Contributions of the Distance-Dependent Reorganization Energy and Proton-Transfer to the Hole-Transfer Process in DNA

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Abstract: A kinetic study of the single-step hole transfer in DNA was performed by measuring time-resolved transient absorption. DNA molecules with various sequences were designed and conjugated with naphthalimide (NI) and phenothiazine (PTZ) to investigate the sequence and distance dependence of the single-step hole transfer between guanines (Gs). Hole injection into DNA was accomplished by excitation of the NI site with a 355 nm laser pulse, and the kinetics of the hole-transfer process were investigated by monitoring the transient absorption of the PTZ radical cation ($PTZ^{\cdot+}$). Kinetic analysis of the time profile of $PTZ^{\cdot+}$ based on the kinetic model showed that the distance dependence of the hole-transfer process was significantly influenced by the DNA sequence. Results of temperature- and isotope-effect experiments demonstrated that the activation energy increased as the number of bridge bases separating the Gs increased. This is because of the distance-dependent reorganization energy and contribution of the proton-transfer process to the hole transfer in DNA.

Keywords: charge transfer · DNA · proton transfer · reorganization energy · time-resolved spectroscopy

Introduction

Charge-transfer phenomena in DNA have been the subjects of recent extensive research efforts.[1–4] In particular, the hole-transfer process in DNA has attracted considerable attention because of its relevance to the development of DNA-based electrochemical biosensory and nanoelectronic devices,[5–7] and its involvement in DNA-oxidative lesions.[8,9] Additionally, there has been much interest in whether the hole transfer through DNA has relevant biological consequences.[4,10–14]

A hole generated in DNA has been shown to migrate through π-stack arrays over long distances.[15] Long-distance hole transfer in DNA has been demonstrated experimentally by the results of strand-cleavage studies in duplexes possessing multiple guanine (G)-containing sites. The model of a multi-step hole-hopping mechanism, in which G, with the lowest oxidation potential among the four nucleobases, acts as a charge carrier, is the most widely adopted.[16–20] In addition, it has been demonstrated to occur by hole hopping between adenines (As) (A-hopping) if two Gs are separated by more than four adenine:thymine (A:T) base pairs.[21]

An alternative mechanism in which a delocalized hole migrates by a polaron-like mechanism has been proposed by Schuster and co-workers.[22,23] Recently, an experimental and theoretical investigation has shown that DNA-base dynamics, occurring over a range of time scales relevant to the charge transfer, modulate the electronic coupling between a donor and acceptor.[24,25] Based on the results of the influence of the base dynamics, Barton and co-workers have proposed that a hole might migrate over long distances by domain hopping, in which the charge is transiently delocalized over sequence-dependent domains defined by the local structure.[26,27]

The mechanism of the hole-transfer process in DNA has been intensively studied both theoretically[28–30] and experimentally.[1–3] Hole transfer through DNA π-stack arrays has been investigated mainly by strand-cleavage analysis using polyacrylamide gel electrophoresis (PAGE), which has provided information about the relative rates of hole migration with respect to the reaction with water as a function of base sequence.[31] However, the PAGE technique is not suitable for the investigation of kinetics and dynamics in real time.

Time-resolved measurements provide significant information on the kinetics and dynamics for the hole-transfer process in DNA. Although photoinduced electron transfer between a nucleobase and an excited molecule has been well

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characterized,[32–38] there are only a few reports concerning hole-transfer dynamics in DNA, as this is a slower process than photoinduced electron transfer.[39–41] Lewis and Wasielewski et al. designed DNA conjugates possessing a stilbene linker, and measured the rate constants of the hole transfer from G to GG by means of femtosecond time-resolved transient absorption measurements.[39,40] By monitoring the decay of the stilbene radical anion, they observed the hole-transfer process between the Gs. However, they could not examine hole-transfer at a rate slower than $\sim 10^5\ s^{-1}$, due to the rapid recombination between the stilbene radical anion and the G radical cation ($G^{\bullet+}$).[40]

Regardless of the intense research efforts, many factors, such as DNA structural dynamics, reorganization, and proton-transfer, which is considered to affect the hole-transfer process, still remain unclear. In fact, the number of theoretical studies has outpaced reports of experimental work. In many cases, theoretical predictions have preceded the availability of direct experimental measurements of hole-transfer dynamics. Therefore, experimental work based on the time-resolved measurements is a prerequisite for the further understanding of this subject.

Recently, we demonstrated that A-hopping occurs very rapidly ($>10^8\ s^{-1}$)[42] and can be applied to generate a long-lived charge-separated state in DNA.[43,44] Furthermore, we directly observed the long-distance hole transfer through DNA by utilizing the A-hopping to inject a long-lived hole.[45] In this paper, we report the kinetic study for the single-step hole transfer as a function of distance and temperature, and demonstrate that the change in the reorganization energy, which is dependent on the distance between the Gs, causes a considerable decrease in the hole-transfer rate. In addition, the deprotonation of $G^{\bullet+}$ to produce the deprotonated G radical ($G^{\bullet}(-H^+)$), and the protonation of $G^{\bullet}$ by a water molecule, could play an important role in hole-transfer.

Results and Discussion

Charge separation through A-hopping: The photophysical properties of naphthalimide (NI) have been previously characterized by Kelly et al.[46,47] The chemical structure of NI, and the sequences of DNA modified with NI, are shown in Figure 1a and b, respectively. NI is likely to stack easily with neighboring nucleobases because of its hydrophobicity and planar structure. Furthermore, because it shows a moderate absorption at wavelengths longer than 300 nm, and DNA shows no absorption at wavelengths longer than 300 nm, NI can be selectively excited with a 355 nm laser pulse.

From the reduction potential of −1.01 V (vs NHE: normal hydrogen electrode) and singlet-state energy of 3.4 eV for NI,[47] the reduction potential of NI in the singlet excited state is calculated to be 2.4 eV vs NHE, which means that NI in the singlet excited state can oxidize all four nucleobases ($E_{ox}=1.47$ and 1.7 V vs NHE for G and A, respectively).[48,49] Therefore, upon excitation of NI, charge

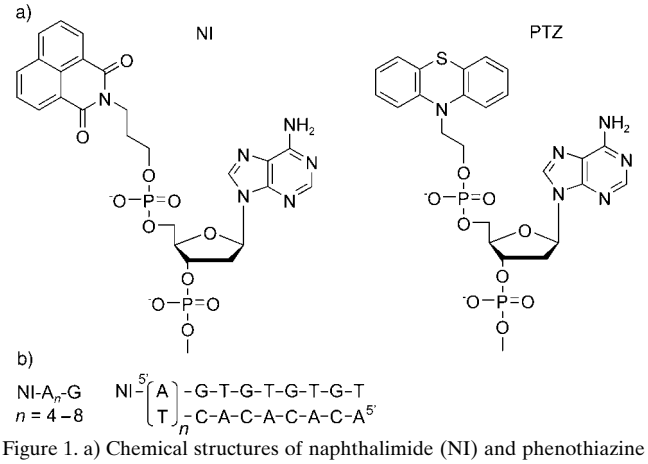
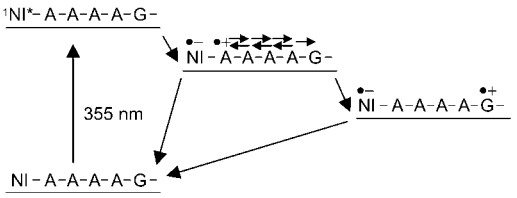


Figure 1. a) Chemical structures of naphthalimide (NI) and phenothiazine (PTZ) attached to the 5′-end of DNA. b) Sequences of the DNA modified with NI.

separation between NI and the neighboring nucleobase is expected to occur. As soon as a hole resides on As, a part of it can migrate to G by hopping between the As to give the charge-separated state between NI and the nearest G (Scheme 1).



Scheme 1. Kinetic scheme for the charge-separation process through A-hopping after the excitation of NI with a 355 nm laser pulse.

The mechanism of the hole transfer between As has been demonstrated experimentally. Giese et al. have shown that a hole generated on A over a successive A sequence is carried by the bridge base A as the A radical cation.[21,31] As for the kinetics, we have shown that the A-hopping occurred very rapidly with a small distance dependence due to the multi-step process,[42] and a long-lived charge-separated state can be generated by utilizing A-hopping in the charge-separation process.[43]

DNA conjugates containing the NI–A_n–G sequence were designed to investigate how the number of As affects the charge-separation process by A-hopping (Figure 1b). Figure 2a shows the transient absorption spectrum observed at 200 ns for NI–A_6–G, following the excitation of NI with a 355 nm laser pulse. A strong absorption at around 400 nm and weak absorption at around 500 nm were observed. These two absorption bands can be assigned to the NI radical anion ($NI^{\bullet-}$),[46] and $G^{\bullet+}$ or $G^{\bullet}(-H^+)$, respectively.[43,50] This result clearly shows that the charge separation between NI and G occurred upon excitation of NI. In addition, $NI^{\bullet-}$ in the charge-separated state persisted longer than several hundred microseconds (Figure 2a, inset), indicating the for-

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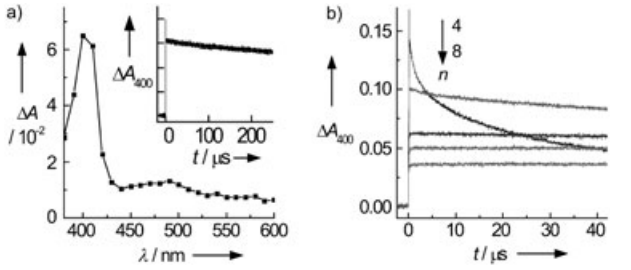

Figure 2. a) Transient absorption spectrum for NI–A_6–G, observed 200 ns after laser flash excitation (100 μM DNA in 20 mM Na phosphate buffer (pH 7.0) and 100 mM NaCl). Inset; time profile monitored at 400 nm. b) Decay profiles for NI–A_n–G (n = 4–8) monitored at 400 nm, following excitation of NI with a 355 nm laser pulse.

mation of a long-lived charge-separated state between NI and the G in DNA. Similar results were observed for DNA conjugated with naphthaldiimide,[43] which can oxidize A. These results indicate that the excitation of a photosensitizer, capable of oxidizing the nearest neighboring A as well as G, causes the long-lived charge-separated state in DNA through A-hopping.

To elucidate what effect the number of As between NI and the nearest G had on the charge-separation yield and the charge-recombination process, the time profiles of the transient absorption monitored at 400 nm were examined for NI–A_n–G (Figure 2b). The yields of formation of the charge-separated state, which were determined from the absorption of $NI^{\bullet-}$ observed following excitation, are shown in Table 1. The yields decreased as the distance between NI and G increased. Because this distance dependence was weak, it indicates that the charge-separation process between NI and G occurred through A-hopping. Except for the case of the NI–A_4–G sequence, the charge-separated state persisted for several hundred microseconds. Because NI is close to G in the NI–A_4–G sequence, charge recombination between $NI^{\bullet-}$ and $G^{\bullet+}$ occurred within this time scale by means of a superexchange mechanism.

Table 1. Quantum yields of charge separation (Φ_{cs}) between NI and the nearest G through A-hopping in NI-conjugated DNA.

DNA	Φ_{cs}[a] [%]	DNA	Φ_{cs}[a] [%]
NI–A_4–G	3.3	NI–A_7–G	1.3
NI–A_5–G	2.6	NI–A_8–G	0.90
NI–A_6–G	1.6		

[a] Determined from the transient absorption of the triplet benzophenone as an actinometer during the 355 nm laser flash photolysis.

It has been mentioned that the hole-escape reaction is endothermic, because the increase in the interionic distance reduces the Coulombic attractive interaction in the ion pair. In an earlier study, Lewis and Wasielewski demonstrated from the time-resolved spectroscopic measurements of the diphenylacetylene-conjugated hairpin DNA that, due to the Columbic interaction, a hole cannot escape from the contact radical ion pair produced after the charge separation.[51] However, we have shown that once a hole resides on A, the hole can migrate along the As in an essentially distance-independent fashion to give the long-lived charge-separated state.[43] The discrepancies between these two experimental studies may correspond to the different yields of the hole-escape reaction from the contact radical ion pair. The charge-separation yields determined through A-hopping in this system were very low, only a few %. Although the initial charge-separated state between $NI^{\bullet-}$ and $A^{\bullet+}$ is likely to recombine, a part of a hole could escape from the contact radical ion pair and could migrate to the nearest hole acceptor.

Miyasaka et al. reported the hole-escape reaction from the contact radical ion pairs and the hole-migration process in a poly(*N*-vinylcarbazole) system.[52] Based on the results of the time-resolved dichroism measurement, they demonstrated that a hole-shift reaction from the initial charge-separated state could be enhanced by delocalization of the cationic site over the carbazole units. A previous computational investigation based on the tight-binding model indicates that the wave function of the hole trapped on the nucleobase could extend over several sites.[29] Such a delocalization of a hole on successive A sequences, in which the As were directly stacked over each other, might be desirable to achieve a thermodynamically unfavorable hole-shift reaction, rather than a thermodynamically favorable charge recombination.

Strategy for observing the hole-transfer process: Recently, we showed that direct observation of the long-distance hole transfer that occurred within the time scale of microseconds to milliseconds could be accomplished by a time-resolved measurement, utilizing the A-hopping to generate a long-lived hole.[45]

To observe the hole-transfer process, we used DNA conjugated with a hole-probe molecule and recorded time-resolved transient absorption measurements. The sequences of DNA examined in this study are shown in Figure 3. NI, used as a photosensitizer to inject a hole into the DNA, was attached at one 5′-end of the DNA, and phenothiazine (PTZ), used to monitor the migration of the hole, was attached at the opposite 5′-end. PTZ is a suitable molecule for probing hole-transfer in this system, because the ground-state absorption of PTZ at a wavelength shorter than 320 nm allows

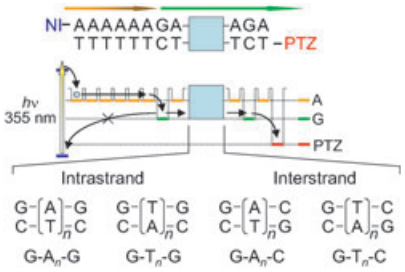

Figure 3. Schematic illustration of the charge-separation process following excitation of the NI site with a 355 nm laser pulse, and the subsequent hole-transfer process in DNA.

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us to selectively excite the NI site with a 355 nm laser pulse, and the oxidation potential of PTZ is less than that of the four nucleobases. In addition, the PTZ radical cation ($PTZ^{\cdot+}$), which is generated by the hole-transfer process, shows a strong absorption at around 520 nm and almost no spectral overlap of $NI^{\cdot-}$.[45]

Excitation of the NI site with a 355 nm laser pulse resulted in the formation of a separated state between NI and the nearest G, due to A-hopping within the laser pulse (<5 ns), as mentioned above. Regardless of the presence or absence of PTZ, the charge-separation yields were almost consistent, meaning that the charge-separated state was formed between NI and the nearest G. Once a hole is trapped at G far away from NI, the charge recombination can no longer occur, as this proceeds through a superexchange mechanism that is strongly dependent on the distance. The oxidation potential of PTZ ($E_{ox}=0.76$ V vs NHE) is quite low compared with four nucleobases; therefore, a hole on G is expected to migrate to PTZ. Accordingly, by monitoring the formation of $PTZ^{\cdot+}$, we can explore directly the kinetics and dynamics of the hole-transfer process.

Consistent with our strategy, the transient absorption for $NI^{\cdot-}$ and $G^{\cdot+}$ was observed upon excitation of NI with a 355 nm laser pulse for the G–T_1–G sequence, and then a broad absorption at around 520 nm assigned to $PTZ^{\cdot+}$ emerged on the time scale of several microseconds (Figure 4 and inset). No effect of the DNA concentration (20–100 μM) on the formation rates was observed, thus confirming that the formation of $PTZ^{\cdot+}$ can be assigned to the intramolecular hole-transfer process.

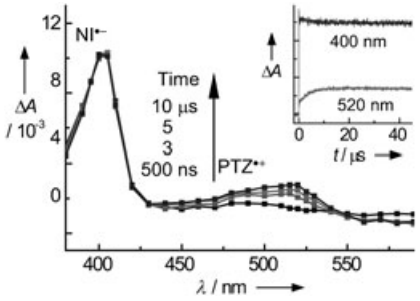

Figure 4. Transient absorption spectra for the G–T_1–G sequence observed at 500 ns, 3, 5, and 10 μs after excitation of the NI site with a 355 nm laser pulse. Inset; time profiles for $NI^{\cdot-}$ and $PTZ^{\cdot+}$ absorption monitored at 400 and 520 nm, respectively.

Distance dependence of the single-step hole-transfer process: The time profiles of the transient absorption of $PTZ^{\cdot+}$ following excitation were monitored, and corresponded to the hole-transfer process. The time profiles of $PTZ^{\cdot+}$ monitored at 520 nm for G–A_n–G ($n=1$–3) are shown in Figure 5a. Apparently, the formation rate constant decreased as the distance between the Gs increased. The formation profiles of $PTZ^{\cdot+}$ correspond to the hole transfer from the G nearest NI, to PTZ, and include several kinds of hole-transfer steps. Therefore, the rate constants of the single-step hole transfer between Gs (k_{ht}) through various intervening base pairs are determined from the kinetic modeling (see Experimental Section), and are shown in Table 2.

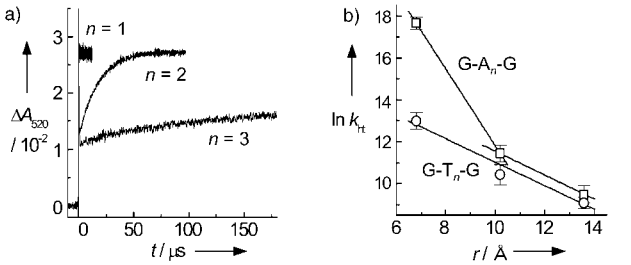

Figure 5. a) Time profiles of the transient absorption of $PTZ^{\cdot+}$ monitored at 520 nm for G–A_n–G ($n=1$–3) at 23 °C. b) Plots of $\log k_{ht}$ versus r for G–A_n–G (□) and G–T_n–G (○). The distance between base pairs is assumed to be 3.4 Å.

Table 2. Rate constant (k_{ht}), activation energy (E_a), and reorganization energy (λ) for single-step hole transfer.

Sequence	n	k_{ht}[a] [s^{-1}]	E_a[b] [eV^{-1}]	λ[c] [eV^{-1}]
G–A_n–G	1	4.8×10^7	0.18	0.72
	2	9.7×10^4	0.43	1.7
	3	1.4×10^4	–	–
G–T_n–G	1	4.6×10^5	0.35	1.4
	2	3.6×10^4	0.50	2.0
	3	9.1×10^3	–	–
G–A_n–C	1	1.4×10^6	0.30	1.2
	2	4.5×10^4	0.53	2.1
G–T_n–C	1	1.6×10^6	0.25	1.0
	2	3.1×10^4	0.50	2.0

[a] Rate constants (k_{ht}) were obtained from the kinetic modeling at 23 °C. Experimental errors are within ±20%. [b] Values of E_a for $n=3$ could not be obtained due to the detection limit. [c] Calculated by assuming that $\Delta G=0$ eV.

In the case of $n=1$, k_{ht} increased in the order G–A_1–G > G–T_1–C ≈ G–A_1–C > G–T_1–G, which was in accordance with a previous study[36] and was explained by the order of the energy gap between G and the bridge base. Because A has a lower oxidation potential than T, the electronic coupling between A and G is greater than that between T and G. The order of the single-step hole-transfer rate is consistent with this prediction.

The distance dependence of the hole transfer is conventionally evaluated by the following Equation (1), in which k_{et} is the electron-transfer rate constant between donor and acceptor, k_0 is the preexponential factor, β is the attenuation factor of k_{ht}, and r is the distance between the hole donor and acceptor (between Gs).

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

Plots of $\log k_{ht}$ versus r for G–A_n–G and G–T_n–G provide the β value (Figure 5b). Interestingly, significant decreases in $\log k_{ht}$ from $n=1$ to $n=2$ ($\beta=1.8$ $Å^{-1}$) and a slight decrease from $n=2$ to $n=3$ ($\beta=0.6$ $Å^{-1}$) were found for G–

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A_n–G, whereas a linear relationship was obtained for the G–T_n–G sequence ($\beta = 0.6$ Å^{-1}). The slope of the plot of $n = 2$–3 for G–A_n–G ($\beta = 0.6$ Å^{-1}) was almost same as that for G–T_n–G. The difference in the r dependence indicates that the mechanism changed as the r between the Gs increased for G–A_n–G. The β value was 1.0 Å^{-1} for both interstrand sequences G–A_n–C and G–T_n–C. The various β values obtained for each sequence suggest that the r dependence of the single-step hole transfer varies considerably with variation in sequence. The sequence dependence of the hole-transfer rate shows an especially fast hole transfer for the GAG sequence compared to the other sequence.

Distance-dependent reorganization energy: To elucidate the mechanism of the r dependence of the hole-transfer process, which is affected by the bridge sequence, we investigated the kinetics and dynamics of the single-step hole transfer as a function of temperature (T). The activation energy (E_a) was obtained from the temperature dependence of k_{ht} at different values of T, according to the following semiclassical Marcus Equation (2),[53] in which A and E_a are the preexponential factor and activation energy, respectively.

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

Time profiles of the transient absorption of PTZ$^{\bullet+}$ monitored at 520 nm between 10 and 30 °C for G–A_2–G are shown in Figure 6a. The rise time of the transient absorption of PTZ$^{\bullet+}$ increased as T increased, as expected according to electron-transfer theory. A similar increase in the formation rates were observed for the other sequences.

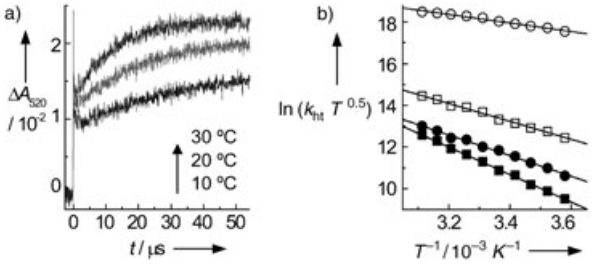

Figure 6. a) Temperature dependence of the time profiles of transient absorption of PTZ$^{\bullet+}$ for G–A_2–G, monitored at 520 nm at 10, 20, and 30 °C. b) Plots of $\log(k_{ht}T^{0.5})$ versus T^{-1} for G–A_n–G ($n = 1$; ○, $n = 2$; ●) and G–T_n–G ($n = 1$; □, $n = 2$; ■).

A plot of $\log(k_{ht}T^{0.5})$ versus T^{-1} provides the E_a value for the hole transfer between Gs (Figure 6b, Table 2). In all four sequences, E_a increased as the r between Gs increased, suggesting that E_a was dependent upon r.

The E_a value is related to the total reorganization energy (λ) according to the following Equation (3), in which ΔG is the thermodynamic driving force, and λ is the reorganization energy.

$$E_a = \frac{(\Delta G + \lambda)^2}{4\lambda} \tag{3}$$

By making the simplifying assumption that the hole transfer between Gs occurs at $\Delta G = 0$, it was possible to estimate λ, which is comprised of the nuclear (λ_v) and solvent (λ_s) reorganization energies (Table 2). Although it is well known that the r-dependent E_a can be attributed to the r dependence of both ΔG and λ, a considerable increase in E_a would be attributed to the r-dependent λ_s, because ΔG and λ_v for the hole-transfer process are considered to be slightly or not at all r dependent. The distance dependence of the reorganization energy has been both experimentally and theoretically studied.[28,54] Michel-Beyerle et al. reported an increase in E_a for the charge-shift reaction from the excited acridine derivative, which was incorporated into DNA, to G or deazaG.[54,55] However, the effect of the distance dependence of λ on the hole transfer between Gs has not been proved experimentally. Our results clearly show that reorganization of the water molecules surrounding DNA is closely involved in the hole-transfer process between Gs, and the increase in λ_s, rather than the decrease in electronic coupling associated with hole transfer, causes the decrease in hopping rates.

Proton-coupled hole transfer: The variation in the increase in λ for the four sequences cannot be explained by considering only the difference in λ_s, because the degree of increase in λ_s is considered to be independent of the intervening bases. For instance, a considerable increase in λ (ca. 1 eV) was found for G–A_n–G, whereas λ for G–T_n–G only moderately increased (ca. 0.6 eV). Giese et al. have demonstrated that proton transfer is coupled with hole transfer, and causes the decrease in the hole-transfer efficiency between Gs.[56–58] To examine the effect of the proton-transfer process, a kinetic isotope-effect experiment was carried out. If the deprotonation of G$^{\bullet+}$ contributes to hole-transfer, an isotope effect would be expected. In fact, the decrease in k_{ht} for the G–A_2–G sequence was observed upon changing the solvent from H_2O to D_2O ($k_H/k_D = 1.2 \pm 0.05$), whereas no isotope effect was observed for the G–A_1–G sequence ($k_H/k_D = 1.0 \pm 0.05$). This different isotope effect can be explained by comparing k_{ht} to the rate of deprotonation of G$^{\bullet+}$ by a water molecule.[59] Recently, the deprotonation rate of G$^{\bullet+}$:C or G$^{\bullet}$:C(+H$^+$) was determined to be in the range of 10^6–10^7 s^{-1}.[60] Because the hole transfer for G–A–G occurs faster than deprotonation, it is reasonable that no isotope effect was observed for G–A–G. In contrast, if the hole-transfer process is competitive with or slower than deprotonation, a considerable decrease in k_{ht} is induced because of the contribution of the protonation of G$^{\bullet}$ and increase in λ. By considering the hole transfer from G$^{\bullet}$ to G for G–A_2–G, the λ value was recalculated from the steady-state potential of 1.05 V for G$^{\bullet}$ and the oxidation potential of 1.34 V for G, to be 1.06 eV, according to Equation (3). The difference in the λ value between G–A_1–G and G–A_2–G is 0.34 eV. This value, which is attributed to the distance-dependent reor-

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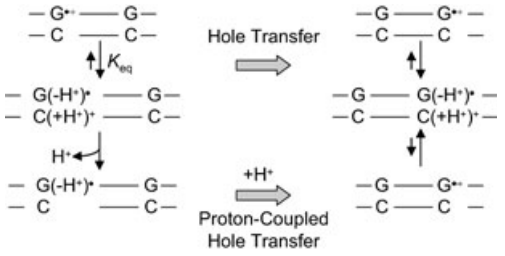
ganization energy, is in good agreement with previous results and reasonably supports our interpretation.

Accordingly, the hole transfer in DNA would consist of two hole-transfer processes from $G^{\bullet+}$ to the next G, and from $G^{\bullet}$ to the next G concomitant with a concerted proton transfer (proton-coupled hole transfer). If the hole transfer occurs faster than the deprotonation of $G^{\bullet+}$, a hole migrates to the next G by a hopping method. Once a proton escapes from $G^{\bullet+}$ to the solvent, the proton-coupled hole transfer should overcome the thermodynamically unfavorable hole transfer from $G^{\bullet}$ to the next G. This is because the steady-state potential for the proton-coupled electron transfer from G to $G^{\bullet}$ is lower than the potential for the oxidation to $G^{\bullet+}$ (Scheme 2). The proton-transfer process, which may be closely related to hole transfer, could rationalize the rapid hole transfer reported by several groups.[61–63]



Scheme 2. Proton-coupled hole transfer in DNA.

A similar isotope effect on the kinetics of the oxidation of deoxyguanosine monophosphate (dGMP) by the 2-aminopurine (2-AP) radical in H_2O and D_2O was reported by Shafirovich and Geacintov.[63,64] They showed that the electron-transfer reaction is coupled to the deprotonation of the dGMP radical cation, demonstrating that such a proton-coupled electron-transfer step leads to a lowering of the overall free energy of reaction, thus favoring electron transfer.[65] The contribution of proton coupling and the considerable increase in λ were reported for intra- and intermolecular electron transfer. In a ruthenium(Ru)–tyrosine dyad system, a drastic change in λ during electron transfer from tyrosine to Ru was revealed by recording temperature-dependent transient absorption measurements. This indicated that protonation and deprotonation on the tyrosine group are coupled with the electron-transfer process.[66] Furthermore, Thorp et al. demonstrated that the deprotonation of G participates in the collisional DNA oxidation by a Ru complex.[67] They deduced that the escape of a proton from the base pair occurs by a "breathing" reaction lasting 1–100 ms. Considering that hole-transfer occurs faster than this "breathing" process, it is likely that local structural fluctuation, facilitating the acceptance and release of a proton, affects the hole-transfer rate.

Conclusion

We have reported the kinetics of the single-step hole transfer from $G^{\bullet+}$ to the next G in DNA as a function of the sequence between Gs, based on time-resolved transient absorption measurements. The r dependence of the hole transfer, characterized by the β value, was strongly influenced by the intervening sequence. From the temperature dependence and isotope effect of the hole-transfer process, it was demonstrated that the increase in E_a, induced by the change in the solvent reorganization energy and contribution of proton transfer, rather than the decrease in electronic coupling, caused the decrease in k_{ht}. A significantly high λ value, relative to the photoinduced charge transfer through the DNA π-stack array, demonstrates that hole hopping is closely associated with the dynamic motion of the DNA bases and the environmental medium.

Our results suggest that the local dynamic motion of DNA, allowing the release and acceptance of a proton from the exterior aqueous environment, may play an important role in the hole-transfer process in DNA. Furthermore, effective long-range hole transport through DNA, leading to the development of a DNA molecular wire, may be accomplished by designing artificial nucleobases to regulate the proton-transfer process.[68]

Experimental Section

DNA synthesis: Oligonucleotides conjugated with naphthalimide and phenothiazine were prepared by using conventional phosphoramidites chemistry and a DNA synthesizer (Applied Biosystems) according to procedures reported previously.[69,70] Synthetic oligonucleotides were purified by using an HPLC system (Jasco, Tokyo) with a reverse-phase C-18 column and an acetonitrile/ammonium formate (50 mM) gradient.

Time-resolved transient absorption measurements: Experiments were performed at 23 °C with DNA duplex (100 μM) in sodium phosphate buffer (20 mM, pH 7.0) and NaCl (100 mM). Nanosecond transient absorption measurements were performed by using the laser flash photolysis (LFP) technique. All samples were deoxygenated by purging with argon. Quantum yields of the charge separation were determined by using benzophenone as a reference standard. The third-harmonic oscillation (355 nm, full width at half maximum of 4 ns, 20 mJ per pulse) from a Q-switched neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (Surelight, Continuum, Santa Clara, CA) was used to excite the NI site selectively. This was possible because NI showed strong absorption at around 355 nm ($\varepsilon_{355} \approx 8 \times 10^3\,M^{-1}cm^{-1}$) and PTZ showed no absorption at wavelengths longer than 320 nm. A xenon flash lamp (XBO-450, Osram, Berlin) was focused into the sample solution as the probe light for the transient absorption measurements. Time profiles of transient absorption in the UV/Vis region were measured by using a monochromator (G250, Nikon) equipped with a photomultiplier (R928, Hamamatsu Photonics, Hamamatsu City, Japan) and digital oscilloscope (TDS-580D, Tektronix). Typically, 16 laser shots with the laser pulse on and off were averaged by using a digital oscilloscope. Significant sample degradation was not observed under these experimental conditions. The kinetic signals obtained were analyzed based on kinetic modeling to determine the single-step hole-transfer rate.

Kinetic analysis procedures: Analysis of the experimental data for all sequences was performed by using Matlab software according to kinetic modeling. A kinetic model of multi-step hole transfer in DNA is shown in Scheme 3.

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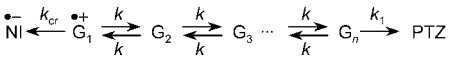


Scheme 3. Kinetic scheme for the multi-step hole transfer in DNA. The hole-hopping rates between Gs (k) are assumed to be identical. The rate constants of charge recombination and hole transfer from $G^{\bullet+}$ to PTZ are represented by k_{cr} and k_1, respectively.

The charge-recombination process (k_{cr}) can be ignored because the charge-separated state persisted over several hundred microseconds. If the hole trapping of $G^{\bullet+}$ with H_2O or O_2 is ignored, simultaneous differential equations can be obtained [Eq. (4)], in which $[G_i]$ (i = 1–n) and [PTZ] correspond to the hole population at each G and PTZ site, respectively, k is the hole-transfer rate constant between Gs, and k_1 is the rate constant for hole transfer from $G^{\bullet+}$ to PTZ.

$$\begin{aligned}
\frac{d[G_1]}{dt} &= -k[G_1]+k[G_2] \\
\frac{d[G_2]}{dt} &= k[G_1]-2k[G_2]+k[G_3] \\
\frac{d[G_n]}{dt} &= k[G_{n-1}]-(k+k_1)[G_n] \\
\frac{d[PTZ]}{dt} &= k[G_n]
\end{aligned} \tag{4}$$

Fitting the result for 5′-NI–A_6–$(GA)_{12}$-3′/3′-T_6–$(CT)_{12}$–PTZ-5′ according to Equation (4) is presented in Figure 7a, from which the rate constants of $k = 6\times10^7\ s^{-1}$ and $k_1 = 7\times10^7\ s^{-1}$ can be derived.

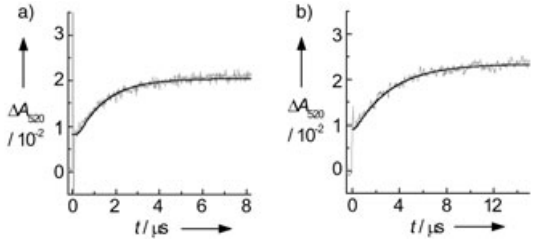
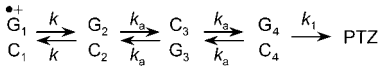


Figure 7. Time profiles of the transient absorption of $PTZ^{\bullet+}$ monitored at 520 nm, and fitting results (solid lines) of experimental data with the kinetic modeling for sequences of a) 5′-NI–A_6–$(GA)_{12}$-3′/3′-T_6–$(CT)_{12}$–PTZ-5′ and b) G–A–C.

To maintain the reliability of the fitting procedure, values of k and k_1 were fixed. A representative kinetic model of the G–A–C sequence is shown in Scheme 4, and the fitting results are shown in Figure 7b. Values of k_a correspond to the hole-transfer rate constants shown in Scheme 4. Kinetic analysis yielded identical k_a values of $1.5\times10^6\ s^{-1}$. Similar analyses were conducted for other sequences to obtain the rate constant of the single-step hole transfer.



Scheme 4. Kinetic scheme for the multi-step hole transfer in the G–A–C sequence.

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