Inorganic Chemistry
Communication

Inorg. Chem. **2009,** *48,* 4637–4639 **4637**
DOI:10.1021/ic900102r

DNA Condensation Induced by Ruthenium(II) Polypyridyl Complexes $[Ru(bpy)_2(PIPSH)]^{2+}$ and $[Ru(bpy)_2(PIPNH)]^{2+}$

Bin Sun,[†,‡] Jing-Xin Guan,[‡] Li Xu,[†] Bo-Le Yu,[†] Long Jiang,[†] Jun-Feng Kou,[†] Li Wang,[†] Xi-Dong Ding,[‡] Hui Chao,*[,†,‡] and Liang-Nian Ji*[,†,‡]

[†]*School of Chemistry and Chemical Engineering and* [‡]*School of Physics and Engineeringand MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, State Key Laboratory of Optoelectronic Materials and Technologies, Sun Yat-Sen University, Guangzhou 510275, People's Republic of China*

Received January 20, 2009

Two novel DNA-intercalating ruthenium(II) complexes, $[Ru(bpy)_2(PIPSH)]^{2+}$ and $[Ru(bpy)_2(PIPNH)]^{2+}$, have been synthesized and characterized. Gel retardation assay, atomic force microscopy, and dynamic light scattering studies show that both complexes can induce the condensation of originally circular plasmid pBR322 DNA to particulate structures under neutral conditions.

DNA in viruses and cells exists in a highly condensed, tightly packed state.[1] The condensation of DNA is essential for biological processes such as DNA transcription and replication and receives additional impetus from an interest in gene therapy.[1,2] During the past decade, many studies have been devoted to the in vitro condensation of DNA and various condensing agents have been identified, such as polyamines, polycationic lipids, neutral polymers, and chitosan.[3] On the contrary, although it has been known for a long time that mononuclear complex $[Co(NH_3)_6]^{3+}$ is able to condense DNA from very dilute aqueous solutions[4] and several other metal complexes have been recently reported to exhibit good condensing abilities to DNA,[5] their vast potential as DNA concentrators still remains largely untapped.

Ruthenium(II) complexes with polypyridyl ligands, because of a combination of easily constructed rigid chiral structures spanning all three spatial dimensions and a rich photophysical repertoire, have prominent DNA binding properties. Some of them have been investigated as nucleic acid probes, DNA-mediated electron transfer, anticancer drugs, and DNA-footprinting and sequence-specific cleaving agents.[6] However, studies involving the condensation of DNA induced by ruthenium(II) polypyridyl complexes are rare. Only recently was $[Ru(DIP)_2(L-L)]^{2+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline; L−L = 4,4′-dicarboxy-2,2′-bipyridine) found to internalize a plasmid carrying the enhanced green fluorescent protein gene.[5] In the present work, we report the remarkable ability of two novel ruthenium(II) complexes, $[Ru(bpy)_2(PIPSH)]^{2+}$ (**1**) and $[Ru(bpy)_2(PIPNH)]^{2+}$ (**2**) [bpy = 2,2′-bipyridine; PIPSH = 2-(4-benzothiazolyl)phenylimidazo[4,5-*f*][1,10]phenanthroline; PIPNH = 2-(4-benzoimidazolyl)phenylimidazo[4,5-*f*][1,10]phenanthroline], to condense the free DNA to particulate structures. These are, to the best of our knowledge, the first examples of DNA-intercalating ruthenium(II) polypyridyl complexes as DNA concentrators.

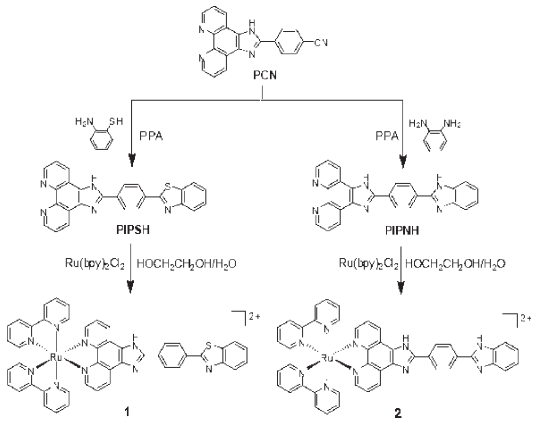
The syntheses of complexes **1** and **2** were achieved as shown in Scheme 1. PIPSH and PIPNH were prepared through condensation of 2-[4-cyanophenyl]imidazo[4,5-*f*][1,10]phenanthroline (PCN)[7] with 2-aminobenzenethiol or *o*-phenylenediamine in refluxing polyphosphoric acid at a molar ratio of 1:1, respectively. The ruthenium(II) complexes were obtained in satisfactory yields (44−62%) by the direct reaction of ligands with appropriate mole ratios of the precursor complex *cis*-$Ru(bpy)_2Cl_2$ in ethylene glycol−water (9:1, v/v). Synthesized as their chloride salts, both complexes were found to be water-soluble. The reaction of sodium methoxide with **1** in methanol yielded the deprotonated complex **3**, and the deprotonated complex can completely revert to the corresponding protonated complex by the

*To whom correspondence should be addressed. E-mail: ceschh@mail.sysu.edu.cn (H.C.), cesjln@mail.sysu.edu.cn (L.-N.J.).

(1) (a) Cerritelli, M. E.; Cheng, N.; Rosenberg, A. H.; McPherson, C. E.; Booy, F. P.; Stephen, A. C. *Cell* **1997**, *91*, 271–280. (b) Daban, J. R. *Biochemistry* **2000**, *39*, 3861–3866.
(2) (a) Luo, D.; Saltzman, W. M. *Nat. Biotechnol.* **2000**, *18*, 33–37. (b) Hamley, L. W.; Castelletto, V. *Angew. Chem., Int. Ed.* **2007**, *46*, 4442–4455.
(3) (a) Ravi Kumar, M. N. V.; Muzzarelli, R. A. A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. J. *Chem. Rev.* **2004**, *104*, 6017–6084. (b) Jeong, J. H.; Kim, S. W.; Park, T. G. *Prog. Polym. Sci.* **2007**, *32*, 1239–1274.
(4) Widom, J.; Baldwin, R. L. *J. Mol. Biol.* **1980**, *144*, 431–453.
(5) (a) Zheng, J. P.; Li, Z.; Wu, A. G.; Zhou, H. L.; Bai, H. Y.; Song, Y. H. *Biochem. Biophys. Res. Commun.* **2002**, *299*, 910–915. (b) Gómez-Segura, J.; Prieto, M. J.; Font-Bardia, M.; Solans, X.; Moreno, V. *Inorg. Chem.* **2006**, *45*, 10031–10033. (c) Musatkina, V. E.; Amouri, H.; Lamoureux, M.; Chepurnykh, T.; Cordier, C. *J. Inorg. Biochem.* **2007**, *101*, 1086–1089. (d) Meng, X. G.; Liu, L.; Zhou, C. S.; Wang, L.; Liu, C. L. *Inorg. Chem.* **2008**, *47*, 6572–6574.
(6) (a) Zeglis, B. M.; Pierre, V. C.; Barton, J. K. *Chem. Commun.* **2007**, 4565–4579. (b) Schuster, G. B.; Landman, U. *Top. Curr. Chem.* **2004**, *236*, 139–161. (c) Elias, B.; Kirsch-De Mesmaeker, A. *Coord. Chem. Rev.* **2006**, *250*, 1627–1641. (d) Gao, F.; Chao, H.; Ji, L. N. *Chem. Biodivers.* **2008**, *5*, 1962–1979.
(7) Deng, H.; Li, H.; Xu, H.; Ji, L. N. *Acta Chim. Sin.* **2002**, *60*, 2159–2166.

© 2009 American Chemical Society
Published on Web 4/10/2009

Scheme 1. Syntheses of Complexes **1** and **2** from PCN



addition of an acid. Although single crystals of **1** and **2** have not yet been obtained, the crystal structure of deprotonated complex **3** was known (Figure S2 in the Supporting Information). The phenyl ring is essentially coplanar with the imidazo[4,5-*f*][1,10]phenanthroline and benzothiazole moieties (dihedral angles are 3.2° and 3.4°, respectively), so PIPS has a large planar aromatic area and possesses intercalating potential for the base pairs of double-helical DNA. Stacking interactions between the PIPS ligands were observed in the crystal unit (Figure S3 in the Supporting Information).

In order to assess the DNA binding behaviors of complexes **1** and **2**, the absorption titrations were carried out (Figure S4 in the Supporting Information). As the calf-thymus DNA concentration is increased, for complex **1**, hypochromism in the metal-to-ligand charge-transfer (MLCT) band reaches as high as 19.3% at 463 nm with a red shift of 6 nm at a ratio of [DNA]/[Ru] of 2.94. For complex **2**, upon addition of DNA, the MLCT band at 461 nm exhibits hypochromism of about 29.4% with a 6 nm red shift at a ratio of [DNA]/[Ru] of 2.94. These spectral characteristics obviously suggest that complexes **1** and **2** bind to DNA via an intercalative mode, which was further confirmed with viscosity experiments (Figure S5 in the Supporting Information). The intrinsic binding constants K_b of complexes **1** and **2** were $(2.3 \pm 0.4) \times 10^6$ and $(9.3 \pm 0.3) \times 10^6$ M^{-1}, respectively. The values are comparable to those of $[Ru(bpy)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*] phenazine, $> 10^6$ M^{-1}),[8] $[Ru(bpy)_2(pip)]^{2+}$ (pip = 2-phenylimidazo[4,5-*f*][1,10]phenanthroline, 4.7×10^5 M^{-1}),[9] and the known DNA intercalator ethidium bromide (EB; 1.4×10^6 M^{-1}).[10]

The EB displacement assays and melting experiments also confirmed the good DNA binding abilities of complexes **1** and **2**. By comparing the fluorescence spectra of EB bound to DNA in the absence and in the presence of ruthenium(II) complexes (Figure S6 in the Supporting Information), we found that the addition of **1** and **2** to DNA pretreated with EB causes obvious reductions of 57.9% and 60.1% in fluorescence intensity, respectively. On the other hand, the intercalation of probe molecules into the DNA double helix usually increases the helix melting temperature. Herein, the

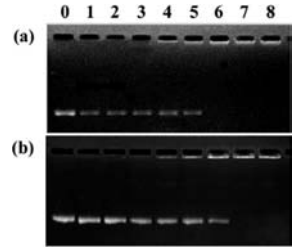


Figure 1. Agarose gel electrophoresis assay to investigate the DNA condensation induced by complexes **1** (a) and **2** (b): lane 0, DNA alone; lanes 1−8, DNA + complex. The DNA concentration is 5 ng/μL. The different concentrations of complex from lane 1 to lane 8 are 2, 4, 6, 8, 10, 20, 40, and 80 μM, respectively.

melting temperatures of CT-DNA in the presence of **1** and **2** were determined by monitoring the absorption of DNA bases at 260 nm as a function of the temperature (Figure S7 in the Supporting Information). Results showed that the melting temperature of free DNA (60.72 °C) increased 10.85 °C for complex **1** and 12.06 °C for complex **2** at a concentration ratio of [Ru]/[DNA] = 0.1, respectively. These data provided an additional support to the stronger intercalation abilities of the complexes into the double-helical DNA.

The abilities of two ruthenium(II) complexes to condense DNA were assessed by gel retardation assay. As shown in Figure 1, when an increase in the concentration of **1** and **2** (varied from 0 to 80 μM), the amount of supercoiled closed circular pBR322 DNA (form I) diminished gradually, and the retardation of DNA was more and more obvious. When the concentration reached 20 μM for **1** and 40 μM for **2**, no form I of plasmid pBR322 DNA was detected, but the strongly compacted DNA remained in the gel loading wells. The results showed that supercoiled plasmid DNA was condensed immediately upon the addition of each of the ruthenium(II) complexes at pH 7.2. The complex doses used here were on the micromolar scale and were much less than those of $Co(NH_3)_6^{3+}$ (at least 1 mM) to promote DNA condensation.[11] Moreover, this DNA condensation does not require the addition of Mg^{2+}.[12] In a control experiment, the complexes $[Ru(bpy)_3]^{2+}$, $[Ru(phen)_3]^{2+}$, $[Ru(bpy)_2(pip)]^{2+}$, and $[Ru(bpy)_2(dppz)]^{2+}$ did not show obvious condensation effects on DNA under identical conditions or even with increasing complex concentrations up to 500 μM (Figure S8 in the Supporting Information). Investigations into the mechanism behind this phenomenon are still ongoing.

To gain detailed structural information about the condensates, atomic force microscopy (AFM) studies were performed in an aqueous solution (50 mM Tris−HCl, 18 mM NaCl, pH 7.2) on an unmodified mica surface. Figure 2 shows typical AFM images of supercoiled pBR322 DNA in the absence and presence of ruthenium (II) complexes. Without ruthenium(II) complexes, the free DNA existed as loose clews or relaxed circles, with little twisting of the strands (Figure 2a). This structure is characteristic of uncondensed DNA morphology.[13] Upon interaction with **1** (40 μM), DNA was induced to form small

(8) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960–4962.
(9) Wu, J. Z.; Ye, B. H.; Wang, L.; Ji, L. N.; Zhou, J. Y.; Li, R. H.; Zhou, Z. Y. *J. Chem. Soc., Dalton Trans.* **1997**, 1395–1401.
(10) Lepecp, J. B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87–106.
(11) Pelta, J.; Livolant, F.; Sikorav, J.-L. *J. Biol. Chem.* **1996**, *271* 5656–5622.
(12) Conwell, C. C.; Hud, N. V. *Biochemistry* **2004**, *43*, 5380–5387.
(13) (a) Yang, J.; Takeyasu, K.; Shao, Z. F. *FEBS Lett.* **1992**, *301*, 173–176. (b) Liu, Y.; Yu, Z. L.; Zhang, Y. M.; Guo, D. S.; Liu, Y. P. *J. Am. Chem. Soc.* **2008**, *130*, 10431–10439.

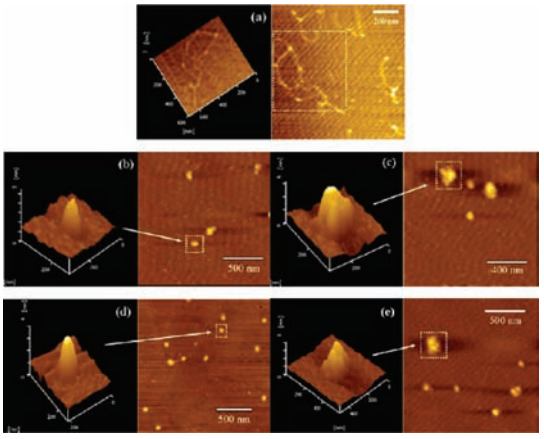


Figure 2. AFM images of pBR322 DNA (1 ng/μL) and its condensation induced by ruthenium(II) complexes on mica in tapping mode in air: (a) DNA; (b) DNA + 40 μM **1**; (c) DNA + 80 μM **1**; (d) DNA + 40 μM **2**; (e) DNA + 80 μM **2**.

nanoparticles with an average diameter of ca. 109 nm (Figure 2b). With in increase in the concentration of **1** (80 μM), larger nanoparticles with about 224 nm diameter were obtained (Figure 2c). Similar DNA condensation behaviors in the presence of **2** were also observed (about 95 nm diameter in Figure 2d and 197 nm diameter in Figure 2e). This phenomenon clearly demonstrated the good DNA condensation ability of **1** and **2**. Dynamic light scattering (DLS) further proves that the structures observed with AFM correspond indeed to the solution structures. From DLS measurements at a scattering angle of 90°, the effective hydrodynamic diameters of the DNA particles condensed by 40 μM **1**, 80 μM **1**, 40 μM **2**, and 80 μM **2** were about 179.9, 368.6, 176.3, and 351.2 nm, respectively (Figure 3).

The behavior of counterions in solution plays a key role in the DNA condensation. It is noted that monovalent and divalent cations alone are inefficient in condensing naked DNA.[14] DNA condensation is believed to be induced primarily by an electrostatic neutralization of the negatively charged DNA backbone with multivalent cations such as spermidine^{3+} and $Co(NH_3)_6^{3+}$, by which repulsive energies are decreased sufficiently to allow for tight packing.[14] However, the mechanism of condensation of supercoiled DNA by complexes **1** and **2** appears to be quite different from that induced by multivalent cations. Like some dinuclear copper (II) complexes[5] and organic intercalating aromatic cations,[15] the driving force of the DNA condensation induced by two ruthenium(II) complexes may be due to not only the electrostatic interactions between the divalent cations and the negatively charged phosphates in DNA but also the high DNA binding affinities of complexes as verified by spectroscopic and melting studies.

(14) Bloomfield, V. A. *Biopolymers* **1997**, *44*, 269–282.
(15) (a) Kapuscinski, J.; Darzynkiewicz, Z. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7368–7372. (b) Kuyper, C. L.; Brewood, G. P.; Chiu, D. T. *Nano Lett.* **2003**, *3*, 1387–1389.

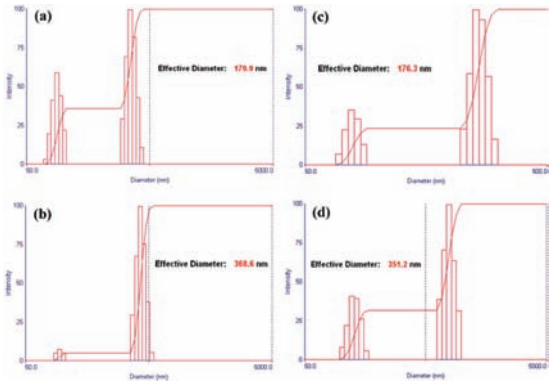


Figure 3. Hydrodynamic diameter distributions *f*(Dh) of pBR322 DNA particles condensed by 40 μM **1** (a), 80 μM **1** (b), 40 μM **2** (c), and 80 μM **2** (d) at a scattering angle of 90° at 25 °C. The DNA concentration is 1 ng/μL.

Recently, some ruthenium(II) complexes containing highly π-deficient polyazaaromatic ligands, such as 1,4,5,8-tetraazaphenanthrene or 1,4,5,8,9,12-hexaazatriphenylene, were found to form adducts with DNA upon visible irradiation.[6] However, in our case, the imidazole-containing ligands PIPSN and PIPNH are poorer π acceptors and better π donors. Similar to $[Ru(bpy)_2(dppz)]^{2+}$, despite their very high affinity for DNA, complexes **1** and **2** display no photoreactivity toward DNA because it is not sufficiently photooxidizing to produce the guanine radical cation. It was testified by continuous irradiation experiments that no change was observed in the absorption spectra of ruthenium(II) complexes under visible irradiation in the presence of CT-DNA (Figures S9 and S10 in the Supporting Information).

In summary, two ruthenium(II) complexes, **1** and **2**, have been synthesized and characterized. Spectroscopic and melting studies suggest that the two complexes possess high affinities for DNA. The most interesting observation is that both complexes can induce the condensation of originally circular plasmid pBR322 DNA to particulate structures under neutral conditions. The present results should be of value for the further understanding of the interaction between DNA and ruthenium(II) polypyridyl complexes, as well as offer the promising potential to control gene expression and delivery with metal complexes.

Acknowledgment. This work was supported by the 973 Program of China (2007CB815306), NSFC (Grants 20771105 and 20871122), NCET (Grant NCET-06-0718), Key Project (108103), and SRF for ROCS of the Ministry of Education.

Supporting Information Available: Synthesis and characterization of the complexes, experimental conditions, Figures S1−S10, and crystallographic data in CIF format for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.