Bioorganic & Medicinal Chemistry 19 (2011) 6881–6884

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Electron transfer through RNA: Chemical probing of dual distance dependence

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ARTICLE INFO

Article history:
Received 31 August 2011
Revised 14 September 2011
Accepted 14 September 2011
Available online 28 September 2011

Keywords:
Electron transfer
RNA
Pyrene
5-Bromouracil
Chemical probing

ABSTRACT

Electron transfer (ET) through RNA duplexes possessing 2′-*O*-pyrenylmethy uridine (Upy) and 5-bromouracil (BrU) as an electron donor and accepter set was investigated. Reductive decomposition of the BrU resulted from the ET over long distances (up to ten AU base pairs) was detected in the RNA conjugates. The RNA mediated ET from the pyrene to BrU showed dual distance dependence. This is well consistent with the previous observation for ET from Upy to nitrobenzene in RNA. In contrast, little or no reductive decomposition of the BrU was observed in the DNA conjugates when the Upy and BrU were separated by more than four AT base pairs.

1. Introduction

Electron transfer (ET) as well as hole transfer in DNA has attracted considerable interest, because of their potential application in DNA-based nanodevices,[1–3] and the relevance to the biological consequences such as DNA damages.[4–6] Through biochemical and spectroscopic studies, it has been shown that both positive and negative charges injected into DNA can migrate over long distances.[7,8] The efficiency of ET through DNA can be modulated by base sequences and its structural dynamics. In addition, the long-lived charge separated state has been realized in the DNA possessing a donor–accepter pair.[9–12] These findings are particularly important in the development of an electrical DNA sensor and a photo-energy conversion device.

Initial evidence of ET through DNA over long distances was provided by using reductive cleavage of thymine dimers and reduced flavins as an electron donor.[13] Several research groups reported the long-distance ET in DNA that has been evident from the use of various electron acceptor and donor pairs.[14–20] It is now accepted that the excess electron migrates by multi-step hopping on pyrimidine bases.[13,16] This mechanism was based on the trend of reduction potentials of nucleobases (T ≈ U, dU >C >>A >G).[21,22] Pyrimidine bases (T (U) and C) are reduced more easily than purine bases (G, A). Hence, pyrimidine bases act as charge carriers in excess electron transfer process.

Until very recently, little attention has been paid for the charge transfer in an RNA duplex. RNA duplexes have the base stacking overlaps and dynamics that are significantly different from those of DNA–DNA as well as of DNA–RNA duplexes. RNA duplexes may therefore be an attractive medium for charge transfer. We have disclosed that excess electron can move through RNA π-stacks from pyrene to nitrobenzene over significant distance with dual distance dependence.[23] This study relied on pyrene fluorescence quantum yields to access the distance dependence. While our results provide an important insight in developments of RNA-based nanodevices, proof of the distance dependence in RNA-mediated electron transfers should be given. Here, we report electron transfer (ET) in RNA duplexes in which distance dependence of ET has been analyzed from the chemical assay using a system consisting of pyrene-modified uridine (Upy) as a photo-excitable electron donor and 5-bromouridine (BrU) as a kinetic electron trap. Reductive decomposition of the BrU resulted from the ET over long distances (up to ten AU base pairs) was detected in the RNA duplexes. The RNA mediated ET from the photo-excited pyrene to BrU showed dual distance dependence, which is well consistent with the fluorescence quenching experiments. In contrast, little or no reductive decomposition of the BrU was observed in the DNA duplexes when the Upy and BrU were separated by more than four AT base pairs.

2. Results and discussion

Our experiments for charge injection and transport in RNA and DNA involves the use of continuing $(rA)_n$–$(U)_n$ and $(dA)_n$–$(dT)_n$ sequences as a bridge, pyrene (Py) as a photo-excitable electron donor, and 5-bromouridine (BrU) as an electron acceptor (Fig. 1). Py was introduced via one-carbon linker at the specific 2′-O-position of U- or T-strand. BrU was site-specifically incorporated into the same strand. Since the free energies for electron transfer from

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doi:10.1016/j.bmc.2011.09.027

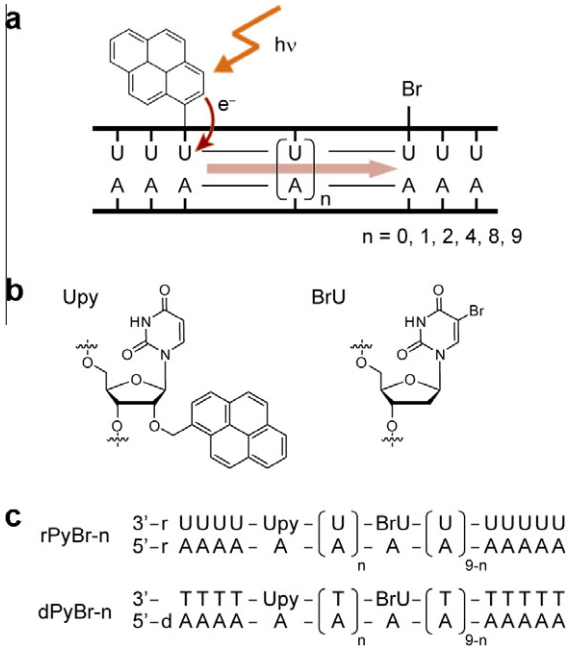

Figure 1. (a) Scheme of excess electron transfer from Py to BrU mediated by RNA duplex. (b) Chemical structures of pyrene-conjugated uridine (U_{Py}) and 5-bromo-2′-deoxyuridine (BrU) used as an electron injector and a kinetic electron trap, respectively. (c) Sequences for RNA (rPyBr-n) and DNA (dPyBr-n) designed for analysis of the excess electron transfer. Py is separated from BrU by a variable number of A-U (RNA) or A-T (DNA) base pairs. 2′-*O*-methyl-substituted nucleotides (rA, U) are used for the RNA duplex.

excited-state pyrene (Py*) to uracil base and from Py* to BrU can be estimated to be ca. −0.5 and −0.5 ∼ −1.0 eV,[24,25] respectively, the electron injection and the subsequent electron transfer processes are expected to be exergonic. As a consequence of the electron transfer, one electron reduction of BrU occurs.[26] The resulting uracil radical anion rapidly leads to the spontaneous decomposition of BrU. By quantifying the decompositions of BrU, the electron transfer reactions form Py* to BrU in RNA and DNA can easily be monitored.[18,27,28]

Table 1 summarizes the melting temperatures (T_ms) for Py- and BrU-modified RNA (rPyBr-n) and DNA (dPyBr-n) duplexes used in the present study. Judging from the T_m values, the doubly modifications of RNA and DNA duplexes have little effect on the stability of duplexes. The CD spectra show that the global conformations of the RNA and the DNA duplexes are A- and B-forms, respectively (Supplementary data). The induced CD observed at around 340 nm for the DNA indicates that the pyrene is intercalated into the base pairs of DNA. No induced CD was observed for RNA at the same wavelength region, which is consistent with that the pyrene is located outside the duplex. The local structures of the pyrene covalently attached via the one carbon liker into RNA and DNA have already been established[29] and are consistent with the present RNA and DNA duplexes.

Table 1
Melting temperatures (T_m) and apparent relative decomposition rates (k_{app}) of rPyBr-n and dPyBr-n

	rPyBr-n		dPyBr-n	
n	T_m[a](°C)	k_{app}[b](%min^{-1})	T_m[a](°C)	k_{app}[b](%min^{-1})
–	49.9[c]	–	48.0[c]	–
0	49.9	110	47.8	260
1	49.8	25	48.1	76
2	50.0	2.8	48.1	0.67
4	49.9	0.30	47.9	<0.01
8	49.9	0.19	48.0	<0.01
9	50.0	0.12	48.1	<0.01

[a] 5 μM duplexes in 20 mM Na phosphate buffer at pH 7 and 100 mM NaCl.
[b] Decomposition rates (k_{app}) were obtained from the slope of the decomposition yields vs irradiation time.
[c] T_m values for unmodified RNA or DNA.

The photo-induced electron transfer reactions were carried out at room temperature by illumination of UV-light (365 nm) for the solutions containing the Py- and BrU-modified duplexes (rPyBr-n and dPyBr-n: 10 μM) in a phosphate buffer (pH 7). The resulting solutions were enzymatically digested by treatment with phosphodiesterase and alkaline phosphatase, and then analyzed by reverse-phase HPLC. The some examples for the HPLC analysis are shown in Figure 2. The amount of intact BrU was decreased with the photoirradiations, by which the yields of the BrU-decomposition could be obtained. Plots of the reaction efficiency against the irradiation time are shown in Figure 3. The reaction yields linearly increased with increase in irradiation time, which can provide the apparent electron transfer rates (k_{app}) as summarized in Table 1. For both the RNA and DNA containing Py and BrU of short distances (less than two base pairs), the photoreaction proceeds efficiently. When Py is separated from BrU by more than four base pairs, the reaction of the BrU resulted from the electron transfer indeed occurred in the RNAs. In contrast, littler or no reactions of the BrU were observed in the DNAs containing Py and BrU with longer than four base pairs.

The electron transfer rates (k_{app}) dependent on the distance (r) between Py as an electron donor and BrU as an accepter were examined. Figure 4 shows the plots of ln k_{app} against r, where the base pair distances are assumed to be 2.8 Å for RNA and 3.4 Å for DNA, respectively. In RNA, the analysis of the plot gave two β values: β_1 is 0.7 Å^{-1} at the short distances (<10 Å) and β_2 is 0.1 Å^{-1} at long distances (>10 Å). The electron transfer from photo-excited Py* to BrU is weakly depended on their distance when the number of intervener rA-U base pairs is more than four. The dual distance dependence in the electron transfer through RNA is thus evident. In DNA, from the analysis of ln k_{app} versus r plot, the β value of 0.8 Å^{-1} was obtained at short distances. As described above, the electron tranfer to BrU from Py* hardly occur at the long distances (>10 Å), thereby a single β value could be obtained for the distance dependent electron transfer from Py* to BrU in our DNA system.

The difference in the decomposition yields of BrU between DNA and RNA conjugates may be rationalized by considering the relative position of the Py in the duplex (Fig. 5). As mentioned above, the position of the Py attached to 2′-O position in the duplex depends on the structural conformation of the duplex. The Py intercalates into the duplex in B-form conformation, whereas the Py is located outside of the duplex in A-form conformation. Namely, there are differences in distance and stacking interaction between

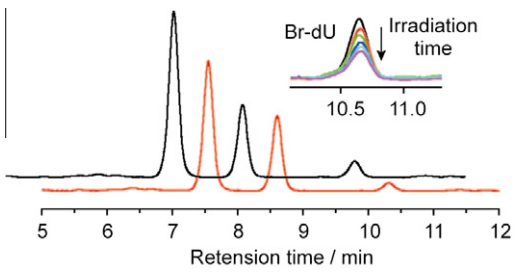

Figure 2. HPLC profiles monitored at 280 nm for the reaction mixtures (rPyBr-2) digested by enzymatic reaction before (black) and after photoirradiation (red). Inset: 0–20 min irradiation. The HPLC peaks observed at 7.6, 8.6, 10.6 min were assigned to uridine, adenine, and 5-bromo-2′-deoxyuridine.

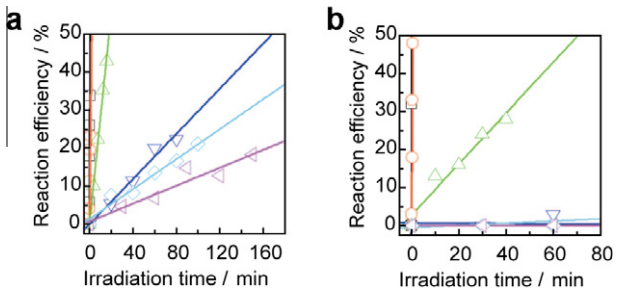

Figure 3. Reaction efficiency of BrU decomposition through excess electron transfer for (a) rPyBr-n and (b) dPyBr-n (Black: $n = 0$, red: $n = 1$, green: $n = 2$, blue: $n = 4$, sky blue: $n = 8$, magenta: $n = 9$). The reaction efficiency was calculated from ratio of peak areas of BrU before and after photoirradiation.

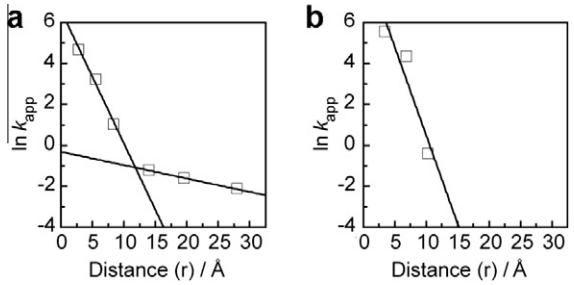

Figure 4. Distance dependence of apparent decomposition rates (k_{app}) for (a) rPyBr and (b) dPyBr. Distance between the Py and BrU are calculated assuming the distance of 3.4 Å between bases for DNA and 2.8 Å for RNA.

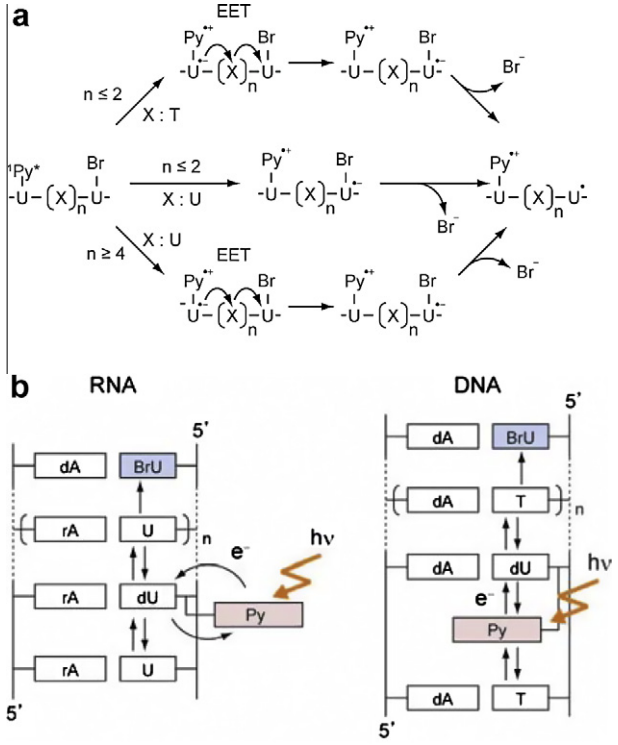

Figure 5. Plausible mechanism of electron injection and excess electron transfer in DNA (right) and RNA (left). EET and BET represent excess electron transfer and back electron transfer, respectively.

the Py and nucleobases. In DNA conjugates, the intercalated Py is directly stacking with pyrimidine bases (T and U). The escape of the excess electron from the contact ion pair ($Py^{\cdot+}$-$dU^{\cdot-}$ or $Py^{\cdot+}$-$T^{\cdot-}$) is ineffective because of very rapid charge recombination in the ion pair (picosecond time scale), resulting in no reductive decomposition of BrU in DNA conjugates at long distances. In the case of hole transfer in DNA, the escape of a positive charge (hole) from the contact ion pair can be achieved in specific sequences where hole transfer is very rapid and competitive with charge recombination.[11,30,31] In addition, the excess electron generated in an opposite side to BrU may cause inefficiency for the reduction of BrU. Unlike DNA conjugates, the Py in RNA conjugates is spatially separated from nucleobases without direct interaction, leading to the slow charge recombination. This slow charge recombination in RNA conjugates allow an excess electron on uridine to escape from initial charge separated state and lead to the efficient excess electron transfer over long distances. Probably, differences in stacking interaction and conformational dynamics between DNA and RNA are also related to the efficiency of excess electron transfer.[32–35] Previous spectroscopic studies demonstrated that A-form RNA:DNA hybrids is better medium for charge transfer compared with DNA hybrids because of increased structural flexibility and base motion of DNA:RNA hybrids on the nanosecond timescale,[33] which is consistent with our results in RNA conjugates. Therefore, slow charge recombination due to the spatial separation between positive and negative charges, and fast electron transfer in A-form conformation is the origin of excess electron transfer observed in RNA.

3. Conclusion

We have shown dual dependence of electron transfer (ET) from photo-excited pyrene (Py^*) to 5-bromouridine in RNA. The result is consistent with the previous analysis of ET from Py^* to nitrobenzene through the π-stacks of RNA. We anticipate that, in addition to DNA, RNA is promising material for several applications such as in functional nanodevices

4. Experimental

4.1. General method

DNA and RNA oligonucleotides were synthesized on an ABI DNA Synthesizer using standard solid-phase phosphoroamidite chemistry. All DNA and RNA synthesis reagents were obtained from Glen Research. 2′-OMe RNA phosphoramidites were used for RNA synthesis. Pyrene-modified oligonucleotides were synthesized according to previous reports.[36,37] 5′-*O*-dimethoxytrityl-2′-*O*-(1-pyrenylmethyl) uridine 3′-*O*-(2-cyanoet'yl)-*N*,*N*′-diisopropyl-phosphoramidite was used for the modification of pyrene. The DNA and RNA samples were prepared by annealing the mixtures of complementary strands in 20 mM Na phosphate buffer (pH 7.0) and 100 mM NaCl. The duplex concentration was 5 μM.

4.2. Photoreaction experiment

The DNA or RNA samples (10 μM in 20 mM Na phosphate buffer (pH 7) and 100 mM NaCl were prepared by mixing equimolar amounts of the complementary strand and gradually annealing with cooling from 80 °C to room temperature. The photoreaction of Py and BrU-containing DNA and RNA duplex were irradiated using a UV transilluminator (365 nm) at various times. The irradiated samples were digested by adding enzymatic solution of the snake venom phosphodiesterase and alkaline phosphatase at 37 °C. The digested samples were analyzed by reverse phase HPLC (JASCO) on a MS-II ODS 4.6 mm × 150 mm column using the following conditions: flow rate, 1.0 mL/min, elution with a solvent mixture of CH_3CN/ammonium formate (50 mM), and linear

gradient over 15 min from 2 to 8% CH_3CN. The decomposition efficiency of BrU was determined from the peak areas of BrU before and after photoirradiation.

Acknowledgment

This research was supported in part by a Grand-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS).

Supplementary data

Supplementary data (UV absorption spectra, CD spectra, fluorescence spectra) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.027.

References and notes

1. Guo, X.; Gorodetsky, A. A.; Hone, J.; Barton, J. K.; Nuckolls, C. *Nat. Nanotechnol.* **2008**, *3*, 163.
2. Xu, B. Q.; Zhang, P. M.; Li, X. L.; Tao, N. J. *Nano Lett.* **2004**, *4*, 1105.
3. Porath, D.; Bezryadin, A.; de Vries, S.; Dekker, C. *Nature* **2000**, *403*, 635.
4. Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.
5. Cadet, J.; Douki, T.; Ravanat, J.-L. *Acc. Chem. Res.* **2008**, *41*, 1075.
6. Kawai, K.; Osakada, Y.; Fujitsuka, M.; Majima, T. *Chem. Biol.* **2005**, *12*, 1049.
7. Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.
8. Giese, B. *Annu. Rev. Biochem.* **2002**, *71*, 51.
9. Lewis, F. D.; Liu, X. Y.; Liu, J. Q.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. R. *Nature* **2000**, *406*, 51.
10. Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159.
11. Kawai, K.; Kodera, H.; Osakada, Y.; Majima, T. *Nat. Chem.* **2009**, *1*, 156.
12. Takada, T.; Kawai, K.; Fujitsuka, M.; Majima, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14002.
13. Behrens, C.; Burgdorf, L. T.; Schwogler, A.; Carell, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 1763.
14. Ito, T.; Rokita, S. E. *J. Am. Chem. Soc.* **2004**, *126*, 15552.
15. Ito, T.; Rokita, S. E. *J. Am. Chem. Soc.* **2003**, *125*, 11480.
16. Behrens, C.; Carell, T. *Chem. Commun.* **2003**, 1632.
17. Cichon, M. K.; Haas, C. H.; Grolle, F.; Mees, A.; Carell, T. *J. Am. Chem. Soc.* **2002**, *124*, 13984.
18. Elias, B.; Shao, F.; Barton, J. K. *J. Am. Chem. Soc.* **2008**, *130*, 1152.
19. Wagner, C.; Wagenknecht, H. A. *Chem. Eur. J.* **2005**, *11*, 1871.
20. Shao, F.; Barton, J. K. *J. Am. Chem. Soc.* **2007**, *129*, 14733.
21. Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541.
22. Steenken, S.; Telo, J. P.; Novais, H. M.; Candeias, L. P. *J. Am. Chem. Soc.* **1992**, *114*, 4701.
23. Maie, K.; Miyagi, K.; Takada, T.; Nakamura, M.; Yamana, K. *J. Am. Chem. Soc.* **2009**, *131*, 13188.
24. Daublain, P.; Thazhathveetil, A. K.; Wang, Q.; Trifonov, A.; Fiebig, T.; Lewis, F. D. *J. Am. Chem. Soc.* **2009**, *131*, 16790.
25. Kaden, P.; Mayer-Enthart, E.; Trifonov, A.; Fiebig, T.; Wagenknecht, H. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1636.
26. Tashiro, R.; Ohtsuki, A.; Sugiyama, H. *J. Am. Chem. Soc.* **2010**, *132*, 14361.
27. Sugiyama, H.; Tsutsumi, Y.; Saito, I. *J. Am. Chem. Soc.* **1990**, *112*, 6720.
28. Chen, T.; Cook, G. P.; Koppisch, A. T.; Greenberg, M. M. *J. Am. Chem. Soc.* **2000**, *122*, 3861.
29. Nakamura, M.; Fukunaga, Y.; Sasa, K.; Ohtoshi, Y.; Kanaori, K.; Hayashi, H.; Nakano, H.; Yamana, K. *Nucleic Acids Res.* **2005**, *33*, 5887.
30. Takada, T.; Kawai, K.; Cai, X. C.; Sugimoto, A.; Fujitsuka, M.; Majima, T. *J. Am. Chem. Soc.* **2004**, *126*, 1125.
31. Lewis, F. D.; Daublain, P.; Cohen, B.; Vura-Weis, J.; Shafirovich, V.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2007**, *129*, 15130.
32. Odom, D. T.; Barton, J. K. *Biochemistry* **2001**, *40*, 8727.
33. O'Neill, M. A.; Barton, J K. *J. Am. Chem. Soc.* **2002**, *124*, 13053.
34. Sartor, V.; Henderson, P. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 11027.
35. Kawai, K.; Osakada, Y.; Sugimoto, A.; Fujitsuka, M.; Majima, T. *Chem. Eur. J.* **2007**, *13*, 2386.
36. Yamana, K.; Iwase, R.; Furutani, S.; Tsuchida, H.; Zako, H.; Yamaoka, T.; Murakami, A. *Nucleic Acids Res.* **1999**, *27*, 2387.
37. Yamana, K.; Zako, H.; Asazuma, K.; Iwase, R.; Nakano, H.; Murakami, A. *Angew. Chem., Int. Ed.* **2001**, *40*, 1104.