Synthesis and Spectroelectrochemistry of Ir(bpy)(phen)(phi)$^{3+}$, a Tris(heteroleptic) Metallointercalator

Christine Stinner,[†] Matthew D. Wightman,[‡] Shana O. Kelley,[†,§] Michael G. Hill,*[,‡] and Jacqueline K. Barton*[,†]

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, and Department of Chemistry, Occidental College, Los Angeles, California 90041

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A tris(heteroleptic) phenanthrenequinone diimine (phi) complex of Ir(III), Ir(bpy)(phen)(phi)$^{3+}$, was synthesized through the stepwise introduction of three different bidentate ligands, and the Λ- and Δ-enantiomers were resolved and characterized by CD spectroscopy. Like other phi complexes, this tris(heteroleptic) iridium complex binds avidly to DNA by intercalation. Electrochemical studies show that Ir(bpy)(phen)(phi)$^{3+}$ undergoes a reversible one-electron reduction at $E_0 = -0.025$ V in 0.1 M TBAH/DMF (versus Ag/AgCl), and spectroelectrochemical studies indicate that this reduction is centered on the phi ligand. The EPR spectrum of electrochemically generated Ir(bpy)(phen)(phi)$^{2+}$ is consistent with a phi-based radical. The electrochemistry of Ir(bpy)(phen)(phi)$^{3+}$ was also probed at a DNA-modified electrode, where a DNA binding affinity of $K = 1.1 \times 10^6$ M^{-1} was measured. In contrast to Ir(bpy)(phen)(phi)$^{3+}$ free in solution, the complex bound to DNA undergoes a concerted two-electron reduction, to form a diradical species. On the basis of UV−visible and EPR spectroscopies, it is found that disproportionation of electrochemically generated Ir(bpy)(phen)(phi)$^{2+}$ occurs upon DNA binding. These results underscore the rich redox chemistry associated with metallointercalators bound to DNA.

Introduction

Metallointercalators have been used extensively to probe the structural and electronic properties of DNA.[1] 9,10-Phenanthrenequinone diimine (phi) complexes of Rh(III) bind tightly to DNA via intercalation and serve as scaffolds for the molecular recognition of DNA functionalities.[2] Recently the crystal structure of a phi complex of Rh(III) bound site-specifically to a DNA octamer was determined at high resolution.[3] In this structure, the phi ligand is found to be deeply intercalated within the B-form DNA base pair stack. Owing in part to this stacking within the DNA helix, intercalated phi complexes of rhodium(III) also function efficiently as electron acceptors and photooxidants in long-range electron-transfer reactions mediated by the π-stack of DNA.[4,5]

Despite their rich photochemistry, phi complexes of rhodium have limited utility as electrochemical probes of DNA owing to irreversible reductions on the (much longer) electrochemical time scale. We have been interested in applying electrochemistry to probe both intercalation and electron transport in DNA.[6−8] Electrochemical measurements using DNA-modified electrodes have been found to be useful in probing metal/nucleic acid interactions.[9] In an effort to characterize more fully the reduced forms of intercalated phi complexes and more generally to probe redox reactions of metallointercalators electrochemically, we have prepared a tris(heteroleptic) iridium(III) analogue. As the reduction potential of Ir(III) is expected to be negative compared to the corresponding Rh(III) couple, we sought to generate a clean phi-radical anion complex without interference from Ir(II).

Here we report the synthesis and characterization of the three-ligand α-diimine iridium complex Ir(phi)(bpy)(phen)$^{3+}$. This

* To whom correspondence should be addressed.
[†] California Institute of Technology.
[‡] Occidental College.
[§] Present Address: Department of Chemistry, Boston College, Chestnut Hill, MA 02467.

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species can be resolved into its Δ- and Λ-isomers and provides a convenient electrochemical probe of electron transfer through the double helix. Interestingly, the electrochemistry and UV−vis/EPR spectroelectrochemistry of Ir(phi)(bpy)(phen)$^{3+}$ reveal a reversible one-electron, phi-centered reduction in aqueous solution, but a two-electron process to give a phi-Ir(II) diradical when intercalated into DNA. These results underscore the dramatic changes in electronic environment that occur upon intercalation and highlight the need for molecular methods that assay reporter molecules bound directly to the double helix.

Experimental Section

Materials. [Ir(phen)Cl_4]H and [and K_3[Co(L-cysu)$_3$] (cysu = cysteinesulfinato(2-)S,N] were synthesized as described previously.[10,11] 2,2′-Bipyridine, 9,10-phenanthrene quinone, 1,10-phenanthroline, trifluormethylsulfonic acid (98%) (tfl), and Sephadex cation and anion-exchange resins were obtained from Aldrich. K_3IrCL_6 was purchased from Johnson & Matthey.

Instrumentation. ^{1}H NMR spectra were recorded on a General Electric QE Plus 300 MHz instrument and ^{13}C NMR spectra on an AM Bruker 500 MHz instrument using DMSO-d_6 (2.49 ppm) as an internal standard. Ultraviolet−visible (UV−vis) absorption spectra were obtained on an HP 8452 UV−vis spectrophotometer. Mass spectral data were collected at the facilities of University of California, Riverside (FAB and electrospray), and the Macromolecular Resources Center of Colorado State University, Department of Biochemistry, Fort Collins. Mass spectra were recorded on a Finnigan LCQ electrospray mass spectrometer. CD spectra were recorded on a Jasco J 500A spectropolarimeter. Reverse phase HPLC was carried out with an HP 1050 system on a C_{18} column. EPR spectra were recorded on a Bruker ESP 300 spectrometer.

Electrochemical experiments were performed using a Bioanalytical System (BAS) Model CV 50 W electrochemical analyzer. Cyclic voltammetry (CV) was carried out at ambient temperature with a normal three-electrode configuration consisting of a modified gold disk working electrode, a Pt wire auxiliary electrode, and either a saturated calomel reference electrode (SCE, Fisher Scientific) or a AgCl/Ag reference electrode containing 1.0 M KCl. The working compartment of the electrochemical cell was separated from the reference compartment by a modified Luggin capillary. Potentials are reported versus Ag/AgCl or SCE as indicated and are not corrected for the junction potential. Bulk electrolyses were carried out at a carbon-mesh electrode in the same cell maintained at 0° C.

UV−visible spectroelectrochemistry was carried out in an optically transparent thin-layer cell, consisting of vapor-deposited platinum working and pseudoreference electrodes and a Pt-wire auxiliary electrode.[12] Heterogeneous electron-transfer rates were determined by cyclic voltammetry and analyzed as described in the literature.[13] The preparation of the derivatized duplexes and the modification of the electrodes were carried out according to published procedures.[6]

Synthesis and Characterization. *cis*-[Ir(bpy)(phen)Cl_2]Cl was prepared as described in the literature[14] with several modifications. A 0.5 g (1 mM) sample of [Ir(phen)Cl_4]H (0.001 mol, 1 equiv) and 0.16 g (1 equiv) of 2,2′-bipyridine were mixed in 25 mL of glycerol and were heated to reflux (>200 °C) for 15 min. The red solution became yellow as the boiling point was reached. After refluxing for 15 min, the reaction mixture was immediatly cooled in ice, and 10 mL of water was added. The solution was loaded on a cation exchange column (Sephadex CM-C25, H^+-form) and washed with water to remove the glycerol. The product was eluted with a 1:1 mixture of 0.1 N HCl/acetonitrile and recrystallized from 10 mL of 0.1 N HCl at +4 °C. Yield: 0.44 g (70%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.35 (t, 1H, 6.7 Hz), 7.70 (d, 1H, 6.0 Hz), 7.85 (dd, 1H, 8.3 and 5.5 Hz), 8.21 (d, 2H, 5.0 Hz), 8.16 (d, 1H, 7.8 Hz), 8.38−8.60 (m, 4H), 8.83 (dd, 2H, 8.0 and 2.1 Hz), 8.97 (d, 1H, 8.0 Hz), 9.14 (d, 1H, 8.2 Hz), 9.73 (d, 1H, 5.7 Hz), 9.86 (d, 1H, 5.4 Hz). MS (FAB): 599 (75%, $M^+ - H$), 564 (25%, $M^+ - H - Cl$). UV−vis [water, nm (ϵ, mM^{-1} cm^{-1})]: pH 7, 226 (36.5), 274 (39.4), 304 (18.4), 316 (16.9), 358 (3.31), isobestic points 374 (2.98), 358 (3.31).

***rac*-[Ir(bpy)(phen)(phi)]tfl_3.** A 135 mg (0.21 mmol, 1 equiv) sample of [Ir(bpy)(phen)Cl_2]Cl was stirred in 10 g of trifluoromethylsulfonic acid (98%) for 8 h at 70 °C. The reaction mixture was cooled to ambient temperature and added dropwise to 250 mL of cold diethyl ether. The beige precipitate of [Ir(bpy)(phen)tfl_2]tfl was separated by centrifugation and was suspended in a concentrated ammonia solution. After refluxing the reaction mixture for 10 h, the solvent was removed under vacuum. The crude yellow reaction product was treated with 0.38 mg (1 equiv) (0.21 mM) of 9,10-phenanthrene quinone without further purification and was stirred in a mixture of 5 mL of 0.4 M NaOH and 15 mL of acetonitrile for 20 h at room temperature. The yellow solution turned red-brown, indicating the formation of a phi complex. After neutralization with 10 mL of 0.1 M hydrochloric acid, the reaction mixture was loaded on a cation exchange column (Sephadex SP-C25, H^+-form) and the red-brown product was eluted with 0.5 M HCl. The resulting solution was evaporated and dried under vacuum. Yield: 50 mg (0.042 mM, 20% referred to [Ir(bpy)(phen)Cl_2]Cl. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.52 (t, 2H, 6.5 Hz), 7.65 (t, 2H, 6.9 Hz), 7.80 (m, 2H, 7.7 Hz), 7.95 (t, 1H, 6.5 Hz), 8.02 (dd, 1H, 8.3 and 5.5 Hz), 8.13 (d, 1H, 5.5 Hz), 8.24 (dd, 1H, 5.5 and 8.2 Hz), 8.32 (dd, 1H, 7.8 Hz), 8.45−8.58 (m, 6H), 8.70 (dd, 2H, 9.2 Hz), 8.92 (d, 1H, 8.0 Hz), 8.95−9.08 (m, 3H), 9.15 (d, 1H, 8.1 Hz), 14.80 and 15.05 (s, 2H, 2 =NH). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 124.5 (2 C), 125.2, 125.3, 127.2, 127.7, 128.1, 128.6, 128.9 (2 C), 129.7, 129.9, 130.0, 131.1, 134.0 (2 C), 136.1 (2 C), 140.6, 141.2, 141.5, 141.9, 142.0, 142.1, 142.3, 143.0 (2 C), 144.5, 145.4, 146.8, 150.8, 151.8, 153.7, 154.5, 156.2, 156.7, 175.3, 175.6. MS (ESI): m/z ($M - 2H^+$) 734 amu. UV−vis [water, nm (ϵ, mM^{-1} cm^{-1})]: pH 7, 226 (51.0), 272 (38.4), 304 (21.9), 396 (9.92).

Enantiomer Separation. The enantiomers of [Ir(bpy)(phen)(phi)]-tfl_3 were separated on a Sephadex CM-C25 column by elution with a 0.7 mM K_3[Co(L-cysu)$_3$] solution in water. After the Λ-enantiomer (which is eluted first) was visibly separated from the Δ-enantiomer, the column was washed with deionized water and carefully emptied onto a flat surface. The colored portions of the column were divided, and the complex was eluted from the resin with 0.1 N HCl/acetonitrile (1:1) and evaporated to dryness. Δ-[Ir(bpy)(phen)(phi)]tfl_3, CD [water, 5×10^{-5} M, nm (ϵ)]: 271 (110), 298 (−156), 326 (−211) 353 (1). Δ-[Ir(bpy)(phen)(phi)]tfl_3, CD [water, 5×10^{-5} M, nm ($\Delta\epsilon$)]: 271 (156), 326 (211), 353 (−1).

Results and Discussion

Synthesis and Characterization. The complexes Ir(phen)$_2$-phi^{3+} and Ir(phi)$_2$phen^{3+} were synthesized by the direct coordination of phi to Ir(III)(phen)$_x$ starting materials, using methodologies developed for the analogous phi complexes of rhodium(III).[15] Here, we employed a new route that features condensation of 9,10-phenanthrenequinone with *cis*-bisammine compounds[16] to form the coordinated phi ligand in a templated synthesis. Ir(bpy)(phen)(phi)$^{3+}$ was synthesized from a (bis)-heteroleptic iridium complex containing bpy and phen ligands in a *cis*-configuration (Scheme 1). The (bis)chloride complex is converted into the more reactive trifluoromethylsulfonate (triflate) compound, which, in turn, yields the corresponding *cis*-bisammine species. As iridium coordination complexes

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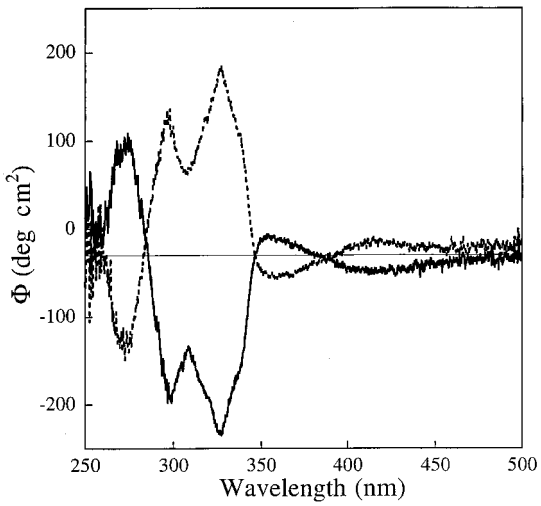

Figure 1. CD spectra of Λ- (dashed line) and Δ-Ir(bpy)(phen)(phi)$^{3+}$ (50 μM) in water.

Scheme 1

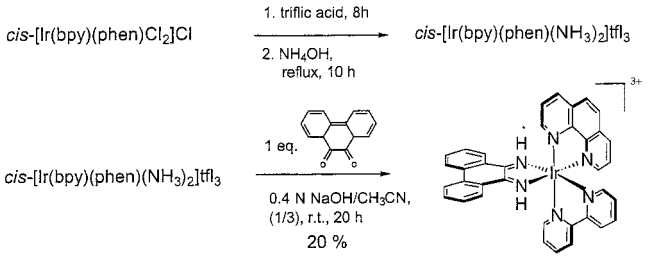

exhibit a lower reactivity than rhodium compounds, it is essential for the synthesis of the bisammine precursor that triflate be introduced as a leaving group. The *cis*-bisammine iridium complex reacts further in a condensation reaction with 1 equiv of 9,10-phenanthrene quinone to form the red Ir(bpy)(phen)(phi)$^{3+}$ product. The reaction is carried out at ambient temperature in a basic solution of acetonitrile/0.4 N NaOH (ratio 1:3). After cation exchange chromatography, the trisheteroleptic complex is >99% pure as verifed by HPLC. The Λ- and Δ-enantiomers were resolved on a cation exchange column using a chiral solvent system containing K_3[Co(L-cysu)$_3$] (cysu = cysteinesulfinato(2−)S,N].[10] The two enantiomers were characterized by CD spectroscopy (Figure 1).

UV−visible absorption spectroscopy reveals that racemic Ir(bpy)(phen)(phi)$^{3+}$ binds avidly to double-stranded DNA. Pronounced hypochromism in the phi-centered $\pi-\pi^*$ transition (with an isobestic point at 425 nm) is observed with the addition of DNA to solutions of the iridum complex (Figure S1). The percentage of hypochromism plotted against the nucleotide−iridium (R_{Nuc}:R_{Ir}) ratio increases linearly until reaching a saturation at 35% with $R_{nuc:Ir}$ = 25. At saturation, the phi-centered absorption is red shifted by 10 nm. This hypochromic shift of the $\pi-\pi^*$ transition suggests preferential intercalative stacking of the phi ligand into DNA, consistent with studies using the analogous rhodium(III) complexes.[1,2]

Electrochemistry. The cyclic voltammogram (CV) of Ir(bpy)(phen)(phi)$^{3+}$ displays a reversible one-electron reduction with $E^{\circ\prime} = -0.025$ V in 0.1 M TBAH/DMF (versus AgCl/Ag) (Figure 2). This potential is very similar to that of the analogous Rh(III) complex, implying that the reduction is phi-centered. The electrochemistry in aqueous solution (pH 7) shows the same reversible one-electron reduction, as well as a second irreversible feature at slightly more negative potentials. This second process

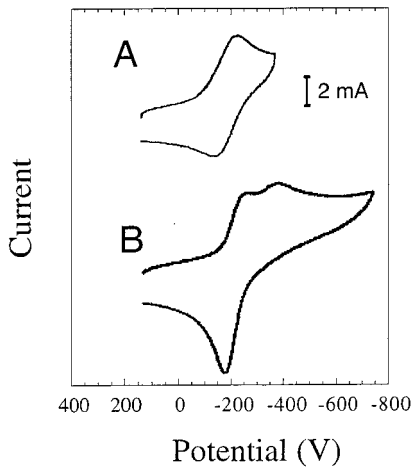

Figure 2. (A) Cyclic voltammogram of 1 mM Ir(bpy)(phen)(phi)$^{3+}$ in 0.1 M TBAH/DMF, scan rate = 100 mV/s. Potentials are reported versus AgCl/Ag. (B) Cyclic voltammogram of 1 mM Ir(bpy)(phen)(phi)$^{3+}$ in aqueous solution (100 mM sodium phosphate, pH 7).

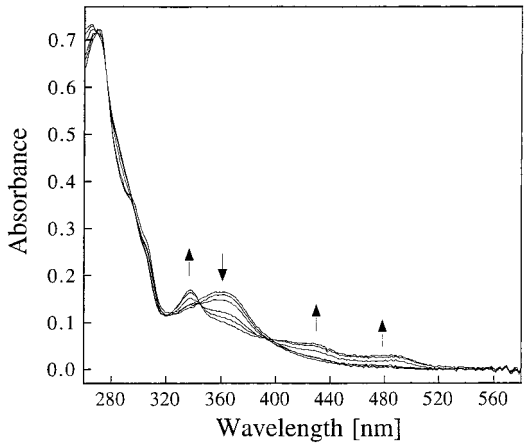

Figure 3. Spectroelectrochemical reduction of 0.1 mM Ir(bpy)(phen)(phi)$^{3+}$ in 0.1 M TBAH/DMF. Data were recorded during a slow linear potential sweep (scan rate = 5 mV/s).

is chemically reversible (as demonstrated by sequential bulk reduction and reoxidation by two electrons to regenerate Ir(bpy)(phen)(phi)$^{3+}$) and is somewhat pH sensitive; at pH >8, the potential of the second process is shifted sufficiently positive that a single, two-electron reduction occurs. UV−visible spectroscopy (Figure S2) also reveals that the electronic spectrum of Ir(bpy)(phen)(phi)$^{3+}$ is sensitive to pH and solvent, presumably due to protonation/deprotonation of the phi nitrogens (pK_a(complex) ≈ 7.5), suggesting that proton-transfer reactions may be the cause of the quasi-reversible response. This pH sensitivity has been seen extensively with phi complexes of Rh(III).[2,16] Regardless of pH, however, oxidation of the doubly reduced Ir(bpy)(phen)(phi)$^{+}$ species occurs in a single two-electron step.

Spectroelectrochemical studies were carried out to characterize the reduced complex. The UV−visible spectral changes that occur upon reduction of Ir(bpy)(phen)(phi)$^{3+}$ in 0.1 M TBAH/DMF are shown in Figure 3. As the $\pi-\pi^*$ band at 360 nm disappears, new transitions at 335, 430, and 485 nm due to Ir(bpy)(phen)(phi)$^{2+}$ grow in. Oxidation of this solution regenerates quantitatively the Ir(III) starting material, indicating that the reduction is reversible on the spectroelectrochemical time scale. Indeed, unlike the corresponding Rh(III) complex, the reduced iridium species is stable at ambient temperature in nonaqueous solvents for minutes under an anaerobic atmosphere.

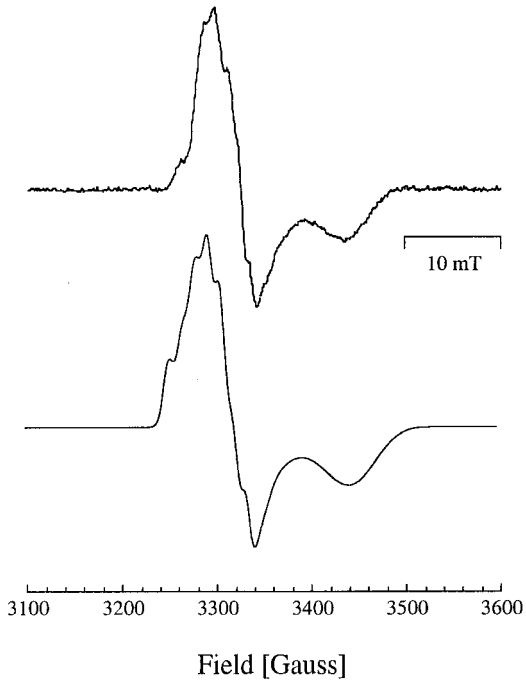

Figure 4. X-band EPR spectrum of 0.1 mM Ir(bpy)(phen)(phi)$^{2+}$ in 0.1 M TBAH/CH_3CN at 77 K (top). The spectrum was simulated (bottom) with $g_1 = 2.019$, $g_2 = 1.994$, $g_3 = 1.926$, $|A_1|$ (^{14}N) $= 10 \times 10^{-4}$ cm^{-1}, and $|A_2|$ (^{14}N) $= 15 \times 10^{-4}$ cm^{-1}.

Analogous spectral changes are observed during the spectroelectrochemical reduction in water (pH 7), with the exception that the one-electron reduced radical is thermally unstable on time scales greater than several minutes, disproportionating to yield a solution containing Ir(bpy)(phen)(phi)$^{3+}$ and (presumably) Ir(bpy)(phen)(phi)$^{+}$.[17]

EPR Spectroscopy. The ambient-temperature X-band EPR spectrum of Ir(bpy)(phen)(phi)$^{2+}$ in acetonitrile displays an isotropic signal at $g = 1.978$, characteristic of an organic-centered radical.[18] The frozen-solution spectrum (77 K, Figure 4) is rhombic, with $g_1 = 2.019$, $g_2 = 1.994$, and $g_3 = 1.926$.[18] Computer simulations indicate hyperfine coupling to two nonequivalent nitrogens, with $|A_1|$ (^{14}N) $= 10 \times 10^{-4}$ cm^{-1} and $|A_2|$ (^{14}N) $= 15 \times 10^{-4}$ cm^{-1}.[19] Although the isotropic g value is slightly lower than g_e, the small g_1 and g_2 values of the anisotropic spectrum, along with no resolvable iridium hyperfine, suggest only a very small degree of spin delocalization from the phi ligand to the metal.[20] Rapid bulk electrolysis of Ir(bpy)(phen)(phi)$^{3+}$ by one electron in aqueous solution (0° C) yields a solution with essentially the same EPR features. In fluid solution, the EPR signal disappears over time, consistent with the slow disproportionation of Ir(bpy)(phen)(phi)$^{2+}$ to yield two spin-paired species. Neither this solution nor one containing the doubly reduced iridium complex produced by bulk electrolysis is EPR active at temperatures as low as 77 K.

(17) A two-electron spectroelectrochemical reduction of Ir(bpy)(phen)(phi)$^{3+}$ in aqueous solution was carried out to characterize spectroscopically the Ir(bpy)(phen)(phi)$^{+}$ species. The resulting solution exhibited spectral properties essentially unchanged from the Ir(bpy)(phen)(phi)$^{2+}$ radical.

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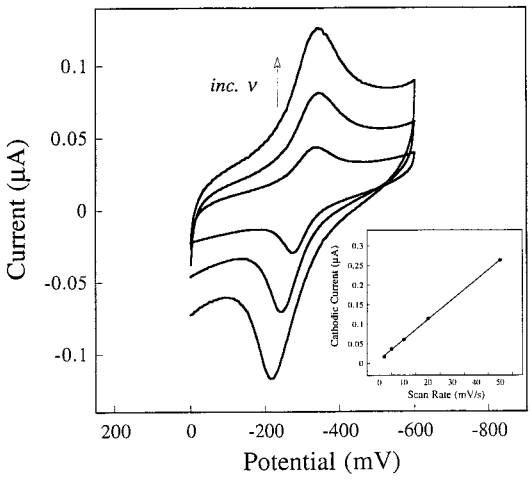

Figure 5. Cyclic voltammetry of 1 μM *rac*-Ir(bpy)(phen)(phi)$^{3+}$ in 100 mM sodium phosphate, pH 7, at a DNA-modified electrode (SH-5′ AGTACAGTTATCGCG 3′ and complement) at scan rates of 5, 10, and 20 mV s^{-1}. Inset: Plot of i_{pc}vs scan rate.

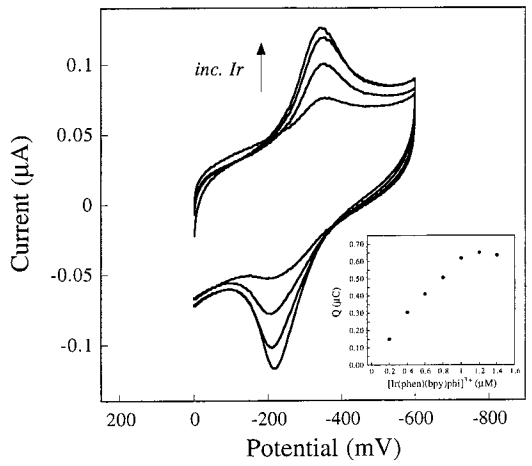

Figure 6. Cyclic voltammetry of *rac*-Ir(bpy)(phen)(phi)$^{3+}$ (100 mM sodium phosphate, pH 7) at a gold electrode modified with DNA (SH-5′ AGTACAGTTATCGCG 3′ and complement). Inset: Plot of charge vs [Ir], where [Ir] is the bulk iridium concentration in solution.

Cyclic Voltammetry at a DNA-Modified Electrode. To assay the redox properties of a metal complex intercalated within the double helix, the electrochemistry of Ir(bpy)(phen)(phi)$^{3+}$ was investigated at a DNA-modified electrode. Using gold electrodes derivatized with 15-base pair DNA duplexes containing a hexylthiol linker (sequence = 5′SH-$(CH_2)_6$-p-AGTACAGTTATCGCG3′),[6] Ir(bpy)(phen)(phi)$^{3+}$ exhibits a chemically reversible reduction at −250 mV versus SCE (Figure 5). This signal appears at sub-micromolar concentrations and exhibits features characteristic of a surface-bound species (e.g., linear plots of cathodic peak current versus scan rate).[21] No signal is observed under these same conditions at an unmodified gold electrode, indicating that the iridium complex is bound to the DNA. Similar results have been obtained using both organic intercalators and electrostatically bound metal complexes.[7]

The affinity of Ir(bpy)(phen)(phi)$^{3+}$ for the DNA-modified surface was determined using cyclic voltammetry. Figure 6 shows the cyclic voltammetry of Ir(bpy)(phen)(phi)$^{3+}$ recorded during a coulometric titration at the modified electrode. This complex binds reversibly, as immersion of an iridium-saturated

(21) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley and Sons: New York, 1980.

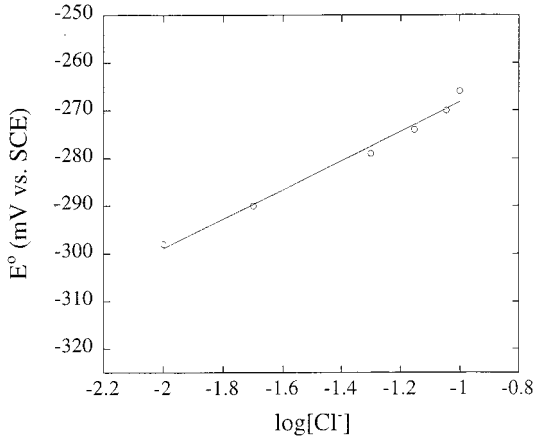

Figure 7. Plot of $E^{\circ\prime}$ for 5 μM Ir(bpy)(phen)(phi)$^{3+/+}$ vs log[Cl$^-$] at a DNA-modified electrode. Aliquots of a saturated KCl solution were added to a solution containing 2 mM sodium phosphate buffer, pH 7.

surface in pure buffer results in the slow dissociation of the complex. The data can be fit to a Langmuir isotherm[19] to give an association constant, K, of 1.1×10^6 M^{-1}. This value is within an order of magnitude of that for binding of Rh(phen)$_2$(phi)$^{3+}$ ($K = 1 \times 10^7$ M^{-1}) to calf thymus DNA in solution;[2] substitution of phen for the less hydrophobic bpy in Ir(bpy)(phen)(phi)$^{3+}$ may account for the slightly lower binding constant.

Interestingly, intercalated into DNA, Ir(bpy)(phen)(phi)$^{3+}$ undergoes a single two-electron reduction at pH 7, as opposed to the sequential one-electron processes found in organic and aqueous solutions. To confirm the two-electron nature of the reduction, the electrochemistry of Ir(bpy)(phen)(phi)$^{3+}$ was investigated as a function of ionic strength at the DNA-modified surface. Owing to the polyanionic phosphate backbone, the individual DNA helices exclude anions from the electrode surface. Because the DNA monolayers effectively act as cation exchange films, a change in the ionic strength of the electrolyte solution (aqueous KCl in our case) should change the ionic work potential at the electrode surface, according to eq 1 (where n is the number of electrons transferred).[22−24]

$$E = E^\circ + 0.059/n \log[\mathrm{Cl}^-] \qquad (1)$$

Indeed, a plot of $E^{\circ\prime}$ versus log[Cl$^-$] for Ir(bpy)(phen)(phi)$^{3+}$ yields a straight line (Figure 7) with slope of 30 mV/log[Cl$^-$], consistent with a two-electron reduction. Notably, with this two-electron value, and assuming a 1:1 iridium:duplex binding stoichiometry, coulometric measurements yield a DNA surface coverage (Γ) of 55 pmol duplex/cm^2. A 1:1 intercalator:DNA stoichiometry has been previously found for organic probe molecules, and the calculated surface coverage is essentially the same as that measured independently via radioactive labeling experiments.[6]

EPR of Ir(bpy)(phen)(phi)$^{2+/+}$ Intercalated into DNA. Samples of Ir(bpy)(phen)(phi)$^{2+}$ intercalated into DNA were prepared by bulk electrolysis of 0.1 mM Ir(bpy)(phen)(phi)$^{3+}$ in tris buffer, pH 7 (0 °C), followed by addition of calf thymus DNA such that the nucleotide-to-iridium ([Nu]/[Ir]) ratio was

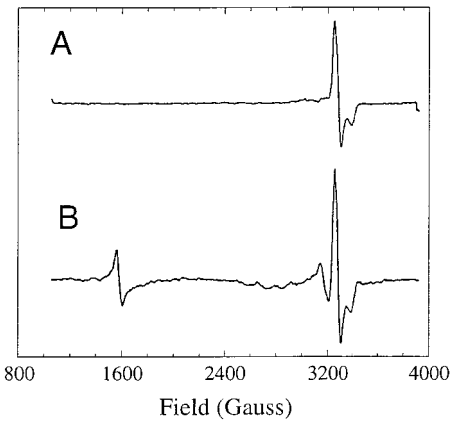

Figure 8. Frozen-solution (77 K) spectrum of electrochemically reduced Ir(bpy)(phen)(phi)$^{3+}$ (0.1 mM Tris HCl, pH 7) before (A) and after (B) addition of calf thymus DNA ([Nu]/[Ir] = 10).

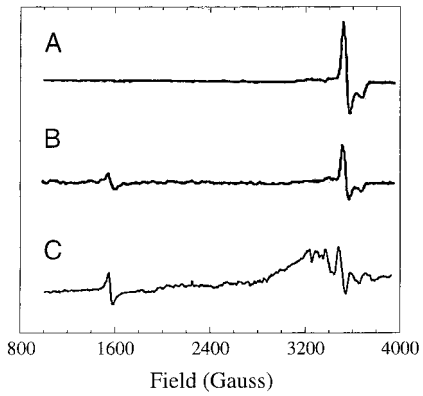

Figure 9. Frozen-solution (77 K) spectra of electrochemically generated Ir(bpy)(phen)(phi)$^{2+}$ (0.1 mM) in the presence of calf thymus DNA ([Nu]/[Ir] = 10). Samples were frozen immediately (A) and 2 min (B) and 5 min (C) after addition of the DNA.

10. Once loaded into degassed EPR tubes, the samples were frozen in liquid nitrogen, and spectra were recorded. The frozen solution spectrum of DNA-bound Ir(bpy)(phen)(phi)$^{2+}$ recorded immediately after the addition of calf thymus DNA exhibits a signal very similar to the one obtained with no added DNA, plus a low field resonance and additional features near $g = 2$ (Figure 8). These additional features are characteristic of a diradical species. Notably, the UV−vis spectrum of Ir(bpy)(phen)(phi)$^{2+}$ shows a characteristic bathochromic shift upon addition of calf thymus DNA; semiquantitative titrations of Ir(bpy)(phen)(phi)$^{2+}$ with DNA yield a dissociation constant approximately equal to that of Ir(bpy)(phen)(phi)$^{3+}$ and certainly well below 0.1 mM.

We considered the possibility that the low-field signal originated from DNA-mediated long-range coupling of multiple spin one-half radicals within the double helix. We therefore carried out the same EPR experiment at a series of [Nu]/[Ir] ratios ranging from 5 to 20. Within this model (and assuming approximately random binding of the iridium complex to DNA), the intensity of the half-field signal ought to decrease as the average distance between the bound radicals is increased. No such dependence was observed. Instead, at all [Nu]/[Ir] ratios investigated, the low-field signal became more pronounced as the time delay was increased between adding the DNA to the Ir(bpy)(phen)(phi)$^{2+}$ and freezing the sample. This effect is illustrated in Figure 9, where the sample was frozen immediately or 2 min and 5 min after mixing calf thymus DNA ([Nu]/[Ir] = 10) with 0.1 mM Ir(bpy)(phen)(phi)$^{2+}$.

(22) (a) Shi, C. N.; Anson, F. C. *J. Phys. Chem. B* **1999**, *103*, 6283. (b) Shi, C. N.; Anson, F. C. *J. Phys. Chem. B* **1998**, *102*, 9850−9854.
(23) Delville, M. H.; Tsionsky, M.; Bard, A. J. *Langmuir* **1998**, *14*, 2774−2779.
(24) Shafer, H. O.; Derback, T. L.; Koval, C. A. *J. Phys. Chem. B* **2000**, *104*, 1025−1029.

These results imply a DNA-induced chemical reaction that results in the observed spin-coupled EPR signal. Given the two-electron electrochemical response of Ir(bpy)(phen)(phi)$^{3+}$ at the DNA-modified surface, one plausible explanation is disproportionation of Ir(bpy)(phen)(phi)$^{2+}$ upon intercalating into DNA to yield Ir(bpy)(phen)(phi)$^{3+}$ plus a single diradical species, Ir(bpy)(phen)(phi)$^{+}$. Several observations point to this conclusion. Even though bulk reduction of Ir(bpy)(phen)(phi)$^{3+}$ by two electrons results in an EPR-silent species in aqueous solution, addition of calf thymus DNA yields instantaneously a spectrum virtually identical to that shown in Figure 9c. Indeed, spin-counting experiments indicate roughly twice the number of unpaired electrons for a calf thymus DNA/iridium mixture prepared by reducing 0.1 mM Ir(bpy)(phen)(phi)$^{3+}$ by two (versus one) electrons.

UV−visible spectroscopic measurements also support disproportionation. Addition of calf thymus DNA to electrochemically generated Ir(bpy)(phen)(phi)$^{2+}$ results in the rapid disappearance of Ir(bpy)(phen)(phi)$^{2+}$ and the appearance of characteristic bands due to Ir(bpy)(phen)(phi)$^{3+}$ at half their original intensity.

It is noteworthy in this context that disproportionation of an intercalated radical in DNA has been documented previously.[25] A pulse radiolysis study of the one-electron reduction of daunorubicin in DNA showed the disproportionation of the daunorubicin semiquinone. This facile disproportionation when bound to DNA was attributed to electron migration within the DNA duplex. It is interesting that in both cases such disproportionation was observed with an intercalator, which is known to be well coupled into the base pair stack.[26] Whether long-range charge transport through the DNA helix can best account for these results, however, needs still to be determined.

Conclusions. The condensation of 9,10-phenanthrene quinone with a *cis*-configurated bisammine precursor provides a very straightforward method for the synthesis of a tris(heteroleptic) diimmine complex of iridium. The electrochemistry of Ir(bpy)(phen)(phi)$^{3+}$ reveals a reversible first reduction that is phi ligand-centered, confirmed by EPR spectroscopy. Probed at a DNA-modified electrode, the iridium(III) metallointercalator exhibits efficient electron transport. Interestingly, upon intercalation into DNA, the phi-radical species becomes unstable with respect to disproportionation; whether this disproportionation involves long-range electron exchange through the double helix or some other process remains unclear. Nevertheless, the profound changes in the electrochemical response of Ir(bpy)(phen)(phi)$^{3+}$ that occur upon intercalation highlight the significant differences in electronic environment between the interior of the double helix and fluid solution, and the rich redox chemistry on DNA that may arise with metallointercalators.

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Supporting Information Available: Figures depicting the electronic spectrum of Ir(bpy)(phen)(phi)$^{3+}$ as a function of DNA binding and pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Houleelevin C.; Gardesalbert M.; Rouscilles, A.; Ferradinin, C.; Hickel, B. *Biochemistry* **1991**, *30*, 8216−8222.

(26) Electrostatic association of an oxoruthenium(IV) cleavage reagent to DNA has also been found to induce a disproportionation reaction of the bound metal complex. See: Welch, T. W.; Ciftan, S. A.; White, P. S.; Thorp, H. H. *Inorg. Chem.* **1997**, *36*, 4812.