DNA Mediated Resonance Energy Transfer from 4′,6-Diamidino-2-Phenylindole to [Ru(1,10-Phenanthroline)$_2$L]$^{2+}$

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ABSTRACT The binding site of Δ- and Λ-[Ru(phenanthroline)$_2$L]$^{2+}$ (L being phenanthroline (phen), dipyrido[3,2-*a*:2′3′-*c*] phenazine (DPPZ), and benzodipyrido[3,2-*a*:2′3′-*c*]phenazine (benzoDPPZ)), bound to poly[d(A-T)$_2$] in the presence and absence of 4′,6-diamidino-2-phenylindole (DAPI) was investigated by circular dichroism and fluorescence techniques. DAPI binds at the minor groove of poly[d(A-T)$_2$] and blocks the groove. The circular dichroism spectrum of all Ru(II) complexes are essentially unaffected whether the minor groove of poly[d(A-T)$_2$] is blocked by DAPI or not, indicating that the Ru(II) complexes are intercalated from the major groove. When DAPI and Ru(II) complexes simultaneously bound to poly[d(A-T)$_2$], the fluorescence intensity of DAPI decreases upon increasing Ru(II) complex concentrations. The energy of DAPI at excited state transfers to Ru(II) complexes across the DNA via the Förster type resonance energy transfer. The efficiency of the energy transfer is similar for both [Ru(phen)$_2$DPPZ]$^{2+}$ and [Ru(phen)$_2$benzoDPPZ]$^{2+}$ complexes, whereas that of [Ru(phen)$_3$]$^{2+}$ is significantly lower. The distance between DAPI and [Ru(phen)$_3$]$^{2+}$ is estimated as 0.38 and 0.64 Förster distance, respectively, for the Δ- and Λ-isomer.

INTRODUCTION

Charge transfer along the DNA stem has been extensively studied since the stacking of π-electrons of the DNA basepairs was found to provide an effective medium for charge transfer (Boon and Barton, 2002, for review). The biological importance of the charge transfer in DNA was highlighted by the discovery of the oxidative damage done to DNA from a distance in the cell nucleus (Núñez et al., 2001, 2002). In the study of charge transfer along DNA, Ru(II) and Rh(II) complexes have been frequently used as an electron donor and acceptor set. For instance, the luminescence intensity of a DNA-intercalated donor bis (phenanthrolone)dipyrido[3,2-*a*:2′3′-*c*]phenazineruthenium (II) ([Ru(phen)$_2$DPPZ]$^{2+}$, Fig. 1) was efficiently quenched by an intercalated acceptor bis(9,10-phnanthrenequinone-diimine)(phenanthrolone)-rhodium(III) compared to a non-intercalative acceptor [Ru(NH$_3$)$_6$]$^{3+}$(Murphy et al., 1993, 1994). In a duplex modified with a Ru(II)/Rh(III) pair, the luminescence quenching stretched at a distance as long as 41 Å. On the other hand, when DNA was modified by nonintercalating drugs, the electron transfer was significantly less efficient (Meade and Kayyem, 1995).

The interactions of DNA with transition metal complexes containing planar polycyclic ligands have been widely studied (Nordén et al., 1996, for review). Although the exact binding mode of the parent [Ru(phenanthroline)$_3$]$^{2+}$ complex ([Ru(phen)$_3$]$^{2+}$, Fig. 1) has been a controversial issue (Haworth et al., 1991; Rehmann and Barton, 1990; Eriksson et al., 1992, 1994; Satyanarayana et al., 1992, 1993; Lincoln and Nordén, 1998; Wilhelmsson et al., 2002), when one of the phenanthroline is replaced by a larger DPPZ or benzodipyrido[3,2-*a*:2′3′-*c*]phenazine (BDPPZ, Fig. 1), the extended ligand is almost certainly intercalated between the DNA basepairs (Hiort et al., 1993; Dupureur and Barton, 1994; Haq et al., 1995; Lincoln et al., 1996; Choi et al., 1997; Tuite et al., 1997; Holmlin et al., 1998; Collins, et al., 1999; Greguric et al., 2002). However, whether the intercalation occurs from the minor groove or from the major groove is still unclear.

We recently demonstrated that the spectral properties of 4′,6-diamidino-2-phenylindole (DAPI, Fig. 1) and [Ru(phen)$_2$DPPZ]$^{2+}$ remained when they are simultaneously bound to poly[d(A-T)$_2$] (Yun et al., 2003), indicating that the binding mode of the [Ru(phen)$_2$DPPZ]$^{2+}$ complex did not change whether the minor groove of DNA is blocked by a minor groove binding drug, DAPI or not (Eriksson et al., 1993; Kim et al., 1996, for DAPI-DNA interaction). It was also found that a strong resonance energy transfer occurred from DAPI to [Ru(phen)$_2$DPPZ]$^{2+}$ ''across'' the DNA stem (Yun et al., 2003). In this work, we expand the previous results by systematic investigation of the efficiency of energy transfer across the poly[d(A-T)$_2$] stem by varying the size of the intercalated ligand in the [Ru(phen)$_2$L]$^{2+}$ complex where L (Fig. 1) being phenanthroline, DPPZ, and BDPPZ. Although the mechanism of the luminescence quenching in the DAPI and [Ru(phen)$_2$L]$^{2+}$ complex pair is probably different from that observed from electron transfer through the stacked basepairs, our study may provide a clue for the full understanding of the electron and/or energy transfer in the DNA system.

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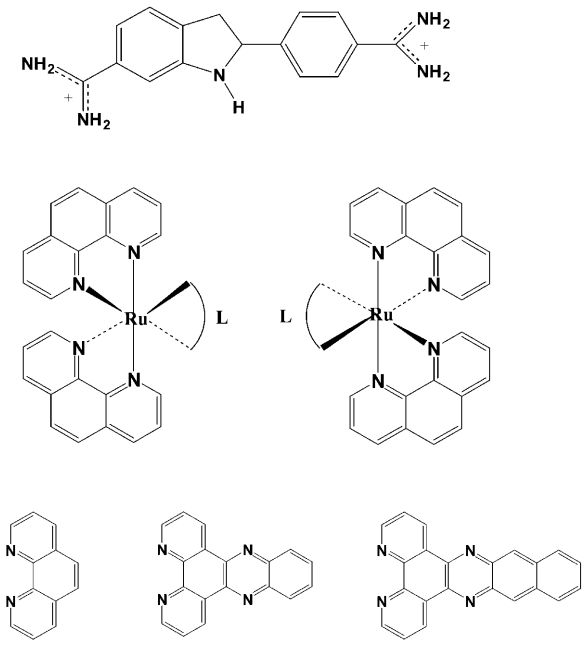

FIGURE 1 Molecular structure of 4′,6-diamidino-2-phenylindole, Δ- and Λ-Ru(II) complexes, and ligand, namely, phenanthroline, DPPZ, and BDPPZ.

MATERIALS AND METHODS

Materials

Poly[d(A-T)$_2$] was purchased from Pharmacia (Seoul, Korea), and was dissolved in 5 mM cacodylate buffer, pH 7.0, containing 100 mM NaCl and 1 mM EDTA, followed by several rounds of dialysis against 5 mM cacodylate buffer, pH 7.0. This was the buffer used throughout this work. [Ru(phen)$_2$L]$^{2+}$ complexes were prepared by the reported procedure (Hiort et al., 1993). Concentrations of DNA, DAPI, and metal complexes were determined using extinction coefficients of $\varepsilon_{262\ nm} = 6600\ cm^{-1}M^{-1}$, $\varepsilon_{342\ nm} = 27000\ cm^{-1}M^{-1}$, $\varepsilon_{445\ nm} = 19000\ cm^{-1}M^{-1}$, $\varepsilon_{439\ nm} = 20000\ cm^{-1}M^{-1}$, and $\varepsilon_{440\ nm} = 22000\ cm^{-1}M^{-1}$, for poly[d(A-T)$_2$], DAPI, [Ru(phen)$_3$]$^{2+}$, [Ru(phen)$_2$DPPZ]$^{2+}$, and [Ru(phen)$_2$BDPPZ]$^{2+}$, respectively (Hirot et al., 1993).

Luminescence, absorption, and circular dichroism measurements

Steady-state fluorescence spectra were recorded on a Jasco FP-777 and absorption spectra on a Jasco V550 (Tokyo, Japan). In the course of titration, small aliquots of the titrant were added to the sample solution and volume corrections were made. The emission intensities of DAPI in the presence of poly[d(A-T)$_2$] were monitored through excitation and emission wavelengths at 360 nm and 450 nm, respectively. The slit widths for both excitation and emission were 3 nm. At these wavelengths, changes in DAPI fluorescence can be monitored without interference with the Ru(II) complex luminescence. Intrinsic circular dichroism (CD) of Ru(II) complex as well as induced CD of DAPI, which is induced upon binding to DNA, were recorded on a Jasco J-715 spectropolarimeter as was described by Nordén and his co-workers (Nordén et al., 1992; Nordén and Kurucsev, 1994). The fluorescence decay time of DAPI was measured using an IBH 5000U Fluorescence Life Time System (Glasgow, UK). The LED source of a nanoLED-03, which produces an excitation radiation at 370 nm with full width at half-maximum of ~1.3 ns, was used to excite poly[d(A-T)$_2$] bound DAPI. The silt widths for both excitation and emission were 16 nm for fluorescence decay measurement.

RESULTS

Circular dichroism

CD spectra of Δ- and Λ-[Ru(phen)$_2$BDPPZ]$^{2+}$ at various Ru(II) complex concentrations in the presence of the DAPI-poly[d(A-T)$_2$] are shown in Fig. 2, *a* and *b*, and those of Δ- and Λ-[Ru(phen)$_3$]$^{2+}$ in Fig. 3, *a* and *b*. The [Ru(phen)$_2$DPPZ]$^{2+}$ enantiomers exhibited similar behavior (data not shown). The concentration of DAPI in this solution corresponds to one DAPI molecule per five basepairs, at which all available sites in the minor groove were saturated (Larsen et al., 1989; Eriksson et al., 1993). When DAPI is bound to the minor groove of poly[d(A-T)$_2$], it produces a positive induced CD signal in the 300 ~ 400-nm region as it was reported (Eriksson

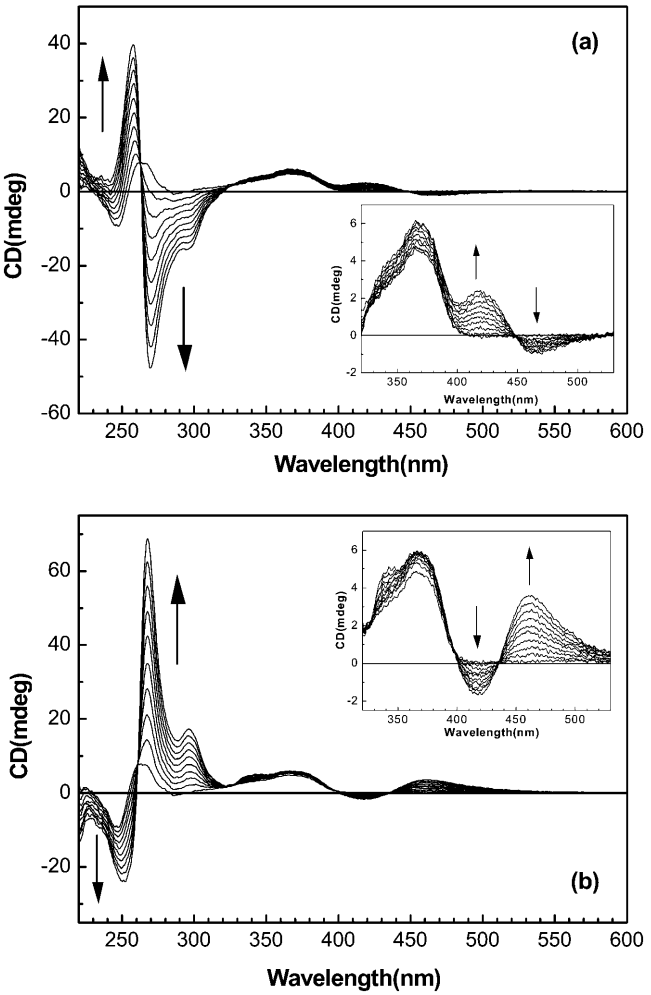

FIGURE 2 CD spectra of (*a*) Δ- and (*b*) Λ-[Ru(phen)$_2$BDPPZ]$^{2+}$ complex in the presence of the DAPI-poly[d(A-T)$_2$]. [DAPI] = 3 μM, [polynucleotide] = 30 μM in base. The concentrations of Ru(II) complexes were 0.0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, and 2.7 μM.

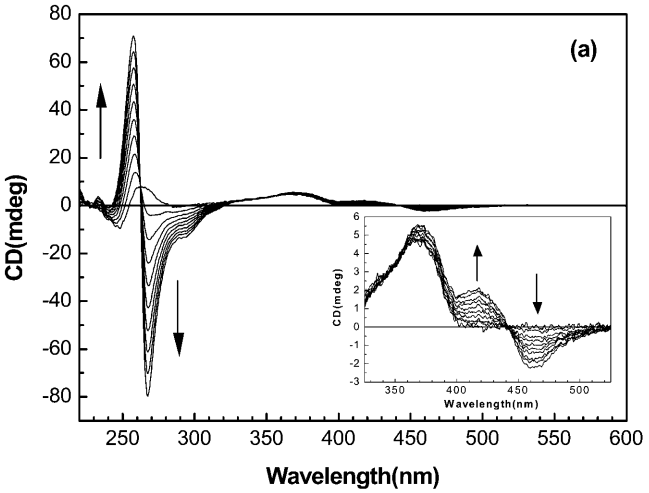

FIGURE 3 CD spectra of (*a*) Δ- and (*b*) Λ-$[Ru(phen)_3]^{2+}$ complex in the presence of the DAPI-poly[d(A-T)$_2$]. Concentrations are the same as in Fig. 2.

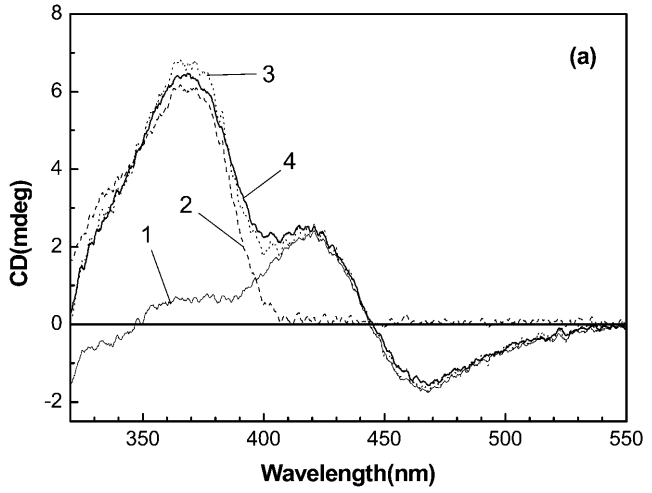

FIGURE 4 CD spectrum of (*a*) Δ- and (*b*) Λ-$[Ru(phen)_2BDPPZ]^{2+}$ (*thin solid curve*; *curve 1*) and DAPI (*dashed curve*; *curve 2*) bound to poly [d(A-T)$_2$], sum of curves 1 and 2 (*thick solid curve*; *curve 4*), and CD spectrum of the $[Ru(phen)_2BDPPZ]^{2+}$-DAPI-poly[d(A-T)$_2$] complex (*thick solid curve*; *curve 3*). [DAPI] = 3 μM, [polynucleotide] = 30 μM, [Ru(II) complex] = 3 μM.

et al., 1993; Kim et al., 1996, for DAPI-DNA interaction). Several isodichroic points in all the CD spectra were observed for all the metal complexes, indicating a homogeneous binding mode even in the presence of DAPI.

CD spectra of DAPI-poly[d(A-T)$_2$], Δ-$[Ru(phen)_2BDPPZ]^{2+}$-poly[d(A-T)$_2$], and Δ-$[Ru(phen)_2BDPPZ]^{2+}$-DAPI-poly[d(A-T)$_2$] complexes above 320 nm (where the CD signal does not interfere with DNA CD) are compared in Fig. 4 *a*. The CD spectra for the Λ-isomer are displayed in Fig. 4 *b*. For each isomer, the sum (*curve 3*) of the respective CD spectrum of $[Ru(phen)_2BDPPZ]^{2+}$-poly[d(A-T)$_2$] (*curve 1*) and DAPI-poly[d(A-T)$_2$] (*curve 2*) is similar to the CD spectrum of $[Ru(phen)_2BDPPZ]^{2+}$-DAPI-poly[d(A-T)$_2$] (*curve 4*). This result shows that no major conformational change occur for the poly[d(A-T)$_2$] bound drugs: neither for DAPI nor for the metal complexes. It also indicates neither a significant displacement nor the release of bound drugs occur as a result of the simultaneous binding of the Ru(II) complex and DAPI at high drug to DNA ratios. Similarity in the CD spectra of both isomers of $[Ru(phen)_3]^{2+}$ and $[Ru(phen)_2DPPZ]^{2+}$ bound to poly[d(A-T)$_2$] in the presence and absence of DAPI were observed (data not shown), leading to a similar conclusion.

Decrease in fluorescence intensity of DAPI-poly[d(A-T)$_2$] upon Ru(II) complexes binding

The fluorescence intensity of DAPI-poly[d(A-T)$_2$] decreased with increasing concentration of Ru(II) complexes as shown in Fig. 5 *a*. However the plot of the ratio of the fluorescence intensity of DAPI-poly[d(A-T)$_2$] in the absence of Ru(II) complex to the presence (I_0/I versus [Q], a normal Stern-Volmer plot; Lakowicz, 2001) did not result in a straight line but an upward bending curve, indicating that the quenching does not follow a simple static or dynamic mechanism. The

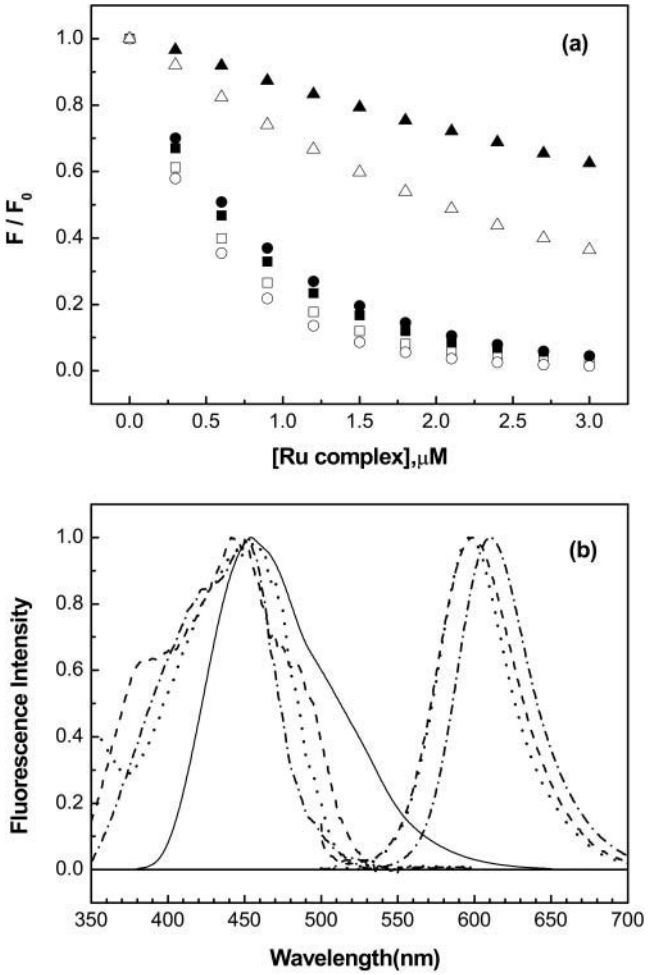

FIGURE 5 (*a*) Decrease in fluorescence intensity of DAPI-poly[d(A-T)$_2$] with respect to the Ru(II) complex concentrations. Circles represent [Ru(phen)$_2$BDPPZ]$^{2+}$, squares [Ru(phen)$_2$DPPZ]$^{2+}$, and triangles [Ru(phen)$_3$]$^{2+}$. Open symbols denote Λ-isomer and closed symbols Δ-isomer. Excitation at 360 nm and emission at 450 nm. Slit widths are 3 nm for both excitation and emission. (*b*) Fluorescence emission spectrum of the DAPI-poly[d(A-T)$_2$] complex (excitation: 360 nm) and excitation and emission spectrum of Ru(phen)$_2$BDPPZ]$^{2+}$ (*dotted curves*), [Ru(phen)$_2$DPPZ]$^{2+}$ (*dashed curves*), and [Ru(phen)$_3$]$^{2+}$ (*dash-dot curves*) that bound to the complex. All spectra are normalized to unity. [poly[d(A-T)$_2$]] = 30 μM in base. [Ru complex] = [DAPI] = 3.0 μM.

decrease in DAPI fluorescence intensity is considerably more efficient for the Λ-isomer compared to the Δ-isomer of all three Ru(II) complexes. It was also noticed that the efficiency of the quenching of DAPI fluorescence decreases, in the order of the size of the ligand: the extent of the decrease in DAPI fluorescence is similar for [Ru(phen)$_2$DPPZ]$^{2+}$ and [Ru(phen)$_2$BDPPZ]$^{2+}$, whereas the quenching efficiency is lowest for Δ-[Ru(phen)$_3$]$^{2+}$ complexes. In the absence of poly[d(A-T)$_2$], quenching of the DAPI fluorescence by any Ru(II) complex was not observed, ensuring that the simultaneous assembly of DAPI and the metal complex on to DNA is necessary for fluorescence quenching. The fluorescence of Ru(II) complex at appropriate excitation and emission wavelengths for DAPI fluorescence (360 nm and 450 nm, respectively) is also negligible. Therefore, the decrease in fluorescence intensity is certainly from the DAPI molecule. The fluorescence emission spectra of DAPI-poly[d(A-T)$_2$] and the Ru(II) complexes, as well as excitation spectra of the Ru(II) complexes are depicted in Fig. 5 *b*. A large overlap between the emission band of the DAPI-poly[d(A-T)$_2$] complex and the excitation spectra of the Ru(II) complexes is observable, suggesting that the mechanism behind the decrease in DAPI fluorescence involves, at least in part, the resonance energy transfer type.

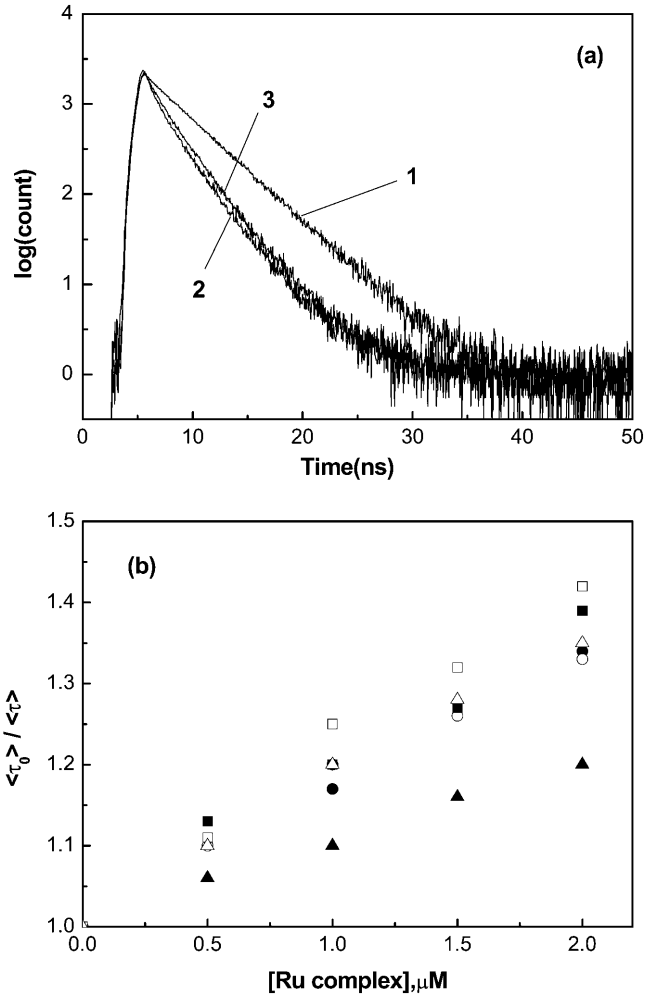

FIGURE 6 (*a*) Decay profile of DAPI-poly[d(A-T)$_2$] in the absence (*curve 1*) and presence of [Ru(phen)$_2$DPPZ]$^{2+}$ (*curve 2*) and [Ru(phen)$_3$]$^{2+}$ (*curve 3*). Excitation at 370 nm by LED source and emission at 460 nm. Slit widths were 16 nm for both excitation and emission. [DAPI] = [Ru(II) complexes] = 3.0 μM. [poly[d(A-T)$_2$]] = 30 μM in base. (*b*) The ratio of average decay time of DAPI-poly[d(A-T)$_2$] (defined in the text) in the absence of Ru(II) complex to their presence. Symbol assignment is the same as in Fig. 5.

Fluorescence decay time of DAPI-poly[d(A-T)$_2$] in the presence of Ru(II) complexes

For further understanding of the fluorescence quenching of DAPI-poly[d(A-T)$_2$] by the Ru(II) complexes, fluorescence decay times of DAPI-poly[d(A-T)$_2$] were measured in the absence and presence of Ru(II) complexes. In the absence of the Ru(II) complex, DAPI-poly[d(A-T)$_2$] exhibited two decay components being $\tau_1 = 0.64 \pm 0.081$ ns and $\tau_2 = 3.88 \pm 0.019$ ns, with their relative amplitude $a_1 = 3.86 \pm 0.41\%$ and $a_2 = 96.14 \pm 0.41\%$, respectively (Fig. 6 *a*, *curve 1*), the long decay component dominants. The fluorescence decay time of DNA-free DAPI at various pH (Szabo et al., 1985) and of DAPI-poly[d(A-T)$_2$] (Cavatorta, et al., 1985; Barcellona and Gratton, 1989) have been reported. Although the result from the three components analysis agreed with that reported by Szabo et al. (1985), neither the residuals nor χ^2 improved by the three components analysis compared to the two components analysis in our condition. Furthermore, the results from our two components analysis agrees with that reported by Barcellona and Gratton (1989). Therefore, the two components result will be used for further discussion (see Discussion). In the presence of Ru(II) complexes, both long component of the fluorescence decay time and its amplitude of DAPI-poly[d(A-T)$_2$] have a tendency to decrease. For instance, the decay time of the long component of DAPI-poly[d(A-T)$_2$] was 2.97 ns with its relative amplitude at 62.59% compared with 3.88 ± 0.019 ns, with its relative amplitude at 96.14 ± 0.41% in the absence of the Ru(II) complex. A Stern-Volmer type plot of the ratio of the average decay time in the absence of the Ru(II) complex to its presence relative to the Ru(II) complex concentration is depicted in Fig. 6 *b*, where the average decay time is defined by, for two components decay, $\bar{\tau} = (a_1\tau_1^2 + a_2\tau_2^2)/(a_1\tau_1 + a_2\tau_2)$ (Lakowicz, 2001). Although the average decay time decreases by ~40% in the presence of 2 μM of the Ru(II) complex, this effect alone is obviously not enough to explain decrease in the fluorescence intensity of DAPI-poly[d(A-T)$_2$].

DISCUSSION

Binding site of Ru(II) complexes to poly[d(A-T)$_2$]

As it was mentioned, the location, i.e., the major versus minor groove, from where the ligand of $[Ru(phen)_2L]^{2+}$ complex intercalates is still a debated question. When the major groove of the poly(dA)·poly(dT) is blocked by the third strand, poly(dT), spectral properties of both Δ- and Λ-$[Ru(phen)_2DPPZ]^{2+}$ enantiomers remained very similar (Jenkins et al., 1992; Choi et al., 1997). These results suggested that the extended ligand intercalated from the minor groove where the insertion of the $[Ru(phen)_2DPPZ]^{2+}$ does not interfere with the third strand in the major groove (Choi et al., 1997). However, it also could be assumed that the $[Ru(phen)_2DPPZ]^{2+}$ complexes bind to the new major groove of the triplex from where it produces similar spectral properties with those bound to the duplex (Jenkins et al., 1992). The fluorescence decay time of $[Ru(phen)_2DPPZ]^{2+}$, that bound to T4 DNA, in which the cytosine residues are glycosylated at the 5-CH_2-OH position in the major groove, was similar to that of calf thymus DNA. This supports the minor groove binding of the $[Ru(phen)_2DPPZ]^{2+}$ complexes (Eimer et al., 1997), as have some recent 1H NMR studies shown (Collins et al., 1999; Greguric et al., 2002).

However, there is also evidence for $[Ru(phen)_2DPPZ]^{2+}$ intercalation from the major groove. The luminescence intensity of the $[Ru(phen)_2DPPZ]^{2+}$ complex decreased in the presence of a major groove binding Δ-α-[Rh[(*R*,*R*)-Me_2trien]phi]$^{3+}$, although that increased by adding a minor groove binding drug, distamycin (Holmlin et al., 1998). This observation indicates that the releasing of the $[Ru(phen)_2DPPZ]^{2+}$ complex upon binding of Δ-α-[Rh[(*R*,*R*)-Me_2trien]phi]$^{3+}$ and, hence, supporting the major groove binding of $[Ru(phen)_2DPPZ]^{2+}$. The invariant CD spectra of Ru(II) complexes in the presence and absence of a minor groove binding drug DAPI (Fig. 4) indicate that the complexes bind in the major groove. The binding mode of DAPI is not affected by the presence of Ru(II) complexes, even at a high drug to DNA base ratio employed in this work. It is also noteworthy that, from several observed isodichroic points at different Ru(II) complex to DNA base ratios (Figs. 1 and 2), the binding mode of the Ru(II) complexes to poly[d(A-T)$_2$] is homogeneous in our condition.

Resonance energy transfer across the DNA stem

Excited state energy of DAPI transfers to the Ru(II) complexes that bind to poly[d(A-T)$_2$] at the opposite site (Fig. 5 *b*). When the ratio of fluorescence intensity in the absence of quencher to its presence with respect to the quencher concentration, i.e., I_0/I versus [Q], is plotted (a Stern-Volmer plot), a simple dynamic or static, or a combination of both, mechanism (Lakowicz, 2001) could not explain the decrease in fluorescence intensity of DAPI. The distance is an important factor for the efficiency of the resonance energy transfer. In our case, the ligand DPPZ and BDPPZ exhibited a similar efficiency, indicating that both ligands are within the Förster distance, whereas the phenanthroline ligand is within a distance such that the efficiency decreased to 62% and 36% for Δ- and Λ-$[Ru(phen)_3]^{2+}$, respectively. According to the theory, the efficiency (E) depends on the distance (r) between the donor and acceptor through $E = R^6/(R^6 + r^6)$ (Lakowicz, 2001), where R is the Förster distance, the distance between DAPI and Δ-$[Ru(phen)_3]^{2+}$ is 0.38 R and that of Λ-$[Ru(phen)_3]^{2+}$ is 0.64 R. However, the difference in distance from the minor groove to Δ- and Λ-$[Ru(phen)_3]^{2+}$, both of which intercalate from the major groove, is not conceivable. The difference in energy transfer efficiency between Δ- and Λ-isomer was observed for all three Ru(II) complexes, although this

difference is the most pronounced for $[Ru(phen)_3]^{2+}$. Therefore, the difference in energy transfer efficiency between Δ- and Λ-isomer should be understood as a reflection of difference in the relative orientation. Indeed, from our previous linear dichroism study (Yun et al., 2003), the roll angle, defined by a clockwise roll around the complex twofold axis of Δ-$[Ru(phen)_2DPPZ]^{2+}$, was slightly modified compared to Λ-isomer as a result of the presence of DAPI.

Although we do not have a full explanation, increase in the Ru(II) complex concentration results in a decrease of the long fluorescence decay component of DAPI. The decrease in the fluorescence decay time is one of the results of the interaction between Ru(II) complexes and DAPI that is simultaneously bound to poly[d(A-T)$_2$], because there was no interaction detected between them in the absence of DNA. This phenomenon might be related to some kind of dynamic motion of DNA.

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