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Electrochemical Studies of the Interaction of Metal Chelates with DNA. 4. Voltammetric and Electrogenerated Chemiluminescent Studies of the Interaction of Tris(2,2′-bipyridine)osmium(II) with DNA

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Cyclic voltammetric and electrogenerated chemiluminescent data were used to study the binding of tris(2,2′-bipyridine)-osmium(II), $Os(bpy)_3^{2+}$, an electrostatic binder, to calf thymus DNA. The oxidized form of the osmium complex, $Os(bpy)_3^{3+}$, has a stronger association to DNA than the reduced form, $Os(bpy)_3^{2+}$, as indicated by the negative shift of $E^{\circ\prime}$ of the CV waves ($K_{3+}/K_{2+} = 3.35$). The calculated binding constant, K_{2+}, and binding site size, s, for the $Os(bpy)_3^{2+}$–DNA system depended slightly on whether a mobile or a static equilibrium was assumed. In 10 mM NaCl, 10 mM Tris pH 7, $K_{2+} = (7.3 \pm 0.4) \times 10^3$ M^{-1} and $s = 3$ base pairs (mobile) and $K_{2+} = (5.0 \pm 0.2) \times 10^3$ M^{-1} and $s = 3$ base pairs (static). Electrogenerated chemiluminescence (ECL) was produced upon oxidation of $Os(bpy)_3^{2+}$ at a Pt electrode in a solution containing 10 mM $C_2O_4^{2-}$ and 10 mM phosphate at pH 5. Addition of DNA caused a decrease in the emission intensity (I); a plot of I vs relative DNA concentration yielded $K_{2+} = (6.5 \pm 0.5) \times 10^3$ M^{-1} and $s = 3$ base pairs. The osmium complex produced ECL when bound to the DNA molecule with an efficiency of 30% that of the unbound chelate.

INTRODUCTION

We report studies of the interaction of $Os(bpy)_3^{2+}$ and calf thymus DNA using voltammetric and electrogenerated chemiluminescent data. The application of electrochemistry and its use in characterizing metal chelate–DNA interactions have been described in previous publications (*1–4*). The interest for these types of systems is based on the use of metal complexes as probes for DNA structure (*5–7*) and as cleaving agents for DNA sequence determination (*8–11*). They can also be used as footprinting reagents to study drug–DNA interactions (*12*) and as potential antitumor agents (*13–15*).

Electrogenerated chemiluminescence (ECL) refers to the phenomenon where luminescence is produced in a redox reaction of electrogenerated reactants. ECL investigations have been based on radicals of aromatic compounds and on Ru(II) complexes (*16–22*). ECL has also been used to study the interaction of $Os(bpy)_3^{2+}$ with several surfactants in the presence of oxalate (*23*). Electrochemical oxidation of $Os(bpy)_3^{2+}$ in the presence of oxalate produces the excited state, $Os(bpy)_3^{2+*}$, which emits light. In a previous study, ECL measurements were applied to study the binding of $Ru(phen)_3^{2+}$ to DNA (*4*). In this study the addition of DNA decreased the ECL intensity, I, and a study of I as a function of relative DNA concentration allowed estimation of binding constant and binding site size. A flow-injection ECL system was employed and this required the use of surfactants (Triton X-100 and Tween-20) to prevent bubble formation in the ECL cell. Their presence, however, affected the binding of the metal complex with DNA and also complicated the analysis of the results.

In this paper we investigate the $Os(bpy)_3^{2+}$–DNA system in surfactant-free solutions by both voltammetry and ECL. $Os(bpy)_3^{2+}$ is structurally similar to the tris(bipyridine) complexes of Ru(II), Co(III), and Fe(II) and presumably binds to DNA mainly by electrostatic attraction, although the possibility of partial intercalation of one of the bipyridyl ligands cannot be totally dismissed (*24–26*). We have chosen a complex that primarily binds to the exterior of DNA to compare its ECL behavior with that of $Ru(phen)_3^{2+}$, which is a partial intercalator. As opposed to the Ru complex, which did not produce appreciable ECL when intercalated between the DNA bases, the Os complex externally bound to DNA did produce ECL, although with a diminished efficiency compared to the unbound species.

EXPERIMENTAL SECTION

Materials. Tris(2,2′-bipyridyl)osmium(II) was synthesized according to previously reported procedures (*27*). Stock solutions were prepared with 10 mM NaCl, 10 mM Tris pH 7 (buffer 1) or 10 mM $Na_2C_2O_4$, 10 mM phosphate pH 5 (buffer 2). Calf thymus (CT) DNA was purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. DNA concentration per nucleotide phosphate, NP, was determined spectrophotometrically assuming $\epsilon_{260} = 6600$ M^{-1} cm^{-1}. Stock solutions were stored at 4 °C and discarded after no more than 3 days. All other chemicals (NaCl, $Na_2C_2O_4$, Tris, HCl, KH_2PO_4) were of reagent grade and used as received. Solutions were prepared by using purified water from a Millipore Milli-Q system.

Instrumentation and Procedures. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a Bioanalytical Systems (BAS) (West Lafayette, IN) Model BAS-100 electrochemical analyzer. Voltammetric measurements were carried out in a one-compartment cell using a glassy carbon (area, 0.088 cm^2) or a Pt disk (area, 0.023 cm^2) working electrode, a Pt flag counter electrode, and a saturated calomel reference electrode (SCE). DPV studies were done under the following conditions: pulse amplitude, −50 mV; pulse width, 50 ms; sweep rate (v), 4 mV/s; sample width, 17 ms; pulse period, 1 s.

ECL measurements were carried out with an Origen 1 Analyzer (Igen, Inc., Rockville, MD) controlled by an IBM PS/2 computer, Model 25. The technical details of this flow-injection ECL apparatus have been described previously (*4*). The working and counter electrodes were two platinum disks (area, 0.07 cm^2) and the reference electrode was Ag/AgCl (3 M NaCl). The luminescence was monitored with a Hamamatsu R1104 photomultiplier tube operated at 1000 V. The earlier problem of bubble formation in the flow-injection system was overcome in this work by circulating a buffered solution containing surfactants between experiments. Traces of surfactants left on the tubing walls appear sufficient to avoid formation of bubbles. ECL measurements were also performed with a larger, conventional, three-electrode cell arrangement to compare the results with those obtained with the Origen 1 cell configuration, which shows appreciable uncompensated resistance. For this purpose a Princeton Applied Research (PAR) Model 173 potentiostat/175 universal programmer in combination with a Hamamatsu C1230 photomultiplier tube, operated at 1000 V, was used. In this arrangement a Pt disk (area, 0.03 cm^2) served as the working electrode and the counter and reference electrodes were the same as those used for the voltammetric studies.

Ultraviolet–visible spectra were obtained on a Hewlett-Packard Model 8450A spectrophotometer and fluorescence spectra (un-

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Table I. Cyclic Voltammetric Data for $Os(bpy)_3^{2+}$ in the Presence of DNA[a]

υ, V/s	R	E_{pa},[b] mV vs SCE	E_{pc},[b] mV vs SCE	ΔE, mV	$E^{\circ\prime}$, V vs SCE	i_{pc}/i_{pa}	i_{pa}/i_{pa} (R = 0)
0.010	0	662 (2)	595 (2)	67	0.629	0.77	1
	21	637 (2)	568 (1)	69	0.603	0.90	0.69
	40	633 (3)	563 (3)	70	0.598	0.70	0.62
	74	630 (3)	563 (1)	67	0.597	0.82	0.60
0.100	0	663 (2)	598 (1)	65	0.631	0.73	1
	21	638 (1)	567 (1)	71	0.603	0.76	0.73
	40	631 (2)	567 (1)	64	0.599	0.75	0.60
	74	630 (1)	565 (1)	65	0.598	0.82	0.59
0.500	0	663 (2)	596 (1)	67	0.630	0.76	1
	21	641 (2)	567 (3)	74	0.604	0.72	0.73
	40	630 (1)	565 (1)	65	0.598	0.82	0.59
	74	635 (2)	562 (2)	73	0.599	0.76	0.59

[a] Supporting electrolyte, buffer 1. Working electrode, glassy carbon, 0.088 cm^2, $[Os(bpy)_3^{2+}]$ = 0.10 mM. [b] Number in parentheses represents the standard deviation in mV for three measurements.

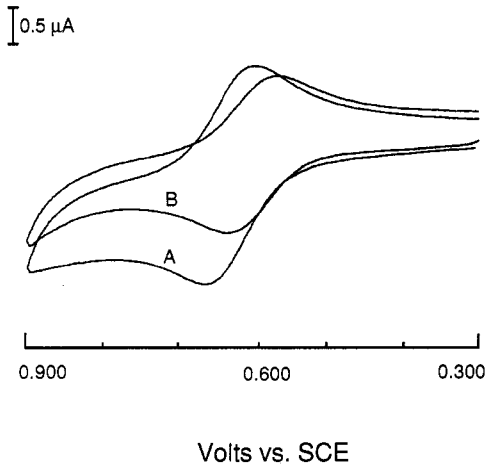


Figure 1. Cyclic voltammograms of 0.1 mM $Os(bpy)_3^{2+}$ (A) in the absence and (B) in the presence of 3.0 mM NP: sweep rate, 100 mV/s; supporting electrolyte, buffer 1; working electrode, glassy carbon (area, 0.088 cm^2).

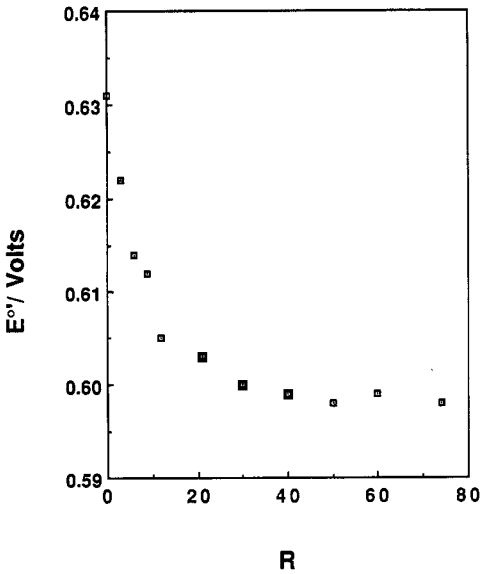


Figure 2. Effect of R ([NP]/[$Os(bpy)_3^{2+}$]) on $E^{\circ\prime}$: [$Os(bpy)_3^{2+}$] = 0.1 mM; supporting electrolyte, buffer 1; working electrode, glassy carbon (area, 0.088 cm^2).

corrected) on a SLM-AMINCO (Urbana, IL) spectrophotofluorimeter, Model SPF-500.

All glassware employed was silanized with a 5% solution of trimethylchlorsilane (Petrarch Systems, Bristol, PA) in toluene. All experiments were carried out at 25 °C. Non-linear regression analysis used to fit the voltammetric and ECL titration data were performed by using the SAS statistical software (The SAS Institute, Cary, NC) on an IBM 3081D computer.

RESULTS

CV of $Os(bpy)_3^{2+}$ in the Presence of DNA. Typical CV curves for 0.1 mM $Os(bpy)_3^{2+}$ in buffer 1, (A) in the absence and (B) in the presence of CT DNA are shown in Figure 1. In the absence of DNA, the anodic peak potential, E_{pa}, appeared at 0.664 V and the cathodic, E_{pc}, at 0.599 V. The separation of anodic and cathodic peak potentials, ΔE_p, is 65 mV, suggesting a quasireversible one-electron transfer reaction. The formal potential, $E^{\circ\prime}$, obtained from the average of E_{pa} and E_{pc} was 0.631 V. CV at different scan rates, υ (50–500 mV/s), showed that both the anodic peak current, i_{pa}, and the cathodic peak current, i_{pc}, were proportional to $\upsilon^{1/2}$ at both Pt and glassy carbon electrodes, showing that significant adsorption of $Os(bpy)_3^{2+}$ and its oxidized form do not occur. Adsorption of the closely related $Ru(bpy)_3^{2+}$ has similarly not been reported. Addition of CT DNA to the $Os(bpy)_3^{2+}$ solution results in (1) a shift of the anodic and cathodic peak potentials to more negative values and (2) a decrease of the anodic and cathodic currents. Typical voltammetric data of $Os(bpy)_3^{2+}$ at different DNA concentrations, given in terms of the relative concentration, R, and the concentration of nucleotide phosphate, [NP], where R = [NP]/[$Os(bpy)_3^{2+}$], are summarized in Table I. In the presence of 3.0 mM NP, E_{pa} = 0.633 V and E_{pc} = 0.566 V, yielding $E^{\circ\prime}$ = 0.600 V. Thus $E^{\circ\prime}$ shifted by 31 mV to more negative potentials. This shift indicates that the oxidized form, $Os(bpy)_3^{3+}$, interacts with DNA more strongly than the reduced form, $Os(bpy)_3^{2+}$. The peak separation ΔE = 67 mV shows that DNA does not affect the kinetics of electron transfer. The effect of R on the $E^{\circ\prime}$ value is shown in Figure 2.

Furthermore, in the presence of an excess of DNA, the anodic peak current, i_{pa}, decreased to 64% of that in its absence. The current decreases because the diffusion coefficient of the osmium complex when bound to DNA is much smaller than that of the free complex. The free and bound diffusion coefficients, D_f and D_b, for $Os(bpy)_3^{2+}$ were determined as reported previously (2): $D_f = (3.9 \pm 0.4) \times 10^{-6}$ cm^2/s (DNA absent) by CV and $D_b = (8.2 \pm 0.8) \times 10^{-7}$ cm^2/s by DPV at R = 1000.

The voltammetric titration (measurement of i_{pa} at different R values) of $Os(bpy)_3^{2+}$ with DNA is shown in Figure 3. The experimental data were fit to eqs 1, 2, and 3 by a nonlinear regression analysis (2), where i_T is the total anodic peak

$$i_T = B(C_f D_f^{1/2} + C_b D_b^{1/2}) \quad \text{(static equilibria)} \quad (1)$$

$$i_T = BC_T(X_f D_f + X_b D_b)^{1/2} \quad \text{(mobile equilibria)} \quad (2)$$

$$B = (2.69 \times 10^5) A n^{3/2} \upsilon^{1/2}$$

$$C_b = \{b - (b^2 - 2K^2[\text{NP}]C_T/s)^{1/2}\}/2K \quad (3)$$

$$b = 1 + C_T K + K[\text{NP}]/2s$$

Table II. CV and ECL Titration of $Os(bpy)_3^{2+}$ with CT DNA

technique	model	experimental values[c]		best fit[d]			
		10^6D_f, cm^2/s	10^6D_b, cm^2/s	10^6D_f, cm^2/s	10^6D_b, cm^2/s	$10^{-3}K_{2+}$, M^{-1}	s (bp)
CV[a]	mobile	3.9 (0.4)	0.82 (0.08)	3.9	1.0	7.3 (0.4)	3
	static	3.9 (0.4)	0.82 (0.08)	3.9	1.0	5.0 (0.2)	3
ECL[b]						6.5 (0.5)	3

[a] Supporting electrolyte, buffer 1. [b] Supporting electrolyte, buffer 2. [c] Values in parentheses represent $\pm 2\sigma$ for five measurements. [d] Value in parentheses represent 95% confidence interval of value determined by nonlinear regression calculations.

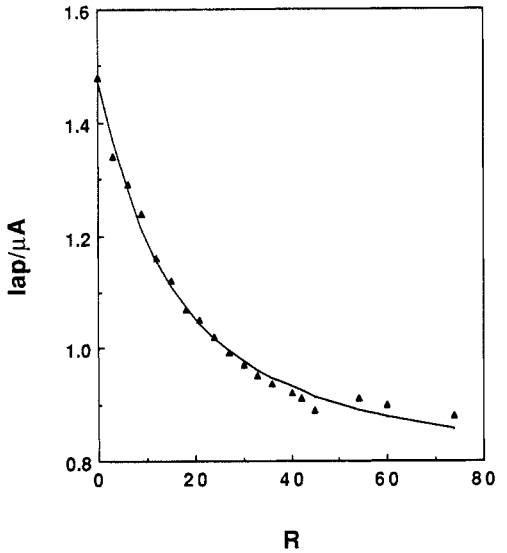


Figure 3. Cyclic voltammetric titration of 0.1 mM $Os(bpy)_3^{2+}$ with CT DNA: sweep rate, 100 mV/s; supporting electrolyte, buffer 1; working electrode, glassy carbon (area, 0.088 cm^2).

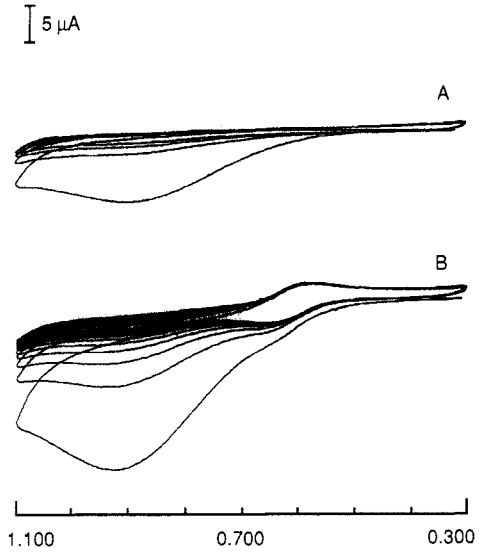
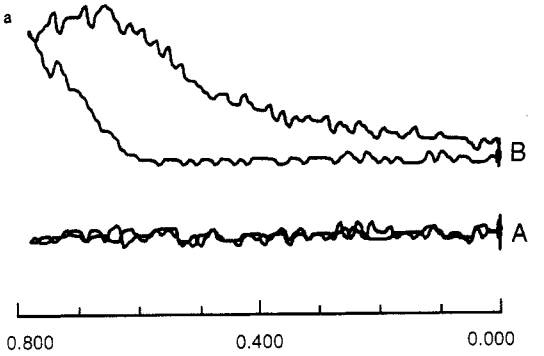


Figure 4. Cyclic voltammograms of 10.0 mM $C_2O_4^{2-}$ (A) in the absence and (B) in the presence of 1.0 mM $Os(bpy)_3^{2+}$: sweep rate, 100 mV/s; supporting electrolyte, buffer 2; working electrode, platinum disk (area, 0.023 cm^2).

current, A is the area of the working electrode, n is the number of electrons involved in the redox reaction, υ is the scan rate, C_b is the concentration of the bound complex, C_f is the concentration of the free complex, C_T is the total concentration of the complex, [NP] is the total concentration of nucleotide phosphate, K is the binding constant of the interaction of $Os(bpy)_3^{2+}$ with DNA, and s is the binding site size of $Os(bpy)_3^{2+}$ in base pairs. The analysis was performed assuming



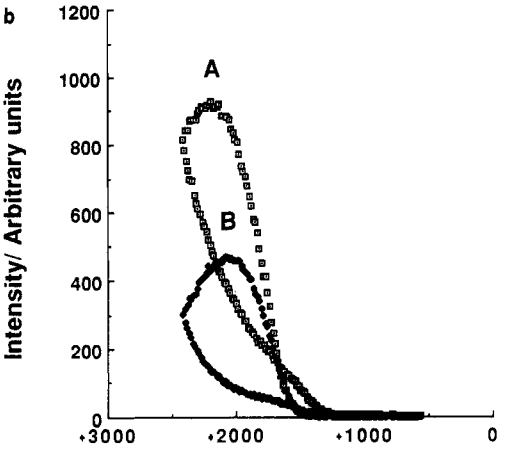


Figure 5. (a) ECL emission of a solution (A) without and (B) with 1.0 mM $Os(bpy)_3^{2+}$: sweep rate 100 mV/s; supporting electrolyte, buffer 2; working electrode, platinum disk (area, 0.03 cm^2). (b) ECL spectra of 0.1 mM $Os(bpy)_3^{2+}$ (A) in the absence and (B) in the presence of 2.4 mM NP: sweep rate, 438 mV/s; supporting electrolyte, buffer 2.

both static and mobile equilibria. In Figure 3 the solid line represents the best curve for mobile equilibrium. A summary of the titration results is given in Table II.

CV and ECL of $Os(bpy)_3^{2+}$–$C_2O_4^{2-}$ System. The CV of $C_2O_4^{2-}$ in buffer 2 (A) without and (B) with $Os(bpy)_3^{2+}$ is shown in Figure 4. The peak potential for the irreversible oxidation of $C_2O_4^{2-}$ occurred at about 0.90 V while that of $Os(bpy)_3^{2+}$ occurred at 0.66 V. This CV shows that the oxidation of $Os(bpy)_3^{2+}$ at the electrode surface occurs before that of $C_2O_4^{2-}$, as required for the production of the excited state, $Os(bpy)_3^{2-*}$, and ECL emission. However, no catalytic effect was observed on the oxidation wave of $Os(bpy)_3^{2+}$ in the presence of oxalate. Maximum ECL intensity for this system was observed at 0.67 V (Figure 5a).

ECL of $Os(bpy)_3^{2+}$ in the Presence of DNA. Typical ECL response for 0.1 mM $Os(bpy)_3^{2+}$ in buffer 2, (A) in the absence and (B) in the presence of DNA, is shown in Figure 5b. The presence of 2.4 mM nucleotide phosphate decreased

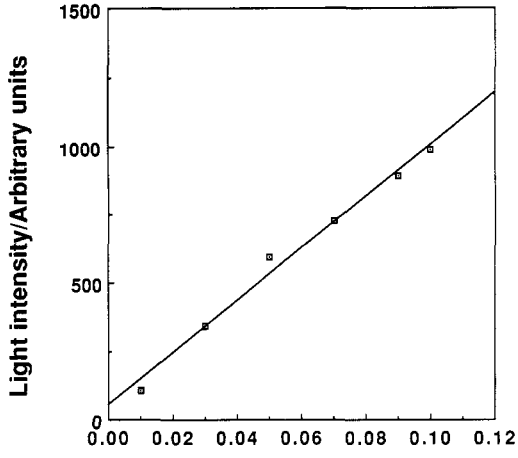


Figure 6. ECL dependence on $Os(bpy)_3^{2+}$ concentration: sweep rate, 438 mV/s; supporting electrolyte, buffer 2.

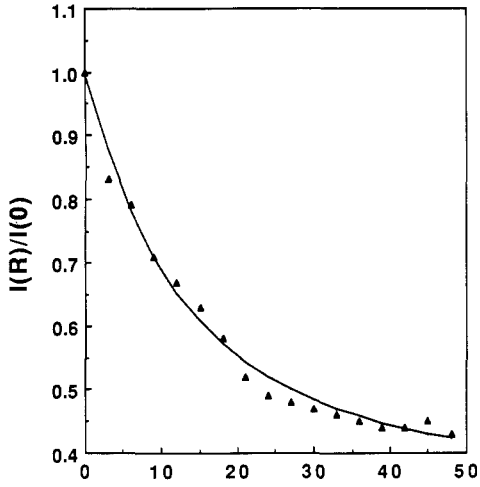


Figure 7. ECL titration of 0.1 mM $Os(bpy)_3^{2+}$ with CT DNA: sweep rate, 438 mV/s; supporting electrolyte, buffer 2.

the ECL intensity to 50% of that in its absence. The ECL peak intensity in the absence of DNA, $I(0)$, was a linear function of the $Os(bpy)_3^{2+}$ concentration over the range 10^{-4} to 10^{-5} M, as shown in Figure 6, so that

$$I(0) = AC_T \quad (4)$$

where A is a proportionality constant. In the presence of DNA, the total ECL peak intensity, $I(R)$, includes the contribution of both bound and free complex and can be expressed as (*4*)

$$I(R) = AC_f + qAC_b \quad (5)$$

where q ($0 \leq q \leq 1$) is another constant that accounts for the effect of DNA binding on ECL intensity. Combining eqs 4 and 5, we obtain

$$I(R)/I(0) = 1 - C_b(1 - q)/C_T \quad (6)$$

Equations 1–3 were used to fit the ECL titration curve in Figure 7 in the same way as in the voltammetric studies. The results of the regression calculations are tabulated in Table II.

Absorbance and Photoluminescence Spectra of $Os(bpy)_3^{2+}$ in the Presence of DNA. The absorbance and photoluminescence spectra of $Os(bpy)_3^{2+}$ (A) in the absence and (B) in the presence of DNA are shown in Figure 8 and Figure 9, respectively. The absorbance of $Os(bpy)_3^{2+}$ was

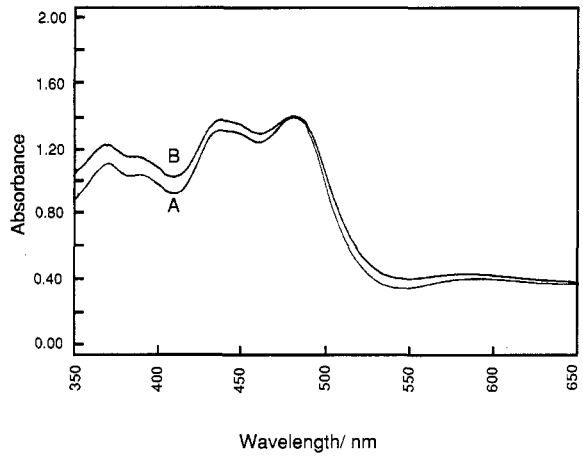


Figure 8. Absorbance spectra of 0.10 mM $Os(bpy)_3^{2+}$ (A) in the absence and (B) in the presence of 3.0 mM NP, buffer 2.

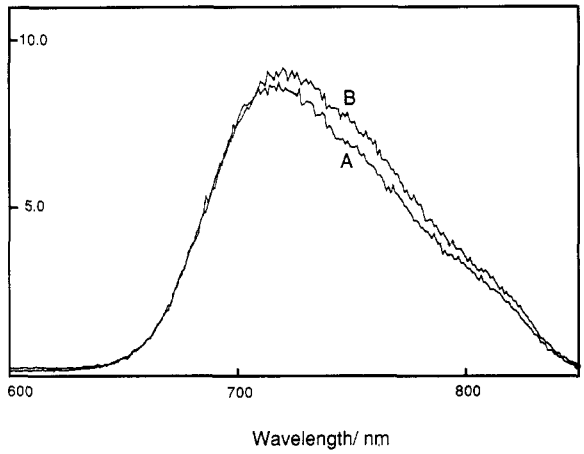
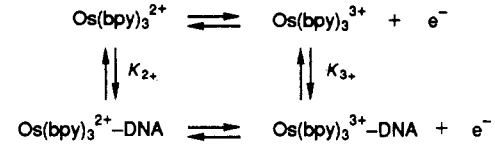


Figure 9. Photoluminescence spectra of 0.05 mM $Os(bpy)_3^{2+}$ (A) in the absence and (B) in the presence of 1.5 mM NP: buffer 2; excitation wavelength, 470 nm.

Scheme I



essentially unaffected by its interaction with DNA; however, the luminescence spectrum changed slightly. The emission intensity was increased slightly by addition of DNA with perhaps a very small shift to longer wavelengths. This intensity increase can probably be attributed to a slight decrease in quenching of the $Os(bpy)_3^{2+}$ in the presence of DNA; note that these solution were not deaerated.

DISCUSSION

The binding model used to analyze the CV and ECL titration data assumes that all DNA sites are equivalent and independent and that the DNA molecule can be saturated with $Os(bpy)_3^{2+}$ molecules. This simple model has been shown to yield good estimates of binding constants in the study of the thermodynamics of metal chelate–DNA interactions (*1–3*). We have described previously (*2*) the equilibria between DNA and a redox couple according to Scheme I. From CV data, for example, we can extract information about both the reduced and the oxidized forms of the complex. The 31-mV negative shift in $E^{\circ\prime}$ observed in the CV wave of the complex in the presence of DNA indicated that $K_{3+}/K_{2+} = 3.35$. The stronger affinity for the more positively charged redox form is expected in this type of electrostatic interaction. Note that

with intercalative binding with these types of metal chelates, the +2 form is bound more strongly than the +3 form, probably because of the importance of hydrophobic interactions (2). The voltammetric determination of the binding site size, s, for $Os(bpy)_3^{2+}$ agrees quite well with those obtained for $Co(bpy)_3^{3+}$ and $Fe(bpy)_3^{2+}$. The binding constant K_{2+}, however, was somewhat larger than for $Fe(bpy)_3^{2+}$ in the same supporting electrolyte solution (10 mM NaCl, 10 mM Tris, pH 7.0).

The ECL titration analysis was based on the measurement of the light emitted by the excited species, $Os(bpy)_3^{2+*}$, which also is in equilibrium with DNA. This excited species is formed by the mechanism in eqs 7–13 (22).

$$Os(bpy)_3^{2+} \rightleftarrows Os(bpy)_3^{3+} + e^- \quad (7)$$

$$Os(bpy)_3^{3+} + C_2O_4^{2-} \rightarrow Os(bpy)_3^{2+} + C_2O_4^{-\bullet} \quad (8)$$

$$C_2O_4^{-\bullet} \rightarrow CO_2 + CO_2^{-\bullet} \quad (9)$$

$$Os(bpy)_3^{3+} + CO_2^{-\bullet} \rightarrow Os(bpy)_3^{2+*} + CO_2 \quad (10)$$

or

$$Os(bpy)_3^{2+} + CO_2^{-\bullet} \rightarrow Os(bpy)_3^{+} + CO_2 \quad (11)$$

$$Os(bpy)_3^{+} + Os(bpy)_3^{3+} \rightarrow Os(bpy)_3^{2+*} + Os(bpy)_3^{2+} \quad (12)$$

$$Os(bpy)_3^{2+*} \rightarrow Os(bpy)_3^{2+} + h\nu \quad (13)$$

The ECL titration curve reached a constant value $I(R)/I(0) = 0.45$ at $R \geq 35$. This behavior suggests that the bound complex contributes to ECL. To fit the experimental results q and n were fixed and K was calculated. This process was repeated until the regression coefficient was minimized. The best fit resulted when $q = 0.3$, indicating that the ECL efficiency of the bound complex is 30%. It seems that $Os(bpy)_3^{2+}$, even though it is bound to the phosphate backbone, is able to react with $C_2O_4^{2-}$, as opposed to results with intercalated $Ru(phen)_3^{2+}$, where $q = 0.02$ (4). The negative phosphate groups repel $C_2O_4^{2-}$ from penetrating the DNA molecule to react with intercalated $Ru(phen)_3^{2+}$, while $Os(bpy)_3^{2+}$ bound on the outside is more accessible. However, because of the smaller diffusion coefficient of bound chelate, the ECL intensity will be smaller than that for an equal concentration of free complex. The photoluminescence study demonstrates that the decrease in ECL intensity cannot be attributed to any change in luminescence efficiency.

A disadvantage of the ECL method proposed in an earlier study (4) was the need for surfactants to carry out the flow-injection ECL studies. Surfactants can associate hydrophobically with DNA and therefore can affect the interaction between DNA and the complex of interest. Thus the establishment of conditions that allow operation in the absence of surfactants is a significant improvement in the method. Of course, the ECL approach can be used only with a special group of chelates that can be made to emit under ECL conditions. However, they are particularly advantageous, because very low concentrations (down to 10^{-9} M or below) of metal chelate can often be employed, allowing studies at large R values, even with small DNA concentrations.

The ECL titration results agree very well with those from CV, considering the difference in pH and ionic strength between the ECL (10 mM $Na_2C_2O_4^-$, 10 mM phosphate pH 5.0) and CV (10 mM NaCl, 10 mM Tris pH 7.0) solutions.

We have demonstrated that both cyclic voltammetric and electrogenerated chemiluminescent techniques are useful in studying the binding of complexes to DNA. CV for example offers the advantage over ECL of providing information about the reduced as well as the oxidized form. The absorbance and luminescence behavior of the osmium complex in the presence of DNA resembles that for $Ru(bpy)_3^{2+}$ (24) supporting the implication of the same type of interaction. Note that the very small changes observed in the spectral response would make spectroscopic studies of the metal chelate–DNA interaction very difficult with the Os–bpy complex. On the other hand the electrochemical and ECL studies can be carried out easily.

CONCLUSIONS

ECL, in addition to being useful in studying the binding of luminescent species with DNA, can also characterize their type of interaction (for example, ionic vs hydrophobic association). Species that bind to the interior of the DNA molecule by intercalation produce little ECL, while those that bind to the exterior of DNA by electrostatic interactions do show ECL, although at lower levels than the free complex.

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