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A Nucleobase that Releases Reporter Tags upon DNA Oxidation

Akimitsu Okamoto, Kazuo Tanaka, and Isao Saito*

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, and SORST, Japan Science and Technology Corporation, Kyoto 615-8510, Japan

Received August 27, 2003; E-mail: saito@sbchem.kyoto-u.ac.jp

DNA biosensors offer considerable promise for extracting information from target genes in a quick and simple manner. Various DNA probes that give signals in a sequence-specific fashion, as represented by molecular beacons, have been widely used.[1] However, there are very few DNA probes that can release useful functional molecules.[2] A molecular releasing system that is triggered by external stimulation such as oxidation or photoirradiation would be a useful tool for gene analysis.

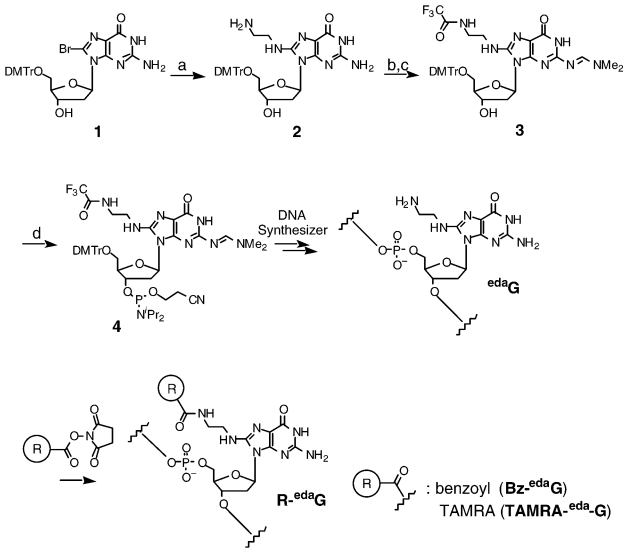
Herein, we report a novel nucleoside, ethylenediamine-modified G (^{eda}G), and a new molecular releasing system controllable by one-electron oxidation of oligodeoxynucleotides (ODNs). Reporter units tethered to ^{eda}G were easily released from ODNs by mild oxidation. In a long-range hole transport experiment, DNA duplex containing ^{eda}G efficiently and stoichiometrically released a fluorescent tag.

The synthesis of ^{eda}G-containing ODN is outlined in Scheme 1. 8-BromoG (**1**) protected by a 4,4′-dimethoxytrityl group[3] was converted to **2** by refluxing in ethylenediamine, and the amino groups were protected to afford **3**. ^{eda}G phosphoramidite **4** was incorporated into ODNs by a conventional method. By the postsynthetic modification of ^{eda}G-containing ODNs using *N*-hydroxysuccinimidyl ester of carboxylic derivatives such as benzoic acid (Bz) and tetramethylrhodamine (TAMRA), reporter units were incorporated into the amino side chain of ^{eda}G in the ODNs.

We initially examined the photooxidation of single-stranded ODN containing Bz-^{eda}G, **ODN1(Bz-^{eda}G)** 5′-d(TATAAT**X**TAATAT)-3′ (**X** = Bz-^{eda}G), in the presence of a photosensitizer riboflavin.[4] A sample solution of 10 μM **ODN1(Bz-^{eda}G)** in 50 mM sodium cacodylate (pH 7.0) was irradiated at 366 nm in the presence of 50 μM riboflavin at 0 °C. Photoirradiation of **ODN1(Bz-^{eda}G)** resulted in a rapid decomposition of ODN ($t_{1/2}$ = 6.2 min). The major isolable photoproducts of the oxidation of **ODN1(Bz-^{eda}G)** were identified by mass spectrometric analysis as ODN fragments **5**, cleaved via an abasic site, ([M − H]$^-$, calcd. 1968.32, found 1967.68 by MALDI-TOF), 5′-phosphate end **6** ([M − H]$^-$, calcd. 1870.22, found 1870.11 by MALDI-TOF), and benzamide **7** possessing a guanidinium group (M^+, 207 and its fragments 148, 105, 77, and 59 by LC-ESI/MS/MS) (Scheme 2). The identified products strongly suggest that the rapid decomposition of ^{eda}G may proceed via the G cation radical decomposition mechanism proposed earlier[5] to result in a release of a functional unit as typically represented by **7**.

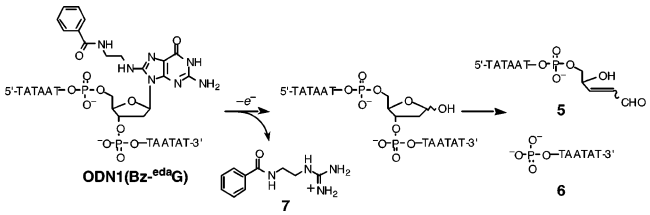
The site-selective oxidation of ^{eda}G-containing ODN was also observed with other one-electron oxidants. Ir(IV) is a highly selective oxidant that reacts exclusively with oxidized nucleobases such as 8-oxoG and 8-oxoA.[6] The oxidation potential ($E_{1/2}$) of Bz-^{eda}G is 0.59 V (vs NHE),[7] which is close to that of 8-oxoG (0.56 V,[8] 0.60 V[9]). The ^{eda}G-containing ODN **ODN1(X)** (**X** = Bz- and TAMRA-^{eda}G) was mixed with Ir(IV).[6,10] A solution of single-stranded **ODN1(X)** (10 μM) in sodium cacodylate buffer (pH 7.0) was incubated at room temperature in the presence of sodium

Scheme 1 [a]



[a] Reagents and conditions: (a) ethylenediamine, 130 °C, 7 h; (b) ethyl trifluoroacetate, triethylamine, methanol, 0 °C, 2 h; (c) DMF-dimethylacetal, DMF, room temperature, 2 h, 63% (three steps); (d) $(^iPr_2N)_2PO(CH_2)_2CN$, tetrazole, acetonitrile, room temperature, 2 h, quant.

Scheme 2



hexachloroiridate(IV) (20 μM). Oxidation with Ir(IV) resulted in a rapid degradation of **ODN1(X)** at the site of modified ^{eda}G (57% consumption for Bz-^{eda}G and 89% for TAMRA-^{eda}G in 15 min incubation, as determined by HPLC).

The method using ^{eda}G constitutes a facile strategy for detecting long-range hole transport through DNA without complicated and unwieldy analyzing processes such as quantification of oxidative guanine damage of labeled DNA[11] or the analysis of photodynamics.[12] We examined the detection of TAMRA released from **ODN2** containing a TAMRA-tethered ^{eda}G via long-range hole transport through DNA (Figure 1). The reaction sample containing the **ODN2**/**ODN2′(U*)** duplex possessing a cyanobenzophenone-modified uridine (**U***)[13] as a hole injector was irradiated at 312 nm (Figure 1a).[14] The photolyzed ODN was then removed from the sample solution by passing through a centrifugal filter (Microcon YM-3). A strong fluorescence at 576 nm was observed from the filtrate of the **ODN2**/**ODN2′(U*)** sample after photoirradiation, and the fluorescence after 60 min of irradiation was 7 times stronger

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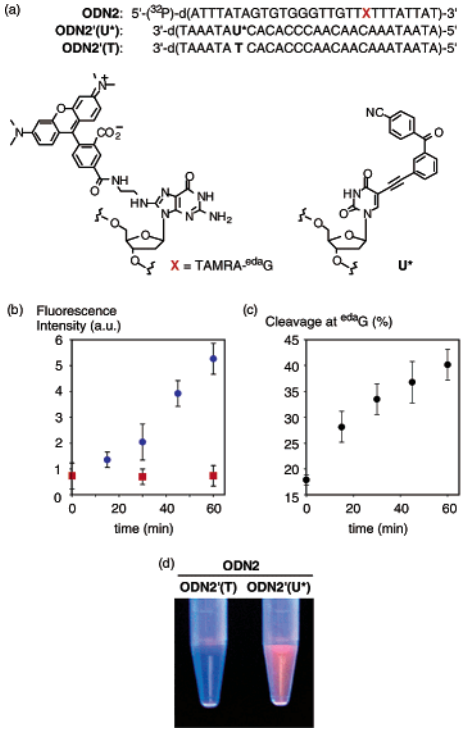

Figure 1. Release of TAMRA from TAMRA-^{eda}G via long-range DNA oxidation. (a) Sequences of duplex ODNs. (b) Fluorescence intensity of the reaction samples after photoirradiation and removal of ODN. The duplexes in 10 mM sodium cacodylate (pH 7.0) were irradiated ($\lambda = 312$ nm) at 0 °C followed by centrifugation with a centrifugal filter (Microcon YM-3). Fluorescence spectra were measured at 550 nm excitation. Fluorescence intensities at 576 nm were designated by ● (blue) for **ODN2**/**ODN2′(U*)** duplex and by ■ (red) for **ODN2**/**ODN2′(T)**. (c) Cleavage of TAMRA-^{eda}G via hole transport. ^{32}P-labeled duplex in 10 mM sodium cacodylate (pH 7.0) was irradiated ($\lambda = 312$ nm) at 0 °C followed by a hot piperidine treatment. The relative damaging extents show the percentage of strand breakages at the ^{eda}G site relative to the total strand cleavage obtained by densitometric analysis. (d) Fluorescence image of the samples given by **ODN2**/**ODN2′(T)** duplex (left) and **ODN2**/**ODN2′(U*)** duplex (right) after 312 nm irradiation (60 min) followed by removal of ODN by filtration. The fluorescence image of the filtrate was taken using a transilluminator (312 nm).

than that of the control **ODN2**/**ODN2′(T)** without **U*** (Figure 1b). The fluorescence intensity of the **ODN2**/**ODN2′(U*)** sample increased in proportion to the irradiation time. The change of fluorescence intensity showed a good correlation with the strand cleavage at ^{eda}G site, which was independently quantified by PAGE for the experiment using the **ODN2**/**ODN2′(U*)** (Figure 1c).[15] In addition, in the PAGE analysis for the photoirradiated duplex, it was observed that lesions at the GGG sites, located between **U*** and ^{eda}G, were strongly suppressed. Thus, ^{eda}G acts as a very efficient hole trap, and the hole generated in the duplex by **U*** is selectively trapped at the ^{eda}G site via a long-range hole transport to result in the release of TAMRA from the duplex.

The fluorescence from the photoirradiated sample was visually detectable. As shown in Figure 1d, a strong visible emission was observable with the filtrate of the photoirradiated **ODN2**/**ODN2′-(U*)** sample, whereas the emission from the filtrate of photoirradiated **ODN2**/**ODN2′(T)**, a control sample, was negligible. The incorporation of TAMRA-^{eda}G into the duplex makes it possible to detect hole transport through DNA without PAGE analysis.

In conclusion, we have developed a novel nucleosbase, ^{eda}G, that efficiently releases reporter tags upon one-electron oxidation. The ^{eda}G-selective degradation of ODNs can be achieved by various mild oxidizing agents. This oxidant-dependent molecular releasing technique is useful not only for drug releasing systems but also for the release of a fluorescent tag after gene analysis.

Supporting Information Available: Detailed experimental data of ^{eda}G and the related ODNs (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The decrease of the fluorescence intensity of TAMRA by photobleaching was less than 5% after 312 nm photoirradiation for 90 min.

(15) The 18% of TAMRA-^{eda}G in intact ODN was also damaged by hot piperidine treatment (90 °C, 20 min).

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