It also must have a rather reactive nucleophile at its active center (probably a cysteine[1c]). These two factors in combination explain why efforts to employ such conjugated analogues have only succeeded at inactivating PDC, but failed to inactivate pyruvate oxidase, pyruvate dehydrogenase, pyruvate-ferredoxin oxidoreductase, or benzoylformate decarboxylase. Furthermore, addition of excess TDP had no effect on our observations, whereas it enhanced the regain of activity by benzoylformate decarboxylase when treated with [*p*-(bromomethyl)benzoyl]formic acid.[6] This reflects the stronger binding of the coenzyme by PDC compared to benzoylformate decarboxylase.

These conjugated pyruvic acid analogues have become useful in metabolic studies as well. In a recent report (*E*)-4-(4-chlorophenyl)-2-oxo-3-butenoic acid proved to be the most efficient reagent to demonstrate the participation of pyruvic acid in the formation of N^6-acetyl-N^6-hydroxylysine.[13]

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Voltammetric Studies of the Interaction of Metal Chelates with DNA. 2. Tris-Chelated Complexes of Cobalt(III) and Iron(II) with 1,10-Phenanthroline and 2,2′-Bipyridine

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Abstract: Voltammetric methods were used to probe the interaction (electrostatic or intercalative) of metal complexes, $ML_3^{3+/2+}$ (M = Fe, Co; L = 1,10-phenanthroline, 2,2′-bipyridine), with calf thymus DNA. Binding constants (K_{n+}) and binding site sizes (s) were determined from voltammetric data, i.e., shifts in potential and changes in limiting current with addition of DNA. The exact magnitude for the parameters depends on whether the $ML_3^{3+/2+}$/DNA reaction is assumed to be static (S) or mobile (M) within the characteristic time of a voltammetric experiment. $Co(phen)_3^{3+/2+}$ binds via intercalation with K_{3+} = 1.6 (±0.2) × 10^4 M^{-1} (S, s = 6 bp) to 2.6 (±0.4) × 10^4 M^{-1} (M, s = 5 bp). The 2+ ion interacts more favorably via hydrophobic interaction with the nucleotide bases than does the 3+ ion. Both forms of the $Fe(phen)_3^{2+/3+}$ couple bind with approximately the same affinity, K_{2+} = 7.1 (±0.2) × 10^3 M^{-1} (S, s = 5 bp) and 1.47 (±0.04) × 10^4 M^{-1} (M, s = 4 bp). $Co(bpy)_3^{3+/2+}$ shows appreciable electrostatic binding in 50 mM NaCl solution [K_{3+} = 9.4 (±1.5) × 10^3 M^{-1} (S) to 1.4 (±0.3) × 10^4 M^{-1} (M), s = 3 bp in each case], whereas $Fe(bpy)_3^{2+/3+}$ does not bind at these ionic strengths. At lower ionic strength (10 mM NaCl, 10 mM Tris, pH 7.1), binding of $Fe(bpy)_3^{2+/3+}$ is enhanced [K_{2+} = 1.1 (±0.6) × 10^3 M^{-1} nS, s = 4 bp) to 1.4 (±0.1) × 10^3 M^{-1} (M, s = 3 bp)].

We describe here voltammetric studies of the interaction of the coordination complexes $Co(bpy)_3^{3+}$, $Fe(bpy)_3^{2+}$ (bpy = 2,2′-bipyridyl), $Co(phen)_3^{3+}$, and $Fe(phen)_3^{2+}$ (phen = 1,10-phenanthroline) with calf thymus DNA. We extend our previously reported studies of $Co(phen)_3^{3+}$–DNA interactions via electrochemical methods[1] and describe the dependence of the electrochemical behavior on the nature of the ligands coordinated to the metal center.

A number of metal chelates have been used as probes of DNA structure, in solution,[2] as agents for mediation of strand scission of duplex DNA,[3] and as chemotherapeutic agents.[4] Ruthenium(II) complexes with phen and related ligands have been studied extensively as structural probes[5] and mediators of DNA cleavage reactions.[6] Enantioselective interactions of phen and bpy complexes of iron(II) have also been used as structural probes,[7] and FenII) chelated by EDTA[8] and other complexing agents,[9] tethered

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to a second moiety which interacts with duplex DNA by intercalation and/or groove binding, have been intensively studied in the photoactivated cleavage of DNA. Enantioselective binding and DNA strand scission have also been reported for metallointercalation systems based on Pt(II),[10a-f] Zn(II),[10g] Cu(II), and Cu(I).[11,12] We have chosen to concentrate this work on complexes of Co(III) and Fe(II), which have not received as much attention as the Ru(II) systems but possess the same interesting characteristics of metallointercalation and DNA cleaving properties.[13,14] The electrochemical behavior of the bpy and phen complexes of Co(III)/(II) and Fe(II)/(III) are well-understood,[15] providing well-behaved systems for the study of the interactions of these species with DNA, via electrochemical methods.

The application of electrochemical methods to the study of metallointercalation and coordination of metal ions and chelates to DNA provides a useful complement to the previously used methods of investigation, such as UV–visible spectroscopy. Small molecules which are not amenable to such methods, either because of weak absorption bands or because of overlap of electronic transitions with those of the DNA molecule, can potentially be studied via voltammetric techniques. Multiple oxidation states of the same species as well as mixtures of several interacting species can be observed simultaneously. Equilibrium constants (K) for the interaction of the metal complexes with DNA can be obtained from shifts in peak potentials, and the number of base pair sites involved in binding (s) via intercalative, electrostatic, or hydrophobic interactions can be obtained from the dependence of the current passed during oxidation or reduction of the bound species on the amount of added DNA. In some cases it should also be possible to obtain kinetic data from current and potential measurements, since voltammetric methods are sensitive to chemical reactions (e.g., ligand dissociation) coupled to the electron-transfer steps.

A number of studies have addressed the electrochemistry of DNA, via redox reactions of the purine and pyrimidine bases.[16] However, compared to spectroscopic methods, electrochemical methods have received little attention for studies of the interaction of small molecules with DNA. Several anthracycline antibiotics, which bind to DNA via intercalative and electrostatic interactions with the sugar–phosphate backbone,[17] have been studied electrochemically, as have the intercalating dyes methylene blue, neutral red, and cresyl violet.[18] Osmium tetraoxide has been examined as a polarographic marker for single-stranded DNA.[19] Binding of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) to DNA was analyzed via polarographic determination of the free *cis*-DDP, following reaction with DNA.[20] The adduct $(NH_3)_5Ru^{III}$–DNA was examined in the single-stranded form by differential pulse voltammetry,[21] and the binding of aquo ions of Cu^{2+} and Cd^{2+} was investigated by polarographic methods.[22] However, while some of these earlier studies described measurements of K and s, they did not consider either diffusion of an equilibrium mixture of free and bound electroactive species or the effects of binding to DNA on the thermodynamics of electron transfer.

In this paper, we describe the application of electrochemical measurements of the redox couples $Co(phen)_3^{3+/2+}$, $Fe(phen)_3^{2+/3+}$, $Co(bpy)_3^{3+/2+}$, and $Fe(bpy)_3^{2+/3+}$ in the presence of DNA to quantify the binding of the metal complexes to double-stranded DNA. Binding is interpreted in terms of the interplay of electrostatic interactions with the charged sugar–phosphate backbone and intercalative (hydrophobic) interactions within the DNA helix (i.e., the stacked base pairs).

Experimental Section

Materials. Calf thymus DNA was obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. The solid Na^+ salt was stored at 4 °C. Solutions of DNA (ca. 10^{-5} M in nucleotide phosphate, NP) in 50 mM NaCl/5 mM Tris, pH 7.1, gave ratios of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of ca. 1.8–1.9, indicating that the DNA was sufficiently free of protein.[23] Subjecting the DNA to phenol extraction[24] failed to improve the value of A_{260}/A_{280}. Furthermore, cyclic voltammetry of concentrated DNA solutions (ca. 5.0 mM NP) at a hanging mercury drop electrode (PAR Model 303 static mercury drop electrode) failed to exhibit any faradaic processes at potentials between 0.0 and −1.0 V vs saturated calomel electrode (SCE) but showed a 10%–15% reduction in charging current, compared to that of the DNA-free supporting electrolyte. Significant contamination of the DNA solution by protein would be apparent in CV experiments, due to faradaic processes arising from reduction of Hg–S bonds formed upon adsorption of proteins, possessing sulfur-containing amino acids, to the Hg drop.[25] Several of the known histones which may be expected to be contaminants of calf thymus DNA contain electroactive –SH groups or disulfide bonds.[26] Concentrated stock solutions of DNA (5.0–12.0 mM NP) were prepared in the supporting electrolyte of interest, and concentrations were deter-

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Table I. Voltammetric Behavior of $Co(phen)_3^{3+/2+}$ in the Presence of DNA[a]

ν/V·s^{-1}	R[b]	E_{pc}/V[c]	E_{pa}/V	ΔE_p/mV	$E_{1/2}$/V	i_{pa}/i_{pc}	i_{pc}/i_{pc} (R = 0)
0.01	0	0.107 (1)	0.172 (2)	65	0.140	1.05	1
	30	0.122 (2)	0.180 (4)	58	0.151	1.05	0.46
	50	0.122 (3)	0.183 (2)	61	0.153	1.1	0.34
	70	0.123 (2)	0.187 (3)	64	0.155	0.97	0.32
0.10	0	0.107 (1)	0.173 (2)	66	0.140	1.15	1
	30	0.122 (1)	0.180 (2)	58	0.151	1.0	0.52
	50	0.122 (2)	0.182 (1)	60	0.152	0.99	0.41
	70	0.120 (2)	0.182 (1)	62	0.151	0.87	0.39
1.0	0	0.110 (3)	0.179 (2)	68	0.145	1.17	1
	30	0.124 (2)	0.183 (2)	59	0.154	1.09	0.54
	50	0.116 (5)	0.187 (4)	71	0.152	0.95	0.44
	70	0.099 (4)	0.181 (3)	82	0.140	0.72	0.47

[a] Supporting electrolyte, 50 mM NaCl + 5 mM Tris, pH 7.1. [b] $[Co(phen)_3^{3+}] = 1.0 \times 10^{-4}$ M. [c] Numbers in parentheses are standard deviations for five measurements.

Table II. Voltammetric Behavior of $Fe(phen)_3^{2+/3+}$ in the Presence of DNA[a]

ν/V·s^{-1}	R[b]	E_{pc}/V[c]	E_{pa}/V	ΔE_p/mV	$E_{1/2}$/V	i_{pa}/i_{pc}	i_{pc}/i_{pc} (R = 0)
0.05	0	0.830 (1)	0.895 (1)	65	0.862	0.8	1
	39	0.829 (1)	0.902 (3)	73	0.865	0.7	0.52
	69	0.825 (1)	0.900 (3)	75	0.863	0.6	0.44
0.10	0	0.833 (3)	0.898 (4)	65	0.865	0.8	1
	39	0.828 (2)	0.904 (1)	76	0.866	0.7	0.51
	69	0.827 (1)	0.902 (1)	75	0.864	0.7	0.44
0.5	0	0.835 (1)	0.900 (5)	65	0.867	0.7	1
	39	0.835 (1)	0.898 (1)	63	0.866	0.7	0.48
	69	0.835 (1)	0.902 (3)	67	0.868	0.7	0.39

[a] Supporting electrolyte, 50 mM NaCl + 5 mM Tris, pH 7.1. [b] $[Fe(phen)_3^{2+}] = 8.0 \times 10^{-5}$ M. [c] Numbers in parentheses are standard deviations for five measurements.

mined by UV absorbance at 260 nm, on 1:100 dilutions. The extinction coefficient, ϵ_{260}, was taken as 6600 M^{-1} cm^{-1}.[27] Stock solutions were stored at 4 °C and discarded after no more than 4 days, unless treated with one or two drops of $CHCl_3$, which prolonged the useful life of DNA solutions for up to 7–10 days. The presence of $CHCl_3$ had no effect on voltammetric results.

Tris(1,10-phenanthroline)cobalt(III) perchlorate trihydrate $[Co(phen)_3(ClO_4)_3 \cdot 3H_2O]$, tris(2,2′-bipyridyl)cobalt(III) perchlorate trihydrate $[Co(bpy)_3(ClO_4)_3 \cdot 3H_2O]$, tris(1,10-phenanthroline)iron(II) perchlorate $[Fe(phen)_3(ClO_4)_2]$, and tris(2,2′-bipyridyl)iron(II) dichloride $[Fe(bpy)_3Cl_2]$ were prepared according to previously reported procedures.[28] Starting materials, 1,10-phenanthroline monohydrate (phen), 2,2′-bipyridine (bpy), $CoCl_2 \cdot 6H_2O$, and $FeSO_4$, were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received. The complexes were recrystallized twice from water and dried overnight, under vacuum, at 25 °C. Stock solutions of the metal complexes were prepared before each series of experiments and were discarded afterward.

Potassium octacyanomolybdate(IV) dihydrate $[K_4Mo(CN)_8 \cdot 2H_2O]$ was prepared as described previously.[29] Potassium ferricyanide $[K_3Fe(CN)_6]$, was purchased from Aldrich and used without further purification.

All other chemicals used for preparation of supporting electrolytes (NaCl, Tris, HCl) were reagent grade. Solutions were prepared in high-purity water (ρ = 18 MΩ·cm) from a Millipore Milli-Q water purification system.

Instrumentation. Cyclic voltammetry (CV) was performed with a Bioanalytical Systems (West Lafayette, IN) Model BAS-100 electrochemical analyzer or with a Princeton Applied Research (PAR) Model 173 potentiostat/175 universal programmer with data storage and manipulation via a Norland Corp. (Fort Atkinson, WI) Model 3001 digitial oscilloscope. Differential pulse voltammetry (DPV) and chronocoulometry (CC) employed the BAS-100 electrochemical analyzer, with the following parameters (DPV): pulse amplitude = −50 mV, pulse width = 50 ms, sweep rate (ν) = 4 mV/s, and pulse period = 1 s. Typically, CV peak potentials were reproducible to better than ±5 mV (at moderate sweep rates) and DPV peak potentials to ±2 mV. Cell resistances were measured with a Yellow Springs Instruments Model 35 conductance meter. Ultraviolet–visible spectra were obtained on a Hewlett-Packard Model 8450A spectrophotometer.

Procedures. All voltammetric experiments were performed in single-compartment cells of volume 5–15 mL. The working electrodes were glassy carbon disks (Bioanalytical Systems, West Lafayette, IN) with a geometric area of 0.071 cm^2 [for Co(III)/(II) systems] or 0.088 cm^2 [for Fe(II)/(III) systems]. The working electrode was polished prior to each series of experiments with 0.25-μm diamond paste (Buehler, Lake Bluff, IL) on a nylon buffing pad and then subjected to ultrasonic cleaning for ca. 5 min, in 95% ethanol. The use of 0.05-μm alumina, in place of diamond paste, proved unsatisfactory. Erratic peak potentials were obtained in CV of Co(III)/(II) redox couples under these conditions, as well as some irreproducibility in current measurement. A saturated calomel electrode (SCE), prepared according to Adams,[30] was used in all experiments. A Pt flag served as counter electrode.

Supporting electrolytes were 50 mM NaCl/5 mM Tris (denoted buffer 1) or 10 mM NaCl/10 mM Tris (denoted buffer 2), adjusted to pH 7.1 with HCl. Assembled cells had a resistance (working electrode to counter electrode) of 500 Ω (buffer 1) and 770 Ω (buffer 2). Thus, the maximum distortion in peak potential, due to IR drop, was 2 mV (in buffer 1) and 3 mV (in buffer 2) at the maximum currents passed during voltammetric experiments (ca. 3.5 μA).

All glassware for solution preparation and electrochemical experiments was silanized with a 5% (v/v) solution of trimethylchlorosilane (Petrarch Systems, Bristol, PA) in toluene. Where appropriate, solutions were deoxygenated via purging with N_2 gas for 15 min prior to measurements; during measurements, a stream of N_2 was passed over the solution. N_2 was passed through an O_2 scrubbing system[31] and saturated with the aqueous supporting electrolyte before entering the electrochemical cell.

Nonlinear regression analysis of titration data, presented below, was performed with SAS statistical software (The SAS Institute, Inc., Cary, NC) on an IBM 3081D computer system.

All experiments were carried out at the ambient temperature of the laboratory (23–25 °C). All measurements, unless specified otherwise, are the average of at least three to five replicate measurements.

Results

$Co(phen)_3^{3+/2+}$ and $Fe(phen)_3^{3+/2+}$. Typical CV behavior of 0.10 mM $Co(phen)_3^{3+}$ in the absence and presence of DNA is shown in Figure 1 and that of 0.076 mM $Fe(phen)_3^{2+}$, under similar conditions, in Figure 2. Summaries of voltammetric results are given in Tables I and II. The supporting electrolyte for all experiments was buffer 1. CV of $Co(phen)_3^{3+/2+}$ (ν = 100 mV/s) in the absence of DNA (Figure 1A) featured reduction of 3+ to

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(28) (a) Dollimore, L. S.; Gillard, R. D. *J. Chem. Soc., Dalton Trans.* **1973**, 933. (b) Musumeci, S.; Rizzarelli, F. S.; Sammartano, S.; Bonomo, R. P. *Inorg. Chim. Acta* **1973**, *7*, 660. (c) Gaudiello, J. G.; Sharp, P. R.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 6373.

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(31) Rusling, J. F. *J. Electroanal. Chem.* **1981**, *125*, 447.

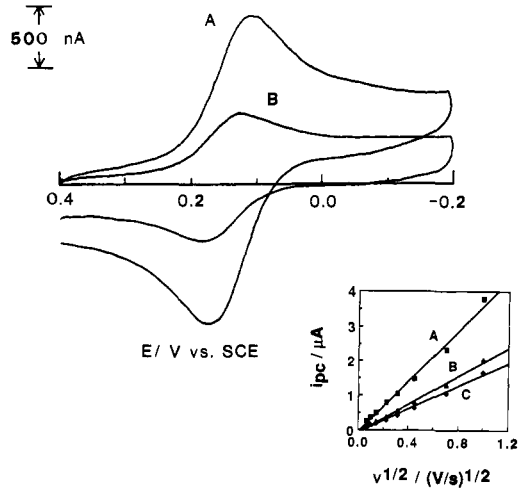

Figure 1. Cyclic voltammograms of 1.0×10^{-4} M $Co(phen)_3^{3+}$ in the (A) absence and (B) presence of 5.0 mM nucleotide phosphate (NP). Sweep rate, 100 mV/s. Supporting electrolyte, buffer 1. Inset: Effect of DNA on the diffusion of $Co(phen)_3^{3+}$ at (A) $R = 0$, (B) $R = 30$, and (C) $R = 50$.

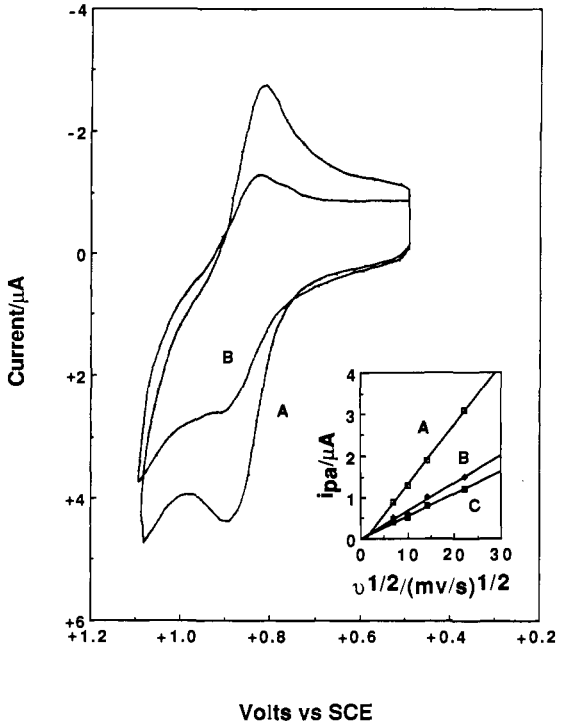

Figure 2. Cyclic voltammograms of 7.6×10^{-5} M $Fe(phen)_3^{2+}$ (A) in the absence and (B) presence of 3.39 mM NP. Supporting electrolyte, buffer 1. Sweep rate, 500 mV/s. Inset: Effect of DNA on the diffusion of $Fe(phen)_3^{2+}$ at (A) $R = 0$, (B) $R = 41.2$, and (C) $R = 72.1$.

the 2+ form at a cathodic peak potential, E_{pc}, of 0.107 V vs SCE. Reoxidation of 2+ occurred, upon scan reversal, at 0.174 V. The separation of the anodic and cathodic peak potentials, ΔE_p, 67 mV, indicated a quasireversible, 1-e^- redox process. The formal potential, $E^{\circ\prime}$ (or voltammetric $E_{1/2}$), taken as the average of E_{pc} and E_{pa}, was 0.140 V, in the absence of DNA. In the presence of 5.0 mM nucleotide phosphate, NP (Figure 1B), at the same concentration of $Co(phen)_3^{3+}$, $E_{pc} = 0.125$ V and $E_{pa} = 0.183$ V. Thus, both the anodic and cathodic peak potentials shifted to more positive values vs a solution without DNA ($E_{1/2} = 0.154$ V). The value of ΔE_p in the presence of DNA was 58 mV, showing that reversibility of the electron-transfer process was maintained or even improved under these conditions. $E_{1/2}$, in this case, shifted to more positive potentials by 14 mV.

CV peak potentials were independent of sweep rate, ν, over the range 5–1.0 V/s, with ΔE_p in the range of 62–68 mV, in the

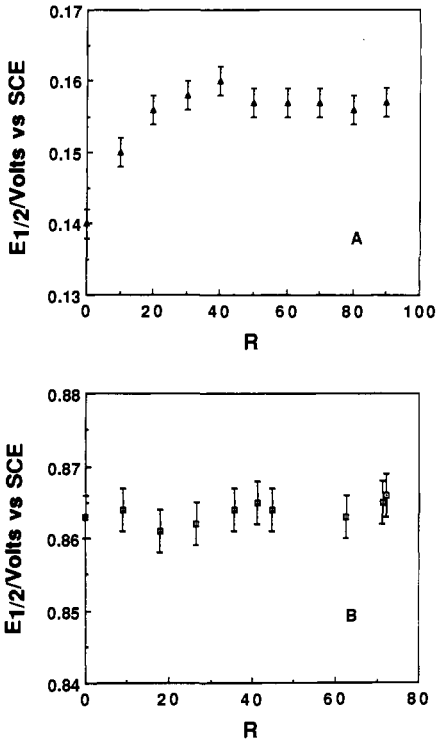

Figure 3. Dependence of $E_{1/2}$ on the ratio NP:metal complex, R, for (A) 1.0×10^{-4} M $Co(phen)_3^{3+}$ (by DPV) and (B) 7.6×10^{-5} M $Fe(phen)_3^{2+}$ (by CV). Supporting electrolyte, buffer 1.

absence of DNA. In the presence of DNA (0 < concentration of nucleotide phosphate, [NP] ≤ 7.0 mM) and at intermediate ν (5 mV/s ≤ ν ≤ 200 mV/s), ΔE_p was somewhat smaller (58–61 mV) with E_{pc} and E_{pa} independent of ν. At higher ν and in the presence of DNA (e.g., 1.0 V/s), slight broadening of ΔE_p was observed, possibly due to the onset of kinetic complications (ΔE_p > 70 mV) as well as a slight dependence of E_p on ν (e.g., E_{pc} = 0.123 V at 0.01 V/s and [NP] = 7.0 mM, while $E_{pc} = 0.099$ V at 1.0 V/s and [NP] = 7.0 mM).

Typical behavior of $Fe(phen)_3^{2+/3+}$ (ν = 500 mV/s) is shown in A and B of Figure 2 for a solution without DNA and in the presence of 3.4 mM NP, respectively. In the absence of DNA, $Fe(phen)_3^{2+}$ was oxidized to the 3+ ion at $E_{pa} = 0.898$ V and reduced, upon scan reversal, at $E_{pc} = 0.828$ V; $E_{1/2} = 0.863$ V (ΔE_p = 70 mV). In the presence of DNA (R = 45), $E_{1/2}$ was 0.864 V and ΔE_p was 68 mV. Reversibility of the electron transfer was maintained in the presence of DNA, but there was no apparent shift in $E_{1/2}$. Peak potentials were independent of scan rate (50 ≤ ν ≤ 500 mV/s), and ΔE_p values were between 63 and 76 mV.

The positive shift in $E_{1/2}$ with increasing ratio of total concentration of NP to total concentration of Co(III), R, suggests a difference in the binding properties of the Co(III) and Co(II) species to DNA. The absence of a shift in $E_{1/2}$ of the Fe(II)/(III) couple, however, suggests that the two halves of this redox couple interact with DNA to about the same extent. Figure 3 shows results for experiments in which the [NP] was varied over a wide range for 0.1 mM $Co(phen)_3^{3+}$ (Figure 3A) and 0.076 mM $Fe(phen)_3^{2+}$ (Figure 3B). $E_{1/2}$ values were determined from the DPV peak potential (in Figure 3A), E_p, by the relation[32a]

$$E_{1/2} = E_p + \Delta E/2 \quad (1)$$

where $E_{1/2}$ is the equivalent of the average of E_{pc} and E_{pa} in CV experiments and ΔE is the pulse amplitude (−50 mV). These $E_{1/2}$ values were in good agreement with those determined from CV experiments. The average of E_{pc} and E_{pa} was used to determine $E_{1/2}$ in Figure 3B. The limiting shift in $E_{1/2}$ of +17 mV for $Co(phen)_3^{3+}$ was taken as the difference between the $E_{1/2}$ at R

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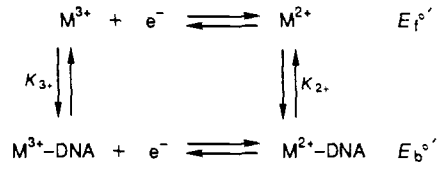
Table III. Voltammetric Behavior of $Mo(CN)_8^{4-/3-}$ and $Co(phen)_3^{3+/2+}$ in the Presence of DNA[a]

couple	R[b]	E_{pc}/V	E_{pa}/V	$E_{1/2}$/V	ΔE_p/mV	$i_{pc}/\mu A$	$i_{pa}/\mu A$	$i_{pc}/i_{pc}(R=0)$	$i_{pa}/i_{pa}(R=0)$
Mo	0	0.560	0.482	0.521	78	0.4	1.02		1
	54.5	0.564	0.485	0.525	79	0.38	0.99		0.97
Co	0	0.104	0.170	0.137	66	1.3	1.1	1	
	54.5	0.115	0.183	0.149	68	0.6	0.4	0.36	

[a] Supporting electrolyte, 50 mM NaCl + 5 mM Tris, pH 7.1. Sweep rate, 100 mV/s. [b] $[Co(phen)_3^{3+}] = 1.1 \times 10^{-4}$ M; $[Mo(CN)_8^{4-}] = 1.1 \times 10^{-4}$ M.

Scheme I



= 0 and the average of the $E_{1/2}$ values corresponding to the plateau of the $E_{1/2}$–R curve ($R \geq 30$). $E_{1/2}$ for $Fe(phen)_3^{2+}$ did not change appreciably, over the same range of [NP].

The net shift in $E_{1/2}$ can be used to estimate the ratio of equilibrium constants for the binding of the 3+ and 2+ ions to DNA. This is analogous to the treatment of the association of small molecules with micelles.[33] For a Nernstian electron transfer, in a system in which both the oxidized and reduced forms associate with a third species in solution (DNA), Scheme I can be applied. Here, M^{3+} and M^{2+} represent the oxidized and reduced forms of the metal complex and M^{n+}–DNA denotes a metal complex bound to the DNA molecule. $E_f^{\circ\prime}$ and $E_b^{\circ\prime}$ are the formal potentials of the 3+/2+ couple, in the free and bound forms, respectively. K_{3+} and K_{2+} are the corresponding binding constants for the 3+ and 2+ species to DNA. Consideration of the Nernst equations for the reversible redox reactions of the free and bound species and the corresponding equilibrium constants for binding of each oxidation state to DNA yields, for a 1-e^- redox process

$$E_b^{\circ\prime} - E_f^{\circ\prime} = 0.059 \log (K_{2+}/K_{3+}) \quad (2)$$

Thus, for a limiting shift of +17 mV, K_{2+}/K_{3+} for $Co(phen)_3^{3+/2+}$ is 1.94; i.e., the 2+ ion is apparently bound about twice at strongly as the 3+ ion. However, in the case of $Fe(phen)_3^{2+/3+}$, $K_{2+}/K_{3+} = 1$, and each oxidation state interacts with DNA to the same extent. The implications of these results are discussed in a subsequent section.

Previously,[1] we reported K_{2+}/K_{3+} to be 4.8, corresponding to a shift in $E_{1/2}$ of +40 mV, over the range $0 \leq R \leq 300$. However, we have observed that the apparent $E_{1/2}$ of $Co(phen)_3^{3+/2+}$, in the absence of DNA, depends on the concentration of electroactive species. Since different concentrations of $Co(phen)_3^{3+}$ were used in the previous study to gain access to a very large range of R values (0.10 mM for $0 \leq R \leq 100$ and 0.01 mM for $100 < R \leq 300$), ca. 15–20 mV of the previously reported potential shift can be attributed to a dependence of $E_{1/2}$ on the concentration of $Co(phen)_3^{3+}$. The remainder of the shift is accountable via binding to DNA, within the error of the measurement. Diffusion coefficients in the free and bound limits are comparable at different concentrations of $Co(phen)_3^{3+}$, however, since the ratio i_p/C (where i_p is the DPV peak current and C is the total concentration of electroactive species) is essentially constant over the concentration range 10^{-6} M $\leq C \leq 10^{-4}$ M.

In addition to changes in formal potential upon addition of DNA, the voltammetric current decreases, as shown in the insets of Figures 1 and 2. For $Co(phen)_3^{3+}$ (inset of Figure 1) plots of cathodic peak current, i_{pc}, vs $\nu^{1/2}$ were linear with y intercepts = 0, within the error of the measurement, as expected for reversible e^- transfer.[32b] The slope of the i_{pc}–$\nu^{1/2}$ plot decreased with increasing R, indicating a reduction in the apparent diffusion coefficient of $Co(phen)_3^{3+}$ as [NP] increased. The slope of inset curve A of Figure 1 ($R = 0$) gives the diffusion coefficient of the free $Co(phen)_3^{3+}$, D_f, of 3.7 (±0.6) × 10^{-6} cm^2/s. Curves B and C correspond to apparent D values of 1.14 × 10^{-6} cm^2/s (R = 30) and 7.5 × 10^{-7} cm^2/s (R = 50), respectively. For $Fe(phen)_3^{2+}$ (see inset of Figure 2) similar measurements gave D_f = 4.9 (±1) × 10^{-6} cm^2/s. In the limit of very large R, where binding of the metal complex to DNA is more nearly quantitative (see below), DPV measurements [R = 1000, 8.5 × 10^{-6} M $Co(phen)_3^{3+}$] gave a diffusion coefficient of the bound species, D_b, of 2.6 (±0.6) × 10^{-7} cm^2/s. Similar measurements on $Fe(phen)_3^{2+}$ (R = 1111, 1.0 × 10^{-5} M in complex) gave D_b = 9.0 (±0.8) × 10^{-8} cm^2/s. Thus, the apparent diffusion coefficient of $Co(phen)_3^{3+}$ decreased by a factor of 14.2 and that of $Fe(phen)_3^{2+}$ by 55, upon addition of a large excess of DNA. These two experimental limits represent diffusion of the free and bound metal complex, respectively. In CV experiments at 0.1 mM Co(III), $50 \leq R \leq 70$ (ν = 100 mV/s), the value of i_{pc} was ca. 40% of that in the absence of DNA. The ratio of anodic to cathodic peak currents, i_{pa}/i_{pc}, was close to 1 under all experimental conditions, indicating that the 3+ and 2+ species are both stable on the time scale of the CV measurement. The ratio i_{pa}/i_{pc} was not affected by the presence of O_2 in the solution, indicating that the 2+ ion was not reoxidized via dissolved O_2 on the time scale of the experiment. For $Fe(phen)_3^{2+}$, i_{pa} decreased to ca. 43% of that in the absence of DNA, for solutions where $40 \leq R \leq 70$. The ratio i_{pa}/i_{pc} was 0.7–0.8 at all ν and [NP].

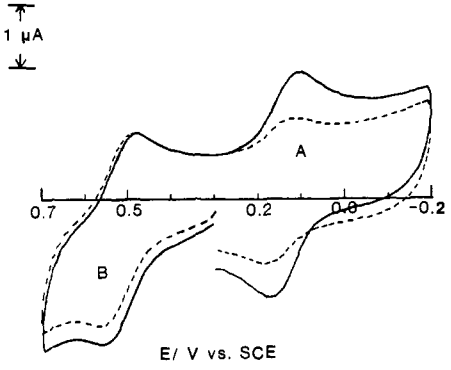


Figure 4. Cyclic voltammetry of a mixture of $Co(phen)_3^{3+}$ (redox couple A, 1.1 × 10^{-4} M) and $Mo(CN)_8^{4-}$ (redox couple B, 1.1 × 10^{-4} M), in the absence (solid curve) and the presence (dashed curve) of 5.45 mM NP. Sweep rate, 100 mV/s. Scan initiated at +0.30 V vs SCE.

We interpret the change in current upon DNA addition in terms of the diffusion of an equilibrium mixture of free and DNA-bound metal complex to the electrode surface. These changes can be used to quantify the binding of the metal complex to DNA and are addressed in more detail in a subsequent section.

Effect of DNA on Diffusion of Anions. To show that the decrease in i_{pc} is due to diffusion of the $M(phen)_3^{3+}$–DNA adduct and not merely to diffusion of free metal complex in a solution of increased viscosity, nor to blockage of the electrode surface by an adsorbed layer of DNA that could possibly form at the electrode surface, experiments were carried out on a mixture of $Co(phen)_3^{3+}$ and $Mo(CN)_8^{4-}$, at the same concentration (0.11 mM, in buffer 1), in the absence and presence of DNA (R = 54.5), as shown in Figure 4. Results are summarized in Table III. $Mo(CN)_8^{4-}$ should not interact with DNA, because of coulombic repulsion between its high negative charge and the negatively charged sugar–phosphate backbone of DNA. In the absence of DNA (solid curve), $Mo(CN)_8^{4-/3-}$ (couple B) gave $E_{1/2}$ = 0.521 V and $Co(phen)_3^{3+/2+}$ (couple A) gave $E_{1/2}$ = 0.137 V. Upon addition of DNA (dashed curve), $E_{1/2}$ for $Mo(CN)_8^{4-/3-}$ was 0.525 V and

(33) Kaifer, A. E.; Bard, A. J. *J. Phys. Chem.* **1985**, *89*, 4876.

Table IV. Voltammetric Behavior of $Co(bpy)_3^{3+/2+}$ in the Presence of DNA[a]

ν/V·s^{-1}	R[b]	E_{pc}/V[c]	E_{pa}/V	ΔE_p/mV	$E_{1/2}$/V	i_{pa}/i_{pc}	$i_{pc}/i_{pc}(R=0)$
0.01	0	0.056 (3)	0.125 (3)	69	0.090	0.7	1
	30	0.052 (2)	0.118 (7)	66	0.085	0.56	0.57
	50	0.052 (1)	0.122 (5)	70	0.088	0.42	0.48
0.10	0	0.053 (1)	0.118 (1)	65	0.086	0.5	1
	30	0.040 (1)	0.105 (4)	65	0.073	0.24	0.7
	50	0.035 (1)	0.105 (3)	69	0.070	0.17	0.58
	70	0.035 (1)	0.109 (1)	74	0.072	0.17	0.56
1.0	0	0.027 (5)	0.110 (8)	83	0.069	0.41	1
	30	0.016 (2)	0.095 (1)	78	0.056	0.17	0.67
	50	−0.002 (5)	0.097 (2)	99	0.048	0.12	0.58

[a] Supporting electrolyte, 50 mM NaCl + 5 mM Tris, pH 7.1. [b] $[Co(bpy)_3^{3+}] = 1.0 \times 10^{-4}$ M. [c] Numbers in parentheses are standard deviations for three measurements.

Table V. Voltammetric Behavior of $Fe(bpy)_3^{3+/2+}$ in the Presence of DNA[a]

ν/V·s^{-1}	R[b]	E_{pc}/V[c]	E_{pa}/V	ΔE_p/mV	$E_{1/2}$/V	i_{pa}/i_{pc}	i_{pc}/i_{pc} ($R=0$)
0.05	0	0.822 (1)	0.887 (1)	65	0.856	0.9	1
	47	0.777 (1)	0.843 (1)	66	0.810	0.9	0.6
	66	0.783 (1)	0.845 (1)	62	0.814	0.8	0.51
0.10	0	0.822 (4)	0.884 (1)	62	0.853	0.8	1
	47	0.780 (1)	0.845 (1)	65	0.812	0.8	0.61
	66	0.781 (2)	0.847 (4)	66	0.814	0.9	0.52
0.5	0	0.820 (1)	0.892 (4)	72	0.855	0.9	1
	47	0.775 (1)	0.850 (1)	75	0.812	0.7	0.64
	66	0.775 (1)	0.850 (1)	75	0.812	0.9	0.48

[a] Supporting electrolyte, 10 mM NaCl + 10 mM Tris, pH 7.1. [b] $[Fe(bpy)_3^{2+}] = 8.0 \times 10^{-5}$ M. [c] Numbers in parentheses are standard deviations for five measurements.

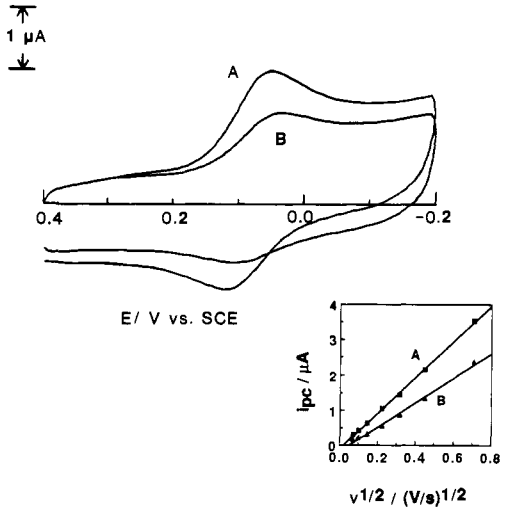


Figure 5. Cyclic voltammetry of 1.0×10^{-4} M $Co(bpy)_3^{3+}$ (A) in the absence and (B) in the presence of 5.0 mM NP. Inset: Effect of DNA on diffusion of $Co(bpy)_3^{3+}$, at (A) $R = 0$ and (B) $R = 50$. Supporting electrolyte, buffer 1. Sweep rate, 100 mV/s.

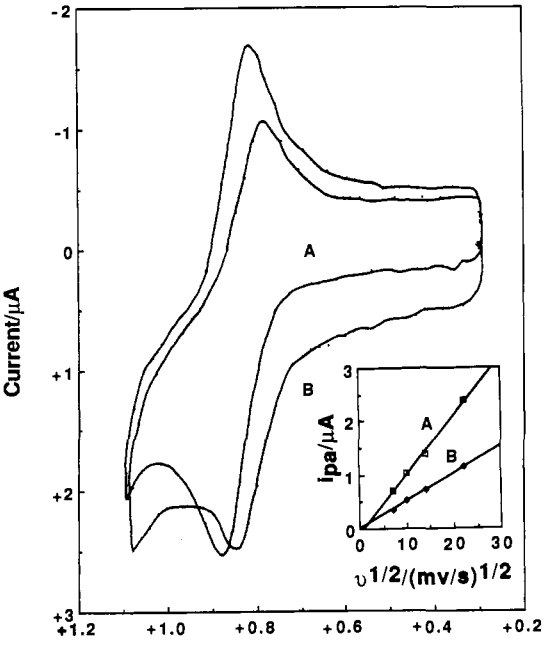


Figure 6. Cyclic voltammetry of 8.1×10^{-5} M $Fe(bpy)_3^{2+}$ in the (A) absence and (B) presence of 3.84 mM NP. Supporting electrolyte, buffer 2. Sweep rate, 500 mV/s. Inset: Effect of DNA on diffusion of $Fe(bpy)_3^{2+}$ at (A) $R = 0$ and (B) $R = 66$.

for $Co(phen)_3^{3+}$ was 0.149 V; i.e., the ΔE_p values of both couples were not significantly affected by the presence of DNA. However, a large difference was observed in the behavior of the currents of the two redox couples. In the presence of DNA, i_{pc} for Co(III)/(II) decreased to 36% of that in the absence of DNA, while i_{pa} for the Mo(IV)/(V) couple was 97% of that in the absence of DNA, when correction was made for the change in background which occurred upon addition of DNA. No significant changes in the steady-state current were observed when the potential was cycled continuously for up to 45 min after the initial scan. Similar results were obtained for $Fe(CN)_6^{3-/4-}$ solutions; addition of DNA only slightly affected the current. Thus, a decrease in currents in CV experiments can be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule. Enhanced viscosity of DNA solutions apparently has only a small effect on diffusion, and there is no significant obstruction of the glassy carbon surface via adsorption of DNA, as opposed to Hg electrodes, where adsorption of DNA at negative potentials is significant.[34]

$Co(bpy)_3^{3+/2+}$ and $Fe(bpy)_3^{2+/3+}$. CV behavior of $Co(bpy)_3^{3+}$ (1.0×10^{-4} M) in the absence and presence of DNA is shown in Figure 5 and that of $Fe(bpy)_3^{2+}$ (8.1×10^{-5} M) in Figure 6. Voltammetric results are presented in Tables IV and V. In the absence of DNA (Figure 5A), reduction of the Co(III) species to Co(II) occurred at $E_{pc} = 0.052$ V and reoxidation at $E_{pa} = 0.118$ V ($\nu = 100$ mV/s); $E_{1/2} = 0.085$ V. The peak potential separation

(34) (a) Miller, I. R. *J. Mol. Biol.* **1961**, *3*, 229. (b) Miller, I. R.; Bach, D. *Biopolymers* **1966**, *4*, 705. (c) Frei, Y. F.; Miller, I. R. *J. Phys. Chem.* **1965**, *69*, 3018.

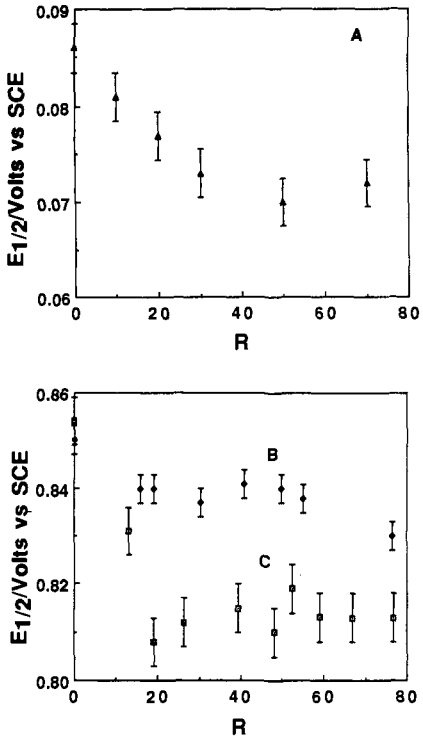


Figure 7. Dependence of $E_{1/2}$ from cyclic voltammetry, on R, for (A) 1.0×10^{-4} M $Co(bpy)_3^{3+}$ (buffer 1), (B) 7.6×10^{-5} M $Fe(bpy)_3^{2+}$ (buffer 1), and (C) 8.1×10^{-5} M $Fe(bpy)_3^{2+}$ (buffer 2). $E_{1/2}$ was determined from the average of E_{pc} and E_{pa}.

was 66 mV, indicating a fairly reversible electron transfer, as in the case of $Co(phen)_3^{3+}$. In the presence of 5 mM NP (Figure 5B), E_{pc} shifted to 0.036 V and E_{pa} to 0.106 V, yielding $E_{1/2}$ = 0.071 V (ΔE_p = 70 mV). Thus, the apparent $E_{1/2}$ shifted to more negative potentials by 14 mV in the presence of DNA. CV of $Fe(bpy)_3^{2+/3+}$, at the same supporting electrolyte concentration as for $Co(bpy)_3^{3+/2+}$ (buffer 1), gave a small shift in $E_{1/2}$. For example, at R = 0 $E_{1/2}$ = 0.852 V (ΔE_p = 63 mV), and at R = 41 $E_{1/2}$ = 0.840 V (ΔE_p = 70 mV). However, more extensive measurements (see below) suggest that the shift in $E_{1/2}$ is probably less than 10 mV. The peak current for oxidation of the Fe(II) species did not decrease under these conditions, indicating no detectable binding of $Fe(bpy)^{2+/3+}$ to DNA in buffer 1.

To determine whether any binding of $Fe(bpy)_3^{2+}$ could be detected at lower salt concentrations, experiments were carried out in 10 mM NaCl–10 mM Tris, pH 7.1 (buffer 2), (Figure 6). At R = 0, $E_{1/2}$ was 0.851 V (ΔE_p = 70 mV), and at R = 47.4, $E_{1/2}$ = 0.812 V (ΔE_p = 70 mV). Thus, at this lower ionic strength, $E_{1/2}$ shifted to more negative potentials by 39 mV. The anodic peak current for oxidation of $Fe(bpy)_3^{2+}$ decreased to 61% of that in the absence of DNA. These trends suggest much stronger binding of $Fe(bpy)_3^{2+}$ to DNA at lower ionic strength. Under these conditions, E_{pc} and E_{pa} were independent of ν, and ΔE_p's were in the range 62–75 mV.

The behavior of $E_{1/2}$ of $Co(bpy)_3^{3+}$ with varying [NP] is shown in Figure 7A and for $Fe(bpy)_3^{2+}$ in Figure 7B,C, for $0 \le R \le 80$. Little change in $E_{1/2}$ occurred after ca. R = 30, as in the case of $Co(phen)_3^{3+}$. The ratio of binding constants for $Co(bpy)_3^{3+}$ and $Co(bpy)_3^{2+}$ K_{2+}/K_{3+} = 0.6, and so for the bpy complex, the 3+ ion is bound ca. 1.7 times as strongly as the 2+ ion. At low ν ($\le$100 mV/s), E_p's and ΔE_p were essentially independent of ν, but at higher values of ν, e.g., 500 mV/s to 1.0 V/s, ΔE_p values were in the range 83–99 mV and increased with increasing ν, indicating the onset of kinetic complications to the electron-transfer steps.[35] $E_{1/2}$ of $Fe(bpy)_3^{2+/3+}$ was essentially unaffected by the presence of DNA in buffer 1 (Figure 7B) but shifted to more negative values by 41 mV in buffer 2, giving K_{2+}/K_{3+} = 0.21. Again, little change in $E_{1/2}$ occurred after R = 30.

The behavior of i_{pc} for reduction of $Co(bpy)_3^{3+}$ is shown in the inset of Figure 5 and that for oxidation of $Fe(bpy)_3^{2+}$ in the inset of Figure 6. For $Co(bpy)_3^{3+}$, in the absence of DNA, i_{pc} was linear with $\nu^{1/2}$ for $5 \le \nu \le 500$ mV/s, with zero intercept within the error of the measurement. From this, the free diffusion coefficient for $Co(bpy)_3^{3+}$ was calculated as $D_f = 5.0\ (\pm 0.6) \times 10^{-6}$ cm^2/s. Increasing the concentration of DNA caused suppression of the slope of i_{pc}–$\nu^{1/2}$, as observed for $Co(phen)_3^{3+}$. The bound diffusion coefficient, D_b, was determined to be $3.2\ (\pm 1.0) \times 10^{-7}$ cm^2/s, from DPV measurements at 8.0×10^{-6} M $Co(bpy)_3^{3+}$ and R = 1000. Thus the apparent diffusion coefficient decreased by a factor of 15.6. Similar results were obtained for i_{pa}–$\nu^{1/2}$ plots for oxidation of $Fe(bpy)_3^{2+}$ (inset of Figure 6), in buffer 2. D_f was 2.8 ($\pm$0.6) $\times 10^{-6}$ cm^2/s from the slope of $i_{pa}/\nu^{1/2}$ at R = 0. D_b was estimated as $1.8\ (\pm 0.6) \times 10^{-7}$ cm^2/s at R = 1037 and 1.0×10^{-5} M complex.

The ratio i_{pa}/i_{pc} was less than 1, for $Co(bpy)_3^{3+/2+}$. Rigorous deaeration failed to affect i_{pa}/i_{pc}. This ratio would be 1 for an uncomplicated redox process. At low ν ($\le$10 mV/s, R = 0), i_{pa}/i_{pc} was ca. 0.7 and decreased with increasing ν. In the presence of DNA, i_{pa}/i_{pc} decreased with increasing ν and also with increasing [NP]. These results suggest that $Co(bpy)_3^{3+}$ is adsorbed on the glassy carbon electrode[36] and that the presence of DNA may enhance this process. A solution of 0.11 mM $Co(bpy)_3^{3+}$ was subjected to a 250-ms potential step (chronocoulometry) into the diffusion-controlled region of Figure 5A. The intercept on the charge axis was 0.75 μC. A 0.1 mM $Co(phen)_3^{3+}$ solution, however, gave a straight line with approximately the same slope, but which intersected the charge axis at the same point as did a blank experiment, in which only supporting electrolyte was used (0.55 μC). The excess charge between the intercepts of $Co(bpy)_3^{3+}$ and blank experiments (corresponding to a surface excess, Γ, of 2.95×10^{-11} mol/cm^2), and the lack thereof in the case of $Co(phen)_3^{3+}$, provides evidence for an adsorptive complication to the $Co(bpy)_3^{3+}$ voltammetry and the uncomplicated behavior of $Co(phen)_3^{3+}$.[37] $Co(bpy)_3^{3+}$ is probably weakly adsorbed at glassy carbon, under these conditions, since $i_{pa}/i_{pc} < 1$, but no adsorption postwave is observed.[36] Even if no correction is made for the adsorptive component of the total current for reduction of $Co(bpy)_3^{3+}$, the data indicate that $Co(bpy)_3^{3+/2+}$ is moderately strongly bound to DNA, since i_{pc} decreased to 56% of that in the absence of DNA, for a solution with R = 70 (100 mV/s). Equilibrium binding data for $Co(bpy)_3^{3+}$ thus probably represent lower limits of binding parameters, becuase the small surface excess of oxidized form at the electrode increases the current measured at any R. The features of the voltammetric behavior of $Co(bpy)_3^{3+}$ and $Fe(bpy)_3^{2+}$ are summarized in Tables IV and V.

Titration of Metal Complexes with DNA. The development of a method to quantify the binding of the electroactive metal complexes to DNA is composed of two parts. First, an equation based on an equilibrium binding model is derived, to compute the relative concentrations of free and DNA-bound complex in bulk solution, as a function of [NP]. Then, voltammetric equations relating the measured value of i_{pc} or i_{pa} to mass transfer of the mixture of free and bound metal to the electrode surface must be chosen, depending on whether the exchange of free and bound species is static or mobile (see below) on the time scale of the CV experiment.

We consider the binding of an electroactive metal center, M, to a binding site, S, composed of s base pairs (bp), residing on the duplex DNA strand (resulting in a bound species, M–S):

$$M + S = M\text{–}S \tag{3}$$

The microscopic equilibrium constant for binding is

$$K = C_b/C_fC_s \tag{4}$$

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where C_b, C_f, and C_s represent the equilibrium concentrations of bound metal, free metal, and free binding sites, respectively. The total concentration of metal, C_t, is

$$C_t = C_b + C_f \quad (5)$$

and the total concentration of sites along a DNA molecule with an average total number of base pairs L is

$$xC_{DNA} = C_b + C_s \quad (6)$$

where

$$x = L/s \quad (7)$$

$$C_{DNA} = [NP]/2L \quad (8)$$

Here, s is the binding site size (in base pairs) of the small molecule interacting with DNA; C_{DNA} is the total concentration of DNA strands; x is the average number of binding sites per molecule of DNA. Solution of eq 4–6 for the concentration of bound complex as a function of [NP], making the appropriate substitutions, eq 7 and 8, to eliminate DNA strand concentration, yields

$$C_b = \frac{b - \left(b^2 - \frac{2K^2C_t[NP]}{s}\right)^{1/2}}{2K} \quad (9a)$$

$$b = 1 + KC_t + K[NP]/2s \quad (9b)$$

K represents the microscopic binding constant for each site; the successive binding constants for 1, 2, ..., n metal complexes to a DNA molecule will differ from K by appropriate statistical factors. This is analogous to the well-known situation of proton binding constants in a molecule containing several, noninteracting, basic sites. Equations 9a and 9b are valid for the assumption of noncooperative, nonspecific binding to DNA with the existence of one type of discreet binding site. While more complex analyses of the binding equilibria are possible,[38] the simple model outlined here adequately describes the voltammetric results.

Under the assumption of reversible, diffusion-controlled electron transfer, two limiting cases may be described for the current in CV, from eq 9, depending on whether the interconversion of free and bound metal, in Scheme I, is treated as static[39] (no interconversion during a scan, eq 10) or mobile[40] (rapid interconversion during a scan, eq 11), on the time scale of the CV experiment:

$$i_{pc} = B(D_f^{1/2}C_f + D_b^{1/2}C_b) \quad (10)$$

$$i_{pc} = BC_t(D_fX_f + D_bX_b)^{1/2} \quad (11)$$

where i_{pc} is total cathodic current for reduction of the bound and free metal complexes and X_f and X_b are the free and bound mole fractions, respectively, of the complex in solution ($X_f = C_f/C_t$ and $X_b = C_b/C_t$). B represents the appropriate, concentration-independent terms in the voltammetric expression. For a Nernstian reaction in CV at 25 °C,[32] $B = 2.69 \times 10^5 n^{3/2}Av^{1/2}$, where n is the number of electrons transferred per metal complex and A is the surface area of the glassy carbon disk.

It is of interest to examine the behavior of X_b as R is varied over a wide range (at constant C_t) and also to examine the voltammetric behavior, in terms of i_{pc} vs R:

$$R = [NP]/C_t \quad (12)$$

which is predicted by the model under the limiting conditions of eq 10 and 11. Evans has noted the experimental conditions under which each limiting case is justified.[41] Below, we discuss the more appropriate limiting case for this work.

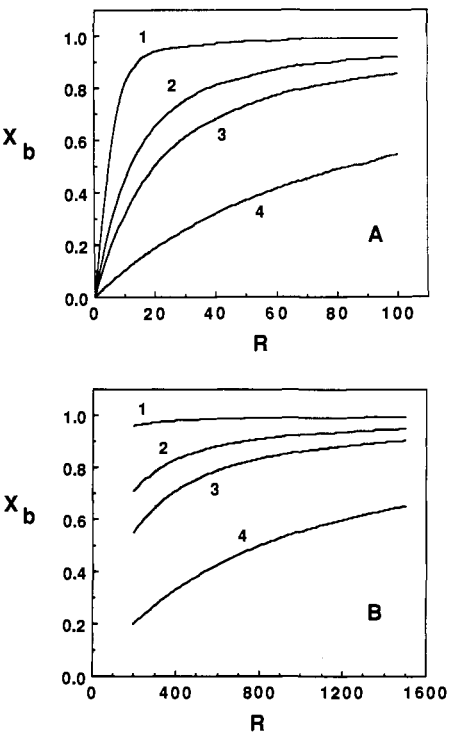

Figure 8. (A) Fraction of metal complex bound to DNA (X_b) as a function of R at $C_t = 1.0 \times 10^{-4}$ M complex. Curves 1–4 represent $K = 1 \times 10^5$, 1×10^4, 5×10^3, and 1×10^3 M^{-1}, respectively, for $s = 4$ bp. (B) Fraction of metal complex bound to DNA as a function of R, for $C_t = 1.0 \times 10^{-5}$ M complex. Other parameters are as given in (A).

The characteristic behavior of the equilibrium binding model is shown in Figure 8. The fraction of electroactive species bound to DNA, X_b, is shown as a function of R, for a binding site size (s) of 4 bp. Curves 1–4 represent binding constants, K, of 1×10^5, 1×10^4, 5×10^3, and 1×10^3 M^{-1}, respectively, with $C_t = 1.0 \times 10^{-4}$ M (CV conditions, Figure 8A) or 1.0×10^{-5} M (DPV conditions, Figure 8B). The steepness of the initial rise in X_b with increasing R increases as K becomes larger. At low K, e.g., 1×10^3 M^{-1} (Figure 8A, curve 4), X_b barely reaches 0.5, over the range $0 \le R \le 80$. For K between 5×10^3 M^{-1} and 1.0×10^4 M^{-1}, X_b reaches 0.8–0.9 over the same range of R. Thus, under the conditions required for CV measurements, the metal complex–DNA mixture is composed of 80–90% bound complex and 10–20% free complex, for typical values of K. The largest change in X_b with increasing R occurs at $R \le 30$. This is consistent with our observations that little change occurs in $E_{1/2}$ at $R > 30$. Figure 8B shows that for both phen complexes and $Co(bpy)_3^{3+}$ (see Table VI) the lower limit on X_b is ca. 0.8–0.9 (with D_b measured at $R \ge 1000$). $Fe(bpy)_3^{2+}$ binding is much weaker, even at reduced ionic strength, where X_b is ca. 0.5. Thus, the values of all D_b measured by DPV contain some contribution from equilibrium free metal. In most cases, reasonable estimates can be obtained. However, the smaller the actual value of K for a system, the less exact the estimates of D_b will be, due to limitations imposed by the maximum value of R that can be attained, as governed by the detection limits of the DPV method and the solubility of DNA. Several other physical contributions to D_b may exist which can potentially increase its experimentally determined value (see below), which are not related to free electroactive species.

It is also necessary to examine the behavior of the two limiting models, eq 10 and 11. In the limit of no interconversion of bound and free species (in each oxidation state), on the time scale of the CV experiment, the total current is always smaller ($R > 0$) than that in the case of rapid (mobile) interconversion. Thus, assumption of rapid interconversion will yield values of K that are

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Table VI. Cyclic Voltammetric Titration of Co(III)/(II) and Fe(II)/(III) Complexes[a,b]

		experiment[c]		regression[d]					
complex	model	10^6D_f/cm^2·s^{-1}	10^7D_b/cm^2·s^{-1}	10^6D_f/cm^2·s^{-1}	10^7D_b/cm^2·s^{-1}	$10^{-3}K_{3+}$/M^{-1}	s/bp	K_{2+}/K_{3+}	$10^{-3}K_{2+}$/M^{-1}
$Co(phen)_3^{3+}$	static	3.7 (0.3)	2.6 (0.3)	4.2	2.6	16 (2)	6	1.94	30
	mobile	3.7 (0.3)	2.6 (0.3)	4.2	2.6	26 (4)	5	1.94	51
$Co(bpy)_3^{3+}$	static	5.0 (0.3)	3.2 (1.0)	4.5	12	9.4 (1.5)	3	0.58	5.4
	mobile	5.0 (0.3)	3.2 (1.0)	4.5	12	14 (3)	3	0.58	8.4
$Fe(phen)_3^{2+}$	static	4.9 (0.5)	0.9 (0.04)	4.9	0.9	7.1	5	1	7.1 (0.2)
	mobile	4.9 (0.5)	0.9 (0.04)	4.9	0.9	14.7	4	1	14.7 (0.4)
$Fe(bpy)_3^{2+}$	static	2.8 (0.3)	1.8 (0.3)	2.8	1.8	5.0	4	0.21	1.1 (0.6)
	mobile	2.8 (0.3)	1.8 (0.3)	2.8	1.8	6.6	3	0.21	1.4 (0.1)

[a] $[Co(phen)_3^{3+}] = 1.0 \times 10^{-4}$M; $[Co(bpy)_3^{3+}] = 1.0 \times 10^{-4}$ M. Supporting electrolyte, 50 mM NaCl + 5 mM Tris, pH 7.1. Sweep rate, 100 mV/s. [b] $Fe(phen)_3^{2+}$: 3.43×10^{-5} mol of NP titrated with 0.69 mM complex. Supporting electrolyte, 50 mM NaCl + 5 mM Tris, pH 7.1. $Fe(bpy)_3^{2+}$: 3.83×10^{-5} mol of NP titrated with 0.75 mM complex. Supporting electrolyte, 10 mM NaCl + 10 mM Tris, pH 7.1. Sweep rate, 500 mV/s. [c] Numbers in parentheses are standard deviations of experimental measurements. [d] Numbers in parentheses represent 95% confidence interval of parameter estimate from nonlinear regression.

larger than those predicted by the static limit, given the same diffusional parameters and binding site size. For our systems, the two limiting cases give experimental K values which differ by no more than a factor of 2.0. It is difficult to determine exactly the more appropriate model for the measured current, since the reduction processes of free and bound material are not well resolved. Thus, we present results for calculations of both limiting cases.

Finally, some qualitative aspects of the influence of K, s, and D_b on the modeling of the binding equilibrium are presented, to determine how to choose the best-fit parameters in the most judicious fashion. The end point of the titration curve becomes more pronounced as K is increased. At binding constants of $<10^3$ M^{-1}, the curve is barely distinguishable from a straight line, and no useful information can be obtained in this binding regime. The degree of decrease in current, compared to a solution without added DNA, is also dependent on D_b. Changes in the magnitude of D_b cause no major changes in the curvature of the titration curve in the region of the end point, but they change the point at which the current no longer decreases significantly (e.g., in experiments where decrease in current is plotted as a function of increasing R, at constant C_t). The ratio D_f/D_b and the accuracy with which D_b can be measured will ultimately by the limiting factors in the definition of the binding curve and thus in the ability to fit curves for values of K and s. It is always desirable to have systems in which D_f/D_b values are as large as possible. In our studies, experimentally determined ratios are between 14 and 55; these are large enough to yield useful titration curves. The determination of s presents the most difficulty, since it (1) changes the degree of curvature at the end point, (2) changes the point at which the current stops decreasing significantly, and (3) changes the position of the end point along the R axis. Effect 3 is expected, since s determines the end point of the titration experiment. However, effects 1 and 2 suggest that s is correlated with both K and D_b and thus cannot reliably be fitted simultaneously with the other parameters of interest. Our approach was to set D_f and D_b (adjusted only within the limits of their experimental determination) and regress current–[NP] (or current–C_t) data onto eq 8 and either 10 or 11, for integer values of s. The best fit of the parameters to the experimental data was taken as those corresponding to the value of s, which yielded the minimum sum of squares deviation, between the experimental and calculated currents, with s varied over a sufficiently wide range (e.g., 1 bp $\leq s \leq$ 20 bp).

Results for the titration of 0.1 mM $Co(phen)_3^{3+}$ and 0.1 mM $Co(bpy)_3^{3+}$ with DNA are shown in Figure 9. The supporting electrolyte in both cases was buffer 1, and the best-fit curves shown are for the mobile equilibrium limit. Complete results of nonlinear regression analysis of the titration data are summarized in Table VI. The values of K_{2+} were obtained from the K_{2+}/K_{3+} ratio determined from the shift of $E_{1/2}$. Analogous titration experiments are shown in Figure 10 for experiments with $Fe(phen)_3^{3+}$ (Figure 10A) and $Fe(bpy)_3^{3+}$ (Figure 10B). The curves shown are for mobile exchange; complete results are given in Table VI. In these cases the experiment was performed differently from those in Figure 9; a solution initially containing only DNA was titrated

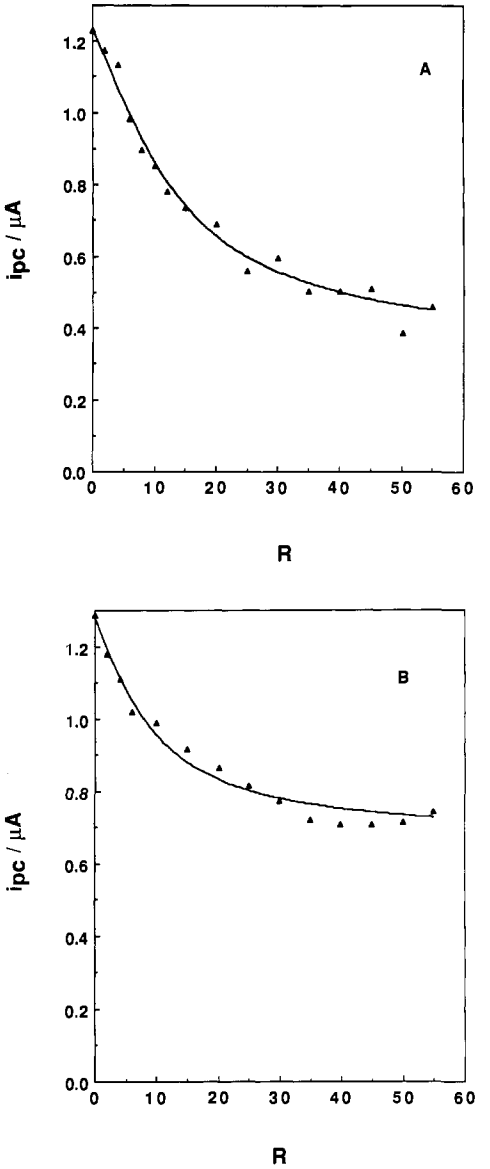

Figure 9. Titration of (A) 1.0×10^{-4} M $Co(phen)_3^{3+}$ and (B) 1.0×10^{-4} M $Co(bpy)_3^{3+}$ with DNA. Points represent experimental data, and the solid curve represents the results for best-fit parameters with consideration of the mobile equilibrium limiting case, as given in the text. Sweep rate, 100 mV/s. Supporting electrolyte, buffer 1.

with the metal complex. Exactly the same information about binding is obtained from this experiment. Binding of the Fe complexes is weaker in each ligand case than that of the corresponding Co complexes. These differences probably reflect different contributions to binding by both electrostatic and inter-

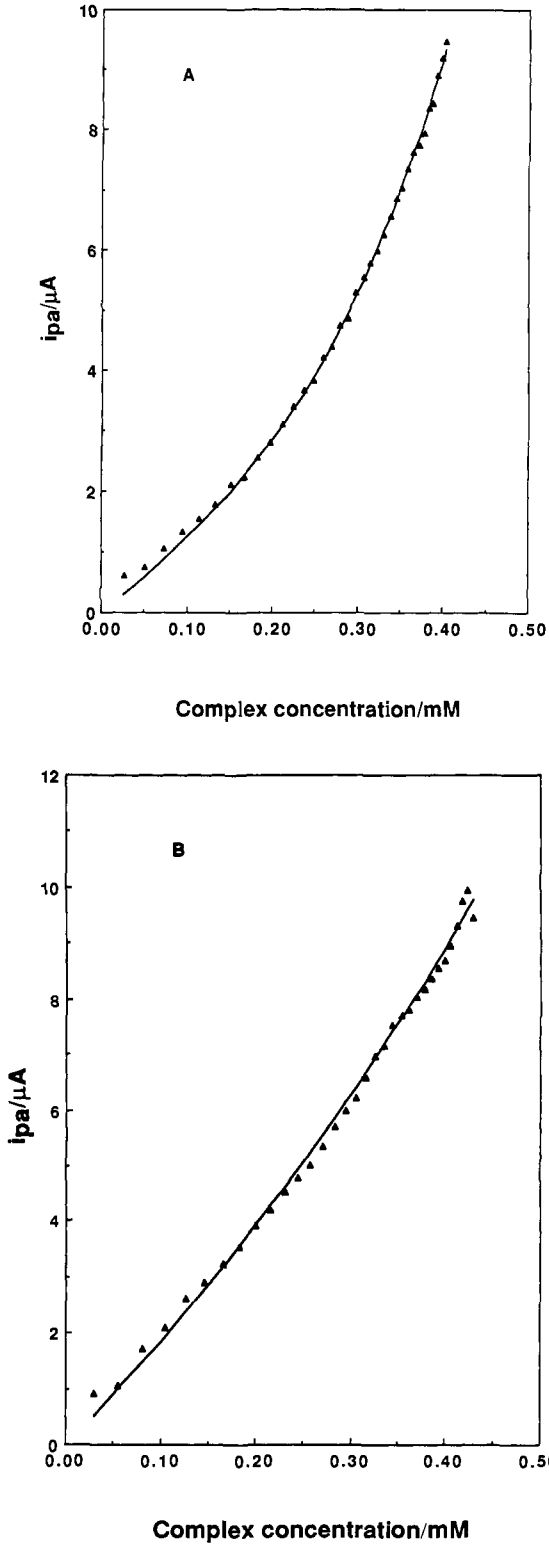

Figure 10. Titration of (A) 3.43×10^{-5} mol of NP with 0.68 mM $Fe(phen)_3^{2+}$ and (B) 3.83×10^{-5} mol of NP with 0.75 mM $Fe(bpy)_3^{2+}$. Points represent experimental data, and the solid curve represents the best fit for the binding parameters given in the text, with consideration of the mobile equilibrium limiting case. Supporting electrolyte, buffer 2. Sweep rate, 500 mV/s. Initial solution volume, 5 mL.

calative components of the interactions of these complexes with DNA, as discussed in detail in the following section.

Discussion

Differentiation of Intercalative and Coulombic Interactions. The shifts in the formal potential, $E^{\circ\prime}$, of the $M(phen)_3^{3+/2+}$ and $M(bpy)_3^{3+/2+}$ redox couples can be understood in terms of the predominant mode of interaction of each complex with the DNA strand.

As shown by Barton,[2a,d] tris-chelated transition-metal complexes with phen ligands bind to DNA by intercalation, with partial insertion of one of the ligands between adjacent base pairs on the DNA duplex strand. The remaining ligands are disposed along the major groove of the DNA molecule, and therefore, the complex can interact electrostatically with the sugar–phosphate backbone and hydrophobically with the environment in the region of the paired nucleotide bases.

The configuration of the metal complex, when in contact with the DNA helix, has been deduced for $Ru(phen)_3^{2+}$ and $Ru(DIP)_3^{2+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline).[5a-c] Similar binding has been suggested for $Fe(phen)_3^{2+}$.[7] The structurally analogous complexes of Co(III) probably bind by partial ligand intercalation and dispose themselves along the DNA molecule in a similar fashion, since their enantiospecificity toward B-form vs Z-form DNA and ability to unwind supercoiled, closed circular DNA are directly analogous to the ruthenium(II) cases.[13] The noncovalent interaction of the intercalating ligand with the DNA base pairs[2b,c] and the concomitant disposition of the remaining ligands along the groove[5h] brings the $Co(phen)_3^{3+}$ complex into close contact with an environment that is hydrophobic compared to the region of the charged sugar–phosphate backbone. The hydrophilic coat/hydrophobic core structure of the DNA molecule has been discussed previously.[42] The interplay between electrostatic and hydrophobic (intercalative) interactions therefore can be important in the overall binding of a charged species which possesses a planar, aromatic moiety.[2c,43]

The limiting shift in $E^{\circ\prime}$ of +17 mV for the $Co(phen)_3^{3+/2+}$ couple ($0 \leq R \leq 90$) shows, via eq 2, that the 2+ ion is bound to DNA 1.94 times more strongly than the 3+ ion. This suggests that the intercalated complex interacts predominantly with the hydrophobic interior of the DNA strand, so that reduction of the 3+ ion is facilitated compared to that of a solution that does not contain DNA. Electrochemical effects influenced by hydrophobic interactions of charged, electroactive molecules with hydrophobic or amphiphilic host matrices have been described previously, e.g., for the interactions of viologens and metal chelates with micelles[33,40c,44] and perfluorosulfonated (Nafion) polymers.[45] For example, the 1+ form of 1-decyl-1′-methyl-4,4′-bipyridinium binds more strongly to Triton X-100 micelles than the 2+ form.[44a] $Os(bpy)_3^{2+}$ binds to SDS (sodium dodecyl sulfate) micelles more strongly than $Os(bpy)_3^{3+}$,[44b] and MV^+ (MV = methylviologen, 1,1′-methyl-4,4′-bipyridinium) binds more strongly than MV^{2+} to SDS.[33] $Ru(bpy)_3^{2+}$ associates more strongly than the 3+ cation with Nafion.[45] Hydrophobic interactions have also been implicated in the association of CoL_3^{2+} (where L = bpy or 4,4′-dimethyl-bpy) with CTAB (cetyltrimethylammonium bromide) micelles.[46] These examples show that hydrophobic interactions, in which reduction of the total charge on the electroactive species yields stronger binding in the hydrophobic domain of the host matrix, can be important in describing the binding of a charged molecule to an amphiphilic matrix, and in many cases these interactions can overcome simple coulombic interactions (e.g., between a positively charged metal complex and a negatively charged SDS micelle or in this case the negatively charged sugar–phosphate backbone of DNA). The nature of the solvent can have substantial effects on electron-transfer thermodynamics.[47] The DNA strand may be considered as a local "solvent" environment, as far as bound metal

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complex is concerned, which differs from the bulk medium in dielectric constant and charge distribution. In fact, an increase in the hydrophobic character of the bulk solvent via addition of alcohols can effectively decrease the binding of the strongly intercalating ethidium cation to DNA.[48] However, since many systems that demonstrate intercalation also show ionic strength dependent binding,[5c,g,7,49] there is generally an interplay between electrostatic and hydrophobic interactions, even in systems in which intercalation is evident. The degree to which hydrophobic interactions predominate over electrostatic ones is likely to be dictated by structural, geometric, and charge considerations for the binding molecule. Since the $Fe(phen)_3^{2+/3+}$ couple shows weaker binding than the corresponding Co(III)/(II) couple and no preference of either the oxidized or reduced form for DNA, under the same experimental conditions, for $Fe(phen)_3^{2+/3+}$ the intercalative component of binding is probably less important than in $Co(phen)_3^{3+/2+}$. One possible explanation of this effect is that the intercalating phen ligand penetrates between adjacent base pairs on DNA to a smaller extent in the Fe(II) case than in the Co(III) case.

Tris-chelated metal complexes possessing bpy ligands bind to DNA predominantly via electrostatic intercalations with the negatively charged deoxyribose–phosphate backbone.[5c,e,i,7] The smaller size of the bpy ligand vs phen and slight nonplanarity of the ligand preclude effective intercalation between adjacent base pairs on DNA.[5e] For example, $Ru(bpy)_3^{2+}$ does not unwind poly[d(G-C)] or poly[d(A-T)], and its excited-state luminescence is rapidly quenched by ferrocyanide in the presence of DNA, as opposed to that of $Ru(phen)_3^{2+}$, which is protected from quenching while intercalated into DNA.[5e] These observations, in addition to the ionic strength dependence of the binding of bpy-ligated complexes to DNA,[5c,i] support the conclusion that the bpy complexes reside primarily at the outer, hydrophilic coat of the DNA helix, with no significant intercalative component to the binding process. The shift of $E^{\circ\prime}$ for $Co(bpy)_3^{3+/2+}$ to more negative potentials by −14 mV ($K_{2+}/K_{3+} = 0.6$, buffer 1) and of $Fe(bpy)_3^{2+/3+}$ by −41 mV ($K_{2+}/K_{3+} = 0.21$, buffer 2) indicates that the 3+ ion is bound more strongly than the 2+ ion in each case. This is consistent with electrostatic binding of $M(bpy)_3^{3+/2+}$ to DNA via the anionic phosphate residues with release of Na^+ counterions from the DNA strand.[50]

Comparison to Previous Studies. Binding of a number of other metal chelates with DNA has been studied previously. For example, the $Ru(phen)_3^{2+}$ complex, whose binding constant K_{2+} has been measured by equilibrium dialysis (6.2×10^3 M^{-1} in 50 mM NaCl[5a] and 2.13×10^3 M^{-1} in 100 mM NaCl[5g]), binds less strongly than the corresponding Fe and Co species. However, the Pt(I) complex, $Pt(en)(phen)^+$ binds more strongly; $K_+ = 1.8 \times 10^5$ M^{-1} in 100 mM NaCl.[51] The binding of $Ru(bpy)_3^{2+}$, which like the bpy complexes of Co and Fe involves mainly electrostatic interactions, has been studied at different ionic strengths. No detectable binding of $Ru(bpy)_3^{2+}$ to DNA was observed in 50 mM NaCl,[5e] while at 10 mM phosphate buffer some weak binding has been observed,[5c] but not precisely quantitated. K_{2+} for $Ru(bpy)_3^{2+}$ is 2×10^5 M^{-1} at an ionic strength less than 5 mM,[51] with s = 1.5 bp. Other workers have reported K_{2+} of $Ru(bpy)_3^{2+}$ to be 3.0×10^6 M^{-1} (s = 10 bp) at 1.0 mM NaCl and 1.4×10^6 M^{-1} (s = 25 bp) at 10.0 mM NaCl.[7] In general, the values of s for the bpy complexes are smaller than those of the corresponding phen complexes, perhaps reflecting the slightly smaller size of the bpy complexes.

The relative magnitudes of the binding constants for the structurally similar complexes here agree with the concept that interactions involving intercalation are usually stronger than the corresponding purely electrostatic ones under equivalent experimental conditions. The binding constants obtained here are the averages of the behavior expected for pure Δ and Λ enantiomers of the complexes, especially in the case of $M(phen)_3^{n+}$. Binding of racemic $Ru(phen)_3^{2+}$ to DNA was intermediate between that of the Δ and Λ enantiomers.[2a,d] The binding constants are also smaller than those obtained for the classical intercalators, e.g., ethidium and proflavin, where complete insertion of the planar molecules between base pairs is possible.[2b] For example, K_+ for ethidium is 7×10^7 M^{-1} in 40 mM Tris-HCl buffer, pH 7.9,[49b] and 1.4×10^6 M^{-1} in 40 mM NaCl–25 mM Tris-HCl,[49c] and proflavin binds with $K = 4.1 \times 10^5$ M^{-1} (*Escherichia coli* DNA, 50% GC content) in 0.1 M Tris-HCl.[52]

Mass Transfer and Equilibrium Considerations. To our knowledge, no kinetic data are available for determining the appropriate limiting case (static or mobile) for exchange of free and bound metal complex, in each oxidation state, and the more appropriate eq 10 or 11, for use in the analysis. We suggest, however, that the mobile limit (rapid interconversion of free and bound complex on the time scale of the voltammetric measurement) may be the more accurate representation of these systems, since no appreciable kinetic limitations to the redox reactions were observed in i_{pc}–$\nu^{1/2}$ trends or in the dependence of ΔE_p on ν. Kinetic contributions from rate-limiting exchange of free and bound complex would be expected to give significantly broader ΔE_p values than those observed here,[44,53] on the basis of the proposed reaction scheme. The diffusion coefficients of bound metal complex, measured by DPV, are somewhat larger than those previously reported for analogous samples of calf thymus DNA, where diffusion coefficients have been previously reported as 9.2×10^{-8} cm^2/s[34b] and 1.3×10^{-8} cm^2/s.[54] The increase in magnitude of D_b can be explained by the existence of a small equilibrium concentration of free metal complex. This will exaggerate the value of D_b. Since the binding constants of systems such as those studied here are rather small (typically $\leq 3.0 \times 10^4$ M^{-1}), the presence of equilibrium free metal will always compromise the determination of D_b, although relatively reliable estimates may be obtained. Since the DNA samples are probably polydisperse, diffusion of metal complex bound to small, more rapidly diffusing fragments may also contribute to enhancement of D_b. Facilitated diffusion along the DNA strand,[55] which has not been examined in this work, cannot be ruled out as a further contribution to D_b.

Conclusions

Electrochemical methods have been used to probe the mode of interaction of $M(phen)_3^{3+/2+}$ and $M(bpy)_3^{3+/2+}$ with DNA, where M = Co or Fe. Shifts in $E^{\circ\prime}$ can be used to differentiate intercalative interactions, which involve hydrophobic interactions with the interior of the DNA molecule, from electrostatic ones, which involve the outer anionic coat of DNA. Binding parameters for multiple oxidation states can be obtained from the dependence of peak current on the ratio of nucleotide phosphate to metal from titration experiments. $Co(phen)_3^{3+}$ binds intercalatively to DNA, with the reduced (2+) form associating more strongly than the oxidized form. For $Fe(phen)_3^{2+}$, no charge preference was found. $Co(bpy)_3^{3+}$ and $Fe(bpy)_3^{2+}$ bind via electrostatic interaction, where the oxidized (3+) form is bound more strongly. $Fe(bpy)_3^{2+}$ displays a marked ionic strength dependence of its binding to DNA. No binding is observed in 50 mM NaCl, whereas $Co(bpy)_3^{3+}$ binds moderately, under these conditions.

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Registry No. $Co(phen)_3^{3+}$, 18581-79-8; $Fe(phen)_3^{2+}$, 14708-99-7; $Co(bpy)_3^{3+}$, 19052-39-2; $Fe(bpy)_3^{2+}$, 15025-74-8.

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