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Communications

Regulation of DNAzyme function by hypoxic irradiation that induces one-electron reduction of 2-oxoalkyl group on thymine base

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ABSTRACT: We characterized the one-electron reduction of oligodeoxynucleotides with a 2-oxopropyl group on a thymine base ($d^{oxo}T$) and applied the reaction to the radiolytic activation of DNAzyme function. We designed a system in which the DNAzyme function of cleaving mRNA was suppressed by introduction of $d^{oxo}T$ into the strand of DNAzyme. Hypoxic X-irradiation led to recovery of the cleavage ability because the 2-oxopropyl group was removed to form unmodified DNAzyme. We characterized the DNAzyme function by monitoring the fluorescence change of fluorophore- and quencher-labeled target strands. We confirmed that the DNAzyme function could be regulated by hypoxic X-irradiation and the reaction of $d^{oxo}T$.

Stimuli-responsive DNAs offer favorable properties for biological applications such as gene analysis and therapy.[1–8] These functional DNAs normally remain inert but exhibit their functions once appropriate stimuli are applied. Several stimuli including addition of metal ion,[2–4] binding of borax,[5] and photoirradiation[6–8] have been used to recover their original activities. However, the precise control of the function still remains a major issue. Herein, we demonstrate the control of the function of DNAzyme using hypoxic X-irradiation as an external stimulus.[9–13] Because DNAzyme can cleave target RNA,[14–19] it is expected to enable a new generation of gene-targeting smart drugs. X-irradiation is desirable for activating biomaterials and controlling drug efficacy[9] because X-rays have high live-body permeability. Recently, we reported that the 2-oxoalkyl group on a thymine base ($d^{oxo}T$) was removed by one-electron reduction induced by hypoxic X-irradiation to form unmodified thymine. The $d^{oxo}T$-bearing oligodeoxynucleotides (ODNs) were confirmed to be reduced upon hypoxic X-irradiation, releasing 2-oxopropyl group and producing the corresponding uncaged ODNs that could hybridize with their complementary strand into ordinary duplex. In this study, we employed the unique reaction of $d^{oxo}T$ for controlling DNAzyme function. We modified the DNAzyme 17E,[20] which cleaves target strands in the presence of zinc ion, so that the function of DNAzyme 17E could be regulated by hypoxic X-irradiation.

To allow the regulation of DNAzyme activity to cleave its target strands, we designed the system, in which the $d^{oxo}T$ was introduced at a crotch in the loop region of DNAzyme 17E. We expected that the modified DNAzyme 17E (ODN 1) would not cleave the target strand (DNA/RNA chimeric oligomer: ODN 3) because of the effect of steric hindrance induced by the 2-oxopropyl group. By contrast, when ODN 1 was X-irradiated under hypoxic conditions, the 2-oxopropyl group should be removed from ODN 1 to form unmodified ODN 2, which would catalyze cleavage of the target strand. Thus, the DNAzyme catalysis to cleave RNA should function as usual under X-irradiation conditions.

Initially, to verify the reaction of the 2-oxopropyl group in the DNA strand, we prepared ODN 4 by automated DNA synthesis and performed one-electron reduction for ODN 4 in the presence of complementary ODN 6 in the X-radiolysis of argon-purged aqueous solution containing excess 2-methyl-2-propanol as the scavenger of oxidizing hydroxyl radicals. Under these radiolysis conditions, hydrated electrons (e_{aq}^-) are generated as the major active species.[21] Figure 2 shows a representative profile for the radiolytic reduction of ODNs by e_{aq}^- under these radiation conditions. X-irradiation of ODNs under hypoxic conditions gave a single new product, the yield of which increased as a function of increasing radiation dose. To identify the product, we purified it by HPLC, and analyzed it using mass spectrometry. The molecular weight of the product was identical to the unmodified ODNs (ODN 5). Thus, removal of the 2-oxopropyl group in DNA oligomers occurred under hypoxic X-irradiation. The G values, which indicate the number of molecules produced or changed per 1 J of radiation energy absorbed by the reaction system, were 73 and 24 nmol/J for the consumption of ODN 4 and the formation of the corresponding unmodified ODN 5, respectively.

We next examined a radiolysis of the ODNs in aerobic aqueous solution to elucidate the effect of molecular oxygen on radiolytic reactivity. The radiolytic removal of the 2-oxopropyl group from $d^{oxo}T$ in aerobic conditions was markedly inefficient, in contrast to the prompt formation of unmodified ODN under hypoxic conditions. It has been demonstrated that molecular oxygen scavenges hydrate electrons, which hampers radiolytic reduction. Thus, it is reasonable to conclude that the radiolytic removal of the 2-oxopropyl group from ODN 4 occurred via reduction by e_{aq}^- under hypoxic conditions, which is consistent with previous results.[10]

We next conducted the cleavage of a fluorophore-labeled DNA/RNA chimeric oligomer (ODN 3) by ODN 1, the activity of which was regulated by X-irradiation. The ODN 1 contained a fluorophore, fluorescein, at the 5'-end of the strand, and the quencher dabcyl was connected at the 3'-end of the strand. ODN 3 showed weakened fluorescence because of energy transfer between two molecules, whereas the fluorescence emission could be recovered after cleavage. Thus, we could evaluate the activity of ODN 1 as a DNAzyme by monitoring the fluorescence of ODN 3. The X-irradiation of ODNs was carried out in HEPES buffer containing 100 mM NaCl and 100 μM $ZnCl_2$ at ambient temperature under hypoxic or aerobic conditions, followed by further incubation of the mixture for 1 h. After the reaction, cleavage of ODN 3 was analyzed by fluorescence spectroscopy. As shown in Figure 3, strong fluorescence was observed from ODN 3 after 25 Gy X-irradiation under hypoxic conditions, whereas only a background level of fluorescence was observed in ODN 3 without X-irradiation. The increasing radiation dose resulted in an enhancement of fluorescence intensity. There results clearly indicate that the 2-oxopropyl group in ODN 1 efficiently suppressed the cleavage of ODN 3, while X-irradiation induced the recovery of DNAzyme activity of ODN 1 by removal of 2-oxopropyl group, resulting in a cleavage of the DNA/RNA chimeric substrate.

In conclusion, we characterized the radiolytic removal of the 2-

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oxopropyl group in DNA to construct a radiation-activated DNAzyme, which could cleave DNA/RNA chimeric substrate. The 2-oxoalkyl group on the thymidine base in the ODNs was efficiently removed by hypoxic X-irradiation, whereas the removal of the 2-oxopropyl group was suppressed under aerobic conditions. We characterized the cleavage of the DNA/RNA chimeric substrate, which was labeled with a fluorophore and quencher, by monitoring fluorescence emission. We confirmed that the function of DNAzyme was suppressed by introduction of the 2-oxopropyl group on the thymidine base in ODN 1, and that the hypoxic X-irradiation led to the recovery of cleavage ability because of removal of the substituent.

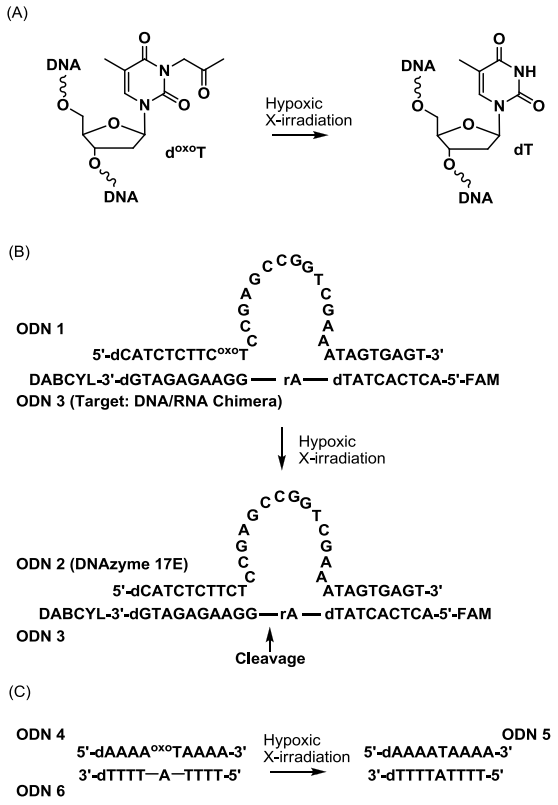

Figure 1. Chemical structures and sequences of oligodeoxynucleotides used in this study. (A) One-electron reduction of thymine base possessing 2-oxopropyl group ($d^{oxo}T$) upon hypoxic X-irradiation. (B) Regulation of DNAzyme function by hypoxic X-irradiaiton. (C) One-electron reduction of oligodeoxynucleotides possessing $d^{oxo}T$ unit.

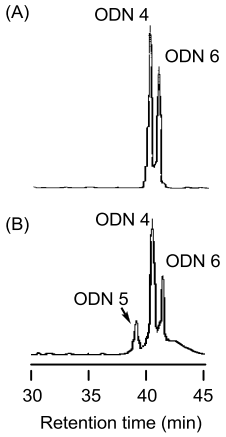

Figure 2. HPLC profiles for the one-electron reduction of ODN 4 in the presence of ODN 6 in the hypoxic radiolysis of aqueous solution containing 10% 2-methyl-2-propanol. (A) Before irradiation. (B) After irradiation (900 Gy).

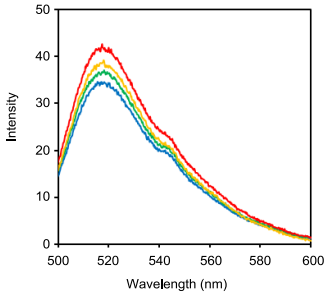

Figure 3. Regulation of DNAzyme activity by hypoxic X-irradiation. The fluorescence spectra of the reaction samples consisted of ODN 1 and ODN 3 after hypoxic X-irradiation (green: 0 Gy, orange: 25 Gy, red: 50 Gy) were measured using excitation at 485 nm. The emission spectrum of ODN 3 (blue) without any treatment was also measured.

KEYWORDS: X-irradiation, DNAzyme, Artificial oligonucleotides

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