Interactions of Flexible Diamine-bridged Dinuclear Ruthenium(II)–2,2′-Bipyridine Complexes with Mononucleotides and DNA

Yasuo Nakabayashi,* Nobuaki Iwamoto, Hiroyuki Inada, and Osamu Yamauchi
Unit of Chemistry, Faculty of Engineering, Kansai University, 3-3-35 Yamate-cho, Suita, Osaka 564-8680

(Received January 13, 2006; CL-060050; E-mail: yasuon@ipcku.kansai-u.ac.jp)

A series of new flexible diamine-bridged dinuclear ruthenium(II) complexes $[\{RuCl(bpy)_2\}_2(\mu\text{-BL})]^{2+}$ (bpy = 2,2′-bipyridine; BL = $H_2N(CH_2)_nNH_2$ (n = 4 ($[\mathbf{1}]^{2+}$), 8 ($[\mathbf{2}]^{2+}$), 12 ($[\mathbf{3}]^{2+}$))) were prepared. Time-course spectroscopic experiments revealed that these dinuclear ruthenium(II) complexes selectively bind to guanine residues in calf thymus (CT) DNA and the degree of binding of $[\mathbf{2}]^{2+}$ or $[\mathbf{3}]^{2+}$ to CT-DNA relative to $[\mathbf{1}]^{2+}$ is high.

The design of molecules that target particular DNA sequences is one of the major challenges in the field of molecular recognition. Ruthenium complexes are known for their antitumor activity.[1] The potential application of ruthenium complexes with polypyridine ligands in the design and development of photophysical and stereoselective probes of nucleic acid structure has been explored extensively in recent years. However, these works have focused primarily on mononuclear complexes, with di- or polynuclear complexes attracting limited attention.[2–9] It is believed that dinuclear ruthenium complexes can overcome both acquired and intrinsic resistance to the antitumor drug cisplatin (*cis*-$[PtCl_2(NH_3)_2]$), because they are capable of forming a completely different range of DNA adducts compared to cisplatin and its analogues.[10] In this study, we have investigated the syntheses of new flexible diamine-bridged dinuclear ruthenium(II) complexes, $[\{RuCl(bpy)_2\}_2(\mu\text{-BL})]^{2+}$ (bpy = 2,2′-bipyridine; BL = $H_2N(CH_2)_nNH_2$ (n = 4 ($[\mathbf{1}]^{2+}$), 8 ($[\mathbf{2}]^{2+}$), 12 ($[\mathbf{3}]^{2+}$))) and the interactions of these ruthenium(II) complexes with mononucleotides and DNA using spectroscopic (UV–vis, ^{1}H NMR, and CD) methods.

A series of new dinuclear ruthenium(II) complexes $[\{RuCl(bpy)_2\}_2(\mu\text{-BL})](PF_6)_2\cdot nH_2O$, ($[Ru_2\text{-BL}](PF_6)_2\cdot nH_2O$),[11] or mononuclear complex $[RuCl(n\text{-butylamine})(bpy)_2]PF_6\cdot 0.5H_2O$ ($[\mathbf{4}](PF_6)_2\cdot 0.5H_2O$)[11] were synthesized from a mixture of *cis*-$[RuCl_2(bpy)_2]\cdot 2H_2O$[12] (0.52 g, 1.0 mmol) and BL (0.45 mmol) or *n*-butylamine (0.07$_3$ g, 1.0 mmol) and were metathesized to the corresponding chloride salts.

The mononuclear ruthenium complexes of structural formulas [RuCl(bpy)(terpy)]Cl, *cis*-$[RuCl_2(bpy)_2]$, and *mer*-$[RuCl_3(terpy)]$ (terpy = 2,2′:6′,2″-terpyridine) have coordinated to DNA preferentially at guanine residues.[13] To investigate the specificity of the binding of $[Ru_2\text{-BL}]^{2+}$ or $[\mathbf{4}]^{+}$ to nucleobases, we have studied reactions of the ruthenium(II) complexes with various mononucleotides in 5 mM Tris-HCl/50 mM NaCl buffer solution at pH 7.4 by UV–vis and ^{1}H NMR spectroscopy. For reactions of 40 μM $[Ru_2\text{-BL}]^{2+}$ with 0.4 mM mononucleotides, changes in UV–vis spectra in the case of disodium guanosine-5′-monophosphate (GMP) and disodium thymidine-5′-monophosphate (TMP) were observed under metal-to-ligand charge-transfer (MLCT) absorption bands of $[\mathbf{3}]^{2+}$ initially centered at 345 and 488 nm shifted to shorter wavelength (hypsochromism) with hyperchromism for GMP and hypochromism for TMP with time. The addition of $AgNO_3$ into the reaction mixture of $[Ru_2\text{-BL}](PF_6)_2\cdot nH_2O$ and GMP or TMP gave white precipitates, revealing that $[Ru_2\text{-BL}]^{2+}$ released Cl^- ions during the reaction. No pH change of the reaction solution of $[\mathbf{3}]^{2+}$ (20 μM) with GMP (40 μM) was observed after 3 h, while the pH of reaction solution of $[\mathbf{3}]^{2+}$ (20 μM) with TMP (40 μM) decreased from 7.89 (initial) to 6.64 after 3 h, compatible with N3 coordination to ruthenium(II) with concomitant N3H deprotonation.[14] On the other hand, no change in UV–vis spectra in the case of disodium adenosine-5′-monophosphate and disodium cytidine-5′-monophosphate was observed. Similar behaviors were obtained for $[\mathbf{1}]^{2+}$, $[\mathbf{2}]^{2+}$, and $[\mathbf{4}]^{+}$. These facts suggest that the reactions of the $[Ru_2\text{-BL}]^{2+}$ or $[\mathbf{4}]^{+}$ with GMP or TMP proceed via Cl^- ion replacement by GMP or TMP. We recorded the time-course ^{1}H NMR spectra at 37 °C to elucidate the reaction process of $[Ru_2\text{-BL}]^{2+}$ (1 mM) and GMP or TMP (2 mM). A new H8 signal appeared within 15 min at δ/ppm = 8.9, which was shifted to high frequency relative to free GMP (δ/ppm = 8.2) and increased in intensity with time. A new TMP CH_3 signal (δ/ppm = 1.6) appeared at lower frequency compared to that for free TMP (δ/ppm = 1.9). For free TMP, the pK_a value of 9.99[15] is attributed to deprotonation of N3H. It is generally known that N7 of GMP in the predominant binding site for the metal coordination. Sadler et al. reported the ^{1}H NMR spectral changes for the 1:1 binding reaction between the organometallic ruthenium(II) arene complex $[RuCl(\eta^6\text{-Bip})(en)]PF_6$ (Bip = biphenyl, en = ethylenediamine) and GMP.[15] The chemical shift of the H8 similarly occurred downfield by 0.5 ppm relative to free GMP upon metalation to the N7 moiety.

In competitive binding experiments for $[Ru_2\text{-BL}]^{2+}$ (40 μM) with GMP versus TMP (0.4 mM, each), the only GMP selectively coordinated to $[Ru_2\text{-BL}]^{2+}$, as evidenced by both the increase in absorption peak intensities and hypsochromic shifts at ca. 345 nm, resulting from substitution of Cl^- ligands by GMP. For reactions of 40 μM $[Ru_2\text{-BL}]^{2+}$ with 0.6 mM CT-DNA, the similar pattern of UV–vis absorption spectral changes for the reactions with GMP as shown in Figure 1 was observed, indicating that $[Ru_2\text{-BL}]^{2+}$ selectively bind to N7 of G bases in DNA. In Watson–Crick double helical DNA, N3 of T bases would not be expected to be available for binding to ruthenium(II) because N3 is involved in hydrogen bonding in base pairs. After the reaction at 37 °C for 3 h, the MLCT bands of $[\mathbf{1}]^{2+}$, $[\mathbf{2}]^{2+}$, $[\mathbf{3}]^{2+}$, and $[\mathbf{4}]^{+}$ at ca. 345 nm exhibited hyperchromic shifts of 3.3, 6.3, 7.6, and 5.2% and hypsochromic shifts of 1, 5, 7, and 2 nm, respectively.

As illustrated in Figure 2, the CD signals of CT-DNA at 275 and 248 nm progressively decreased in intensity with bathochromic shifts as increasing the amounts of $[\mathbf{1}]^{2+}$ (A). Similar changes occurred for $[\mathbf{2}]^{2+}$ and $[\mathbf{3}]^{2+}$ although the degrees for the decrease of the intensity of CD bands were different. The de-

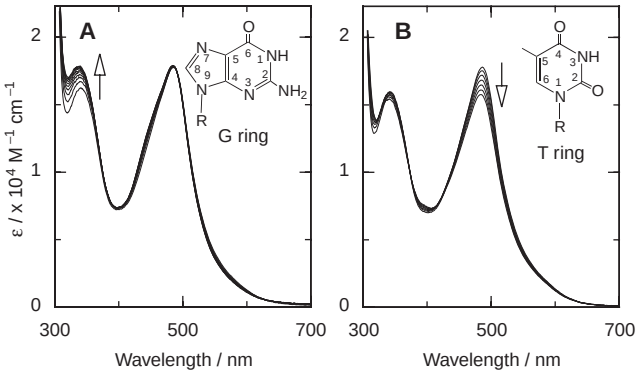

Figure 1. UV–vis spectral changes scanned at every 15 min for 40 μM $[\mathbf{3}]^{2+}$ containing 0.4 mM GMP (A) and TMP (B) in 5 mM Tris-HCl/50 mM NaCl (pH 7.4).

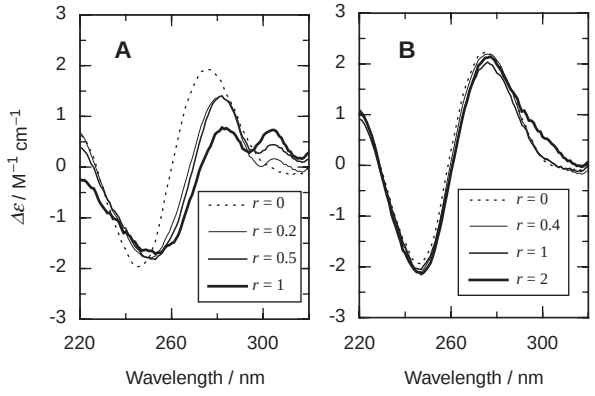

Figure 2. CD spectra of 20 μM CT-DNA after addition of $[\mathbf{1}]^{2+}$ (A) and $[\mathbf{4}]^{+}$ (B) at 37 °C for 3 h. r = [complex]/[DNA].

crease of the intensity of CD bands suggests that the unwinding of the DNA helix and the loss of its helicity.[16] On the other hand, little change in CD spectra in the case of $[\mathbf{4}]^{+}$ (B) was observed, indicating that the helicity of DNA remains in the B-type. In order to clarify the degrees of ruthenium(II) complexes to CT-DNA, ethanol precipitation experiments were performed as described by Barton and Lolis.[17] Solutions of CT-DNA were reacted with $[Ru_2\text{-BL}]^{2+}$ at [complex]/[CT-DNA] = 0.1 for 5 h at 37 °C, and the CT-DNA was precipitated. Levels of bound and free $[Ru_2\text{-BL}]^{2+}$ were determined from the concentrations of $[Ru_2\text{-BL}]^{2+}$ remaining in the supernatant. The degrees of binding of $[\mathbf{1}]^{2+}$, $[\mathbf{2}]^{2+}$, $[\mathbf{3}]^{2+}$, and $[\mathbf{4}]^{+}$ to CT-DNA were 57 ± 3, 68 ± 5, 73 ± 2, and 55 ± 2%, respectively. The higher degree of binding of $[\mathbf{2}]^{2+}$ or $[\mathbf{3}]^{2+}$ to CT-DNA relative to $[\mathbf{1}]^{2+}$ was observed, which may be explained by the length of bridging ligands of the complexes: the probability of forming bifunctinal adducts of $[\mathbf{2}]^{2+}$ or $[\mathbf{3}]^{2+}$ with G residues in DNA is higher than that of $[\mathbf{1}]^{2+}$ as the bridging ligand for $[\mathbf{2}]^{2+}$ or $[\mathbf{3}]^{2+}$ is longer than that for $[\mathbf{1}]^{2+}$.

In conclusion, we have shown here that $[Ru_2\text{-BL}]^{2+}$ ions selectively bind to guanine residues in DNA and affect the ability to DNA-binding.

This work was supported in part by Japan-U.S. Cooperative Cancer Research Program from Japan Society for the Promotion of Science and a Grant-in-Aid for Scientific Research (No. 16350036) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, to which our thanks are due.

References and Notes

1 M. J. Clarke, *Coord. Chem. Rev.* **2002**, *232*, 69.
2 F. M. Foley, F. R. Keene, J. G. Collins, *J. Chem. Soc., Dalton Trans.* **2001**, 2968.
3 C. Metcalfe, S. Spey, H. Adams, J. A. Thomas, *J. Chem. Soc., Dalton Trans.* **2002**, 4732.
4 I. Claustro, G. Abate, E. Sanchez, J. H. Acquaye, *Inorg. Chim. Acta* **2003**, *342*, 29.
5 C.-W. Jiang, *Eur. J. Inorg. Chem.* **2004**, 2277.
6 J. A. Smith, J. G. Collins, B. T. Patterson, F. R. Keene, *Dalton Trans.* **2004**, 1277.
7 A. A. Holder, S. Swavely, K. J. Brewer, *Inorg. Chem.* **2004**, *43*, 303.
8 F. Liu, K. Wang, G. Bai, Y. Zhang, L. Gao, *Inorg. Chem.* **2004**, *43*, 1799.
9 P. U. Maheswari, V. Rajendiran, M. Palaniandavar, R. Parthasarathi, V. Subramanian, *Bull. Chem. Soc. Jpn.* **2005**, *78*, 835.
10 K. van der Schilden, F. Garcia, H. Kooijman, A. L. Spek, J. G. Haasnoot, J. Reedijk, *Angew. Chem., Int. Ed.* **2004**, *43*, 5668.
11 $[\mathbf{1}](PF_6)_2\cdot 3H_2O$: Anal. Calcd for $C_{44}H_{50}N_{10}O_3Cl_2P_2F_{12}Ru_2$: C, 39.74; H, 3.79; N, 10.53%. Found: C, 39.98; H, 3.51; N, 10.46%. ^{1}H NMR in CD_3CN, δ_H (270 MHz): 9.85 (2H, d, J = 5.0 Hz), 8.95 (2H, d, J = 5.6 Hz), 8.41 (4H, d, J = 7.8 Hz), 8.25 (4H, d, J = 8.4 Hz), 8.07 (4H, m), 7.72 (10H, m); 7.41 (2H, d, J = 6.2 Hz), 7.06 (4H, t, J = 6.4 Hz), 2.97 (4H, t, J = 7.3 Hz), 1.05 (4H, m). UV–vis in CH_3CN, λ_{max} (10^{-4} ε (M^{-1} cm^{-1})): 354 nm (1.54), 512 nm (1.54). $[\mathbf{2}](PF_6)_2\cdot 2H_2O$: Anal. Calcd for $C_{48}H_{56}N_{10}O_2Cl_2P_2F_{12}Ru_2$: C, 42.14; H, 4.13; N, 10.24%. Found: C, 42.33; H, 3.76; N, 10.32%. ^{1}H NMR in CD_3CN, δ_H (270 MHz): 9.91 (2H, d, J = 5.6 Hz), 9.00 (2H, d, J = 5.0 Hz), 8.41 (4H, d, J = 7.8 Hz), 8.25 (4H, d, J = 7.8 Hz), 8.07 (4H, m), 7.72 (10H, m), 7.43 (2H, d, J = 5.0 Hz), 7.06 (4H, t, J = 6.7 Hz), 3.06 (4H, t, J = 7.2 Hz), 1.21 (4H, m) 0.9–1.0 (8H, br). UV–vis in CH_3CN, λ_{max} (10^{-4} ε (M^{-1} cm^{-1})): 356 nm (1.57), 517 nm (1.61). $[\mathbf{3}](PF_6)_2\cdot H_2O$: Anal. Calcd for $C_{52}H_{62}N_{10}OCl_2P_2F_{12}Ru_2$: C, 44.42; H, 4.44; N, 9.96%. Found: C, 44.45; H, 4.26; N, 9.91%. ^{1}H NMR in CD_3CN, δ_H (270 MHz): 9.91 (2H, d, J = 5.6 Hz), 9.01 (2H, d, J = 5.0 Hz), 8.42 (4H, d, J = 7.8 Hz), 8.25 (4H, d, J = 8.4 Hz), 8.07 (4H, m), 7.72 (10H, m), 7.43 (2H, d, J = 5.0 Hz), 7.06 (4H, t, J = 6.7 Hz), 3.07 (4H, t, J = 7.3 Hz), 1.25 (4H, m); 1.0–1.1 (16H, br). UV–vis in CH_3CN, λ_{max} (10^{-4} ε (M^{-1} cm^{-1})): 358 nm (1.57), 518 nm (1.62). $[\mathbf{4}]PF_6\cdot 0.5H_2O$: Anal. Calcd for $C_{24}H_{28}N_5O_{0.5}ClPF_6Ru$: C, 42.64; H, 4.17; N, 10.36%. Found: C, 42.52; H, 3.85; N, 10.44%. ^{1}H NMR in CD_3CN, δ_H (270 MHz): 9.91 (1H, d, J = 5.0 Hz), 9.02 (1H, d, J = 5.6 Hz), 8.43 (2H, d, J = 7.8 Hz), 8.26 (2H, d, J = 5.6 Hz), 8.08 (2H, m), 7.73 (5H, m), 7.44 (1H, d, J = 5.0 Hz), 7.06 (2H, t, J = 6.7 Hz), 3.06 (2H, t, J = 7.3 Hz), 1.25 (2H, quint, J = 8.4 Hz), 1.03 (2H, sext, J = 7.8 Hz), 0.68 (3H, t, J = 7.3 Hz). UV–vis in CH_3CN, λ_{max} (10^{-4} ε (M^{-1} cm^{-1})): 360 nm (0.78), 523 nm (0.80). Notwithstanding several attempts it turned out to be impossible to obtain single crystal suitable for X-ray structural determination.
12 D. P. Rillema, D. G. Taghdiri, D. S. Jones, C. D. Keller, L. A. Worl, T. J. Meyer, *Inorg. Chem.* **1987**, *26*, 578.
13 O. Nováková, J. Káspárková, O. Vrána, P. M. van Vliet, J. Reeddijk, V. Brabec, *Biochemistry* **1995**, *34*, 12369.
14 B. Lippert, *Coord. Chem. Rev.* **2000**, *200–202*, 487.
15 H. Chen, J. A. Parkinson, R. E. Morris, P. J. Sadler, *J. Am. Chem. Soc.* **2003**, *125*, 173.
16 K. Karidi, A. Garoufis, N. Hadjiliadis, J. Reedijk, *Dalton Trans.* **2005**, 728.
17 J. K. Barton, E. Lolis, *J. Am. Chem. Soc.* **1985**, *107*, 708.

Published on the web (Advance View) June 3, 2006; doi:10.1246/cl.2006.722