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Hole Transfer in LNA and 5-Me-2′-deoxyzebularine-Modified DNA

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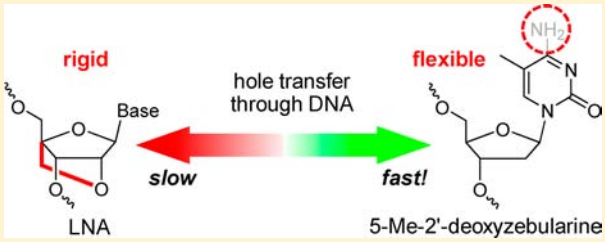
ABSTRACT: We report the measurement of hole-transfer rate constants (k_{ht}) in locked nucleic acid (LNA) and 5-Me-2′-deoxyzebularine (**B**)-modified DNA. LNA modification, which makes DNA more rigid, caused a decrease of more than 2 orders of magnitude in k_{ht}, whereas **B** modification, which increases DNA flexibility, increased k_{ht} by more than 20-fold. The present results clearly showed that hole-transfer efficiency in DNA can be increased by increasing DNA flexibility.



INTRODUCTION

The DNA double helix mediates a hole (a positive charge) transfer of over 10 nm.[1−3] This renders DNA an interesting bottom-up material for the design of nanoelectronic sensors and devices. The hole-transfer process in DNA has therefore attracted wide attention.[4−9] However, extensive studies conducted in the past decade clearly show that the hole transfer in DNA is strongly influenced by the DNA sequence[10−12] and that the hole transfer in natural DNA is inherently slow except for special sequences, such as poly(dA) and poly(dG),[13−15] which limits the potential applications of duplex DNA as a conducting molecule in molecular-scale devices.

Several factors affect the hole-transfer dynamics in DNA. Gel electrophoresis strand cleavage experiments,[16] conductivity measurements,[17−20] and theoretical calculations[21,22] of DNA and PNA show that hole-transfer efficiency is sensitive to the HOMO level of nucleobases. We recently reported that the hole-transfer rate (k_{ht}) depends strongly on the HOMO energy gap between the bases and can be finely tuned over three orders of magnitude by varying the HOMO energy of the bases.[23−26] Okamoto et al. demonstrated that the increase in the π-stacking surface area between neighboring bases increases the charge-transfer efficiency.[4] The k_{ht} is thought to be controlled by the frequency of occurrence of DNA conformations that are particularly amenable to hole transfer.[27−34] Changes in the molecular conformation due to thermal fluctuations, which take place over a wide range of time scales (fs−μs), can significantly alter the base-stacking between neighboring bases and, in turn, the k_{ht}. Lewis and co-workers depicted that the higher mobility of 7-deazaadenine may partially account for the observed faster k_{ht} between 7-deazaadenines compared to that between Gs or As.[35]

In the present study, to further investigate the influence of the π-stacking surface area between neighboring bases and the local conformational flexibility of DNA on the hole-transfer dynamics, we synthesized DNA modified with locked nucleic acid (LNA: G_L, C_L, A_L, T_L) and 5-Me-2′-deoxyzebularine (**B**) (Figure 1). LNA is a structurally rigid bicyclic nucleotide,[36] in which the ribose moiety is modified with an extra bridge connecting the 2′ oxygen and 4′ carbon, locking the ribose in the C3′-endo conformation. X-ray analysis clearly showed that LNA confers a more efficient base stacking than natural B-DNA due to the smaller helical twist.[37] Bartik and co-workers determined the base-pair lifetime in the LNA/DNA hybrid by measuring the imino proton exchange time.[38] The base-pair

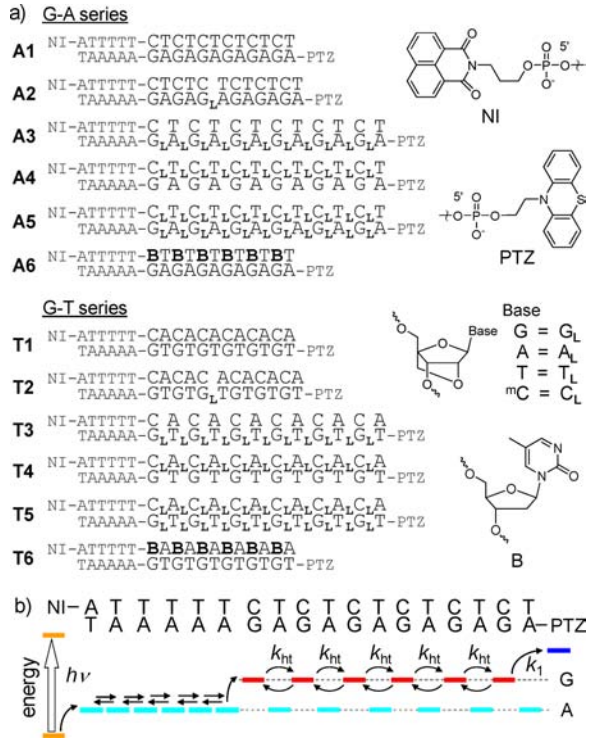


Figure 1. (a) Chemical structure of LNA and **B**, photosensitizer NI, hole-acceptor PTZ, and DNA sequences used in this study. (b) A schematic representation of charge injection by the hole transfer between As and sequential hole transfer between Gs followed by trapping at the hole acceptor PTZ.

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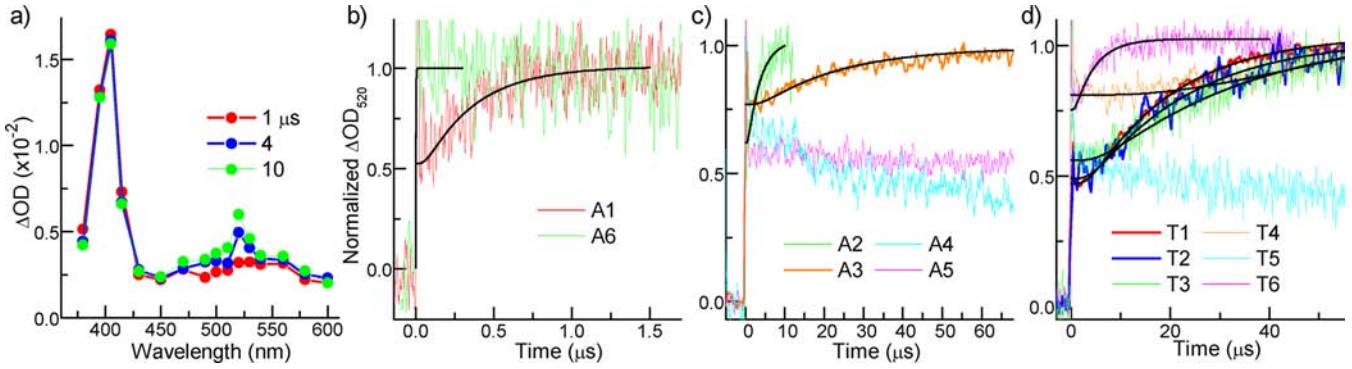

Figure 2. (a) The transient absorption spectrum of **A2**. Time profiles of the transient absorption of $PTZ^{\bullet+}$ monitored at 520 nm during the laser flash photolysis of (b,c) G-A and (d) G-T repeating sequences. The smooth black curves are the fit derived from the kinetic model using k_{ht} values depicted in Table 1.

lifetime of the G-C pairs in the LNA/DNA hybrid are larger than those of the corresponding pair in the DNA/DNA duplex, demonstrating that base-pair breathing rates decrease upon LNA modifications. **B** is an analog of C that forms a reduced number of hydrogen bonds between G. As the lifetime of the A-T pair with two hydrogen bonds is smaller than those of the G-C pair with three hydrogen bonds,[38] replacement of C with **B** would lead to faster breathing of base pair and thus increases DNA flexibility. To our surprise, despite the increase in the π-stacking surface area between neighboring bases, LNA modification completely suppressed the charge transfer through DNA in the time range of <100 μs. On the other hand, replacement of C with **B** significantly increased k_{ht}. These results further confirmed that the local conformational motion of DNA plays a key role in charge transfer in DNA, clearly showing that k_{ht} can be increased by increasing DNA flexibility.

EXPERIMENTAL SECTION

DNA Synthesis. Cyanoethyl phosphoramidite of *N*-(3-hydroxypropyl)-1,8-naphthalimide and 10-(2-hydroxyethyl)phenothiazine were synthesized as previously reported.[39−42] LNA and 5-Me-2′-deoxyzebularine-modified DNA were purchased from Gene Design Inc. All other reagents for DNA synthesis were purchased from Glen Research. DNA was synthesized on an Applied Biosystems DNA synthesizer and purified by reverse phase HPLC and lyophilized. All of the DNA studied here were characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOFF) mass spectra, and their concentrations were determined by complete digestion with nuclease P1 and AP to 2′-deoxyribonucleosides. Duplex solutions (DNA 80 μM, 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM sodium phosphate buffer (pH 7.0)) were prepared by mixing equimolar amounts of the desired DNA complements and gradually annealing with cooling from 80 °C to room temperature.

Melting Temperature Measurements. The thermal denaturation profile was recorded on a JASCO V-530 spectrometer equipped with a Peltier temperature controller (ETC-505T). The absorbance of the DNA sample (at a strand concentration of 2 μM in 100 mM NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate (pH 7.0)) was monitored at 260 nm from 15 to 90 °C with a heating rate of 1 °C/min. The T_m value was determined as the maximum in a plot of $\Delta A_{260}/\Delta T$ versus temperature.

Laser Flash Photolysis. The nanosecond transient absorption measurements were performed using the laser flash photolysis technique.[2,23−26] Briefly, the third-harmonic oscillation (355 nm, fwhm of 4 ns, 8−12 mJ/pulse) from a Q-switched Nd:YAG laser (Continuum, Surelight) was used for the excitation light, which was expanded to a 1 cm diameter. The light from a xenon flash lamp (Osram, XBO-450) was focused into the sample solution for the transient absorption measurement. Time profiles of the transient absorption in the UV−vis region were measured with a monochromator (Nikon, G250) equipped with a photomultiplier (Hamamatsu Photonics, R928) and digital oscilloscope (Tektronics, TDS-580D). The time profiles of temperature-dependence study were measured by using a JASCO Peltier temperature-controlled cell holder (EHC-716). The time profiles were obtained from the average of 32 laser shots.

Kinetic Modeling. The rate constants of the single-step hole transfer between Gs (k_{ht}) were determined from the kinetic modeling.[11,26] Analysis of time profiles based on the multistep hopping mechanism was performed with numerical analysis by using Matlab software. Kinetic model of multistep hole transfer process is shown in Figure 1b. Charge recombination process can be ignored because the charge separated state persists over several hundred microseconds when naphthalimide (NI) and the nearest G are separated by six A-T base pairs which works as an insulator.[43,44] According to Figure 1b, simultaneous differential equations are described as eq 1, where $[G_i\ (i = 1-6)]$ corresponds to the hole population at each G site, k_{ht} is hole transfer rate constants between Gs, and k_1 is hole transfer from $G_6^{\bullet+}$ to PTZ.

$$\begin{aligned}
\frac{d[G_1]}{dt} &= -k_{ht}[G_1] + k_{ht}[G_2] \\
\frac{d[G_2]}{dt} &= k_{ht}[G_1] - 2k_{ht}[G_2] + k_{ht}[G_3] \\
&\vdots \\
\frac{d[G_6]}{dt} &= k_{ht}[G_5] - (k_{ht} + k_1)[G_6] \\
\frac{d[PTZ]}{dt} &= k_1[G_6]
\end{aligned} \tag{1}$$

RESULTS AND DISCUSSION

The k_{ht} through the LNA and **B**-modified DNA was determined by nanosecond time-resolved transient absorption measurements as previously reported.[2,23−26] The photosensitizer NI was attached to six consecutive A-T base pairs at one end of the duplex in order to inject a hole onto the G nearest the NI via rapid hole transfer between As upon laser irradiation (<10 ns). Once a hole is trapped at G far from $NI^{\bullet-}$, charge recombination mainly proceeds by a strongly distance-dependent superexchange mechanism, thus forming a long-lived charge-separated state (>100 μs). The hole transfer through the DNA by sequential hole transfer between Gs was monitored by the formation of the radical cation of the hole acceptor PTZ ($PTZ^{\bullet+}$) attached at the other end of DNA, which shows a peak at around 520 nm (Figure 2).[2,23−26] The

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rate constant of each hole-transfer step was determined based on kinetic modeling. Two types of sequences, G-A (**An**: n = 1−6) and G-T (**Tn**: n = 1−6) repeating sequences, were investigated in which a hole moved faster through G-A repeating sequence **A1** than through G-T repeating sequence **T1** due to the closer HOMO level between G and A compared with that between G and T.[26] The LNA modification resulted in a significant drop in k_{ht}, and hole transfer was completely suppressed when all the nucleotides in the G-A or G-T repeating region were modified with LNA ($k_{ht} < 10^5$ s^{-1}). In sharp contrast, the replacement of C with **B** resulted in a significant increase in k_{ht}, and in the case of **A6**, the hole transfer occurred faster than the time resolution of our setup ($k_{ht} > 10^9$ s^{-1}) close to the limit of the natural sequences, i.e., k_{ht} in poly(dA) and ply(dG) sequences (1.2×10^9 and 4.3×10^9 s^{-1}, respectively).[15] Thus, in the case of G-A repeating sequences, LNA modification resulted in a k_{ht} decrease of more than 2 orders of magnitude, whereas **B** modification caused more than a 20-fold increase in k_{ht}.

O'Neill and Barton reported that complete freezing of the conformational motion of DNA bases at 77K results in the strong suppression of photo-induced electron-transfer quenching of photoexcited 2-aminopurine.[29] The present results further demonstrated that restriction of the DNA backbone results in a significant drop in the charge-transfer efficiency of DNA. The temperature dependence of k_{ht} was measured

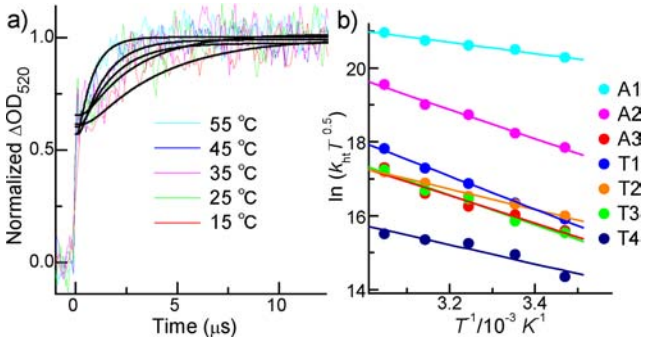

Figure 3. (a) Time profiles of the transient absorption of $PTZ^{\bullet+}$ monitored at 520 nm during the 355 nm laser flash photolysis of **A2** at various temperatures. (b) Plots of ln ($k_{ht}T^{0.5}$) versus T^{-1}.

(Figure 3) and the activation energy (E_a) was determined using the conventional Arrhenius model:

$$k_{ht}\sqrt{T} = A\exp\left(-\frac{E_a}{k_B T}\right) \quad (2)$$

where A is a preexponential factor. In the G-A sequences, the LNA modification resulted in an increase of the E_a, suggesting that greater DNA conformational motion is required for LNA-modified DNAs to adopt a conformation amenable to hole transfer (Table 1). On the other hand, LNA modification in the G-T sequences resulted in a decrease in the E_a value. The hole transfer occurs more than 50-fold slower in the G-T repeat sequence **T1** compared with that in the G-A repeat sequence **A1**. The alignment of the DNA bases of the G-T repeat sequence in B-DNA (**T1**) may be in conformation that has lower hole-transfer activity compared with that in the LNA/DNA hybrid (**T2**−**4**). Thus, LNA modification may result in a decrease in E_a. This is consistent with the finding that single

Table 1. Hole-Transfer Rate (k_{ht}), Activation Energy (E_a), and the UV Melting Temperature (T_m)

DNA	k_{ht} (s^{-1})[a]	E_a (eV)[b]	T_m (°C)
A1	3.8×10^7	0.17	59
A2	3.8×10^6	0.34	65
A3	6.0×10^5	0.32	78
A4	$<1 \times 10^5$	—	79
A5	$<1 \times 10^5$	—	77
A6	$>1 \times 10^9$	—	33
T1	7.0×10^5	0.40	65
T2	6.0×10^5	0.24	69
T3	4.0×10^5	0.35	80
T4	1.0×10^5	0.23	81
T5	$<1 \times 10^5$	—	81
T6	3.0×10^6	—	33

[a]Rate constants were obtained from the kinetic modeling at 22 °C. [b]Calculated according to eq 2 using the k_{ht} values measured at various temperatures.

LNA modification caused a 10-fold decrease in k_{ht} in the G-A sequence (**A2**), while it only slightly affected k_{ht} in the G-T sequence (**T2**). Otherwise, the results may suggest that the conformational motion required to take the optimum hole-transfer active conformation differs between the G-A and G-T sequences. The effects of LNA modification on k_{ht} and E_a values were more pronounced with top-strand modification in both G-A and G-T sequences. These results further shed light on the importance of local rather than global DNA conformational motion on the hole-transfer dynamics in DNA.

■ CONCLUSIONS

In summary, an increase in the rigidity of DNA by LNA modification resulted in the suppression of hole transfer, whereas the replacement of C with **B**, which increases the flexibility of base pairing, resulted in a significant increase in hole-transfer efficiency. These results further supported the notion that hole transfer in DNA is a conformationally gated process and that charge-transfer dynamics in peptide that take place under similar physiological conditions are regulated by the dynamics of the conformational changes of peptides.[45,46] The present results clearly demonstrated that hole-transfer efficiency in DNA can be increased by increasing DNA flexibility by weakening the base pair association, which may help in the design of artificial bases of those exhibits with higher hole-transport properties than those of natural bases.

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Notes

The authors declare no competing financial interest.

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