http://dx.doi.org/10.5012/bkcs.2013.34.10.2895

Direction of Intercalation of a *bis*-Ru(II) Complex to DNA Probed by a Minor Groove Binding Molecule 4',6-Diamidino-2-phenylindole

Yoon Jung Jang, Raeyeong Kim, Nataraj Chitrapriya, Sung Wook Han,† Seog K. Kim,* and Inho Bae‡,*

*Department of Chemistry, Yeungnam University, Gyeong-buk 712-749, Korea. *E-mail: seogkim@yu.ac.kr*
†School of Herb Medicine Resource, Kyungwoon University, Kumi, Gyeong-buk 730-852, Korea
*‡Department of Physics, Yeungnam University, Gyeong-buk 712-749, Korea. *E-mail: ihbae@yu.ac.kr*
Received May 21, 2013, Accepted July 4, 2013

Direction of intercalation to DNA of the planar dipyrido[3,2-*a*:2',3'-*c*]phenazine ligands (dppz) of a *bis*-Ru(II) complex namely, [Ru(1,10-phenanthroline)$_2$dipyrido[3,2-*a*:2',3'-*c*]phenazine]$^{2+}$ linkered by a 1,3-*bis*(4-pyridyl)propane, was investigated by probing the behavior of 4',6-diamidino-2-phenylindole (DAPI) that bound deep in the minor groove. *Bis*-intercalation of DPPZ resulted in a little blue shift and hyperchromism in DAPI absorption band, and a large decrease in DAPI fluorescence intensity which accompined by an increase in the dppz emission intensity. Diminishing the intenisty of the positive induced circular dichroism (CD) and linear dichroism (LD) were also observed. These spectral changes indicated that insertion of dppz ligand caused the change of the binding mode of DAPI, which probably moved to the exterior of DNA from the minor groove and interacted with the phospghate groups of DNA by electrostatic interaction. At the surface of DNA, DAPI binds at the phosphate groups of DNA by electrostatic attraction. Consequently, this observation indicated that the dppz ligand intercalated from the minor groove.

Key Words : *Bis*-Ru(II)-bpp complex, Intercalation, DNA, DAPI, Polarized spectroscopy

Introduction

The binding of small molecules to DNA is key to understanding the mode of action of many drugs. Since Barton *et al*.'s pioneering studies of the DNA-binding properties of Δ- and Λ-[Ru(phen)$_3$]$^{2+}$ some 30 years ago,[1] many ruthenium complexes such as [Ru(phen)$_2$L]$^{2+}$ have been synthesized and investigated as structure-specific DNA probes,[2-4] direct imaging of DNA in living cell,[5] and electron transfer.[6-9] Moreover, their exceptional photo physical and photochemical features make them suitable as DNA light-switching sensors,[10,11] as agent to target photo-oxidation in DNA[12,13] and as probes for DNA conductivity.[14]

Various binding mode of the mononuclear complexes to DNA have been proposed.[1,2,7,13-16] Initially the [Ru(phen)$_3$]$^{2+}$ complex was suggested to bind to surface of the minor groove of DNA and to intercalate from the major groove with one of its 1,10-phenanthroline sit between the DNA base pairs.[17] However, possibility of classic intercalation of any phenanthroline ligands has been ruled out by viscosity measurements.[16] Although partial intercalation from the major groove was suggested by molecular modeling and energy minimization work,[18] a recent NMR and circular dichroism study showed that the interaction between the [Ru(phen)$_2$L]$^{2+}$ complex and DNA occurs in the minor groove of DNA without separation of the stacked bases.[14] However, when one of the ligand of the [Ru(phen)$_2$L]$^{2+}$ complex is replaced by either dipyrido[3,2-*a*:2',3'-*c*]phenazine (referred to as DPPZ) or benzodipyrido[3,2-*a*:2',3'-*c*]phenazine (referred to as BDPPZ), the extended ligand undoubtedly intercalates between the DNA base pairs.[11,19-23] Much effort has been taken by several research groups to determine whether the binding took place from the major or minor groove. The NMR studies with hexamer have consider that interaction of the Ru-dppz into DNA from the minor groove, whereas spectroscopic results with triple helical poly(dA)[poly(dT)]$_2$ establishing the intercalation of dppz ligand of [Ru(phen)$_2$dppz] preferentially from the major groove of DNA.[24] Very recently Niyazi and co-workers have also suggested that ruthenium complexes bound to two oligonucleotide duplexes from the minor groove by means of the crystal structures. The dppz ligand of ruthenium complex intercalates symmetrically and perpendicularly from the minor groove of the d(CCGGTACCGG)$_2$ duplex at the central TA/TA step, but not at the central AT/AT step of d(CCGGATCCGG)$_2$.[25] However, the emphasis of these studies have concerned only on mononuclear complexes.

In recent times, binuclear ruthenium complexes have been started to receive attention, which in general show greater selectivity due to increased size and higher binding affinity due to increased charge.[26-29] Of these, the binuclear ruthenium complex [μ-(linker)L$_2$(dipyrido[3,2-*a*:2',3'-*c*]phenazine)$_2$-(phenanthroline)$_2$Ru(II)$_2$]$^{2+}$ with linker: 1,3-*bis*-(4-pyridyl)-propane and L: PF$_6$ (hereafter referred to as *bis*-Ru(II)-bpp) complex in Figure 1, is of particular interest in the context of potential biological activity because it has been shown to bind DNA base-pairs by intercalation of two dppz moieties by thorough spectroscopic and thermodynamic study.[30] However, whether the metal complex intercalates from the major groove or from the minor groove has not been clarified yet. In this work, we investigated spectral properties of a DNA-minor groove binding drug, 4,6-diamidino-2-phenyl-

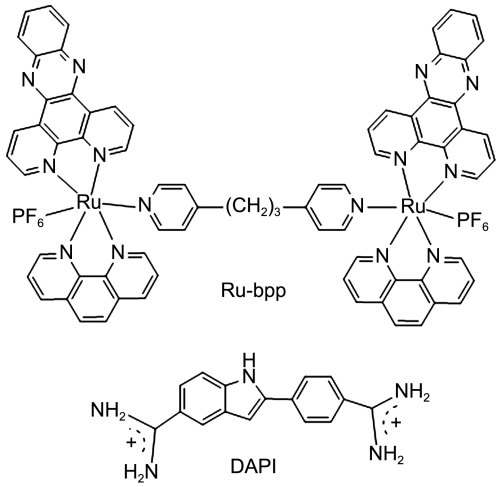

Figure 1. Chemical structure of the [μ-(linker)$(PF_6)_2$(dipyrido-[3,2-*a*:2',3'-*c*]phenazine)$_2$ (phenanthroline)$_2$Ru(II)$_2$]$^{2+}$ with linkers: 1,3-bis-(4-pyridyl)-propane), abbreviated as *bis*-Ru-bpp and 4',6-diamidino-2-phenylindole (DAPI).

indole (DAPI, Figure 1) in the presence of *bis*-Ru(II)-bpp under assumption that if the insertion of dppz ligand occurs at the major groove, alteration of the spectral properties of DAPI changes little because it is in the opposite direction of DNA while if the intercalation occurs from the minor groove, appearance of spectra of the minor groove binding DAPI change in a large extent.

Experimental

Materials and Measurements. Calf thymus DNA (referred to as DNA) was purchased from Worthington (Lakewood, NJ, USA) and purified by dissolution (exhaustive shaking at 4 °C) in a 5.0 mM cacodylate buffer at pH 7.0, containing 100 mM NaCl and 1.0 mM EDTA, followed by several rounds of dialysis at 4 °C against 5.0 mM cacodylate buffer, pH 7.0. The latter buffer was used throughout this work. Other chemicals were purchased from Aldrich or Merck and used without purification. The *bis*-Ru(II)-bpp complex was synthesized by reported method.[30] The mixing ratio, $R = 0.1$ indicates five *bis*-Ru(II)-bpp complexes (or 10 dppz moieties) and 10 DAPI per 100 DNA bases or phosphate. The concentrations of DNA and of the *bis*-Ru(II)dppz complex and DAPI were determined spectrophotometrically using their proper extinction coefficients: $\varepsilon_{258nm} = 6700\ M^{-1}cm^{-1}$, $\varepsilon_{372nm} = 29680\ M^{-1}cm^{-1}$, $\varepsilon_{342nm} = 27000\ M^{-1}cm^{-1}$ for DNA , *bis*-Ru(II)dppz complex and DAPI, respectively.

Absorption spectra were recorded on a Cary 100 (Varian, Australia). Circular and linear dichroism spectra (referred to as CD and LD, respectively) were measured on a J715 and J810 (Jasco, Tokyo, Japan) spectropolarimeter, respectively. CD spectra were averaged over an appropriate numbers of scan when necessary. A Wada-type inner-rotating flow cell[31] was used to align the DNA sample for LD measurement, as it was described by Norden and coworkers.[32-34] Fluorescence spectra were recorded using a Jasco FP-777 fluorometer. The fluorescence emission spectra of DAPI in the presence and absence of *bis*-Ru(II)-bpp complex was recorded with an excitation of 360 nm, the maximum excitation for DNA-bound DAPI. Emission wavelengths of 463 and 625 nm, for DAPI and *bis*-Ru(II)-bpp complex respectively, were used in the fluorescence experiment.

Results

Absorption Spectroscopy. Figure 2 shows the absorption spectrum of DNA-bound DAPI and the *bis*-Ru(II)-bpp complex. Absorption spectra of the DAPI and the *bis*-Ru(II)-bpp complex bound simultaneously to DNA is also shown. DNA-bound DAPI produced an absorption peak at 362 nm (Fig. 2(a), spectrum c), while the *bis*-Ru(II)-bpp complex exhibited absorption maxima at 380 and 440 nm with a shoulder at 480 nm in the metal-to-ligand charge transfer (MLCT) region (Fig. 2(a), spectrum b). When they bound to DNA simultaneously, the absorption peak at 380 nm slightly shifted to short wavelength (to 375 nm) and the absorbance increased. Although the shape of absorption spectrum of the

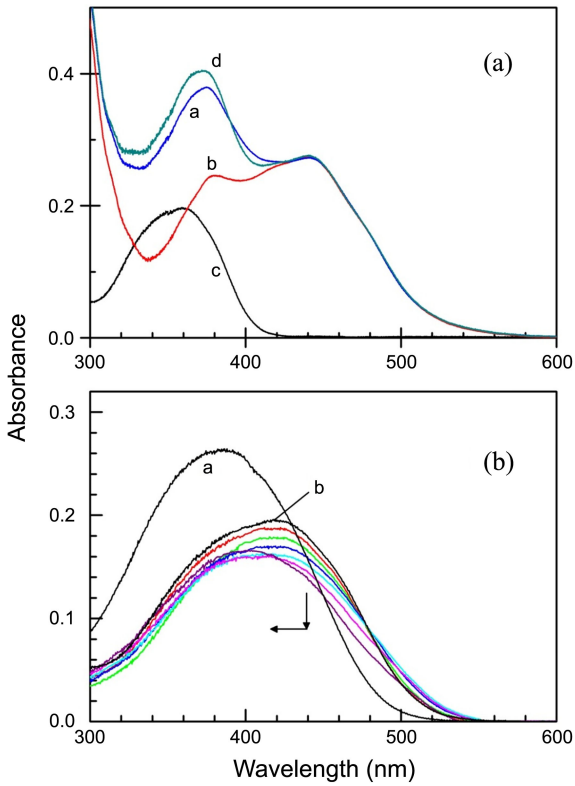

Figure 2. (a) Absorption spectra of *bis*-Ru-bpp and DAPI bound to DNA (curve b and curve c, respectively) and the DNA-DAPI-*bis*-Ru-bpp complex (curve a). Sum of curves b and c is denoted by curve d. [DNA] = 100 μM, [DAPI] = 6 μM and [*bis*-Ru-bpp] = 18 μM. (b) Absorption spectrum of DAPI in the absence and presence of DNA (curve a and b, respectively), and the change in absorption spectrum of DNA-bound DAPI with increasing *bis*-Ru-bpp complex concentration (to arrow direction), which were constructed by subtraction of the absorption spectra the DNA-*bis*-Ru-bpp complex from that of the DNA-DAPI-*bis*-Ru-bpp complex. [DNA] =100 μM, [DAPI] =10 μM, [*bis*-Ru-bpp] = 0-18 μM. The *bis*-Ru-bpp complex increased with an increment of 3 μM.

DAPI-*bis*-Ru(II)-bpp-DNA complex in the 300-600 nm (Fig. 2(a), spectrum a) seemed to be similar to the sum of the absorption spectrum of DNA-bound DAPI and *bis*-Ru(II)-bpp complex (Fig. 2(a), spectrum d), the absorption spectrum of the DAPI-*bis*-Ru(II)-bpp-DNA complex showed slightly lower absorbance at 300-400 nm region compared to curve d, suggesting that there is an interaction between DNA-bound DAPI and the *bis*-Ru(II)-bpp complex. On the other hand, absorption spectrum in the long wavelength region (420-600 nm) which is the MLCT band of the DNA-bound *bis*-Ru(II)-bpp complex remained, indicating that the binding mode of *bis*-Ru(II)-bpp complex is not disrupted by the DNA-bound DAPI. Absorption maximum at 342 nm of DAPI in the absence of DNA in aqueous environment shifted to ~360 nm with a large hypochromism upon binding to DNA (Fig. 2(b), spectrum a and b, respectively). Upon increasing the concentration of the *bis*-Ru(II)-bpp complex to 18 μM, the significant blue shift is observed in the DAPI band compare to lower concentration of the *bis*-Ru(II)-bpp complex (from ~360 nm to ~350 nm). These results imply that DAPI molecule maybe relocated from the minor groove of DNA. However, the changes in the maximum absorbance is the considerably different even at the lower concentration of the *bis*-Ru(II)-bpp complexed with the DAPI-DNA complex. The fact that the band around ~350 nm, which is originates from the DAPI-DNA-*bis*-Ru(II)-bpp complex still intact, indicated that the DAPI molecule remains interact with double helical DNA even in the presence of *bis*-Ru(II)-bpp complex.

Circular Dichroism. CD spectra of the DNA-bound DAPI in the presence of various *bis*-Ru(II)-bpp complex concentrations are shown in Figure 3. The concentration of DAPI in the complex was 10 μM and that of the DNA was 100 μM, corresponding to one DAPI molecule per five base pairs, at which all accessible DNA sites in the minor groove were saturated. When DAPI is bound to the minor groove of poly[d(A-T)$_2$] or native DNA, it produces a positive induced CD signal in the 300-400 nm region although DAPI does not contain any chiral center.[35] Origin of this induced CD signal has been thought to be the interactions between electric transition of DAPI and chirally arranged DNA bases. Upon increasing the concentration of the *bis*-Ru(II)-bpp complex, the intensity of the positive CD signal around at 370 nm decreased, whereas intensity of the positive CD signal corresponding to intercalation of *bis*-Ru(II)-bpp complex around at 468 nm increased.[30] When the concentration of the *bis*-Ru(II)-bpp complex increased to 18 μM, the intensity of the positive CD signal at DAPI absorption region almost disappeared, suggesting that DAPI was displaced as a result of intercalation of the *bis*-Ru(II)-bpp complex. This displacement, however, do not necessarily means the release of DAPI from DNA because the absorption spectrum of DNA-bound DAPI did not complete recover by intercalation of the *bis*-Ru(II)-bpp complex. Increase in the intensity of positive CD signal at the long wavelength was proportional to the concentration of the *bis*-Ru(II)-bpp complex. Furthermore, the wavelength in which CD signal appears coincides with the MLCT absorption band of the *bis*-Ru(II)-bpp complex. These observations indicated that the presence of DAPI at the minor groove of DNA does not affect the binding mode of *bis*-Ru(II)-bpp complex.

Linear Dichroism. LD spectra of DAPI (10 μM) (curve a), *bis*-Ru(II)-bpp(18 μM) (curve c) complex in the presence of the 100 μM of DNA and the DAPI-*bis*-Ru(II)-bpp-DNA complex (curve b) are shown in Figure 4. LD signal in the DNA absorption region for the DNA-DAPI adduct produced a large negative LD signal in the DNA absorption region as it was expected from the set-up adopted in this work, suggesting that DNA base transitions or base planes are aligned normal to the flow direction which coincides with DNA helix axis. In the DAPI absorption region, a positive LD signal was observed. The positive LD signal in the DAPI absorption region indicated that the long molecular axis of DAPI tilt in a large extent relative to the DNA helix axis. The angle between the long axis of DAPI and DNA helix axis has been reported from the similar LD spectrum of the DNA-DAPI adduct as 45°, which is identical to the angle of

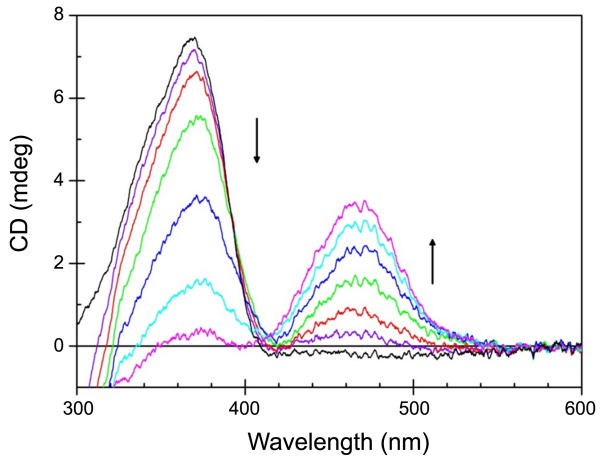

Figure 3. CD spectra of the DNA-DAPI-Ru-bpp complex adduct. [DAPI] = 10 μM, [DNA] = 100 μM in nucleobase. The concentrations of the *bis*-Ru-bpp complex increased to the arrow direction from 0 to 18 μM with an increment of 3 μM.

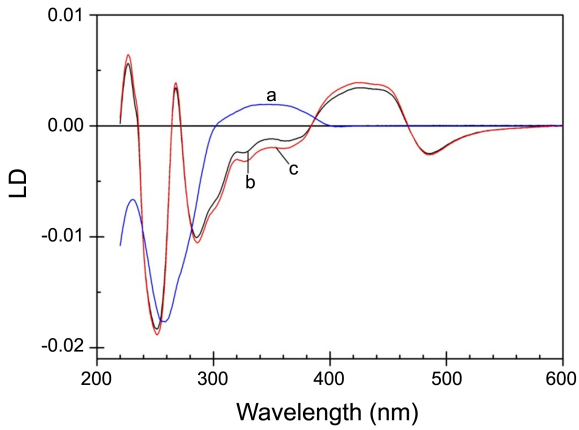

Figure 4. LD spectra of 18 μM concentration of the DNA-DAPI (curve a), the DNA-*bis*-Ru-bpp complex (curve b) and the DAPI-*bis*-Ru-bpp-DNA complex (curve c) adducts. [DAPI] = 10 μM, [DNA] = 100 μM in nucleobase. [*bis*-Ru-bpp complex] = 18 μM.

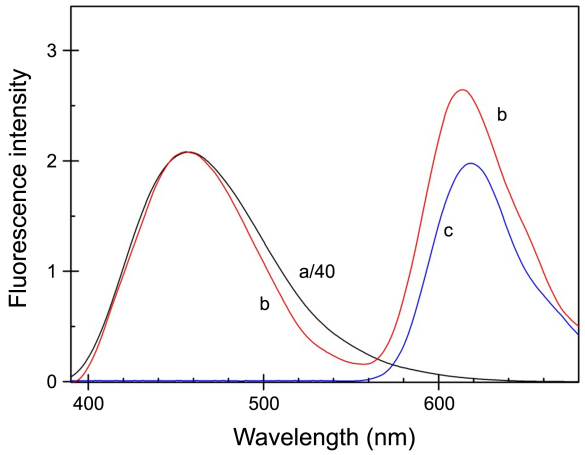

Figure 5. Emission spectrum of the DNA-DAPI adduct (curve a), DNA-DAPI-*bis*-Ru-bpp complex (curve b) and DNA-*bis*-Ru-bpp complex adduct (curve c). The samples were excited at 360 nm. Slit widths were 5nm for both excitation and emission. [*Bis*-Ru-bpp] = 1.2 μM, [DAPI] = 1 μM, [DNA] = 10 μM in nucleobase.

the minor groove.[35] This observation support strongly that DAPI binds at the minor groove of DNA. On the other hand, the DNA-*bis*-Ru(II)-bpp complex adduct produced complicated LD signal both in DNA and MLCT absorption region. The shape of LD is identical to published results for the DNA-*bis*-Ru(II)-bpp complex adduct, in which both dppz ligands of the *bis*-Ru(II)-bpp complex were intercalated.[30] The complication originated from the various direction of intra-ligand electric transition moment in the DNA absorption region and the electric transition corresponding to MLCT band in the long wavelength. Closer analysis on LD spectrum is not the scope of this study. The DNA-DAPI- *bis*-Ru(II)-bpp complex mixture exhibited the LD spectrum very resemble to that of the DNA-*bis*-Ru(II)-bpp complex adduct (Fig. 4, spectrum b). This observation suggested that the DAPI molecule in the minor groove extruded from the binding site in addition to CD spectrum. However, it should be noted again that DAPI was not completely removed from DNA because the absorption spectrum of DAPI in the presence of DNA was not identical to that in the aqueous solution.

Fluorescence. Fluorescence spectra of the DNA-bound DAPI and *bis*-Ru(II)-bpp complex and the DAPI-*bis*-Ru(II)-bpp-DNA complex adduct were recorded for 10 times diluted sample compared to the absorption, CD and LD measurement to avoid the inner-filter effect. The fluorescence emission spectrum of the DNA-*bis*-Ru(II)-bpp complex adduct has the maximum at 457 nm which is in agreement with reported value (Figure 5) for minor groove bound DAPI.[35] The emission spectrum with its center at 618 nm was apparent for the DNA-*bis*-Ru(II)-bpp complex adduct, which is nonfluorescent in the aqueous solution, suggesting that at least a part of the *bis*-Ru(II)-bpp complex is in the nonpolar environment, supporting the intercalative binding mode of the dppz ligands. When DAPI and the *bis*-Ru(II)-bpp complex were bound to DNA simultaneously, a large decrease in the fluorescence intensity of the DNA-DAPI was observed. At the concentration adopted in this work, the fluorescence intensity of DNA-bound DAPI decreased by 40 times by the presence of the *bis*-Ru(II)-bpp complex. Decrease in the fluorescence intensity reflects the change in the environmental polarity of DAPI: from nonpolar minor groove to polar environment. In addition, considering that the DNA-bound *bis*-Ru(II)-bpp complex exhibited a large absorption band in the DAPI emission region, the decrease in the DNA-bound DAPI emission intensity involves, at least in part, Förster type resonance energy transfer. On the other hand, the emission intensity of the *bis*-Ru(II)-bpp complex somewhat increased. In the Förster type resonance energy transfer mechanism, the energy donor molecule and accepter molecule are required to be near. The energy transferred from excited donor may or may not enhance the emission intensity of the acceptor molecule. In the DAPI-*bis*-Ru(II)-bpp-DNA complex adduct case, the excited energy of DAPI contributes to the emission intensity of the DNA-bound *bis*-Ru(II)-bpp complex, because the intensity in the DNA-bound *bis*-Ru(II)-bpp complex emission region somewhat increased.

Discussion

The effect of the binding of the *bis*-Ru(II)-bpp complex on the spectral properties of the DNA-bound DAPI can be summarized as small change in absorption spectrum, diminishing CD and LD, and a fluorescence quenching in a large extent. As it was mentioned, positive CD spectrum of DAPI which binds deep in the minor groove was induced by the interaction of electric transition moment of DNA along the long molecular axis and the chirally arranged DNA bases. Disappearance of DAPI CD spectrum upon the intercalation of dppz ligands of the *bis*-Ru(II)-bpp complex indicated the extrusion of DAPI from the minor groove. Furthermore, LD in the DAPI absorption region also disappeared as a result of the dppz ligand intercalation, suggesting that DAPI cannot be oriented in the flow. The results from both polarized spectroscopies indicated extrusion of DAPI from the minor groove. When DAPI was extruded from the minor groove, it can be either free in the aqueous solution or attached to DNA at which it is apart from the DNA bases and free to rotate. However, the fact that absorption spectrum of extruded DAPI does not coincide with that in the aqueous solution (absorption spectrum of DAPI in the presence of the *bis*-Ru(II)-bpp complex was observed ~350 nm while that in the aqueous solution was at 342 nm) strongly against complete release of DAPI from DNA. When it bind to single stranded poly(dA), DAPI exhibited a similar absorption spectrum that observed from this study and the zero CD in the DAPI absorption region.[36] Considering that the single stranded poly(dA) does not possess any groove or intercalative site, DAPI was concluded to bind to phosphate group of poly(dA). The binding may be stabilized by the electrostatic interaction between positively charged DAPI and negatively charged phosphate groups of the single stranded poly(dA). Similarly, DAPI is extruded from the minor groove as a result of the

bis-Ru(II)-bpp complex intercalation and bind at the phosphate group of the double stranded DNA *via* electrostatic interactions. When DAPI bind at the phosphate group, it is free to rotate because the binding occurs at one point therefore, it would not be oriented in the flow. Existing of DAPI near the *bis*-Ru(II)-bpp complex is also evidenced by the Förster type resonance energy transfer, in which the energy donor and acceptor are required to vicinity.

The binding mode of the *bis*-Ru(II)-bpp complex to DNA has been well-established. The negative band observed in the dppz ligand region (Fig. between 300-400 nm) suggested the planar dppz ligand near parallel to the plane of DNA base-pairs, *i.e*., near perpendicular to the local DNA helix axis, both in the presence and absence of DAPI. Closer analysis of this type of LD has been reported[30] and would not be proper to repeat in here. Appearance of the fluorescence emission spectrum of the *bis*-Ru(II)-bpp complex suggested that the complex locates at least in part in the nonpolar environment. Increase in fluorescence intensity for the various Ru(II) complex upon binding to DNA has been well documented and this effect has been called as "the light switch effect".[1,8,11] The only site in which the dppz ligand can be perpendicular to the local DNA helix axis and protected from polar water molecule is intercalation site. The presence of DAPI did not interfere the insertion of dppz ligand. Furthermore, the direction of insertion of the dppz ligand conceivably is the minor groove. The rest of the moiety of the *bis*-Ru(II)-bpp complex particularly the bridge moiety locates in or near the minor groove, and have DAPI be removed from the minor groove.

Conclusion

Intercalation of the dppz ligands of the *bis*-Ru(II)-bpp complex to the DNA-DAPI adduct resulted in a removing of the DAPI molecule from its original binding site namely the minor groove. DAPI then bind at the surface of DNA probably to the phosphate group by the electrostatic interaction between the positive charge of DAPI and the negative charge of the phosphate group. On the other hand, the presence of DAPI did not interfere the intercalation of dppz ligand of the *bis*-Ru(II)-bpp complex to DNA. Therefore, the dppz ligands insert vary likely from the minor groove.

Acknowledgments. This study was supported by an Internal Research Grant of Yeungnam University. Dr. Jang acknowledges support for this study from the National Research Foundation (Grant no. 2012-0004253).

References

1. Barton, J. K. *Science* **1986**, *233*, 727-734.
2. Zeglis, B. M.; Pierre, V. C.; Barton, J. K. *Chem. Commun*. **2007**, 4565-4579.
3. Metcalfe, C.; Thomas, J. A. *Chem. Soc. Rev*. **2003**, *32*, 215-224.
4. Moucheron, C. *New J. Chem*. **2009**, *33*, 235-245.
5. Gill, M. R.; Jorge, G.-L.; Foster, S. J.; Smythe, C.; Battaglia, G.; Jim, A.; Thomas, A. *Nature Chem*. **2009**, *1*, 662-667.
6. Svensson, F. R.; Matson, M.; Li, M.; Lincoln, P. *Biophys. Chem*. **2010**, *149*, 102-106.
7. Westerlund, F.; Pierard, F.; Eng, M. P.; Norden, B.; Lincoln, P. *J. Phys. Chem. B*. **2005**, *109*, 17327-17332.
8. Lee, B. W.; Moon, S. J.; Youn, M. R.; Kim, J. H.; Jang, H. G.; Kim, S. K. *Biophys. J.* **2003**. *85*, 3865-3871.
9. Poteet, S. A.; Majewski, M. B.; Breitbach, Z. S.; Griffith, C. A.; Singh, S.; Armstrong, D. W.; Wolf, M. O.; MacDonnell, F. M. *J. Am. Chem. Soc*. **2013**, *135*, 2419-2422.
10. Friedman, A. E. *J. Am. Chem. Soc*. **1990**, *112*, 4960-4962.
11. Hiort, C.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc*. **1993**, *115*, 3448-3454.
12. Smith, J. A.; George, M. W.; Kelly, J. M. T. *Coord. Chem. Rev*. **2011**, *255*, 2666-2675.
13. Elias, B. *Chem. Eur. J*. **2007**, *14*, 369-375.
14. Barton, J. K.; Olmon, E. D.; Sontz, P. A. *Coord. Chem. Rev*. **2011**, *255*, 619-634.
15. Eriksson, M.; Leijon, M.; Hiort, C.; Nordn, B.; Graslund, A. *Biochemistry* **1994**, *33*, 5031-5040.
16. Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573-2584.
17. Rehmann, J. P.; Barton, J. K. *Biochemistry* **1990**, *29*, 1701-1709.
18. Haworth, I. S.; Elcock, A. H.; Freeman, J.; Rodger, A.; Richards, W. G. *J. Biomol. Str. Dyn.* **1991**, *99*, 23-44.
19. Jenkins, Y.; Barton, J. K. *J. Am. Chem. Soc*. **1992**, *114*, 8736-8738.
20. Jenkins, Y.; Freidman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809.
21. Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc*. **1994**, *116*, 10286-10287.
22. Haq, I.; Lincoln, P.; Suh, D.; Nordén, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc*. **1995**, *117*, 4788-4796.
23. Lincoln, P.; Broo, A.; Nordén, B. *J. Am. Chem. Soc*. **1996**, *118*, 2644-2653.
24. Choi, S. D.; Kim, M. S.; Kim, S. K.; Lincoln, P.; Tuite, E.; Nordén, B. *Biochemistry* **1997**, *36*, 214-223.
25. Niyazi, H.; Hall, J. P.; O'Sullivan, K.; Winter, G.; Sorensen, T.; Kelly, J. M.; Cardin, C. J. *Nature Chem*. **2012**, *4*, 621-628.
26. Smith, J. A.; Morgan, J. L.; Turley, A. G.; Collins, J. G.; Keene, F. R. *Dalton Trans*. **2006**, 3179-3187.
27. Chao, H.; Yuan, Y. X.; Zhou, F.; Ji, L. N.; Zhang, J. *Transit. Met. Chem.* **2006**, *31*, 465-469.
28. Liu, F. R.; Wang, K. Z.; Bai, G. Y.; Zhang, Y. G.; Gao, L. H. *Inorg. Chem*. **2004**, *43*, 1799-1806.
29. O'Reilly, F.; Kelly, J.; Kirsch-De Mesmaeker, A. *Chem. Commun*. **1996**, 1013-1014.
30. Kwon, B.-H.; Choi, B.-H.; Lee, H. M.; Jang, Y. J.; Lee, J.-C.; Kim, S. K. *Bull. Korean Chem. Soc*. **2010**, *31*, 1615-1620.
31. Wada, A., Kozawa, S. *J. Polym. Sci*. **1964**, *2*, 853-864.
32. Norden, B.; Seth, S. *Appl. Spectrosc.* **1985**, *39*, 647-655.
33. Norden, B.; Kubista, M.; Kurucsev, T. *Q. Rev. Biophys*. **1992**, *25*, 51-170.
34. Norden, B.; Kurucsev, T. *J. Mol. Recognit*. **1994**, *7*, 141-156.
35. Eriksson. S.; Kim, S. K.; Kubista, M.; Norden, B. *Biochemistry* **1993**, *32*, 2987-2998.
36. Jung, K. S.; Kim, M. S.; Lee, G.-J.; Cho, T.-S.; Kim, S. K.; Yi, S. Y. *Bull. Korean Chem. Soc*. **1997**, *18*, 510-514.