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Hole transfer through DNA monitored by transient absorption of phenothiazine radical cation

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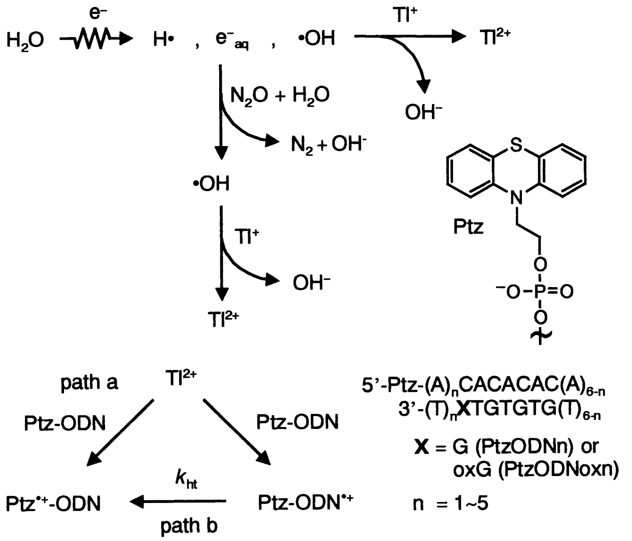
Abstract—The hole transfer through DNA was monitored by the transient absorption of radical cation of phenothiazine ($Ptz^{+\bullet}$). The occurrence of hole transfer from $G^{+\bullet}$ generated in DNA to Ptz was observed in the time range of 100 μs. The hole transfer to Ptz was significantly suppressed by introducing oxG as a hole trap in DNA, and the trapping reaction of $oxG^{+\bullet}$ by water was suggested to occur with the rate faster than $\sim 10^5\ s^{-1}$. © 2001 Elsevier Science Ltd. All rights reserved.

DNA-mediated electron and hole transfer is noticed for its possibility of applications as biosensors and bioelectronic devices.[1] Especially, the behavior of the hole in DNA has been extensively studied since the reaction of the hole with either oxygen or water causes mutation which leads to diseases.[2] The distance and thermodynamic driving force (ΔG) dependences of single-step photo-induced electron transfer in DNA have been reported in detail in previous reviews by Lewis et al.[3] However, there are a few reports on the spectroscopic measurement for hole transfer process in DNA occurring after the hole generation in DNA.[4] We have recently reported a new method for the study of hole transfer through DNA, which is a combination of pulse radiolysis and site specific labeling of oligonucleotide (ODN) with pyrene.[5] Since the hole is irreversibly generated in DNA and free from charge recombination, our system provides a unique system for the observation of the processes over 1 μs. Hole transfer through DNA was investigated by monitoring the transient absorption of radical cation of pyrene ($pyrene^{+\bullet}$) during pulse radiolysis of pyrene-conjugated ODN. The results demonstrated a distance dependence of the hole transfer from G radical cation ($G^{+\bullet}$) generated in ODN to pyrene and the hole transfer through DNA over the period of 100 μs was revealed. Here, to investigate the driving force dependence of the hole transfer in DNA, pulse radiolysis of phenothiazine (Ptz)-conjugated ODN was measured.

The radical cation of Ptz ($Ptz^{+\bullet}$) has a distinct absorption peak at $\lambda_{max} = 520$ nm with a large molar extinction coefficient ($\varepsilon = 9\times10^3\ M^{-1}\ cm^{-1}$).[6] 5′-Ptz-conjugated ODNs (PtzODNn) were synthesized by automated solid-phase phosphoramidite method as reported.[7] Introduction of Ptz at a terminal site caused an increase in T_m for PtzODN1 ($T_m = 35.3$°C) compared to



Scheme 1. Kinetic scheme for generation of oxidizing reagent Tl^{2+} during pulse radiolysis, hole generation and transfer in Ptz-conjugated ODN. Sequences and chemical structure of 5′-Ptz-conjugated ODN.

Keywords: DNA; hole transfer; pulse radiolysis; phenothiazine.

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unmodified ODN1 ($T_m = 33.5°C$). This stabilization was attributed to end-capping with π-electron overlap between the Ptz and bases.[8] The oxidation potential of Ptz ($E^0 = 0.76$ V versus NHE in CH_3CN)[7] is lower than those of pyrene ($E^0 = 1.40$ V versus NHE in CH_3CN) and G ($E^0 = 1.47$ V versus NHE in CH_3CN).[5] In addition, the oxidation potential of Ptz is even lower than that of 8-oxo-7,8-dihydroguanine (oxG: $E^0 = 1.09$ V versus NHE in CH_3CN). Therefore, hole transfer from both $G^{+\bullet}$ and $oxG^{+\bullet}$ to Ptz is expected to occur, in contrast to the pyrene-conjugated ODN where hole transfer from $pyrene^{+\bullet}$ to oxG was observed.

Since the oxidation of Ptz moiety occurs with simply adding $K_2S_2O_8$, Tl_2SO_4 was used as an oxidant source instead of $K_2S_2O_8$ in this study.[9] Oxidizing reagent Tl^{2+} was generated during pulse radiolysis (28 MeV, 8 ns) of N_2O-saturated (~22 mM) aqueous solution containing 2 mM Tl_2SO_4, 20 mM pH 7.0 Na phosphate buffer, and 0.2 mM (strand conc.) ODN. The hydrated electrons (e^-_{aq}) react with N_2O to give $^\bullet OH$, and Tl^{2+} was produced within 20 ns by the reaction of Tl^+ with $^\bullet OH$. Since Tl^{2+} is powerful enough to oxidize all four DNA bases ($E^0 = 2.2$ V versus NHE in H_2O), random oxidation of DNA whole strand is expected. However, the hole was trapped by G with the lowest oxidation potential among the four DNA bases. Thus, the hole was generated in PtzODN from the one-electron oxidation with Tl^{2+} to give $G^{+\bullet}$ or $Ptz^{+\bullet}$ (Scheme 1).

The distance dependence of hole transfer was investigated systematically by changing the number of A-T base pairs separating the Ptz and G-region. A transient absorption with a peak at 410 nm assigned to Tl^{2+} was observed after the electron pulse during the pulse radiolysis (Fig. 1). Together with decay of Tl^{2+}, formation of the transient absorption with a maximum peak at 520 nm assigned to $Ptz^{+\bullet}$ was observed. In the case of PtzODN1, formation rate of $Ptz^{+\bullet}$ and decay rate of Tl^{2+} observed were almost same (Fig. 2A). Therefore, hole transfer from $G^{+\bullet}$ to Ptz through DNA is faster than the diffusional process for PtzODN1 ($k_{ht} > k_{obs} = 1.4\times10^5\ s^{-1}$). After completion of the diffusional process within 20 µs, that is after the consumption of Tl^{2+} and formation of $Ptz^{+\bullet}$ (path a) or $G^{+\bullet}$, secondary formation of $Ptz^{+\bullet}$ was observed for PtzODN3 in the time scale of 100 µs (Fig. 2B). This secondary formation of $Ptz^{+\bullet}$ was attributed to the hole transfer from $G^{+\bullet}$ to Ptz through DNA (Scheme 1, path b). The time profile of PtzODN3 was fitted by double exponentials. The faster component was assigned to the direct oxidation of Ptz by path a, while the slower component was assigned to the hole transfer process by path b. This gave the rate constant of the hole transfer $k_{ht} = 2\times10^4\ s^{-1}$ for PtzODN3. No secondary formation was observed for PtzODN5, which shows that hole transfer for this ODN proceeds much more slowly. As a consequence, the yield of $Ptz^{+\bullet}$ was the lowest for PtzODN5 (Table 1).[5]

Next, in order to determine the driving force dependence of the hole transfer dynamics in DNA, pulse radiolysis of Ptz-conjugated ODNs with one oxG sub-

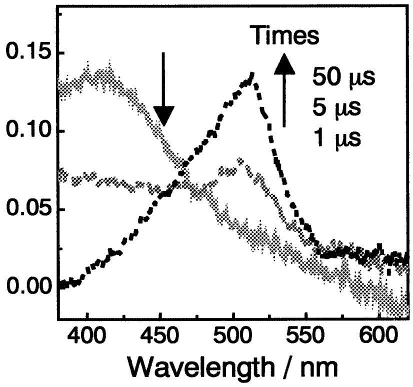


Figure 1. Transient absorption spectra of PtzODN1 obtained at 1, 5, and 50 µs during pulse radiolysis of N_2O-saturated aqueous solution containing 0.2 mM PtzODN1 (strand. conc.), 20 mM pH 7.0 Na phosphate buffer, and 1 mM Tl_2SO_4.

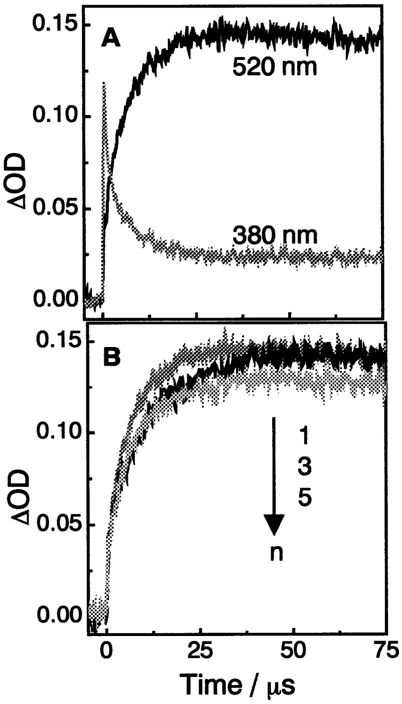


Figure 2. (A) Time profiles of transient absorption monitored at 520 and 380 nm for PtzODN1. (B) Formation of the transient absorption of $Ptz^{+\bullet}$ observed at 520 nm for PtzODN1, PtzODN3 and PtzODN5.

Table 1. ΔOD_{520} at 100 µs and observed reaction rate constants for oxidation of PtzODN with Tl^{2+} monitored at 520 nm

ODN	ΔOD_{520}	k_{obs} ($10^5\ s^{-1}$)
PtzODN1	0.14	1.4
PtzODN3	0.14	1.4, 0.2
PtzODN5	0.12	1.3

stituted for G (PtzODNoxn) as a hole trap was performed. The yield of $Ptz^{+\bullet}$ of PtzODNoxn was lower than that of PtzODN1 (Fig. 3, Table 2). The number (n) of intervening A-T base pairs between the Ptz and oxG showed little effect on the yield and formation rate of $Ptz^{+\bullet}$. These results demonstrate that the hole transfer from the hole generated in ODNoxn region to Ptz did not occur in the case of PtzODNoxn. Apparent hole transfer kinetics in solution is determined by a competition between hole transfer through DNA and irreversible trapping of radical cation (Scheme 2).[2,10] Therefore, the reaction of $oxG^{+\bullet}$ with water is faster than the hole transfer from $oxG^{+\bullet}$ to Ptz, and the rate is suggested to be faster than $\sim 10^5$ s^{-1}.

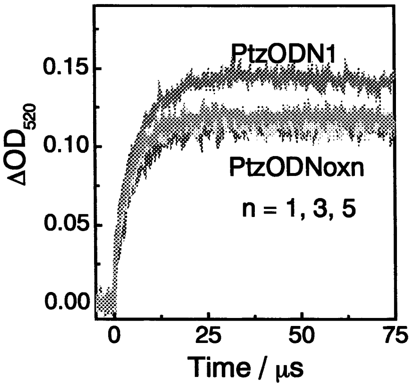
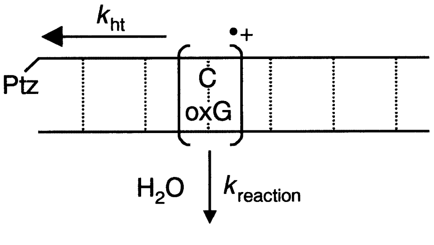


Figure 3. Time profiles of transient absorption monitored at 520 nm for PtzODN1, and PtzODNoxn ($n=1$, 3, 5). Three time profiles were overlapped for PtzODNoxn ($n=1$, 3, 5).

Table 2. ΔOD_{520} at 100 μs and observed reaction rate constant for oxidation of PtzODNoxn with Tl^{2+} monitored at 520 nm

ODN	ΔOD_{520}	k_{obs} (10^5 s^{-1})
PtzODNox1	0.11	1.8
PtzODNox2	0.11	2.0
PtzODNox3	0.11	2.0
PtzODNox4	0.11	2.0
PtzODNox5	0.12	2.0



Scheme 2.

In the present study, the hole transfer through DNA was investigated by monitoring the formation of $Ptz^{+\bullet}$. Our results suggest that the reaction rate of $oxG^{+\bullet}$ and water ($>10^5$ s^{-1}) is much faster than that of $G^{+\bullet}$ and water ($<10^4$ s^{-1}).[5] Thus, not only the low oxidation potential of oxG but also the high reactivity $oxG^{+\bullet}$ with water may contribute on the depth of oxG as a hole sink in DNA.[11]

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