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RNA-Mediated Electron Transfer: Double Exponential Distance Dependence

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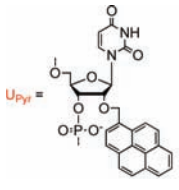
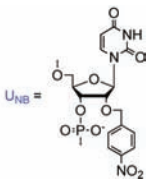
DNA-mediated charge transfer (CT) has attracted considerable interest, because of its potential applications in nanoscale devices. Both positive and negative charges injected into DNA bases can move through DNA over significant distances, where the nature of the charge donor and acceptor and the structural dynamics of DNA significantly affect DNA-mediated CT.[1] In addition, the intervening base sequences and the integrity of the base stacks have been shown to be crucial factors that modulate the DNA-mediated CT, which led to electrochemical devices that detect the perturbations of DNA stacks such as a mismatch base pair.[2] Recently, it has been found that the photoinduced charge migration in DNA possessing a donor−acceptor pair has resulted in the long-lived charge separated state.[3] This finding should offer important insights for the development of photoenergy conversion devices such as solar cells.

In contrast to the DNA-mediated CT, little attention has been paid to CT in an RNA duplex. However, RNA duplexes have base stacking overlaps and dynamics that are significantly different from those of DNA−DNA as well as of DNA−RNA duplexes.[4] RNA duplexes are therefore another choice of attractive medium for CT. In this report, we describe the first example of electron transfer in RNA duplexes consisting of a pendant donor and acceptor pair. An important finding is that long-range excess electron transfer occurs through RNA π-stacks with double exponential distance dependence. This new finding will contribute to uncover the mechanism of RNA-mediated electron transfer and open the way for development of RNA-based devices that conduct electron migrations.[5]

Experimental design for our study of charge injection and transport in RNA involves the use of continuing $(\mathrm{rA})_n-(\mathrm{U})_n$ sequences as a bridge, pyrene (Pyr) as a photoexcitable electron donor, and nitrobenzene (NB) as an electron acceptor. Pyr and NB were site-specifically introduced into the 2′-*O*-position of the U-strand with one carbon linker. We previously showed that 2′-*O*-pyrene-modified RNA duplexes exhibit strong fluorescence while retaining the A-form conformation and the thermal stability similarly to those of unmodified RNAs.[6] Since the free energies for electron transfer from excited-state pyrene (Pyr*) to uracil base and from Pyr* to NB can be estimated to be ca. −0.5 and −1.09 eV,[7] respectively, the electron injection and the subsequent electron transfer processes are expected to be exergonic (Scheme 1). The photoexcitable and energetic properties of Pyr-NB-modified $(\mathrm{rA})_n-(\mathrm{U})_n$ duplexes would permit us to study the electron transfer between the donor and the acceptor by a simple fluorescence quenching technique.





Scheme 1. Schematic Diagram of Electron Transfer between Pyr and NB on RNA

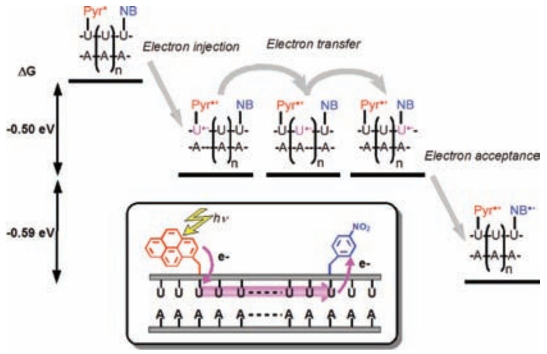


Table 1. Melting Temperatures (T_m) and Fluorescence Quantum Yields (Φ) of Oligoribonucleotide Duplexes

Duplexes with rA$_{20}$	T_m / °C[a]	Φ[b]
P0: 5′-r$\mathbf{U}_{19}U$-3′	47.1	
P5: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathbf{U}_{14}U$-3′	48.1	0.152
NB10: 5′-r$\mathbf{U}_{9}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{9}U$-3′	41.0	
P5NB6: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{13}U$-3′	43.9	0.008
P5NB7: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathbf{U}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{12}U$-3′	40.0	0.022
P5NB8: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathbf{UU}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{11}U$-3′	42.0	0.075
P5NB10: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathbf{UUUU}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{9}U$-3′	42.1	0.104
P5NB12: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathbf{UUUUUU}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{7}U$-3′	42.1	0.117
P5NB15: 5′-r$\mathbf{U}_{4}\mathrm{U}_{\mathrm{Pyr}}\mathbf{UUUUUUUUU}\mathrm{U}_{\mathrm{NB}}\mathbf{U}_{4}U$-3′	43.2	0.123

[a] Melting temperatures at 260 nm were determined for duplexes (2.5 μM) in a pH 7 phosphate buffer containing 0.1 M NaCl and 0.001 M EDTA. [b] Fluorescence quantum yields of the Pyr-NB-modified $\mathrm{rA}_{20}-\mathrm{U}_{20}$ duplexes were determined based on quinine sulfate ($\Phi = 0.55$) in 0.1 N H_2SO_4 as a standard. **U** and *U* are 2′-*O*-methyluridine and uridine, respectively.

The sequences of Pyr−NB-modified 20-mer $\mathrm{rA}_{20}-\mathrm{U}_{20}$ and T_m and quantum yields (Φs) of the duplexes are summarized in Table 1. Figure 1a shows the steady state fluorescence spectra for **P5** and **P5NB*n*** (where $n = 6$, 7, 8, 10, 12, or 15 position) measured in a pH 7 buffer under the identical duplex concentration (2.5 μM). All the Pyr-NB-modified duplexes (**P5NB*n***) exhibited the pyrene monomer fluorescence originated from the pyrenyl $^1(\pi, \pi^*)$ excited state that is similar to that from the single pyrene-modified duplex (**P5**). Notably, no emission from other excited products such as charge transfer complex $[\mathrm{Pyr}^{\bullet+}-\mathrm{U}^{\bullet-}]$ was observed. The quantum yields of **P5NB*n*** were substantially low ($\Phi = 0.008-0.123$) when compared with that ($\Phi_0 = 0.152$) of **P5**. As a result of the steady state fluorescence and under the assumption that the electron injection and the electron acceptance are the same in all RNA duplexes, the observed difference in Φ directly reflects differences in the efficiency of the electron transfer from the donor Pyr* to the nitrobenzene acceptor.

Figure 1b indicates the plot of $\ln[(\Phi_0/\Phi) - 1]$ against the bridge distance[4a] between the donor and the acceptor. We obtain dual

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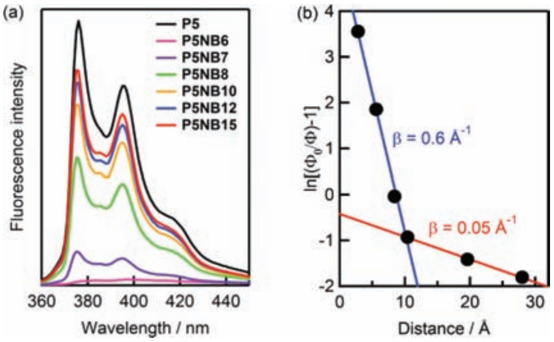

Figure 1. (a) Fluorescence spectra ($\lambda_{ex} = 345$ nm) of Pyr−NB-modified rA$_{20}$−U$_{20}$ (2.5 μM) measured at room temperature (22 °C) in the same buffer used for the melting studies. (b) Distance dependence of electron transfer between Pyr and NB on the RNA.

straight lines. Therefore, the distance dependence of the electron transfer rate between the pyrene donor and nitrobenzene acceptor separated by rA−U bridges of various lengths has the following double exponential form, which gave two β values of 0.6 Å^{-1} for the short distances (<10 Å) and 0.05 Å^{-1} for the long distances (>10 Å).

$$k(r) = k_0[\exp(-\beta_1 r) + \mathrm{C}\exp(-\beta_2 r)],\ \beta_1 = 0.6\ \text{Å}^{-1},\ \beta_2 = 0.05\ \text{Å}^{-1}$$

The T_m data and absorption and CD spectral observations (Supporting Information) indicate the apparent lack of the ground-state interactions between the donor, the bridge, and the acceptor. All the doubly modified RNA duplexes **P5NB*n*** showed the T_m values that are lowered by 3−7 °C when compared with that of **P0**. The T_m data strongly support that little stacking interactions of the Pyr and NB groups with the nucleotide bases in the duplexes occur. **P5NB*n***s exhibit the CD profiles that are essentially similar to those of **P0** and **P5**. Little or no induced CD was observed for **P5** and **P5NB*n*** at around the pyrene absorption (300−360 nm). The induced CD data are therefore consistent with the fact that the Pyr group does not strongly interact with the neighboring bases. Importantly, the lack of induced CD observed for **P5NB6** strongly suggests that the ground-state electronic interactions between the Pyr and the neighboring NB do not occur in the duplex. The shapes and absorption maxima of the UV−vis spectra of **P5NB*n*** are very close to those of **P5**. This is indicative of the ground-state electronic interactions between the Pyr and the NB not occurring even in the nearest neighbor modification (**P5NB6**), which is consistent with the CD spectral observation.

Notably, there are differences between our RNA-mediated electron transfer and the charge transfer in DNA, although both charge transfer studies have used pyrene as an electron donor. In sharp contrast to the 2′-*O*-Pyr-NB-modified RNA, the modified DNA duplexes possessing a 5-pyrenylethynyl group at the 5-position of the uracil base (5-Pyr-U) and 5-halouracil (5-XU) exhibited the exciplex emission at ~460 nm due to the charge transfer product [Pyr$^{\bullet+}$−U$^{\bullet-}$].[8] The quantum yields of the exciplex emission do not depend on the 5-XU trap location, which is indicative of the excess electron in the charge transfer state in DNA being restrained from hopping to the nearby 5-XU trap. Because our RNA system contains an energetically stronger electron acceptor than 5-XU,[7] highly efficient electron transfer up to ~28 Å can occur between a pendant pyrene donor and a nitrobenzene acceptor through (rA)$_n$−(U)$_n$ bridges in spite of little ground-state electronic interactions between the donor, the bridge, and the acceptor. In contrast to the Pyr-modified rA$_{20}$−U$_{20}$ (**P5**), the quantum yield of the single pyrene modified dA$_{20}$−T$_{20}$ was very low (0.0028) due to the pyrene intercalation as expected from our previous findings.[6] Nevertheless, fluorescence quenching occurs in doubly modified Pyr−NB−dA$_{20}$−T$_{20}$, which was dependent on the distance of the (dA)$_n$−(T)$_n$ bridge.

The double exponential distance dependence has been obtained in the charge recombination in DNA hairpins possessing the stilbene donor and acceptor pair with two β values of 0.97 and 0.42 Å^{-1},[3a] where the superexchange mechanism at shorter distances and the crossover from the superexchange to the hopping mechanisms at longer distances are involved. Small β values ranging from 0.10 to 0.3 Å^{-1} have been reported for the long-range excess electron transfer in DNA.[9] In our electron transfer (ET) system through the RNA π-stacks, the β-value 0.6 Å^{-1} at short distances is consistent with the single-step electron transfer. The small β-value 0.05 Å^{-1} for the RNA-mediated ET at long distances shows the possibility of electron hopping. The local structural dynamics may play some role in the ET.

We have shown for the first time that an excess electron can move through RNA π-stacks over a significant distance with dual distance dependence. The results are important in designs of fluorescent RNA probes as well as RNA-based devices.

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Supporting Information Available: Experimental details, and the data for electron transfer between pyrene and nitrobenzene through rA$_{20}$-U$_{20}$ at high salt conditions and through dA$_{20}$-T$_{20}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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