# Chiral Discrimination in the Binding of Tris(phenanthroline)ruthenium(II) to Calf Thymus DNA: An Electrochemical Study

Sethuraman Mahadevan and Mallayan Palaniandavar*

Department of Chemistry, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India.
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The binding of Δ-, Λ-, and *rac*-$[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) and Δ-, Λ-, and *rac*-$[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridyl) with calf thymus DNA has been examined by cyclic and differential pulse voltammetric techniques to obtain structural insight into the noncovalent binding of the enantiomers to DNA. The insignificant shift in $Ru^{II}/Ru^{III}$ peak potentials on the addition of DNA suggests that both the oxidized and reduced forms bind to DNA to the same extent. Interestingly, DNA selectively decreases the peak currents of Δ-$[Ru(phen)_3]^{2+}$ but not those of the Λ-enantiomer; *rac*-$[Ru(phen)_3]^{2+}$ exhibits an intermediate behavior, thus suggesting that the Δ-form exhibits significant selectivity for B-DNA. The binding constants ($K_{2+}$) and binding site sizes (*s*) have been determined from the decrease in the peak currents. The binding constant ($K_{2+}$) of Δ-$[Ru(phen)_3]^{2+}$ is on the order of $10^4$ $M^{-1}$ which is less than that for proven intercalators. In contrast, the electrochemical behavior of all three forms of $[Ru(bpy)_3]^{2+}$ remains almost unaffected in the presence of DNA, suggesting that the complexes might reside on the hydrophilic coat of the DNA helix.

## INTRODUCTION

The study of the consequences of incorporation of stereochemistry (chirality) into small inorganic complexes that bind to DNA has received enormous attention and exploitation. Chiral octahedral ruthenium complexes containing aromatic ligands like phenanthroline (phen) and bipyridyl (bpy) have the potential as possible nonradioactive probes of DNA secondary structure (*1*). Various spectroscopic and photophysical techniques and other methods have been employed to understand the mode of their binding to DNA (*2*−*8*). Such a knowledge would help to identify the features of DNA, which are being recognized, and is essential for the further development of compounds of practical and sensitive probes of DNA structure. Additional thermodynamic information is needed to determine how useful the ruthenium enantiomers are as probes of DNA structure.

Satyanarayana et al. have reviewed what is known concerning the DNA binding details of the most well-studied $[Ru(phen)_3]^{2+}$ complex (*9*). On the basis of electric dichroism studies, Yamagishi et al. showed that Δ- and Λ-$[Ru(phen)_3]^{2+}$ are oriented differently relative to the DNA helix axis and that the Δ-form is bound to DNA with one of its phen ligands intercalated between the base pairs (*8*) while the Λ-form is not intercalated but instead is bound by an electrostatic mechanism (*10*). On the basis of unwinding studies (*11*) using closed circular DNA, Barton et al. suggested that the Δ-enantiomer possesses greater affinity for the DNA duplex. They also showed that the hypochromic effect in the absorption spectra of bound Δ- and Λ-enantiomers is small compared to those of other known intercalators and that the increase in fluorescence lifetime in the presence of DNA is higher for the Δ- than for the Λ-enantiomer. However, equilibrium dialysis and fluorescence lifetime measurements (*4*) showed that both intercalation and surface binding were used by both enantiomers to bind to DNA. The recent NMR experiments (*6*, *12*) on the interaction of ruthenium enantiomers with the oligonucleotides $d(GTGCAC)_2$ and $d(CGCGCG)_2$ led to the conclusion that the Δ-form prefers intercalation while the Λ-form prefers surface binding in the minor groove. In contrast, linear dichroism (*7*) and two-dimensional (2D) NMR studies (*13*) revealed that both enantiomers employ nonintercalative modes to interact with DNA within the minor groove.

Very recently, Chaires et al. made a reinvestigation of the interaction of $[Ru(phen)_3]^{2+}$ with DNA (*9*) using absorbance and fluorescence methods, circular dichroism, viscosity, and competition dialysis and showed that both Δ- and Λ-$[Ru(phen)_3]^{2+}$ weakly bind to DNA by a single mode. However, the two isomers had different effects on the hydrodynamic properties of DNA as measured by viscosity which revealed that they differ in their individual binding modes. The binding of both enantiomers to DNA was found to be very weak in comparison to that of proven intercalators, with binding constants of $10^4$ $M^{-1}$. Further, both of them have been shown to bind only weakly to DNA by a predominantly electrostatic, entropically driven binding mode. The observation of modest changes in optical properties upon binding led to the conclusion that stereoselectivity is modest.

Thus, there is controversy surrounding the DNA binding mechanism of Δ- and Λ-$[Ru(phen)_3]^{2+}$, and so our recent interest in the quantitative evaluation of binding of copper complexes (*14*) to DNA using electrochemical techniques prompted us to employ this technique to understand the binding of $[Ru(phen)_3]^{2+}$ to DNA. Voltammetric techniques have so far not been exploited for noncovalent labeling of enantioselective interaction of chiral metal complexes with DNA, though they have been successfully used recently to probe the interaction of metal chelates (*15*−*18*) and a viologen-derived compound (*19*) with DNA. In fact, DNA-binding complexes and ligands that possess redox activity are scarce. We report here for the first time the results of a voltammetric investigation of the stereoselective interaction of enantiomeric and racemic $[Ru(phen)_3]^{2+}$ and $[Ru(bpy)_3]^{2+}$ with calf thymus DNA.

* Author to whom correspondence should be addressed.
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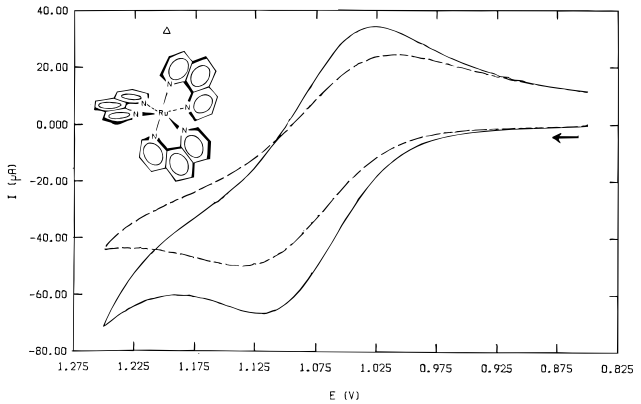


**Figure 1.** Cyclic voltammograms of $5.0 \times 10^{-4}$ M Δ-Ru(phen)$_3^{2+}$ in the absence (—) and in the presence (– –) of 12.5 mM NP: supporting electrolyte, 50 mM NaCl + 5 mM Tris-HCl (pH 7.1); and scan rate, 100 mV/s.

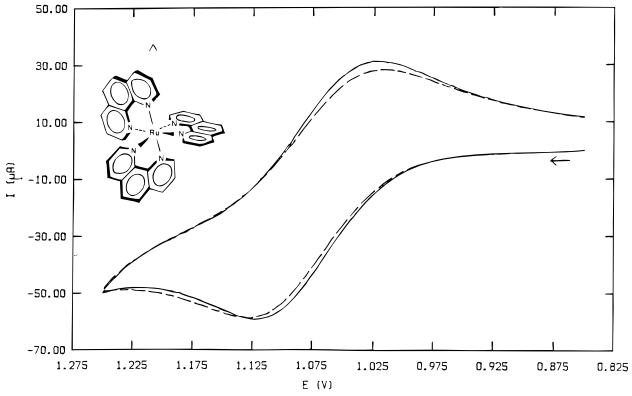


**Figure 2.** Cyclic voltammograms of $5.0 \times 10^{-4}$ M Λ-Ru(phen)$_3^{2+}$ in the absence (—) and in the presence (– –) of 12.5 mM NP: supporting electrolyte, 50 mM NaCl + 5 mM Tris-HCl (pH 7.1); and scan rate 100 mV/s.

## EXPERIMENTAL PROCEDURES

**Materials.** Disodium salt of calf thymus DNA (SIGMA) was stored at 4 °C and used as received. Solutions of DNA in 50 mM NaCl/5 mM Tris-HCl (pH 7.1) (buffer 1) gave a ratio of UV absorbance at 260 and 280 nm, $A_{260}/A_{280}$, of ≈1.9, indicating that the DNA was sufficiently pure and free of protein (*20*). Concentrated stock solutions of DNA were prepared in 50 mM NaCl/5 mM Tris-HCl (pH 7.1), and the concentration of nucleotide phosphate was determined by UV absorbance at 260 nm on 1:100 dilutions. The extinction coefficient, $\epsilon_{260}$, was taken as 6600 $M^{-1}$ $cm^{-1}$ (*21*). Stock solutions were stored at 4 °C and used after no more than 4 days.

Tris(phenanthroline)ruthenium(II) diperchlorate ([Ru(phen)$_3$](ClO$_4$)$_2$) (*22*) and tris(bipyridyl)ruthenium(II) diperchlorate ([Ru(bpy)$_3$](ClO$_4$)$_2$) (*23*) were synthesized and enantiomers separated (*24*) as described previously.

**Methods and Instrumentation.** All cyclic (CV) and differential pulse (DPV) voltammetry experiments were performed in a single compartment cell with a three-electrode configuration on an EG&G PAR 273 potentiostat/galvanostat equipped with an IBM PS/2 computer and a glassy carbon disk, and the reference electrode was a saturated calomel electrode. A platinum plate was used as the counter electrode. The supporting electrolyte was buffer 1. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to the measurements; during the measurements, a stream of $N_2$ was passed over the solution. All experiments were carried out at 25 ± 0.2 °C, maintained by a Haake D8-G circulating bath.

## RESULTS AND DISCUSSION

**Electrochemical Behavior of [Ru(phen)$_3$]$^{2+}$ and [Ru(bpy)$_3$]$^{2+}$.** Typical CV behaviors of Δ- and Λ-[Ru(phen)$_3$]$^{2+}$ in buffer 1 as the supporting electrolyte are shown in Figures 1 and 2. A summary of the voltammetric results is given in Table 1. The formal potentials of the Ru(II)/Ru(III) couple, $E^{\circ\prime}$ (or voltammetric $E_{1/2}$), taken as the average of the anodic peak potential, $E_{pa}$, and the cathodic peak potential, $E_{pc}$, determined from CV, are in good agreement with those from DPV. In DPV, the $E_{1/2}$ was determined from the peak potential, $E_p$, using the relationship (*25a*)

$$E_{1/2} = E_p + \Delta E/2 \quad (\Delta E \text{ is the pulse amplitude})$$

The enantiomers as well as the racemic form exhibited the same $E_{1/2}$ (1.074 V vs SCE) values. The CV peak potentials and $\Delta E_p$ ($E_{pa}-E_{pc}$) values were independent of scan rate over the range 0−0.5 V/s, and the $\Delta E_p°$ (obtained by the extrapolation of $\Delta E_p$ to a zero scan rate) values are almost the same (84−88 mV) (aqueous 0.1 M $NaClO_4$ solution of 1 mM (hydroxymethyl)ferrocene under identical experimental conditions gave the $\Delta E_p°$ value of 73 mV with $E_{1/2}$ 0.176 V vs SCE).

**Table 1. Voltammetric Behavior of Ru(phen)$_3^{2+}$ Enantiomers in the Presence of DNA**

| complex | $v$ (V $s^{-1}$) | $R$ | $E_{pc}$ (V) | $E_{pa}$ (V) | $\Delta E_p$ (mV) | $E_{1/2}$ (V) | $i_{pc}/i_{pa}$ | $i_{pa}/i_{pa}$ ($R = 0$) |
|---|---|---|---|---|---|---|---|---|
| Δ-Ru(phen)$_3^{2+}$ | 0.01 | 0 | 1.032 | 1.118 | 86 | 1.075 | 1.1 | 1 |
| | | 25 | 1.030 | 1.118 | 88 | 1.074 | 1.0 | 0.72 |
| | 0.05 | 0 | 1.030 | 1.120 | 90 | 1.075 | 1.1 | 1 |
| | | 25 | 1.028 | 1.118 | 90 | 1.073 | 1.0 | 0.73 |
| | 0.10 | 0 | 1.030 | 1.118 | 88 | 1.074 | 1.1 | 1 |
| | | 25 | 1.032 | 1.120 | 88 | 1.076 | 1.0 | 0.71 |
| Λ-Ru(phen)$_3^{2+}$ | 0.01 | 0 | 1.028 | 1.116 | 88 | 1.072 | 1.0 | 1 |
| | | 25 | 1.028 | 1.118 | 90 | 1.073 | 1.0 | 0.98 |
| | 0.05 | 0 | 1.028 | 1.120 | 92 | 1.074 | 1.1 | 1 |
| | | 25 | 1.026 | 1.120 | 94 | 1.073 | 1.0 | 0.98 |
| | 0.10 | 0 | 1.028 | 1.118 | 90 | 1.073 | 1.1 | 1 |
| | | 25 | 1.030 | 1.122 | 88 | 1.076 | 1.0 | 0.96 |
| *rac*-Ru(phen)$_3^{2+}$ | 0.01 | 0 | 1.028 | 1.118 | 90 | 1.074 | 1.1 | 1 |
| | | 25 | 1.030 | 1.122 | 92 | 1.076 | 1.0 | 0.87 |
| | 0.05 | 0 | 1.032 | 1.122 | 90 | 1.076 | 1.1 | 1 |
| | | 25 | 1.028 | 1.120 | 92 | 1.079 | 1.1 | 0.89 |
| | 0.10 | 0 | 1.032 | 1.120 | 92 | 1.076 | 1.1 | 1 |
| | | 25 | 1.030 | 1.120 | 90 | 1.075 | 1.0 | 0.86 |

**Table 2. Voltammetric Behavior of Ru(bpy)$_3^{2+}$ Enantiomers in the Presence of DNA**

| complex | $v$ (V $s^{-1}$) | $R$ | $E_{pc}$ (V) | $E_{pa}$ (V) | $\Delta E_p$ (mV) | $E_{1/2}$ (V) | $i_{pc}/i_{pa}$ | $i_{pa}/i_{pa}$ ($R = 0$) |
|---|---|---|---|---|---|---|---|---|
| Δ-Ru(bpy)$_3^{2+}$ | 0.01 | 0 | 1.022 | 1.100 | 78 | 1.061 | 1.04 | 1 |
| | | 25 | 1.022 | 1.100 | 78 | 1.061 | 1.10 | 0.98 |
| | 0.05 | 0 | 1.020 | 1.102 | 82 | 1.061 | 1.09 | 1 |
| | | 25 | 1.020 | 1.100 | 80 | 1.060 | 1.18 | 0.96 |
| | 0.10 | 0 | 1.024 | 1.102 | 78 | 1.063 | 1.17 | 1 |
| | | 25 | 1.022 | 1.104 | 82 | 1.063 | 1.19 | 0.95 |
| Λ-Ru(bpy)$_3^{2+}$ | 0.01 | 0 | 1.020 | 1.100 | 80 | 1.060 | 1.07 | 1 |
| | | 25 | 1.024 | 1.102 | 78 | 1.063 | 1.09 | 0.94 |
| | 0.05 | 0 | 1.020 | 1.102 | 82 | 1.061 | 1.09 | 1 |
| | | 25 | 1.020 | 1.100 | 80 | 1.060 | 1.10 | 0.96 |
| | 0.10 | 0 | 1.022 | 1.100 | 78 | 1.060 | 1.13 | 1 |
| | | 25 | 1.026 | 1.102 | 76 | 1.064 | 1.16 | 0.97 |
| *rac*-Ru(bpy)$_3^{2+}$ | 0.01 | 0 | 1.022 | 1.102 | 80 | 1.062 | 1.06 | 1 |
| | | 25 | 1.022 | 1.100 | 78 | 1.061 | 1.08 | 0.95 |
| | 0.05 | 0 | 1.020 | 1.100 | 80 | 1.060 | 1.09 | 1 |
| | | 25 | 1.024 | 1.104 | 80 | 1.064 | 1.12 | 0.94 |
| | 0.10 | 0 | 1.026 | 1.104 | 78 | 1.065 | 1.13 | 1 |
| | | 25 | 1.024 | 1.102 | 78 | 1.063 | 1.14 | 0.97 |

The voltammetric results for Δ-, Λ-, and *rac*-[Ru(bpy)$_3$]$^{2+}$ are collected in Table 2. Both the enantiomers and the racemic form exhibited the same $E_{1/2}$ (1.060 V vs SCE) and $\Delta E_p°$ (76 mV) values.

The $i_{pa}$ vs $v^{1/2}$ plots for all the present complexes give straight lines passing through the origin, and the ratio

of the cathodic to anodic peak currents, $i_{pc}/i_{pa}$, is nearly unity, suggesting that the redox systems undergo reversible one-electron transfer (*25b*) under the present conditions. The closeness of the latter ratio to 1 under all our experimental conditions indicates that both the 2+ and 3+ species are stable on the time scale of the CV measurements.

**Redox Behavior of $[Ru(phen)_3]^{2+}$ and $[Ru(bpy)_3]^{2+}$ in the Presence of Calf Thymus DNA.** On the addition of DNA ($R$ = [NP]/[Ru] = 25) to all three forms of $[Ru(phen)_3]^{2+}$, $\Delta E_p$ remains almost unaffected, $i_{pc}/i_{pa}$ is nearly unity, and the shift in $E_{1/2}$ is less than 5 mV. At higher scan rates, however, a slight increase in $\Delta E_p$ is observed, possibly due to the onset of kinetic complications. The electrochemical responses of all three forms of $[Ru(bpy)_3]^{2+}$ are also unaffected by the addition of DNA. These changes are reminiscent of small changes (*9*) in the magnitudes of optical properties of bound Δ- and Λ-enantiomers compared to those of other known intercalators. Further, $[Ru(bpy)_3]^{2+}$ has been found (*26*) to bind to DNA but at a low salt concentration. Beyond $R$ = 25, the solution became highly viscous, rendering the location of CV peaks difficult.

Analogous to the treatment of the association of small molecules with micelles (*27*), the net shift in $E_{1/2}$ can be used to estimate the ratio of equilibrium constants for the binding of the Ru(II) and Ru(III) complexes to DNA using the equation

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

where $E_b^{\circ\prime}$ and $E_f^{\circ\prime}$ are the formal potentials of the 3+/2+ couple in the free and bound forms, respectively, and $K_{2+}$ and $K_{3+}$ are the corresponding binding constants for the binding of 2+ and 3+ species to DNA, respectively. The ratio of binding constants of Ru(II) and Ru(III) species estimated is nearly 1 for all three forms of phen and bpy complexes, suggesting that both Ru(II) and Ru(III) forms interact with DNA to the same extent. This is in contrast to the observation (*19*) that $viol^{2+}$ species is bound ca. 70 times more strongly than the $viol^{\bullet+}$ species to DNA, indicating the importance of the electrostatic component in the interaction of the bis-9-acridinyl derivative containing a viologen linker chain with DNA.

**Changes in Peak Current Values in the Presence of DNA and Determination of Binding Constants.** The addition of DNA, though it causes no change in $E_{1/2}$ and $\Delta E_p$, diminishes the peak currents appreciably for the Ru(II)/Ru(III) couple of Δ-$[Ru(phen)_3]^{2+}$ (Figure 1). The plot of the anodic peak current, $i_{pa}$, vs $v^{1/2}$ in the presence of DNA is linear, with the $y$ intercept equal to zero, within the error of the measurements, as expected for a reversible electron transfer (*25b*). The slope of this plot decreases with an increase in $R$, indicating a reduction in the apparent diffusion coefficient of Δ-$[Ru(phen)_3]^{2+}$. Thus, the slope of line A of Figure 3a ($R$ = 0) gives the diffusion coefficient of free Δ-$[Ru(phen)_3]^{2+}$, $D_f$, of $(3.08 \pm 0.40) \times 10^{-5}$ cm$^2$/s, while line B corresponds to an apparent $D$ value of $(1.75 \pm 0.20) \times 10^{-5}$ cm$^2$/s ($R$ = 25). In contrast, no significant change in peak currents is observed for the Λ-isomer (Figure 2). For the racemic one, as expected, a decrease lesser than that for the Δ-isomer is observed (Figure 3b). Thus, the apparent reduction in anodic peak current for the addition of excess DNA ($R$ = 25) is 26% for the Δ-isomer, while it is only 12% for the racemic one. The decrease in current upon the addition of DNA may be interpreted in terms of diffusion of an equilibrium mixture of free and DNA-bound ruthenium complex to the electrode surface. This suggests that Δ-$[Ru(phen)_3]^{2+}$ binds to DNA more strongly

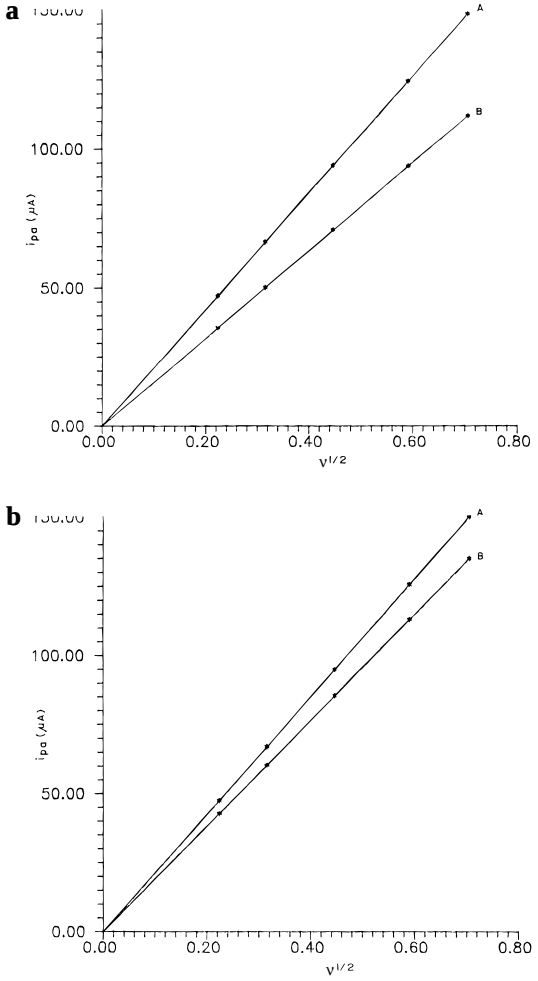


**Figure 3.** Effect of DNA on the diffusion of (a) Δ-$Ru(phen)_3^{2+}$ at (A) $R$ = 0 and (B) $R$ = 25 and (b) *rac*-$Ru(phen)_3^{2+}$ at (A) $R$ = 0 and (B) $R$ = 25.

than the Λ-isomer. The observation of enantioselectivity is consistent with that by Barton et al. (28); for DNA-mediated long range electron transfer from photoexcited $[Ru(phen)_3]^{2+}$ enantiomers to isostructural complexes of Co(III), Rh(III), and Cr(III) bound along the DNA strand, the rate constant for the Λ-enantiomer is from 1.5 to 2 times greater than that for the Δ-enantiomer, suggesting the preferential overall binding of the latter to DNA.

From the dependence of peak currents on the value of $R$ (Figure 4), the binding parameters for binding of ruthenium species to DNA have been calculated (Table 3), assuming both static and mobile equilibria using the treatment given by Bard et al. (16) by

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

$$i_t = BC_t(X_f D_f + X_b D_b)^{1/2} \text{ (mobile equilbiria)} \quad (2)$$

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

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

$$b = 1 + C_t K + K[NP]/2s$$

fitting the experimental data into eqs 1−3 by a nonlinear regression analysis, where $i_t$ is the total anodic peak current, $A$ is the area of the working electrode, $n$ is the number of electrons involved in the redox reaction, $\nu$ is the scan rate, $C_b$ is the concentration of the bound

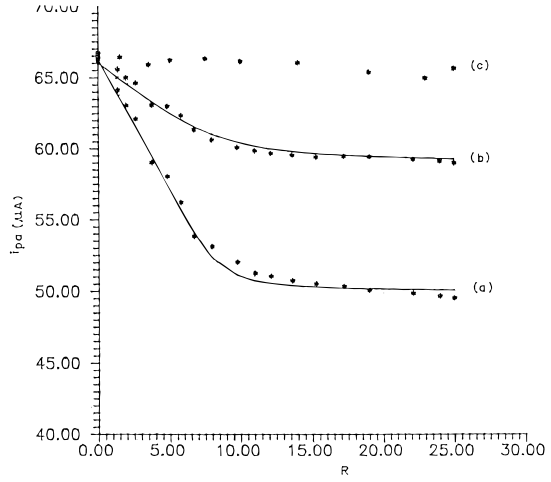


**Figure 4.** Voltammetric titration [measurement of $i_{pa}$ at different $R$ (=[NP]/[Ru]) values] of (a) $5 \times 10^{-4}$ M Δ-Ru(phen)$_3^{2+}$, (b) $5 \times 10^{-4}$ M *rac*-Ru(phen)$_3^{2+}$, and (c) $5 \times 10^{-4}$ M Λ-Ru(phen)$_3^{2+}$ with CT DNA. The points represent experimental data while the solid lines the best fit for the mobile equilibrium-limiting case.

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, and $K$ is the binding constant for the interaction of [Ru(phen)$_3$]$^{2+}$ with DNA. The insignificant changes in peak current for Λ-[Ru(phen)$_3$]$^{2+}$ and for all the forms of [Ru(bpy)$_3$]$^{2+}$ did not permit the calculation of the binding parameters.

**Binding Mechanism for Ruthenium Enantiomers; Δ-[Ru(phen)$_3$]$^{2+}$ Is a Nonclassical Intercalator.** In the original work of Barton et al. utilizing luminescence and NMR methods, two major DNA binding modes are described (*4*, *6*): one intercalative favored by Δ-[Ru(phen)$_3$]$^{2+}$ and the other a "surface bound" mode favored by Λ-[Ru(phen)$_3$]$^{2+}$. The novel EPR and time-resolved luminescence studies (*29*) on nitroxide-labeled Ru(II) polypyridyl complexes reveal, in addition to these two independently different modes of binding, the presence of unbound complex species in the atmospheric collar of ions, which is not entirely independent of or is loosely associated with the polyanionic DNA.

In the present study, the magnitude of the binding constant, $K_{2+}$, determined for the Δ-isomer is found to be greater than that for the racemic form, suggesting the preferential enantioselective interaction of the former with B-DNA. The interaction may be primarily intercalative, involving the hydrophobic interior of the DNA molecule, and it is diffulct to rule out the other two modes of interaction involving the outer anionic coat of DNA. The intercalation may be partial as suggested earlier (*11*, *26*, *30*), stacking one phen in between the adjacent base pairs in the DNA helix and the remaining two phen ligands pointing outward and aligned along the right-handed major groove. Interestingly, the same binding site size ($s$ = 4) is obtained for both Δ- and *rac*-forms, supporting the involvement of the same binding mode. The values of $K$ and $s$ are in reasonable agreement with those obtained by optical spectroscopy ($K = 5.5 \times 10^3$ M$^{-1}$; $s$ = 5 bp) (*5*), fluorescence measurements ($K = 4.9 \times 10^4$ M$^{-1}$) (*9*), electrochemiluminescence investigation ($K = 8.1 \times 10^3$ M$^{-1}$; $s$ = 4 bp) (*31*), and DNA-melting studies ($K = 5.5 \times 10^4$ M$^{-1}$) (*9*) and are in the same range as those determined (*16*) for the corresponding Co(III) ($K = 2.6 \times 10^4$ M$^{-1}$; $s$ = 5 bp) and Fe(II) ($K = 1.5 \times 10^4$ M$^{-1}$; $s$ = 4 bp) complexes.

Further, the binding constants obtained here are lower than those obtained for the classical intercalators, e.g., ethidium and proflavin, compounds with proven DNA binding mode where complete insertion of the planar molecules between base pairs is possible. For example, $K_+$ for ethidium is $7 \times 10^7$ M$^{-1}$ in 40 mM Tris-HCl buffer (pH 7.9) (*32a*) and $1.4 \times 10^6$ M$^{-1}$ in 40 mM NaCl/25 mM Tris-HCl (*32b*), and proflavin binds with $K = 4.1 \times 10^5$ M$^{-1}$ (*Escherichia coli* DNA, 50% GC content) in 0.1 M Tris-HCl (*33*). The overall nonplanar structure of the present tris(phen) complexes, which contrasts with the fused ring aromatic structures of intercalating agents, make classical intercalation or groove binding either impossible or extremely unlikely. Hence, for steric reasons, these compounds cannot fully intercalate into DNA, and so binding is expected to occur by the partial insertion of a portion of the compound between the adjacent DNA base pairs. This is consistent with the molecular-modeling and energy minimization calculations (*34*). Thus, the present phen complexes may be considered to fall among a third class (*9*) of partially intercalating or nonclassical DNA binding agents.

The noncovalent interaction of one of the phen ligands with the DNA base pairs and the concomitant disposition of the remaining ligands along the groove brings the Δ-[Ru(phen)$_3$]$^{2+}$ complex into close contact with an environment that is hydrophobic compared to the region of the charged sugar−phosphate backbone. 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 like a phen ring. Generally, there is 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 there is no preference for either the oxidized or the reduced form of Δ- and *rac*-[Ru(phen)$_3$]$^{2+}$ species for DNA, charge appears to be less important and the intercalative component of binding the most important.

It is worth noting that the $i_{pa}$ vs $R$ plot (Figure 4) for both *rac*- and Δ-[Ru(phen)$_3$]$^{2+}$ levels off even at low $R$ (≈10) values. This is interesting because, for the corresponding Fe(II) and Co(III) complexes, the leveling off takes place at a higher $R$ (≈30) value (*16*). This is similar to the decrease in the hydrodynamic property (viscosity)

**Table 3. Cyclic Voltammetric Titration of Ru(phen)$_3^{2+}$ with DNA[a]**

| | | experiment[b] | | regression[c] | | | |
|---|---|---|---|---|---|---|---|
| complex | model | $10^5 D_f$ (cm$^2$ s$^{-1}$) | $10^5 D_b$ (cm$^2$ s$^{-1}$) | $10^5 D_f$ (cm$^2$ s$^{-1}$) | $10^5 D_b$ (cm$^2$ s$^{-1}$) | $10^{-4} K_{2+}$ (M$^{-1}$) | $s$ (bp) |
| *rac*$^-$ | static | 3.11 (0.4) | 2.52 (0.5) | 3.05 | 2.41 | 1.3 (0.8) | 4 |
| | mobile | 3.11 (0.4) | 2.52 (0.5) | 3.05 | 2.41 | 1.8 (1.4) | 4 |
| Δ$^-$ | static | 3.08 (0.4) | 1.75 (0.2) | 3.03 | 1.73 | 6.1 (0.6) | 4 |
| | mobile | 3.08 (0.4) | 1.75 (0.2) | 3.03 | 1.73 | 10.4 (1.0) | 4 |

[a] $D_f$ = free diffusion coefficient. $D_b$ = bound diffusion coefficient. $K$ = binding constant. $s$ = binding site size in base pairs (bp). [b] Numbers in parentheses are standard deviations of experimental measurements. [c] Numbers in parentheses represent the 95% confidence interval of the parameter estimate from nonlinear regression.

of DNA at a low binding ratio, which is consistent with the bending or kinking of the DNA helix upon binding of the Δ- or *rac*-form to DNA by partial intercalation (*9*). So the decrease in peak current may be due to the involvement of the Δ-enantiomer in the formation of kinks in the DNA helix. As more kinks are formed with increased binding, a rodlike superhelical structure would result since the kinks would not be phased (*35*); this leads to the formation of a plateau, a stage beyond which further binding of complex is restricted. It is interesting to note that the Λ-form has been reported (*9*) not to encourage kinking. However, it should be pointed out that there is no direct evidence for the metal-promoted kink formation.

**The Electrochemical Technique Is More Sensitive than Optical Methods for the Study of the Interaction of Redox Species with DNA.** The DNA strand may be considered a local "solvent" environment as far as bound metal complex is concerned. Since the nature of the solvent can have substantial effects on electron transfer thermodynamics and since the Δ-enantiomer bound via partial intercalation differs in dielectric constant and charge distribution from the corresponding complex in bulk medium, the binding could be very easily detected by the decrease in peak currents which depend on the concentration and diffusion coefficient of the redox active species. Thus both Δ- and Λ-$[Ru(phen)_3]^{2+}$ bind to DNA by different binding modes which consequently manifest themselves in peak currents rather than in redox potentials. On the other hand, optical changes of $[Ru(phen)_3]^{2+}$ enantiomers upon DNA binding are unlike those observed for proven intercalators. Unlike the case of single chromophore intercalating ligands (such as ethidium bromide and daunomycin), assigning the binding mode from alterations in the absorption spectra for a compound which has three symmetry equivalent chromophores, only one of which can undergo a significant perturbation in the presence of DNA, is a much more difficult task (*9*). Thus, the peak currents but not the peak potentials are better estimates of the enantioselective interaction of redox active tris-chelated species than the spectroscopic techniques. Hence, the differing effect of DNA on the CV peak currents of $[Ru(phen)_3]^{2+}$ enantiomers provides a simple and direct method to monitor binding events. Further, one would be optimistic that electrochemical techniques would be useful in studying site or base specificity; Δ-$[Ru(phen)_3]^{2+}$ would prefer GC bp while the Λ-enantiomer AT bp, leading to a substantial decrease in peak currents. Thus, though some AT preference with the latter enantiomer has been described (*4*), a high level of base specificity is not expected nor has been observed so far for the weak overall binding by tris(phen)metal complexes. Further, these enantiomers are expected to discriminate against radically different conformations of DNA (i.e., B- vs Z-DNA); Δ-$[Ru(phen)_3]^{2+}$ would selectively bind to B-DNA as in the present case while Λ-enantiomer to Z DNA, leading to appreciable decrease in peak currents; however, no evidence for enantioselective binding of the Λ-form to Z-DNA has been obtained so far.

**Binding Mechanism for Ruthenium Enantiomers; Both Δ- and Λ-$[Ru(bpy)_3]^{2+}$ Bind Electrostatically.** Our observation that the peak currents of neither the racemic nor the enantiomeric forms of $[Ru(bpy)_3]^{2+}$ are affected significantly by the addition of DNA is consistent with the findings that they do not unwind poly[d(G-C)] or poly[d(A-T)] (*5*) and that their 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 (*3*). Further, it has been shown that the tris-chelated metal complexes possessing bpy ligands reside primarily at the outer hydrophilic coat of DNA, predominantly via electrostatic interaction with the negatively charged deoxyribose−phosphate backbone (*26*, *36*). The smaller size and higher nonplanarity of the bpy compared to the phen ligand preclude effective intercalation between the adjacent base pairs of DNA.

It is really surprising to see that $[Ru(bpy)_3]^{2+}$ is not strongly bound to DNA at 50 mM NaCl; a similar observation has been made previously (*3*). However, at 10 mM phosphate buffer, some weak binding has been observed (*26*) but not precisely quantitated. Further, at an ionic strength less than 5 mM, the $K_{2+}$ for $[Ru(bpy)_3]^{2+}$ has been reported to be $2 \times 10^5$ $M^{-1}$ (*37*). Other workers have reported the $K_{2+}$ to be $3 \times 10^6$ $M^{-1}$ at 1 mM NaCl and $1.4 \times 10^6$ $M^{-1}$ at 10 mM NaCl (*38*). Similarly, $[Fe(bpy)_3]^{2+}$ binds at 10 mM NaCl but does not at 50 mM NaCl (*16*). Barton et al. have also observed that at high NaCl concentrations the binding of the Ru(II)−phen complex is diminished (*29*). Thus, it appears that an increase in ionic strength causes a decrease in the extent of binding. The effective attraction between the oppositely charged species *viz* polyanionic DNA and the Ru(II) complex cation is known to be reduced at higher ionic strengths (*39*). Haq et al. have also very recently shown (*40*) the salt dependence of binding of $[Ru(phen)_2dppz]^{2+}$ to DNA, the binding constant decreasing with an increase in NaCl concentration. They have used polyelectrolyte theory (*41*) to illustrate that the counterion release arising from an increased phosphate spacing resulting from the intercalation of the complex becomes unfavorable at high salt concentrations.

At 10 mM NaCl, the binding constant $K_{2+}$ follows the trend $[Ru(bpy)_3]^{2+}$ ($1.4 \times 10^6$ $M^{-1}$) (*38*) ≫ $[Os(bpy)_3]^{2+}$ ($7.3 \times 10^3$ $M^{-1}$) (*17*) > $[Fe(bpy)_3]^{2+}$ ($1.4 \times 10^3$ $M^{-1}$) (*16*), illustrating that the Ru(II) complex appears to exhibit selective binding with DNA. Similarly, at 50 mM NaCl, $[Co(bpy)_3]^{3+}$ ($K_{3+}$, $1.4 \times 10^4$ $M^{-1}$) (*16*) binds strongly while Ru(II) and Fe(II) complexes do not (*26*, *16*). This is obviously because of the higher charge on and the smaller size of the Co(III) complex and illustrates the importance of the electrostatic component of binding. Since 1 equiv of a divalent ion is known to have a greater influence on DNA binding than 2 equiv of a monovalent ion (*39*), it is expected that the trivalent Co(III) complex would be more strongly bound than the divalent Ru(II) complex.

## CONCLUSION

Our studies show that Δ-$[Ru(phen)_3]^{2+}$ prefers to bind to DNA relatively strongly by partial rather than classical intercalation and consequently decreases the currents of redox peaks, while Λ-$[Ru(phen)_3]^{2+}$ binds weakly by a predominantly electrostatic mode with no effect on current response. The extent of binding of *rac*-$[Ru(phen)_3]^{2+}$ is intermediate between those of the enantiomers. In contrast, the enantiomers of $[Ru(bpy)_3]^{2+}$ bind weakly at 50 mM NaCl but do not show any selectivity, suggesting that the middle ring in phen has a role to play possibly by facilitating partial intercalation. So we are tempted to place Δ-$[Ru(phen)_3]^{2+}$ among the nonclassical DNA binding agents. Thus, the present study shows that it is possible to use the potentially sensitive and versatile electrochemical method as a new tool to study the enantioselective interaction of enantiomers of $[Ru(phen)_3]^{2+}$ with DNA and hence to examine the subtle features of the polynucleotide structure.

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