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Selective Binding and Reverse Transcription Inhibition of Single-Strand poly(A) RNA by Metal TMPyP Complexes

Zhu-Xin Zhou, Feng Gao,* Xing Chen, Xiang-Jing Tian, and Liang-Nian Ji

MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P. R. China

Supporting Information

ABSTRACT: Ni-, Cu-, and Zn-TMPyP are capable of binding to single-strand poly(A) RNA with high preference and affinity and inhibiting the reverse transcription of RNA by both M-MuLV and HIV-1 reverse transcriptase. With 10 nM azidothymidine, the IC_{50} value of M-TMPyP could be lowered to 10^{-1} μM order.

DNAs have been the focus of drug targeting for decades.[1] The more recent discovery of micro-RNAs and emerging knowledge of their critical roles in essential cellular activities have led to a paradigm shift from DNA to RNA as the focus of drug targeting to control genetic activity. Because the genetic codes of most viruses are stored in viral RNA, such as human immunodeficiency virus (HIV) and hepatitis C virus, the recent antiviral drug design is focused on targeting RNA reverse transcriptase (RT), by which to inhibit the duplication of viral RNA.[2] The polyadenylic ribonucleic acid [poly(A)] tail, consisting of 200−250 adenine bases at the 3′ end of mRNA, plays a significant role for the initiation of translation, maturation, and stability of mRNA as well as in the production of alternate forms of protein.[3] Poly(A) also plays important roles in HIV-1 first (minus)-strand DNA transfer,[4] during the reverse transcription of HIV-1 viral RNA, where tRNA3Lys serves as the primer.[5] Therefore, a study on RNA-binding behaviors of small molecules will be of value in these fields.

Water-soluble *meso*-tetrakis(4-*N*-methylpyridinium) porphyrin (TMPyP) and its metal derivatives have been found capable of interacting with a variety of double-strand (ds) DNA, G-quadruplex DNA, yeast-transfer RNA (tRNA), and other biological macromolecules.[6] Their ds DNA binding modes could be partial intercalation, groove binding, and outside binding with self-stacking of the porphyrins.[7] During the preparation of this paper, Cu-TMPyP was reported to bind "externally" to single-strand (ss) DNA.[8] However, the possibility of interaction between metal complexes of TMPyP (M-TMPyP) and completely ss RNA and conceivable binding mode(s) are still unrevealed. Therefore, we contrastively discuss in this paper the binding properties of Ni, Cu, and Zn complexes of TMPyP (Chart 1) to three different RNAs—ss poly(A) RNA, tRNA, and total RNA—which are mainly in ds form with ss regions, and their effects on the reverse transcription of ss RNA by RT.

The interactions between M-TMPyP and RNA were first studied by absorption spectra titration (Figure S1 in the Supporting Information, SI). The spectral changes and intrinsic binding constants K_b were calculated[9] and summarized in Table 1.

Chart 1

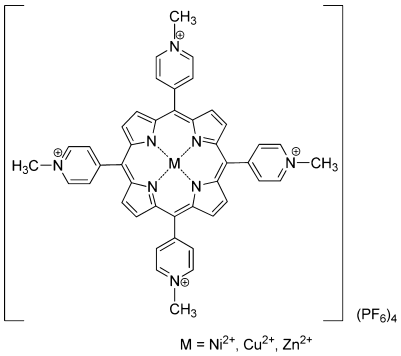

Table 1. Hypochromism (H) and Red Shift ($\Delta\lambda$) of the Soret Band in the Absorption Spectra of M-TMPyP (5 mM Tris-HCl, 50 mM NaCl, pH 7.0) upon the Addition of RNA and Calculated Binding Constants (K_b)

metal	RNA	K_b (M^{-1})	H (%)	$\Delta\lambda$ (nm)
Zn	total RNA	$(1.99 \pm 0.16) \times 10^6$	31.3	12
	tRNA	$(1.81 \pm 0.15) \times 10^6$	33.4	12
	poly(A)	$(2.00 \pm 0.12) \times 10^8$	64.0	19
Cu	total RNA	$(2.26 \pm 0.15) \times 10^6$	45.6	9
	tRNA	$(2.52 \pm 0.18) \times 10^6$	46.5	9
	poly(A)	$(8.17 \pm 0.63) \times 10^8$	67.3	17
Ni	total RNA	$(1.67 \pm 0.14) \times 10^6$	34.5	9
	tRNA	$(1.90 \pm 0.11) \times 10^6$	31.1	10
	poly(A)	$(3.57 \pm 0.21) \times 10^8$	53.9	20

M-TMPyP showed typical metalloporphyrin spectra[10] with an intense absorption at about 400−450 nm (Soret band) and two prominent bands (Q bands) at 500−550 nm with less intensities. Upon the addition of RNA, large hypochromism effects in the Soret band were observed, with moderate red shifts. All M-TMPyP have similar spectral changes and K_b values for total RNA and tRNA, suggesting a similar binding mode and affinity. This can be explained by the structural similarity of these two RNAs containing both ds and ss regions. For ss poly(A) RNA, spectral changes (53.9−67.3% hypochromism and 17−20 nm

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red shift) are apparently higher and the calculated K_b values were 2 orders of magnitude higher than those of total RNA and tRNA. This preferred ss poly(A) RNA binding behavior has also been observed in ss poly(dA) DNA over CT-DNA[11] and $(dA)_{10}$ DNA over several ss and ds DNAs, such as $(dT)_{10}$, $(dU)_8$, $(dC)_{10}$, and $(dT)_{16}$.[8] Small molecules that bind selectively and with high affinity to ss nucleic acid have been considered to be rare.[12]

Circular dichroism (CD) spectral titration was also performed by using a fixed RNA concentration to which increments of M-TMPyP solutions were added. The CD intensity of the positive band at 270 nm for total RNA and tRNA greatly decreased with red shifts (Figure S2a–f in the SI), which could be attributed to an intercalation mode like proflavine.[13] However, for ss poly(A) RNA (Figure 1), binding of Cu-TMPyP turned the positive band

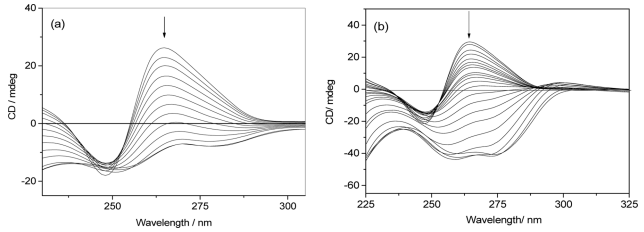

Figure 1. CD spectral titration of Cu-TMPyP (a) and Ni-TMpPyP (b) to ss poly(A) RNA in a 5 mM Tris-HCl buffer with 50 mM NaCl (pH 7.0). Arrows show spectral changes with increasing amounts of M-TMPyP.

at 270 nm into a weak negative band at 280 nm. CD spectra of poly(A) showed two strong negative bands at 275 and 255 nm upon the addition of Ni-TMPyP. Zn-TMPyP also induced similar CD spectral changes (Figure S2g in the SI), but the new negative bands were weak. ss DNA has been found to be better than ds DNA at internalizing a binding molecule encumbered by bulky substituents, by stacking between a part of the binding molecule and DNA bases.[14] Therefore, it is not surprising that the changes in CD for poly(A) are greater than those for total RNA and tRNA. However, no report has depicted a CD band reversal of poly(A) RNA, in either structural transition or ligand binding, to the best of our knowledge. Therefore, we have tried to explore other possible binding modes, besides intercalation (either partially or completely).

Axial coordination of iron(II) porphyrins plays an important role in the function of heme. The planar M-TMPyP complexes possess adequate space for axial coordination, which makes remarkable contributions to potential interactions with bases of nucleic acids or nucleotide. To test the binding abilities of M-TMPyP complexes, thermodynamics-based absorption spectral titrations of four nucleoside monophosphates (AMP, UMP, CMP, and GMP) were performed. Upon the addition of nucleoside monophosphate (NMP), the absorption spectra of M-TMPyP showed remarkable hypochromism effects and red shifts (Figure S3 in the SI), as the electrons from guests transferred to the porphyrin ligand through the connection to the center metal cation, increasing the $a_{2u}(\pi)$ orbital energy and reducing the excitation energy between the $a_{2u}(\pi)$ and $e_g(\pi^*)$ orbitals. The axial binding constants were estimated from $\ln K + n \ln c_L = \ln(A_0 - A_e)/(A_e - A_\infty)$, where K is the equilibrium binding constant, c_L is the guest (NMP) concentration, n is the coordination number, and A_0, A_e, and A_∞ refer to the absorbance corresponding to the guest concentration of 0, c_L, and relative infinity.[15] As shown in Table S1 in the SI, all M-TMPyP can form 1:1 adducts with different NMPs. The binding constants K are about 10 times higher than those of TMPyP, without central metals, binding to four deoxynucleoside monophosphates (dNMPs) by $\pi-\pi$ interactions.[16] This suggests that M-TMPyP binds NMP via both zinc phosphate coordination and $\pi-\pi$ stacking like the reported binding modes of zinc(II) salophen with a square-planar coordination center similar to that of AMP.[17] AMP and GMP possessed larger binding constants, which can be attributed to their double-ring structures and which lead to stronger $\pi-\pi$ overlap upon complexation.[16]

Upon comparison of the binding of RNA to NMP by M-TMPyP above, it can basically be confirmed according to relative literatures that (1) M-TMPyP prefers to bind total RNA and tRNA by intercalation, at least partially, into the ds regions and (2) M-TMPyP can form 1:1 adducts with NMP by axial coordination. Some inferences could be drawn that M-TMPyP binds ss poly(A) RNA through, but not limited to, an intercalative mode. According to the unexpectedly large changes in CD and absorption spectra, the strand of poly(A) RNA might undergo conformation changes over a wide range, which was hypothesized to arise from the simultaneous interactions of partial intercalation of M-TMPyP and its axial coordination with the phosphate backbone of RNA. Although M-TMPyP can also bind nucleotides of ss RNA loops in total RNA and tRNA, the binding is weaker than that of intercalation with ds RNA stems.

To explain the result that Cu-TMPyP performed as the most potent ss poly(A) RNA binder, density functional theory (DFT) calculation was presented by *Gaussian 03*[18] at the B3LYP/LanL2DZ level. According to the calculation results (Figures S4 and S5 in the SI), the lowest unoccupied molecular orbital (LUMO) of Cu-TMPyP is apparently lower than those of Ni- and Zn-TMPyP by 6.9 and 9.1 kJ/mol. The lower energy level of LUMO rendered Cu-TMPyP favor interactions with the highest occupied molecular orbital of RNA.

The inhibition of moloney murine leukemia virus reverse transcriptase (M-MuLV RT) by M-TMPyP has been examined. As shown in gel electrophoresis (Figure 2), the amount of

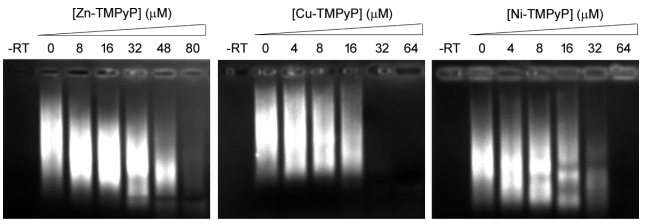

Figure 2. Gel electrophoresis analysis of M-MuLV RT inhibition of M-TMPyP in different concentrations, using poly(A) RNA as the template and $(dT)_{18}$ as the primer in 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, and 10 mM DTT.

produced cDNA decreased as the concentration of M-TMPyP increased and nearly disappeared when M-TMPyP reached certain concentrations. The IC_{50} value (concentration of M-TMPyP that prevented 50% of the RNA from reverse transcribing to cDNA) of Cu-TMPyP (12 μM) is lower than those of Ni-TMPyP (16 μM) and Zn-TMPyP (40 μM), indicating an inhibitory activity trend of Cu-TMPyP > Ni-TMPyP > Zn-TMPyP. Because the concentration of the poly(A) substrate in RT inhibition was 144 μM in nucleoside, it implied that, to inhibit 50% activity of M-MuLV RT, ss poly(A) needed a bound M-TMPyP molecule every 12, 9, and 3.6 nucleosides for Cu-, Ni-, and Zn-TMPyP, respectively.

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We also tested the HIV-1 RT inhibitory activity of M-TMPyP by colorimetric enzyme immunoassay, together with azidothymidine (AZT), the first clinical drug for HIV. The IC_{50} values of M-TMPyP (12, 8.0, and 25 μM for Ni-, Cu-, and Zn-TMPyP) were higher than that of AZT (0.050 μM) but were comparable with those of some HIV-1 RT inhibitors.[19] When we mixed M-TMPyP and AZT with a concentration much lower than their individual IC_{50} values, the inhibitory activity was greatly improved (Figure 3). It showed that 1.0 μM Cu-TMPyP could increase the HIV-1 RT inhibition percentage (Inh %) of 10 nM AZT from 13% to 93%. In other words, even 10 nM AZT could lower the IC_{50} value of M-TMPyP to 10^{-1} μM order. This indicated that anti-HIV activity could be enhanced by using different types of drugs together, like the AZT/dideoxycytidine combination.[20]

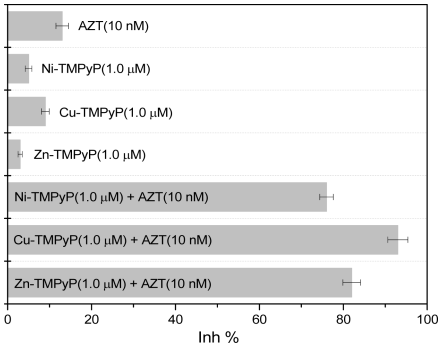

Figure 3. HIV-1 RT inhibitory percentage (Inh %) of drugs and their mixtures.

In conclusion, the versatile nucleic acid binders M-TMPyP (M = Cu, Ni, and Zn) bind ss poly(A) RNA with high preference, compared to total RNA and tRNA. The binding mode has been hypothesized to be partial intercalation and phosphate binding, which lead to long-range conformational change. M-TMPyP, especially Cu-TMPyP, has shown good inhibitory effects on the reverse transcription of RNA by both M-MuLV and HIV-1 RT. A combination of M-TMPyP and AZT may greatly improve the inhibitory activity, giving them another prospect of practical application. Because molecular recognition of RNA by small molecules is an area that is currently of great interest, our results may provide new insight for the design and development of small-molecule-based RNA-targeting therapeutic agents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, absorption spectral titration of RNAs and NMP, RNA CD titration by M-TMPyP, DFT calculation data, plots of RT inhibition quantitation, and table of NMP binding data of M-TMPyP. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: gaofeng9@mail.sysu.edu.cn.

Notes

The authors declare no competing financial interest.

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