Excess Electron Transfer in DNA Studied by Pulse Radiolysis and γ-Radiolysis of Naphthalimide and Iodouridine Modified ODN

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An excess electron was attached to naphthalimide (NI) modified 12-mer oligodeoxynucleotides (ODNs) and the electron transfer in DNA was investigated by monitoring the transient absorption of NI radical anion ($NI^{\bullet -}$) during the pulse radiolysis. Formation of the transient absorption of $NI^{\bullet -}$ was observed according to the reaction of e_{aq}^- with NI-modified ODN. Only 25% of e_{aq}^- reacting with NI-modified ODN were observed by the transient absorption of $NI^{\bullet -}$, which corresponds to electron transfer over no more than three base pairs, suggesting a low mobility of an electron attached to DNA. Electron transfer in DNA was also studied by γ-radiolysis of ODN containing 5-iododeoxyuridine (^{I}U) as a second electron acceptor. Electron transfer in DNA was estimated by the protection of dehalogenation of ^{I}U offered by NI during the γ-radiolysis of NI- and ^{I}U-modified ODNs where the spacing between the NI and ^{I}U was varied. The protection effect became very low by the insertion of three or four A−T base pairs between NI and ^{I}U. The results driven from both pulse radiolysis and γ-radiolysis experiments were consistent with the low mobility of an excess electron in DNA, which is in strong contrast to the occurrence of the long-range hole transfer in DNA.

Introduction

Generation and motion of electrons and holes in DNA have attracted considerable interest, because they are related to mutation and strand scission of DNA, which lead to carcinogenesis and aging.[1,2] Furthermore, DNA-mediated electron transfer is interesting from the viewpoint of using DNA as a building block for nanoelectronic and biosensoric devices.[3] The kinetics of the hole transfer in DNA has been well established, and the single step hole transfer rate constants from G to GG, and GG to G in DNA have been determined by Lewis et al. to be in the range of 10^6-10^8 s^{-1}.[4] Recently, we have demonstrated the occurrence of hole transfer in DNA over the period of 100 μs on the basis of the pulse radiolytic study of pyrene- and phenothiazine-modified oligodeoxynucleotides (ODNs).[5−9] Direct observation of the hole transfer in DNA has been also reported by Barton et al.[10] and Shafirovich et al.[11] on the basis of transient absorption measurements. On the other hand, there are few direct transient spectroscopic observations of the excess electron transfer in DNA.

The excess electron transfer in DNA has been mainly studied by radiation chemistry using the reactions of the solvated electron (e_{aq}^-) with DNA during radiation-induced reactions. Excess electron transfer in DNA in glasses, ices, and solids at low temperatures (from 4 to 195 K) has been studied by Sevilla et al. during the γ-radiolysis.[12−16] They have reported the occurrence of single-step tunneling with overall distance decay constant β of approximately 0.9 $Å^{-1}$. As for the experiments at room temperature, the electron transfer from the DNA radical anion to the randomly intercalated electron acceptors has been investigated during the pulse radiolysis.[17−19] However, in those studies, noncooperative random binding of the electron acceptors to DNA is a prerequisite, and contribution of the unbounded free electron acceptors should be also taken into account. Furthermore, since radical anion of the electron acceptor is hydrophilic compared to the unreduced form, it is necessary to give consideration to the dissociation of the electron acceptor radical anion from the hydrophobic base stacks in DNA, which leads to increase the absorbance due to the recovery of hypochromism. Here, to overcome these ambiguities, we synthesized ODNs covalently bonded with naphthalimide (NI) as an electron acceptor at the defined position. The electron transfer from the DNA radical anion to NI was investigated by monitoring the transient absorption of radical anion of NI ($NI^{\bullet -}$) during pulse radiolysis of the NI-modified ODNs at 23 °C. The excess electron transfer in DNA has been also studied by γ-radiolysis experiments using bromouracil as an electron acceptor.[20,21] Therefore, 5-iododeoxyuridine (^{I}U) was incorporated to ODN as a second electron acceptor in which elimination of iodine ion and formation of uracil radical occur with the electron attachment. Several ODNs with different distances between NI and ^{I}U were synthesized and the electron transfer in DNA was investigated from the protection of the reaction of ^{I}U by NI during the γ-radiolysis of NI- and ^{I}U-modified ODNs at 0 °C. It has been demonstrated that, in contrast to the occurrence of the long-range hole transfer up to 200 Å[22−26] over a period of 100 μs[5] in DNA, excess electron transfer occurs for only three or four base pairs and accomplishes within 1 μs in DNA.

Experimental Section

Measurement of Melting Temperature. Thermal denaturation profiles were obtained with Jasco V-530 spectrophotometer equipped with a Peltier temperature controller. Absorbance of the ODN sample (4 μM duplex in 20 mM phosphate buffer (pH 7.0)) was monitored at 260 nm (A_{260}) from 10 to 70 °C with a heating rate of 1 °C min^{-1}. The T_m value was determined as the maximum in a plot of $\Delta A_{260}/\Delta T$ versus temperature.

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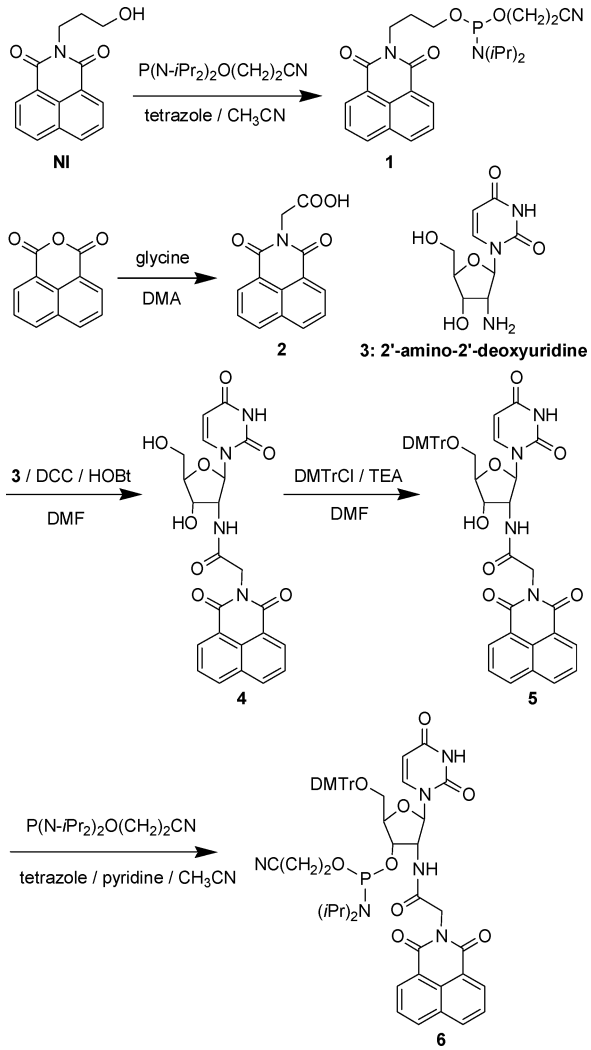
Pulse Radiolysis. An excess electron in DNA was generated from electron attachment during the pulse radiolysis (28 MeV, 8 ns, 0.7 kGy pulse^{-1}) of Ar-saturated aqueous solution containing 100 mM *t*-BuOH and 20 mM Na phosphate buffer (pH 7.0), and ODN at 23 °C. A xenon flash lamp (Osram, XBO-450), which was synchronized with the electron pulse, was focused through the sample as a probe light for the transient absorption measurement. Time profiles of the transient absorption were measured with a monochromator (CVI, DK-240) equipped with a photomultiplier (Hamamatsu Photonics, R928) and digital oscilloscope (Tektronix, TDS 580D). For the time-resolved transient absorption spectral measurement, the monitor light was focused into a quartz optical fiber, which transported the light to a gated-multichannel spectrometer (Unisoku, TSP-601−02).

γ-Radiolysis. Aliquots (10 μL) of oligonucleotide solutions containing 100 mM *t*-BuOH, 20 mM Na phosphate buffer (pH 7.0), and 0.1 mM ODN (unless otherwise stated) in 0.5-mL tubes were irradiated with ^{60}Co γ-ray for 1.8 kGy at 0 °C and subjected to HPLC analysis.[27] The reaction of $^{\mathrm{I}}$U was determined as the average of three measurements.

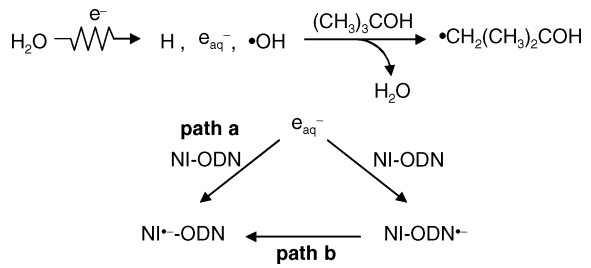
Results and Discussion

For direct observation of an excess electron generated in DNA, we synthesized NI-modified ODNs, since radical anion of NI (NI$^{\bullet-}$) has a large molar extinction coefficient ($\epsilon_{400} \approx 4 \times 10^4$ M^{-1} cm^{-1}) at a distinct absorption peak ($\lambda_{max} = 400$ nm) (Scheme 1). Introduction of NI at 5′-end of ODN (ODN **8**) and 2′ sugar position of uridine (ODN **10**) caused an increase in T_m compared to that of unmodified ODN (ODN **7**). Thus, NI was attached to DNA without disturbance of the duplex stability. Because the reduction potential of NI ($E_{red} = -0.84$ V vs NHE in H_2O)[28] is less negative than those of C or T ($E_{red} \approx -1.1$ V vs NHE in H_2O),[29] electron transfer from T$^{\bullet-}$ and C$^{\bullet-}$ to NI is expected to occur. An excess electron in DNA was generated from electron attachment during the pulse radiolysis, and the electron transfer in DNA was investigated by monitoring the transient absorption of NI$^{\bullet-}$ (Scheme 2). Time-resolved absorption spectra observed during the pulse radiolysis of ODN **8** were shown in Figure 1. Concomitant with the decay of the broad transient absorption of e_{aq}^-, formation of the transient absorption was observed with a maximum peak at 400 nm, which was assigned to NI$^{\bullet-}$ (Figure 1, inset). Formation of NI$^{\bullet-}$ was accomplished within 1 μs, and NI$^{\bullet-}$ was observed to 500 μs and out to that time it was stable. NI$^{\bullet-}$ can be formed by two processes, direct electron attachment at NI (Scheme 2, path a) and electron transfer from T$^{\bullet-}$ and C$^{\bullet-}$ generated in the ODN moiety to NI (path b). To clarify the electron-transfer process in DNA, pulse radiolyses of ODN **7** and **8** were examined at several ODN concentrations. The decay of e_{aq}^- and formation of NI$^{\bullet-}$ were monitored by the transient absorption at 630 and 400 nm, respectively, and analyzed by the first-order rate equation to give the decay rate constant of k_{630} for e_{aq}^- and the formation rate constant of k_{400} for NI$^{\bullet-}$. A linear dependence of k_{630} on the ODN concentration was observed for all ODNs studied here, and k_{630} was in good agreement with k_{400} (Table 1). These results suggest that either electron transfer occurs from nucleotide radical anion to NI conjugated at the 5′-end faster than the diffusional collision process between e_{aq}^- and ODN (Scheme 2, path b), or the low contribution of the electron transfer from the nucleotide radical anion to NI, that is, NI$^{\bullet-}$ forms mainly from direct collisonal process (Scheme 2, path a). The bimolecular rate constant of e_{aq}^- with ODN (k_e) was obtained from the slope of the linear

SCHEME 1



SCHEME 2



plots of k_{630} versus [ODN]. The value of k_e for ODN **8** was similar to that for unmodified ODN **7**, suggesting that the electron attachment occurs competitively at NI and ODN moiety for ODN **8**. This is consistent with the result of pulse radiolysis of the 1:1 mixture of ODN **8** and unmodified ODN **7** (Table 1, ODN **9**) where the yield of NI$^{\bullet-}$ ($\Delta\Delta OD_{400}$) decreased by half though similar k_{630} was observed compared to that of ODN **8** alone. The yield of e_{aq}^- reacted with ODN (ϕ) is described by eq 1

$$\phi = k_e[\mathrm{ODN}]/(k_e[\mathrm{ODN}] + k_{buffer}) \qquad (1)$$

where k_{buffer} is the decay rate of e_{aq}^- in the absence of ODN. Taking the ϵ of NI$^{\bullet-}$ ($\epsilon_{400} \approx 4 \times 10^4$ M^{-1} cm^{-1}) and e_{aq}^- ($\epsilon_{630} \approx 2 \times 10^4$ M^{-1} cm^{-1}) into account, only 25% of the electrons reacted with NI−ODN were observed as the transient

TABLE 1: Melting Temperatures (T_m), Decay Rate Constant of e_{aq}^- (k_{630}), Formation Rate Constant of $NI^{\bullet-}$ (k_{400}), Bimolecular Rate Constant of e_{aq}^- with ODN (k_e), and the Yield of $NI^{\bullet-}$ ($\Delta\Delta OD_{400}$)

	ODNs	T_m (°C)	k_{630} (S^{-1})[a]	k_{400} (S^{-1})[a]	k_e (M^{-1} S^{-1})[b]	$\Delta\Delta OD_{400}$ at 2 μs[a]
7	$d(AC)_6/d(TG)_6$	42	4.2×10^6		1.5×10^{10}	
8	NI-$d(AC)_6/d(TG)_6$	45	3.8×10^6	3.6×10^6	1.3×10^{10}	0.21
9	**7** + **8**[c]		3.7×10^6	3.8×10^6		0.11
10	$d(AC)_6/d(TG)_2GU_{NI}(TG)_3$	46				

[a] From th pulse radiolysis of Ar-saturated aqueous solutions containing 100 mM *t*-BuOH, 20 mM phosphate buffer (ph 7.0), and 0.2 mM ODN. [b] Determined from the linear plot of k_{630} vs [ODN] in the range of 0.05−0.2 mM. [c] Mixture (1:1) of **7** and **8**.

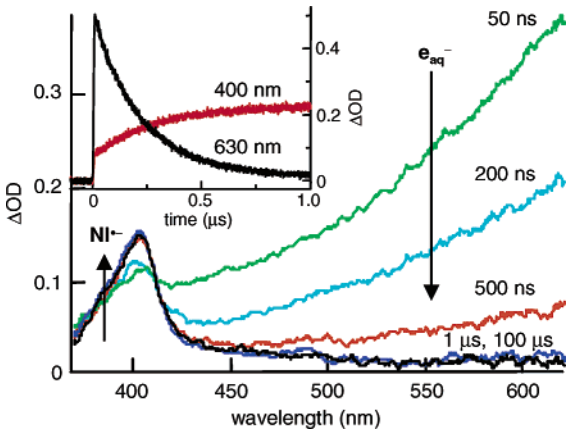


Figure 1. Transient absorption spectra observed at various times after an electron pulse during the pulse radiolysis of ODN **8**. The inset shows the time profiles of the transient absorption monitored at 630 and 400 nm. The peak at 400 nm is assigned to $NI^{\bullet-}$, while the broad band over 400 nm is to e_{aq}^-.

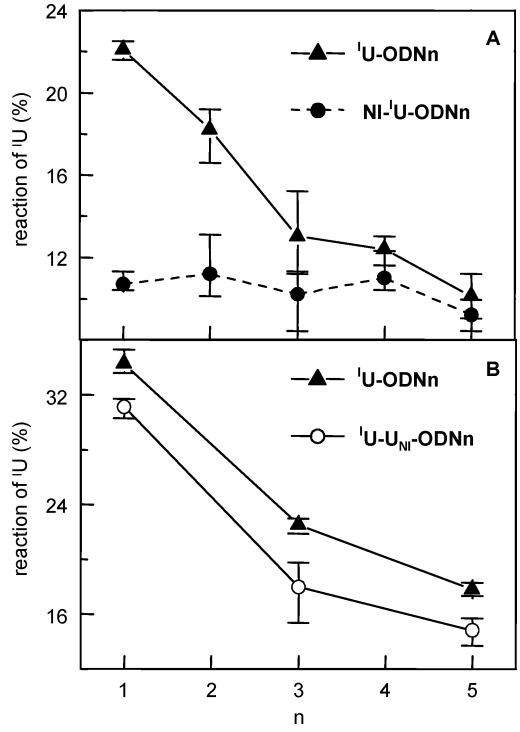
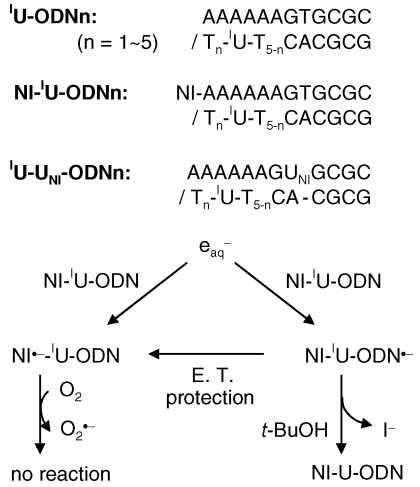


Figure 2. Plots of reaction yield of ^{I}U vs *n* during the γ-radiolysis of aqueous solutions of (A) **^{I}U−ODNn** and **NI−^{I}U−ODNn**, (B) **^{I}U−ODNn** and **^{I}U−U_{NI}−ODNn**.

absorption of $NI^{\bullet-}$. This value corresponds to the electron attached at three base pairs (25% of 12-mer ODN), assuming nonselective and random electron attachment to NI−ODN. Thus, the mean electron-transfer distance can be estimated as less than three base pairs. These results suggest a low contribution of the electron-transfer process on the formation of $NI^{\bullet-}$, and a low mobility of the attached electron in DNA.

SCHEME 3



To further investigate the electron transfer in DNA, ^{I}U was incorporated to ODN as a second electron acceptor. The reactivity of ^{I}U in the absence of NI was compared with that in the presence of NI, and the electron transfer in DNA was discussed in terms of the protection effect of NI on the deiodination of ^{I}U during the γ-radiolysis of doubly NI- and ^{I}U-modified ODN (Scheme 3). The mobility of the attached electron in DNA was examined systematically by changing the number of A−T base pairs (*n*) separating the ^{I}U and NI moieties (**NI−^{I}U−ODNn**, **^{I}U−U_{NI}−ODNn**). γ-Irradiated ODNs were digested with snake venom phosphodiesterase/nuclease P1/alkaline phosphatase and the consumption of ^{I}U was quantified by HPLC (Figure 2).[30] For the ODN containing only ^{I}U (**^{I}U−ODNn**), higher reactivity was observed for ^{I}U incorporated at the end of ODN, and the reactivity of ^{I}U decreased with increasing the distance from the end of ODN to the ^{I}U moiety. These results demonstrate a relatively favorable electron attachment at the end of ODN, probably due to the unfavorable electron repulsion between phosphate group of ODN and e_{aq}^- at the side of ODN. In the presence of NI, deiodination of ^{I}U was suppressed, and the protection effect of NI was calculated according to eq 2

$$\text{Protection effect (\%)} = (1 - (\text{deiodination of } ^I\text{U in the presence of NI})/(\text{deiodination of } ^I\text{U in the absence of NI})) \times 100 \quad (2)$$

Interestingly, highest suppression effect of NI at the 5′-end was observed for $n = 1$ ODN and the protection effect greatly decreased as the distance between ^{I}U and NI increased (Figure 3). When NI was incorporated at the inside of ODN (**^{I}U−U_{NI}−ODNn**), the protection effect of NI was the lowest for $n = 1$, demonstrating that the distance between NI and ^{I}U plays a crucial role on the protection effect.[31] In both series of ODNs, the protection effect became very low by the insertion of three or four A−T base pairs between NI and ^{I}U. Thus, NI exerts its

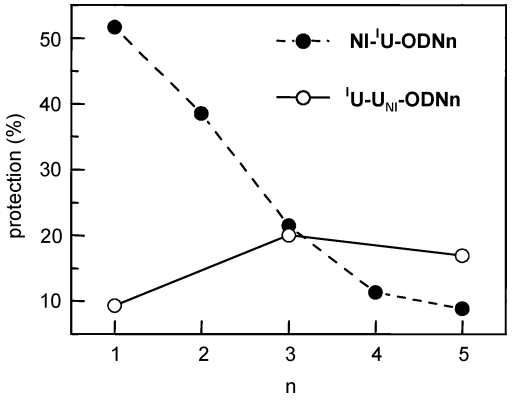


Figure 3. Plots of the protection of the reaction of ^{I}U by NI vs *n* during the γ-radiolysis of aqueous solutions of **NI**−^{I}**U**−**ODNn** and ^{I}**U**−$\mathbf{U_{NI}}$−**ODNn**.

influence across only three or four base pairs, suggesting the low mobility of excess electron in DNA. Our results further confirmed the results driven from Sevilla et al.[12−16] and early γ-radiolysis experiments using bromouracil as an electron acceptor[20,21] in which low mobility of electrons in DNA has been demonstrated.

The measured short migration distances of the attached electron might be explained in part by fast irreversible trapping of electrons, due to irreversible protonation of $T^{\bullet-}$ or $C^{\bullet-}$, in contrast to the long lifetime of $G^{\bullet+}$ or deprotonated $G^{\bullet}$ in DNA, which plays an important role in long-range hole transfer in DNA.[5] Although the reaction of $T^{\bullet-}$ and $C^{\bullet-}$ has been well studied at low temperature,[32−34] there are few reports on the kinetics of the reaction of $T^{\bullet-}$ and $C^{\bullet-}$ in DNA at room temperature.[35] Thus, the lifetime of $T^{\bullet-}$ or $C^{\bullet-}$ in DNA at physiological temperature should be addressed in the future study.

Conclusions

In the present study, the distance dependence of excess electron transfer in DNA following one electron reduction of DNA was investigated in detail using both pulse radiolysis (transient absorption measurements) and γ-radiolysis (product analysis) of NI- and ^{I}U-modified ODNs. Formation of $NI^{\bullet-}$ according to the decay of e_{aq}^{-} was observed during the pulse radiolysis of NI-modified ODN, while no secondary formation build-up in the transient absorption of $NI^{\bullet-}$ was observed after the consumption of e_{aq}^{-}. It may be possible to assume that the electron-transfer reactions occur faster than that observed during the pulse radiolysis experiments but are not detected due to the time taken for the bimolecular reaction of e_{aq}^{-} with DNA. However, the yield of $NI^{\bullet-}$ was only 25% of the electron reacted with NI−ODN, which corresponds to the electron attached at three base pairs. Thus, electron transfer was observed over no more than three base pairs. Similar results were also obtained from the γ-radiolysis experiments where NI exerts its effect as an electron acceptor for only three or four base pairs. The results clearly demonstrated the low mobility of the excess electron in DNA which is in strong contrast to the occurrence of the long-range hole transfer in DNA.

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Supporting Information Available: Synthesis of naphthalimide and iodouridine modified ODN (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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