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Neither Δ- nor Λ-Tris(phenanthroline)ruthenium(II) Binds to DNA by Classical Intercalation†

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ABSTRACT: Equilibrium binding studies and viscosity experiments are described that characterize the interaction of Δ- and Λ-$[Ru(o\text{-phen})_3]^{2+}$ with calf thymus DNA. The mode of binding of these compounds to DNA is a matter of controversy. Both isomers of $[Ru(o\text{-phen})_3]^{2+}$ were found to bind but weakly to DNA, with binding constants of 4.9 (±0.3) × 10^4 M^{-1} and 2.8 (±0.2) × 10^4 M^{-1} determined for the Δ and Λ isomers, respectively, at 20 °C in a solution containing 5 mM Tris-HCl (pH 7.1) and 10 mM NaCl. We determined that the quantity $\delta \log K/\delta \log [Na^+]$ equals 1.37 and 1.24 for the Δ and Λ isomers, respectively. Application of polyelectrolyte theory allows us to use these values to show quantitatively that both the Δ and Λ isomers are essentially electrostatically bound to DNA. Viscosity experiments show that binding the Λ isomer does not alter the relative viscosity of DNA to any appreciable extent, while binding of the Δ isomer *decreases* the relative viscosity of DNA. From these viscosity results, we conclude that neither isomer of $[Ru(o\text{-phen})_3]^{2+}$ binds to DNA by classical intercalation.

The use of chiral metal complexes to probe the structure of DNA is an active area of research at the interface of chemistry and biology. Pioneering studies by Barton and co-workers (Barton et al., 1984, 1986; Kumar et al., 1985) have shown that optically active isomers of tris(phenanthroline)-ruthenium(II) (Figure 1) bind to DNA with distinctive characteristics. The binding model proposed from their studies is one in which *each* isomer, Δ- and Λ-$[Ru(o\text{-phen})_3]^{2+}$,[1] exhibits *two* types of interaction with right-handed DNA. Both intercalation and outside (or surface) binding were proposed as binding modes for each isomer, with intercalation deemed to be the predominant one (Long & Barton, 1990). The major groove was proposed as the site of interaction for both types of binding, although recent NMR evidence (Rehman & Barton, 1990a,b) suggests that surface binding may take place in the minor groove of DNA.

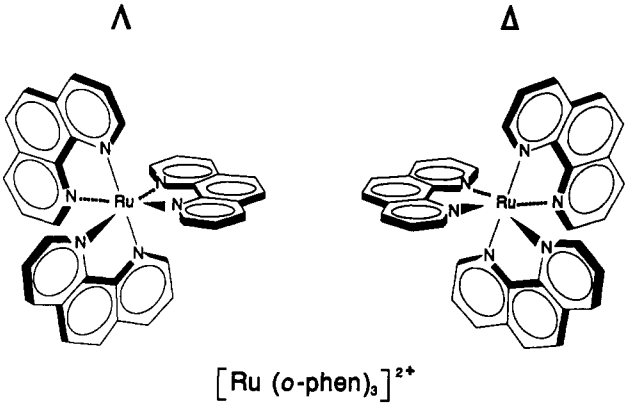

FIGURE 1: Structures of Δ- and Λ-$[Ru(o\text{-phen})_3]^{2+}$.

Studies by Hiort et al. (1990) have questioned important elements of the binding mechanism proposed by Barton and co-workers (Barton et al., 1984, 1986; Kumar et al., 1985). Observed isosbestic points in linear dichroism spectra suggested

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[1] The accepted abbreviation of tris(phenanthroline)ruthenium(II) is $[Ru(o\text{-phen})_3]^{2+}$. This will be further shortened to Δ-, Λ-, and *rac*-Ru to denote the optically active isomers and the racemate, respectively.

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that each isomer has only a single mode of DNA binding. Further, from consideration of the orientation of the bound metal complexes relative to the helical axis of DNA, coupled with the small magnitude of spectroscopic changes upon binding, Hiort et al. (1990) concluded that neither isomer is bound by intercalation to DNA.

Molecular modeling and energy minimization studies have also been reported for the ruthenium–DNA complexes (Howorth et al., 1991) and suggest that steric effects might block binding of Δ- and Λ-Ru in the minor groove of DNA. The results of energy minimization agreed with Barton and co-workers (Barton et al., 1984, 1986; Kumar et al., 1985) that each isomer can bind to DNA by two different modes. In addition to groove binding, each isomer can bind by *partial* insertion of one of its phenanthroline rings between neighboring base pairs of DNA. The latter mode is distinct from classical intercalation, in which a planar aromatic chromophore is *fully* inserted between adjacent DNA base pairs, resulting in a pronounced extension of the helix (Lerman, 1961; Wilson & Jones, 1982).

The potential importance of these compounds as structural probes of DNA and the controversy surrounding their binding mode have led us to reinvestigate their interactions with DNA. We report the binding constants of the isomers toward calf thymus DNA as determined by fluorescence measurements and by equilibrium dialysis. These studies show that the ruthenium isomers bind to DNA far more weakly than expected for intercalators. From a study of the NaCl dependence of their binding, and subsequent analysis by polyelectrolyte theory, we conclude that the isomers are essentially electrostatically bound to DNA, in sharp contrast to the proven intercalators daunomycin and ethidium, whose binding is stabilized by a substantial contribution from nonelectrostatic molecular forces (i.e., van der Waals interactions, hydrogen bonding). Moreover, viscosity measurements (perhaps the most critical test for the classical intercalation model in the absence of X-ray or NMR structural data) show that neither Λ- nor Δ-Ru bind by classic intercalation. Our data help to clarify the controversy surrounding the mode of binding of these compounds to DNA.

MATERIALS AND METHODS

Ruthenium Compounds. The synthesis and resolution of the ruthenium compounds were as earlier described (Dwyer & Gyarfas, 1949; Dwyer et al., 1947). The values of $\Delta\epsilon$ (the difference in molar extinction coefficients for the absorbance of left- and right-handed circularly polarized light) at 462 nm for the two enantiomers as their perchlorate salts in BPE buffer were (+)-Λ-Ru = 20.3 ± 1 M^{-1} cm^{-1} and (−)-Δ-Ru = 22.2 ± 1 M^{-1} cm^{-1}. Comparison of these values with the literature (Hiort et al., 1990) suggests that the enantiomeric purity of our samples was >95%. Concentrations of the ruthenium compounds were determined optically using ϵ^{M}_{447} = 19 000 M^{-1} cm^{-1} (Lin et al., 1976).

DNA Samples. DNA was purchased from Sigma Chemical Co. (lot no. 42F 9555) or Boehringer Mannheim, Inc., Biochemical Division (lot no. 11540223-23), and was sonicated and purified as previously described (Chaires et al., 1982).

Equilibrium Binding Studies. Binding constants for the interaction of Δ- and Λ-Ru were obtained by fluorescence titration and equilibrium dialysis experiments. Fluorescence studies utilized a Perkin-Elmer 650-40 fluorescence spectrometer. Fixed amounts of ligand were titrated with increasing amounts of calf thymus DNA, over a range of DNA concentrations from 10^{-6} to 10^{-3} M. An excitation wavelength of 455 nm was used, and total fluorescence emission was monitored by collecting all emitted light passing through a 495-nm cutoff filter. The fraction of ligand bound was calculated (assuming a linear spectral response) from the relation $C_b = C_t[(F - F^0)/(F^{max} - F^0)]$, where C_t is the total ligand concentration, F the observed fluorescence emission intensity at a given DNA concentration, F^0 the intensity in the absence of DNA, and F^{max} the fluorescence of the totally bound ligand. We found F^{max}/F^0 to be 2.25 and 2.05 for Δ- and Λ-Ru, respectively. Binding data were cast into the form of a Scatchard plot of r/C_f vs r, where r is the binding ratio $C_b/[DNA]_t$ and C_f is the free ligand concentration. All experiments were conducted at 20 °C in a buffer containing 5 mM Tris-HCl (pH 7.1) and NaCl concentrations as indicated. Equilibrium dialysis experiments were conducted as follows. A 1-mL sample of calf thymus DNA (0.148 mM bp) was dialyzed against 15 mL of buffer plus varying amounts of added ligand. SpectraPor 4 dialysis tubing (MWCO 12 000–14 000) was used, and solutions were dialyzed for 24–48 h with continuous agitation. Free ruthenium concentrations were determined from the dialysate by absorbance measurements at 447 nm. Total ruthenium concentration (C_t) was determined from the retentate by absorbance measurements at the isosbestic point, 464 nm, assuming ϵ^{M}_{464} = 13 600 M^{-1} cm^{-1} (Barton et al., 1984). The amount of bound drug was then calculated by difference, $C_b = C_t - C_f$.

Viscosity Experiments. Viscosity experiments used an Ostwald-type viscometer, immersed in a thermostated water bath maintained at 27 (±0.1) °C. Titrations were conducted without removing the DNA samples from the viscometer by using a Stoelting Co. Model 51218M microsyringe assembly to add ligand, followed by bubbling with nitrogen to ensure mixing. The amount of bound ligand was determined by parallel optical titration experiments, using identical sample and titrant volumes and concentrations. DNA samples approximately 200 bp in length were prepared by sonication (Chaires et al., 1982) for these studies, in order to minimize complexities arising from DNA flexibility. For the viscometer used, a buffer flow time, t^0, of 76.34 (±0.06) s was observed. Relative viscosities for DNA in the presence and absence of ligand were calculated (neglecting possible changes in axial ratios) from the relation $\eta = (t - t^0)/t^0$, where t is the observed flow time. A DNA concentration range of 1.0–2.8 mM bp was investigated, concentrations that yielded flow times of ≥90 s.

RESULTS AND ANALYSIS

Figure 2 shows binding isotherms obtained for Δ- and Λ-Ru. Data were obtained by fluorescence titration of fixed amounts of ligand with increasing amounts of DNA and by equilibrium dialysis. The agreement between the data obtained by the two methods is excellent, a fact that validates the assumption of a linear spectral response made in the analysis of fluorescence data. Qualitative inspection of Figure 2 indicates that Δ-Ru binds more tightly to calf thymus DNA under these conditions than does Λ-Ru. Quantitative analysis of these data was performed by nonlinear least-squares fitting of the data of Figure 2 to the simple neighbor exclusion model of McGhee and von Hippel (1974), yielding estimates of K (the binding constant for the interaction of a ligand with an isolated DNA binding site) and n (the exclusion parameter expressed in base pairs). For Δ-Ru, this analysis yields values of K = 4.9 (±0.3) × 10^4 M^{-1} and n = 3.7 (±0.2) bp. Values obtained for Λ-Ru are K = 2.8 (±0.2) × 10^4 M^{-1} and n = 3.4 (±0.3) bp. Fits of the data of Figure 2 to more complicated binding models

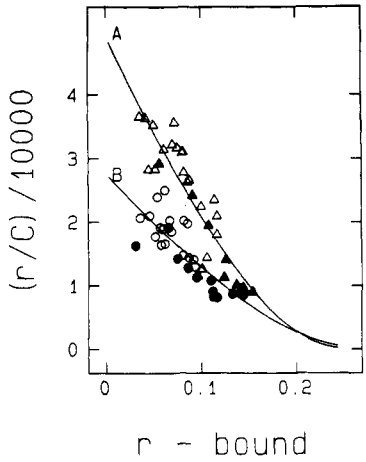

FIGURE 2: Binding isotherms for the interaction of Δ-Ru (A) and Λ-Ru (B) with calf thymus DNA. Filled symbols refer to data obtained by equilibrium dialysis, while open symbols refer to data obtained by fluorescence titration. The lines are the best fit, obtained by nonlinear least-squares analysis, to the simple McGhee–von Hippel neighbor exclusion model (McGhee & von Hippel, 1974). Experiments were conducted at 20 °C, in a solution containing 5 mM Tris-HCl (pH 7.1) and 10 mM NaCl.

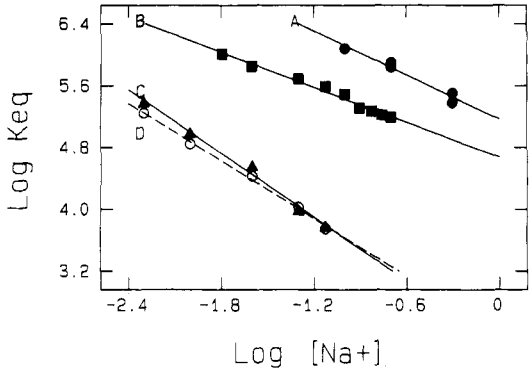

FIGURE 3: Salt dependence of the equilibrium binding constants for DNA binding ligands. Data are shown for the proven intercalators daunomycin (A) and ethidium (B) and for Δ-Ru (C) and Λ-Ru (D). The lines indicate the best linear least-squares fit to the data and provide the following slopes: ethidium, –0.75; daunomycin, –0.84; Δ-Ru, –1.37; Λ-Ru, –1.24.

involving cooperative ligand–ligand (McGhee & von Hippel, 1974) or electrostatic (Friedman & Manning, 1984) interactions were considered, but these models did not significantly improve the fits and were therefore rejected. The binding constants extracted from the data of Figure 2 show that Δ-Ru binds 1.8 times more tightly to calf thymus DNA than does Λ-Ru, which is at best only modest enantiomeric selectivity. The exclusion parameters are essentially the same for the two isomers and indicate that upon binding to DNA each isomer removes 3–4 bp as potential binding sites from the DNA lattice. These binding data are fully accounted for by a single binding mode as described by the McGhee–von Hippel neighbor exclusion model. There is no indication in these data, and no statistical justification, for assuming more complicated binding models involving multiple binding modes characterized by different binding constants.

Figure 3 shows the salt dependence of Δ- and Λ-Ru binding to DNA and comparative data for the proven intercalators ethidium and daunomycin. The clear dependence of the binding constants for these compounds upon Na^+ concentration is a consequence of the linkage of ligand and Na^+ binding to DNA and may be analyzed by polyelectrolyte theory (Record et al., 1978). From that theory, the slope of the lines in Figure 3 provide an estimate of $Z\psi$, where Ψ is the fraction of

Table I: Comparative Energetics of Ligand Binding to DNA[a]

compound	K_{obs}/ 10^4 M^{-1}	$\Delta G°$, kcal/mol	$Z\Psi$	$K°_t$/ 10^4 M^{-1}	$\Delta G°_t$, kcal/mol
ethidium	49.4	–7.7	0.75	6.1	–6.5
daunomycin	490.0	–9.0	0.84	42.2	–7.6
Δ-Ru	0.97	–5.4	1.38	0.02	–3.1
Λ-Ru	1.07	–5.5	1.24	0.03	–3.4

[a] K_{obs} is the binding constant for the interaction of a ligand molecule with an isolated DNA binding site. $\Delta G°$ is the binding free energy, calculated from the relation $\Delta G° = -RT \ln K_{obs}$. $Z\Psi$ is the absolute value of the slope obtained from the plots of Figure 3. $K°_t$ and $\Delta G°_t$ are the thermodynamic binding constant and free energy, respectively, calculated according to the theory of Record et al. (1978). These values characterize the nonelectrostatic contribution to the binding reaction. All values refer to 20 °C and to a solution containing 5 mM Tris-HCl (pH 7.1) and 50 mM NaCl.

counterions associated with each DNA phosphate ($\Psi = 0.88$ for double-stranded B-form DNA) and Z is the charge on the ligand. The data of Figure 3 indicate that ethidium and daunomycin both carry a charge of approximately 1, which is consistent with the known structures of these compounds. Both Δ- and Λ-Ru carry a net charge of 2. Consequently, the slopes of the lines through the data for these compounds in Figure 3 are greater than 1, being 1.37 and 1.24 for the Δ and Λ isomers, respectively. These values are less than the theoretically expected value of $Z\psi = (2)(0.88) = 1.76$. Such lower values could arise from coupled anion release (from the ligand) or from changes in ligand or DNA hydration upon binding. A value of $Z\psi = 2.2$ has been reported for the interaction of *rac*-Ru with calf thymus DNA (Barton et al., 1986), a value that exceeds the theoretical prediction for a ligand with a net positive charge of 2. Knowledge of $Z\psi$ allows for a quantitative estimation of the nonelectrostatic contribution to the DNA binding constant (and free energy) for these compounds. Record et al. (1978) have shown that

$$\ln K_{obs} = \ln K°_t + Z\xi^{-1}(\ln(\gamma_{\pm}\delta)) + Z\Psi(\ln(M^+)) \quad (1)$$

where Z and Ψ are as defined above, K_{obs} is the apparent binding constant at a monovalent cation concentration of M^+, $K°_t$ is the "thermodynamic" binding constant, $\gamma_{\pm}$ is the mean activity coefficient at cation concentration M^+, and the remaining terms are constants for double-stranded B-form DNA, $\xi = 4.2$ and $\delta = 0.56$. The quantity $K°_t$ is the equilibrium binding constant corrected for the free energy of ion release and refers to a standard state in which all reactants, including ions, have unit activity. The magnitude of $K°_t$ indicates the extent to which nonelectrostatic forces stabilize the ligand–DNA complex.

Table I summarizes the energetics of ligand binding to DNA for ethidium, daunomycin, and the ruthenium compounds. The striking observation emerging from these data is that the binding constants for both Δ- and Λ-Ru are 1–2 orders of magnitude *lower* than those measured for the proven intercalators ethidium and daunomycin. Such a difference corresponds to an approximate 2 kcal/mol *less favorable* binding free energy. Calculated values of $K°_t$ are large in magnitude for both daunomycin and ethidium, indicating that nonelectrostatic forces (e.g., van der Waals interactions, hydrogen bonding) play a prominent role in stabilizing the binding complex. In sharp contrast, $K°_t$ values for both Δ- and Λ-Ru are negligible when compared to K_{obs}, implying that binding of these compounds to DNA is essentially electrostatic in nature. All values in Table I refer to 50 mM Na^+, a concentration where experimental data are available for the four compounds being compared. At higher Na^+ concentrations, the difference in the relative binding affinities of Δ-

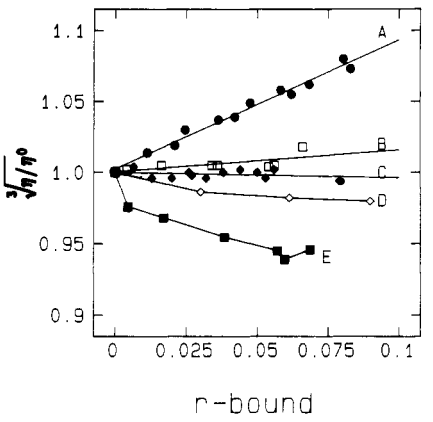

FIGURE 4: Effect of increasing amounts of bound ligand on the relative specific viscosity of calf thymus DNA. Viscosity is presented as $(\eta/\eta^0)^{1/3}$ in accord with the theory of Cohen and Eisenberg (1969). Data are shown for the proven intercalator ethidium (A) and the proven groove binder Hoechst 33258 (C) and for Δ-Ru (E), Λ-Ru (B), and *rac*-Ru (D).

and Λ-Ru and proven intercalators is more pronounced. Under physiological salt concentrations (200 mM Na^+), for example, the ruthenium compounds would bind to DNA with an affinity on the order of 10^3 M^{-1}, whereas ethidium and daunomycin bind with an affinity of 10^5–10^6 M^{-1}. Compared to proven intercalators, Δ- and Λ-Ru form but weak complexes with DNA, complexes that are essentially electrostatic in nature.

Figure 4 shows the results of a comparative viscosity study designed to explore the mode of binding of ruthenium compounds to DNA. Hydrodynamic data provide perhaps the most critical test for intercalative binding in the absence of X-ray crystallographic or NMR structural data (Lerman, 1961; Dougherty & Pilbrow, 1984; Wilson & Jones, 1982), yet no such data have been reported for any of the ruthenium compounds. Figure 4 shows that the intercalator ethidium increases the relative specific viscosity, as expected for the lengthening of the DNA double helix resultant from intercalation. The slope of the ethidium data in Figure 4 (0.91) is near the slope of 1.0 predicted from the theory of Cohn and Eisenberg (1969). In contrast to ethidium, the data of Figure 4 show that the groove-binding antibiotic Hoechst 33258 does not appreciably alter DNA viscosity. Neither the Δ- nor Λ-Ru shows the effect on DNA viscosity expected from the classical intercalation model. The data for the Λ isomer shown in Figure 4 are very similar to that obtained for Hoechst 33258, suggesting a nonintercalative, and possibly a groove, binding mode. Binding of the Δ isomer *decreases* the relative specific viscosity of DNA. Such behavior may be explained by a binding mode that produces bends or kinks in the DNA helix (Kapicak & Gabbay, 1975). Also shown in Figure 4 are results obtained for *rac*-Ru, which fall between the data obtained on the individual isomers, and which must represent average hydrodynamic properties, weighted by the amounts of each isomer bound to DNA. These viscosity data indicate unambiguously that Δ- and Λ-Ru do not behave as classic intercalators and must bind by some other mode.

DISCUSSION

The mode of binding of Δ- and Λ-Ru to DNA is a matter of controversy (Barton et al., 1984, 1986; Kumar et al., 1985; Rehman & Barton, 1990a,b; Hiort et al., 1990 ; Howorth et al., 1991), a controversy that is important to resolve in order to critically evaluate the utility and potential of these compounds as probes of DNA structure. We present here new data and analysis that helps to clarify how Δ- and Λ-Ru bind to DNA. We conclude from our studies that neither isomer binds to DNA by a mode that is consistent with the features of the classic intercalation model as first proposed by Lerman (1961) and subsequently refined [see Wilson and Jones (1982) for an insightful review].

The criteria for establishing the mode of binding of ligands to DNA have been extensively reviewed (Dougherty & Pilbrow, 1984; Wilson & Jones, 1982; Waring, 1970), and a recent commentary (Long & Barton, 1990) has discussed the relative merits of selected criteria in specific connection with the binding of Δ- and Λ-Ru to DNA. Not all criteria should be equally weighted, however, in attempting to establish whether or not a ligand binds to DNA by intercalation. Optical or photophysical probes generally provide necessary, but not sufficient, clues to support an intercalation binding model. We regard hydrodynamic measurements that are sensitive to length increases (i.e., viscosity, sedimentation, rotational diffusion as measured by transient electric dichroism) as the least ambiguous and most critical tests of the intercalation model in solution. The classical intercalation model (Lerman, 1961) *demands* that the DNA helix lengthen as base pairs are separated to accommodate the bound ligand. Hydrodynamic methods are best suited to detect such changes and, in the absence of crystallographic structural data, are essential evidence to support an intercalation model. No such measurements have been reported until now to characterize the interaction of Δ- and Λ-Ru with DNA, although changes in the orientation factor S in linear dichroism experiments (Hiort et al., 1990) have strongly suggested changes in the hydrodynamic properties of DNA upon binding of Δ-Ru.

In Figure 4, we confirm that the proven intercalator ethidium increases DNA viscosity to almost exactly the extent predicted for intercalation into rodlike DNA (Cohen & Eisenberg, 1969). In sharp contrast, the known groove-binding ligand Hoechst 33258 causes essentially no change in DNA viscosity, reflecting the fact that groove binding need not result in a length increase in the helix. Neither Δ- nor Λ-Ru increases DNA viscosity upon binding (Figure 4). From this observation, we can only conclude that neither isomer binds by classical intercalation. The two ruthenium isomers show distinctive behavior in the viscosity experiment. The Λ isomer behaves very much like Hoechst 33258, indicating that it might bind in one of the DNA grooves without distortion of the helical structure. In contrast, the Δ isomer *decreases* DNA viscosity. While such behavior is unusual, it is not without precedent. In studies of stereoselective ligand binding to DNA that were ahead of their time, Kapicak and Gabbay (1975) reported similar effects with several aromatic-substituted diammonium cations. Viscosity decreases were explained by a model in which ligands bound by a *partial*, nonclassical intercalation mode, in which the planar aromatic moiety could not be fully inserted between base pairs. The result of such binding was proposed to be a static bend (or kink) in the DNA helix, which would reduce its effective length and, concomitantly, its viscosity. We believe a similar model can explain the results we observe for Δ-Ru in Figure 4. Because of the bulky structure of Δ-Ru, a single phenanthroline ring cannot completely intercalate. From the structure of the ruthenium compounds it is evident that the hydrogen atoms located at position 1 (8) of the *nonintercalated* phenanthroline rings would effectively block full intercalation of the remaining ring. Partial intercalation may occur, however, which would act as a "wedge" to pry apart one side of a base pair stack but not fully separate the stack as required by the classical intercalation model. The possible result is a static bend or kink in the helix. Since viscosity is exquisitely

sensitive to length changes, a reduction in helix end-to-end distance resulting from bending is readily measured. By design, our viscosity experiments were conducted on DNA samples sonicated to lengths below the persistence length to DNA, so that we might avoid possible complexities arising from changes in DNA flexibility. We note that recent molecular modeling studies of the interaction of Δ- and Λ-Ru with DNA (Haworth et al., 1991) showed that partial intercalation was energetically feasible, but full, classical, intercalation was not. A marked decrease in the orientation of the Δ-Ru–DNA complex compared to free DNA in linear dichroism experiments led Hiort et al. (1990) to suggest that Δ-Ru might kink the helix, although it was argued that this isomer was groove bound. Our data and interpretation are fully consistent with these observations.

The measured binding affinities of Δ- and Λ-Ru toward DNA, and the salt dependency of their binding (Figure 3), are consistent with the binding modes we propose. In the classical intercalation model, the planar, aromatic portion of the bound ligand is sandwiched between two base pairs, forming a stack stabilized by extensive van der Waals contacts. Crystallographic structures of certain intercalation complexes show that extensive hydrogen-bonding interactions may also contribute to the stability of the complexes (Wang, 1987). Intercalative binding is thus stabilized by nonelectrostatic molecular interactions. From the salt dependence of binding, we may calculate the nonelectrostatic contribution to the binding constant and free energy. The proven intercalators daunomycin and ethidium are shown in Table I to possess a substantial nonelectrostatic contribution to their binding free energy. In contrast, Δ- and Λ-Ru are found to be essentially electrostatically bound to DNA, a finding inconsistent with the nature of intercalative binding. Indeed, the magnitude of their binding constants is several orders lower than that of those observed for the proven intercalators daunomycin and ethidium and is of the same order of magnitude of the binding constant reported for the binding of Mg^{2+} to DNA (Daune, 1974).

Changes in DNA superhelicity have been reported to result from the binding of both Δ- and Λ-Ru (Barton, et al., 1984) and of *rac*-Ru (Kelly et al., 1985). These reports require reconciliation with our interpretation that these compounds are not classical intercalators. First, we regard helix unwinding as a necessary, but insufficient, criterion for intercalation. Known intercalators unwind DNA to widely varying extents (Waring, 1970). Daunomycin, on one extreme, unwinds DNA by only 10°, perhaps because extensive unwinding is inhibited by molecular interactions involving drug constituents lying in the minor groove. In spite of its low unwinding angle, daunomycin causes a DNA length increase of the magnitude expected for an intercalator (Fritzche et al., 1982), and its intercalation into DNA has been confirmed by X-ray crystallography (Wang, 1987). The DNA helix can thus lengthen without extensive unwinding. Can the DNA helix be unwound without lengthening? Results obtained for two interesting and unusual DNA binding compounds suggest that it can and that helix unwinding is therefore not a stringent criterion for intercalation. Irehidiamine A is a steroidal diamine whose structure makes intercalation sterically unfeasible. Irehidiamine A unwinds supercoiled DNA but *does not* lengthen linear DNA as measured by sedimentation velocity (Waring, 1970). Crystal violet, a propeller-shaped molecule whose shape precludes classical intercalation, was found to unwind supercoiled DNA (with an unwinding angle of 9.8°), yet was found to *decrease* the intrinsic viscosity of linear DNA (Wakelin et al., 1981). From the results obtained on these two compounds, it thus appears possible for nonintercalators to unwind supercoiled DNA without lengthening the helix, by an as yet unknown mechanism. We therefore believe that the observed unwinding of supercoiled DNA by $[Ru(o\text{-phen})_3]^{2+}$ (Barton et al., 1984; Kelly et al., 1985) does not provide unambiguous proof of an intercalative binding mode.

Two studies have attempted to measure the angle between the DNA helix axis and the C_3 axis of the bound ruthenium isomers (Yamagishi, 1984; Hiort et al., 1990). Hiort et al. (1990) concluded from the magnitude of spectroscopic changes that neither isomer bound by intercalation, although Λ-Ru was found by linear dichroism to be oriented with one phenanthroline ring nearly perpendicular to the helix axis. Yamagishi (1984), on the other hand, proposed that Δ-Ru intercalated while Λ-Ru was externally bound, based on orientation angles obtained by electric dichroism. Given the complexity of the Ru chromophore, and the possibility we propose that Δ-Ru may bend or kink the DNA helix, unambiguous interpretation of dichroism data is difficult. These methods, by themselves, cannot definitively establish the DNA binding modes of these compounds.

In order for a DNA structural probe to be used with confidence, its binding thermodynamics and the structure of its complex with DNA must be thoroughly understood. Metal complexes of the type studied here have generated much excitement as possible structural probes of DNA. The results we present serve to clarify how tris(phenanthroline)ruthenium(II) isomers interact with DNA, an essential step toward critically evaluating the utility of these compounds as practical and sensitive probes of DNA structure.

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