Interaction of Ruthenium(II)[(1,10-phenanthroline)$_2$benzodipyrido[3,2-a:2',3'-c]-phenazine]$^{2+}$ with Single Stranded Poly(dA) and Poly(dT): Turning off the Light Switch

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The spectral properties, namely the circular dichroism, electric absorption and luminescence properties, of Λ- and Δ-[Ru(II)(1,10-phenanthroline)$_2$benzodipyrido[*b*:3,2-*h*:2',3'-*j*]phenazine]$^{2+}$ ([Ru(phen)$_2$BDPPZ]$^{2+}$) in the presence and absence of single stranded poly(dA) and poly(dT) were compared in this work. In the presence of single stranded DNAs, hypochromism in the absorption spectrum and significant changes in the circular dichroism spectrum in the ligand absorption band were apparent, indicating the strong interaction of the [Ru(phen)$_2$BDPPZ]$^{2+}$ complex with the single stranded DNAs. The luminescence intensity of the Ru(II) complex decreased stoichiometrically with increasing concentrations of the single stranded DNAs. All of these spectral changes were independent of the configuration of the Ru(II) complex and the nature of the DNA bases. Therefore, it is conceivable that both enantiomers of the [Ru(phen)$_2$BDPPZ]$^{2+}$ complex interact electrostatically with the negatively charged phosphate groups of DNA. However, the spectral properties of [Ru(II)(1,10-phenanthroline)$_3$]$^{2+}$ were not altered even in the presence of single stranded DNAs. Therefore, the size of the ligand involved in the interaction of the metal complex with the phosphate group of DNA may play an important role, even when the nature of the interaction is electrostatic.

Key Words : Ru complex, DNA, Transition metal, Optical spectroscopy, Phenanthroline

Introduction

Water-soluble Ru(II) complexes containing planar polycyclic aromatic ligands are known to be excellent non-covalent probes for the structure and dynamics of nucleic acids.[1-4] Among the most well-known of such metal complexes are [Ru(II)(1,10-phenanthroline)$_3$]$^{2+}$, [Ru(II)-(1,10-phenanthroline)$_2$dipyrido[3,2-*a*:2',3'-*c*]phenazine]$^{2+}$ and [Ru(II)(1,10-phenanthroline)$_2$benzodipyrido[*b*:3,2-*h*:2',3'-*j*]phenazine]$^{2+}$ (referred to as [Ru(phen)$_3$]$^{2+}$, [Ru(phen)$_2$-DPPZ]$^{2+}$, and [Ru(phen)$_2$BDPPZ]$^{2+}$, respectively). Upon binding to double stranded DNA, both the DPPZ and BDPPZ ligands intercalate almost certainly between the DNA base-pairs.[5-10] In the case of the [Ru(phen)$_2$DPPZ]$^{2+}$ complex, a remarkable enhancement in the luminescence intensity has been reported upon its intercalation into double stranded DNA.[5-8] This observation is known as the "light switch effect". The origin of the light switch effect is believed to be the removal of the water molecules surrounding the [Ru(phen)$_2$DPPZ]$^{2+}$ complex caused by its intercalation. Recent photophysical investigations showed that two energetically close MLCT bands, whose relative energies are sensitive to the environmental polarity, are involved in the light switch mechanism.[11-14] Although the intercalation of the large DPPZ ligand between the DNA base pair has been deemed to be the origin of the light switch effect, it has recently been reported that the luminescence intensity can also be largely enhanced by the association of the [Ru(phen)$_2$DPPZ]$^{2+}$ complex with a single stranded DNA which cannot provide any intercalation pocket.[15,16] In the complex formed between [Ru(phen)$_2$DPPZ]$^{2+}$ and single stranded oligonucleotides, the hydrophobic environment for the [Ru(phen)$_2$DPPZ]$^{2+}$ complex may be provided by a cavity formed from the single stranded DNA.[15] The formation of a luminescent complex involving the [Ru(phen)$_2$DPPZ]$^{2+}$, DNA base, and phosphate group was also suggested as a possible origin of the light switch effect of the single stranded DNA.[16]

Compared to the [Ru(phen)$_2$DPPZ]$^{2+}$-DNA complex, the physicochemical properties of the Ru(II) complex with the extended ligand, BDPPZ (Figure 1), and its DNA complex have been less well investigated, although the DBPPZ ligand also intercalates between the DNA base pairs to form a complex with a double stranded DNA.[9] One of the possible reasons for this could be the less drastic enhancement in the

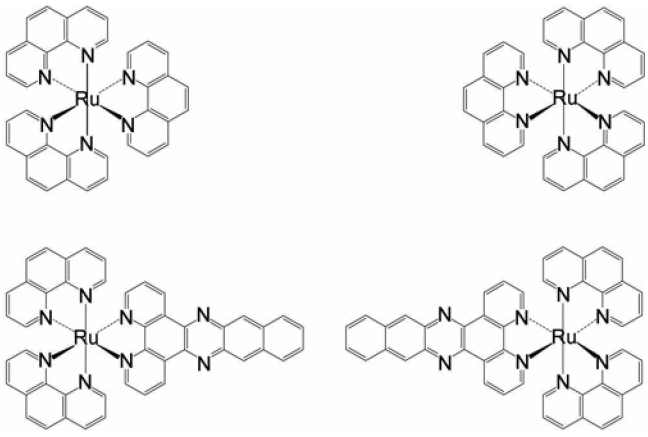

Figure 1. Chemical Structures of Λ- (left) and Δ-[Ru(phen)$_3$]$^{2+}$ and [Ru(phen)$_2$BDPPZ]$^{2+}$.

luminescence intensity of $[Ru(phen)_2BDPPZ]^{2+}$ when it is associated with a double stranded DNA. However, in the course of our study to further understanding of the light switch effect, in contrast with $[Ru(phen)_2DPPZ]^{2-}$, we observed a pronounced decrease in the luminescence intensity of $[Ru(phen)_2BDPPZ]^{2+}$ when it forms a complex with single stranded poly(dA) and poly(dT), which can therefore be referred to as "turning the light switch off". The binding mode and the changes in the luminescence intensity of $[Ru(phen)_3]^{2-}$ and $[Ru(phen)_2BDPPZ]^{2-}$ are compared and the possible origin of the decrease in the luminescence intensity is discussed in this article.

Experimental

Materials. Single-stranded poly(dA) and poly(dT) were purchased from Amersham Biosciences (NJ, USA), and were dissolved in a buffer containing 100 mM NaCl, 1 mM EDTA and 5 mM cacodylate buffer at pH 7.0. The solution was dialyzed several times against 5 mM cacodylate buffer at pH 7.0. All of the samples used in this work were prepared in the same buffer. The homochiral Ru(II) complexes were synthesized by the reported method.[7,17,18] Their concentrations were determined spectrophotometrically using the extinction coefficients, ε_{257nm} = 8600 $M^{-1}cm^{-1}$, ε_{264nm} = 8520 $M^{-1}cm^{-1}$, ε_{445nm} = 19000 $M^{-1}cm^{-1}$, and ε_{440nm} = 22000 $M^{-1}cm^{-1}$ for the poly(dA), poly(dT), $[Ru(phen)_3]^{2+}$ and $[Ru(phen)_2BDPPZ]^{2-}$ complexes, respectively. Therefore, the concentration of DNA indicates the concentration of DNA base or phosphate.

Spectroscopic Measurements. The luminescence intensity of the Ru(II) complexes were recorded on a Jasco FP 777 spectrofluorimeter. The absorption and circular dichroism (CD) spectra were recorded on a Cary 100 spectrometer and a Jasco J810 spectropolarimeter, respectively. In the titrations, aliquots of concentrated polynucleotide solution were added to 5 μM of the Ru(II) complex solution and the necessary volume corrections were made. The luminescence quantum yield was measured by comparing the area of the emission spectrum of the $[Ru(phen)_2BDPPZ]^{2+}$ complex in various solutions to that of the $[Ru(phen)_2DPPZ]^{2-}$ complex, whose quantum yield has been reported to be 0.0046.[11] The $[Ru(phen)_2DPPZ]^{2+}$ complex was excited at the corresponding excitation maximum in each solution.

Results

The emission spectrum of Λ-$[Ru(phen)_2BDPPZ]^{2-}$ with increasing poly(dT) concentration is depicted in Figure 2. When $[Ru(phen)_2BDPPZ]^{2-}$ was excited at 440 nm, its luminescence intensity gradually decreased with increasing concentration of poly(dT). This decrease in the luminescence intensity with increasing polynucleotide concentration is in contrast with $[Ru(phen)_2DPPZ]^{2+}$, which shows the light switch effect.[5-8] The shape of the emission spectrum remained even at a high poly(dT) concentration, indicating that the

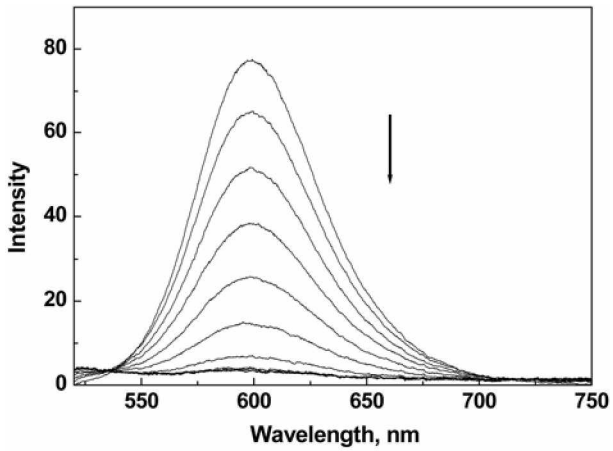

Figure 2. Emission spectrum of Λ-$[Ru(phen)_2BDPPZ]^{2+}$ with increasing poly(dT) concentration. The complex was excited at 440 nm and the slit widths were 5/5 nm for both excitation and emission. The other set, namely Λ- and Δ-$[Ru(phen)_2BDPPZ]^{2-}$-poly(dA), as well as the Δ-$[Ru(phen)_2BDPPZ]^{2+}$-poly(dT) mixtures, exhibited a similar decrease. [Ru(II) complex] = 5 μM. [poly(dT)] = 1-10 μM, increasing in the direction of the arrow with increments of 1 μM.

luminescence originated only from the DNA-unbound $[Ru(phen)_2BDPPZ]^{2-}$: if the $[Ru(phen)_2BDPPZ]^{2-}$-poly(dT) complex is luminescent, a change in the shape of the emission spectrum would be expected. The presence of poly(dA) gave a similar result (data not shown). A similar decrease in the luminescence intensity was observed also for Δ-$[Ru(phen)_2BDPPZ]^{2-}$ in the presence of single stranded poly(dA) and poly(dT) (data not shown). In Figure 3, the decreases in the luminescence intensity at the emission maximum with respect to the increasing concentration of the single stranded poly(dA) and poly(dT) are depicted. Both Δ- and Λ-$[Ru(phen)_2BDPPZ]^{2-}$ (panels (a) and (b), respectively) exhibited a gradual decrease in their luminescence intensity at the emission maximum with increasing polynucleotide concentration. The luminescence quenching by poly(dA) seems to be more efficient than that afforded by poly(dT) for both enantiomers. The luminescence intensities were completely quenched at a polynucleotide concentration of 5-6 μM, indicating that the binding stoichiometry of the Ru(II) complex and polynucleotide was 1:1. In contrast, the change in the luminescence intensity of $[Ru(phen)_3]^{2-}$ in the presence of the polynucleotide is small. In other words, no light switch on or off effect was observed in the case of $[Ru(phen)_3]^{2-}$.

As shown in Figure 4(a), the absorption spectrum of Λ-$[Ru(phen)_3]^{2-}$ was not altered by adding polynucleotide, suggesting that the interaction of the Λ-$[Ru(phen)_3]^{2-}$ complex with poly(dT), if any, is very weak. The Δ-enantiomer exhibited the same result (data not shown). On the other hand, the changes in the absorption spectrum of the Λ-$[Ru(phen)_2BDPPZ]^{2-}$ complex in the presence of poly(dT) was drastic. A large decrease in the ligand absorption band and short wavelength range in the MLCT band, as well as an increase in the tail region of the MLCT band, were apparent

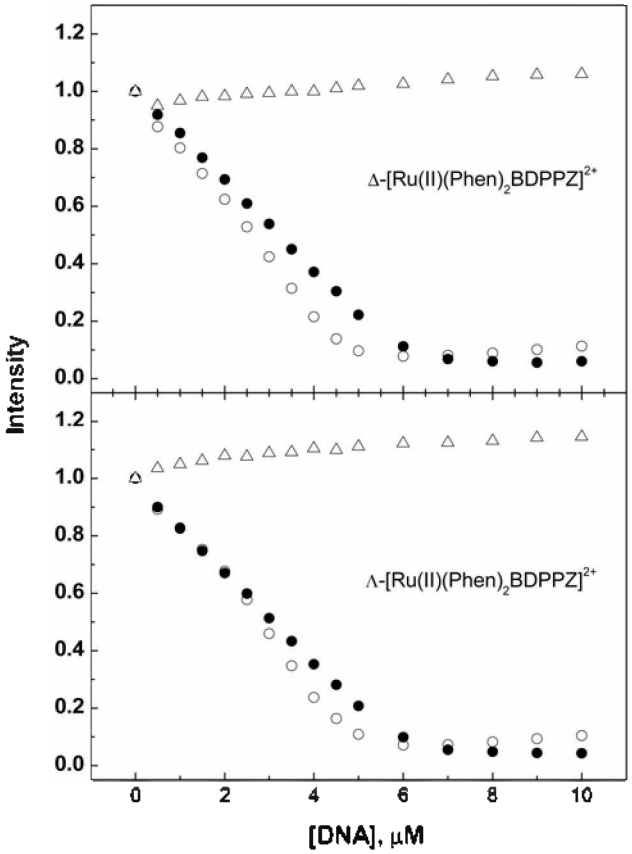

Figure 3. Decrease in luminescence intensity of Δ- and Λ-[Ru(phen)$_2$BDPPZ]$^{2+}$ at the emission maximum with respect to the increasing poly(dA) and poly(dT) concentration. The complex was excited at 440 nm and the luminescence intensity was detected at 599 nm. The slit widths for both excitation and emission were 5 nm. Open circles: poly(dA), closed circles: poly(dT). The changes in the luminescence intensity of Δ- and Λ-[Ru(phen)$_3$]$^{2+}$ in the presence of poly(dT) are also shown as open triangles. The addition of poly(dA) did not alter the luminescence intensity of the Δ- and Λ-[Ru(phen)$_3$]$^{2-}$ complexes as it was observed for poly(dT), and hence, are not shown.

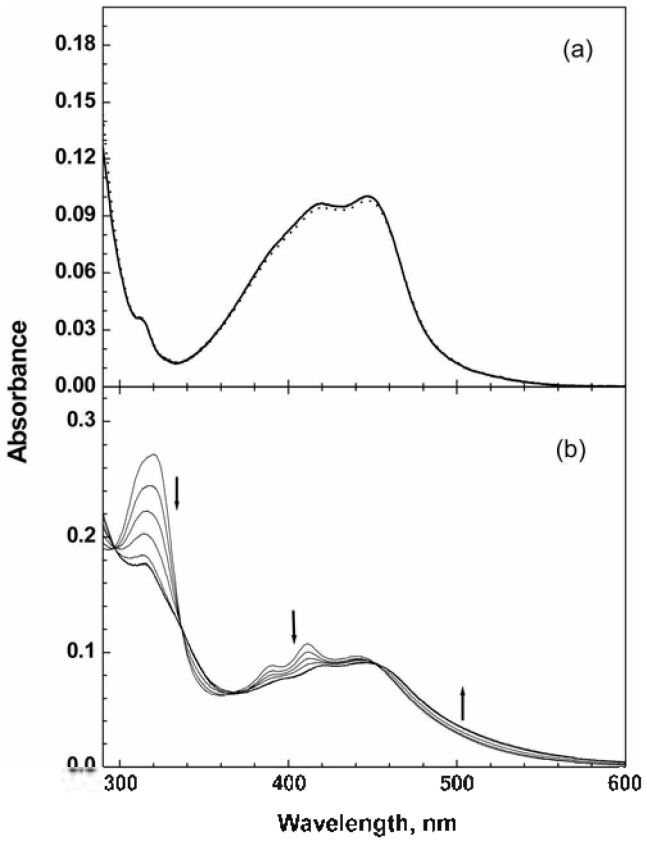

Figure 4. Absorption spectrum of Λ-[Ru(phen)$_3$]$^{2+}$ (panel (a)) and Λ-[Ru(phen)$_2$BDPPZ]$^{2-}$ (panel(b)) in the presence of poly(dT). [Ru(II) complex] = 5 μM. In panel (a), the absorption spectra of Λ-[Ru(phen)$_3$]$^{2-}$ in the absence (solid curve) and presence of 14 μM poly(dT) (dotted curve) are compared. In Panel (b), the concentration of poly(dT) increases gradually in the direction of the arrow. [Poly(dT)] = 2, 4, 6, 8, 12, 14 μM.

as the concentration of poly(dT) was increased. This observation suggests that Λ-[Ru(phen)$_2$BDPPZ]$^{2-}$ can form a complex with poly(dT) in the ground state. Although the data are not shown here, the other set, namely the Δ-[Ru(phen)$_2$BDPPZ]$^{2-}$-poly(dT), Λ-[Ru(phen)$_2$BDPPZ]$^{2-}$-poly(dA) and Δ-[Ru(phen)$_2$BDPPZ]$^{2-}$ complexes, exhibited similar results, suggesting that the formation of the ground state complex was not affected by the nature of the DNA bases. A similar conclusion can be drawn from the CD measurement (Figure 5). As is exemplified for the Λ-[Ru(phen)$_3$]$^{2-}$-poly(dT) set in Figure 5 panel (a), the CD spectrum of [Ru(phen)$_3$]$^{2+}$ was not altered when the single stranded polynucleotide was added. This observation suggests that, in addition to the absorption spectra and luminescence measurement, the interaction of [Ru(phen)$_3$]$^{2+}$ with single stranded DNA is negligible. On the other hand, large alterations, especially in the ligand absorption region, were observed for both Δ- and Λ-[Ru(phen)$_2$BDPPZ]$^{2+}$ upon their association with poly(dT) (Figures 5(b) and (c), respectively). A large change in the absorption spectrum occurred in both the phenanthroline and extended BDPPZ ligand absorption region. The addition of poly(dA) to Δ- and Λ-[Ru(phen)$_2$BDPPZ]$^{2-}$ resulted in a similar change(data not shown). It is noteworthy that the shape of the MLCT band remains the same when [Ru(phen)$_2$BDPPZ]$^{2-}$ forms a complex with single stranded DNA, which is in contrast with the case of double stranded DNA.[9]

The change in the luminescence intensity of any given compound is often attributed to the change in the polarity of environment of that compound. The luminescence quantum yield of [Ru(phen)$_2$DPPZ]$^{2+}$, an analogous compound of [Ru(phen)$_2$BDPPZ]$^{2-}$, has been reported to depend directly on the polarity of the solution.[11] As the polarity of the solution, in which [Ru(phen)$_2$DPPZ]$^{2+}$ is dissolved, increases, the quantum yield increases. Therefore, the environmental polarity, as well as the formation of hydrogen bonds with the solvent molecules, has been suggested as the possible reason for the light switch effect. However, the luminescence quantum yield of [Ru(phen)$_2$BDPPZ]$^{2+}$ decreased as the solution's polarity increased (Figure 6), which is in contrast with the case of [Ru(phen)$_2$DPPZ]$^{2+}$. As shown in Figure 6, the quantum yield decreased almost linearly with increasing

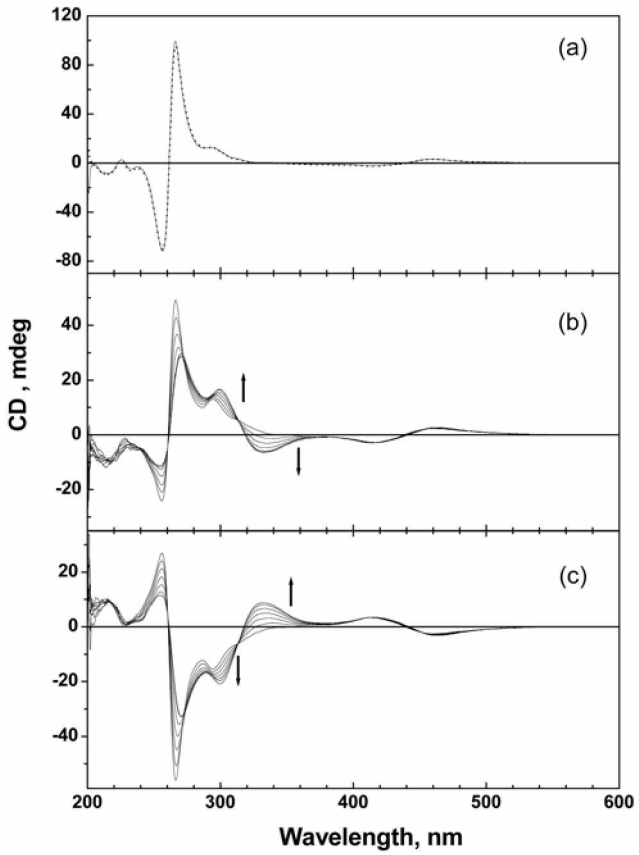

Figure 5. CD spectrum of Λ-$[Ru(phen)_3]^{2+}$ (panel (a)), Λ-$[Ru(phen)_2BDPPZ]^{2+}$ (panel(b)), and Δ-$[Ru(phen)_2BDPPZ]^{2+}$ (panel(c)) in the presence of poly(dT). [Ru(II) complex] = 5 μM. In panel (a), the CD spectra of Λ-$[Ru(phen)_3]^{2+}$ in the absence (solid curve) and presence of 12 μM poly(dT) (dotted curve) are compared. In Panel (b) and (c), the concentration of poly(dT) increases gradually in the direction of the arrow. [Poly(dT)] = 2, 4, 6, 8, 10, 12 μM.

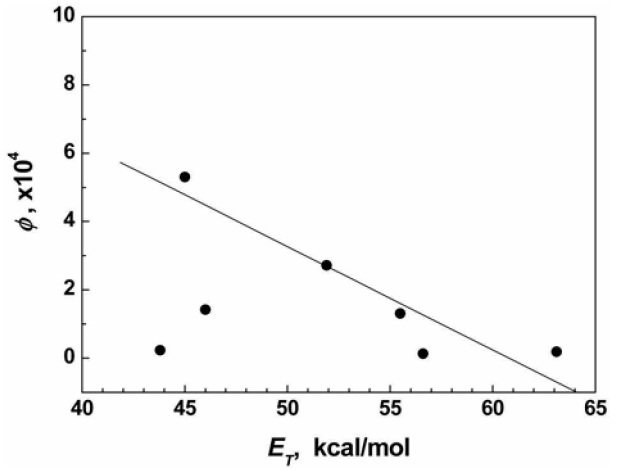

Figure 6. The luminescence quantum yield of $[Ru(phen)_2BDPPZ]^{2+}$ with respect to the polarity of solution. From left to right on the *x*-axis, the solutions were DMF, DMSO, MeCN, ethanol, methanol, formamide and water.

polarity, except in the cases of DMF and MeCN. It should also be noted that the quantum yield of $[Ru(phen)_2BDPPZ]^{2+}$ is lower than that of $[Ru(phen)_2DPPZ]^{2+}$. For instance, the quantum yield of $[Ru(phen)_2BDPPZ]^{2+}$ is 17 times lower than that of $[Ru(phen)_2DPPZ]^{2+}$ in ethanol.

Discussion

Binding mode of $[Ru(phen)_2BDPPZ]^{2+}$ complex to single stranded DNAs. The spectral changes observed upon the binding of both the Δ- and Λ-$[Ru(phen)_2BDPPZ]^{2+}$ complexes to poly(dA) and poly(dT) can be summarized as a decrease in the luminescence intensity, hypochromism in the absorbance, and a large alteration in the CD spectrum. The hypochromism and change in the CD spectrum are particularly pronounced in the ligand absorption region. The decrease in the luminescence intensity was almost proportional to the concentrations of poly(dA) and poly(dT) and the binding stoichiometry was 1:1. All of these observations indicate that the Ru(II) complex can form a non-luminescence ground state complex with single stranded DNA. The fact that the formation of the complex was not affected by the nature of the bases indicates that the binding target of the Ru(II) complex is the phosphate group. Therefore, it is conclusive that the positively charged Ru(II) complex forms a complex with the negatively charged DNA phosphate group *via* electrostatic interaction. This conclusion is in contrast with the case of $[Ru(phen)_2DPPZ]^{2+}$. The binding and the "light switch effect" of the $[Ru(phen)_2DPPZ]^{2+}$ complex was affected by the length and nature of the base.[15,16] Therefore, it was concluded that for the $[Ru(phen)_2DPPZ]^{2+}$ complex both the base and phosphate group are involved in the luminescent complex.

Origin of the "light switch effect". In the case of the $[Ru(phen)_2DPPZ]^{2+}$ complex, the origin of the light switch effect has been well studied for double stranded DNAs. The $[Ru(phen)_2DPPZ]^{2+}$ complex has two MCLT bands. The relative energy levels of these two MLCT bands depend on the environmental polarity. In a nonpolar environment, *i.e.*, in the DNA intercalation pocket, the luminescent energy level is lower than that of the non-luminescent level and, therefore, it can be inferred the luminescence develops from the lower energy level, resulting in the "light switch effect".[12-14] Direct evidence for the enhancement of the luminescence intensity of the $[Ru(phen)_2DPPZ]^{2+}$ complex by decreasing the solution's polarity was also provided.[11] However, the mechanism for the light switch effect of the $[Ru(phen)_2DPPZ]^{2+}$ complex upon its association with single stranded DNAs is not clear yet, because the single stranded DNA cannot provide any intercalation pocket, *i.e.*, non-polar environment for the incoming Ru(II) complex.[15,15] In the case of the $[Ru(phen)_2BDPPZ]^{2+}$ complex, the luminescence quantum yield decreases proportionally with respect to the solution's polarity except in the cases of DMF and MeCN. Although the reason for these two exceptions is unclear, the "light switch off" effect of the $[Ru(phen)_2BDPPZ]^{2+}$ complex may be attributed to the increase in the environmental polarity upon its association with the negatively charged phosphate groups of the single stranded

DNAs.

Dependence of the "light switch effect" on the ligand size. It is noteworthy that the spectral changes of the Λ- and Δ-[Ru(phen)$_3$]$^{2+}$ complexes were not significantly altered in the presence of poly(dA) and poly(dT), indicating that the interaction of both the Λ- and Δ-[Ru(phen)$_3$]$^{2+}$ complexes with single stranded DNA is negligibly small. The luminescence intensity of the [Ru(phen)$_3$]$^{2+}$ complex was not significantly changed. On the other hand, the Δ- and Λ-[Ru(phen)$_2$DPPZ]$^{2+}$ complexes exhibited different spectral changes, as well as the DNA base-dependent light switch effect, upon their association with single stranded DNAs. The spectral changes of the [Ru(phen)$_2$BDPPZ]$^{2+}$ complex in the presence of single stranded DNAs are also pronounced. However, the change was the same for both enantiomers. The decrease in the luminescence was also independent of the nature of the DNA bases. Therefore, it is conclusive that the gradual increase in the ligand size affects the interaction of the Ru(II) complexes with the single stranded DNA. The size of the [Ru(phen)$_2$DPPZ]$^{2+}$ complex may allow it to fit into the cavity[15] formed by the single stranded DNA or to interact with both the DNA base and the phosphate groups.[16] while the [Ru(phen)$_2$BDPPZ]$^{2+}$ complex is too large for these interactions.

Conclusions

Both enantiomers of the [Ru(phen)$_2$BDPPZ]$^{2+}$ complex interact with the phosphate group of single stranded DNAs, thereby exhibiting the light switch turning off effect caused by the increase in the environmental polarity. The interactions of the [Ru(phen)$_3$]$^{2+}$ complexes with single stranded DNAs are negligibly small.

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