J|A|C|S
ARTICLES
Published on Web 07/29/2006

Photoinduced Reductive Repair of Thymine Glycol: Implications for Excess Electron Transfer through DNA Containing Modified Bases

Takeo Ito,* Akiko Kondo, Satoru Terada, and Sei-ichi Nishimoto*

Contribution from the Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Kyoto Daigaku Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

Received February 23, 2006; E-mail: takeoit@scl.kyoto-u.ac.jp; nishimot@scl.kyoto-u.ac.jp

Abstract: Photoinduced reduction of thymine glycol in oligodeoxynucleotides was investigated using either a reduced form of flavin adenine dinucleotide ($FADH^-$) as an intermolecular electron donor or covalently linked phenothiazine (PTZ) as an intramolecular electron donor. Intermolecular electron donation from photoexcited flavin ($^*FADH^-$) to free thymidine glycol generated thymidine in high yield, along with a small amount of 6-hydroxy-5,6-dihydrothymidine. In the case of photoreduction of 4-mer long single-stranded oligodeoxynucleotides containing thymine glycol by $^*FADH^-$, the restoration yield of thymine was varied depending on the sequence of oligodeoxynucleotides. Time-resolved spectroscopic study on the photoreduction by laser-excited *N*,*N*-dimethylaniline (DMA) suggested elimination of a hydroxyl ion from the radical anion of thymidine glycol with a rate constant of $\sim 10^4\ s^{-1}$ generates 6-hydroxy-5,6-dihydrothymidine (6-HOT$^\bullet$) as a key intermediate, followed by further reduction of 6-HOT$^\bullet$ to thymidine or 6-hydroxy-5,6-dihydrothymdine (6-HOT). On the other hand, an excess electron injected into double-stranded DNA containing thymine glycol was not trapped at the lesion but was further transported along the duplex. Considering redox properties of the nucleobases and PTZ, competitive excess electron trapping at pyrimidine bases (thymine, T and cytosine, C) which leads to protonation of the radical anion ($T^{-\bullet}$, $C^{-\bullet}$) or rapid back electron transfer to the radical cation of PTZ ($PTZ^{+\bullet}$), is presumably faster than elimination of the hydroxyl ion from the radical anion of thymine glycol in DNA.

Introduction

A wide variety of genotoxic agents, such as ionizing radiation, ultraviolet radiation, and chemical oxidants, induce chemical modifications of DNA bases.[1] 5,6-Dihydroxy-5,6-dihydrothymine (thymine glycol) is a major oxidative DNA damage structure that generates in DNA as a consequence of oxidative stress.[2] Exposure of chromatin DNA of a cultured human cell to γ-radiation (116 Gy) caused a significant increase of thymine glycol formation in a yield of $\sim 6.6 \times 10^5$ DNA bases.[3] Although thymine glycol is a lethal lesion in DNA, it is subjected to cellular repair process in vivo and removed by excision repair enzymes, such as endonuclease III (*Endo III*).[4] Reactive oxygen species including the hydroxyl radical (OH$^\bullet$) generated as a result of water radiolysis accounts for the formation of thymine glycol in DNA (reaction 1). In addition, direct interaction between radiation quanta and DNA molecules gives rise to electron-loss centers and the counterpart electrons at the early stage of the DNA damage reaction induced by ionizing radiation (reaction 2)

$$H_2O \rightarrow OH^\bullet, H^\bullet, e_{aq}^- \quad (1)$$

$$DNA \rightarrow DNA^{+\bullet} + e^- \quad (2)$$

Recent studies have shown that the radical cations generated as such migrate hundreds of angstroms through DNA and cause oxidation of DNA bases as a result of irreversible trapping by the nucleobases.[5] Due to lower HOMO energy of guanine (G) than those of the other bases, G sites undergo site-specific oxidation to form an alkaline-labile 8-oxoguanine structure, and

(1) (a) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987. (b) Cadet, J.; Vigny, P. In *Bioorganic Photochemistry: The Photochemistry of Nucleic Acids*; Morrison, H., Ed.; John Wiley & Sons: New York, 1990; pp 1−272. (c) Taylor, J. S. *Acc. Chem. Res.* **1994**, *27*, 76−82. (d) Taylor, J. S. *Pure Appl. Chem.* **1995**, *67*, 183−190. (e) *Radiation Damage in DNA: Structure/Function Relationship at Early Times*; Zimbrick, J. D., Fuciarelli, A. F., Eds.; Battelle Press: Columbus, OH, 1995. (f) Greenberg, M. M.; Barvian, M. R.; Cook, G. P.; Goodman, B. K.; Matray, T. J.; Tronche, C.; Venkatesan, H. *J. Am. Chem. Soc.* **1997**, *119*, 1828−1839. (g) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1151.

(2) (a) Frenkel, K.; Goldstein, M. S.; Teebor, G. W. *Biochemistry* **1981**, *20*, 7566−7571. (b) Wallace, S. S. *Free Rad. Biol. Med.* **2002**, 33, 1−14.

(3) Dizdaroglu, M. *Mutat. Res.* **1992**, *275*, 331−342.

(4) (a) Demple, B.; Linn, S. *Nature* **1980**, *287*, 203−208. (b) Breimer, L. H.; Lindahl, T. *J. Biol. Chem.* **1984**, *259*, 5543−5548. (c) Dizdaroglu, M.; Laval, J.; Boiteux, S. *Biochemistry* **1993**, *32*, 12105−12111. (d) D'Ham, C.; Romieu, A.; Jaquinod, M.; Gasparutto, D.; Cadet, J. *Biohcemistry* **1999**, *38*, 3335−3344. (e) Ide, H. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *68*, 207−221.

(5) (a) Giese, B. *Annu. Rev. Biochem.* **2002**, *71*, 51−70. (b) Giese, B.; Biland, A. *Chem. Commun.* **2002**, 667−672. (c) Delaney, S.; Barton, J. K. *J. Org. Chem.* **2003**, *68*, 6475−6483. (d) In *Long-Range Charge Transfer in DNA I*; Schuster G. B., Ed.; Topics in Current Chemistry; Springer-Verlag: New York, 2004; Vol. 236. (e) In *Long-Range Charge Transfer in DNA II*; Schuster G. B., Ed.; *Topics in Current Chemistry*; Springer-Verlag: New York, 2004; Vol. 237. (f) Kawai, K.; Takada, T.; Nagai, T.; Cai, X. C.; Sugimoto, A.; Fujitsuka, M.; Majima, T. *J. Am. Chem. Soc.* **2003**, *125*, 16198−16199. (g) *Charge Transfer in DNA*; Wagenknecht, H.-A., Ed.; Wiley-VCH: Weinheim, Germany, 2005.

10.1021/ja061304+ CCC: $33.50

thereby such oxidative damage from a distance possibly influences the distribution of oxidative lesions in DNA.[6] Experiments have also shown that hole transfer in DNA is extremely sensitive toward structural changes caused by modified nucleobases or mismatched base pairs.[7] Several mechanisms for hole migration depending on the energetics of the nucleobases and on the structural characteristics of the system under investigation were proposed in the past decade. The mechanisms include single-step superexchange, multistep hopping, phonon-assisted polaron hopping, and ion-gated hopping.[5]

A counterpart of the hole transfer process, which has been referred to as excess electron transfer (EET), has been studied by electron spin resonance (EPR) of γ-irradiated frozen solutions of DNA[8] and also recently demonstrated by means of direct injection of electrons into DNA from photoexcited flavins,[9] aromatic amines,[10] pyrenes,[11] phenothiazine[12] or other chemically synthesized electron donors[13] linked to the DNA duplexes. Intensive investigations so far have suggested that excess electrons hop more efficiently over T−A base pairs than G−C base pairs and are trapped by electron acceptors, such as a cyclobutane pyrimidine dimer (CPD)[9] or 5-bromo-2′-deoxyuridine (BrdU).[10] Comprehensive knowledge about such an interaction between electrons and DNA is essential for investigating the roles of proteins which contain redox-active cofactors. For example, DNA photolyase transfers a single electron to a CPD site in a DNA duplex upon photoexcitation of a flavin chromophore ($FADH^-$) in the enzyme and initiates redox repair of the cyclobutane ring of CPD.[14] Recent collaborative work by David, Barton, and co-workers has demonstrated rapid scanning of DNA lesions by electronic long-range communication between DNA-bound repair enzymes.[15] From a viewpoint of cancer radiotherapy, such a reductive electron generated in the radiolysis is operative as a reactive species under hypoxic conditions in solid tumor tissues, and thus it is highly possible that such a charge transfer in DNA occurs at the earliest stage of radiation-induced DNA damage processes, which affects the distribution of the base lesions.

In a series of studies on the chemical reactivity of thymine glycol, our group has shown previously that thymine glycol is chemically repaired to the original thymine structure by reduction with radiolytically generated electrons (e_{aq}^-, reaction 1) or photoexcited aromatic amines. From the results of quantitative product analysis, it was predicted that thymine glycol undergoes one-electron reduction to generate a 6-hydroxy-5,6-dihydrothymin-5-yl radical (6-HOT•), followed by the second one-electron reduction to thymine.[16] So far, there have been no reports of an enzyme that photochemically catalyzes the reductive repair of thymine glycol in DNA via the similar redox mechanism, but interaction between the reductive electron and the thymine glycol lesion may change the DNA-mediated electron transfer characteristics. Indeed, it has been recently demonstrated that redox properties of intervened artificial nucleobases influence the efficiency of EET in DNA.[9e] Furthermore, knowledge of the fundamental properties of damaged DNA bases is important to develop novel devices for electrochemical detection of DNA lesions.

We describe herein our first attempt to investigate the redox reactivity of thymine glycol lesions in DNA and the influence of such a base modification on the efficiency of EET through the duplex DNA. As described below, we have prepared a series of oligodeoxynucleotides containing a single thymine glycol residue in each sequence by well-established methods and examined the photoreduction of the single strands by photoexcited $FADH^-$ ($^*FADH^-$). In addition, we have synthesized DNA−phenothiazine (PTZ) conjugates for photochemical injection of an electron into the duplex DNA containing thymine glycol and studied EET through the lesion by employing gel electrophoresis. On the basis of our findings, we discuss the possibility of reductive repair of thymine glycol in DNA and implications of DNA mediated EET with respect to DNA base damage reactions.

Results

Photoinduced Reductive Repair of Thymine Glycol in DNA. First of all, we examined the reactivities of free thymidine glycol and oligodeoxynucleotides containing thymine glycol toward the photoexcited reduced form of flavin, $^*FADH^-$ in anoxic aqueous solution. The mechanistic roles of the flavin chromophore in DNA photolyase have been well established by employing varieties of analytical methods[14] including laser flash photolysis.[17] The excited state of $FADH^-$ has enough reducing power ($E_{ox}^* = -2.84$ V against SCE) to initiate single-electron transfer to the pyrimidine dimers. Flavin derivatives have been used by Carell and co-workers for photochemical studies on EET through DNA duplexes, where they incorporated

(6) For example, see: (a) Seidel, C. A. M.; Schulz, A.; Sauer, H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553 and references therein. (b) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618. (c) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686−12687. (d) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, 118, 7063−7068. (e) Shafirovich, V.; Dourandin, A.; Geacintov, N. E. *J. Phys. Chem. B* **2001**, *105*, 8431−8435. (f) Shafirovich, V.; Cadet, J.; Gasparutto, D.; Dourandin, A.; Huang, W. D.; Geacintov, N. E. *J. Phys. Chem. B* **2001**, *105*, 586−592. (g) Misiaszek, R.; Crean, C.; Joffe, A.; Geacintov, N. E.; Shafirovich, V. *J. Biol. Chem.* **2004**, *279*, 32106−32115.

(7) (a) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096−1100. (b) Kelley, S. O.; Boon, E. M.; Barton, J. K.; Jackson, N. M.; Hill, M. G. *Nucleic Acids Res.* **1999**, *27*, 4830−4837.

(8) (a) Messer, A.; Carpenter K.; Forzley, K.; Buchanan, J.; Yang, S.; Razskazovskii, Y.; Cai, Z.; Sevilla, M. D. *J. Phys. Chem. B* **2000**, *104*, 1128−1136. (b) Cai, Z.; Li, X.; Sevilla, M. D. *J. Phys. Chem. B* **2002**, *106*, 2755−2762.

(9) (a) Schwogler, A.; Burgdorf, L. T.; Carell, T. *Angew. Chem., Int. Ed.* **2000**, *39*, 3918−3920. (b) Behrens, C.; Burgdorf, L. T.; Schwogler, A.; Carell, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 1763−1766. (c) Cichon, M. K.; Haas, C. H.; Grolle, F.; Mees, A.; Carell, T. *J. Am. Chem. Soc.* **2002**, *124*, 13984−13985. (d) Breeger, S.; Hennecke, U.; Carell, T. *J. Am. Chem. Soc.* **2004**, *126*, 1302−1303. (e) Manetto, A.; Breeger, S.; Chatgilialoglu, C.; Carell, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 318−321.

(10) (a) Ito, T.; Rokita, S. E. *J. Am. Chem. Soc.* **2003**, *125*, 11480−11481. (b) Ito, T.; Rokita, S. E. *Angew. Chem., Int. Ed.* **2004**, *43*, 1839−1842. (c) Ito, T.; Rokita, S. E. *J. Am. Chem. Soc.* **2004**, *126*, 15552−15559.

(11) (a) Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Chem.—Eur. J.* **2002**, *8*, 4877−4883. (b) Kaden, P.; Mayer A.; Trifonov, A.; Fiegig, T.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1636−1639.

(12) Wagner, C.; Wagenknecht, H.-A. *Chem.—Eur. J.* **2005**, *11*, 1871−1876.

(13) (a) Giese, B.; Carl, B.; Barl, T.; Carell, T.; Behrens, C.; Hennecke, U.; Schiemann, O.; Feresin, E. *Angew. Chem., Int. Ed.* **2004**, *43*, 1848−1851. (b) Lewis, F. D.; Liu, X.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2002**, *124*, 11280−11281.

(14) Sancar, A. *Chem. Rev.* **2003**, *103*, 2203−2238.

(15) (a) Boon, E. M.; Livingston, A. L.; Chmiel, N. H.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12543−12547. (b) Yavin, E.; Boal, A. K.; Stemp, E. A.; Boon, E. M.; Livingston, A. L.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3546−3551. (c) Yavin, E.; Boal, A. K.; Lukianova, O. A.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Biochemistry* **2005**, *44*, 8397−8407.

(16) (a) Ide, H.; Otsuki, N.; Nishimoto S.; Kagiya, T. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1387−1392. (b) Nishimoto, S.; Ide, H.; Otsuki, N.; Nakamichi, K.; Kagiya, T. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1127−1134.

(17) (a) Kim, S. T.; Heelis, P. F.; Okamura, T.; Hirata, T.; Mataga, N.; Sancar, A. *Biochemistry* **1991**, *30*, 11262−11270. (b) Kim, S. T.; Volk, M.; Rousseau, G.; Heelis, P. F.; Sancar, A.; Michelbeyerle, M. E. *J. Am. Chem. Soc.* **1994**, *116*, 3115−3116.

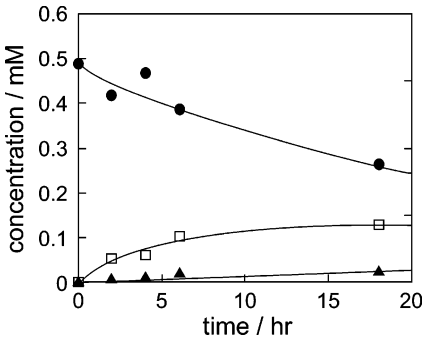
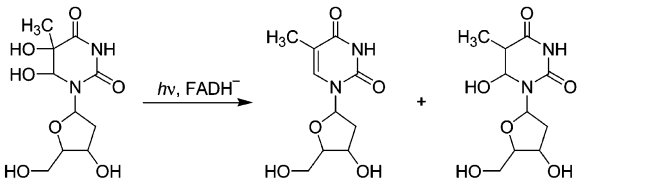


Figure 1. Photoinduced reduction of thymidine glycol (0.5 mM) by $FADH^-$ (0.2 mM) in the presence of EDTA (20 mM) in Ar-purged phosphate buffer solution (pH 7.0): (●) decomposition of thymidine glycol, (□) formation of thymidine, and (▲) 6-hydroxy-5,6-dihydrothymidine.

Scheme 1. Photoinduced Reduction of Thymidine Glycol



both the flavin derivative and a thymine cyclobutane photodimer into DNA and investigated the electron-transfer process from $*FADH^-$ to the photodimer.[9]

A deoxygenated aqueous solution of thymidine glycol (0.5 mM) was photoirradiated with a high-pressure Hg arc through a Pyrex glass filter (> 300 nm)[18,19] in the presence of FAD (0.2 mM) and excess EDTA (20 mM) to generate a reduced form of FAD ($FADH^-$) in situ.[9e,20,21] As analyzed by HPLC, the photoirradiated solution gave two major products, which were assigned to thymidine and 6-hydroxy-5,6-dihydrothymidines (6-HOT) by coeluting the authentic samples (Scheme 1). Formation of the structures was observed in the photosensitized reduction of thymine glycol by a series of aromatic amines, and also in the radiation-induced reduction of thymine glycol, where either hydrated electrons (e_{aq}^-) and/or carbon dioxide radical anions ($CO_2^{-\bullet}$) are generated as reducing species.[16] About 20% of thymidine glycol was decomposed after 6-h photoirradiation, and 91% of the products accounted for thymidine (Figure 1), which is comparable with the yield of thymine from thymine glycol as sensitized by *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) (72%).[16a,22] The generation of 6-HOT supports the previous prediction that 5,6-dihydro-6-hydroxythymidin-5-yl radical (6-HOT•) is an intermediate in the reaction. In addition to the two major products, a few minor products were observed by the HPLC analysis (data not shown). Considering the possible pathway for generation

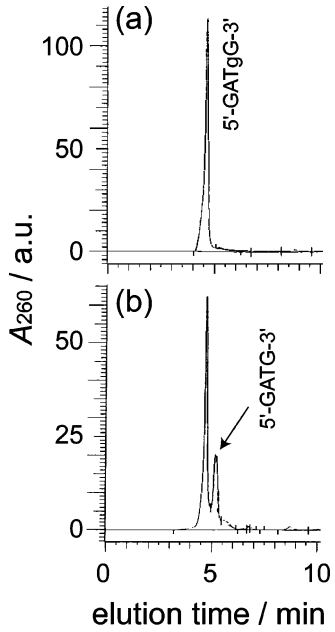


Figure 2. Typical HPLC profiles of 5′-GATgG-3′ (a) before and (b) after 90-min UV irradiation as eluted with 12% aqueous acetonitrile solution.

Table 1. Photoinduced Reductive Repair of Thymine Glycol in Single-Stranded Oligodeoxynucleotides[a]

sequence of XX(Tg/T)X	decomposition of XX(Tg)X/%	yield of XXTX/%	selectivity/%
5′-GG(Tg/T)G-3′	62	<1	<1
5′-GA(Tg/T)G-3′	62	37	60
5′-GG(Tg/T)A-3′	61	41	67
5′-GA(Tg/T)A-3′	85	46	54
5′-CG(Tg/T)A-3′	54	15	28

[a] The single-stranded DNA (66 μM) in phosphate buffer Solution (pH 7) was exposed to UV light in the presence of FAD (0.1 mM) and EDTA (20 mM) for 90 min at 24 °C. The amounts of decomposed oligodeoxynucleotide and the restoration were quantified based on the absorbance at 260 nm on the HPLC profiles (Figure 2).

of the 5-yl radical, the minor products may include 5-hydroxymethyl-2′-deoxyuridine[23] and/or the stereoisomers of 6-HOT, but they were not identified in this study.

Next, we prepared 4-mer long oligodeoxynucleotides containing thymine glycol by chemical oxidation of the corresponding thymine-containing DNA in the presence of $KMnO_4$ and examined $FADH^-$ sensitized reduction of the sequences under anaerobic conditions. The Ar-saturated oligodeoxynucleotide (66 μM) solutions were exposed to UV light (λ > 300 nm) in the presence of FAD (0.1 mM) and EDTA (20 mM) and analyzed by the use of HPLC (Figure 2). Restoration of thymine from thymine glycol in the oligodeoxynucleotides after 90-min irradiation was quantitated with reference to authentic DNA samples (Table 1). Compared with the reaction of free thymidine glycol, the selectivity of thymine restoration for each sequence was relatively low, probably because of some side reactions, such as reduction of pyrimidine bases. Interesting sequence dependence was observed as can be seen in Table 1; the solution of oligodeoxynucleotide containing only G and thymine glycol (5′-GGTgG-3′) gave a trace of a repaired sequence (5′-GGTG-3′). On the other hand, G and adenine (A) containing oligodeoxynucleotides (5′-GATgG-3′, 5′-GGTgA-3′, and 5′-GATgA-

(18) Thymine glycol absorbs light at wavelengths shorter than 300 nm, and UV light through the cutoff filter selectively generates the excited state of flavin.
(19) Heyroth, F. F.; Loofbourow, J. R. *J. Am. Chem. Soc.* **1934**, *56*, 1728−1734.
(20) An excess amount of EDTA generates a two-electron reduced form of FAD. Restoration of thymidine from thymidine glycol was negligible in the photolysis of FAD without EDTA.
(21) Traber, R.; Kramer, H. E. A.; Hemmerich, P. *Biochemistry* **1982**, *21*, 1687−1693.
(22) Photolysis (λ > 300 nm, 45 min) of thymine glycol (1 mM) in the presence of TMPD (1 mM) in aqueous buffer solution (pH 7).
(23) Berger, M.; Sarrazin, F.; Cadet, J. *J. Chim. Phys. Phys. Chim. Biol.* **1993**, *90*, 853−861.

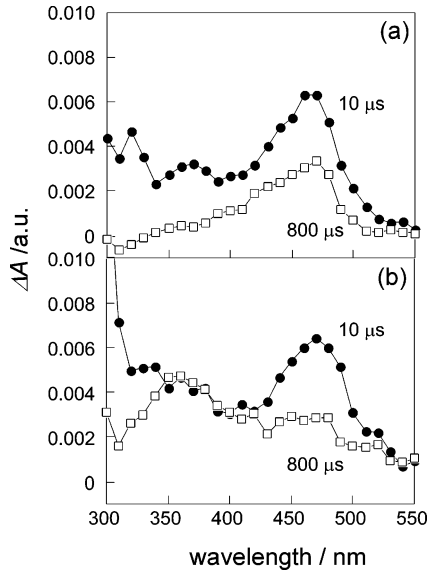


Figure 3. Transient absorption spectra of the intermediates as observed 10 μs and 800 μs after the 266-nm laser photolysis of DMA (0.2 mM) in phosphate buffer solution (pH 7.0) in the (a) absence or (b) presence of thymidine glycol (3.0 mM).

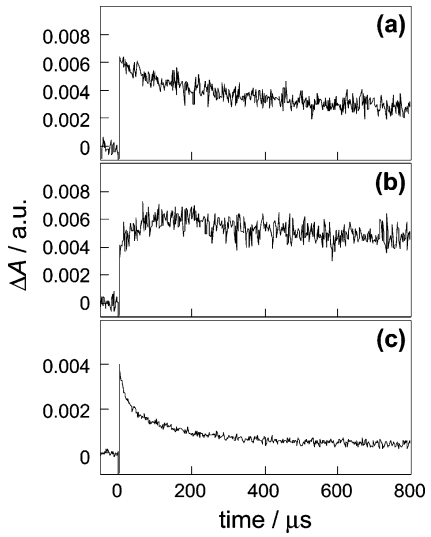


Figure 4. Time courses of transient absorption at (a) 460 and (b) 360 nm upon 266-nm laser photolysis of DMA (0.2 mM) in the presence of thymidine glycol (3.0 mM). (c) Time course of transient absorption at 360 nm upon 266-nm laser photolysis of DMA (0.2 mM).

3′) showed higher reactivity (37−46%) compared to that of cytidine (C) containing DNA (5′-CGTgA-3′, 15%). In consideration of reduction potentials of DNA bases and the high reducing power of *FADH$^-$, short-range EET in the single strands might be responsible for the difference in the restoration yield.

Laser Flash Photolysis Study on the Repair Mechanism. Our previous product studies on the photoreduction of thymine glycol were indicative that the reductive repair proceeds via elimination of the hydroxyl ion from the radical anion of thymine glycol, thereby 6-HOT$^\bullet$ is generated as an intermediate;[16] however, the involvement of the 5-yl radical has not been directly observed yet. In this context, we next performed a time-resolved spectroscopic study of radical intermediates in the photoinduced reduction of thymidine glycol. Since FAD has multiple redox states and the corresponding protonated and deprotonated structures depending on the pH of the solutions, spectral overlaps of the flavin chromophores prevented direct observation of 6-HOT$^\bullet$ in the photoreduction of thymidine glycol by *FADH$^-$. As is well-known, *N*,*N*-dimethylaniline (DMA) is a photoinducible electron donor with a high reducing power (E_{ox}* = −3.3 V),[24] and the radical cation of DMA (DMA$^{+\bullet}$) shows a characteristic transient absorption band at around 460 nm (Figure 3a).[25] Laser excitation at 266 nm of an Ar-saturated aqueous solution of thymidine glycol (3.0 mM) in the presence of DMA (0.2 mM) at pH 7 resulted in the intense absorption of DMA$^{+\bullet}$ at 460 nm, as shown in Figure 3b. The radical cation decays relatively slowly (Figure 4a), and thereafter an absorption band with λ_{max} = 360 nm emerged (Figure 4b), as was evidently observed 800 μs after excitation (Figure 3b). The transient spectrum of the slower decayed intermediate at around 360 nm shown in Figure 3b was quite similar to that of the 6-hydroxy-5,6-dihydrothymin-5-yl radical which was obtained in the previous pulse radiolysis study of 5-bromo-6-hydroxy-5,6-dihydrothymine.[26] Therefore, the intermediate observed 800 μs after the pulse is assigned to 6-HOT$^\bullet$ generated by elimination of the hydroxyl ion from the radical anion of thymidine glycol. Due to the well-established oxidizing ability of the 6-hydroxy-5,6-dihydrothymin-5-yl radical,[27] it is expected that structurally similar 6-HOT$^\bullet$ undergoes further one-electron reduction to the corresponding anion by the ground-state DMA (reaction 3).

$$\text{6-HOT}^\bullet + \text{DMA} \rightarrow \text{6-HOT}^- + \text{DMA}^{+\bullet} \qquad (3)$$

If one-electron reduction of 6-HOT$^\bullet$ occurs during the DMA-sensitized reaction, we could observe a slow growing component of DMA$^{+\bullet}$ along with the rapid buildup due to laser-induced ionization of DMA. A not so simple decay profile in Figure 4a might be indicative of involvement of such a process, but unfortunately, analysis of the decay kinetics of DMA$^{+\bullet}$ based on the time profile at 460 nm was not successful because of spectral overlap of the radical cation and the excited state of DMA, as has been suggested previously.[28]

The rate constant of electron transfer from photoexcited DMA (DMA*) to thymidine glycol was thus conveniently estimated by Stern−Volmer analysis of the fluorescence quenching. Upon excitation of DMA (0.3 mM) in anoxic aqueous buffer solution (pH 7) at 300 nm, corresponding fluorescence at around 320−450 nm was observed, and the intensity of fluorescence of DMA (λ_{em} = 360 nm) decreased with increasing concentration of thymidine glycol (0−3.3 mM) in the DMA solution. The Stern−Volmer treatment of the data yields a linear plot (Supporting Information) with a slope of $k_q\tau = 0.11 \times 10^3$ M^{-1}, which is deduced to be $k_q = 3.8 \times 10^{10}$ M^{-1} s^{-1} using a reported singlet lifetime of DMA, $\tau = 2.8 \times 10^{-9}$ s.[29] This almost diffusion

(24) Scannell, M. P.; Fenick, D. J.; Yeh, S.-R.; Falvey, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 1971−1977.
(25) (a) Habersbergerová, A.; Janovský, I.; Teplý, J. *Radiat. Res. Rev.* **1968**, *1*, 109−181. (b) Land, E. J.; Porter, G. *Trans. Faraday Soc.* **1963**, *59*, 2027−2037. (c) Ito, T.; Shinohara, H.; Hatta, H.; Fujita, S.; Nishimoto, S. *J. Phys. Chem. A* **2000**, *104*, 2886−2893
(26) Deeble, D. J.; von Sonntag, C. *Z. Naturforsch.* **1985**, *40c*, 925−928.
(27) (a) Jovanovic, S. V.; Simic, M. G. *J. Am. Chem. Soc.* **1986**, *108*, 5968−5972. (b) Steenken, S. *Chem. Rev.* **1989**, *89*, 503−520.
(28) (a) Cadogan, K. D.; Albrecht, A. C. *J. Phys. Chem.* **1969**, *73*, 1868−1877. (b) Land, E. J.; Richards, J. T.; Thomas, J. K. *J. Phys. Chem.* **1972**, *76*, 3805−3812.
(29) *Handbook of Fluorescence Spectra of Aromatic Molecules*, 2nd ed.; Beriman, I. B., Ed.; Academic Press: New York, 1971; p 473.

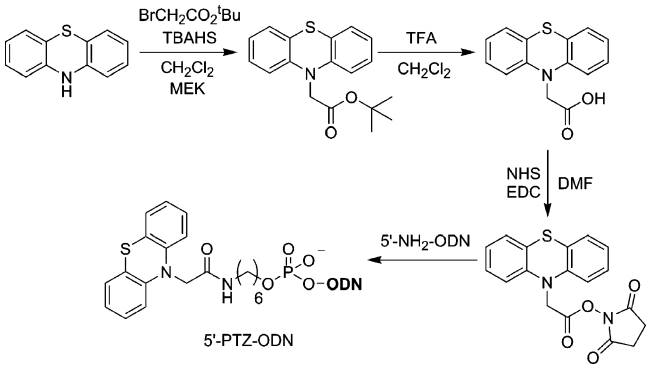
Chart 1. Sequences of Oligodeoxynucleotides Used in This Study

PTZ-ODN 1 : 5'-**PTZ**-ACA A ATC GAC TGC-3'
Tg-ODN 1 : 3'- TGT $\mathbf{T_g}$ TAG CTG ACG-5'

PTZ-ODN 2 : 5'-**PTZ**-ACT A TCC GAC TGC-3'
Tg-ODN 2 : 3'- TGA $\mathbf{T_g}$ AGG CTG ACG-5'

PTZ-ODN 3 : 5'-**PTZ**-AGA G AA CCT GCG TGA CCG-3'
Tg-BrdU-ODN : 3'- TC $\mathbf{T_g}$ C^{Br}UT GGA CGC ACT GGC-5'
BrdU-BrdU-ODN : 3'- $TC^{Br}U$ C^{Br}UT GGA CGC ACT GGC-5'

Scheme 2. Synthesis of Phenothiazine Containing Oligodeoxynucleotides



controlled rate constant is consistent with the observed fast buildup of the absorption of $DMA^{+\bullet}$ at 460 nm (Figure 4a).

Excess Electron Transfer through Thymine Glycol in DNA. The experimental data obtained from the photoinduced reduction of thymine glycol in 4-mer long oligodeoxynucleotides suggest potential electron hopping between vicinal nucleobases in the single-stranded DNA. We investigated the effect of thymine glycol in duplex DNA on the efficiency of DNA-mediated EET by the use of polyacrylamide gel electrophoresis. Commercially available thymidine glycol phosphoramidite was site-specifically incorporated into oligodeoxynucleotides using a DNA synthesizer (Tg-ODN 1, 2 and Tg-BrdU-ODN, Chart 1). To avoid unnecessary oxidation of nucleotides by the photoexcited oxidized form of flavin (*FAD), phenothiazine (PTZ) was employed as a photoinducible electron donor and was covalently linked to the complimentary strand with a 5′-amino linker by the standard amine-*N*-hydroxysuccinimide coupling (PTZ-ODN 1−3, Scheme 2). In a separate study, we confirmed that photoexcited PTZ has enough reducing power ($^*E_{ox} = -2.7$ V)[30] to reductively repair thymidine glycol by photoirradiating ($\lambda > 300$ nm) a deaerated thymidine glycol solution in the presence of free PTZ (data not shown). 5′-End ^{32}P-radiolabeled Tg-ODN 1 was annealed with the complimentary electron donor strand (PTZ-ODN 1), and the duplex was exposed to UV light (365 nm) under an anoxic atmosphere at 4 °C. The irradiated samples were then treated with hot piperidine and subjected to polyacrylamide gel electrophoresis. Since the thymine glycol structure is intrinsically unstable in a basic solution at a high temperature,[31] an oligodeoxynucleotide containing a repaired thymine residue (13-mer) can be detected as a slow-moving band, while a fast-moving band corresponding to a fragment of 9-mer long oligodeoxynucleotide is observed for the thymine-glycol containing DNA.

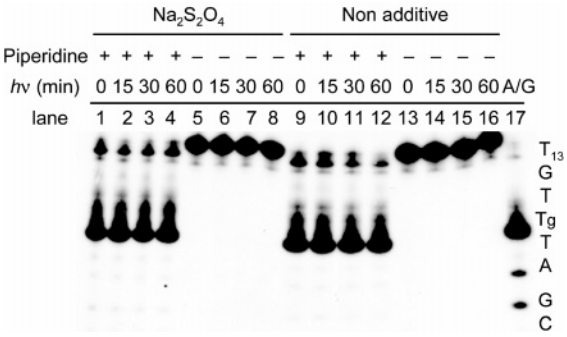


Figure 5. Autoradiogram of a 15% polyacrylamide gel containing 7 M urea showing cleavage products of 5′-^{32}P-labeled Tg-ODN 1. Duplex DNA (Tg-ODN 1/PTZ-ODN 1) was photoirradiated for the indicated periods in the presence (lanes 1−8) or absence (lanes 9−16) of $Na_2S_2O_4$, then analyzed either directly (lanes 5−8 and 13−16) or after subsequent treatment with piperidine (lanes 1−4 and 9−12). Lane 17 is the Maxam−Gilbert A+G sequencing lane.

Figure 5 shows a gel image of photoirradiated samples of Tg-ODN 1/PTZ-ODN 1 as analyzed before (lanes 13−16) and after (lanes 9−12) piperidine treatment. On this gel, however, increase of the full length oligodeoxynucleotide band (T_{13}) was not observed, but most of the irradiated oligodeoxynucleotides were hydrolyzed at the thymidine glycol site (Tg). As was proposed by the mechanistic studies above, a total of two electron reductions would be necessary for complete repair of thymidine glycol. Based on this prediction, photoreaction in the presence of excess sodium dithionite ($Na_2S_2O_4$) as a second electron source was also performed under the same conditions (lanes 1−8), but no remarkable difference on the cleavage pattern was observed. Maximum yield of a reductive repair of thymine glycol was obtained from the single-stranded oligonucleotide 5′-GATgA-3′ as shown above; thus photoreactivity of a duplex bearing the sequence (Tg-ODN 2/PTZ-ODN 2) was also examined under the same conditions, but essentially the same results were obtained (Supporting Information).

In addition, we designed an oligodeoxynucleotide containing both thymine glycol and BrdU in the sequence (Tg-BrdU-ODN), hybridized it with the PTZ-containing complimentary oligodeoxynucleotide (PTZ-ODN 3), and then investigated photoinduced EET through the duplex (Chart 1). As has been used as a chemical probe for EET in DNA,[10] BrdU traps an electron and generates the 2′-deoxyuridyl-5-yl radical by losing the bromide anion, and then the radical in turn abstracts a hydrogen atom from the deoxyribose of a 5′-adjacent nucleotide.[32] Therefore, EET followed by piperidine treatment results in strand cleavage at the 5′-adjacent base of BrdU. Figure 6a is the gel image of a control photoirradiated solution of a duplex containing double BrdU sites (BrdU-BrdU-ODN, Chart 1), showing a cleavage band at the 5′-next of the first BrdU site

(30) Excited-state oxidation potential was calculated using $^*E_{ox} = E_{ox} - E_{00}$ with the oxidation potential of PTZ ($E_{ox} = 0.59$ V; Mecklenburg, S. L.; Peek, B. M.; Schoonover, J. R.; McCafferty, D. G.; Wall, C. G.; Erickson, B. W.; Meyer, T. J. *J. Am. Chem. Soc.* **1993**, *115*, 5479−5495) and zero excitation energy (E_{00}) evaluated from λ_{00} (= 381 nm) in aqueous solution. $^*E_{ox} = -2.0$ V has been adopted for phenothiazine incorporated in DNA in a recent study by Wagenknecht (see ref 12).

(31) (a) Iida, S.; Hayatsu, H. *Biochim. Biophys. Acta* **1971**, *228*, 1−8. (b) Cadet, J.; Ducolomb, R.; Teoule, R. *Tetrahedron* **1977**, *33*, 1603−1607. (c) Cadet, J.; Teoule, R. *J. Chromatogr.* **1975**, *115*, 191−194.

(32) (a) Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025−10030. (b) Cook, G. P.; Chen, T.; Koppisch, A. T.; Greenberg, M. M. *Chem. Biol*. **1999**, *6*, 451−459. (c) Fujimoto, K.; Ikeda, Y.; Ishihara, S.; Saito, I. *Tetrahedron Lett.* **2002**, *43*, 2243−2245.

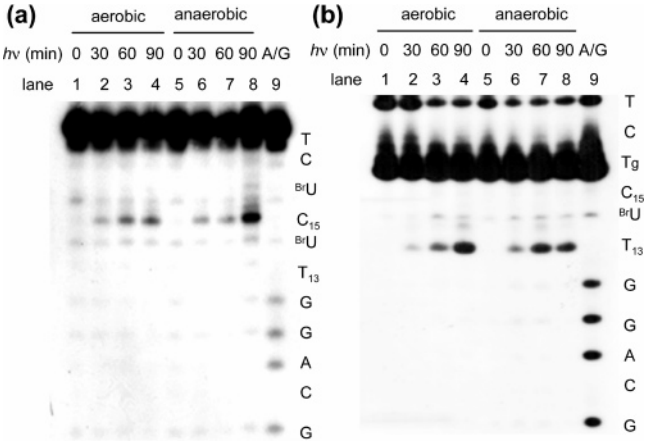


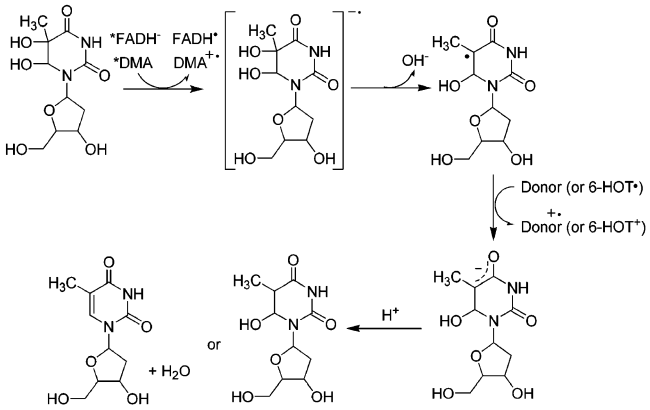
Figure 6. (a) Autoradiogram of a 15% polyacrylamide gel containing 7 M urea showing cleavage products of 5′-^{32}P-labeled BrdU-BrdU-ODN. Duplex DNA (BrdU-BrdU-ODN/PTZ-ODN 3) was photoirradiated for the indicated periods under aerobic (lanes 1−4) or anaerobic atmosphere (lanes 5−8) and then treated with piperidine. Lane 9 is the Maxam−Gilbert A+G sequencing lane. (b) Autoradiogram of a 15% polyacrylamide gel containing 7 M urea showing cleavage products of 5′-^{32}P-labeled Tg-BrdU-ODN. Duplex DNA (Tg-BrdU-ODN/PTZ-ODN 3) was photoirradiated for the indicated periods under aerobic (lanes 1−4) or anaerobic atmosphere (lanes 5−8) and then treated with piperidine. Lane 9 is the Maxam−Gilbert A+G sequencing lane.

(C_{15}), not of the second BrdU site (T_{13}). This result suggests an excess electron injected from PTZ was efficiently trapped at the proximal BrdU site, which prevented further migration to the distal BrdU. Oxygen did not have a significant effect on the electron-transfer efficiency (lanes 1−4), implying electron transfers inside of the DNA duplex rather than becomes detached from and reattaches to the DNA. In the case of the thymine-glycol-containing duplex Tg-BrdU−ODN/PTZ-ODN 3, however, an excess electron migrated to the distal BrdU site beyond the thymine glycol site and induced strand scission at T_{13} (Figure 6b). According to previous studies,[33] DNA replication is inhibited by thymine glycol in the DNA strand, presumably because hydrogen bonding between thymine glycol and the complimentary adenine is less effective than that within the normal A−T base pair. The lower melting temperature of Tg-BrdU-ODN/PTZ-ODN 3 (61.3 °C) compared to BrdU-BrdU-ODN/PTZ-ODN 3 (67.5 °C) suggests slight destabilization of the glycol-containing duplex (Supporting Information), which is inconsistent with the previous results. At this point, it is not clear whether thymine glycol is flipped from the center of the duplex,[34] but the result that an excess electron reached the distal BrdU is noteworthy as an example of EET through a modified DNA base.

Discussion

Reductive Repair Mechanism of Thymidine Glycol. We employed herein $FADH^-$ as an electron donor for photoreduction of thymidine glycol not only because the excited state has a high reducing power for electron donation to thymidine glycol but also because it could be a model reaction of thymine glycol repair by flavin-containing photoenzymes. As in the previous cases of photoreduction of thymine glycol by aromatic amines,[16] efficient restoration of thymidine from thymidine glycol by the reduction with $*FADH^-$ was observed despite structural differences between thymine glycol and thymidine glycol. Transient absorption data obtained by laser flash photolysis indicated that $DMA^{+\bullet}$ is generated immediately after laser excitation followed by relatively slow formation of the intermediate 5-yl radical, 6-HOT$^\bullet$, while the precursor radical anion of thymidine glycol was not detected by the current flash photolysis system. These results and our previous findings are inconsistent with the stepwise two-electron-transfer mechanism, in which the radical anion of thymidine glycol loses the hydroxyl ion and then the resulting radical undergoes further reduction, as shown in Scheme 3. A hypothetical decay profile of 6-HOT$^\bullet$ can be obtained by subtracting the contribution of the transient species of DMA (Figure 4c) from the observed decay profile at 360 nm in Figure 4b. By analyzing the differential time profile (Supporting Information), the built-up component (<100 μs) of 6-HOT$^\bullet$ observed at 360 nm gave a first-order rate constant of $\sim 3 \times 10^4$ s^{-1}, which is much slower than that of debromination from the BrdU radical anion ($k = \sim 10^9$ s^{-1}).[35] As discussed earlier, 6-HOT$^\bullet$ is a relatively stable oxidizing radical as it can oxidize aromatic amines,[27] but the source of the second electron in this reaction is not clear. Taking into account the long lifetime of 6-HOT$^\bullet$ ($\tau_{1/2} > 1$ ms) obtained from the differential time profile (Supporting Information), further reduction by another excited state of the electron donor or disproportionation in 6-HOT$^\bullet$ might be responsible.

Scheme 3. Proposed Reaction Mechanism of Thymidine Glycol Repair by Photoinduced Electron Transfer from Either FAD or DMA



Excess Electron Hopping through Thymidine Glycol in DNA. Several groups have made efforts toward understanding the mechanism of EET in DNA, but our knowledge about EET is still limited to elementary principles. Early investigation into temperature dependent EET in DNA by EPR as well as recent studies on distance dependent EET have suggested that an excess electron hops between nucleobases, especially pyrimidines, by a thermally activated mechanism at ambient temperatures.[8−11] Escape to the surrounding aqueous phase or trapping at pyrimidines to generate dihydropyrimidine derivatives is ex-

(33) For example, see: (a) Hayes, R. C.; Petrullo, L. A.; Huang, H.; Wallace, S. S.; LeClerc, J. E. *J. Mol. Biol.* **1988**, *201*, 239−246. (b) Rouet, P.; Essigmann, J. M. *Cancer Res.* **1985**, *45*, 6113−6118. (c) Ide, H.; Kow, Y. W.; Wallace, S. S. *Nucleic Acids Res.* **1985**, *13*, 8035−8052. (d) Clark, J. M.; Pattabiraman, N.; Jarvis, W.; Beardsley, G. P. *Biochemistry* **1987**, *26*, 5404−5409. (e) Basu, A. K.; Loechler, E. L.; Leadon, S. A.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7677−7681. (f) Mlasklewicz, K.; Miller, J.; Ornstein, R.; Osman, R. *Biopolymers* **1995**, *35*, 113−124. (g) Iwai, S. *Chem.−Eur. J.* **2001**, *7*, 4344−4351.

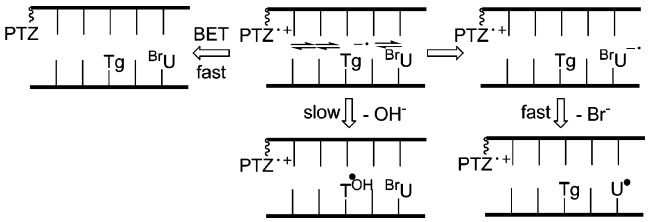
(34) Kao, J. Y.; Goljer, I.; Phan, T. A.; Bolton, P. H. *J. Biol. Chem.* **1993**, *268*, 17787−17793.

(35) Rivera, E.; Schuler, R. H. *J. Am. Chem. Soc*. **1983**, *87*, 3966−3971.

pected as the fate of the excess electron on DNA,[1a,36] but this has not been experimentally demonstrated yet.

The results of photoinduced reduction obtained above show that photoexcited $FADH^-$ can promote efficient reduction of thymine glycol in the single-stranded 4-mer long oligodeoxynucleotides. Interesting is the fact that the yield and the selectivity of repaired thymine are sequence dependent and seem to be correlated with reduction potentials of the nucleobases (G, −3.00 V; A, −2.76 V; C, −2.59 V; T, −2.42 V vs SCE)[37] in the sequence (Table 1). Due to the high reducing power of $*FADH^-$, any nucleobases including thymine glycol in the short single-stranded DNA could undergo direct one-electron reduction; thereafter the reductive electron possibly hops to a vicinal nucleobase until it is irreversibly trapped at high reduction potential sites, or otherwise it may escape to aqueous phase. While the reduction potential of thymine glycol in DNA is not available at this point, it was suggested that 5,6-saturated pyrimidine derivatives have slightly more negative reduction potentials than those of the original pyrimidines.[38] Therefore, C may most easily undergo one-electron reduction among the four nucleobases (A, T, G, C) and thymine glycol. The low yield of 5′-CGTA-3′ from a photoirradiated solution of 5′-CGTgA-3′ (28%) supports the prediction, where trapping of the excess electron by C and competitively fast protonation of the cytosine radical anion prevent further electron transfer to the thymine glycol residue.[8b,10b,12] In fact, kinetic analyses of the reactions of pyrimidine radical anions ($C^{-\bullet}$, $T^{-\bullet}$) in aqueous solution have demonstrated the quite fast protonation of $C^{-\bullet}$ completing on a nanosecond time scale or faster[39] and the comparatively slow protonation of $T^{-\bullet}$ ($k < 10^3\ s^{-1}$).[40] On the other hand, adenine-containing sequences showed high reactivity among the sequences, presumably because adenine is a temporal electron mediator as it can transport an excess electron to thymine glycol or other nucleobases. In contrast, no restoration of 5′-GGTG-3′ was observed in the photoreduction of the 5′-GGTgG-3′ sequence; nevertheless the sequence was decomposed during the photoirradiation. Previous theoretical calculations estimated the electron affinity (EA) of hydroxyl radical adducts of DNA bases, suggesting the 6-hydroxy-5-yl radical $6\text{-HOT}^\bullet$ possesses a high EA of >2 eV.[41] Thus, one explanation for the reactivity of 5′-GGTgG-3′ might be that $6\text{-HOT}^\bullet$ generated by direct reduction of thymine glycol underwent further reduction by the adjacent G sites which have low ionization potentials (IP, 6−7 eV for GG)[6c,d] and then the generated guanine radical cations undergo hydrolysis to afford final oxidation products, such as 8-oxo-2′-deoxyguanine.[42] In addition, such a radical may give rise to cross-linked lesions via coupling to 5′-adjacent guanine, as discussed earlier[41,42] Further product analysis of the electron-transfer reaction in single-stranded DNA would be necessary for understanding the reactivity and the detailed reaction mechanisms.

Scheme 4. Excess Electron Transfer through Thymine Glycol (Tg) in DNA



Carell and co-workers have synthesized varieties of oligodeoxynucleotides with the thymine cyclobutane dimer as a chemical probe of EET through a DNA duplex.[9] In the reaction system, intramolecular electron transfer to a thymine dimer in DNA causes splitting of the C5−C6 cyclobutane ring with a relatively slow rate constant of $k = \sim 10^7\ s^{-1}$, and the single electron further migrates along the duplex after repairing the dimer. Thus, the apparent EET efficiency in the system is less sequence dependent compared to the system using BrdU as the detection probe of EET.[13a] Considering this fact, it is not surprising that we observed efficient excess electron trapping at BrdU rather than at the thymine glycol site (Figure 6). Given that the radical anion of thymine glycol in duplex DNA also loses the hydroxyl ion with a rate constant of $\sim 10^4\ s^{-1}$, most of the excess electrons injected from the covalently linked PTZ reach the thymine glycol residue and migrate either further to BrdU or back to the PTZ radical cation (back electron transfer, BET) before the glycol radical anion irreversibly loses the hydroxyl ion (Scheme 4). On the other hand, in the case of intermolecular electron donation to the 4-mer long single-stranded DNA by free $FADH^-$, electron recombination between the neutral FADH radical ($FADH^\bullet$) and the excess electron on the oligodeoxynucleotide is less expected once charge separation occurs between them. Under such circumstances, an injected excess electron would eventually react with thymine glycol unless it is irreversibly trapped by high electron-affinic cytosines or it escapes to the solution phase.

While biological implications of EET in DNA are still uncertain, recent collaborative work by David, Barton, and co-workers have demonstrated rapid scanning of DNA abasic sites using DNA-mediated electron transfer between DNA-bound repair enzymes, such as *Escherichia coli MutY* and *Endo III*.[15] Inefficient electron transfer through damaged sites prevents relocation of the repair enzymes containing redox active $[4Fe\text{-}4S]^{2+}$ cofactor, thereby increasing the local concentration of repair enzymes in the vicinity of damaged DNA. However our results are indicative that the thymine glycol structure in DNA does not work as an efficient excess-electron trap, although thymine glycol is also a substrate for damage repair by *Endo III*.[4] It is thus likely that not all of the damaged structures induce the mode of electron communication for efficient detection of the substrates, since it seems that DNA-mediated excess electron-transfer efficiency depends on the redox property of the intervened nucleobases as shown above. For investigating such an electron communication between DNA-bound proteins,

(36) (a) Nishimoto, S.; Ide, H.; Nakamichi, K.; Kagiya, T. *J. Am. Chem. Soc.* **1983**, *105*, 6740−6741. (b) Ito, T.; Shinohara, H.; Hatta, H.; Nishimoto, S. *J. Org. Chem.* **1999**, *64*, 5100−5108.
(37) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553.
(38) Scannell, M. P.; Prakash, G.; Falvey, D. E. *J. Phys. Chem. A* **1997**, *101*, 4332−4337.
(39) (a) Visscher, K. J.; de Haas, M. P.; Loman, H.; Vojnovic, B.; Warman, J. M. *Int. J. Radial. Biol.* **1987**, *52*, 745−753. (b) Visscher, K. J.; Hom, M.; Loman, H.; Spoelder, H. J. W.; Verberne, J. B. *Radiat. Phys. Chem.* **1988**, *32*, 465−473. (c) Raytchev, M.; Mayer, E.; Amann, N.; Wagenknecht, H.-A.; Fiebig, T. *ChemPhysChem* **2004**, *5*, 706−712.
(40) (a) Novais, H. M.; Steenken, S. *J. Am. Chem. Soc.* **1986**, *108*, 1−6. (b) Steenken, S. *Chem. Rev.* **1989**, *89*, 503−520.
(41) Colson, A.-O.; Sevilla, M. D. *J. Phys. Chem.* **1995**, *99*, 13033−13037.
(42) (a) Box, H. C.; Budzinski, E. E.; Dawidzik, J. B.; Wallace, J. C.; Iijima, H. *Radiat. Res.* **1998**, *149*, 433−439. (b) Box, H. C.; Dawidzik, J. B.; Budzinski, E. E *Free Radical Biol. Med.* **2001**, *31*, 856−868. (c) Douki, T.; Rivière, J.; Cadet, J. *Chem. Res. Toxicol.* **2002**, *15*, 445−454.

further studies on the EET through other types of modified DNA bases would be necessary.[43]

Conclusions

In this study, we have demonstrated that photoreduction of thymine glycol in short single-stranded DNA by a photoexcited reduced form of flavin affords repaired thymine. A laser flash photolysis study suggested an electron adduct of thymidine glycol loses the hydroxyl ion to afford a 6-hydroxy-5,6-dihydrothymidin-5-yl radical as an intermediate, which is further reduced to thymidine. On the other hand, as investigated with chemically synthesized duplex DNAs containing phenothiazine and thymine glycol, it was demonstrated that a photoinjected excess electron does not induce reductive repair, since elimination of the hydroxyl ion from the radical anion of thymine glycol is relatively slow compared to competitive electron trapping at 5-bromouracil or cytosine. In this context, it is less expected that an oxidative lesion such as thymine glycol undergoes further reduction to thymine in radiation-exposed hypoxic cells. Another new finding is that this type of DNA base lesion does not necessarily prevent excess electron transfer through DNA, while hole transfer along the DNA duplex is remarkably sensitive to base-stacking perturbation induced by intervening base lesions.

Experimental Section

Materials. Flavin adenine dinucleotide disodium salt was used as received from Sigma Chemical. Potassium permanganate, ethylenediaminetetraacetic acid, and *N*,*N*-dimethylaniline were purchased from Nacalai Tesque and used without further purification. Reagents for high-performance liquid chromatography (HPLC) including solvents, acetonitrile, and methanol (HPLC grade) were used as received from Wako Pure Chemical Industries. *cis*-Thymidine glycol and 6-hydroxy-5,6-dihydrothymidines were synthesized and purified following the methods reported previously.[30] The stereostructure was not further determined in this study. 10*H*-Phenothiazine-10-acetic acid was synthesized by the same method as reported.[44] Aqueous solutions for all experiments were prepared using water purified with Corning Mega-Pure System MP-190 (>16 M Ωcm).

Synthesis of 4-mer Long Oligodeoxynucleotides Containing *cis*-Thymine Glycol.[45] Commercially purchased 15 OD_{260} oligodeoxynucleotide (Nisshinbo, Tokyo) was stirred in 4.7 mL of K_2HPO_4 (0.1 M, pH 8.6) buffer solution containing 5.5 mM $KMnO_4$ at 4 °C for 15 min. The reaction was quenched by the addition of allyl alcohol (0.1 mL, Wako), and the mixture was stirred at 4 °C for another 1 h. After centrifugation (1500 rpm) at room temperature for 5 min, the supernatant was desalted with a short column of reversed phase (C18 Sep-Pak, Waters). The DNA was eluted with 2 × 5 mL of 50% CH_3CN/H_2O. The single-stranded DNA solution was injected to a reversed-phase HPLC column (5C18), and the corresponding peak was isolated. A small portion of the isolated DNA was digested with Phosphodiesterase I (Snake Venon, Funakoshi) and Alkaline Phosphatase I (Nacalai) in Tris (0.1 M)−$MgCl_2$ (20 mM) buffer solution at 37 °C for 1 h. Samples were injected on a reversed-phase column (Inertsil ODS-3, GL Science Inc., ϕ 4.6 mm × 250 mm) and analyzed with a Hitachi D-7000 HPLC system. The elution of A, C, G, and *cis*-thymidine glycol was observed from the thymine-glycol-containing DNA by comparing the authentic sample solution of them. Yields of the oligodeoxynucleotides were typically less than 30% as calculated based on their absorbance at 260 nm.

Photosensitized Reduction by *FADH$^-$. Typically, solutions of thymidine glycol (0.1−20 mM) in phosphate buffer containing FAD (0.1−0.2 mM) were purged with Ar before photoirradiation. For effective generation of the reduced form of FAD ($FADH^-$) in situ, EDTA·2Na (20 mM) was added to the solutions. The solutions in sealed Pyrex glass tubes were photoirradiated (λ_{ex} > 300 nm) under magnetic stirring (1000 rpm) at 24 °C with a high-pressure Hg arc (450 W, Eikosha 400). In the case of photoreduction of 4-mer long oligonucleotides containing thymine glycol, an Ar-saturated phosphate buffer solution (2.5 mM, pH 7.0) of FAD (0.1 mM) and EDTA·2Na (20 mM) was preirradiated for 30 min, synthesized 4-mer long DNA (66 μM) was added, and then the solution was exposed to UV light for 90 min. Analytical HPLC was performed with a Shimadzu 10AS HPLC system equipped with a Rheodyne 7725 sample injector. Sample solutions were injected onto a 5 μm C18 reversed-phase column (Wakosil 5C18, ϕ 4.6 mm × 150 mm, Wako). Phosphate buffer solutions (10 mM, pH 3.0) containing various concentrations of methanol (10−25 vol %) were delivered as the mobile phase. The column eluents were monitored by the UV absorbance at 210 or 260 nm.

Nanosecond Laser Flash Photolysis. The laser flash photolysis experiments were carried out with a Unisoku TSP-601 flash spectrometer with a Continuum Surelite-I Nd:YAG (Q-swithched) laser, as described previously.[46] A fourth harmonic emission at 266 nm (ca. 50 mJ per 6 ns pulse) was employed for the photolysis, and the data obtained were analyzed on NEC PC-9801 and IBM ThinkCentre computers. Aqueous solutions of thymidine glycol (3.0 mM) at pH 7.0 containing DMA (0.2 mM) were deaerated by Ar bubbling prior to the laser flash photolysis experiments.

Fluorescence Quenching. Various concentrations of thymidine glycol in phosphate buffer (10 mM, pH 7.0) solution containing DMA (0.3 mM) were purged with Ar for 10 min and sealed in a quartz cuvett with a Teflon cap. Fluorescence spectra were recorded on a Hitachi F-2000 fluorescence spectrophotometer (slit width: 1.0 nm, $\lambda_{ex} = 300$ nm, $\lambda_{em} = 360$ nm). The dynamic quenching rate constant k_q was determined by the Stern−Volmer equation: $I_0/I = 1 + k_q\tau_0$ [thymidine glycol], where I_0 and I are the intensity of the fluorescence at 360 nm, and τ_0 is the lifetime of the excited singlet state of DMA[28] in the absence of thymidine glycol.

Synthesis of Oligodeoxynucleotides Containing Modified Bases. Modified oligonucleotides were synthesized on a 1.0 μmol scale (500 Å CPG column) using an Applied Biosystems 3400 DNA synthesizer. *cis*-Thymidine glycol phosphoramidite and 5-bromo-2′-deoxyuridine phosphoramidite (Glen Research) were site specifically incorporated into the oligonucleotides by trityl-off synthesis. Coupling reactions were allowed to proceed for 2 min (for A, T, G, or C) or 16.7 min (for BrdU or thymidine glycol) and were subsequently followed by standard oxidation and capping steps. Upon completion of the oligodeoxynucleotide synthesis, the CPG column was treated with 28% aqueous ammonium hydroxide for 1 h at room temperature. The resulting aqueous ammonium hydroxide solutions were consolidated and stored at room temperature for 24 h. The aqueous ammonium hydroxide solutions were lyophilized and purified by the use of HPLC (Hitachi D-7000). For deprotecting TBDMS groups on thymine glycol, the solid was treated with triethylamine trihydrofluoride (0.5 mL) at 40 °C for 12 h and desalted on an NAP 10 column (Amersham Biosciences) before HPLC purification. The DNAs were characterized by MALDI-TOF mass spectrometry; *m/z*: calcd for Tg-ODN 1 ($C_{126}H_{164}N_{46}O_{81}P_{12}$) 4014.62, found 4014.12; calcd for Tg-ODN 2 ($C_{128}H_{162}N_{52}O_{78}P_{12}$) 4048.64, found 4048.32; calcd for Tg-BrdU-ODN ($C_{173}H_{221}BrN_{63}O_{111}P_{17}$) 5565.42, found 5563.5; calcd for BrdU-BrdU-ODN ($C_{172}H_{216}Br_2N_{63}O_{109}P_{17}$) 5596.28, found 5595.34.

(43) Recent systematic screenings have found modified base structures that impact electron-transfer efficiency (Boal, A. K.; Barton, J. K. *Bioconjugate Chem.* **2005**, *16*, 312−321).
(44) Tierney, M. T.; Grinstaff, M. W. *J. Org. Chem.* **2000**, *65*, 5355−5359.
(45) Jerkovic, B.; Kung, H. C.; Bolton, P. H. *Analytical Biochemistry* **1998**, *255*, 90−94.
(46) Ito, T.; Shinohara, H.; Hatta, H.; Fujita, S.; Nishimoto, S. *J. Phys. Chem. A* **1999**, *103*, 8413−8420.

Synthesis of Phenothiazine-10-acetic Acid Succinimidyl Ester. Phenothiazine-10-acetic acid (329 mg, 1.28 mmol), *N*-hydroxysuccinimide (177 mg, 1.54 mmol, Nacalai), and ethyl-3-(3-dimethylaminopropyl)carbodiimide (294 mg, 1.54 mmol, Nacalai) were dissolved in dry DMF under a N_2 atmosphere. The solution was stirred at room temperature overnight. The solution was extracted with ethyl ether (30 mL) and water, washed with water (10 mL × 2), and then dried over $MgSO_4$. Solvent was removed under reduced pressure. Silica gel column chromatography (1:0−2:1 hexane/ethyl acetate) gave a white solid (72%). ^{1}H NMR ($CDCl_3$): 7.17−7.08 (m, 4H), 6.96−6.92 (m, 2H), 6.73 (d, J = 8.2 Hz, 2H), 4.85 (s, 2H), 2.87 (s, 4H). ^{13}C NMR: 168.64, 165.48, 143.13, 127.49, 126.90, 123.40, 123.35, 114.63, 48.62, 25.54. HR-FAB MS (matrix: 3-nitrobenzyl alcohol) *m*/*z*: (M^+) calcd for $C_{18}H_{14}N_2O_4S$ 354.0674, found 354.0671.

Synthesis of Oligodeoxynucleotides Containing Phenothiazine. Oligodeoxynucleotides with an amino-linker C6 at the 5′-termini (ODN 1−3) were commercially synthesized (Sigma) and desalted by a size exclusion chromatograph column (MicroBioSpin 6, Biorad). To an acetonitrile solution (200 μL) of *N*-hydrosuccinimide derivatized phenothiazine (3.0 mg, 8.4 μmol) was added ODN 1, 2, or 3 (200 μM, 200 μL) and 100 μL of saturated $NaHCO_3$, and the solution was incubated at 37 °C for 24 h. Monitoring of the coupling reaction and purification of the conjugate were carried out by the use of HPLC (Hitachi D-7000) with a 5−50% (over 45 min) gradient of MeCN/100 mM TEAA buffer (pH 5.0). The purified DNAs were characterized by MALDI-TOF mass spectrometry; *m*/*z*: calcd for PTZ-ODN 1 ($C_{146}H_{182}N_{53}O_{77}P_{13}S$) 4346.04, found 4345.81; calcd for PTZ-ODN 2 ($C_{145}H_{183}N_{48}O_{80}P_{13}S$) 4313.00, found 4312.39; calcd for PTZ-ODN 3 ($C_{195}H_{242}N_{76}O_{107}P_{18}S$) 5952.05, found 5951.43.

Photolysis and Polyacrylamide Gel Electrophoresis Analysis of DNA. The oligonucleotides (20 μL, 400 pmol) were 5′-^{32}P labeled with 4 μL of [γ-^{32}P]ATP (Amersham Bioscience, 10 mCi/mL) using T4 polynucleotide kinase (Nippon Gene) following the manufacture's instruction. Labeled DNA samples were recovered by ethanol precipitation, further purified by a 15% polyacrylamide gel, and extracted by the crush and soak method. Duplex DNA (1 μM) containing the labeled and the complementary PTZ-containing strand was annealed in a N_2-saturated buffer (10 mM phosphate buffer (pH 7.0) with 90 mM NaCl) by heating to 90 °C, followed by slow cooling to room temperature. The solution in a 1.5-mL eppendorf tube was exposed to 365 nm UV light with a FTI-20L transilluminator (Funakoshi, Tokyo) at 4 °C. All reaction mixtures were precipitated by addition of herring sperm DNA (1 mg/mL), 3 M sodium acetate (pH 5.2), and ethanol. The precipitated DNA was dissolved in 10 vol % piperidine, heated at 90 °C for 20 min, and then dried under reduced pressure. The radioactivity of the samples was assayed using an Aloka 1000 liquid scintillation counter (Aloka, Tokyo), and the dried DNA pellets were resuspended in loading buffer (80 vol % formamide, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). All reaction mixtures, along with a Maxam−Gilbert G+A sequencing reaction, were heat-denatured at 90 °C for 3 min and quickly chilled on ice. The samples (1.0 μL, (5−20) × 10^3 cpm) were loaded onto a 15% polyacrylamide (acrylamide−bisacrylamide 19:1) gel containing 7 M urea, electrophoresed at 1900 V for approximately 80 min, transferred to a cassette, and stored at −80 °C with Fuji X-ray films (RX-U). Cleavage of the labeled strand was quantified by autoradiography using ATTO Densitograph software (version 3.0).

Acknowledgment. This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Young Scientists (B). We also gratefully acknowledge the Japan Securities Scholarship Foundation and the Japan Atomic Energy Research Institute for financial support of this work. We thank Professors Steven E. Rokita, Hiroshi Ide, and Kazuhito Tanabe for helpful discussions.

Supporting Information Available: Melting curves of oligodeoxynucleotide duplexes, Stern−Volmer plot for fluorescence quenching of DMA by thymidine glycol, time-dependent transient absorption of 6-hydroxy-5,6-dihydrothymidin-5-yl radical, and polyacrylamide gel analysis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA061304+