2-Oxoalkyl caged oligonucleotides: one-electron reductive activation into emergence of ordinary hybridization property by hypoxic X-irradiation

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Ionizing radiation triggers the activation of caged oligodeoxynucleotides (ODNs) with a 2-oxoalkyl leaving group to give the corresponding normal uncaged strands. We designed and synthesized ODNs caged by a 2-oxopropyl group at a given thymine N(3) position ($d^{oxo}T$) to evaluate their one-electron reduction characteristics. Upon hypoxic X-radiolysis in aqueous solution, the caged ODNs released the 2-oxopropyl group to produce the corresponding uncaged ODNs. Digestion by a restriction enzyme *Swa* I revealed that caged ODN pre-irradiated in hypoxia could form an ordinary duplex with its complementary strand.

Introduction

Artificial oligonucleotides (ODNs) that can be externally stimulated to regulate their hybridization properties have been explored for possible application to DNA biosensors, bionanodevices and gene therapy.[1–7] With this view, various attempts were made to either enhance or suppress the bioactivity of ODNs by regulating their recognition ability, *e.g.* binding of a metal ion to mismatched[2] or modified nucleobases,[3] addition of borax and boronic acid to modified riboses,[4] and photochemical methods with nitrobenzene[5] or azobenzene[6] functionality linked to an ODN.

High-energy ionizing radiation seems to be among the potential external triggers for artificial ODNs with bioactive functionality, generating reactive transient species such as ion radicals, free radicals and excited molecules that induce formation or cleavage of chemical bonding.[8] The advantage of ionizing irradiation is its capability to control spatially and temporally the chemical reactions without any additives. Thus, ionizing radiation is an attractive trigger for the control of DNA functions, however, it has not yet been applied to regulation of DNA hybridization properties.

We herein report X-radiolytic activation of ODNs caged by a removable 2-oxoalkyl group, which was previously demonstrated to be an effective functionality for selective hypoxic-radiation activated 5-fluorouracil (5-FU) and 5-fluorodeoxyuridine (5-FdUrd) prodrugs.[9] An activation mechanism has been proposed by which the 2-oxoalkyl group undergoes one-electron reduction by hydrated electrons (e_{aq}^-)[10] generated *via* radiolysis of water to form the corresponding π* anion radical, followed by thermal activation into the σ* anion radical that has a weakened N–C bond between the 5-fluorouracil unit and the 2-oxoalkyl group and is readily hydrolyzed to release the 2-oxoalkyl group.[9b,9e] In this study, we designed and synthesized ODNs caged by a 2-oxopropyl group at a given thymine N(3) position ($d^{oxo}T$), which has a characteristic structure similar to the previous radiolytic reduction activated 5-FdUrd prodrug. These $d^{oxo}T$-bearing ODNs were confirmed to be one-electron reduced upon hypoxic X-irradiation, releasing the 2-oxopropyl group and producing the corresponding uncaged ODNs, which could hybridize into an ordinary duplex with their complementary strand.

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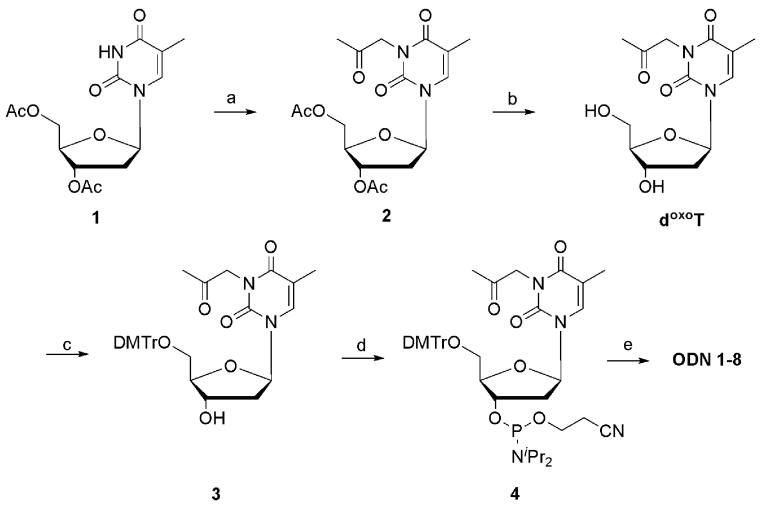
Results and discussion

The synthetic routes to $d^{oxo}T$ and artificial ODNs are shown in Scheme 1. Diacetyl thymidine **1** was coupled with α-bromoacetone and subsequently hydrolyzed under basic conditions to produce $d^{oxo}T$. The resulting $d^{oxo}T$ was incorporated into DNA *via* phosphoramidite **4**, using a DNA synthesizer. The structures of synthesized ODNs as confirmed by MALDI-TOF mass spectrometry are summarized in Table 1.

We initially conducted one-electron reduction of 5-mer **ODN 1** by the X-radiolysis of an argon-purged aqueous solution containing excess 2-methyl-2-propanol as the scavenger of oxidizing hydroxyl radicals.[9b] Under these radiolysis conditions, reducing hydrated electrons (e_{aq}^-) are generated as the major active species. Fig. 1 shows a representative reaction profile of the radiolytic reduction of **ODN 1** by e_{aq}^- under hypoxic conditions. The appearance of single new peak in Fig. 1 was attributable to formation of the corresponding uncaged ODN (5′-AATAA-3′) according to the molecular weight [$(M - H)^-$] of the fractionated sample measured by MALDI-TOF mass spectroscopy[11] and overlap injection of authentic samples in the HPLC analysis. The *G* values (the number of molecules produced or changed per 1 J of radiation energy absorbed by the reaction system) were 137 nmol J^{-1} for the decomposition of **ODN 1** and 113 nmol J^{-1} for the formation of corresponding uncaged ODN.

In contrast to the hypoxic X-radiolysis, the removal of 2-oxopropyl group from $d^{oxo}T$ of **ODN 1** to give uncaged ODN became considerable less efficient in the X-radiolysis under aerobic conditions: the *G* values were evaluated as 17.6 nmol J^{-1} for the decomposition of **ODN 1** and 2.4 nmol J^{-1} for the formation of uncaged ODN. In view of the well documented evidence that molecular oxygen efficiently captures reducing species of e_{aq}^- into superoxide anion radical ($O_2^{-\bullet}$) and thereby inhibits radiolytic reduction,[9d,12] the radiolytic removal of 2-oxopropyl group from $d^{oxo}T$ is likely to occur *via* one-electron reduction by e_{aq}^- almost



Scheme 1 *Reagents and conditions*: (a) NaH, bromoacetone, DMF, 0 °C, 94%; (b) LiOH·H_2O, methanol–water (2 : 1), 0 °C, 94%; (c) 4,4′-dimethoxytrityl chloride, pyridine, 0 °C, 83%; (d) 2-cyanoethyl tetraisopropylphosphorodiamidite, 1*H*-tetrazole, acetonitrile, room temperature, 40 min, quant. (e) automated DNA synthesis.

Table 1 The oligodeoxynucleotides (ODNs) used in this study

	Sequences[a]
ODN 1	5′-d(AA^{oxo}**T**AA)-3′
ODN 2	5′-d(AAAA^{oxo}**T**AAAA)-3′
ODN 3	5′-d(TTTT*ATT*^{oxo}***T****AAAT*TTTTT)-3′
ODN 4	5′-d(AAAAA*ATTTAAAT*AAAAA)-3′
ODN 5	5′-d(^{oxo}**T**A)-3′
ODN 6	5′-d(^{oxo}**T**G)-3′
ODN 7	5′-d(^{oxo}**T**T)-3′
ODN 8	5′-d(^{oxo}**T**C)-3′

[a] Recognition sites of restriction enzyme *Swa* I are shown in italic.

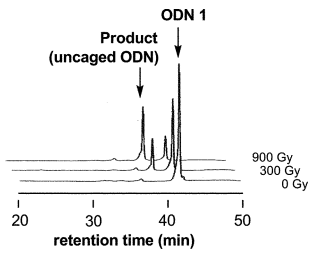


Fig. 1 HPLC profiles for the one-electron reduction of **ODN 1** (110 μM) in the hypoxic X-radiolyses (0, 300 and 900 Gy) of aqueous solution containing 10% 2-methyl-2-propanol.

selectively under hypoxic conditions. Similar reductive removal of the 2-oxopropyl group in a hypoxia selective manner was also confirmed for 9 mer **ODN 2**, although reaction efficiency was lower than that of 5-mer **ODN 1** (Table 2). The lowered efficiency is probably due to the increased competitive reaction of nucleobases around d^{oxo}T structure with e_{aq}^-. Furthermore, as seen in Table 2, a trend seems to be apparent that the radiolytic reductive conversion of caged ODN consisting of dT, dA and d^{oxo}T into the corresponding uncaged analog becomes substantially less efficient upon increasing the ODN chain length. The radiolytic one-electron reduction of **ODN 1–3** by e_{aq}^- may produce primary anion radicals of dT and dA that could transfer the electron to the caged base d^{oxo}T. However, the results shown in Table 2 indicate that this may not occur significantly, thus suggesting that irreversible processes are occurring such as irreversible protonation of the anion radicals. This is in accord with the fact that the thymine anion radical protonates irreversibly at C-6 and the adenine anion radical protonates irreversibly at C-2 and C-8.[13]

Table 2 *G*-values for the decomposition of caged ODNs bearing d^{oxo}T and for the formation of the corresponding uncaged ODNs in the X-radiolysis under hypoxic and aerobic conditions

	Hypoxic conditions		Aerobic conditions	
	Decomposition/nmol J^{-1}	Formation/nmol J^{-1}	Decomposition/nmol J^{-1}	Formation/nmol J^{-1}
ODN 1	137	113	17.6	2.4
ODN 2	106	45	12	0.8
ODN 3	(<62)[a]	(<50)[a]	(<10)[a]	ND[b]
ODN 5	167	149	35	30
ODN 6	167	134	24	15
ODN 7	181	130	35	11
ODN 8	69	49	19	11

[a] Since the signals assigned to **ODN 3** and the corresponding uncaged ODN considerably overlapped each other on the HPLC chart, the *G* values could not be quantified with sufficient accuracy. [b] The formation of uncaged ODN was not detected.

To confirm the removal of the 2-oxoalkyl group from caged ODN with longer chains upon hypoxic irradiation and to characterize its hybridization property, we performed the enzymatic digestion of 18-mer caged **ODN 3** pre-irradiated in hypoxia, which had d^{oxo}T at the center of the *Swa* I recognition site.[14] An equimolar amount of 5′-^{32}P-end labeled **ODN 4**, which is complementary to the uncaged normal analog of **ODN 3**, was added to a solution of caged **ODN 3** after hypoxic X-irradiation and the resulting duplex was digested by *Swa* I at 25 °C for 15 min. Fig. 2 shows a representative gel electrophoresis for elucidating strand cleavage behavior. Without X-irradiation, no cleavage of **ODN 4** was observed (lane 2), indicating that the enzymatic cleavage of **ODN 3–ODN 4** duplex by *Swa* I was inhibited by the presence of obstructive 2-oxopropyl group on the caged **ODN 3** strand. In contrast, the cleavage efficiency of **ODN 4** in the presence of caged **ODN 3** pre-irradiated in hypoxia was remarkably enhanced (lane 3). This result indicates that hypoxic X-irradiation removed 2-oxopropyl group on caged **ODN 3**, thereby resulting in the formation of an ordinary duplex with **ODN 4** that can be a substrate for *Swa* I. Consistent with the inefficient removal of 2-oxopropyl group upon aerobic X-irradiation, the enzymatic cleavage of the duplex derived from caged **ODN 3** pre-irradiated under aerobic conditions was suppressed to a background level (lane 5).

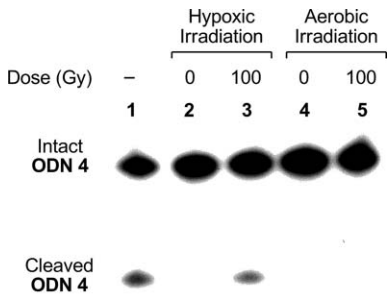


Fig. 2 Autoradiograms of the denaturing sequencing gel for enzymatic digestion of **ODN 4** in the presence of **ODN 3** pre-irradiated under hypoxic or aerobic conditions. The pre-irradiated **ODN 3** was added to the solution of 5′-^{32}P labeled **ODN 4**, and then digested by *Swa* I (10 U) at 25 °C for 15 min. Lane 1, control duplex (5′-TTTTTATTTAAATTTTTT-3′)–**ODN 4**; lanes 2, 3 **ODN 3** pre-irradiated under hypoxic conditions (0 Gy and 100 Gy)–**ODN 4**; lanes 4, 5 **ODN 3** (0 and 100 Gy) pre-irradiated under aerobic conditions–**ODN 4**.

To identify the effect of base sequence on the radiolytic reduction of intramolecular d^{oxo}T, we carried out a comparative one-electron reduction of dinucleotides **ODN 5–8** consisting of d^{oxo}T and four types of natural nucleobase upon hypoxic X-irradiation. As shown in Table 2, the *G* values for the decomposition of caged **ODN 5–7**, in which d^{oxo}T is linked to A, G, or T, respectively, and the formation of corresponding uncaged dinucleotides were similar levels. In contrast, dinucleotides **ODN 8** with a linkage between d^{oxo}T and C showed a considerably lower G value, relative to the other three dinucleotides, suggesting that the counterpart base unit of C could effectively capture e_{aq}^- and thereby inhibit the one-electron reduction of d^{oxo}T to generate dT. It has been reported that cytosine derivatives are reduced by e_{aq}^- faster than the other bases.[15] Furthermore, as suggested by the recent studies on excess electron transfer through DNA,[16] rapid irreversible protonation processes involved in cytosine anion radical may be competitive with electron transfer from one-electron reduced cytosine to the neighboring d^{oxo}T in **ODN 8**.

Conclusion

In summary, we have demonstrated activation of caged ODNs possessing a hindered d^{oxo}T unit by X-radiolytic one-electron reduction. The removal of the 2-oxopropyl group on caged ODN occurred efficiently upon hypoxic X-irradiation, while the reaction efficiency was dramatically decreased upon aerobic irradiation. Artificial DNA derivatives that can be activated to regulate DNA and RNA recognition properties with the aid of external triggers are useful for a variety of applications. As an example, we are trying to make a next generation of DNAzyme and antisense molecules, whose function can be regulated by hypoxic irradiation.

Experimental

General

^{1}H NMR spectra and ^{13}C NMR spectra were measured with JEOL JNM-AL 300 (300 MHz) or JEOL JMN-EX-400 (400 MHz) spectrometers. Coupling constants (*J* values) are reported in Hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ^{1}H NMR, δ = 77.0 in ^{13}C NMR) and residual dimethyl sulfoxide (δ = 2.49 in ^{1}H NMR, δ = 39.5 in ^{13}C NMR) as an internal standard. FAB Mass spectra were recorded on a JEOL JMS-SX102A spectrometer. A Rigaku RADIOFLEX-350 was used for X-radiolysis. High-performance liquid chromatography (HPLC) was performed using a HITACHI L-7100 equipped with a Intersil ODS-3 column (GL science Inc.), and the elution peaks were detected using a UV-Vis detector L-7455 at 260 nm wavelength. A Wakogel C-200 was used for silica gel chromatography. Precoated TLC plates Merck silica gel 60 F_{254} were used for monitoring the reactions and also for preparative TLC. Restriction enzyme, *Swa* I, was purchased from New England BioLabs. The reagents for the DNA synthesizer were purchased from Glen Research. *N*,*N*-Dimethylformamide (DMF) was distilled under reduced pressure. All other reagents and solvents were used as received.

2′-Deoxy-3′,5′-di-*O*-acetyl-3-(2′-oxopropyl)thymidine (2)

2′-Deoxy-3′,5′-di-*O*-acetylthymidine **1** (2.78 g, 8.53 mmol) was added to a suspension of sodium hydride (614 mg, 25.6 mmol) in anhydrous DMF (20 ml) at 0 °C and the mixture was stirred at 0 °C for 15 min. To the resulting mixture was added bromoacetone (3.51 g, 25.6 mmol) and the mixture was stirred at 0 °C for 2.5 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 33% hexane–ethyl acetate) to give **2** (2.61 g, 80%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25 (s, 1H), 6.22 (dd, 1H, *J* = 7.6, 6.1 Hz), 5.14 (m, 1H), 4.69–4.68 (2H), 4.30–4.28 (2H), 4.19 (m, 1H), 2.42 (ddd, 1H, *J* = 14.6, 6.1, 1.5 Hz), 2.19 (s, 3H), 2.15 (dd, 1H, *J* = 14.6, 7.6 Hz), 2.06 (s, 3H), 2.03 (s, 3H), 1.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.2, 170.2, 170.1, 162.5, 150.3, 133.0, 110.3, 85.6, 82.1, 74.0, 63.7, 49.8, 37.5, 27.1, 20.7, 20.7, 13.2; FABMS (NBA)

m/e 383 [(M + H)$^+$]; HRMS calcd. for $C_{17}H_{23}N_2O_8$ [(M + H)$^+$] 383.1449, found 383.1449.

2′-Deoxy-3-(2′-oxopropyl)thymidine (d^{oxo}T)

To a solution of **2** (2.61 g, 6.83 mmol) in methanol (13.4 ml) and water (6.7 ml) was added lithium hydroxide monohydrate (631 mg, 15.04 mmol) and the mixture was stirred at 0 °C for 30 min. The reaction mixture was concentrated *in vacuo* and the crude product was purified by column chromatography (SiO_2, 9% MeOH–$CHCl_3$) to give d^{oxo}T (1.91 g, 94%) as a yellow solid: mp 56–57 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (s, 1H), 6.07 (t, 1H, J = 6.9 Hz), 4.69 (s, 2H), 3.90 (m, 1H), 3.87 (dd, 1H, J = 11.6, 2.8 Hz), 3.75 (dd, 1H, J = 11.6, 2.8 Hz), 2.44–2.36 (1H), 2.24 (ddd, 1H, J = 13.4, 6.9, 3.9 Hz), 2.18 (s, 3H), 1.87 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.0, 162.7, 150.5, 135.2, 110.1, 87.0, 86.9, 71.1, 62.2, 50.0, 40.2, 27.4, 13.2; FABMS (NBA) *m/e* 299 [(M + H)$^+$]; HRMS calcd. for $C_{13}H_{19}N_2O_6$ [(M + H)$^+$] 299.1243, found 299.1242.

2′-Deoxy-5′-*O*-dimethoxytrityl-3-(2′-oxopropyl)thymidine (3)

A solution of d^{oxo}T (544 mg, 1.83 mmol) and 4,4′-dimethoxytrityl chloride (929 mg, 2.74 mmol) was stirred in anhydrous pyridine (3.5 ml) for 50 min at 0 °C. The reaction mixture was diluted with saturated $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 9% MeOH–$CHCl_3$) to give **3** (908 mg, 83%) as a yellow solid: mp 93–94 °C; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.55 (s, 1H), 7.35–7.18 (9H), 6.76 (d, 2H, J = 8.9 Hz), 6.30 (t, 1H, J = 6.4 Hz), 4.67 (d, 2H, J = 1.9 Hz), 4.49 (m, 1H), 3.96 (m, 1H), 3.71 (s, 6H), 3.40 (dd, 1H, J = 10.6, 2.9 Hz), 3.29 (dd, 1H, J = 10.6, 3.4 Hz), 2.43–2.17 (2H), 2.16 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.5, 162.6, 158.2, 150.1, 148.7, 144.0, 136.2, 135.1, 134.9, 134.2, 129.7, 127.7, 127.5, 126.6, 123.6, 112.8, 109.5, 86.4, 86.0, 85.2, 71.1, 63.1, 57.3, 54.7, 49.6, 40.7, 26.7, 17.8, 11.9; FABMS (NBA) *m/e* 601 [(M + H)$^+$]; HRMS calcd. for $C_{34}H_{37}N_2O_8$ [(M + H)$^+$] 601.2550, found 601.2541.

2′-Deoxy-5′-*O*-dimethoxytrityl-3′-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite-3-(2′-oxopropyl)thymidine (4)

A solution of **3** (218 mg, 3.63 mmol), 2-cyanoethyl tetraisopropylphosphorodiamidite (115 µl, 0.362 mmol), and tetrazole (33.1 mg, 0.47 mmol) in acetonitrile (1.0 ml) was stirred at ambient temperature for 40 min. The mixture was filtered off and used without further purification.

Caged ODN synthesis

ODNs caged by a 2-oxopropyl group were synthesized by the conventional phorphoramidite method using an Applied Biosystems 392 DNA/RNA synthesizer. Synthesized ODNs were purified by reversed phase HPLC on a Inertsil ODS-3 column (10 × 250 mm, elution with a solvent mixture of 0.1 M triethylammonium acetate (TEAA), pH 7.0, linear gradient over 60 min from 0% to 30% acetonitrile at a flow rate 3.0 mL min^{-1}). Mass spectra of ODNs purified by HPLC were determined with MALDI-TOF mass spectroscopy (acceleration voltage 21 kV, negative mode) with 2′,3′,4′-trihydroxyacetophenone as matrix, using T_8 ([M − H]$^-$ 2370.61) and T_{17} ([M − H]$^-$ 5108.37) as an internal standard; **ODN 1**, 5′-d(AA^{oxo}TAA)-3′, *m/z* 1494.09 (calcd for [M − H]$^-$ 1494.05); **ODN 2**, 5′-d(AAAA^{oxo}TAAAA)-3′, *m/z* 2803.37 (calcd for [M − H]$^-$ 2802.94); **ODN 3**, 5′-d(TTTTTATT^{oxo}TAAATTTTTT)-3′, *m/z* 5504.41 (calcd for [M − H]$^-$ 5504.62); **ODN 5**, 5′-d(^{oxo}TA)-3′, *m/z* 610.65 (calcd for [M − H]$^-$ 610.49); **ODN 6**, 5′-d(^{oxo}TG)-3′, *m/z* 625.72 (calcd for [M − H]$^-$ 626.49); **ODN 7**, 5′-d(^{oxo}TT)-3′, *m/z* 602.24 (calcd for [M − H]$^-$ 601.48); **ODN 8**, 5′-d(^{oxo}TC)-3′, *m/z* 586.13 (calcd for [M − H]$^-$ 586.47).

Radiolytic reduction

To establish hypoxia, aqueous solutions of **ODN 1–8** (110 µM) containing 10% 2-methyl-2-propanol were purged with argon for 30 min and then irradiated in a sealed glass ampoule at ambient temperature with an X-ray source (4.05 Gy min^{-1}). After the irradiation, the solution was immediately subjected to HPLC analysis.

Melting temperature (T_m) measurement

After the irradiation of the oligomers possessing 2-oxoalkyl groups, the corresponding complementary DNA was added to the samples. T_ms of the duplexes (3.0 µM, duplex concentration) were taken in a 50 mM Tris-HCl (pH 7.9) containing 100 mM NaCl and 10 mM $MgCl_2$. Absorbance *vs.* temperature profiles were measured at 260 nm using a JASCO V-530 UV/VIS spectrometer connected with a ETC-505T temperature controller. The absorbance of the samples was monitored at 260 nm from 5 °C to 70 °C with a heating rate of 1 °C min^{-1}. From these profiles, first derivatives were calculated to determine T_m values.

Preparation of 5′-^{32}P-end labeled DNA oligomers

The DNA oligomers were 5′-end-labeled by phosphorylation with 4 µL of [γ-^{32}P]ATP and 4 µL of T4 polynucleotide kinase using standard procedures.[17] The 5′-end-labeled DNA oligomers were recovered by ethanol precipitation and further purified by 15% preparative nondenaturing gel electrophoresis and isolated by the crush and soak method.[18]

Digestion of irradiated ODN 3 and 5′-^{32}P-labeled ODN 4 by restriction enzyme

According to the protocol described above, radiation (0 and 100 Gy) of **ODN 4** (110 µM) was carried out. After the irradiation, 3 µL of radiated **ODN 3** was added to 12 µL of 3.3 µM ^{32}P-labeled **ODN 4**. Hybridization was achieved by heating the sample at 90 °C for 5 min and slowly cooling to room temperature. The resulting duplex was incubated in 50 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1 µg mL^{-1} BSA with *Swa* I (10 U) at 25 °C for 15 min. After the digestion, the reaction mixture was ethanol precipitated with 800 µL of ethanol. The precipitated DNA was washed with 100 µL of cold ethanol and then dried *in vacuo*. The radioactivity of the samples was then measured using aloka 1000 liquid scintillation counter and the dried DNA pellets were resuspended in 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue). All samples were heat denature at 90 °C for

3 min and quickly chilled on ice. The samples (1 μL, 10×10^3 cpm) were loaded onto 15% polyacrylamide and 7 M urea sequencing gel and electrophoresed at 1900 V for approximately 30 min. The gel was dried and exposed to X-ray film with an intensifying sheet at −80 °C.

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10 Radiolysis of a diluted aqueous solution at around pH 7.0 produces primary water radicals such as oxidizing hydroxyl radicals (•OH), reducing hydrated electrons (e_{aq}^-) and reducing hydrogen atoms (•H) with the *G* values of $G(\bullet OH) = 280$ nmol J^{-1}, of $G(e_{aq}^-) = 280$ nmol J^{-1}, and $G(\bullet H) = 60$ nmol J^{-1}, respectively.
11 The radiolytic product from caged **ODN 1** was identified as the corresponding uncaged ODN (5′-AATAA-3′, *m*/*z* 1494.08, calcd for $[M - H]^-$ 1494.05) by mass spectrometry.
12 Similar effect of oxygen was observed for the activation of 5-FdUrd prodrugs. See K. Tanabe, Y. Makimura, Y. Tachi, A. Imagawa-Sato and S. Nishimoto, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 2321–2324 and ref. 9*d*.
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14 Melting temperatures of **ODN 3**–**ODN 4** duplex and control duplex, d(5′-TTTTTATTTAAATTTAAA-3′)–**ODN 4**, were characterized to be 37.0 and 48.9 °C, respectively.
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