Photoinduced Release of Fluorescent Probe in the Presence of Target DNA: Synergetic Effect of Two Types of Oligonucleotides with Indolequinone–Coumarin Conjugate and Flavin Photosensitizer

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We have synthesized and evaluated two types of functionalized oligodeoxynucleotides (ODNs) that can release a fluorescent coumarin probe via co-hybridization with a target DNA base sequence and subsequent photoirradiation. Photoirradiation of flavin-bearing ODN in the presence of a counterpart ODN with indolequinone–coumarin conjugate and a target complementary DNA strand gave rise to the efficient release of coumarin with a multiple turnover as a result of intermolecular flavin-photosensitized reduction of indolequinone–coumarin conjugate, while the release of coumarin was considerably suppressed in the absence of the target DNA. Concomitant with this photoreaction, we could observe intense fluorescence emission from the sample solution photoirradiated with the target DNA, but weak fluorescence from a control solution without the target DNA.

Modified oligonucleotides (ODNs) possessing a base-sequence dependent molecular releasing functionality have been developed as DNA biosensors that can transmit genetic information.[1–3] Among the various strategies for such a molecular releasing system,[2] much current interest has been focused on the design of artificial DNA that is activated by photoirradiation to release the functional molecule,[3] because of ease of the system control.

We herein propose a novel DNA biosensor, which can release a fluorescent probe efficiently in the presence of complementary strand under conditions of photoirradiation (Figure 1). The proposed system involves two types of modified ODNs possessing a reductively fluorescent probe-releasing function of indolequinone–coumarin conjugate (ODN 1) and a photosensitized reducing function of flavin chromophore (ODN 2), respectively. Indolequinone derivatives have been identified as effective eliminating substituents via photolytic reduction in the presence of reducing photosensitizers such as flavins.[4] Photoirradiation of flavin-bearing ODN 2 in the presence of a counterpart ODN 1 with indolequinone–coumarin conjugate and a target complementary DNA strand (ODN 3) gave rise to the efficient release of coumarin, leading to an intense fluorescence, as a result of photosensitized one-electron reduction of indolequinone–coumarin conjugate located in close proximity to a photosensitizing flavin chromophore. The photosensitized coumarin release occurred with a multiple turnover, in which dissociation of photolyzed ODN 1 and alternative association of fresh ODN 1 repeat on the ODN 2–ODN 3 hybrid, resulting in an enhanced release of excess amount of coumarin. In contrast, the absence of specified target ODN 3 led to less amount of coumarin release, which is attributable to the decreased extent of the photosensitized reduction occurring via diffusion of the two functional ODNs.

The synthesis of the modified ODNs is outlined in Scheme 1. Indolequinone–coumarin conjugate **4** and flavin derivative **6**, both of which had a succinimidyl functional group to be connected to the amino modified DNA, were prepared from 5-hydroxyindole **1** and flavin derivative **5**,[5] respectively. The incorporation of these functional groups into ODNs was achieved by coupling of a modified ODN possessing an amino group with **4** or **6** to give ODN 1 or ODN 2, respectively. The formation of modified ODNs was confirmed by MALDI-TOF mass spectrometry. The structures of ODNs used in this study are shown in Figure 1.

To characterize the functionality of flavin chromophore and indolequinone–coumarin conjugate on the ODNs, we photoirradiated mixtures of 10 μM ODN 1 and 1 μM ODN 2 at 365 nm in the absence and presence of complementary 1 μM ODN 3 in

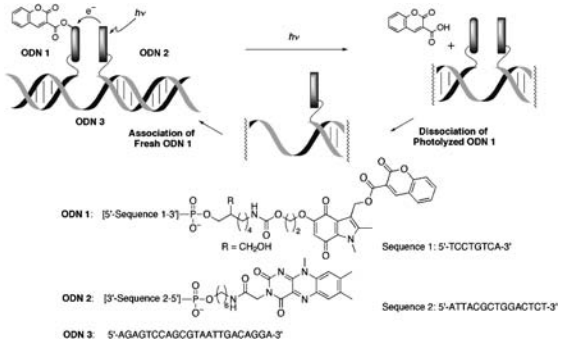
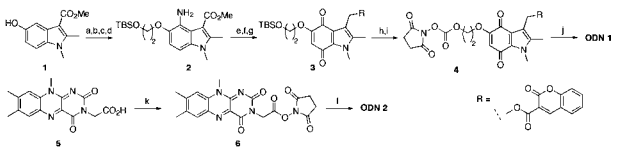


Figure 1. Schematic illustration of the photoactivated fluorescent probe releasing system targeting DNA specific base sequence and the structures of ODNs used in this study.



Scheme 1. Reagents and conditions: (a) 2-Bromo-1-*tert*-butyldimethylsilyloxyethane, NaH, DMF, 37%; (b) HNO_3, AcOH, CH_2Cl_2, 67%; (c) Sn, HCl, EtOH, quant.; (d) TBSCl, imidazole, DMF, 97%; (e) DIBAL-H, CH_2Cl_2, 90%; (f) Fremy's salt, NaH_2PO_4, THF, 38%; (g) Coumarin-3-carboxylic acid, DCC, DMAP, CH_2Cl_2, 60%; (h) PPTS, CH_2Cl_2, MeOH, H_2O, 98%; (i) *N*,*N*′-disuccinimidyl carbonate, Et_3N, DMF, 72%; (j) 3′-amino-linked ODN, $NaHCO_3$, CH_3CN–H_2O; (k) *N*-hydroxysuccinimide, DCC, DMF, 60%; (l) 5′-amino-linked ODN, $NaHCO_3$, CH_3CN–H_2O.

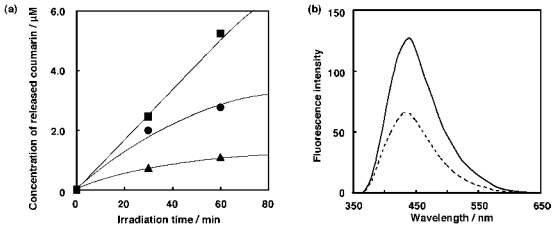


Figure 2. (a) The concentration changes of coumarin upon photoirradiation at 365 nm of flavin chromophore in ODN 2. The photoirradiation of the mixture of ODN 1 and ODN 2 for 0, 30, and 60 min was carried out in the presence (■) or absence (●) of complementary ODN 3. The dark reaction (▲) of ODN 1 was also carried out in the presence of ODN 2 and ODN 3. The reaction was monitored by reversed phase HPLC, using a UV detector at 290 nm. (b) Fluorescence spectra of the reaction mixtures of ODN 1/ODN 2 after photoirradiation for 60 min with (solid line) and without (dashed line) ODN 3, respectively, and removal of ODNs. The sample solutions were irradiated at 20 °C followed by centrifugation (13,000 rpm) with Microcon (YM-3, 3000 MWCO), centrifugal filter devices, at 0 °C for 30 min. The fluorescence of the filtrate was measured with an excitation wavelength of 320 nm using a Shimadzu RF-5300PC fluorescence spectrophotometer.

10 mM sodium phosphate buffer (pH 6.5) containing 0.1 M NaCl and 100 μM EDTA at 20 °C.[6] The time courses of the photoreactions monitored by HPLC are shown in Figure 2a. Photosensitized release of coumarin was suppressed in the absence of complementary ODN 3, in which spontaneous diffusion of the two modified ODNs occurs preferentially.[7] In contrast, the addition of ODN 3 enhanced the coumarin releasing efficiency to a substantial degree, up to a 1.7-fold increase. These results strongly indicate that co-hybridization of ODN 1 and ODN 2 with ODN 3 into a duplex structure gives rise to close contact between indolequinone–coumarin conjugate and flavin chromophore, leading to an efficient flavin-photosensitized one-electron reduction of indolequinone–coumarin conjugate to release coumarin.[8] The coumarin release was confirmed to occur as a turnover reaction process, in which a net amount of 2 μM coumarin was generated by the proposed mechanism during the photosensitization for 60 min in the presence of only 1 μM ODN 2 and 1 μM ODN 3 (see Figure 2a).[9] This result suggests that the hybrid of ODN 2 with ODN 3 could play a catalytic function for the coumarin releasing photoreaction of ODN 1, similar to the previously reported drug release system regulated by nucleic acids hybridization.[2a] In a separate control dark reaction of ODN 1 in the presence of ODN 2 and ODN 3, we confirmed that the release of coumarin was suppressed to the background level and that photoactivation of indolequinone–coumarin conjugate in ODN 1 by flavin chromophore in ODN 2 is responsible for the efficient release of coumarin.

To further identify the functionality of these modified ODNs, we monitored the releases of coumarin from ODN 1, using fluorescence spectrometry after removal of ODNs by a centrifugal filter (Microcon) from the reaction mixtures of ODN 1 and ODN 2 photoirradiated in the presence and absence of ODN 3, respectively. As shown in Figure 2b, an intense fluorescence emission at $\lambda_{max} = 440$ nm was observed for the filtrate obtained from the photoreaction in the presence of ODN 3. In contrast, the fluorescence intensity was suppressed for the sample derived from photoirradiation in the absence of ODN 3, corresponding to inefficient release of coumarin. Thus, the catalytic release of coumarin regulated by duplex formation and photoirradiation could be successfully detected by monitoring the fluorescence intensity.

In summary, we have developed a photoinduced molecular releasing system that targets a prescribed DNA base sequence, using sequence selective duplex formation and subsequent one-electron reduction of indolequinone–coumarin conjugate in ODN 1 via photosensitization by flavin chromophore in ODN 2. Photoirradiation of the modified ODNs in the presence of their complementary strand ODN 3 led to efficient release of coumarin, resulting in an intense fluorescence emission. Compared with previous DNA biosensors,[1–3] a remarkable feature of present system is that the turnover reaction leading to release of an excess amount of coumarin could be achieved by photosensitization. In this view, this system would be applicable to a differentiation of small amount of specified ODN with increment of fluorescence emission.

Our current study focuses on construction of higher sensitive systems, by which one can detect genetic information from quite small amount of genomic DNA, using a family of photofunctionalized ODNs and intense fluorescence probe releasing with multiple turnover.

References and Notes

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6 Melting temperatures of ODN 1/ODN 3 duplex and ODN 2/ODN 3 duplex were characterized to be 27.8 and 58.8 °C, respectively. In view of these thermal denaturation profiles, we carried out photoreactions at 20 °C.

7 We observed moderate release of coumarin from ODN 1 in the presence of ODN 2 but absence of ODN 3 under photoirradiation conditions. This release was probably attributable to a non-specific photoreduction of indolequinone–coumarin conjugate by photoexcited flavin chromophore.

8 Compound **3** has molar extinction coefficient (ε) of 753 M^{-1} cm^{-1} at 365 nm, which is significantly smaller than the corresponding value of 5174 M^{-1} cm^{-1} for compound **5**. Therefore, although direct excitation of the indolequinone–coumarin conjugate in ODN 1 is unavoidable, the flavin chromophore in ODN 2 may preferentially absorb exciting light at 365 nm. In this context, we confirmed that close contact between indolequinone–coumarin conjugate and flavin chromophore resulted in a much enhanced release of coumarin. Thus, we concluded that the intermolecular flavin-photosensitized one-electron reduction of indolequinone–coumarin conjugate is dominant in the release of coumarin.

9 Since prolonged photoirradiation resulted in a decomposition of coumarin, we evaluated photoreactions within ca. 60 min.

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