Inorganic Chemistry

Communication

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First Ruthenium(II) Polypyridyl Complex As a True Molecular "Light Switch" for Triplex RNA Structure: $[Ru(phen)_2(mdpz)]^{2+}$ Enhances the Stability of Poly(U)·Poly(A)*Poly(U)

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Supporting Information

ABSTRACT: Stabilization of triple helical structures is extremely important for carrying out their biological functions. Nucleic acid triple helices may be formed with DNA or RNA strands. In contrast to many studies in DNA, little has been reported concerning the recognition of the RNA triplex by transition-metal complexes. In this article, $[Ru(phen)_2(mdpz)]^{2+}$ (Ru**1**) is the first metal complex able to enhance the stability of the RNA triplex Poly(U)·Poly(A)*Poly(U) and serve as a prominent molecular "light switch" for the RNA triplex.

Triple helical structures were first reported on the basis of fiber diffraction studies of the RNA polymers poly(U)·poly(A)*poly(U).[1] Subsequently, interest in these novel structural variants of nucleic acids has been rapidly growing, especially during the past two decades, due to their potential as tools in molecular biology as well as possible therapeutic agents.[2] However, the poor stability of these structures limits their practical applications under physiological conditions.[3] Thus, stabilization of triple helical structures is extremely important for carrying out their biological functions. The previous reports indicate that stabilization of triple helical RNA structure can be achieved by the action of intercalators,[4] in particular when covalently linked to the third strand,[5] whereas intercalators not covalently linked can either stabilize[6] or destabilize.[7] Furthermore, a more recent discovery of microRNAs and unraveling of their cellular functions led to a paradigm shift from DNA binding to RNA binding agents as potential gene regulators,[8] revealing that RNA triplex formation may also be an important structural motif of these small RNAs that the therapeutic agents may target for gene regulation. Surprisingly and in contrast to the large number of studies in triple helices DNA,[9] RNA triple helices have not been extensively studied.[10] At present, most of such studies have been mainly focused on organic compounds[4] and, to a far lesser extent, on metal complexes.[10]

Previous investigations indicate that many polypyridyl-based ruthenium(II) complexes interacting with DNA are excellent probes for physical properties of DNA.[11] Notably, $[Ru(phen)_2(dppz)]^{2+}$ and $[Ru(bpy)_2(dppz)]^{2+}$ (phen = phenanthroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine; bpy = 2,2′-bipyridine) known as DNA "light switches" have attracted much attention.[12] More recently, our reports indicate that $[Ru(phen)_2(mdpz)]^{2+}$ (Ru**1**, Figure 1) exhibits the "light switch" effect.[11b] However, surprisingly and in contrast to the large number of studies in DNA, little has been reported concerning the recognition of the triple-stranded nucleic acid by transition-metal complexes.

Figure 1. Chemical structure of Ru**1**.

Herein, Ru**1** and poly(U)·poly(A)*poly(U) are chosen for this investigation. The binding properties of Ru**1** to poly(U)·poly(A)*poly(U) are demonstrated by using various biophysical techniques. To the very best of our knowledge, Ru**1** is the first metal complex able to act as a true molecular "light switch" for triple helical RNA and stabilize the Hoogsteen base-paired third strand of the triplex RNA.

Poly(U)·poly(A)*poly(U) is prepared[13] by mixing poly(U) and poly(A)·poly(U) in a 1:1 molar ratio in a pH 7.0 phosphate buffer (6 mmol/L Na_2HPO_4, 2 mmol/L NaH_2PO_4, 1 mmol/L Na_2EDTA, 19 mmol/L NaCl). The formation of the triplex is confirmed by the melting profile (Figure S1A) and CD spectral pattern (Figure S1B). The optical melting profile of the triplex shows a biphasic transition, the first transition representing the displacement of the Hoogsteen base-paired third strand from the triplex and the second transition representing the duplex denaturation to the single-stranded structures. The first T_m (T_{m1}) and the second T_m (T_{m2}) are 37.5 and 46.0 °C, respectively, which are in conformity with the earlier reports.[13] The intrinsic CD spectral pattern of the triplex shows two distinct signals at about 240 and 260 nm, respectively, and further confirms the formation of the triplex.[13]

The extent of enhancement on the RNA triplex poly(U)·poly(A)*poly(U) binding was illustrated in steady-state

Received: January 16, 2012
Published: March 30, 2012

   dx.doi.org/10.1021/ic300093h | *Inorg. Chem.* 2012, 51, 4417−4419

luminescence experiments. Figure 2 showed the relative emission intensities I/I_0 (where I_0 was the emission measured

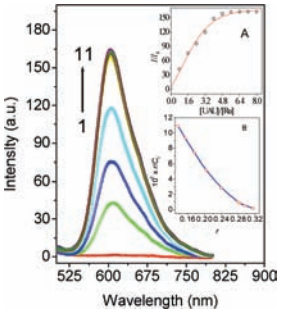

Figure 2. Representative fluorescence emission spectra of Ru**1** treated with poly(U)·poly(A)*poly(U) in pH 7.0 phosphate buffer at 20 °C. [Ru**1**] = 2.0 uM; for curves 1 → 11, [UAU] = 0–17.7 μM, respectively. Insets: (A) plots of I_0/I versus [UAU]/[Ru**1**], (B) Scatchard plots of Ru**1**. The solid line represents the nonlinear least-squares best-fit of the experimental points to the neighbor exclusion model.

in the absence of the RNA triplex) for Ru**1** in the presence of poly(U)·poly(A)*poly(U). The RNA-free Ru**1** showed negligible emission in aqueous solution; namely, its emission was temporarily closed ("turned off"). However, its emission is enhanced ("turned on") upon progressive addition of increasing concentrations of the RNA triplex to its solution to around 131 times that of the initial luminescence, indicating that a light-switch effect is occurring. In comparison with poly(U)·poly(A)*poly(U), poly(U)·poly(A) can only moderately increase the fluorescence of Ru**1** (Figure S2). The large fluorescence changes are indicative of strong association of Ru**1** to the triplex RNA resulting presumably from an effective overlap of the bound molecules with the base triplets. Furthermore, this result also proposes the location of the bound molecule in a hydrophobic environment similar to an intercalated state, because intercalation usually causes an increase in luminescence.[14] According to the Scatchard equation,[15] the results of fluorescence titration data were also converted to Scatchard plots and analyzed according to an excluded site model for a noncooperative binding phenomenon. The values of K_i (K_i is the intrinsic binding constant to an isolated binding site) and n (n is the number of nucleotides excluded by the binding of a single drug complex) are (2.6 ± 0.0016) × 10^7 M^{-1} and 2.9, respectively. Comparing the values (K_i and n) of the triplex–Ru**1** interaction obtained here with those of the poly(U)·poly(A)*poly(U) triplex–alkaloid interaction,[16] it suggests that, to some extent, the extended ligand is advantageous to the interaction between the ligand and the RNA triplex.[17]

The absorption titrations for Ru**1** with the triplex RNA were performed in a phosphate buffer solution at 20 °C (Figure S3). Significant spectral changes were observed upon the progressive addition of increasing concentrations of the triplex to Ru**1** solution, showing several hypochromisms at 266, 292, and 401 nm, respectively. The hypochromicity at 401 nm was estimated to be 30% with a red shift of 7 nm in the visible region, which essentially indicated strong intermolecular interaction involving effective overlap of the π electron cloud of Ru**1** with the base triplets that is speculative of intercalative complexation. Additionally, both polarity effects of the triplex and electron transfer from the base triplets may also contribute to the spectral changes to a certain extent. Two distinct isosbestic points were located at 312 and 476 nm, respectively, which revealed the existence of equilibrium between the free and bound form of Ru**1**. According to the equation,[18] the intrinsic binding constant K_b and the binding site s of Ru**1** to the triplex were determined as (3.6 ± 0.26) × 10^6 M^{-1} and 0.86. The binding constant K_b is close to that of coralyne–triplex interaction {(4.0 ± 0.60) × 10^6 M^{-1}} and is remarkably higher than those of berberine and palmatine–triplex interaction {(1.6 ± 0.40) × 10^4 M^{-1} for berberine, (1.6 ± 0.40) × 10^4 M^{-1} for palmatine}. This may indicate that the binding mode of Ru**1** in the triplex is the same as coralyne binding with the poly(U)·poly(A)*poly(U) triplex via intercalation.[16] The K_b of Ru**1** to the triplex is very close to that of duplex DNA ((4.1 ± 0.50) × 10^6 M^{-1}), but the binding site s of Ru**1** to the triplex is smaller than that of Ru**1** to duplex DNA (s = 2.1).[11b] To further investigate the specific binding of Ru**1**, the absorption titrations for Ru**1** with duplex RNA were performed under the same conditions (Figure S4). The K_b and s of Ru**1** to poly(U)·poly(A) are (2.1 ± 0.26) × 10^5 M^{-1} and 0.35, respectively. Thus, to some extent, Ru**1** preferentially binds to triplex RNA and duplex DNA.

The denaturation curves of the poly(U)·poly(A)*poly(U) triplex and its complex are presented in Figure S5. The quantitative data on the melting temperatures for different values of the C_D/C_P (drug/nucleotide phosphate molar ratio) ratio are summarized in Table 1. Note that the Ru**1**-free RNA

Table 1. Melting Temperatures (°C) for the Investigated poly(U)·poly(A)*poly(U) in the Absence and Presence of Ru1

C_D/C_P	[Na^+] (mM)	T_{m1} (°C) 3 → 2	T_{m2} (°C) 2 → 1
0	35	37.5	46.0
0.01	35	39.3	50.6
0.03	35	39.4	51.2
0.05	35	41.2	51.5
0.06	35	41.1	51.6
0.08	35	41.0	54.5

triplex melts in two well resolved sequential transitions. The lower temperature HG transition reflects dissociation of the RNA triplex to the poly(rA)·poly(rU) duplex and the poly(rU) single strand, while the higher temperature WC transition reflects denaturation of the remaining duplex into its component single strands. The thermal stability of the RNA duplex increases as the C_D/C_P increases from 0 to 0.08 (with a C_D/C_P ratio of 0.08 corresponding to a ΔT_m of +8.5 °C). Comparing a C_D/C_P ratio of 0.08 with that of 0.05, an inspection of Table 1 and Figure S4 also reveals that Ru**1** binding slightly decreases the thermal stability of the RNA triplex at a C_D/C_P ratio of 0.08 (corresponds to a ΔT_m of −0.2 °C), while exerting a thermally stabilizing influence on the triplex at a C_D/C_P ratio of 0.05 (ΔT_m = 3.7 °C). These observations suggest that Ru**1** binds more strongly to the triplex structure than to the duplex structure at C_D/C_P ratios ≤ 0.05, while binding more strongly to the duplex than to the triplex structure at C_D/C_P ratios > 0.05. From this result, we propose that the mdpz ligand of Ru**1** is intercalated with the two phenanthroline ligands located in the minor groove of the polynucleotide poly(U)·poly(A)*poly(U), thus stabilizing the third strand poly(U) by expansion of the stacking interaction; on the other hand, the cationic nature of Ru**1** and the

dx.doi.org/10.1021/ic300093h | Inorg. Chem. 2012, 51, 4417–4419

counterion distribution about the phosphate group caused by dication binding probably account for at least part of the triplex stabilization by Ru**1**. The CD spectrum of the RNA triplex with Ru**1** further confirms that Ru**1** indeed stabilizes the triple helix (Figure S6). Note that this behavior is different from what has been reported in the case of the binding of ethidium,[19] proflavine and its complex Pt-proflavine,[20] and some alkaloids,[4a16] where ethidium, proflavine, and its complex Pt-proflavine were shown to have a destabilizing effect on the third strand poly(U) and a stabilizing effect on the duplex poly(U)·poly(A), while some alkaloids, such as berberine, palmatine and coralyne, could obviously stabilize the Hoogsteen base-paired third strand as well as the Watson–Crick base paired duplex.[4a] To the best of our knowledge, our observation of Ru**1**-induced changes in the thermal stability of the poly(U)·poly(A)*poly(U) triplex provides the first demonstration of metal complex binding to a RNA triplex.

Figure 3 shows the effect of Ru**1** binding on the apparent molecular length of poly(U)·poly(A)*poly(U). Upon the addition of Ru**1**, there is an initial decrease at low concentrations and subsequent increase in the apparent molecular length of the poly(U)·poly(A)*poly(U) triplex, eventually reaching a plateau at a ratio of 0.23. The observed initial decrease in contour length at low concentrations may reflect a Ru**1**-induced conformational change in the triplex, while ascribing the subsequent increase in solution viscosity to the effects of intercalation. Such a conformational change could arise from a Ru**1**-induced kink or bend in the helix, thereby reducing its effective molecular length. Additionally, while a binding induced conformational change results in a decrease in contour length that compensates most of the increase caused by drug intercalation. The slight increase in solution viscosity observed in the contour length profile of the poly(U)·poly(A)*poly(U) triplex may simply reflect a binding-induced increase in the stiffness of the triplex. The relatively small maximum viscosity increase may also arise from the value of *n*, namely the number of base triplets per binding site, and the nature of the intercalative binding to the triple helix. The value of *n* (2.87, from the above luminescence studies) determined here is close to 3, which means that the maximum number of bound ligand molecules is smaller, resulting in a lower maximum extension of the triplex. The result suggests that Ru**1** binds to triplex RNA via an intercalative mechanism.

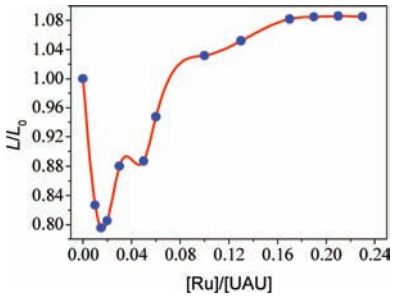

Figure 3. Viscometric Ru**1** titrations of poly(U)·poly(A)*poly(U) at 20 °C. [UAU] = (2.79×10^{-4}) M. Solution conditions are the same as those described in the legend of Figure 2.

In conclusion, the results indicate that the ruthenium(II) complex $[Ru(phen)_2(mdpz)]^{2+}$ can not only stabilize the Hoogsteen base-paired third strand but also can serve as a true molecular "light switch" for triple-stranded poly(U)·poly(A)*poly(U). These results further advance our knowledge of the interaction of metal complexes with triple helical structures and may be useful in developing RNA targeted therapeutics and an RNA probe.

■ ASSOCIATED CONTENT

Supporting Information

The equations used to calculate the binding constants, Figures S1–S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the NSFC (21071120) and the Scientific Research Foundation of Hunan Provincial Education Department (11A117) for financial support.

■ REFERENCES

(1) (a) Felsenfeld, G.; Davies, D. R.; Rich, A. *J. Am. Chem. Soc.* **1957**, *107*, 5528–5529. (b) Stevens, C. L.; Felsenfeld, G. *Biopolymers* **1964**, *2*, 293–398.

(2) Cooney, M.; Czernuszewicz, G.; Postel, E. H.; Flint, S. J.; Hogan, M. E. *Science* **1988**, *241*, 456–459.

(3) Wang, S.; Kool, E. T. *Nucleic Acid Res.* **1995**, *23*, 1157–1164.

(4) (a) Sinha, R.; Kumar, G. S. *J. Phys. Chem. B* **2009**, *113*, 13410–13420. (b) Xi, H. J.; Davis, E.; Ranjan, Ni.; Xue, L.; Hyde-Volpe, D.; Arya, D. P. *Biochemistry* **2011**, *50*, 9088–9113.

(5) Shchyolkina, A. K.; Timofeev, E. N.; Lysov, Yu. P.; Florentiev, V. L.; Jovin, T. M.; Arndt-Jovin, D. J. *Nucleic Acids Res.* **2001**, *29*, 986–995.

(6) Polak, M.; Hud, N. V. *Nucleic Acids Res.* **2002**, *30*, 983–992.

(7) Lehrman, E. A.; Crothers, D. M. *Nucleic Acids Res.* **1977**, *4*, 1381–1392.

(8) (a) Esau, C. C.; Monia, B. P. *Adv. Drug Delivery Rev.* **2007**, *59*, 101–114. (b) Bartel, D. P. *Cell* **2004**, *116*, 281–297.

(9) Arya, D. P. *Acc. Chem. Res.* **2011**, *44*, 134–146.

(10) García, B.; Leal, J. M.; Paiotta, V.; Ruiz, R.; Secco, F.; Venturini, M. *J. Phys. Chem. B* **2008**, *112*, 7132–7139.

(11) (a) Stewart, D. J.; Fanwick, P. E.; McMillin, D. R. *Inorg. Chem.* **2010**, *49*, 6814–6816. (b) Tan, L. F.; Liang, X. L.; Liu, X. H. *J. Inorg. Biochem.* **2009**, *103*, 441–447.

(12) (a) Dupureur, C. M.; Barton, J. K. *Inorg. Chem.* **1997**, *36*, 33–43. (b) Chen, M. J.; Li, H.; Shao, J. Y.; Huang, Y.; Xu, Z. H. *Inorg. Chem.* **2011**, *50*, 2043–2045.

(13) Das, S.; Kumar, G. S.; Ray, A.; Maiti, M. *J. Biomol. Struct. Dyn.* **2003**, *20*, 703–714.

(14) Shi, S.; Zhao, J.; Geng, X. T.; Yao, T. M.; Huang, H. L.; Liu, T. L.; Zheng, L. F.; Li, Z. H.; Yang, D. J.; Ji, L. N. *Dalton Trans.* **2010**, *39*, 2490–2493.

(15) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469–489.

(16) Durand, M.; Seche, E.; Maurizot, J. C. *Eur. Biophys. J.* **2002**, *30*, 625–633.

(17) Choi, S. D.; Kim, M. S.; Kim, S. K.; Lincoln, P.; Tuite, E.; Nordén, B. *Biochemistry* **1997**, *36*, 214–223.

(18) Carter, M. T.; Rodriguez, M.; Bard, A. *J. Am. Chem. Soc.* **1989**, *111*, 8901–8911.

(19) (a) Waring, M. J. *Biochem. J.* **1974**, *143*, 483–486. (b) Lehrman, E. A.; Crothers, D. M. *Nucleic Acids Res.* **1977**, *4*, 1381–1392.

(20) García, B.; Leal, J. M.; Paiotta, V.; Ruiz, R.; Secco, F.; Venturini, M. *J. Phys. Chem. B* **2008**, *112*, 7132–7139.