Interactions of Enantiomeric Ruthenium(II) Complexes with Polynucleotides As Studied by Circular Dichroism, Electric Dichroism Measurements, and Photolysis

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The spectroscopic titrations, circular dichroism (CD), electric linear dichroism (LD), emission measurements, and photoactivated reactions have been used to study the interactions of the enantiomers of $[Ru(bpy)_2phi]Cl_2$ and $[Ru(phen)_2phi]Cl_2$ (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline and phi = 9,10-phenanthrenequinone diimine) with different compositions and conformations of polynucleotides. The investigated polynucleotides are calf thymus DNA, B-poly[d(G-C)]$_2$, B- and Z-poly[d(G-m^{5}C)]$_2$, B-poly[d(A-T)]$_2$, and B-[poly(dA)·poly-(dT)]. These complexes are bound to polynucleotides with the binding constants larger than 10^4 M^{-1}. From the CD and LD spectra, the phi ligands of both enantiomers are concluded to be intercalated between the base pairs of polynucleotides. According to the LD spectra, the opposite enantiomers orient the ancillary two ligands (bpy or phen) in different directions, indicating the presence of chiral discriminations by DNA. Z-poly[d(G-m^{5}C)]$_2$ is found to be transformed into the B-form upon intercalative binding with chiral complexes. There are no emission and photoactivated cleavage activities between enantiomers and polynucleotides.

Introduction

Binding studies of small molecules with DNA are very important in the development of new reagents which bind strongly and selectively to DNA. Metal complexes, natural antibiotics, porphyrins, protein, and oligonucleotide molecules have been investigated for their DNA binding affinities.[1−8] The binding modes are dependent on their sizes, functions, and stereochemical properties. The DNA photocleavage reactions that are activated by metal complexes and covalent interactions between DNA and metal complexes are also the subjects of interest.[9,10] Reversible interactions with DNA have occurred as (1) external surface binding for cations, (2) groove binding for large molecules, and (3) intercalations for the planar molecules or compounds containing planar rings systems. Metal chelates containing Ru, Fe, Cu, Zn, Co, and Rh have been studied previously.[11−16] $[RuL_3]^{2+}$ (L = polypyridyl) and its mixed ligand derivatives, for instance, are concluded to bind with DNA in two different binding interactions: (a) intercalation and (b) binding along the groove.[17] It has been reported that the phi ligands (phi = 9,10-phenanthrenequione diimine) of both Δ- and Λ-$[Ru(bpy)_2phi]^{2+}$ (bpy = 2,2′-bipyridine) complexes show the intercalation between the base pairs of calf thymus DNA.[18] The phi ligand of Δ-$[Rh(phen)_2phi]^{3+}$ (phen = 1,10-phenanthroline) complex intercalates in $[d(GTCGAC)_2]$.[19] The Δ- and Λ-enantiomers of $[Ru(phen)_2\ DPPZ]^{2+}$ complex, where DPPZ = dipyrido[3,2-*a*:2′,3′-*c*]phenazine, intercalate between the base pairs of calf thymus DNA.[20] One of the interesting aspects is the stereoselective effects on the interactions of the enantiomers of these complexes. Such effects would be a key to designing a reagent specific to the base sequences or conformations of polynucleotides.

In this work, $[Ru(bpy)_2phi]Cl_2$ and $[Ru(phen)_2phi]Cl_2$ complexes were synthesized and resolved to opposite enantiomers. The binding interactions between an enantiomer and a duplex polynucleotide such as calf thymus DNA, B-poly[d(G-C)]$_2$, B- and Z-poly[d(G-m^{5}C)]$_2$, B-poly[d(A-T)]$_2$, and B-[poly(dA)·poly-(dT)] have been studied by using spectroscopic titration, circular dichroism (CD), electric linear dichroism (LD), and emission methods. The photoactivated reactions between plasmid DNA and each enantiomer have also been done. Structures are shown in Figure 1.

Experimental Section

Ruthenium Complexes. $[Ru(bpy)_2phi]Cl_2$ and $[Ru(phen)_2phi]Cl_2$ were synthesized from $RuCl_3{\cdot}xH_2O$ (Aldrich) and $K_2[RuCl_5(H_2O)]$ (Johnson Matthey) according to literature methods.[16,21,22] The racemic $[Ru(bpy)_2phi]Cl_2$ complex was resolved by being eluted on a CM-Sephadex column [35 × 1.8 cm (i.d.)] with a saturated antimonyl potassium tartrate solution. The Δ-isomer was eluted first, being followed by the Λ-isomer. The assignment of the Δ-isomer in the CD spectrum can be interpreted by a deep negative ellipticity band located

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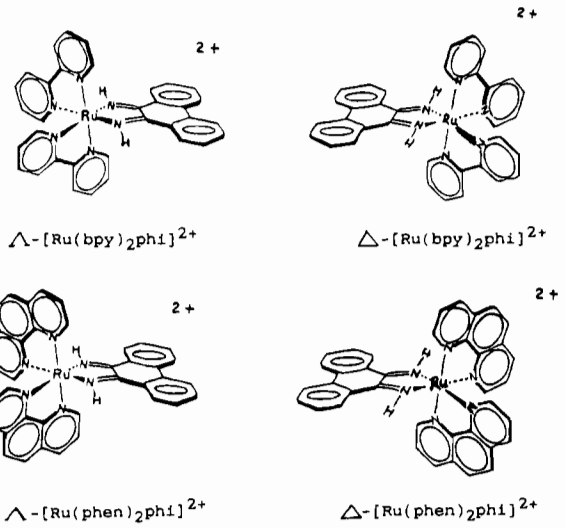

Figure 1. Absolute configurations of chiral ruthenium complexes.

at 287 nm and a positive ellipticity band about 270 nm. This is consistent with the previous results for analogous bisbidentate chelate complexes.[23] $\Delta\epsilon_{535}$ was determined to be 23 and -24 M^{-1} cm^{-1} for Δ- and Λ-enantiomers, respectively. For racemic $[Ru(phen)_2phi]^{2+}$, the same procedure was carried out and $\Delta\epsilon_{535}$ was determined to be 16 and -16 M^{-1} cm^{-1} for Δ- and Λ-enantiomers, respectively. The similar assignment of absolute configuration can be done for Δ- and Λ-enantiomers. The concentrations of $[Ru(bpy)_2phi]2^+$ and $[Ru(phen)_2phi]^{2+}$ were determined spectrophotometrically at 535 nm by using the reported values of extinction coefficients of 48 000 and 51 900 M^{-1} cm^{-1}, respectively.[16]

Double Stranded Nucleic Acids. In some cases, a [poly(dA)·poly(dT)] solution was prepared without further purification or dialysis. The pH of the polynucleotide solution was adjusted to 7.0 with 1 mM sodium cacodylate buffer. In other preparations, the polynucleotide was dissolved in 1 mM sodium cacodylate buffer (pH = 7) and subjected to repeated phenol extractions (three times) and then precipitated by ethanol, dissolved, and dialyzed against a cacodylate buffer. No significant differences in the binding constants beween the purified and nonpurified polynucleotides were observed. Calf thymus DNA, the sodium salt of poly[d(G-C)]$_2$, poly[d(A-T)]$_2$, and [poly(dA)·poly(dT)] were purchased from Sigma. The concentrations of these polynucleotides were determined spectrophotometrically by using the extinction coefficients 6600 (260 nm),[24] 8400 (260 nm),[25] 6600 (260 nm),[26] and 6000 (258 nm),[27] respectively. Poly[d(G-m^{5}C)]$_2$ (Pharmacia Biochemicals) was transformed into Z-forms according to the literature methods.[28] B- and Z-poly[d(G-m^{5}C)]$_2$ were obtained using a 35 mM sodium cacodylate solution and 1.2 mM sodium cacodylate and 30 μM Mg^{2+} solutions, respectively. Their concentrations were determined spectrophotometrically using the extinction coefficient 7000 (260 nm).[29] The formation of B- and Z-form polynucleotides was confirmed from the absorption and CD spectra in the UV region.

Spectroscopic Titration. Spectroscopic titrations were carried out at room temperature to determine the binding strength between a polynucleotide and each enantiomer. Two kinds of solutions were prepared for spectroscopic titration. Solution **A** contained a chiral metal complex and a polynucleotide. Solution **B** contained the same concentration of the metal complex only. In titration, solution **A** was added by units of 0.1 mL to 2 mL of solution **B**.

CD, Emission, and LD Measurements. CD and emission spectra were recorded for a solution of a free enantiomer or a solution containing the enantiomer fully bound to a polynucleotide. LD spectra were measured with an instrument as described previously.[30] The field strength dependence studies of linear dichroism were done at 540 nm by changing the applied field from 540 V/cm to 5.46 kV/cm at the constant pulse width of 400 μs. The wavelength dependence of dichroism was determined by applying a pulse of 5.46 kV/cm × 1 ms.

The Photoactivated Reactions. The photoactivated reactions between each enantiomer and plasmid DNA (pBluescriptII (Stratagene, CA)) were done using 5 mm glass tubes containing a 10 μL solution mixture of plasmid DNA (3×10^{-5} M) and an enantiomer (0.6×10^{-5} M) under the irradiation of 400 nm light at 35° for 3 h. After that, the sample solution was mixed with 2 μL of bromothimol blue gel-loading buffer.[31] The sample solutions (each 10 μL) were electrophoresed for 1 h at 60 V on 1% agarose gel stained with ethidium bromide. The TBE solution was used as an electrophoresis buffer.

Instrumentation. Absorbance, circular dichroism, and emission measurements were done with a spectrophotometer (JASCO-UVIDEC-430A), a spectropolarimeter (JASCO-J-500A), and a spectrofluorometer (JASCO-FP-770), respectively. The instrument used to measure the linear dichroism was constructed in this laboratory.[30] For the CD measurements in the vacuum UV region, the spectropolarimeter (JASCO-J-700) was used.

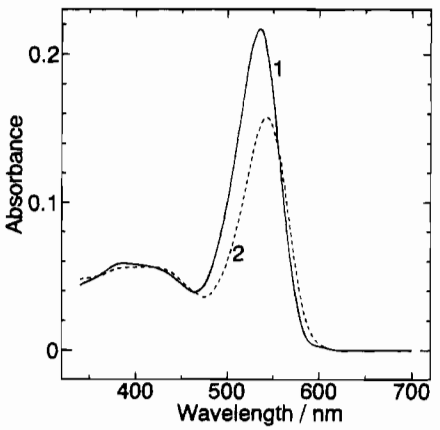

Figure 2. Absorption spectra of (a) Λ-$[Ru(phen)_2phi]^{2+}$ (4.12×10^{-6} M) and (b) Λ-$[Ru(phen)_2phi]^{2+}$ (4.12×10^{-6} M) and Z-poly[d(G-m^{5}C)]$_2$ (2.21×10^{-5} M). In both cases, 1.2 mM sodium cacodylate and 30 μM Mg^{2+} were added.

Results

Spectroscopic Titration. For spectroscopic titrations, a solution of a chiral metal complex and a polynucleotide (solution **A**) was added to a solution of the same concentration of the metal complex only (solution **B**). Solution **A** was added by units of 0.1 mL to 2 mL of solution **B**. The concentrations of the metal complex and salts were kept constant throughout the titration. In the cases of calf thymus DNA, B- and Z-poly-[d(G-m^{5}C)]$_2$, 16 mM sodium cacodylate, 35 mM sodium cacodylate, and 1.2 mM sodium cacodylate and 30 μM Mg^{2+} were used, respectively. In the case of other synthetic polynucleotides, 1 mM sodium cacodylate was used. Curve 1 in Figure 2 (solution **B** of Λ-$[Ru(phen)_2phi]^{2+}$) exhibits the absorption maxima at 535 nm, which is assigned to metal-to-ligand charge-transfer (MLCT) band from a central Ru^{2+} to the phi ligand, and the absorption band around 440–470 nm, which is assigned to MLCT from a central Ru^{2+} to the phen ligands. On adding solution **A** of Z-poly[d(G-m^{5}C)], the absorbance of the MLCT band at 535 nm decreased with the concomitant shift of the peak position towards the longer wavelength. The addition of solution **A** was done until the absorbance at 535

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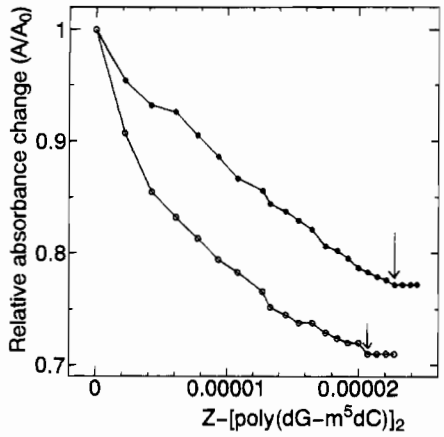

Figure 3. Decreases of relative absorbance changes (535 nm) of Δ-[Ru(phen)$_2$phi^{2+}] (5.07×10^{-6} M) (●) and of Λ-[Ru(phen)$_2$phi^{2+}] (4.12×10^{-6} M) (○) with the addition of solution **A** of Z-poly[d(G-m^{5}C)] (4.66×10^{-5} M). The saturated levels are indicated by arrows.

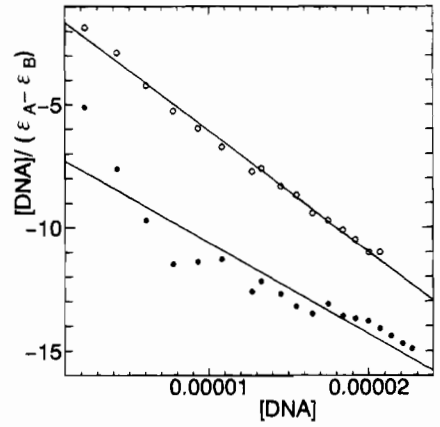

Figure 4. Plot of [DNA]/($\epsilon_A - \epsilon_B$) vs [DNA] for the systems as shown in Figure 3.

nm remained unchanged and the spectrum attained the final curve 2 in Figure 2. At this stage, all binding sites of the polynucleotides were fully bound by chiral ruthenium complex.

Figure 3 shows the plot of the relative absorbance change at 535 nm (A/A_0) against the concentration of the polynucleotide, $[DNA]_0$, where A_0 and A are the initial absorbance of solution **B** {Δ-[Ru(phen)$_2$phi]$^{2+}$ (5.07×10^{-6} M) (●) or Λ-[Ru(phen)$_2$-phi]$^{2+}$ (4.12×10^{-6} M) (○)} and the absorbance of a titrated mixture after addition of solution **A** of Z-poly[d(G-m^{5}C)] (4.66×10^{-5} M), respectively. A/A_0 decreases with the increase of $[DNA]_0$ until it is leveled off in the range of $[DNA]_0$ larger than $[DNA]_s$ as denoted by an arrow in the figure. The numbers of nucleotide phosphate of polynucleotide occupied by one complex (n) was estimated approximately by

$$n = [DNA]_s/[M] \quad (1)$$

in which [M] is the concentration of the chiral metal complex. The spectral change from curve 1 to curve 2 in Figure 2 clearly points out the binding of the metal complex with the polynucleotide.[32]

The intrinsic binding constant of an enantiomer was determined according to eq 2,[33] where [DNA] is the equilibrium

$$[DNA]/(\epsilon_A - \epsilon_B) = [DNA]/(\epsilon_B - \epsilon_F) + 1/K_B(\epsilon_B - \epsilon_F) \quad (2)$$

concentration of polynucleotide and ϵ_A, ϵ_F, and ϵ_B correspond to absorbance/[Ru], the extinction coefficient for a free ruthenium complex and the extinction coefficient for a bound ruthenium complex, respectively. In Figure 4, $[DNA]/(\epsilon_A - \epsilon_B)$ is plotted against [DNA] to give a straight line for each enantiomer. The ratio of the slope to the intercept of the y-axis is equal to the binding constant, K_B.

Table 1 summarizes the titration results in which the red shift (R) of the absorption peak at 535 nm (for both complexes), the average numbers of nucleotide phosphate of polynucleotides occupied by one chiral complex (n), and the intrinsic binding constant (K_B) are given for the enantiomers of [Ru(phen)$_2$phi]$^{2+}$ and [Ru(bpy)$_2$phi]$^{2+}$ bound to polynucleotides.

CD Measurements. The CD spectrum was compared between free and fully bound enantiomers of [Ru(phen)$_2$phi]$^{2+}$ and [Ru(bpy)$_2$phi]$^{2+}$. Figure 5 shows the CD spectra for the systems of Δ- or Λ-[Ru(phen)$_2$phi]$^{2+}$ and calf thymus DNA. In the spectrum, a free Δ-isomer shows the positive band around 520−550 nm. When binding to DNA takes place, the band changes the peak position toward the longer wavelength by about 30 nm with the concomitant increase of the amplitude from $\Delta\epsilon = +1.7$ to $+10$. For Λ-isomer, the negative band around 520−550 nm reverses the sign to positive one on binding to DNA. The peak position shifts towards the longer wavelength by 30 nm and $\Delta\epsilon$ changes from -1.8 to $+6.2$. As a result, the absorption bands around 520−550 nm for both enantiomers have the same positive sign on a DNA strand. The behavior may be attributed to the induction of positive CD due to the asymmetric environment of the right-handed helicity of DNA. The results suggest that the phi ligands of both enantiomers penetrate deeply the DNA double helices. The similar inversion of the sign of the band at 520−550 nm is observed for Λ-[Ru(bpy)$_2$phi]$^{2+}$, which binds to calf thymus DNA, indicating that the phi ligand of the bound complex penetrates the polynucleotide helices. The same effects for the CD spectra are seen when the enantiomers of [Ru(bpy)$_2$phi]$^{2+}$ and [Ru(phen)$_2$phi]$^{2+}$ are bound to B-polynucleotide and Z-poly-[d(G-m^{5}C)]$_2$. In all of the cases, the absorbance band in the region of 350−500 nm shows little change on binding to a polynucleotide.

CD measurements in the vacuum UV region were carried out to know the final conformation of a polynucleotide after intercalative binding with enantiomer. Because of the overlap of CD peaks of bpy or phen ligands and that of DNA, CD spectra in the near-UV region (above 200 nm) cannot be used to determine the final conformation of DNA. Figure 6 shows the CD spectra of (a) free Z-poly[d(G-m^{5}C)]$_2$, (b) Z-poly[d(G-m^{5}C)]$_2$ after intercalative binding with Δ-[Ru(bpy)$_2$phi]$^{2+}$ and (c) B-poly[d(G-m^{5}C)]$_2$ after intercalative binding with Δ-[Ru(bpy)$_2$phi]$^{2+}$. According to the literature, the B-form is characterized by a strong positive peak at 186 nm while the Z-form is characterized by a negative one at 195 nm.[34−36] From the spectra, it is observed that the B-polynucleotide still exists in the B-form after binding, while Z-poly[d(G-m^{5}C)]$_2$ changes to the B-form after binding with Δ-[Ru(bpy)$_2$phi]$^{2+}$ enantiomer. All enantiomers of the present chelates induce the conformational transition from Z- to B-poly[d(G-m^{5}C)]$_2$.

LD Measurements. The orientation of an enantiomer bound to polynucleotide is studied by means of electric linear dichroism

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Table 1. Binding Parameters for Chiral Ruthenium Complexes

	$[Ru(bpy)_2phi]^{2+}$						$[Ru(phen)_2phi]^{2+}$					
	Δ-isomer			Λ-isomer			Δ-isomer			Λ-isomer		
polynucleotide	$K_B \times 10^4$	n	R (nm)	$K_B \times 10^4$	n	R (nm)	$K_B \times 10^4$	n	R (nm)	$K_B \times 10^4$	n	R (nm)
calf thymus[a]	1.3	6.1	8	1.1	6.3	8	3.1	6.7	8	1.3	6.8	8
B-p(dG-dC)$_2$[b]	20.1	3.2	6	126.2	3.0	5	95.0	3.5	4	89.5	3.5	6
B-p(dA-dT)$_2$[b]	126.8	3.5	5	123.5	4.0	5	82.4	3.9	3	77.3	4.1	3
B-(pA-pT)[b]	103.9	3.4	4	85.4	3.9	4	7.2	4.4	4	8.7	4.2	4
B-p(dG-m^{5}dC)$_2$[c]	4.2	2.9	5	163.3	2.4	6	4.0	3.1	6	243.1	2.9	6
Z-p(dG-m^{5}dC)$_2$[d]	90.2	2.8	5	28.3	3.1	5	5.3	4.5	5	43.2	5.0	11

[a-d] In the cases *a*, *b*, *c*, and *d*, 16 mM sodium cacodylate, 1 mM sodium cacodylate, 35 mM sodium cacodylate, and 1.2 mM sodium cacodylate and 30 μM Mg^{2+} were used, respectively. K_B, n, and R are the binding constant, average numbers of nucleotide phosphate occupied by one chiral complex and red shift of the absorption peak at 535 nm (for both complexes), respectively.

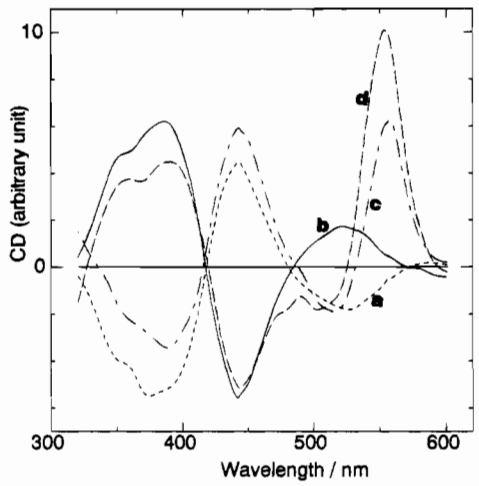

Figure 5. CD spectra of free and bound $[Ru(phen)_2phi]^{2+}$ enantiomers: (a) Λ-isomer (1.83×10^{-5} M); (b) Δ-isomer (1.85×10^{-5} M); (c) Λ-isomer (1.83×10^{-5} M) and calf thymus DNA (1.38×10^{-4} M); (d) Δ-isomer (1.85×10^{-5} M) and calf thymus DNA (1.47×10^{-4} M).

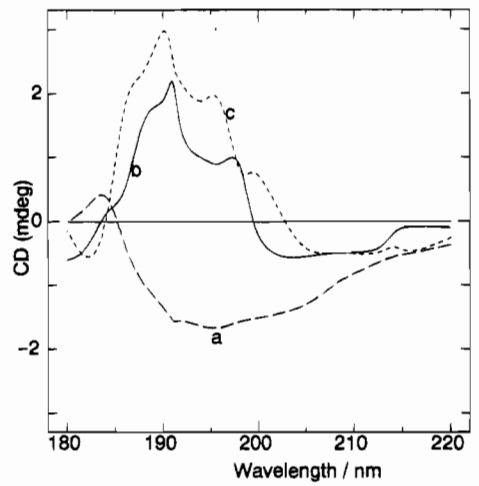

Figure 6. CD spectra in the vacuum UV region for (a) free Z-poly-[d(G-m^{5}C)]$_2$, (b) Z-poly[d(G-m^{5}C)]$_2$ after intercalative binding with Δ-$[Ru(bpy)_2phi]^{2+}$, and (c) B-poly[d(G-m^{5}C)]$_2$ after intercalative binding with Δ-$[Ru(bpy)_2phi]^{2+}$.

(LD) technique. The electric dichroism is measured by applying an electric field pulse of 5.46 kV/cm × 1 ms on a solution of chiral complex-DNA. Under this situation, DNA orients with the helical axis in the direction of electric field. A bound chromophore takes a definite orientation unless it is oriented randomly on a DNA chain. As a result, uniaxial anisotropy is induced in the electronic absorption spectra of the bound chromophore. The induced change of absorbance obeyed the following equation of orientational dichroism:[37]

$$\Delta A/A = (\varrho/6)(1 + 2\cos 2\theta) \quad (3)$$

In this equation $\Delta A/A$ and θ denote the relative absorbance change and the angle between the electric field and the polarization of monitoring light, respectively. ϱ is the reduced linear dichroism defined by

$$\varrho = (\epsilon_{\parallel} + \epsilon_{\perp})/\epsilon \quad (4)$$

in which ϵ, $\epsilon_{\perp}$, and $\epsilon_{\parallel}$ are the extinction coefficients for nonpolarized, perpendicularly polarized, and paralleled polarized lights, respectively.

Figures 7 and 8 show the results for the systems of Δ- and Λ-$[Ru(phen)_2phi]^{2+}$ or Δ- and Λ-$[Ru(bpy)_2phi]^{2+}$ with calf thymus DNA in which ΔA is plotted as a function of wavelength from 380 to 600 nm covering the MLCT absorptions to phen or bpy and phi ligands, respectively. Figures 9–11 show the results of $[Ru(phen)_2phi]^{2+}$ or $[Ru(bpy)_2phi]^{2+}$ with synthetic polynucleotides. General aspects of the dichroism spectra are summarized as below:

(1) The MLCT band due to a phi ligand shows largely negative dichroism. A Λ-isomer exhibits a little more negative amplitude than a Δ-isomer.

(2) The MLCT band due to phen and bpy ligands shows smaller dichroism. The Λ-isomer exhibits a positive dichroism, while a Δ-isomer exhibits nearly zero or zero dichroism.

Reduced linear dichroism (ϱ) in eq 3 is a function of an angle between the transition moment (μ) of electronic absorption and the electric field direction. The MLCT band due to the phi ligand has the μ directed from a Ru(II) atom to the ligand along its short axis. Based on this, ϱ is expressed by the following equation:[37]

$$\varrho = (3/4)(1 + 3\cos 2\Psi_1)\Phi(E) \quad (5)$$

$\Phi(E)$ is the orientation function representing the degree of the fraction of DNA polymer aligned in the electric field (E) direction and Ψ_1 is the angle between the C_2 axis, which is parallel to the transition moment of MLCT band (phi ligand) and the helical axis of DNA.

$\Phi(E)$ is determined from the dependence of the dichroism amplitude on the electric field strength. $\Phi(E)$ is assumed to obey the following equation:[38]

$$\Phi(E) = 1 - 3(\coth X - 1/X)X \quad (6a)$$

$$X = \mu E/kT \quad (6b)$$

μ is the dipole moment of a polynucleotide and k is Boltzmann's

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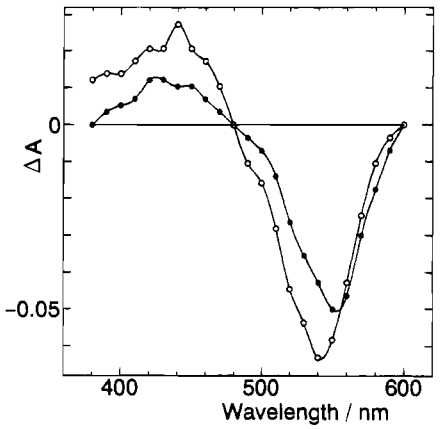

Figure 7. Wavelength dependence of ΔA at $\theta = 0°$ for (●) Δ-[Ru(phen)$_2$phi]$^{2+}$ (1.85×10^{-5} M) and calf thymus DNA (1.47×10^{-4} M) and (○) Λ-[Ru(phen)$_2$phi]$^{2+}$ (1.83×10^{-5} M) and calf thymus DNA (1.38×10^{-5} M).

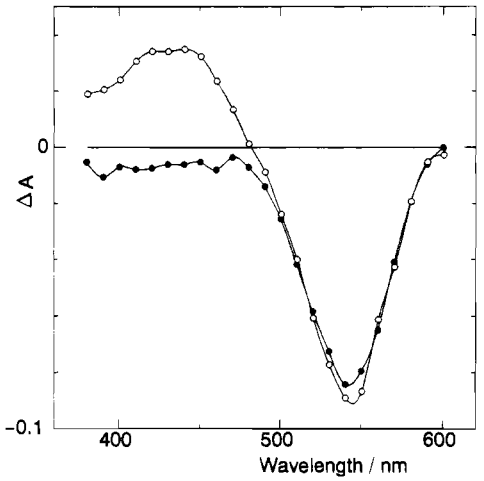

Figure 8. Wavelength dependence of ΔA at $\theta = 0°$ for (●) Δ-[Ru(bpy)$_2$phi]$^{2+}$ (1.66×10^{-5} M) and calf thymus DNA (1.42×10^{-4} M) and (○) Λ-[Ru(bpy)$_2$phi]$^{2+}$ (1.56×10^{-5} M) and calf thymus DNA (1.04×10^{-4} M).

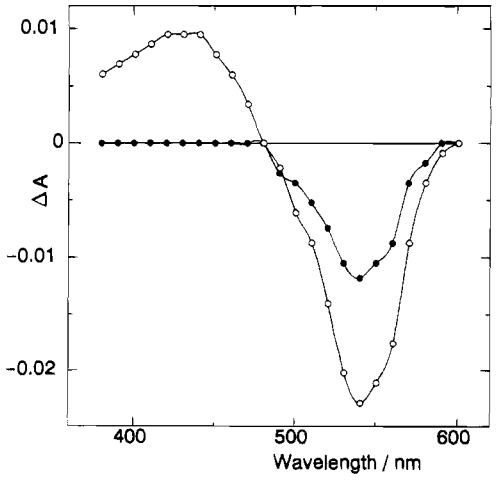

Figure 9. Wavelength dependence of ΔA at $\theta = 0°$ for (●) Δ-[Ru(phen)$_2$phi]$^{2+}$ (6.20×10^{-6} M) and B-poly[d(G-C)]$_2$ (2.14×10^{-5} M) and (○) Λ-[Ru(phen)$_2$phi]$^{2+}$ (5.93×10^{-6} M) and B-poly[d(G-C)]$_2$ (2.19×10^{-5} M).

constant. By comparison of the dependence of ϱ on E for the range of $E = 0.54-5.46$ kV/cm with eq 6a, we choose 5.79×10^{-4} cm/V as the best value of μ/kT as shown in Figure 12. From this value, $\Phi(E)$ is determined to be 0.35 at $E = 5.46$ kV/cm.

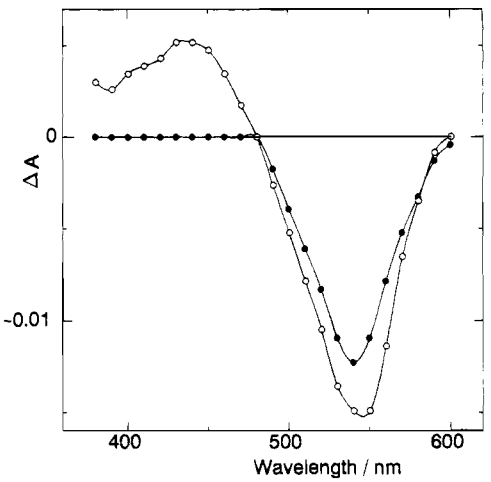

Figure 10. Wavelength dependence of ΔA at $\theta = 0°$ for (●) Δ-[Ru(bpy)$_2$phi]$^{2+}$ (1.1×10^{-5} M) and B-poly[d(G-C)]$_2$ (1.56×10^{-5} M) and (○) Λ-[Ru(bpy)$_2$phi]$^{2+}$ (1.06×10^{-5} M) and B-poly[d(G-C)]$_2$ (1.88×10^{-5} M).

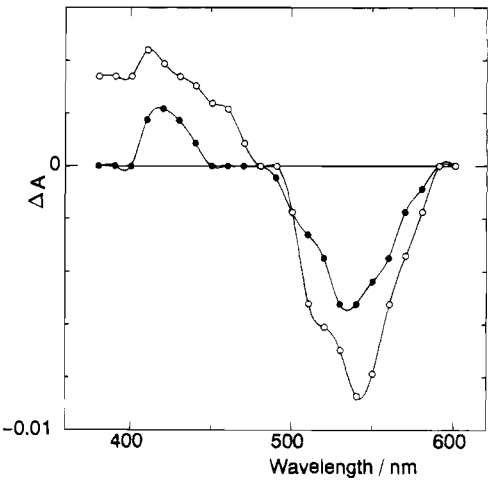

Figure 11. Wavelength dependence of ΔA at $\theta = 0°$ for (●) Δ-[Ru(phen)$_2$phi]$^{2+}$ (6.67×10^{-6} M) and B-poly[d(A-T)]$_2$ (2.72×10^{-5} M) and (○) Λ-[Ru(phen)$_2$phi]$^{2+}$ (6.45×10^{-6} M) and B-poly[d(A-T)]$_2$ (2.72×10^{-5} M).

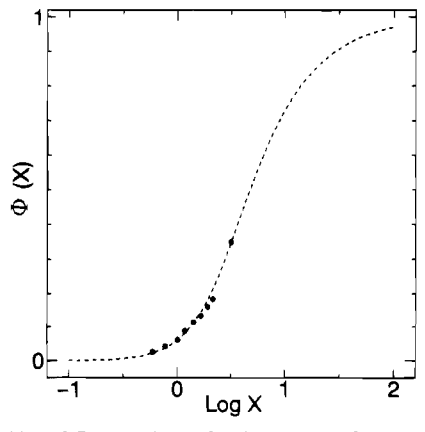

Figure 12. Plot of $\Phi(E)$ vs log X for the system of Δ-[Ru(bpy)$_2$phi]$^{2+}$ and calf thymus DNA. Key: (- - -), theoretical curve calculated from eq 6a; (●) experimental values obtained from $E = 0.54-5.46$ kV/cm. Here X represents $\mu E/kT$ in which μ is the permanent dipole moment of DNA, k, the Boltzmann constant and T, absolute temperature.

The MLCT band due to the phen or bpy ligands is composed of two independent μ's: one (μ_1) is directed along the short axis of one phen or bpy ligand and the other (μ_2) is perpendicular to μ_1.[23] Both μ_1 and μ_2 are located on the plane perpendicular

Table 2. Reduced Linear Dichroism at Complete Orientation (ϱ/Φ) and Orientational Angles, Ψ_1 and Ψ_2 (in deg), for Chiral Ruthenium Complexes Bound to Calf Thymus DNA and Synthetic Polynucleotides (See Text for Definitions of Ψ_1 and Ψ_2)

	$[Ru(bpy)_2phi]^{2+}$												$[Ru(phen)_2phi]^{2+}$											
	Δ-isomer						Λ-isomer						Δ-isomer						Λ-isomer					
	phi		bpy		bpy		phi		bpy		bpy		phi		phen		phen		phi		phen		phen	
polynucleotide	ϱ/Φ	Ψ_1	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_1	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_1	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_1	ϱ/Φ	Ψ_2	ϱ/Φ	Ψ_2
calf thymus	−0.93	77	−0.29	52	−0.15	56	−0.5	70	0.27	69	0.1	64	−0.70	72	0.28	70	0.37	73	−0.87	76	0.45	76	0.32	71
B-p(dG-dC)$_2$	−0.17	63	0	61	0	61	−0.19	64	0.24	68	0.19	67	−0.35	66	0	61	0	61	−0.5	69	0.34	72	0.43	75
B-p(dA-dT)$_2$	−0.16	63	0.13	65	0	61	−0.19	64	0.27	69	0.34	72	−0.12	63	0	61	0.13	65	−0.23	64	0.22	68	0.23	68
B-(pA-pT)	−0.32	66	0.14	65	0.09	64	−0.35	66	0.27	69	0.24	68	−0.15	63	0.17	66	0.17	66	−0.23	64	0.11	64	0.15	65
B-p(dG-m^{5}dC)$_2$	−0.11	62	0	61	0	61	−0.12	63	0.09	64	0.03	61	−0.13	63	0	61	0	61	−0.18	64	0.20	67	0.11	64
Z-p(dG-m^{5}dC)$_2$	−0.17	63	0	61	0	61	−0.15	63	0.27	69	0.27	69	−0.29	65	0	61	0	61	−0.51	70	0.22	68	0.21	67

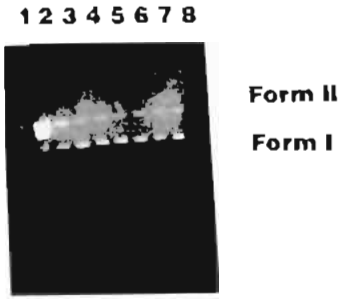

Figure 13. Electrophoresis pattern on 1% agarose gel (with ethidium bromide) of plasmid DNA with ruthenium complexes. Lanes 1, 2, 3, 4, 5, 6, 7, and 8 are DNA standard marker (DNA/HindIII fragments), only plasmid DNA, Δ- and Λ-enantiomers of $[Ru(bpy)_2phi]^{2+}$ and $[Ru(phen)_2phi]^{2+}$, and racemic mixture of both complexes in the absence of light, respectively. Forms I and II are the supercoiled closed circular and nicked circular DNA's, respectively.

to the pseudo-C_3 axis of a complex. Supposing that these transition moments orients in any direction with the same probability on that plane, ϱ is given by the following equation:[39]

$$\varrho = -(3/8)\,(1 + 3\cos 2\Psi_2)\Phi(E), \qquad (7)$$

Ψ denotes the angle between the C_3 axis of a complex and the electric field direction.

Table 2 shows the results of the electric dichroism in which the ϱ/Φ, Ψ_1, and Ψ_2 are given for the enantiomers of $[Ru(phen)_2phi]^{2+}$ and $[Ru(bpy)_2phi]^{2+}$ bound to calf thymus DNA and synthetic polynucleotides. ϱ/Φ is the reduced linear dichroism at complete orientation at wavelengths (nm) of 540 (phi), 450 and 420 (two phen's), and 450 and 420 (two bpy's). Ψ_1 and Ψ_2 are the angles in degrees.

Emission Measurements. Emission measurements were done for the free and bound enantiomers of both complexes. There is no emission property in free and bound enantiomers.

The Photoactivated Reactions. Photoactivated cleavage was investigated as described in the experimental section. No photoinduced plasmid DNA cleavage was observed for each enantiomer. Figure 13 shows the electrophoresis pattern of plasmid DNA with chiral ruthenium complexes. Lanes 1, 2, 3, 4, 5, 6, 7, and 8 are DNA standard marker (DNA/Hind III fragments), only plasmid DNA, Δ- and Λ-enantiomers of $[Ru(bpy)_2phi]^{2+}$ and $[Ru(phen)_2phi]^{2+}$, racemic mixture of both complexes in the absence of light, respectively. Forms I and II are the supercoiled closed circular and nicked circular DNA's, respectively. Since no increase of form II was observed in photolysis, we concluded that there was no photoinduced plasmid DNA cleavage of enantiomers.

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Discussions

The present work has attempted to elucidate the binding structures of chiral metal complexes with natural and synthetic polynucleotides. The photocleavage activities of chiral complexes were also studied.

The binding constant (K_B) is determined for the binding of enantiomeric $[Ru(bpy)_2phi]^{2+}$ and $[Ru(phen)_2phi]^{2+}$ with calf thymus DNA and synthetic polynucleotides. As a result, the enantiomers are bound to DNA with K_B larger than 10^4 M^{-1} (Table 1). These values are more than 10 times larger than those obtained for $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$.[16] Thus the presence of the phi ligand has an effect to increase affinity toward the polynucleotides. The significant stereoselectivity has been observed in the cases of $[Ru(bpy)_2phi]^{2+}$−B-poly[d(G-C)]$_2$, B-poly[d(G-m^{5}C)]$_2$ with $[Ru(bpy)_2phi]^{2+}$ and $[Ru(phen)_2phi]^{2+}$. In these cases large chiral discrimination was seen between the opposite enantiomers.

CD spectra give us the informations on how the helicity of the DNA chain influences the electronic transitions of a bound complex. One of the main conclusions from these measurements is that both $[Ru(bpy)_2phi]^{2+}$ and $[Ru(phen)_2phi]^{2+}$ are bound to B-polynucleotides and Z-poly[d(G-m^{5}C)]$_2$ with their phi ligands intercalated between base pairs. This conclusion is derived from the CD spectra of a bound complex. According to them, the large induction effect by the chain helicity is observed on the spectrum of a bound complex in the MLCT region from a Ru(II) atom to a phi ligand. In the cases of both Δ- and Λ-enantiomers, the binding with a DNA results in the shift of the CD band toward the positive direction. For the Λ-enantiomer, the effect is so prominent that the sign of the band is reversed from the negative sign to the positive one. Based on the results, we conclude that the phi ligands of both complexes are located very closely to the helical backbone. Even if a polynucleotide is in the Z-form as in Z-poly[d(G-m^{5}C)]$_2$, both enantiomers of the present chelates exhibit the intercalative binding. The behavior of intercalation of both enantiomers can be considered as follow. The steric interaction between nonintercalated (ancillary) two ligands and the outer region of DNA is expected to have a prominent factor. Aromatic molecules containing three or four fused rings have no such interaction and they behaved as good intercalators.[40−42] This steric interaction can be relatively small in the case of small ligands like phen or bpy. In fact, Δ-$[Ru(phen)_3]^{2+}$ or Δ-$[Ru(bpy)_3]^{2+}$ isomer which has the same helicity as that of the major groove of DNA showed the intercalative binding properties.[17] In these cases, enantiomeric preference has still exist for

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Δ-isomer with B-DNA. However, when one of the bpy or phen ligand in complex is replaced with a ligand with more extended fused rings such as X in RuL_2X complexes, where X = dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz)[20] or 4,7-phenanthrolino-[6,5-*b*]pyrazine (ppz)[43] or phi,[18] L = bpy or phen, the steric interaction can be reduced by this substitution. Consequently, there are no enantiomeric preferences between Δ- and Λ-enantiomers with B-DNA and both enantiomers showed the intercalative binding.

LD measurements assist the above conclusions by revealing that the phi ligand orients roughly perpendicularly to the helical axis of DNA (Table 2). When the complexes intercalate their phi ligands between the base pairs, the ligands eventually take the direction perpendicular to the helical axis. The orientation of the phi ligand is determined uniquely because the MLCT band from the Ru(II) atom to the phi ligand has the characteristic absorption band at the longer wavelength. As a result, both enantiomers are concluded to intercalate their phi ligands between the base pairs.

As for the orientations of two ancillary (bpy or phen) ligands, the LD spectra show definite difference between the Δ- and Λ-isomers for both $[Ru(bpy)_2phi]^{2+}$ and $[Ru(phen)_2phi]^{2+}$ complexes. For the Λ-isomers (Figures 7−11) the dichroism amplitude at $\theta = 0°$, $\Delta A(\theta)$, is positive in the range 400−450 nm, while $\Delta A(\theta)$ for Λ-isomer is nearly zero or negative in this wavelength region. The absorption bands in this region contain two transitions related to the MLCT from the Ru(II) ion to two bpy or phen ligands.[17] As a result, the plane containing these two transition moments is concluded to orient in the different directions between the Δ- and Λ-isomers, although the differences in angles are very small. These differences may arise from the steric hindrance from the helical periphery of the DNA.

Another interesting aspect in the present study is that the conformation change is induced in Z-poly[d(G-m^5C)]$_2$ when chiral complexes are intercalated in the base pairs. This conclusion is deduced from the CD spectra in vacuum UV region. The CD spectra in vacuum UV region are free from absorption due to ligands and dominated by base pairs interactions only. Therefore they are sensitive indicators of helical geometry of DNA. This is an allosteric Z- to B-transition upon binding of chiral complexes to Z-poly[d(G-m^5C)]. This phenomenon also support the intercalation of phi ligands of chiral complexes. It is well established that intercalators inhibit the transition of B-form to Z-form.[29,44,45]

The base sequences do not seem to affect the binding structures of these complexes greatly from the measurements of binding constants. In accord with this, nearly the same wavelength dependence of the dichroism amplitude is observed when the enantiomers of the complexes are bound to B-poly-[d(G-C)]$_2$ or B- and Z-poly[d(G-m^5C)]$_2$ or B-poly[d(A-T)]$_2$ or B-[poly(dA)·poly(dT)]. Thus it is concluded that the intercalative properties of the present complexes are so strong that the base pair sequences have little effect on the binding strength.

The present complexes show no photoactivated cleavage of plasmid DNA. The reason is that there is no emission from the complexes, thus irradiation does not produce a cleaving agent such as O_2^-.

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