Phototriggered Molecular Release

Phototriggered Drug Release from Functionalized Oligonucleotides by a Molecular Beacon Strategy

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DNA hybridization biosensors offer considerable promise for obtaining sequence information of genes in a fast and simple manner. Various DNA probes that give signals in a sequence-specific fashion, as represented by molecular beacons, have been widely used.[1–3] However, there are very few DNA probes that release functional molecules on recognition of the target sequence.[4,5] The sequence-specific molecule-releasing system that is triggered by external stimulation, such as irradiation would be a very powerful tool for gene analysis.

Herein we report for the first time a molecule-releasing system controllable by an intramolecular quenching based on a molecular-beacon strategy by using photoactive probe oligodeoxynucleotides (ODNs). The photoreaction of the probe ODN containing a photoactive group and a triplet quencher at both ends of the strand was very inefficient when it was in the closed-form ODN (a stem-and-loop structure), whereas irradiation of the open-form ODN hybridized with the complementary DNA resulted in a rapid release of the functional molecule from the ODN (see Figure 1).

ODN sequences for the molecule-releasing system were designed according to the molecular-beacon strategy.[1] Phenacyl ester[6–8] as a photocleavable group via a triplet excited state, and substituted naphthalene[9,10] as a triplet quencher

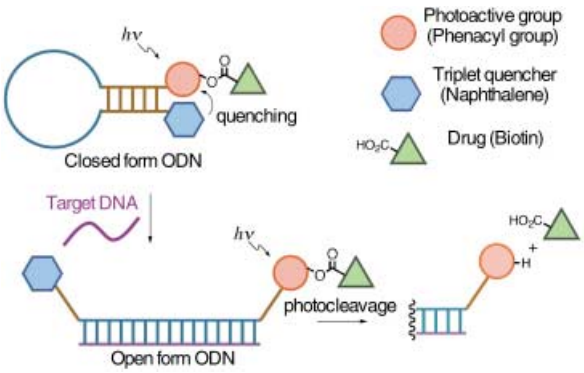

Figure 1. Outline for the phototriggered drug release from functionalized oligonucleotides by a molecular beacon strategy.

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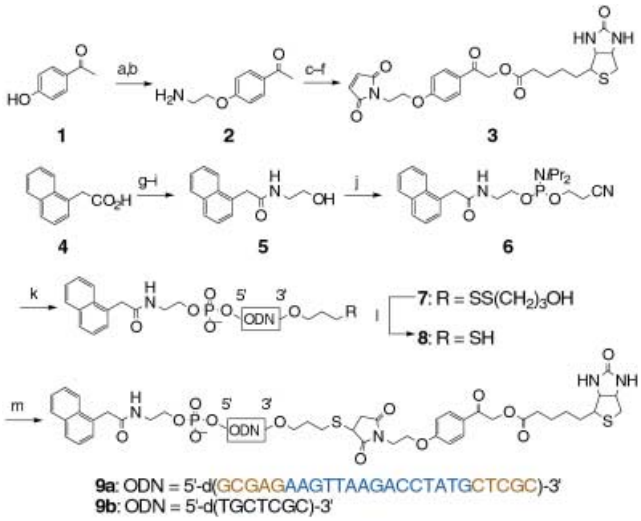
were incorporated into the 3′- and 5′-ends of the ODN, respectively. The stem of the closed-form ODN keeps a phenacyl ester and a naphthalene ring in close proximity to each other, causing the efficient triplet quenching, as in the case of a molecular beacon. In contrast, when the probe ODN is transformed to the open form by hybridization with target DNA,[1] photocleavage of the phenacyl ester proceeds very smoothly under irradiation, because the photoactive phenacyl ester is far away from the naphthalene quencher.

The synthetic route for the functionalized ODN is shown in Scheme 1. The photocleavable phenacyl ester **3**, which has both a maleimide functional group to be connected to thiol-



Scheme 1. Reagents and conditions: a) K_2CO_3, *N*-(2-bromoethyl)phthalimide, acetone, reflux, 10 h, 23%; b) 40% methylamine, 2:1 chloroform-methanol, RT, 5 h, 64%; c) maleic anhydride, toluene, reflux, 5 h; d) sodium acetate, acetic acid, reflux, 1.5 h, 49% in two steps; e) $PhMe_3NBr_3$, THF, RT, 3 h, 35%; f) (+)-biotin, triethylamine, DMF, RT, 2 h, 76%; g) thionyl chloride, reflux, 2 h; h) $TBDMSO(CH_2)_2NH_2$, THF, RT, 12 h, 43% in two steps; i) TBAF, THF, RT, 3 h, 82%; j) $(iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, acetonitrile, RT, 1.5 h, quant.; k) DNA autosynthesizer, then 28% ammonia, 55 °C, 12 h; l) 5 mM dithiothreitol, 25 mM sodium phosphate (pH 8.4), 23 °C, 12 h, quant.; m) 0.5 mM **3**, 250 mM sodium phosphate (pH 7.0), 23 °C, overnight, quant.

modified DNA and a biotin unit as a leaving functional molecule, was prepared from 4-hydroxyacetophenone (**1**). Naphthalene phosphoramidite, **6**, was prepared from 1-naphthylacetic acid (**4**) in four steps. ODNs containing both a naphthalene unit at the 5′ end and a disulfide unit at the 3′ end were synthesized by using a DNA synthesizer by a conventional phosphoramidite method. The disulfide group of the given ODNs **7** was reduced to thiol by dithiothreitol, and then coupled with **3** to give ODNs **9a** and **9b** that were equipped with a photoactive group and a quencher at each end.

We initially investigated the efficiency of the photoreaction of ODN **9a** by irradiation at 312 nm before and after hybridization with the complementary target DNA 5′-d(CATAGGTCTTAACTT)-3′. The irradiation of a solution of **9a** in 15 mM sodium cacodylate (pH 7.0) and 2-propanol (2:1) was

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conducted at 0 °C for 10 seconds, and the disappearance of **9a** was monitored by HPLC. The conversion of closed-form **9a**[11] was relatively inefficient (5% conversion, $k_{obs} = 0.03\ \mu M\ s^{-1}$), whereas irradiation of open form **9a** hybridized with the complementary strand resulted in an efficient degradation (25% conversion, $k_{obs} = 0.17\ \mu M\ s^{-1}$). These results clearly indicate that a structural change caused by hybridization of **9a** with the complementary DNA strongly affects the consumption of **9a**.[12]

To obtain photoproducts, we examined the irradiation of a shorter single-stranded ODN **9b**, and analyzed the reaction products by mass spectrometry. With five minutes of irradiation, **9b** was completely consumed and afforded the product mixture. The molecular weight [(M−H)⁻] of the reaction products given by MALDI-TOF mass spectrometry was 2775.49, thus indicating the formation of acetophenone **10b** (calcd 2775.99) (Scheme 2). Such reductive cleavage of phenacyl ester was also confirmed in the photolysis of a model system, 4-methoxyphenacyl ester of biotin.[6,13,14]

We next quantified the biotin that was released from **9a** by the photolysis of the phenacyl ester. After irradiation at 312 nm of a solution of **9a** (200 pmol), the photoproduct mixture was isolated filtration through a centrifugal filter and mixed with a solution of the complex of avidin and 2-((4′-hydroxyphenyl)azo)benzoic acid (HABA).[15,16] Then, the decrease of the absorbance at 500 nm (A_{500}) originating from the avidin-HABA complex was monitored. The decrease of A_{500} is known to be caused by the displacement of HABA from the avidin-HABA complex by the released biotin.[16] The amount of biotin that was released from **9a** was determined by using a calibration curve of A_{500} given from control experiments (Figure 2). Irradiation of closed-form **9a** resulted in a slight decrease of A_{500}, corresponding to 12% biotin release. In contrast, a drastic decrease of A_{500} corresponding to 84% biotin release was observed in the presence of complementary ODN 5′-d(CATAGGTCTTAACTT)-3′. The efficiency of biotin release was significantly altered by the conformational change of **9a** by hybridization with the target DNA sequence. These results strongly suggest that naphthalene attached to the 5′ end of **9a** acts as an effective intramolecular quencher in closed form **9a** and suppresses photodegradation of the phenacyl ester, which readily occurred in open-form **9a**.

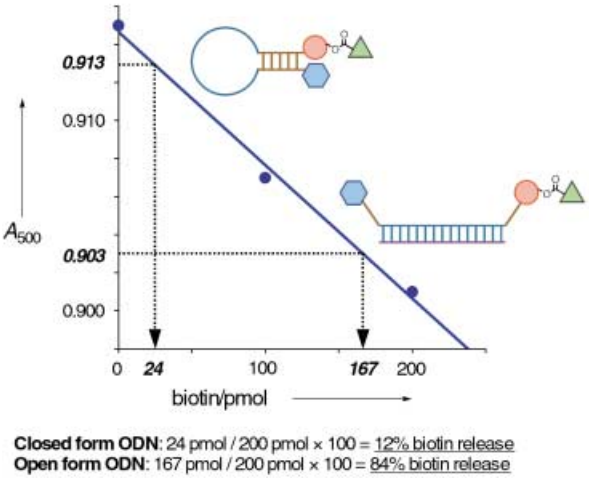
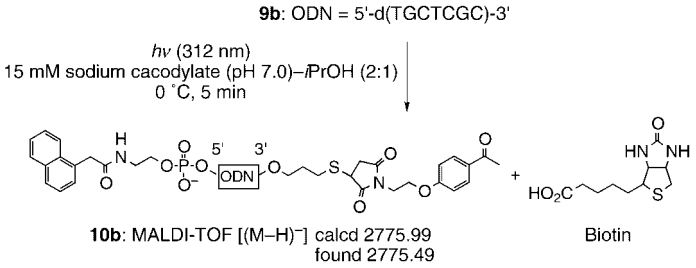


Figure 2. Quantification of biotin released from **9a**. Irradiation (312 nm) of 200 pmol of **9a** in the presence or absence of the complementary ODN 5′-d(CATAGGTCTTAACTT)-3′ was carried out in 15 mM sodium cacodylate (pH 7.0)-*i*PrOH (2:1) at RT with a monochromator (JASCO CRM-FD, 300 W Xe lamp, 40 counts; one count of irradiation approximately corresponds to a surface energy of 0.02 J cm^{-2}). After the reaction mixture was filtered through a Microcon centrifugal filter (YM-3, 3000 NMWL), the filtrate was mixed with a solution of avidin-HABA complex (0.43 mg of ImmunoPure Avidin, PIERCE and 62.5 μg of ImmunoPure HABA, PIERCE) in 86 mM sodium phosphate (pH 7.2) and 130 mM sodium chloride, and then the absorbance at 500 nm (A_{500}) of the mixture was measured. The amount of released biotin was quantified by using a linear fit (the blue solid line, $y = -7.0 \times 10^{-5}x + 0.9147$; $R = 0.9966$) based on A_{500} of an avidin-HABA solution in the presence of 0, 100, and 200 pmol of biotin.



Scheme 2.

In summary, we have demonstrated for the first time a phototriggered molecule-releasing system by using a molecular beacon strategy. Hybridization of the photoactive probe ODN with the complementary target DNA resulted in a rapid photolytic cleavage of phenacyl ester with the release of biotin, although closed form ODN before hybridization suppresses biotin release due to the intramolecular triplet quenching. The drug release occurs effectively by UV irradiation when a specific sequence has been recognized. This new drug-releasing system will facilitate the rational design of a well-controllable prodrug for gene analysis.

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[11] Closed-form **9a** was stable at room temperature. The T_m was 40.4 °C in 10 mM sodium cacodylate (pH 7.0) and 1 mM NaCl.
[12] A control ODN that does not have a 5′ triplet quencher **9a**–naph was also tested. Very rapid degradation was observed for both closed form **9a**–naph and open form **9a**–naph hybridized with the complementary strand (59 % and 55 % conversion, respectively).
[13] A solution of 4-methoxyphenacyl ester of biotin (0.14 mmol) in acetonitrile/2-propanol/water (1:4:2, v/v/v) was irradiated at 312 nm with a transilluminator for 3.5 h at room temperature. The reaction mixture was purified by silica gel chromatography to give 4-methoxyacetophenone and biotin in 59 % yield.
[14] Several peaks were detected by HPLC in the reaction mixture of **9b**, suggesting that the mixture contains several photoproducts in addition to product **10b** as given by MALDI-TOF mass. For a solution containing duplex ODN 5′-d(GCGAGAAGTTAA-GACCTATGCTCGC)-3′/5′-d(CATAGGTCTTAACTT)-3′ and 4-methoxyphenacyl ester of biotin, 8 % consumptions of ODN were observed for 60 s irradiation. Further conversion of **10b** by irradiation was not observed.
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