Observation of Hole Transfer through DNA by Monitoring the Transient Absorption of Pyrene Radical Cation

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A hole generated by one-electron oxidation of DNA is considered to migrate through DNA by hopping between guanines (Gs)[1–5] that exhibit the lowest oxidation potential among the four DNA bases. Interestingly, the occurrence of the multistep long-range hole transfer over a distance of 200 Å has been demonstrated.[3] The hole transfer through DNA and the factors controlling the rates have been extensively studied, since the hole may cause oxidative damage in vivo which leads to mutation.[6] Furthermore, understanding DNA-mediated electron transfer is expected to lead to an additional application for the development of biosensors and bioelectronic devices.[1]

Distance dependence of the electron transfer in DNA has been studied by luminescence quenching experiments,[7–9] and found to be similar to that in proteins.[10] This is a superexchange-type mechanism. On the other hand, long-range hole transfer was evidenced by strand cleavage analysis. After the generation of the radical cation of G (G•+), the hole migrated over a long range through DNA. The long-range hole transfer in DNA is explained by multistep hopping between Gs.[3–5] Lewis et al. have determined the single-step hole-transfer rate constants between G and GG by monitoring the decay of stilbenedicarboxamide radical anion.[2] However, there is no report that provides the kinetics of hole transfer including the multistep process. For the occurrence of long-range hole transfer in DNA using the photochemical method, the radical anion of the electron acceptor accompanied by the generation of G•+ should be consumed. This can be achieved, for example, by reaction with oxygen,[3] to avoid charge recombination diminishing G•+. Furthermore, even once the radical anion is removed, observation of the hole transfer by monitoring the transient absorption of G•+ is difficult due to the low extinction coefficient of G•+, and it is hard to define the site of G•+ since DNA consists of only four bases.

Here, to remove the contribution of the charge recombination process, we used the pulse radiolysis technique instead of the photochemical method, since it is a convenient method for generating either the radical cation or anion of solute molecules up to 10^{-5} M.[11] For the observation of the radical cation generated in DNA, we synthesized oligodeoxynucleotides (ODNs) conju-

Scheme 1
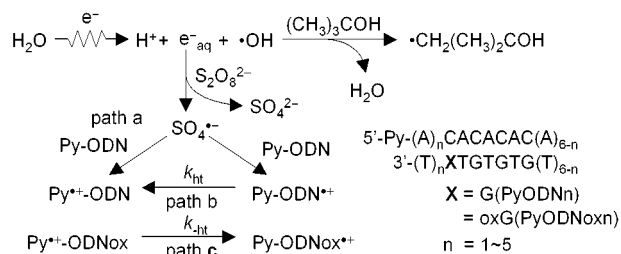
gated with pyrene of which the radical cation (pyrene•+) has a large molar extinction coefficient ($\epsilon \approx 2 \times 10^4$ M^{-1} cm^{-1}) and a distinct absorption peak at $\lambda_{max} = 470$ nm. The efficient amount of radical cation of pyrene-conjugated ODN was generated from one-electron oxidation during the pulse radiolysis in the presence of K$_2$S$_2$O$_8$,[12] and the hole transfer through DNA was monitored by the time-resolved transient absorption of pyrene•+. Since the oxidation potential of pyrene ($E° = 1.40$ V vs NHE in CH$_3$CN)[13] is lower than that of G ($E° = 1.47$ V vs NHE in CH$_3$CN),[14] hole transfer from G•+ to pyrene moiety is expected to occur.[15]

Distance dependence of the hole transfer process from the G-region (5′-GTGTGTG-3′) to the pyrene moiety was studied by pulse radiolysis of 5′-pyrene-conjugated ODNs with a different number of intervening A−T base pairs between the G-region and pyrene moiety (PyODN*n*, *n* = 1−5) (Scheme 1). Formation of the transient absorption with a maximum peak at 470 nm assigned to pyrene•+ was observed after the electron pulse during the pulse radiolysis (Figure 1). In all of the *n* = 1−5 PyODN*n*, pyrene•+ formed by a single-exponential process with the same rate ($k_{obs} = 8 \times 10^5$ s^{-1}) in a time range of 2 μs.[16] After completion of the diffusional process within a few μs, that is after the consumption of SO$_4$•− and formation of pyrene•+ or G•+, interestingly, a larger amount of pyrene•+ was formed for PyODN*n* with a shorter distance separation between the G-region and pyrene moiety in the time scale of 100 μs (Figure 2A). This secondary formation of pyrene•+ was attributed to the hole transfer from G•+ to pyrene through DNA (Scheme 1, path b). Since the hole transfer from the G-region is considered to be much slower for PyODN5 compared to the PyODN*n* (*n* = 1−4),[4] namely in the case of PyODN5, pyrene•+ was formed only from the initial collision process; the time profile of pyrene•+ at 470 nm for PyODN5 was subtracted from those for PyODN*n* (*n* = 2−4). This gave the time profiles of the formation of pyrene•+ from the hole transfer through DNA (Scheme 1, path b) (Figure 2B). The hole transfer rate decreased as the distance between the pyrene moiety and G-region (*r*) increased. This revealed the hole transfer in DNA over a period of 100 μs, with the rate constant k_{ht} in the range of 10^5–10^4 s^{-1}. Since similar results were also observed when the concentration of DNA was decreased by half, the observed results are accounted for by intramolecular processes.

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(12) Since SO$_4$•− is powerful enough to oxidize all four DNA bases ($E° = 2.5$−3.1 V vs NHE in H$_2$O),[11] random oxidation of the whole DNA strand is expected. For a detail experimental conditions, see Supporting Information.
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(15) The evidence that pyrene works as a hole sink was also determined from the γ-radiolysis experiments. See Supporting Information.
(16) This initial formation of pyrene•+ depended linearly on the concentration of PyODN*n* in the range of 0.05−0.2 mM, demonstrating that the formation of pyrene•+ in the time scale of 2 μs resulted from the direct diffusional collision between SO$_4$•− and the pyrene moiety (Scheme 1, path a). This gave the bimolecular reaction rate constant of SO$_4$•− with the pyrene moiety and ODN-region, $k_{Py+ODN} = 4 \times 10^9$ M^{-1} s^{-1}. In contrast, the rate constant of SO$_4$•− with unmodified ODN was determined to be $k_{ODN} = 3 \times 10^9$ M^{-1} s^{-1}. The similar values of k_{Py+ODN} and k_{ODN} demonstrate that the competitive oxidation of pyrene moiety and ODN results in the formation of pyrene•+ and G•+, respectively.
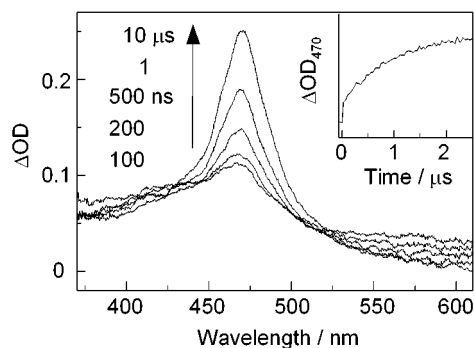
Figure 1. Transient absorption spectra of PyODN1 observed at several times after the electron pulse. The inset shows time profile of the transient absorption peak of pyrene$^{\bullet+}$ at 470 nm, which corresponds to the direct oxidation of pyrene with $SO_4^{\bullet-}$ (Scheme 1, path a).
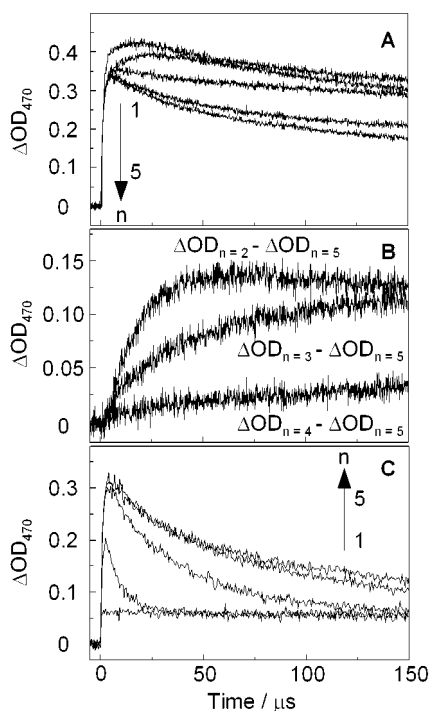

Figure 2. Formation and decay of the transient absorption of pyrene$^{\bullet+}$ monitored at 470 nm. Time profiles for (A) PyODNn ($n = 1-5$), (B) PyODNn ($n = 2-4$) after subtraction of the time profile of PyODN5, and (C) PyODNox$n$ ($n = 1-5$). The transient at 470 nm mainly of pyrene$^{\bullet+}$ for PyODNn decayed by two processes. The initial process in a time range of 100 μs and the longer component with a decay time $>$ 1 ms were observed.

Lewis et al. have reported the hole transfer rate constants from G to GG site and GG site to G across one A−T base pair to be $\sim 5 \times 10^7$ s^{-1} and $\sim 6 \times 10^6$ s^{-1}, respectively.[2] These rate constants are larger by two-three orders than k_{ht} for PyODNn ($n = 2-4$). Our observed smaller rate constant may be explained by the multistep hole transfer process between Gs, liberty from the charge recombination in the present system, and the difference of the driving force between these two systems.

Next, to observe the hole transfer from pyrene$^{\bullet+}$ to inside the DNA, pulse radiolysis of pyrene-conjugated ODNs with one
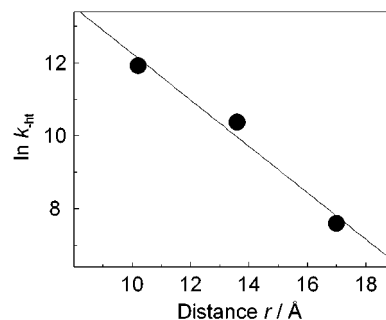

Figure 3. Distance dependence of the hole transfer through DNA. Semilog plot of k_{-ht} against the distance between pyrene and G-region (r). Values of r were calculated assuming an average distance of 3.4 Å between base pairs.

8-oxo-7,8-dihydroguanine (oxG) substituted for G as a hole trap[17] was performed (Figure 2C). When the nearest G to the pyrene moiety was replaced with oxG (PyODNox1), the formation of pyrene$^{\bullet+}$ was significantly suppressed. No secondary formation of pyrene$^{\bullet+}$ was observed for PyODNox$n$ ($n = 2-5$), which again confirms the hole transfer from the G-region to pyrene for the PyODNn ($n = 1-4$). Since the decays of pyrene$^{\bullet+}$ of PyODNox$n$ ($n = 2-4$) were single exponential as the sum of hole transfer from pyrene$^{\bullet+}$ to oxG and the decay of pyrene$^{\bullet+}$ with rate constants k_{-ht} and k_{decay}, respectively, k_{-ht} was found to be 15×10^4, 3×10^4, and 0.2×10^4 s^{-1}, for $n = 2$, 3, and 4 PyODNox$n$, respectively. The rate constant k_{-ht} decreased by increasing the distance between the pyrene and oxG. The linear plots of ln k_{-ht} versus r gave a slope, $\beta = 0.6$ Å^{-1} ($k_{-ht} = A \, e^{-\beta r}$) (Figure 3), which is just equal to the value of $\beta = 0.6$ Å^{-1} for the single-step electron transfer reported by Lewis[7] and Zewail.[8] It should be emphasized that the distance dependence of the single-step hole-transfer process in DNA as a π-stacking system is found to be consistent with those for the single-step electron transfer.

In the present study, we have succeeded in observing the hole-transfer processes by monitoring the formation and decay of pyrene$^{\bullet+}$. Our results strongly suggest that G$^{\bullet+}$, or deprotonated G radical and protonated cytosine base-pair G(−H)$^{\bullet}$:C(+H)$^{+}$,[18] can be present in DNA more than 100 μs and that this long lifetime is at the very origin of the long-range hole transfer observed in DNA.

Supporting Information Available: Experimental conditions for pulse radiolysis, synthesis, and characterization of PyODN, and γ-radiolysis of for PyODN (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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