Pergamon

Tetrahedron Letters 43 (2002) 8083–8085

TETRAHEDRON LETTERS

Regulation of one-electron oxidation rate of guanine and hole transfer rate in DNA through hydrogen bonding

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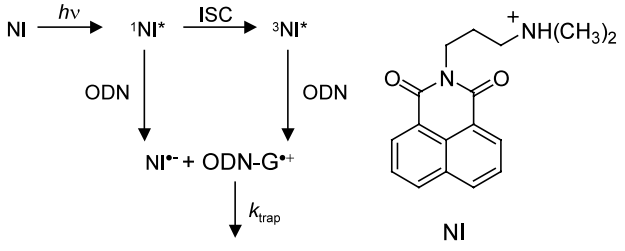
Received 17 June 2002; revised 7 August 2002; accepted 10 September 2002

Abstract—The effects of methyl and bromo groups at C5 of C on the one-electron oxidation rate of G, and on the hole transfer rate in DNA have been investigated. The rates of one-electron oxidation of G and hole transfer from $Py^{\bullet+}$ to 8-oxo-7,8-dihydroguanine were suppressed and enhanced by introducing bromo and methyl groups, respectively, on the C at the complementary strand.

One-electron oxidation of DNA leads to formation of the radical cation of G ($G^{\bullet+}$), the nucleobase with the lowest ionization potential, which migrates via a multi-step hopping in DNA. In the last 5 years, occurrence of the long range hole transfer through DNA up to 200 Å has been demonstrated by Giese,[1] Schuster[2] and Barton.[3] If the hole flow in DNA could be artificially controlled to deposit at the desired site in DNA, it may enable site-selective oxidation and strand scission of DNA which is desired from a therapeutical point of view. Furthermore, the regulation of the transfer rate and direction of the hole generated in DNA is of interest from the perspective of using DNA as a building block for electronic devices.[4] Several G derivatives with different ionization potential have been demonstrated to be useful in making an ionization potential gradient in DNA.[5] Since ionization potential of G is highly dependent on the flanking sequences, it is also possible to design a DNA sequence in which hole flows in one direction.[6–9] Recently, from the experiment in dichloromethane, we have demonstrated that the oxidation potential of G can be controlled by introducing a substituent on base pairing cytosine (C).[10,11] When a methyl group was introduced as an electron donating group at C5 of cytosine (mC), the one-electron oxidation rate of G in G:mC base pair was accelerated. On the other hand, bromo substituent on C as an electron withdrawing group (brC) lead to the suppression of the one-electron oxidation rate of G in G:brC base pair. Here, in order to regulate the hole transfer rate and ultimate oxidation site in DNA, the effects of methyl and bromo groups at C5 of C on the one-electron oxidation rate of G, and the hole transfer rate in DNA have been investigated.



Scheme 1. Chemical structure of NI and mechanistic scheme for photosensitized one-electron oxidation of ODN.

13-Mer oligodeoxynucleotides (ODNs) containing C (ODN-C), mC (ODN-mC), or brC (ODN-brC) as complementary base for G were synthesized. For the one-electron oxidation of G, naphthalimide (NI) was selected as a photosensitizer (Scheme 1).[9,12–14] In the presence of NI, ODN was photo-irradiated with a high-pressure Hg lamp. A 330 nm cutoff filter was used so that all the light was absorbed by the sensitizer but not by the ODN. After photo-irradiation, photooxidized ODNs were digested with sv P. D. E./P1 nuclease/AP and the consumption of G was quantified by HPLC (Table 1).[15] Interestingly, lower consumption of G was observed for ODN-brC compared with those of ODN-C and ODN-mC. The one-electron oxidation of

Keywords: DNA; hydrogen bonding; one-electron oxidation; hole transfer.

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0040-4039/02/$ - see front matter
PII: S0040-4039(02)01949-4

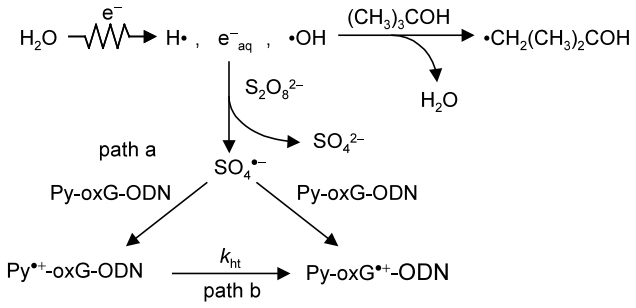
Table 1. Consumption of G in the photosensitized one-electron oxidation of ODNs[a]

ODN	Sequence	−G (%)
ODN-mC	5′-TGTGTGTGTGTGT 3′-AmCAmCAmCAmCAmCAmCA	4.7
ODN-C	5′-TGTGTGTGTGTGT 3′-ACACACACACACA	5.2
ODN-brC	5′-TGTGTGTGTGTGT 3′-AbrCAbrCAbrCAbrCAbrCAbrCA	1.4

[a] Each of the reaction mixtures containing ODN (0.04 mM strand conc.) and NI (0.1 mM) in pH 7.0 sodium cacodylate buffer (20 mM) was photo-irradiated with a high-pressure Hg lamp fitted with a 330 nm cutoff filter for 20 min. The reaction mixture was directly subjected to enzymatic digestion with snake venom P. D. E., P1 nuclease and alkaline phosphatase and the consumption of G was quantified by reverse phase HPLC.

G can occur either with NI in the singlet excited state (^{1}NI*) or with NI in the triplet excited state (^{3}NI*).[13,14] One-electron oxidation process via ^{1}NI* was studied by steady-state fluorescence quenching experiment (Fig. 1A). G selectively quenched the fluorescence intensity of NI, while moderate quenching was observed for C, mC, and brC with a similar extent. Triplet quenching experiments clearly show the selective quenching of ^{3}NI* by G as shown in Fig. 1B. These results preclude the efficient quenching of either ^{1}NI* or ^{3}NI* by brC compared to those of C. Thus, it is clearly demonstrated that the oxidation potential of G is higher in G:brC base pair than in G:C base pair in DNA. Slightly lower consumption of G in the case of ODN-mC compared to that of ODN-C may be explained by the alteration of the interaction between NI and ODN due to the methyl group at cytosine C5. Since methyl and bromo groups are similar in size, such a mechanism may also contribute to the lower consumption of G observed in the case of ODN-brC to some extent.

Next, in order to regulate the hole transfer in DNA, pulse radiolyses of pyrene (Py) and 8-oxo-7,8-dihydroguanine (oxG) containing ODNs with C (PyODN-C), mC (PyODN-mC), or brC (PyODN-brC) as the complementary base for oxG were performed. Since the oxidation potential of pyrene ($E°=1.40$ V versus NHE in CH_3CN) is higher than that of oxG ($E°=1.09$ V versus NHE in CH_3CN), hole transfer occurs from pyrene radical cation ($Py^{\bullet+}$) to oxG.[16] $Py^{\bullet+}$ was generated from one-electron oxidation during the pulse radiolysis in the presence of $K_2S_2O_8$, and the hole transfer from $Py^{\bullet+}$ to oxG in DNA was monitored by the time-resolved transient absorption of $Py^{\bullet+}$ as has been previously reported (Scheme 2).[16,17] Decays of $Py^{\bullet+}$, which correspond to the hole transfer from $Py^{\bullet+}$ to oxG (Scheme 2 path b), were single exponential and the rate constants (k_{obs}) were listed in Table 2. Slightly faster and slower hole transfer rates were observed for PyODN-mC and PyODN-brC, respectively, compared to PyODN-C. It is demonstrated that the hole transfer rate in DNA can be regulated by introducing methyl and bromo groups at the C5 of C.



Scheme 2. Mechanistic scheme for generation of oxidizing reagent $SO_4^{\bullet-}$ during pulse radiolysis, hole generation and transfer in Py and oxG containing ODN.

Differences between the oxidation potentials of G:C base pair and G:mC or G:brC were estimated to be 0.01–0.03 V.[10] In the case of the hole transfer from $Py^{\bullet+}$ to oxG studied here, the high driving force (ca. −0.4 V) lead to the moderate effect of methyl and bromo groups on the hole transfer rate. However, these substituents may exert significant effects on the equilibrium between $G^{\bullet+}$:C and $G^{\bullet+}$:mC, and $G^{\bullet+}$:brC in DNA (Scheme 3).[18–20]

In the present study, suppression of the one-electron oxidation rate of G in the G:brC base pair, and the acceleration and suppression of hole transfer rate from $Py^{\bullet+}$ to 8-oxo-7,8-dihydroguanine was demonstrated by introducing methyl and bromo groups on C, respec-

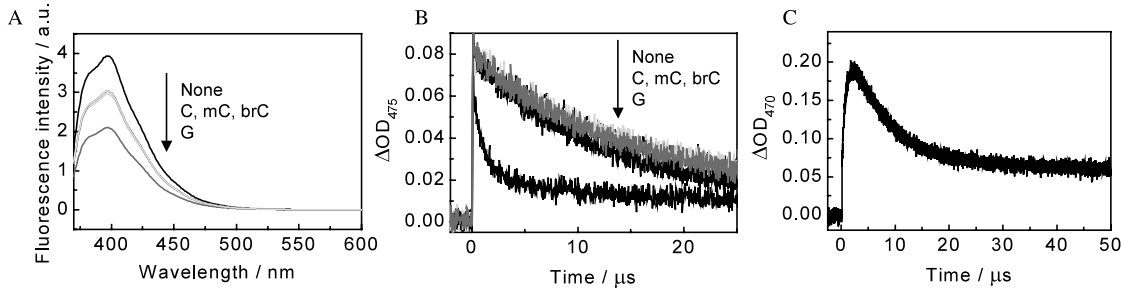
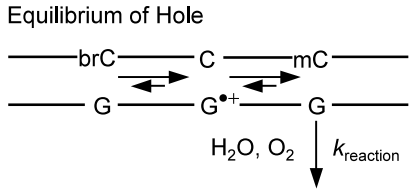


Figure 1. (A) Fluorescence quenching ($\lambda_{ex}=360$ nm) of NI (5 μM) by C, mC, brC and G (8 mM). (B) Time profiles of transient absorption monitored at 475 nm upon 355 nm excitation of Ar-saturated aqueous solution of NI (40 μM) in the presence of C, mC, brC and G (5 mM), respectively. (C) Time profiles of transient absorption monitored at 470 nm for PyODN-C during pulse radiolysis. The measurements conditions were shown in Table 2.

Table 2. Observed rate constant of the hole transfer (k_{obs}) from $Py^{\bullet+}$ to oxG for Py and oxG containing ODNs[a]

ODN	Sequence	k_{obs} (10^4 s^{-1})
PyODN-mC	5′-Py-AAmCACACACAAAA 3′-TToxGTGTGTGTTTT	13
PyODN-C	5′-Py-AACACACACAAAA 3′-TToxGTGTGTGTTTT	11
PyODN-brC	5′-Py-AAbrCACACACAAAA 3′-TToxGTGTGTGTTTT	9.7

[a] Pulse radiolysis was carried out in an Ar-saturated aqueous solution containing 10 mM $K_2S_2O_8$, 100 mM *t*-BuOH, 50 mM pH 7.0 Na phosphate buffer, and 0.2 mM (strand conc.) ODN.



Scheme 3. Schematic representation of hole transfer, site selective oxidation, and strand scission.

tively. Our results suggest that the direction of the hole transfer and ultimate oxidation site in DNA may be controlled by introducing a substituent on cytosine.

Acknowledgements

We thank the members of the Radiation Laboratory of SANKEN, Osaka University for running the linear accelerator. This work has been partly supported by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, Sport and Culture of Japan.

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