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Hole Transfer Kinetics of DNA

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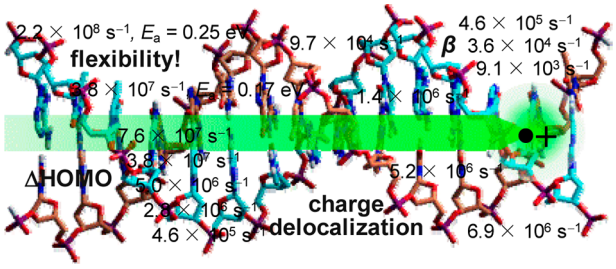

Not long after the discovery of the double-helical structure of DNA in 1952, researchers proposed that charge transfer along a one-dimensional π-array of nucleobases might be possible. At the end of the 1990s researchers discovered that a positive charge (a hole) generated in DNA migrates more than 200 Å along the structure, a discovery that ignited interest in the charge-transfer process in DNA. As a result, DNA became an interesting potential bottom-up material for constructing nanoelectronic sensors and devices because DNA can form various complex two-dimensional and three-dimensional structures, such as smiley faces and cubes. From the fundamental aspects of the hole transfer process, DNA is one of the most well-studied organic molecules with many reports on the synthesis of artificial nucleobase analogues. Thus, DNA offers a unique system to study how factors such as the HOMO energy and molecular flexibility affect hole transfer kinetics.

Understanding the hole transfer mechanism requires a discussion of the hole transfer rate constants (k_{HT}). This Account reviews the k_{HT} values determined by our group and by Lewis and Wasielewski's group, obtained by a combination of the synthesis of modified DNA and time-resolved spectroscopy. DNA consists of G/C and A/T base pairs; the HOMO localizes on the purine bases G and A, and G has a lower oxidation potential and a higher energy HOMO. Typically, long-range hole transfer proceeded via sequential hole transfer between G/C's. The kinetics of this process in DNA sequences, including those with mismatches, is reproducible via kinetic modeling using the determined k_{HT} for each hole transfer step between G/C's. We also determined the distance dependence parameter (β), which describes the steepness of the exponential decrease of k_{HT}. Because of this value, >0.6 Å^{-1} for hole transfer in DNA, DNA itself does not serve as a molecular wire. Interestingly, hole transfer proceeded exceptionally fast for some sequences in which G/C's are located close to each other, an observation that we cannot explain by a simple sequential hole transfer between G/C's but rather through hole delocalization over the nucleobases.

To further investigate and refine the factors that affect k_{HT}, we examined various artificial nucleobases. We clearly demonstrated that k_{HT} depends strongly on the HOMO energy gap between the bases (Δ_{HOMO}), and that k_{HT} can be increased with decreasing Δ_{HOMO}. We reduced Δ_{HOMO} between the two type of base pairs by replacing adenines (A's) with deazaadenines (^{z}A's) or diaminopurines (D's) and showed that the hole transfer rate through the G/C and A/T mix sequence increased by more than 3 orders of magnitude. We also investigated how DNA flexibility affects k_{HT}. Locked nucleic acid (LNA) modification, which makes DNA more rigid, lowered k_{HT} by more than 2 orders of magnitude. On the other hand, 5-Me-2′-deoxyzebularine (B) modification, which increases DNA flexibility, increased k_{HT} by more than 1 order of magnitude. These new insights in hole transfer kinetics obtained from modified DNAs may aid in the design of new molecular-scale conducting materials.

Introduction

Duplex DNA forms a beautiful one-dimensional π-stacked array of base pairs. This array presents a unique structure for the investigation of charge transfer processes. Besides its function in the storage of genetic information, DNA can be used to construct various nanometer-sized two- and three-dimensional nanostructures and thus has the potential as a bottom-up material for electronic circuits. Hence, the mechanism underlying charge transfer through DNA has been extensively studied both experimentally and theoretically

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in the past two decades. From a chemistry point of view, the goal in understanding the charge transfer mechanism is to explain the charge transfer processes based on the charge transfer rate constants, which may vary depending on the DNA sequence. The kinetics of the charge transfer process in DNA was examined by using a synthetic DNA site specifically modified with a fluorophore or a photosensitizer, which can inject a charge at the defined site of DNA upon photoexcitation. The initial reports on the kinetics of charge transfer in DNA dealt with the photoinduced charge separation process between a fluorophore or a photosensitizer (electron acceptor) and guanine (G, electron donor), which exhibits the highest HOMO level (i.e., the lowest oxidation potential) among the four nucleobases.[1] The rate constant for the charge transfer process (k_{CT}) usually follows an exponential dependence on the donor−acceptor distance Δr (eq 1), where β is a distance dependence parameter.

$$k_{CT} = k_0 \exp(-\beta \Delta r) \quad (1)$$

The charge separation rate can be accessed by measuring the fluorescence lifetime of the fluorophore.[2] To firmly establish the charge transfer mechanism, it is necessary to directly observe products of charge transfer, that is, radical ions, and this can be achieved by transient absorption measurements. Two groups, that of Lewis and Wasielewski and that of Barton and Zewail, reported consistent β values of 0.6−0.7 Å^{-1} for the charge separation process in DNA.[3,4] β-values are demonstrated to depend primarily on the nature of the mediating molecules, the so-called bridge, between the donor and the acceptor.[5] For example, β-values of 1.0−1.3 Å^{-1} have been reported for protein-bridged systems, larger values were obtained for solvent water (1.6 Å^{-1}), and much smaller values (<0.1 Å^{-1}) were reported for a *p*-phenylenevinylene bridge, suggesting a molecular-wire-like behavior.[6] It was suggested that DNA can be a more effective medium for charge transfer than proteins but is far less effective than fully conjugated bridges.

The k_{CT} determined for charge separation are essentially affected by the characteristics of the used photosensitizer, such as singlet excitation energy, redox potential, and its orientation within the DNA. In order to determine the intrinsic charge transfer kinetics of DNA, it is crucial to assess the charge transfer rate between nucleobases, i.e., the charge transfer processes in which both the donor and acceptor are nucleobases. At the end of the 1990s, polyacrylamide gel electrophoresis (PAGE) strand cleavage experiments demonstrated that a positive charge (a hole) generated in DNA can migrate along DNA over 200 Å, more than 60 base pairs.[7,8] The nucleobase oxidative products are generated far away from the initial hole injection site where DNA can be cleaved upon appropriate treatment, which can be visualized during PAGE analysis. Oxidation of DNA leads to the exclusive formation of G oxidative products forming distance dependent G-cleavage bands. Hence, a hole was suggested to migrate along DNA as a consequence of multistep hole transfer between G's. PAGE analysis has provided many important fundamental insights into the mechanism underlying hole transfer between nucleobases, such as the sequence and mismatch dependence of relative hole transfer efficiency. However, the mechanism or kinetics of the formation of G oxidative products, which serve as the hallmarks of long-range hole transfer in DNA, is very complicated and not fully understood.[9,10] Thus, PAGE analysis does not provide the kinetics of hole transfer in DNA.

The hole transfer rate between nucleobases (k_{HT}) was first determined by Lewis and Wasielewski's group in 2000 for some special sequences; hole transfer between G and hole sink G-doublets, triplets, or deazaguanine.[11,12] In 2004, we established a system to measure a long-distance hole transfer in DNA over 100 Å which allowed us to determine the k_{HT} essentially in any kind of DNA sequence that had a k_{HT} over a wide range (10^3−10^9 s^{-1}).[13−16] Lewis and Wasielewski's group also measured k_{HT} between adjacent purine bases, G's, adenines (A's), and deazaadenines (^{z}A's), which was proven to be faster than 10^9 s^{-1}.[17,18] While our group determined that hole transfer processes proceed slower than 10^9 s^{-1}, Lewis and Wasielewski's group dealt mainly with processes faster than 10^9 s^{-1}, and thus, between these two groups, the whole kinetic range of k_{HT} in DNA was covered. A negative charge is also demonstrated to migrate along the DNA in a sequence-dependent manner, and our group is now investigating the kinetics.[19−21] Here, we focus on the well-established hole transfer kinetics in DNA and review the k_{HT} values of various sequences. We then discuss the factors that determine k_{HT} values in DNA.

Hole Transfer between Consecutive A's

To access the k_{HT} in DNA on the basis of transient absorption measurements, it is desirable to construct a synthetic DNA system in which k_{HT} can be clearly discriminated from the charge-separation and charge-recombination kinetics. This can be achieved by generating a long-distance charge-separated state with a lifetime long enough to not interfere with the charge recombination kinetics. We found that hole transfer between (A/T)s proceeds faster than 10^8 s^{-1},[22]

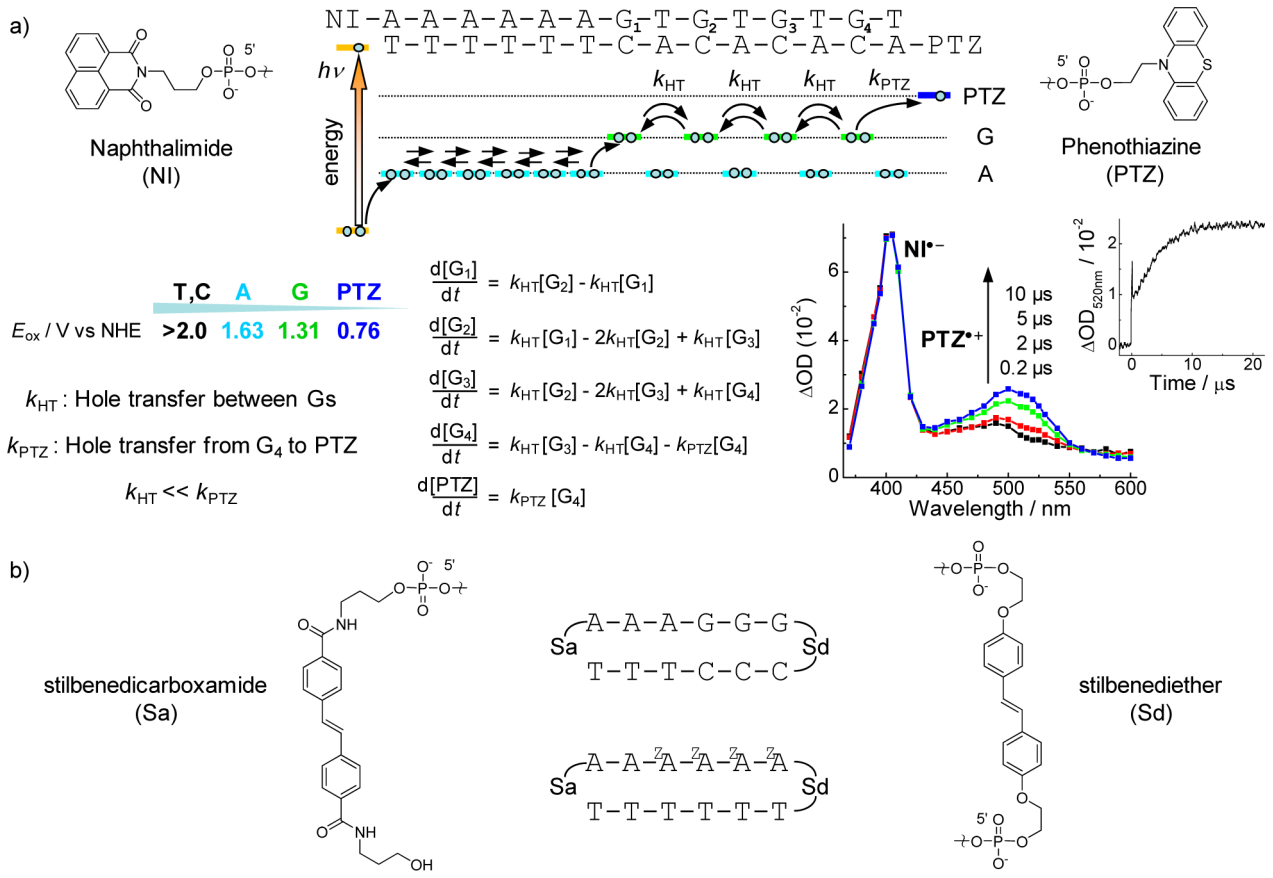

FIGURE 1. (a) Strategy for the kinetic study of long-distance hole transfer process. Schematic illustration for hole injection via hole transfer between A's and subsequent hole transfer between Gs to be trapped at PTZ. Numerical analysis of the formation of $PTZ^{\bullet+}$ was performed according to kinetic modeling using simultaneous differential equations. (b) System employed by Lewis and Wasielewski's group to measured the k_{HT} for adjacent purine bases.

which can compete with charge recombination to produce a long-lived (longer than microsecond) charge-separated state in DNA.[23] We utilized this fast hole transfer between consecutive (A/T)s to measure the k_{HT} between (G/C)s in various sequences, as described in the next section. Later, k_{HT} between adjacent (A/T)s was determined to be $1.2 \times 10^9\ s^{-1}$ by Lewis and Wasielewski.[17]

Multistep Hole Transfer between Gs

Our system for the measurement of k_{HT} is schematically depicted in Figure 1a. We attached naphthalimide (NI) as a photosensitizer at one end of DNA having six consecutive (A/T)s, and a hole acceptor phenothiazine (PTZ) at the other end of DNA having a lower oxidation potential than G. NI shows an absorption peak at a wavelength around 340 nm and can be selectively excited with a 355 nm laser pulse because neither DNA nor PTZ shows absorption at wavelengths longer than 350 nm. In addition, the PTZ radical cation ($PTZ^{\bullet+}$) shows absorption around 520 nm and almost no spectral overlap with $NI^{\bullet-}$, which has a peak at 400 nm. Thus, k_{HT} can be measured by monitoring the formation of the transient absorption of $PTZ^{\bullet+}$ at 520 nm. NI in the singlet excited state ($^1NI^*$) can oxidize both G/C and A/T, and thus, the flash excitation of NI triggers the electron transfer between $^1NI^*$ and the adjacent A to form $NI^{\bullet-}$ and $A^{\bullet+}$. A few percent of the hole escapes from the initial charge recombination via sequential hole transfer between A's, and the hole becomes trapped at G to form $G^{\bullet+}$. Due to the large distance separation between $NI^{\bullet-}$ and $G^{\bullet+}$, charge recombination proceeds very slowly ($<5 \times 10^3\ s^{-1}$) and thus allows the determination of k_{HT} in the range of 10^3–$10^9\ s^{-1}$. Once $G^{\bullet+}$ is formed, hole transfer is expected to occur between Gs and, when it comes up to the other end of the DNA, the hole is trapped at PTZ to form $PTZ^{\bullet+}$.[13] Based on a similar strategy, Lewis and Wasielewski's group measured the k_{HT} between adjacent purine bases by using two stilbene derivatives, which serve as a hole donor and acceptor at either end of a hairpin DNA (Figure 1b).[17,18]

We first tested our system for $(GT)_n$ and $(GA)_n$ sequences with different repeating numbers ($n = 2$–12). The formation

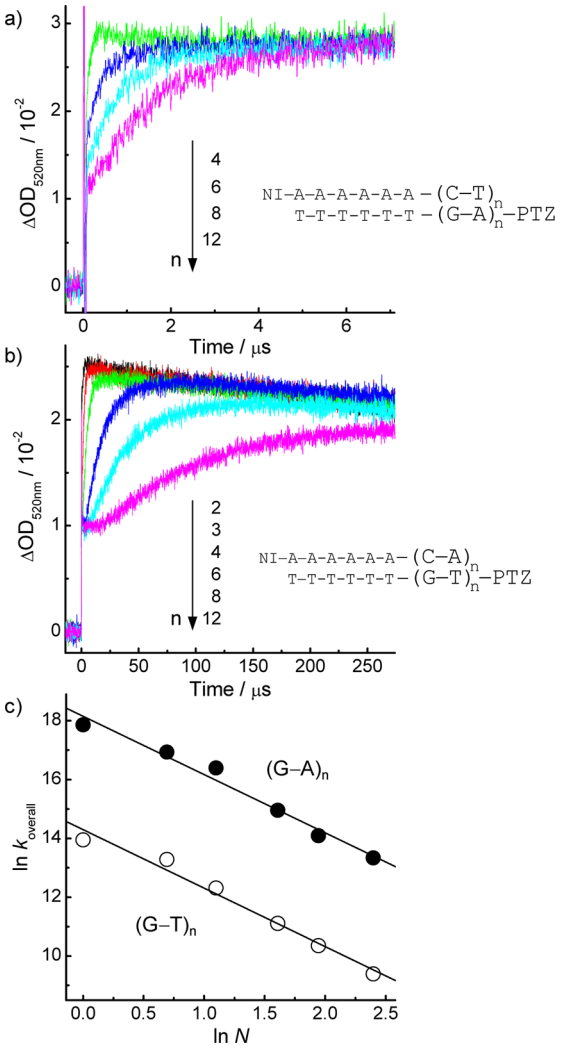

FIGURE 2. Time profiles of the transient absorption monitored at 520 nm assigned to $PTZ^{\bullet+}$ after 355 nm laser flash excitation for (a) $(G\text{-}A)_n$ and (b) $(G\text{-}T)_n$ sequences. (c) Plots of ln $k_{overall}$ versus ln N.[13]

rate of $PTZ^{\bullet+}$ for these sequences gradually decreased as the number of GA and GT repeats increased (Figure 2). Apparent overall hole transfer rate constants ($k_{overall}$), which correspond to the hole transfer rate from one end of DNA to the other for $(GT)_n$ and $(GA)_n$ sequences were obtained by single exponential fits of the formation of $PTZ^{\bullet+}$. If the hole transfer occurs by a random walk process via multistep hole transfer processes between G's, the observed $k_{overall}$ follows eq 2 reported by Jortner and co-workers,[24]

$$k_{overall} = k_{HT}N^{-\eta} \quad (2)$$

where N is a hopping number (= number of G's) and η is a proportional factor that takes the value of 2 in the ideal case of a random walk process.[24] The linear fit of the plots of ln $k_{overall}$ versus ln N gave an η-value of 2.0 ± 0.1 for both $(GT)_n$ and $(GA)_n$ sequences, providing kinetic

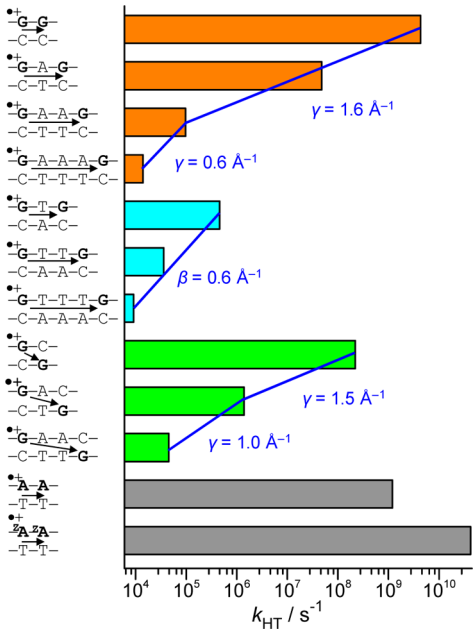

FIGURE 3. Hole transfer rate constants (k_{HT}) and the distance dependence parameter (β).[14−18] γ was used for DNA sequences described with two distance dependence parameters.

evidence that long-distance hole transfer occurs though the multistep hole transfer between G's, consistent with the results of the PAGE experiments.[13]

Distance Dependence of Hole Transfer between G's

As mentioned above, the formation of $PTZ^{\bullet+}$, which corresponds to the hole transfer from one end of DNA to the other, includes multiple hole transfer steps between G's. Based on the results of kinetic modeling, we determined the hole transfer rate constant of each individual hole transfer step between G's (k_{HT}). An example of kinetic modeling and the simultaneous differential equations are shown in Figure 1a, and the measured k_{HT} is summarized in Figure 3[13−16] together with those for adjacent A's, G's, and ^{Z}A's determined by Lewis and Wasielewski.[17,18]

The k_{HT} depended strongly on the sequence. We first discuss the effect of the distance between G/Cs on k_{HT}. The k_{HT} exponentially decreased with increasing distance between (G/C)s, showing that, in general, hole transfer proceeds by superexchange between Gs, and the distance dependence parameter were obtained for the G-A_n-G, G-T_n-G and G-A_n-C/C-T_n-G sequences.[14,15] A linear relationship was obtained for the G-T_n-G sequence ($n = 1-3$) giving a β-value of 0.6 $Å^{-1}$. In the case of the G-A_n-G sequence, while distance dependence parameter was obtained to be 0.6 $Å^{-1}$ for the plots for $n = 2-3$, a large deviation was observed for the plots for $n = 0-2$ sequences (1.6 $Å^{-1}$). The distance dependence parameter

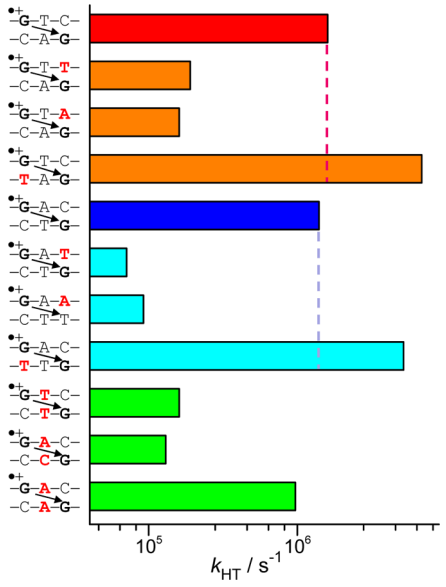

FIGURE 4. k_{HT} in mismatched DNA.[16]

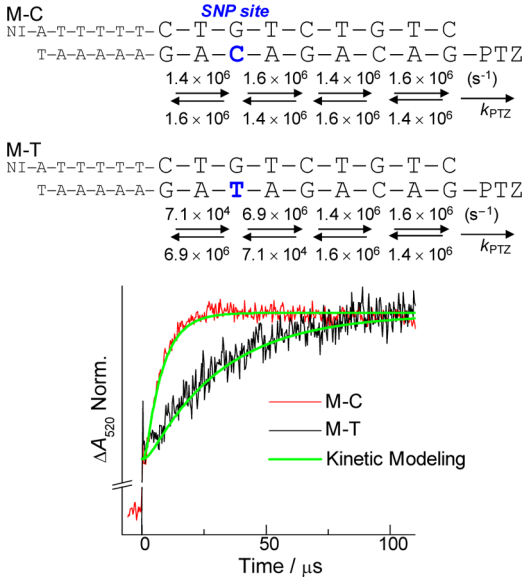

FIGURE 5. Discrimination of SNP by hole transfer kinetics through DNAs. Black and red curves represent time profiles of the transient absorption of $PTZ^{\bullet+}$ at 520 nm during the 355 nm laser flash photolysis, and the green smooth curves are those predicted from the kinetic model using the k_{HT} depicted in Figures 3 and 4.[16]

obtained for interstrand k_{HT} for G-A_n-C/C-T_n-G ($n = 1-2$: 1.0 $Å^{-1}$) was somewhat larger than that for intrastrand hole transfer, and again k_{HT} dropped considerably between $n = 0$ and 1 (1.5 $Å^{-1}$). These results suggest that hole transfer occurs especially fast when G's are located close to each other (G-G, G-A-G, and G-C/C-G), the mechanism of which cannot be explained by a simple superexchange regime. In these sequences, different mechanisms, such as charge delocalization over the bases, should be taken into account to explain the hole transfer kinetics (discussed below).

Giese and co-workers reported that when $(A/T)_n$ sequences between (G/C)s become long ($n \geq 4$), the hole transfer mechanism switches to the hopping mechanism in which A acts as a hole carrier, demonstrating that a hole can move across the $(A\text{-}T)_{16}$ bridge.[25] In our measurements, when the A-T stretch becomes long ($n \geq 4$), the k_{HT} becomes too slow to be measured ($k_{HT} < 5 \times 10^3$ s^{-1}), and thus, we cannot observe the occurrence of a crossover from superexchange to hopping. Shafirovich and co-workers reported that the irreversible reaction of a hole generated in duplex DNA occurs with a rate constant of about 5 s^{-1}.[9] It would be reasonable to infer that, while G-to-G hole transfer across a long (A/T) proceeds very slowly, the long lifetime of a hole in DNA (~0.2 s) allows the hole transfer from G/C to the $(A/T)_n$ stretch, resulting in the crossover of the mechanisms.

Mismatch Dependence of Hole Transfer

The π stacking between the base pairs is considered to be crucially important in the charge transfer processes through DNA, it was suggested that once the π stacking of the DNA bases is perturbed by the presence of a mismatch, the efficiency of charge transfer through the DNA would be significantly decreased. Therefore, the measurement of k_{HT} was expected to offer a unique approach to the detection of a mismatch, which would be especially effective in a long DNA enabling the SNP typing.[26]

The presence of a G/T or G/A mismatch slows the k_{HT} by about an order of magnitude (Figure 4).[16] Replacement of intervening A/T between (G/C)s with a T/T or A/C mismatch slows k_{HT}, while k_{HT} between (G/C)s across an A/A mismatch is similar to that across fully matched sequences. While the presence of an A/A mismatch perturbs the B-form double helix, the replacement of T with A may offer a better bridge between G's since k_{HT} depends strongly on the Δ_{HOMO}, as mentioned below. The k_{HT} from the mismatched G to a matched G is 4- to 5-fold faster than those in the fully matched sequences, which might be explained by its highly flexible nature (discussed below). These results indicate that k_{HT} in mismatched DNA sequences depends on the type of mismatch.

To test the kinetic discrimination of SNPs, k_{HT} was measured in sequences carrying the SNP rs12392145 C to T substitution. The theoretical lines obtained from the kinetic modeling using the k_{HT} described in Figures 3 and 4 correlate well with the experimental results (Figure 5), demonstrating that the hole transfer kinetics between Gs in DNA has been elucidated in considerable detail. These results clearly demonstrated that

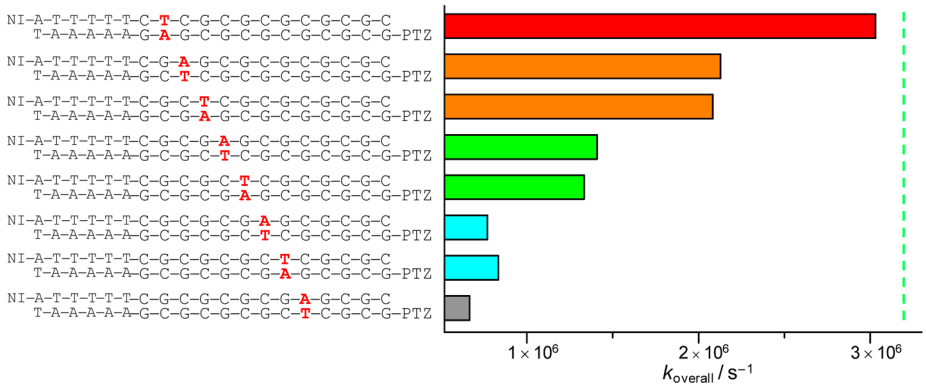

FIGURE 6. Kinetics of hole transfer through G-C repeats intervened by an A/T base pair. The green dashed line is predicted $k_{overall}$ based on kinetic modeling.[27]

SNP information can be transformed into a set of k_{HT}, and that it is possible to detect SNP by measuring k_{HT}.

Charge Delocalization over Bases

As mentioned above, hole transfer proceeded exceptionally fast in some sequences, such as the G-C sequence. To investigate in more detail the origin of the fast hole transfer kinetics observed for the G-C sequence, we further evaluated the $k_{overall}$ across G-C repeats intervened by an A/T, in which the position of the A/T is sequentially changed among G-C repeats (Figure 6). Interestingly, $k_{overall}$ was not identical among the sequences, even though these sequences have exactly the same number and type of hole transfer step between G's.[27] The $k_{overall}$ decreased concomitantly with the increasing length of G-C repeats prior to the A/T. It is also noteworthy that a kind of even−odd effect was observed; that is, the $k_{overall}$ obtained for consecutive even and odd numbers of (G/C)s prior to A/T were almost identical. The kinetic trace obtained from kinetic modeling using k_{HT} described in Figure 3 correlate well with the result obtained for DNA having a single A/T prior to the G-C repeat. Thus, while the sequential hole transfer between G's, where the hole is localized on individual G's, has been reasonably explained our results so far, we encountered an exception when there is a G-C repeat prior to the A/T spacer. The decrease in k_{HT} may be explained by the delocalization of a hole over bases to some extent, which results in the stabilization of the hole.

The hole delocalization over bases and its contribution to the hole transfer mechanism is one of the remaining hot topics in the field.[28−30] Schuster et al. suggested the polaron hopping model for the hole transfer in DNA.[8] Barton and Genereux proposed that hole transfer occurs via the hopping from domain to domain with a size of at least three base pairs.[31] Sugiyama and Saito demonstrated stabilization of the hole over a couple of G's.[32] The delocalization of a hole over (G/C)s may result in an increase of the HOMO compared to that of localized G/C, resulting in a larger Δ_{HOMO} for hole transfer across A/T as well as a decrease in k_{HT}. At the same time, our results suggest that not only the closer distance between Gs but also the delocalization of the hole might contribute to the exceptionally fast k_{HT} obtained for the G-C sequence.[27]

HOMO Energy Gap Dependence of Hole Transfer

Since DNA is one of the most well-studied organic molecules, there are plenty of reports on the synthesis of artificial nucleobase analogues with various properties. Thus, DNA offers a unique system to study the factors that affect the hole transfer kinetics. Next we discuss the sequence-dependent k_{HT} in terms of the HOMO energy gap (Δ_{HOMO}) between the bases by utilizing various natural or artificial bases with different HOMO levels.[33] Wenger recently reported a systematic review about how the donor−bridge−acceptor energetics influence the charge transfer kinetics.[34] According to the semiclassical Marcus theory, k_{HT} is described as

$$k_{HT} = \sqrt{\frac{\pi}{\hbar^2 \lambda k_B T}} |H_{DA}{}^2| \exp\left[-\frac{(\Delta G + \lambda)^2}{4\lambda k_B T} \right] \qquad (3)$$

where λ is the reorganization energy, ΔG is the reaction free energy, k_B is Boltzmann's constant, $\hbar$ is the reduced Planck constant, T is the temperature, and H_{DA} is the electronic coupling between the donor and the acceptor. McConnell developed a mathematical model (eq 3) describing H_{DA} in a system in which the donor and acceptor are separated by a bridge.

$$H_{DA} = \frac{h_{DB} h_{BA}}{\Delta \varepsilon} \qquad (4)$$

H_{DA} depends on the electronic coupling between the donor and the bridge (h_{DB}), the coupling between the

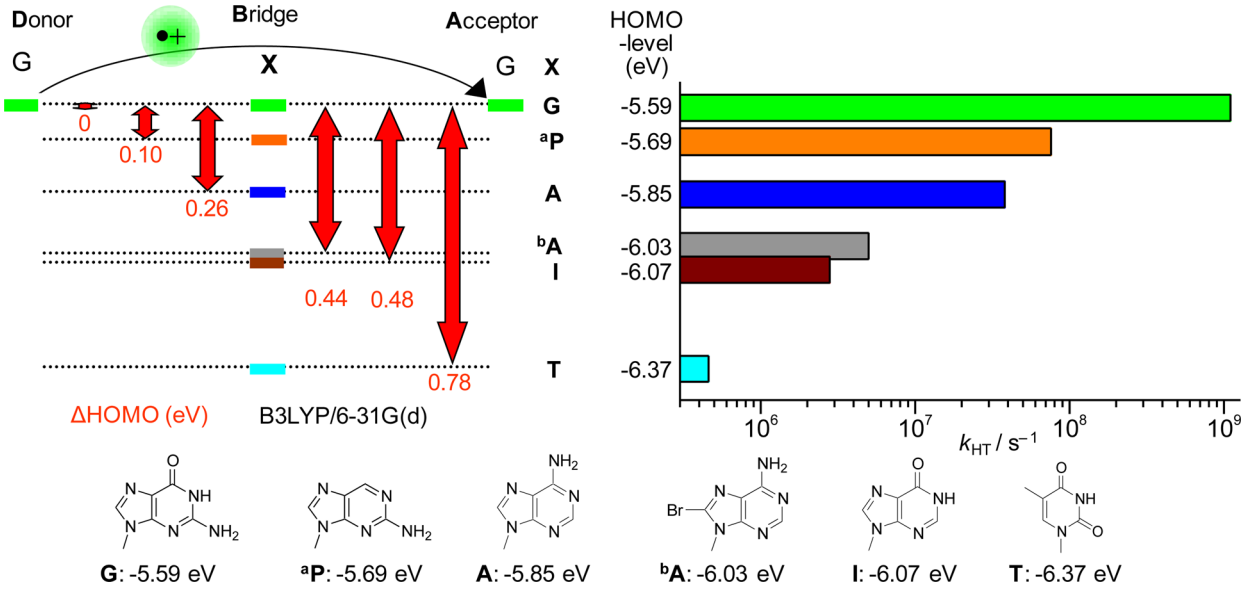

FIGURE 7. HOMO energy gap (Δ_{HOMO}) dependence of k_{HT}.[33]

bridge and the acceptor (h_{BA}), and the tunneling energy gap Δ_{tun}, which is the difference between the energy of the donor–acceptor system at the transition-state configuration and the energy of the bridge-localized states. The Δ_{tun} is reported to be closely related to the hole injection free energy into the bridge[29,34−36] and thus can be approximated using the Δ_{HOMO}, and the k_{HT} is proportional to $(1/\Delta_{HOMO})$.[2] We measured k_{HT} in the following DNAs having bases with different HOMO levels as a bridge between Gs: G, A, T, inosine (I), 2-aminopurine (^{a}P), and 8-bromoadenine (^{b}A). The Δ_{HOMO} varied from 0 to 0.78 eV (Figure 7). When the intervening base between G's was G, the hole transfer was accomplished faster than the time resolution of our setup, showing that k_{HT} was larger than 1×10^9 s^{-1}. The k_{HT} between G's across a single G was obtained by applying the k_{HT} value reported by Lewis and co-workers for adjacent G's (Figure 3)[17] and eq 2, where $N = 2$ and $\eta = 2$ were applied.[24] Interestingly, the formation rate of $PTZ^{\bullet+}$ correlated well with Δ_{HOMO}, and the k_{HT} increased with decreasing Δ_{HOMO}, consistent with the McConnell model. The much larger k_{HT} values obtained for the G-A_n-G sequences compared to the corresponding G-T_n-G sequences can be mainly explained by the Δ_{HOMO}-dependence of the k_{HT}. Larger π–stacking surface area between neighboring bases for G-A_n-G sequences compared with G-T_n-G, sequence dependent local structure, and dynamics may also contribute on the difference of k_{HT} (discussed below).

While we also expected to get some insight into the Δ_{HOMO} by which the hole transfer mechanism switches from superexchange to hopping, no clear turning point was observed. Thermal fluctuations in HOMO energies were demonstrated to range up to 0.4 eV, and thus, the mixing of two mechanisms may take place especially when Δ_{HOMO} is relatively low (<0.4 eV).[29,30,37]

Increasing the Hole Transfer Rate in a Sequence-Independent Manner

In the previous section, we described that the increase in Δ_{HOMO} significantly decreases k_{HT} and thus k_{HT} is inherently small except for G/C-rich sequences. This limits the potential applications of duplex DNA as a conducting molecule in molecular-scale devices in which the use of various sequence patterns is indispensable for constructing nanoarchitectures. It may be possible to increase k_{HT} in a manner that is less dependent on the sequence by decreasing the Δ_{HOMO} between the two base pairs, that is, G/C and A/T. Nakatani and Saito reported that ^{z}A and diaminopurine (D), having a higher HOMO level than A and closer to that of G, serves as a stepping stone for increasing the hole transfer efficiency between (G/C)s separated by an A/T stretch.[1,38] We next replaced A's with ^{z}A's or D's to increase the hole transfer rate.

We tested whether a hole can go over an A/T tract with the assistance of ^{z}A. While the formation of $PTZ^{\bullet+}$ was not observed in the experimental time window of 200 μs for DNA having five consecutive (A/T)s,[14,25] $k_{overall}$ dramatically increased with the increasing number of ^{z}A's, and when all the A's in the A/T tract were replaced with ^{z}A's, the hole

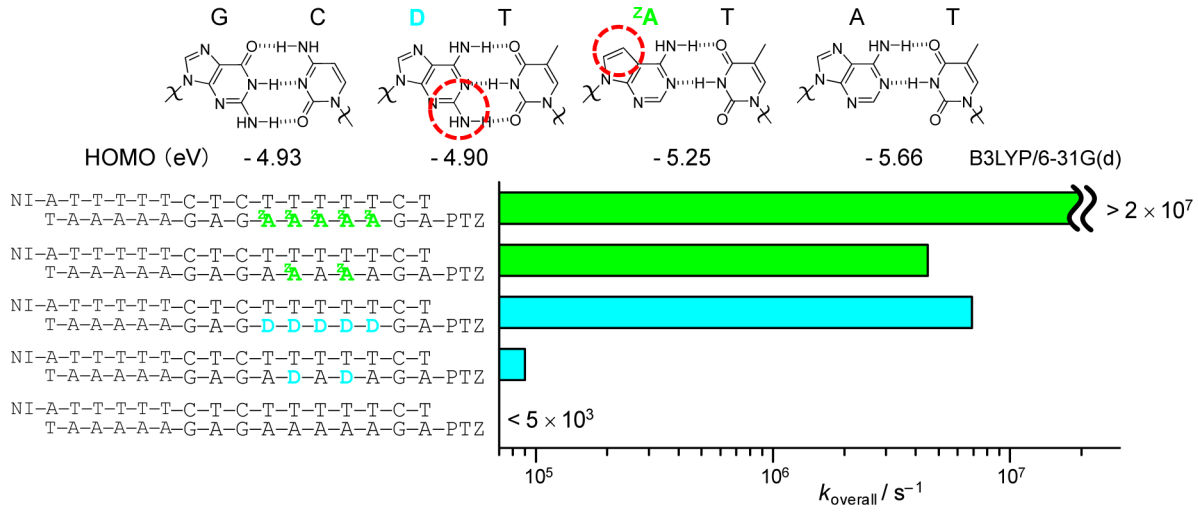

FIGURE 8. Increasing the hole transfer rate in a sequence-independent manner by replacing A's with ^{z}A's or D's.[39,40]

transfer across ca. 50 Å occurred faster than the time resolution of our experimental setup (Figure 8).[39] Amazingly, $k_{overall}$ increased by more than 3 orders of magnitude by the replacement of A's with ^{z}A's. Though less pronounced, a similar effect was achieved by replacing A's with D's.[40] While the theoretical calculation suggested a smaller HOMO energy difference between D/T and G/C than that between ^{z}A/T and G/C, the experimentally derived difference in oxidation potential between D and G is much higher than that between ^{z}A and G.[1,38] Thus, the actual HOMO energy difference between D/T and G/C in the context of a DNA double helix may be higher than that between ^{z}A/T and G/C, resulting in a smaller $k_{overall}$ through DNA replaced with (D/T)s. Alternatively, the greater flexibility of ^{z}A/T compared to D/T may be the reason for the smaller effect of D on $k_{overall}$ (discussed below). In both analogues, the observed fast hole transfer kinetics may also be attributed partially to the charge delocalization over the bases.

Flexibility Dependence of Hole Transfer

Since DNA is a flexible molecule, changes in the molecular conformation due to thermal fluctuations can significantly alter orbital overlap and in turn alter the k_{HT}. Hole transfer through conformationally dynamic DNA is suggested to be affected by the frequency of occurrence of structures that are particularly amenable to hole transfer.[31,41] Lewis and Wasielewski demonstrated that the higher mobility of ^{z}A may partially account for the observed faster k_{HT} between ^{z}A's compared to that between G's or A's.[18] The use of multiple conformations from MD simulations to better simulate the dynamic environment of DNA has now gained popularity in the theoretical calculation of the hole transfer kinetics in DNA.[28−30,42−44] To further investigate the influence of the local conformational flexibility of DNA on k_{HT}, we synthesized DNA modified with locked nucleic acids (LNA) and 5-Me-2′-deoxyzebularine (B) (Figure 9). The base-pair lifetime of the (G/C)s in the LNA/DNA hybrid was reported to be longer than those of the corresponding base pairs in the DNA/DNA duplex. Thus, base-pair breathing rates decrease upon LNA modifications, leading to an increase in the rigidity of DNA.[45] B is an analogue of C that forms a reduced number of hydrogen bonds with G, and the replacement of C with B would lead to faster breathing of the base pair and thus increased DNA flexibility. The LNA modification of all the nucleotides in the G-A or G-T repeat region completely suppressed the hole transfer ($k_{HT} < 10^5$ s^{-1}).[46] While X-ray analysis showed that LNA confers a more efficient base stacking than natural B-DNA due to LNA's smaller helical twist,[47] the loss of flexibility resulted in a significant decrease in k_{HT}. In sharp contrast, the replacement of C with B resulted in a significant increase in k_{HT}. When all C's in the G-A repeat were replaced with B's, the hole transfer occurred faster than the time resolution of our setup ($k_{HT} > 10^9$ s^{-1}). 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}.

The temperature dependence of k_{HT} was measured and the activation energy (E_a) was determined using the conventional Arrhenius model,

$$k_{ht}\sqrt{T} = A\ \exp\left(-\frac{E_a}{k_B T}\right) \qquad (5)$$

where A is a preexponential factor. In the G-A sequences, the LNA modification resulted in an increase in E_a,

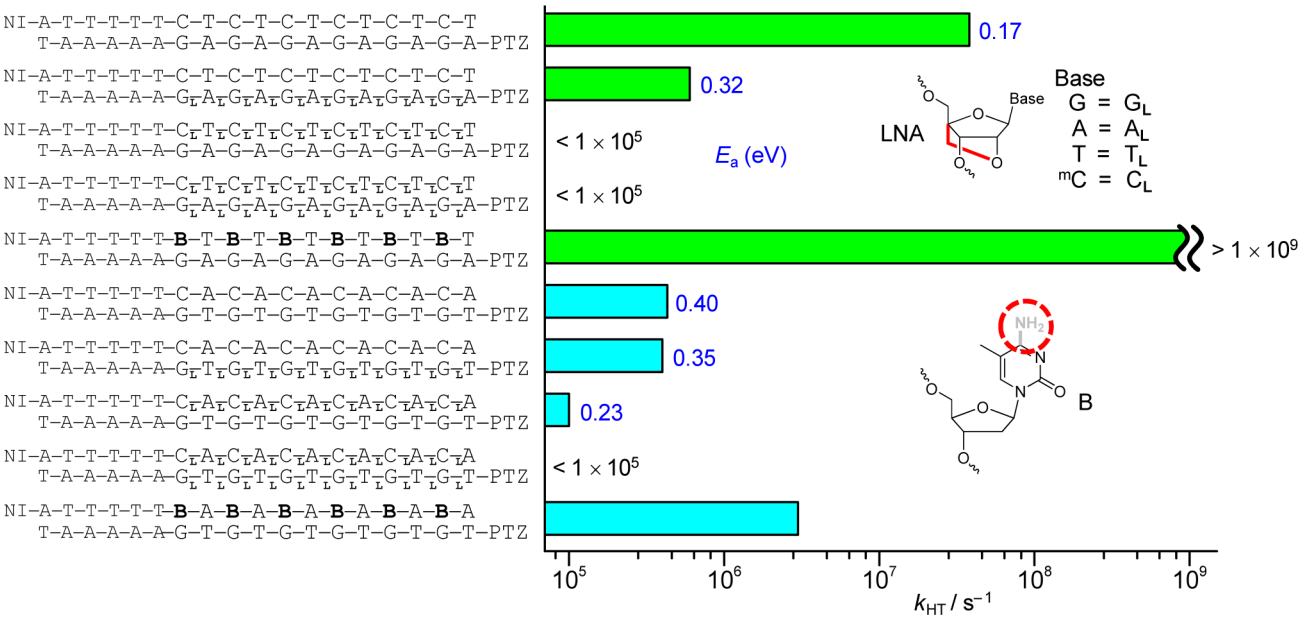

FIGURE 9. Flexibility dependence of k_{HT}. Activation energies (E_a) are shown in blue.[46]

suggesting that greater DNA conformational motion is required for LNA-modified DNAs to adopt a conformation amenable to hole transfer (Figure 9). On the other hand, LNA modification in the G-T sequences resulted in a decrease in E_a. These 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 were more pronounced with top-strand modification in both G-A and G-T sequences. These results suggest that local rather than global DNA conformational motion plays a key role in k_{HT}.

Summary and Outlook

We have summarized the hole transfer kinetics between bases in DNA and discussed the mechanism based on the k_{HT}. In general, hole transfer in DNA was demonstrated to proceed via sequential hole transfer between G/C's; this hole transfer can be well reproduced by kinetic modeling using the determined rate constants for each hole transfer step. β-values of >0.6 Å^{-1} were obtained for photoinduced charge transfer and the hole transfer process in DNA, showing that while DNA is a more effective medium for charge transfer than proteins, it does not serve as a molecular wire. However, exceptionally large k_{HT} values were obtained in some sequences when Gs were located close to each other. In one such sequence, the G-C sequence, we obtained some results suggesting that hole delocalization over bases may be the origin of the observed fast hole transfer kinetics. The contribution of the charge delocalization over bases in the charge transfer mechanism is one of the remaining debates in hole transfer in DNA. In order to further investigate and refine the factors that affect the hole transfer kinetics, we utilized various artificial nucleobases. k_{HT} was demonstrated to increase with a decreasing HOMO energy gap between the nucleobases, and we showed that the k_{HT} through the G/C and A/T mix sequence can be drastically increased by replacing A's with ^{z}A's or D's. While the HOMO levels of the purine bases A and G have been our focus so far, the hole transfer efficiency of DNA would be further improved by adjusting the HOMO levels of the pyrimidine bases C and T to unify the HOMO levels of all four nucleobases. We also demonstrated that a k_{HT} can be increased by increasing the DNA flexibility. Although DNA itself may not be applicable, properly modified artificial DNA may serve as a potential molecule in nanoelectronic sensors and devices.

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FOOTNOTES

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