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Sequence Dependent Binding Modes of the ΔΔ- and ΛΛ-binuclear Ru(II) Complexes to poly[d(G-C)$_2$] and poly[d(A-T)$_2$]

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The binding properties and sequence selectivities of ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ (bip = 4,4'-biphenylene (imidazo [4,4-*f*][1,10]phenanthroline) complexes with poly[d(A-T)$_2$] and poly[d(G-C)$_2$] were investigated using conventional spectroscopic methods. When bound to poly[d(A-T)$_2$], a large positive circular dichroism (CD) spectrum was induced in absorption region of the bridging moiety for both the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes, which suggested that the bridging moiety sits in the minor groove of the polynucleotide. As luminescence intensity increased, decay times became longer and complexes were well-protected from the negatively charged iodide quencher compared to that in the absence of poly[d(A-T)$_2$]. These luminescence measurements indicated that Ru(II) enantiomers were in a less polar environment compared to that in water and supported by minor groove binding. An angle of 45° between the molecular plane of the bridging moiety of the ΔΔ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complex and the local DNA helix axis calculated from reduced linear dichroism (LD^r) spectrum further supported the minor groove binding mode. In the case of ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complex, this angle was 55°, suggesting a tilt of DNA stem near the binding site and bridging moiety sit in the minor groove of the poly[d(A-T)$_2$]. In contrast, neither ΔΔ- nor ΛΛ-[μ-Ru$_2$(phen)$_4$-(bip)]$^{4+}$ complex produced significant CD or LD^r signal in the absorption region of the bridging moiety. Luminescence measurements revealed that both the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes were partially accessible to the I^- quencher. Furthermore, decay times became shorter when bis-Ru(II) complexes bound to poly[d(G-C)$_2$]. These observations suggest that both the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes bind at the surface of poly[d(G-C)$_2$], probably electrostatically to phosphate group. The results indicate that ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ are able to discriminate between AT and GC base pairs.

Key Words : Bis Ru(II) complex, DNA, Sequence selectivity, Polarized spectroscopy, Binding geometry

Introduction

The design of small molecules that target specific sequences of DNA with high affinity is one of the extensively emerging fields in the nucleic acids research.[1-4] A thorough understanding of how to target DNA sites specifically would undoubtedly lead to the development of novel chemotherapeutics and greatly increase the ability of chemists to probe DNA and develop highly sensitive diagnostic agents.[5-7] The difficulties associated with developing a rationale that explains why drugs favor one site over another has stimulated interest in metal complexes that can interact with DNA and in the effects of ligand structure, size and relative disposition of the coordination sphere of the central metal atom on these interactions.[8] Metal complexes are remarkably good mimics of proteins that recognize specific DNA sequences and discriminating sites through a variety of mechanisms.[9-12] These complexes are structurally rigid molecules with well-defined symmetries, which make them particularly well suited for the selective recognition of specific DNA sequences.[13-15]

The chiral discriminations of metal complexes have been previously investigated from the perspective of shape selection.[16-18] However, in contrast to natural small molecules, such as, distamycin, lexitropsins and netropsin, which are known to bind to DNA with sequence selectivity,[19-23] metallo-intercalators show only weak sequence selectivity for DNA duplexes.[24-27] For an example, the well-known intercalator [Ru(phen)$_2$(dppz)]$^{2+}$ has large intercalative binding affinities but its sequence- and enantio-selectivities are poor.[28-31] Metal complexes that can associate within DNA grooves can selectively target particular DNA sequences, but not many groove binding metal complexes have been reported.[32-40] However, Ru(II) and Rh(I) complexes of 2-amino-4-phenyl-amino-6-(2-pyridyl)-1,3,5-triazine have been recently reported to be minor groove binders and to show strong preference for binding to poly[d(A-T)$_2$].[40] Furthermore, the metal complexes formed were stabilized by H-bonding and van der Waals interactions with the AT-rich regions of DNA. Various binding mode for the bis-Ru(II) complex, in which the two [Ru(phen)$_2$(dppz)]$^{2+}$ complexes are connected by various linker, to DNA has been reported.[41-47] The binding mode of bis Ru(II) complex to DNA depends on the nature of the linking moiety, when the linker is rigid and planar, linker moiety can intercalate between DNA base pairs despite the presence of the two bulky phenanthroline ligands.[41-46] In this case, one of the Ru(II) locates in the minor DNA groove and

the other in the major groove. In contrast, when a flexible linker namely, 1,3-bis-(4-pyridyl)-propane was introduced, both dppz ligands of bis-Ru(II) complex intercalated from the minor groove.[47]

In a previous study, we described the syntheses and the non-intercalative binding modes of two bis-Ru(II) complexes, namely, ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ (bip = 4,4'-biphenylene (imidazo [4,4-*f*][1,10]phenanthroline), Fig. 1) with native DNA.[48] For these entities, the linker and both Ru(II) moieties seemed to be located at one of the groove of DNA. In the present study, the sequence-specific binding modes and the relative affinities of ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ to DNA were investigated using two synthetic polynucleotides namely, poly[d(A-T)$_2$] and poly-[d(G-C)$_2$] by combination of conventional spectroscopic methods, including circular and linear dichroism techniques (referred to as CD and LD).

Experimental Procedures

Materials. All reagents and chemicals were purchased from commercial sources and used without further purification. Solvents were purified by standard procedures. The ΔΔ and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ were prepared as previously described.[19] The extinction coefficients of ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ were determined spectrophotometrically to be ε_{460nm} = 34000 $M^{-1}cm^{-1}$. Synthetic polynucleotides were purchased from Sigma Aldrich and were dissolved in 5 mM cacodylate buffer, pH 7.0, which was used throughout this study. The concentrations of polynucleotides were determined spectrophotometrically using molar extinction coefficients: $\varepsilon_{262\ nm}$ = 6600 M^{-1} cm^{-1}, ε_{254nm} = 8400 M^{-1} cm^{-1} for poly[d(A-T)$_2$]and poly[d(G-C)$_2$], respectively. UV/Vis spectra were recorded on a Shimatsu UV1601PC spectrophotometer (Tokyo, Japan).

Fluorescence Measurements. Fluorescence spectra were obtained using on a Jasco FP 777 spectrofluorimeter (Tokyo, Japan). The fluorescence decay profiles of ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ were measured by the single photon counting method, using a streakscope (Hamamatsu Photonics, C10627-03) equipped with a polychromator (Acton Research, SP2300). An ultrashort laser pulse was generated with a Ti:sapphire oscillator (Coherent, Vitesse, fwhm 100 fs) pumped with a diode-pumped solid-state laser (Coherent,Verdi) and the high power (1.5 mJ) pulses are generated with a Ti: sapphire regenerative amplifier (Coherent, Libra, 1 kHz). For excitation of the sample, the output of the Ti:sapphire regenerative amplifier was converted to 450 nm by using an optical parametric amplifier (Coherent, TOPAS). The instrument response function was also determined by measuring the scattered laser light to analyze a temporal profile. This method gives a time resolution of about 50 ps. In the fluorescence quenching experiment, aliquots of the concentrated quencher (KI) solution were added to the [μ-$Ru_2(phen)_4$-$(bip)]^{4+}$-DNA mixture (typically, few μL quencher solution to 3 mL of mixture solution) prior to taking readings and volume correction was performed.

Polarized Spectroscopy. LD and CD spectra were measured either on a J715 or on a J810 spectropolarimeter (Jasco, Tokyo, Japan). A Wada-type inner-rotating flow cell was used to align the DNA sample for LD measurement. As described by Nordén *et al*.,[49,50] the division of measured LD by isotropic absorption spectrum resulted in a dimensionless quantity-reduced linear dichroism (LD^r), which is related to the angle (α) of the electric transition moment of any DNA-bound drug with respect to the local DNA helix axis and the ability of the DNA-drug adduct to orient through:

$$LD^r = 1.5S(3\cos^2\alpha - 1) \quad (1)$$

where *S* is the orientation factor. When the angle α is near 45°, as is the case for electric transition moment of the minor groove binding drug such as hoechst 33258 or 4',6-diamidino-2-phenylindole, positive LD^r is produced in the drug's absorption region.[51,52] In contrast, a negative LD^r in the drug's absorption region with its magnitude comparable or larger than that in the DNA absorption region is expected for the intercalating drug because the in-plane $\pi \rightarrow \pi^*$ transition of intercalated drug is parallel to those of DNA bases.[53]

Results and Discussion

The chemical structure of the ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4$-$(bip)]^{4+}$ are shown in Figure 1. The spectral properties of ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ bound to poly[d(A-T)$_2$] and poly[d(G-C)$_2$] at the [Ru(II) complex]/[DNA] ratios ranging from 0.01 to 0.10 (with an increment of 0.01) were recorded. The resulting spectra for the DNA-bound Ru(II) complex were found to be independent of mixing ratio. Therefore, only that for the [Ru(II) complex]/[DNA] = 0.10 are shown for the reason of clarity, except where specified.

Absorption Spectroscopy: Interaction of bis-Ru(II) Complexes with Synthetic Polynucleotides. Absorption spectra provide a readily available means of scrutinizing interaction between metal complexes and DNA. The absorption spectrum of the free complexes and in the presence of the polynucleotides (the absorption spectrum of the appropriate polynucleotide was subtracted to enable comparisons) is depicted in Figure 2. The binuclear complexes displayed an

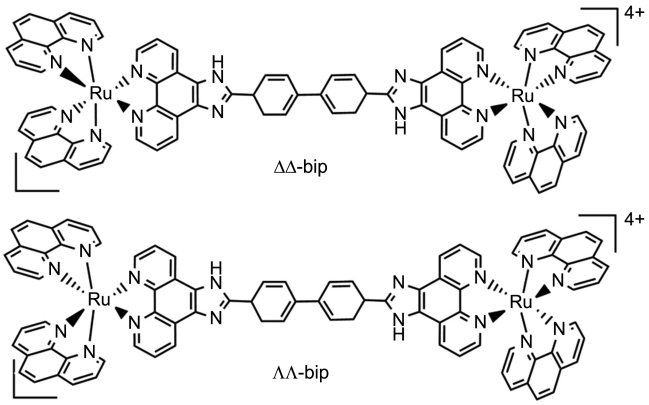

Figure 1. Chemical structure of the binuclear ΔΔ- and ΛΛ-[μ-$Ru_2(phen)4(bip)]^{4+}$ complexes.

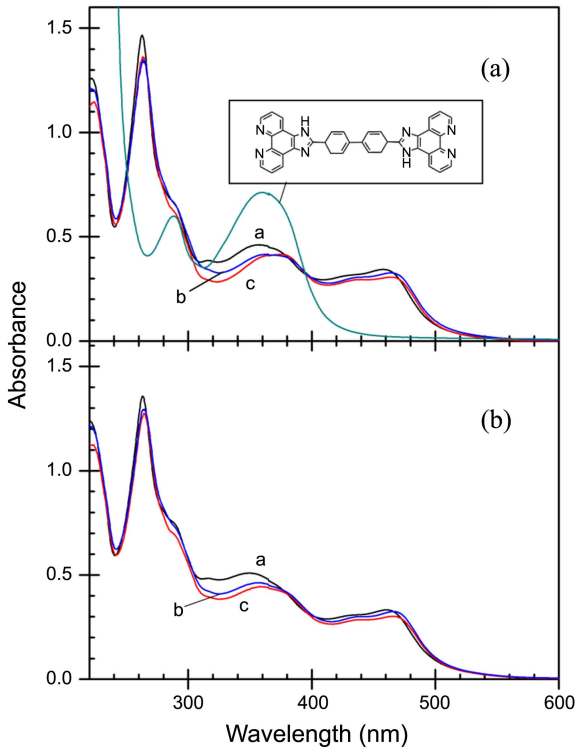

Figure 2. Absorption spectrum of ΔΔ- (panel a) and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ (panel b) in the absence (curve a) and presence of poly[d(G-C)$_2$] (curve b) and poly[d(A-T)$_2$] (curve c). The chemical structure and the absorption spectrum of the bridging moiety in DMF is shown in panel a for easy comparison. [Ru(II) complex] = 10 μM and [polynucleotide] = 100 μM. Absorption spectrum of polynucleotides were subtracted from those of the polynucleotide-Ru(II) complex adduct.

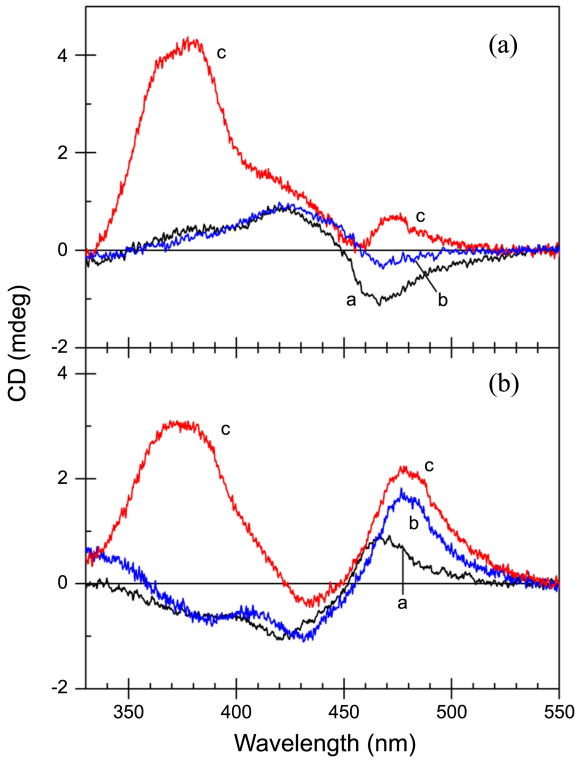

Figure 3. CD spectrum of the ΔΔ- (panel a) and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ (panel b) adducts. The curve assignments and concentrations are as shown in Figure 2.

intra-ligand phenanthroline transition peak at ~260 nm, which is duplicated with the DNA absorption. The bridged bip absorption showed absorption maximum at 288 nm and 360 nm in DMF. Therefore, the band between 320 nm and 400 nm observed from ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ was attributed to $\pi \rightarrow \pi^*$ transition of the bridging (bip) chromophore, although the solution was different. The metal to ligand charge transfer (MLCT) band appeared at lower energy region (460 nm). The addition of the polynucletides (poly[d(A-T)$_2$] or poly[d(G-C)$_2$]) to the ΔΔ- or ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ resulted in the hypochromicity and red shift of the intense $\pi \rightarrow \pi^*$ and of MLCT bands relative to the free metal complexes. These spectroscopic changes unambiguously revealed associations between the chiral binuclear complexes and the polynucletides. The percentage of the hypochromism and red-shift were more prominent for the $\pi \rightarrow \pi^*$ transition of the bridging ligand than the MLCT region. The pronounced spectral changes at the $\pi \rightarrow \pi^*$ transition demonstrated the vital role of bridging part of metal complexes in the binding mode. Differences between the absorption spectral changes (degree of hypochromism and red shift) of ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ with poly[d(A-T)$_2$] were small, and these changes probably reflected a similar mode of association between ΔΔ- and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ with the AT sequence. The addition of double stranded poly[d(G-C)$_2$] caused relatively small changes in the hypochromicities and red shifts of both enantiomers as compared with AT case, indicating that that the bis-Ru(II) complexes interact with GC sequences only weakly as compared with AT sequences.

Circular Dichroism: The Minor Groove Binding Character of the Bridging Moiety. CD spectra provide information about the spectroscopically active chiral compounds and modes of binding between the DNA helix and chiral complexes. The circular dichroism experiment was carried out to investigate the mode of binding and sequence-selectivities (AT *vs* GC sequence) of ΔΔ and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ (Fig. 3). The CD spectral region of 300-550 nm is very informative of the binding modes of enantiomers and polynucleotides and the region below 300 nm is not addressed in this paper because of overlap between the CD signals of phenanthroline ligands and those of polynucleotides 220 and 300 nm. Free ΔΔ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ exhibited positive and negative peaks due to the metal to charge transfer at 418 and 465 nm, respectively, and the spectrum of ΔΔ-enantiomer was symmetric that of the ΔΔ-enantiomer.

Interactions between ΔΔ and ΛΛ-[μ-$Ru_2(phen)_4(bip)]^{4+}$ and duplex poly[d(A-T)$_2$] caused changes in metal to ligand charge transfer bands and also generated an induced CD signal in the bridged ligand absorption region. In the case of ΔΔ-[μ-$Ru_2(phen)_4(bip)]^{4+}$, binding to poly[d(A-T)$_2$] produced a dramatic change in the metal to ligand charge transfer peak position and pattern. In particular, the peak pattern is reversed from the negative sign to the positive one and position shift

towards the longer wavelength from 465 to 475 nm. For ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$, the existing positive MLCT peak shifted toward longer wavelength by 10 nm with a concomitant increase in peak intensity upon binding to poly[d(A-T)$_2$]. As a result, CD signals around 475 nm for both enantiomers had the same positive sign after binding with AT sequence. Observed differences in the MLCT bands of the complexes after adding DNA provided confirmation of their DNA binding. On the other hand, the most prominent change was observed in the bridged ligand absorption region (320-425 nm) of enantiomers in the presence of poly[d(A-T)$_2$] duplex. The addition of poly[d(A-T)$_2$] polynucleotide to ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ solution resulted in strong positive CD bands within the 340-400 nm region. These results are characteristic of minor groove binding mode and demonstrate that both enantiomers bound to the AT sequence at the minor groove of the duplex because observed spectral changes were comparable to those of the some typical minor groove binders, such as 4',6-diamidino-2-phenylindole or Hoechat 33258.[51,52] The ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ leads to similar CD spectral features in the bridged ligand absorption region upon addition of poly[d(A-T)$_2$], but exhibit difference in the CD peak intensities. The higher intensity of induced CD signal of ΔΔ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ confirmed its greater binding affinity to the AT sequence, which is in-line with the above spectral absorption results. These findings suggest that the ΔΔ-enantiomer binds deeper within the minor groove than the ΛΛ-enantiomer.

In contrast, binding to poly[d(G-C)$_2$] only caused superficial changes in the CD spectrum under similar conditions, although these changes were larger for ΛΛ-[μ-Ru_2(phen)$_4$-(bip)]$^{4+}$ than the ΔΔ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ in the MLCT region. Regarding binding to poly[d(G-C)$_2$], the peak intensity of the MLCT band (465 nm) was lower for the ΔΔ-enantiomer, but only a trivial increase was observed for the ΛΛ-enantiomer. Interestingly, both enantiomers produced no CD signal in the bridging moiety absorption region (320-425 nm) when bound to poly[d(G-C)$_2$] duplex, which suggested that the interaction between this moiety and DNA base was trivial. These findings demonstrate that the bridging moiety of the bis-Ru(II) complex does not locate in the minor groove of the GC sequence. Completely different trend was observed for poly[d(G-C)$_2$] and poly[d(A-T)$_2$] in the bridged ligand absorption regions of the two enantiomers. These induced CD results confirmed the sequence selectivity of these bis-Ru (II) enantiomers for the AT sequences.

Reduced Linear Dichroism: Binding Geometry. LD is a technique that can be used with systems that are either intrinsically oriented or oriented during the experiment. LD^r is defined as the wavelength-dependent ratio of measured LD to isotropic absorption spectrum, and is an excellent discriminator of the different binding geometries of small molecules to DNA strands. In order to assess the effect of dihedral angle on DNA-binding geometry and authenticate the results obtained from CD spectroscopy, we measured the LD^r of poly[d(A-T)$_2$] and poly[d(G-C)$_2$] in the presence and absence of the binuclear Ru(II) complexes (Fig. 4). The

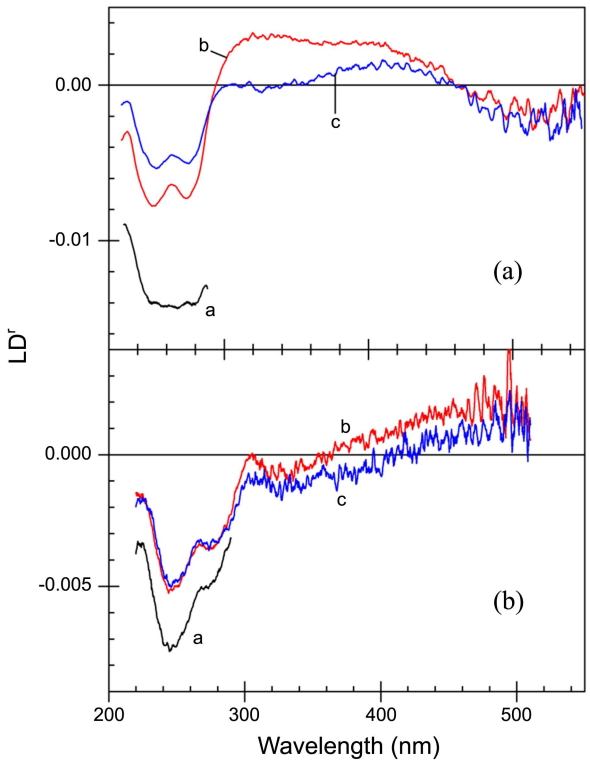

Figure 4. LD^r spectrum of the ΔΔ- and ΛΛ-[μ-Ru_2(phen)4(bip)]$^{4+}$ complexes in the presence of poly[d(A-T)$_2$] (panel a) and poly-[d(G-C)$_2$] (panel b). Curve a denotes the corresponding polynucleotide. Curves b and c represent the ΔΔ- and ΛΛ-enantiomer, respectively. [Ru(II) complex] = 10 μM and [polynucleotide] = 100 μM.

complexes ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ are too small to be orientated, and thus, show no intrinsic LD^r signal. When bound to poly[d(A-T)$_2$], the ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$-(bip)]$^{4+}$ both showed a weak negative LD^r signal in the visible region (approximately at 510 nm) and a positive LD^r band (approximately at 410 nm) from the MLCT transitions of the pheneanthroline ligands. These bisignate LD^r signals reflect the complicated angle of the electric transition moment of the ligand that lies along the line connecting the central Ru(II) and the C_2 axis of the phenanthroline ligands relative to the local DNA helix axis.[54] The in-plane $\pi \rightarrow \pi^*$ transitions of the bridging ligand produced a strong positive LD^r signal between 320 nm and 400 nm for the ΔΔ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$, but this LD^r signal was almost zero for the ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ (curve b and c, respectively in panel a, Fig. 4) when bound to poly[d(A-T)$_2$]. The positive LD^r for the ΔΔ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$-poly[d(A-T)$_2$] adduct resembles those reported for the minor groove binding drugs. The angle, α, was calculated from Eq. (1) (see Experimental Section) by assuming that the average angles of the DNA base relative to the local DNA helix axis was 86°. This angle was 46.8° at 330 nm and 48.2° at 370 nm, which is in agreement with that the angle of the in-plane $\pi \rightarrow \pi^*$ electric transition moment for minor groove binding drugs relative to the local DNA helix axis is near 45°. On the other hand, the ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complex produced

zero LD^r signal in this region when associated with poly[d(A-T)$_2$]. The zero LD^r magnitude in a drug absorption region is resulted by either one of two binding geometries. One possibility is that the electric transition moment of the DNA-bound drug coincides with angle of 55°. The second is due to a random orientation of a DNA-bound drug, which occurs when drugs bind to the negatively charged phosphate group of DNA through single point electrostatic interaction with the positive charge of the bound drug. Judging by the clear LD^r signal in the MLCT band, the former possibility is more feasible in case of the the poly[d(A-T)$_2$]-ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ adduct, *i.e.*, if the Ru(II) complex binds to DNA in a completely random manner, the LD^r signal in the MLCT band should also be zero. Then, the angle of 55° observed for ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ bound to poly[d(A-T)$_2$] suggests that the poly[d(A-T)$_2$] stem near the binding site of the ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complex is strongly tilted. A notable decrease in LD^r magnitude in the DNA absorption region in the presence of ΔΔ- or ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ indicates that both enantiomers sufficiently interact with DNA to decrease its orientation. A decrease in the LD^r magnitude in the DNA absorption region could be caused by either tilting of the DNA stem near the drug-binding site, which reduces DNA contour length or increase its flexibility by reducing the repulsive interaction between the negative charges of the phosphate groups, due to the binding of positively charged drugs. For both ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$, the former is more likely to be the case because the Ru(II) complexes seemed to bind at the minor groove. The significant decrease in LD^r magnitude observed for the poly[d(A-T)$_2$]-ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ compared to ΔΔ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ adduct seemed to agree with the observation that the average angle of the molecular plane of the bridging moiety was more tilted than ΔΔ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ enantiomer.

The LD^r signals induced by poly[d(G-C)$_2$] were strikingly different from those produced by poly[d(A-T)$_2$]. The addition of ΔΔ- or ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes to poly[d(G-C)$_2$] resulted in a significant decrease in LD^r magnitude in the DNA absorption region and these decreases were similar for both enantiomers. In contrast, no significant LD^r signals were observed in the bridging moiety absorption band and in MLCT band (Fig. 4(b)). These results suggest that both ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes bound to poly[d(G-C)$_2$] in a non-specific manner, *i.e.*, they might bind at the DNA surfaces by interacting electrostatically with negatively charged phosphate groups. This binding mode could explain the shapes of CD spectrum. In the bridging moiety absorption region, no CD was induced, suggesting that the interaction between this moiety and DNA bases is weak. The change in the MLCT band in the CD spectrum was also weaker than those bound to poly[d(A-T)$_2$].

Luminescence Measurement: The Environment of bis-Ru(II) Complexes. Fluorescence emission spectrum of the DNA-free ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes and those bound to synthetic polynucleotides are shown in

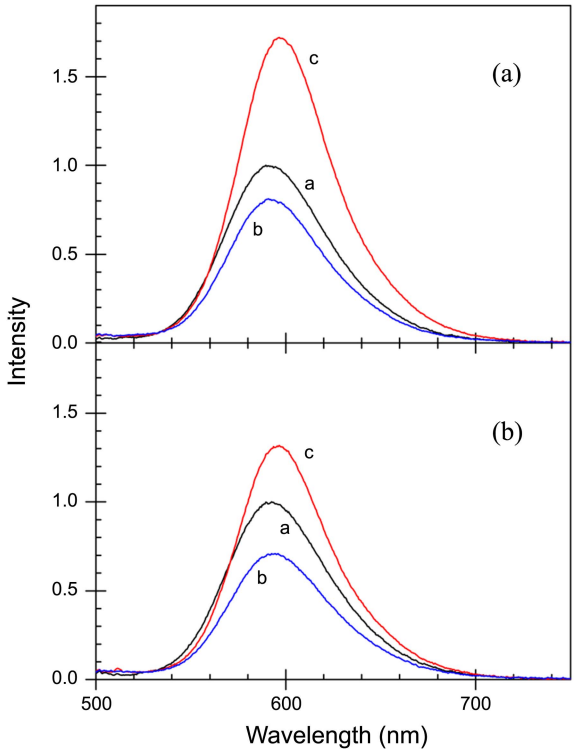

Figure 5. Emission spectrum of the ΔΔ- (panel a) and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complex (panel b) in the presence of poly[d(A-T)$_2$] (curve c) and poly[d(G-C)$_2$] (curve b). Emission spectrum in the absence of polynucleotides are denoted by a. Samples were excited at 458 nm. [Ru(II) complex] = 2 μM and [polynucleotide] = 20 μM.

Figure 5(a) and 5(b), respectively. Both enantiomers emitted luminescence at a maximum wave length of *ca.* 600 nm. Changes in luminescence induced by poly[d(A-T)$_2$] differed significantly from those induced by GC sequence. The addition of poly[d(A-T)$_2$] polynucleotide resulted in an enhancement of emission intensity for both ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$, while it decreased upon binding to poly[d(G-C)$_2$]. Furthermore, enhancement in the luminescence intensity as a result of binding to poly[d(A-T)$_2$] was more pronounced for the ΔΔ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ than for the ΛΛ- enantiomer: luminescence intensity increased by ~1.8 and ~1.3 fold, respectively. Remarkable enhancements in luminescence intensity has been reported for many Ru(II) complexes after binding to double stranded DNA. Possible mechanisms for this enhancement of the luminescence intensity which includes two closed-lying MLCT states with relative energies sensitive to the polarity of the solvent was proposed.[55,56] Therefore, the more pronounced enhancement of luminescence intensity observed for the poly[d(A-T)$_2$]-ΔΔ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ adduct suggests that the environment of the ΔΔ-enantiomer is less polar than ΛΛ- enantiomer. This difference in the environmental polarity may be related to the different extent of DNA tilt at the binding site indicated by LD^r measurement, where binding of the ΛΛ-enantiomer resulted in a larger tilt of the DNA stem than the ΔΔ-enantiomer. Unlike that observed for poly[d(A-T)$_2$], the binding of the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ to poly-

[d(G-C)$_2$] resulted in a decrease in the luminescence intensity, suggesting that the environments of both ΔΔ- and ΛΛ-Ru(II) complexes are more polar when associated with poly[d(G-C)$_2$]. This observation supports our CD and LD^r results. Based on CD measurement, the interaction between both [μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes and poly[d(G-C)$_2$] were concluded to be trivial. Furthermore, LD^r results suggested that both [μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes associates with surface of DNA at the phosphate groups. These provide evidence that these enantiomers demonstrate sequence selectivity for the AT sequence rather than the GC sequence, which also unequivocally supports the results obtained by the CD and LD^r measurement.

The differences in the interaction of ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ with poly[d(A-T)$_2$] and poly[d(G-C)$_2$] are further evidenced by the luminescence quenching experiment when concentration of KI were increased. Iodide ion is known to be a dynamic or collisional florescence quencher and provides a sensitive means of determining the nature of interactions between drugs and DNA. The luminescence intensity of the DNA-free [μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes in solution can be efficiently quenched by anionic quenchers such as the iodide ion, whereas when bound to DNA, they are protected from the anionic water-bound quencher by the array of negative charges along the DNA phosphate backbone. Access to a molecule by quencher is associated with its mode of binding to the DNA helix. The electrostatic barrier caused by negative charges on the phosphate groups at the helix surface may restrict the diffusion of an anionic quencher into the interior of the helix. Therefore, very little or no quenching should be observed, if the binding leads to intercalation or minor groove. In contrast, surface binding mode (electrostatic) between a small molecule and DNA does not prevent quencher access. When interaction mode belongs to major groove binding, the small molecules will be partly protected by DNA, and iodide anions can only partly quench its fluorescence relatively to free molecule. The luminescence quenching responses of the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ to potassium iodide in the absence and presence of poly[d(A-T)$_2$] and poly[d(G-C)$_2$] are compared in Figure 6. KI effectively quenched the luminescences of the both the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes in aqueous solution with quenching constants of 287.6 M^{-1} and 304.8 M^{-1}. However, the corresponding quenching constant for the poly[d(G-C)$_2$] bound ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ were 53.2 M^{-1} and 79.8 M^{-1} respectively. On the other hand, the addition of KI to poly[d(A-T)$_2$]-bound [μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes had an insignificant quenching effect, indicating that KI is accessible to the Ru(II) complexes or the Ru(II) complexes are well protected from incoming KI quencher. This observation concurs with polarized spectroscopic measurements. In the poly[d(A-T)$_2$] adduct case, both the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes are situated in the narrow minor groove, which prevents quencher access. On the other hand, both the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complexes, which were concluded to bind at the surface of the polynucleotide stem from CD and

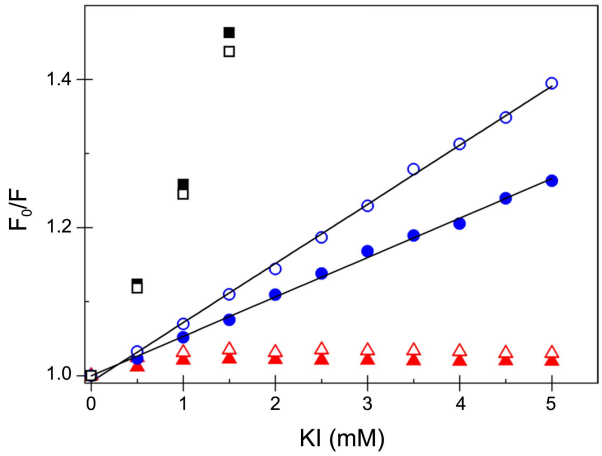

Figure 6. Quenching of luminescence of the ΔΔ- (closed symbols) and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ (open symbols) bound to poly[d(A-T)$_2$] (triangles) and poly[d(G-C)$_2$] (circles) and of luminescence quenching in the absence of polynucleotide (squares). Excitation and emission wavelengths were 458 nm and 600 nm, respectively. . [Ru(II) complex] = 1 μM and [polynucleotide] = 10 μM.

LD, are partially exposed to the aqueous solution allowing quencher access. Furthermore, the access of I$^-$ quencher to ΔΔ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ complex bound to poly[d(G-C)$_2$] was slightly lower than to ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ en-

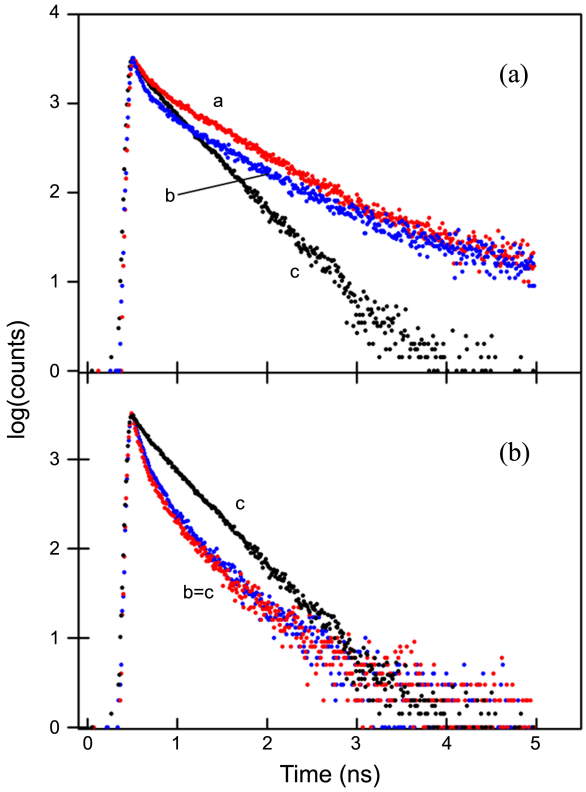

Figure 7. Luminescence decay profile the ΔΔ- and ΛΛ-[μ-Ru$_2$(phen)$_4$(bip)]$^{4+}$ bound to poly[d(A-T)$_2$] (panel a) and poly[d(G-C)$_2$] (panel b). Curves a and b denote ΔΔ- and ΛΛ-enantiomers in the presence of polynucleotides and curve c represents the bis-Ru(II) complex in the absence of polynucleotide. [Ru(II) complex] = 2 μM and [polynucleotide] = 20 μM. The samples were excited at 450 nm.

antiomer. The finding fact that quenching of ΔΔ-[μ-Ru_2-(phen)$_4$(bip)]$^{4+}$ bound to poly[d(G-C)$_2$] is slightly less effective (80% of the polynucleotide-free Ru(II) complex) than quenching of the ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ enantiomer (70%), indicates that the environment of the ΔΔ-enantiomer is slightly less polar (exposed less to aqueous solvent) than that of the ΛΛ-enantiomer when bound to poly[d(G-C)$_2$.

The luminescence decay times of the ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes in the presence and absence of poly[d(A-T)$_2$] or poly[d(G-C)$_2$] are shown in Figure 7(a) and 7(b), respectively. In the absence of DNA, both the ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes exhibited a single decay time of 0.8-0.9 ns. When bound to poly[d(G-C)$_2$], the two decay times of approximately 0.13 ns and 0.84 ns were observed for both ΔΔ- and ΛΛ-enantiomers The short component probably reflects bound species, whereas the long component coincides with that observed in the absence of DNA. This observation is in agreement with decreased luminescence intensity shown by Ru(II) complexes bound to poly-[d(G-C)$_2$]. On the other hand, the decay times of the Ru(II) complexes bound to poly[d(A-T)$_2$] tended to become longer than that in the absence of DNA and were 0.82 ns and 2.339 ns for the poly[d(A-T)$_2$]-ΔΔ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ adduct case, in which the long decay component was easily assigned to the bound species. The poly[d(A-T)$_2$]-ΛΛ-[μ-Ru_2(phen)$_4$-(bip)]$^{4+}$ adduct produced the decay times of 0.12 ns and 1.37 ns. The short component coincides with surface bound spices and long component to partially exposed Ru complex at the bent DNA site. However, further investigations are needed to improve our understanding for the fluorescence decay times.

Conclusions

Present study shows that the synthesized ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes both bind to DNA. Furthermore, binding modes were found to depend on the absolute configuration of the Ru(II) complex and base sequence of DNA. When bound to poly[d(A-T)$_2$], a large positive CD spectrum was induced in the absorption region of the bridging moiety of both ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes. Moreover, the angle between the molecular plane of the bridging moiety relative to the local DNA helix axis was approximately 48° for the ΔΔ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complex, which coincides with the angle of the minor groove, whereas, the angle for the ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complex was 55°. Both enantiomers were found to be located in a relatively non polar environment, and to be protected from the polar quencher. These observations suggest that both ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes bind at the minor groove of poly[d(A-T)$_2$]. In addition, our results indicated a large tilt in the DNA stem near the ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complex binding site. Contrarily, no CD spectrum was induced in the absorption region of bridging moiety and neither of the ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complex was oriented when bound to poly[d(G-C)$_2$]. The polar I^- quencher was partially accessible to poly[d(G-C)$_2$]-bound ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes. These results indicate that ΔΔ- and ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ complexes bind at the minor groove of poly[d(A-T)$_2$] and that the DNA seems to be significantly tilted by ΛΛ-[μ-Ru_2(phen)$_4$(bip)]$^{4+}$ binding. On the other hand, both Ru(II) complexes bound at the surface of poly[d(G-C)$_2$].

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