Phi \geq 0.02\)),\textsuperscript{13} is apparent (Table I). For comparison, \(\Phi_{\text{Ru(bpy)}}\textsuperscript{2+} = 0.042\) for photoluminescence in aqueous solution,\textsuperscript{2} both in the presence of DNA and in its absence; there is no detectable spectroscopic perturbation for Ru(bpy)\textsuperscript{2+} by DNA under physiological conditions. Single photon counting experiments at 25 \(^\circ\)C reveal a biexponential decay of emission from Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} bound to DNA with a short-lived component of 75 ns and a longer lived component of 259 ns.\textsuperscript{14} Emission from R\textsubscript{u}(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} bound to DNA with a short-lived component; there is an absence; there is an emission centered at 632 nm and comparable in intensity to that found in 2-propanol (where \(\Phi \geq 0.02\)),\textsuperscript{13} with Ru(phen);\textsuperscript{1,4} the anionic solution quencher of the ruthenium complex, Fe(CN)\textsubscript{6}\textsuperscript{3-}, preferentially quenches the short-lived component; we therefore assign the longer lived component as the complex, Fe(CN)\textsubscript{6}C, which is less accessible, tightly intercalated form.\textsuperscript{15}

The extent of enhancement on DNA binding is perhaps best illustrated in steady-state luminescence experiments (Figure 1). In the presence of 100 \(\mu\)M poly[d(GC)-(dG)] (B form), intense emission centered at 628 nm is observed, whereas in the absence of polynucleotide, no emission is detectable. We estimate from our limits of detection the enhancement factor for preserving emission in the dppz complex is greater than 1000.

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Figure 1. Steady-state emission spectra of Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} (10 \(\mu\)M) in the absence and presence of B-form (top), Z-form (middle), and A-form (bottom) double-helical nucleic acids. In each case in the absence of polynucleotide, only the base-line spectrum, the same level of emission as detected with pure solvent, is obtained for the ruthenium complex in aqueous solution.\textsuperscript{16} Similar results are evident with Z-form poly[d(GC)-(dG)]; here the relative intensity is still greater and the emission maximum is shifted to 640 nm.\textsuperscript{16} Interestingly, only weak emission (\(I_{\text{em}} = 650\) nm) is apparent in the presence of poly[r(AU)-r(AU)], an A-form helical polymer. Given that Ru(phen)\textsuperscript{2+} does not appear to intercalate into synthetic double-stranded RNA (there is no long-lived luminescent component in emission, for example),\textsuperscript{4} these results suggest that it may be the tightly intercalated interaction that is primarily responsible for preserving emission in the dppz complex.

These results point also to the selective nature of Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} as a molecular switch, responding sensitively to the subtle changes in the structure of the helix. As can be seen in Table I, variations as a function of DNA substrate are detected not only in the intensity of photoluminescence but also in the emission maximum. With B-form polymers, poly[d(GC)-(dG)] and mixed-sequence calf-thymus DNA show emission maxima at 628 and 632 nm, respectively; with the Z- and A-form polymers, the maximum shifts to still longer wavelengths (640 and 650 nm for the Z- and A-form polymers, respectively). It is not clear whether it is the electronic character of the DNA bases and its overlap with the phenazine ring, the accessibility of the phenazine ring to protonation, or some mixture thereof that is responsible for the novel luminescent properties associated with the interaction with the helix.

In summary, we find Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} to be a highly sensitive spectroscopic reporter of double-helical DNA. In aqueous solution, luminescence is detectable only when Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} has intercalated in (or is perhaps otherwise shielded by) the nucleic acid structure. The emission characteristics furthermore sensitively distinguish both in terms of intensity and emission maximum the different helical forms of the polynucleotide. We therefore conclude that Ru(bpy)\textsubscript{3}(dppz)\textsuperscript{2+} can serve as a true molecular "light switch" for DNA structures, and tethered onto oligonucleotides,\textsuperscript{18} the complex may be useful as a sensitive, non-radiometric probe of DNA conformation and structure.
dioactive, luminescent DNA probe in both heterogeneous and homogeneous assays.

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Synthesis and Characterization of the First Example of a Metallacarborane That Incorporates an Alkaline-Earth Metal: The Molecular Structure of closo-1,1,1,1-(MeCN)4·1,2,4-CaC2B10H12

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The organometalllic chemistry of the alkaline-earth metals has received particular attention in recent years.4-10 In this context, high yield synthesis routes to the cyclopentadienyl4,5 and cyclooctatetraenediyl6 complexes of calcium, strontium, and barium have been developed and the molecular structures of (C5Me5)2Ba4 (C8H21BloN3Ca: C, 31.27; H, 6.84; N, 13.68. We have not been able to

<table>
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<th>Number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Numbers accompanying formulas refer to the positions of the heteroatoms within the closo-metalcarborane framework and to the location of the exohedral substituents. Lowest numbers consistent with the molecular geometry are given to carbon in accordance with the inverse periodic order adhered to by the IUPAC Inorganic Nomenclature Committee (see: Adams, R. M. Pure Appl. Chem. 1972, 30, 683). The numbering system used for the nido-carborane anions described herein is identical with that previously employed (see: Dustin, D. F.; Dunks, G. B.; Hawthorne, M. F. J. Am. Chem. Soc. 1973, 95, 725).</td>
</tr>
</tbody>
</table>

Figure 1. The molecular structure of closo-1,1,1,1-(MeCN)4·1,2,4-CaC2B10H12 (1). All hydrogen atoms have been omitted for clarity. Selected interatomic distances (Å): Ca(1)-N(10), 2.471 (5); Ca(1)-N(20), 2.431 (5); Ca(1)-N(30), 2.476 (5); Ca(1)-N(40), 2.508 (5); Ca(1)-C(12), 2.701 (5); Ca(1)-C(3), 2.879 (6); Ca(1)-C(4), 2.895 (5); Ca(1)-B(5), 2.649 (6); Ca(1)-B(6), 2.828 (6); Ca(1)-B(7), 2.935 (6); Ca(1)-B(8), 2.979 (8); Ca(1)-B(9), 2.789 (7); Ca(2)-B(12), 2.814 (8); Ca(2)-B(13), 1.638 (8); Ca(2)-B(14), 1.701 (7); Ca(2)-B(15), 1.692 (8); Ca(2)-B(16), 1.691 (7); Ca(2)-B(17), 2.637 (9); Ca(3)-B(9), 1.840 (8); Ca(3)-B(10), 2.935 (8); Ca(3)-B(16), 1.739 (7). The complex 1 is extremely air- and moisture-sensitive. The presence of the [nido-7,9-C2B10H12]2- fragment in the complex I is supported by the fact that the known [nido-9,12-C2B10H12]2- (H and 1H NMR, vide infra) is produced upon exposure to moisture/H2O.14-16 Complex 1 serves as a source of [nido-7,9-C2B10H12]2- by its reaction with YbI3(MeCN)2 in MeCN to afford the known17 closo-1,1,1,1-(MeCN)4·1,2,4-YbC2B10H12 in quantitative yield. The molecular structure18 of 1 is illustrated in Figure 1, along with selected interatomic distances. The calcium atom asymmetrically caps the open puckered hexagonal face of the [nido-7,9-C2B10H12]2- ligand. Four acetonitrile ligands are bonded to the calcium atom. The Ca-N bond distances range from 2.43 to 2.51 Å, and calcium-carborane distances fall in the range 2.65-2.94 Å. The Ca(1)-C(2) distance of 2.70 Å compares very well with that reported for the complexes [CaH3,1-