Tris(phenanthroline)ruthenium(II): Stereoselectivity in Binding to DNA

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Abstract: The chiral complexes tris(phenanthroline)ruthenium(II), $(phen)_3Ru^{2+}$, bind to DNA by intercalation, and enantiomeric selectivity in binding to a right-handed duplex is found. Spectroscopic, hydrodynamic, and binding parameters are consistent with the intercalative mode of association. Hypochromicity representing a 17% decrease in intensity in the metal to ligand charge-transfer band and enhanced luminescence accompany binding to the duplex. Moreover for a given ruthenium concentration, greater luminescence is found for the Δ isomer in the presence of DNA than for the Λ isomer. The experimental excited-state lifetimes of $(phen)_3Ru^{2+}$ isomers increase identically in the presence of DNA. Therefore the higher emission intensity reflects the larger affinity of the Δ isomer for the helix. An enantiomeric preference for the duplex is evident also in gel electrophoresis experiments with closed circular DNA. Both isomers unwind and rewind supercoiled pColEl DNA, but for a given added ruthenium concentration, more duplex unwinding is apparent with Δ-$(phen)_3Ru^{2+}$. Binding isotherms for racemic $(phen)_3Ru^{2+}$ with calf thymus DNA, obtained by equilibrium dialysis, yield an intrinsic binding constant, $K(0) = 6.2 \times 10^3\ M^{-1}$, and show the metal complex to occupy a four base-pair site at saturation. In addition, dialysates are found to be optically enriched in the less favored Λ enantiomer. Dialysis of Z-DNA against racemic $(phen)_3Ru^{2+}$ does not lead to similar optical enrichment. The results are all consistent with an intercalative model where the stereoselectivity is based upon the different steric interactions between the nonintercalated phenanthroline ligands of the chiral complex and the right-handed DNA phosphate backbone. The enantiomeric selectivity observed illustrates the importance of helix symmetry to drug recognition and provides a route to design probes for right- and left-handed DNA.

The binding of heterocyclic dyes to DNA by intercalation, where the planar aromatic cation stacks between adjacent base pairs of the duplex,[1] has been the subject of considerable investigation.[2-4] Intercalative drugs can be strongly mutagenic, and some, as adriamycin and daunomycin, serve as potent chemotherapeutic agents.[5] The small intercalators such as ethidium or proflavine in addition provide useful chemical probes of nucleic acid structure.[6] Metallointercalators have been particularly useful in probing DNA structure and the intercalation process itself, because the ligands or metal may be varied in an easily controlled manner to facilitate the individual application.[7] Comparisons of the binding of the intercalative 2,2'-bipyridylplatinum(II) reagent with the analogous nonintercalating bis(pyridine)platinum(II) species by fiber X-ray diffraction methods, for example, demonstrated quite simply the requirement for ligand planarity in the intercalation process.[8] The original studies of metallointercalators centered on square-planar platinum(II) complexes containing aromatic terpyridyl or phenanthroline ligands,[9] and single-crystal studies of terpyridylplatinum(II) species stacked with nucleotides showed the platinum complex to insert almost fully between the base pairs.[10,11] More recently the reagent methidiumpropyl-Fe(II)EDTA, which contains a redox-active metal center tethered to an organic intercalator, has been applied in "footprinting" experiments to determine the sequence specificities of small drugs bound to DNA.[12] Bis(phenanthroline)-cuprous ion[13] has similarly been employed in DNA cleavage experiments,[14] and this reagent also presumably binds initially to the DNA by intercalation.

Reagents of high specificity and even stereoselectivity would be desirable in the design both of potent drugs and of structural probes. For the chiral complex $(phen)_3Zn^{2+}$ (phen = 1,10-phenanthroline) an enantiomeric preference in binding to B-DNA has been observed.[15] In order to understand this chiral discrimination in more detail, we have now characterized the binding to the DNA duplex of Δ- and Λ-tris(phenanthroline)ruthenium(II) (Figure 1). As for the tetrahedral $(phen)_2Cu^+$ complex, and in contrast to the square-planar platinum intercalators, the octahedral coordination in the tris(phenanthroline)metal cations can permit a partial insertion of only one coordinated ligand. Thus while one ligand is stacked between base pairs, the remaining nonintercalated phenanthroline ligands should be available to direct the enantiomeric selection.

Ruthenium(II) complexes have been chosen for this study because of (i) the kinetically inert character of the low-spin d^6 species, (ii) their intense metal to ligand charge-transfer (MLCT) band in the visible spectrum and since (iii) many chemical and spectroscopic properties of the poly(pyridine) complexes have been established. The electronic structure of the ground and excited states of tris(bipyridine)ruthenium(II) has been examined in detail.[16] The strong visible absorption band, distinct from the absorption of DNA, in $(phen)_3Ru^{2+}$ as well as its high luminescence provide spectroscopic tools to monitor the intercalative process.[17,18] Most importantly, in contrast to $(phen)_3Zn^{2+}$, which

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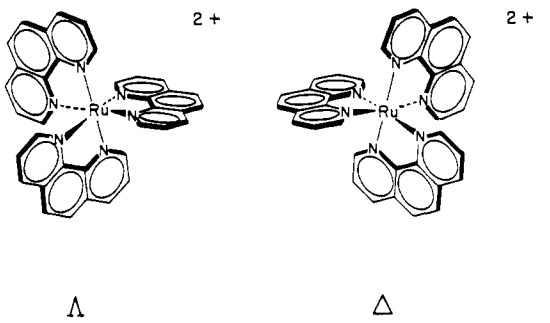

Figure 1. Enantiomers of tris(phenanthroline)ruthenium(II).

is somewhat labile,[19] the ruthenium(II) complexes are essentially inert to racemization.[29] racemization.[20] Optical isomers of $(phen)_3Ru^{2+}$ may be isolated in pure form,[20,21] and the absolute configurations have been assigned.[22] Electric dichroism measurements of $(phen)_3Ru^{2+}$ bound to DNA have already been conducted[23] and support the findings of enantiomeric selectivity.

In this report we demonstrate that tris(phenanthroline)ruthenium(II) complexes bind to DNA by intercalation. Furthermore the direct comparison between enantiomers of spectroscopic features, binding properties, and structural parameters establishes that the Δ enantiomer possesses the greater affinity for a right-handed helix.

Experimental Section

Ruthenium Complexes. $[(phen)_3Ru]Cl_2 \cdot 2H_2O$ was prepared as described by Lin et al.[18] Enantiomers were obtained by successive diastereomeric recrystallizations with antimony D-tartrate anion.[21] At most, two recrystallizations were required to achieve $|[\alpha]_D|$ 1317, after which point additional purification did not yield increased optical activity. Several samples of the Δ and Λ isomers were used in the course of the various binding studies, and, on the basis of comparison to literature values for the specific rotation,[24] all showed a level of optical purity given by $|(C(\Delta\text{-Ru}) - C(\Lambda\text{-Ru}))/(C(\Delta\text{-Ru}) + C(\Lambda\text{-Ru}))| \geq 0.92$. The stereoisomers were isolated as perchlorate salts; and elemental analyses (performed by Galbraith Lab., TN) were as follows: %C, 49.34; %H, 3.29; N, 9.52; calculated for $[(phen)_3Ru](ClO_4)_2 \cdot 2H_2O$, %C, 49.32; %H, 3.22; %N, 9.59. Spectrophotometric and luminescence titrations of racemic $Ru(phen)_3Cl_2$ and equimolar mixtures of Δ- and Λ-$Ru(phen)_3(ClO_4)_2$ with DNA agreed closely, indicating that the presence of perchlorate (≤50 μM) was without effect. Stock ruthenium solutions were either freshly prepared or kept in the dark. Concentrations of $(phen)_3Ru^{2+}$ were determined spectrophotometrically by using ϵ_{447} 19 000 M^{-1} cm^{-1}.[18]

Buffers and Chemicals. Experiments were carried out at pH 7.1 in buffer 1 (5 mM Tris, 50 mM NaCl), buffer 2 (5 mM Tris, 4.0 M NaCl), or buffer 3 (50 mM Tris acetate, 20 mM sodium acetate, 18 mM NaCl pH 7.0). Solutions were prepared with distilled deionized water. Plasticware was used throughout and was cleaned by soaking overnight in 10% HNO_3 followed by exhaustive rinsing. Dialysis membranes were prepared by the following protocol: After they were boiled successively in sodium carbonate, 1% EDTA, and 1% SDS and exhaustively rinsed in deionized water, the membranes were heated to 80 °C in 0.3% sodium sulfite, acidified at 60 °C with 2% sulfuric acid, and thereafter rinsed again with deionized water and 1% EDTA. This procedure serves to minimize metal binding to the membranes.

Nucleic Acids. Calf thymus DNA, obtained from Sigma Chemical Co., was purified by phenol extraction as described previously.[9c] Poly(dGC)·poly(dGC) from P.L. Biochemicals Inc. and plasmid ColE1 from Sigma Chemical Co. were extensively dialyzed in buffer before use.

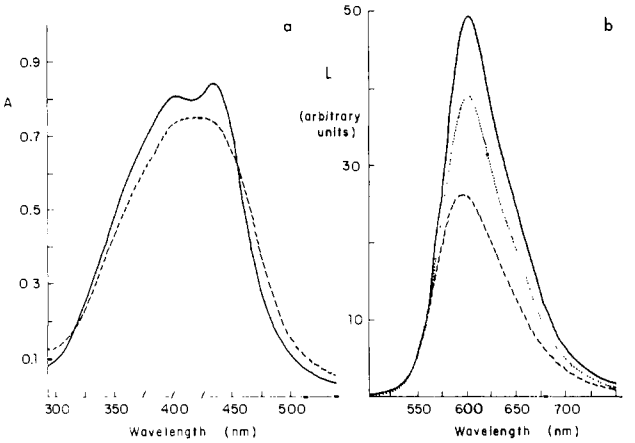

Figure 2. (a) Visible absorption spectra of racemic $(phen)_3Ru^{2+}$ (50 μM) in the absence (—) and presence (– – –) of DNA (1 mM). (b) Luminescence spectra of free (– – –) $(phen)_3Ru^{2+}$ and of Λ-$(phen)_3Ru^{2+}$ (···) and Δ-$(phen)_3Ru^{2+}$ (—) in the presence of DNA (0.3 mM). Ruthenium concentrations were 10 μM. Sample excitation was at 447 nm.

DNA concentrations per nucleotide were determined spectrophotometrically by assuming ϵ_{260} 6600 M^{-1} cm^{-1} for calf thymus DNA[25] and ϵ_{260} 8400 M^{-1} cm^{-1} for poly(dGC).[26]

Spectroscopic Measurements. Absorption spectra were recorded on a Cary 219 spectrophotometer. Absorbance titrations of racemic, Δ- and Λ-$(phen)_3Ru^{2+}$ in buffer 1 were performed by using a fixed ruthenium concentration to which increments of the DNA stock solution were added. Ruthenium was also added to the DNA stock to keep the total dye concentration constant. Luminescence measurements were conducted on a Perkin-Elmer LS-5 fluorescence spectrophotometer at ambient temperature. Samples were excited at 447 nm, and emission was observed between 500 and 700 nm. All experiments were carried out in buffer 1 with $(phen)_3Ru^{2+}$ concentrations typically of 10 μM and DNA phosphate/ruthenium ratios varying from 1 to 50. Lifetime measurements were performed on an Ortec 776 single-photon counter and timer in line with an Apple Computer. The samples were excited with a PRA 510A nanosecond lamp, and emission was observed at 593 nm. Reproducible lifetimes for the bound species in the presence of free ruthenium were obtained by neglecting the first 1.2 μs ($2\tau[Ru(phen)_3^{2+}{}_{free}]$) of the decay curve.

Electrophoresis. Dye gel electrophoresis of supercoiled DNA in 1% agarose was performed in buffer 3 by using the method of Espejo and Lebowitz[6] modified as described previously.[15] Ruthenium concentrations in the gels were carefully determined on the basis of several absorbance readings of the stock concentrations for the enantiomers. Because of the high background luminescence of $(phen)_3Ru^{2+}$, gels were destained for 24 h in buffer prior to staining with ethidium.

Equilibrium Dialysis. Binding isotherms were obtained on the basis of dialysis of calf thymus DNA in buffer 1 against $(phen)_3Ru^{2+}$ at 22 °C. The DNA was dialyzed first exhaustively in buffer to remove small fragments. Thereafter, dialysis against ruthenium was allowed to continue for at least 24 h after which time equilibration was achieved. Each sample consisted of 2 mL of dialysate containing Δ-, Λ-, or *rac*-$(phen)_3Ru^{2+}$, varying in concentration between 50 and 1000 μM, and, within the dialysis bag, 1 mL of 1 mM DNA phosphate. To determine bound and free concentrations, absorbance spectra were taken on dilutions (3–50 μM). Free ruthenium concentrations outside the bag were determined on the basis of absorbance readings at 447 nm. For concentrations of ruthenium inside the bag, in the presence of DNA, readings were obtained at the isosbestic point, where ϵ_{464} 13 630 M^{-1} cm^{-1} (vide infra). Equilibrium dialysis of poly(dGC) was conducted similarly in buffer 2. Measurements of circular dichroism were obtained on a Jasco J-40 automatic recording spectropolarimeter. Because of the irregular baseline of the instrument, all spectra were digitized and replotted after base-line subtraction. Data analyses were performed on an IBM PC and a Digital VAX 11/780.

Results

Spectroscopic Studies. The binding of Λ- and Δ-$(phen)_3Ru^{2+}$ to duplex DNA leads to a decrease and small shift in the visible

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absorption of the ruthenium species and a corresponding increase and shift in luminescence. Figure 2 shows both the absorption spectra and luminescence spectra of $(phen)_3Ru^{2+}$ in the presence and absence of calf thymus DNA. The spectral changes observed here are often characteristic of intercalation.

The hypochromic shift in the broad charge-transfer band of $(phen)_3Ru^{2+}$ as a result of binding to the polynucleotide can be seen in Figure 2A. A decrease of 12% in absorbance at 447 nm is found for the saturating DNA levels employed. Since at these concentrations 70% $(phen)_3Ru^{2+}$ is in the bound form, ϵ(bound)/ϵ(free) = 0.83 at 447 nm. The hypochromic effect is small compared with that found for other intercalators, which may indicate that the charge is not being preferentially localized onto the intercalated ligand. Also for the free ruthenium complex the predominant polarization of the charge-transfer band is perpendicular to the molecular C3 axis[17] rather than parallel to the intercalative plane. Shown in the figure is the change in absorbance for the racemic mixture; also because the observable hypochromic effect is small, significant differences between enantiomers were not obtained. For both isomers a spectral shift of 2 nm to lower energy is found, which supports an electronic stacking interaction of the phenanthroline ligand with the base pairs of the helix. Isosbestic points at 355 and 464 nm are also apparent.

An enhancement in the luminescence of $(phen)_3Ru^{2+}$ on binding to duplex DNA parallels the observed hypochromicity. Figure 2B shows the emission spectra of free $(phen)_3Ru^{2+}$ and of both lambda and delta isomers bound to DNA. These spectra also reveal a shift of 2 nm to longer wavelength with DNA binding. Moreover, in the presence of 0.25 mM DNA phosphate, emission increases of 48% and 87% are observed respectively for Λ- and Δ-$(phen)_3Ru^{2+}$ (10 μM). Note that a signficant fraction of the ruthenium is free in the presence of the DNA at these concentrations, but the associated increase in solution viscosity for higher DNA concentrations precluded studies at saturating binding levels. The greater increase in luminescence seen for the Δ isomer in the presence of DNA over that for the Λ isomer indicates simply that a higher proportion of the Δ isomer is bound, rather than that their modes of association with the helix differ. Measurements of the excited-state lifetimes of enantiomers in the absence and presence of the DNA yielded results consistent with this interpretation. For Δ- and Λ-$(phen)_3Ru^{2+}$, determined separately and as a racemic mixture, identical experimental lifetimes of 2.0 and 0.6 μ s were found respectively in the presence and absence of DNA. Both isomers therefore bind to the helix in a similar fashion, and indeed, if fully bound, would display similar enhancements in luminescence. Substantial increases in fluorescent lifetimes with intercalation have been observed in several instances,[2-4,27] notably for ethidium, and may be explained by the greater rigidity and lower collisional frequency of the molecule when stacked within the helix.

Measurements of Helical Unwinding. Both Λ- and Δ-$(phen)_3Ru^{2+}$ reversibly unwind and rewind supercoiled DNA as a function of increasing concentration of ruthenium(II), and for a given total concentration, a greater unwinding effect is evident for the Δ isomer. Figure 3 illustrates the migration of pColEl DNA through 1% agarose gels containing increasing levels of $(phen)_3Ru^{2+}$. Mobilities are plotted relative to the supercoiled DNA control to permit the inclusion of data from several gel electrophoresis trials. As can be seen in the figure, both isomers unwind the helix. With increasing levels of ruthenium bound, the duplex unwinds, and for a closed circle this unwinding results in first a release of negative supercoils at low levels bound and then the introduction of positive supercoils; the nicked DNA, without similar topological constraints, displays no variation in mobility. The bars in the figure indicate the width of the DNA bands, which vary because of the distribution of topoisomers in the sample. The observed duplex unwinding provides a strong indication of intercalative binding. Control experiments also show the unwinding to be reversible; preincubation of the DNA with ruthenium

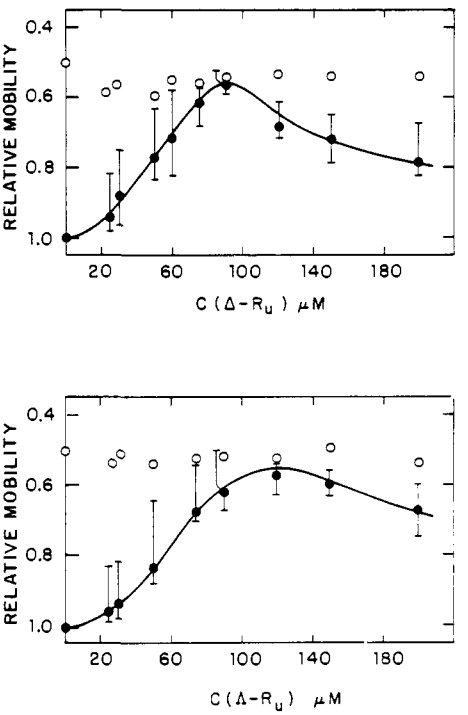

Figure 3. Relative mobilities of closed (●) and nicked (○) circular pColEl DNA in the presence of increasing concentrations of added Δ-(above) and Λ- (below) $(phen)_3Ru^{2+}$. Bars indicate the width of the DNA bands on the basis of the distribution of topoisomers. Comigration points of nicked and closed circular DNAs are seen at 90 and 120 μM for the Δ and Λ isomers, respectively.

complex has no effect on gel mobility. It is interesting to note that no DNA cleavage is observed as a result of binding $(phen)_3Ru^{2+}$, even after irradiation with ultraviolet light (short wavelength) for 1 h.

For a given level of total ruthenium, a higher amount of duplex unwinding is found in the presence of Δ-$(phen)_3Ru^{2+}$ as is evident in Figure 3. The comigration of nicked and closed circular DNAs occurs in the presence of 90 and 120 μM Δ- and Λ-$(phen)_3Ru^{2+}$, respectively. This comigration point represents a fixed amount of helical unwinding. A lower added concentration of Δ-$(phen)_3Ru^{2+}$ is needed to unwind all the negative supercoils in the pColEl DNA. These results therefore also reflect the higher affinity of the Δ isomer over the Λ isomer for the right-handed helix. At a given total concentration of ruthenium, more of the Δ isomer is bound and greater helical unwinding is evident. The alternative explanation for the lower concentration of the Δ isomer at the comigration point would be that the Δ isomer has a larger unwinding angle than the Λ isomer, so that the Δ isomer unwinds the duplex more per drug bound. A particularly large difference between unwinding angles (~30%) would be needed to account for the electrophoresis results, however, and only small variations in unwinding angles are generally observed.[4] Moreover larger, if any, structural perturbations should accompany binding of the Λ isomer to the right-handed helix rather than the Δ isomer. Here then, as well, the results show that the Δ isomer possesses a greater affinity for the DNA duplex.

These data may be used to estimate the intercalative unwinding angle. If we assume for the racemic mixture that the average comigration point of nicked and closed forms occurs with 100 μM ruthenium, then, on the basis of our determination of the binding constant (vide infra), a binding ratio of 0.089 per nucleotide is required to unwind fully the supercoils in the plasmid. Interestingly this value is identical with that calculated for ethidium, since in buffer 3 we observe the comigration of nicked and closed pColEl species in the presence of 5×10^{-7} M dye. Therefore we estimate the unwinding angle for $(phen)_3Ru^{2+}$ to be the same as that for ethidium.[6]

Equilibrium Dialysis Experiments. The results of dialysis of calf thymus DNA with racemic $(phen)_3Ru^{2+}$ at 22 °C in buffer

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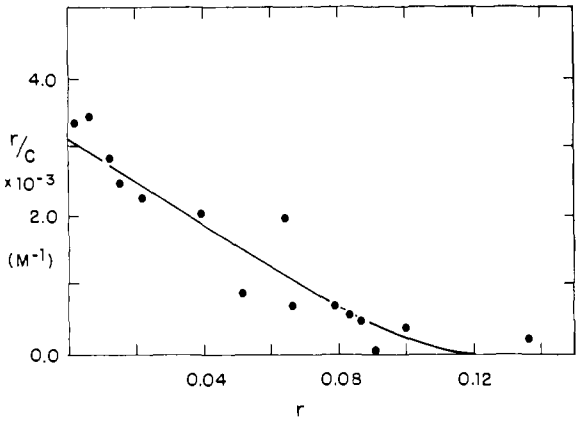

Figure 4. Scatchard plot of $(phen)_3Ru^{2+}$ binding to calf thymus DNA in buffer 1 at 22 °,c, where r is the ratio of bound ruthenium to nucleotide concentrations and C is the concentration of ruthenium free in solution. The solid curve represents the best fit to eq 1.

1 are shown in Figure 4 in the form of a Scatchard plot.[28] The data have been fit by nonlinear least-squares analysis to the following equation governing noncooperative binding to the helix, as derived by McGhee and von Hippel:[29]

$$\frac{r}{C_F} = \frac{K(0)}{2}(1 - 2lr)\left(\frac{1 - 2lr}{1 - 2(l-1)r}\right)^{l-1} \quad (1)$$

where r is the ratio of the bound concentration of ruthenium to the concentration of DNA phosphate, C_F is the concentration of ruthenium free in solution, $K(0)$ is the intrinsic binding constant, and the integer l, which measures the degree of anticooperativity, is the size of a binding site in base pairs. In fitting the data, the binding parameter $K(0)$ was varied for several integer values of l. The best fit, shown as the solid curve in Figure 4, yielded a binding constant $K(0) = 6.2 \times 10^3\ M^{-1}$ (±2%) and an exclusion site size (l) of four base pairs. Data from luminescence titrations were consistent with this curve. The binding constant is quite low in comparison to values of 3×10^5 and $5 \times 10^4\ M^{-1}$ (extrapolated to the ionic strength of our buffer) for ethidium and $[(phen)Pt(en)]^{2+}$, respectively.[30] The lower affinity of $(phen)_3Ru^{2+}$ is not surprising since only partial stacking of the phenanthroline ligand is feasible in this octahedral complex; greater overlap of the phenanthroline with the base pairs may be achieved in the square-planar platinum(II) species. The steric bulk of the nonintercalated ligands determines also the large four base-pair site size compared to a two base-pair (neighbor excluded) site for basically planar reagents.[9,31] Inspection of space-filling models show that the perpendicular phenanthroline ligands each span two base pairs either above or below the intercalatively bound phenanthroline, which is consistent with the binding isotherm.

In these equilibrium dialysis experiments of the racemic mixture, the relative binding of the two enantiomers to the polynucleotide may be determined sensitively on the basis of the degree of optical enrichment of the unbound enantiomer in the dialysate. Figure 5 shows the circular dichroism of a dilution (1.5×10^{-5} M) of the dialysate after equilibration of calf thymus DNA (1 mM) with racemic $(phen)_3Ru^{2+}$ (2×10^{-4} M). Also shown for comparison is the circular dichroism of Δ-$(phen)_3Ru^{2+}$ (0.2 μM). The spectra show clearly that the dialysate has been optically enriched in the less favored Λ isomer. The Δ enantiomer binds preferentially to the right-handed helix. The degree of chiral discrimination may be made more quantitative by comparing the level of optical enrichment (2% for the sample shown) with the overall amount of ruthenium bound. On the basis of a simple competition between the enantiomers for sites along the helix with no cooperativity and, for simplification, describing the binding by each enantiomer in

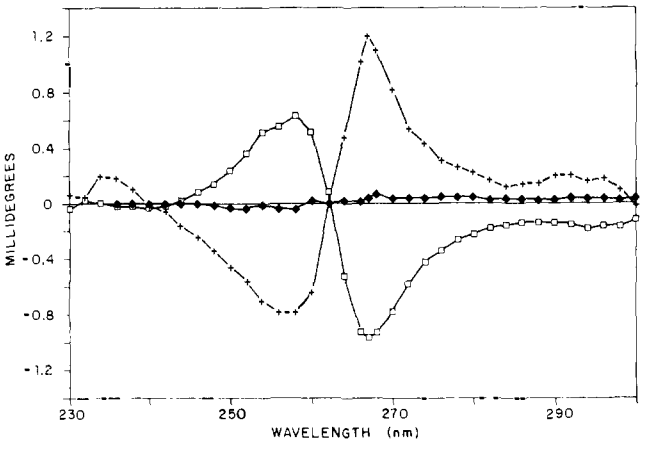

Figure 5. Circular dichroism of (□) Δ-$(phen)_3Ru^{2+}$ and of solutions after dialysis of *rac*-$(phen)_3Ru^{2+}$ against(+) B-DNA and (■) Z-DNA. Dialysis against B-DNA leads to enrichment of the solution in the unbound Λ isomer.

terms of the familiar Scatchard equation, X, the ratio of binding constants $K(\Delta)/K(\Lambda)$ may be calculated as follows:

$$X = (PK(\Delta)(n - r) + 1)\frac{(C_B + \Delta C)}{(C_B - \Delta C)} - PK(\Delta)(n - r) \quad (2)$$

where P is the concentration of DNA phosphate, n is the ratio of drug to DNA phosphate bound at saturation, taken as 0.125, C_B is the total concentration of ruthenium bound, and ΔC is the difference in free concentrations between Δ and Λ isomers as measured by the intensity in the circular dichroism. Measurements of several samples yielded values of 1.1–1.3 for X. Thus the binding affinity of Δ-$(phen)_3Ru^{2+}$ is found to be 10–30% greater than Λ-$(phen)_3Ru^2$ for calf thymus DNA by this method. This value is comparable to the differences seen in luminescence and unwinding experiments. Since the overall binding of $(phen)_3Ru^{2+}$ is small, binding isotherms obtained through equilibrium dialysis tended to show some scatter. A direct comparison of the binding isotherms for the enantiomers in equilibrium dialysis experiments using the pure isomers therefore could not be achieved; significant differences were not evident. Interestingly it appears that the method of optical enrichment yields the most sensitive assay for the differential binding.

Since the enrichment experiment provides the most sensitive method to examine enantiometric discrimination, poly(dGC) in 4M NaCl was also dialyzed against *rac*-$(phen)_3Ru^{2+}$ to test for any enantiomeric preferences in binding to a left-handed DNA helix.[32] At the low binding levels examined, the circular dichroism of the polymer remains inverted, indicating that racemic $(phen)_3Ru^{2+}$ did not induce a Z → B transition. After equilibrium dialysis with bound concentrations comparable to those in earlier experiments using calf thymus DNA, e.g., under conditions where low levels of enrichment could be detected, no optical activity was observed in the dialysate. Therefore, although intercalative binding had occurred, given similar spectral characteristics as in binding to the right-handed helix, no preference in binding was evident. In Figure 5 the essentially base line spectrum of a solution after dialysis against Z-form poly(dGC) has also been included. This lack of discrimination for $(phen)_3Ru^{2+}$ is understandable in view of the shallow, almost grooveless character of the left-handed Z-DNA helix.

Discussion

The present results indicate that tris(phenanthroline)ruthenium(II) binds to DNA by intercalation. The optical changes on binding to DNA agree with those seen for other intercalators. Hypochromicity in the metal to ligand charge-transfer (MLCT) band is observed and represents an overall 17% decrease in in-

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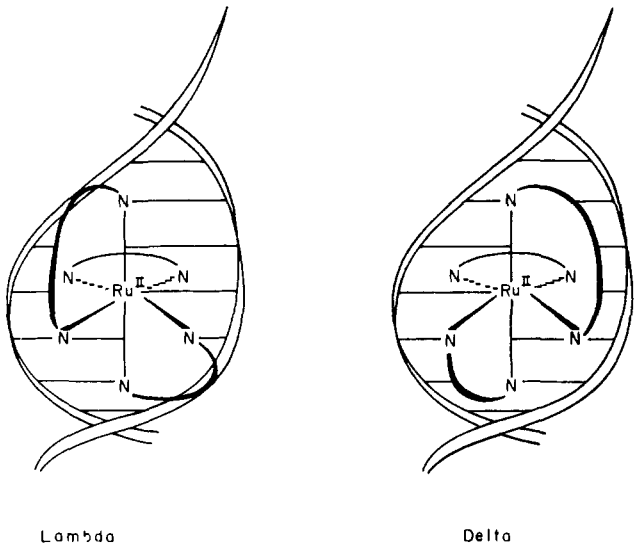

Figure 6. Schematic view of the enantiomers of $(phen)_3Ru^{2+}$ bound to B-DNA, illustrating the basis for the stereoselectivity. Unfavorable steric interactions are seen between the nonintercalated ligands of the Λ isomer and the DNA phosphate backbone. In contrast the Δ isomer fits easily in a right-handed helical groove.

tensity. Stacking interactions with the base pairs lead to hypochromic shifts in the $\Pi \rightarrow \Pi^*$ transitions of organic intercalating dyes, and it is interesting that the Π symmetry of the MLCT preserves the hypochromic effect here. Substantial increases in the luminescence of $(phen)_3Ru^{2+}$ also accompany binding to the duplex. The ehancement in emission and corresponding increased luminescent lifetimes may simply reflect the decreased mobility of the complex when sandwiched into the helix. Emission lifetimes are comparable to those found for $(phen)_3Ru^{2+}$ in sodium lauryl sulfate micelles.[33] In addition to perturbations in the electronic structure of the bound reagent, intercalation leads to hydrodynamic changes in the DNA duplex. With increasing concentrations, $(phen)_3Ru^{2+}$ reversibly unwinds and rewinds superhelical DNA. Although not absolutely definitive,[4] this result provides a very strong indication of intercalation. Surely helical unwinding and lengthening accompany the binding of $(phen)_3Ru^{2+}$. Finally the binding isotherms obtained by equilbrium dialysis yield parameters that are reasonable for the intercalative mode of association. The complex binds to duplex DNA with relatively low affinity and, when bound, encompasses a four base-pair site. The octahedral coordination around the metal precludes effective stacking of the complex between base pairs. If we consider one of the three phenanthroline ligands to insert into the helix, then the other two ligands actually protrude above and below the face of this phenanthroline and decrease the effective area of overlap. Hence only partial insertion is possible, which accounts for the low binding constant. The fact that so small a region of overlap with only partial insertion is necessary for a stabilizing interaction with the duplex is interesting to consider with respect to the binding of aromatic amino acid residues to DNA. The four base-pair site size is similarly consistent with our structural model for the bound complex, where one ligand intercalates and the remaining two ligands span the groove of the helix. A site size of four base pairs is understandable since the internuclear distance of 10.4 Å between distal hydrogen atoms on the ligands not only exceeds the 10.2 Å of a single interbase pair site but must result in partial blockage of the next neighboring base pair both above and below.

Intercalation of tris(phenanthroline)ruthenium(II) into the duplex imposes different steric constraints on Δ and Λ isomers, and it is this difference that determines the enantiomeric selectivity. Perhaps the strongest evidence in support of intercalation is the chiral discrimination we observe. The Δ enantiomer, a right-handed propeller-like structure, displays a greater affinity than Λ-$(phen)_3Ru^{2+}$ for the right-handed DNA helix. Figure 6 illustrates the basis for the enantiomeric selectivity. With one phenanthroline ligand intercalated, the two nonintercalated ligands of the Δ isomer fit closely along the right-handed helical groove. The nonintercalated ligands of the Λ enantiomer, in contrast, are repelled sterically by the phosphate backbone of the duplex. The disposition of the left-handed Λ enantiomer is opposed to the right-handed helical groove. The stereoselectivity seen here is in the direction proposed originally for $(phen)_3Zn^{2+}$ and supports our assignment of the absolute configurations for the zinc enantiomers. No stereoselectivity is apparent in the association of $(phen)_3Ru^{2+}$ with Z-DNA. This left-handed helix does not contain a groove of size and depth comparable to that in B-DNA, and therefore comparable or actually mirror image steric constraints are not expected. Instead the base pairs in the Z-DNA helix are pushed outward toward the solvent, resulting in at most a very wide and shallow major "groove". Hence Z-DNA provides a poor template for this discrimination.

Although it is Δ-$(phen)_3Ru^{2+}$ that binds preferentially to B-DNA, the Λ enantiomer does intercalate into the right-handed helix. The ratio of affinities of Δ and Λ isomers for B-DNA is 1.1–1.5, depending upon the method of analysis. Luminescence enhancements and unwinding experiments with supercoiled DNA suggest the Δ isomer to bind 30–50% more strongly. It is interesting that supercoiling does not alter the selectivity. Optical enrichment assays, which can be extremely sensitive and reflect a direct competition between enantiomers for the helix, yield values of 10–30% greater affinity of Δ-$(phen)_3Ru^{2+}$ for calf thymus DNA. A more precise determination of relative affinities is difficult because the binding constant of either enantiomer for the helix is low. In fact, then, the Λ enantiomer can bind to the right-handed helix, although the phosphate backbone limits access. The addition of bulky substituents onto the phenanthroline rings, in severely blocking interactions of the left-handed enantiomer with the duplex, is necessary to prevent completely intercalation of the Λ isomer.

The present study provides an example of stereospecific interactions with DNA. The stereoselectivity observed is governed by the handedness of the DNA helix. The assymmetric duplex structure serves as a template which discriminates in binding the small molecules on the basis of their chirality. It is interesting that the change in symmetry of the metal complex alone yields a significant difference in its recognition by the helix. The comparison of spectroscopic and binding characteristics of isomers of $(phen)_3Ru^{2+}$ has afforded a detailed description of the structural basis for the enantiomeric selectivity observed first for $(phen)_3Zn^{2+}$.[15] The difference in biological activities of tris(phenanthroline)metal enantiomers is, perhaps, also a function of this stereoselectivity.[34] Indeed the interaction of $(phen)_3Ru^{2+}$ with DNA illustrates how stereospecificity may be incorporated into the design of drugs that bind to the duplex and suggests a means to design reagents that can distinguish the handedness of the DNA helix.[35] Certainly these stereospecific interactions underscore the ability of small intercalating drugs to recognize differences in nucleic acid structure.

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Registry No. $Ru(phen)_3Cl_2$, 23570-43-6; Δ-$Ru(phen)_3(ClO_4)_2$, 27778-24-1; Λ-$Ru(phen)_3(ClO_4)_2$, 52436-98-3; poly(dGC), 36786-90-0.

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