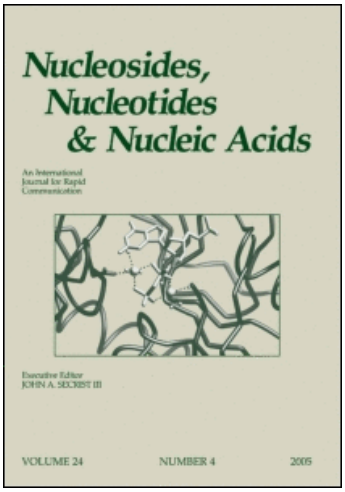

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DEVELOPMENT OF THE NOVEL DRUG RELEASING SYSTEM TRIGGERED BY HYBRIDIZATION WITH TARGET SEQUENCE

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□ *We have already established the strategy of synchronous activation by hybridization, in which the highly reactive cross-linking agent, 2-amino-6-vinylpurine nucleoside analog, can be generated from its stable precursors, the phenylsulfide derivatives, by a hybridization-promoted activation process with selectivity to cytosine. In this study, this in situ activation system was applied to the method for the drug releasing system triggered by hybridization with the target sequence.*

Keywords Cross-link; drug releasing system; oligonucleotides; synchronous activation; 2-amino-6-vinylpurine; FRET

INTRODUCTION

A number of genetic disorders have been identified as the major cause of diseases. Oligonucleotides (ODNs) and their analogs have been exploitable in the development of diagnostic and therapeutic tools for such diseases. Many chemically reactive appendages bound to ODN are available for specified applications. Recently, the attempts to develop DNA sequence-specific drug releasing systems by modified ODNs have been reported. In these systems, the drug is released from the conjugate with ODN by hybridization to target DNA[1] or hybridization and subsequent UV-irradiation.[2,3] Therefore, it is expected that the drug is toxic selectively for the cells containing the target gene sequence.

Previously, we established the new strategy of synchronous activation by hybridization, in which the highly reactive cross-linking agent 2-amino-6-vinylpurine nucleoside analog can be generated from its stable precursors,

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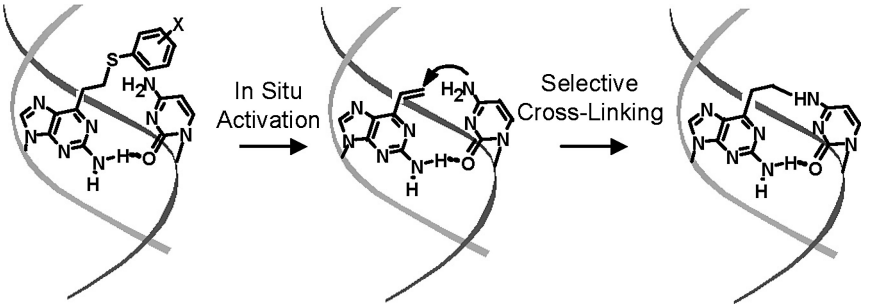

FIGURE 1 In situ activation of the sulfide derivative of 2-amino-6-vinyl purine for selective cross-linking with cytosine.

the phenylsulfide derivatives, by a hybridization-promoted activation process with selectivity to cytosine (Figure 1).[4,5] Recently, we have successfully shown the intracellular ability of this hybridization-promoted alkylation system by demonstrating efficient and selective antisense activity.[6] In these reactions, the sulfide groups are released from modified ODN triggered by hybridization. In this study, we wish to report the new concept for the drug releasing system triggered by hybridization with a target gene.

RESULTS AND DISCUSSION

For the proof of our concept, we first designed two strategies for visualization of the releasing system in cells. In the first one, the fluorescent group is connected to 2-amino vinylpurine with a sulfide bond and the release of sulfide group can be detected by increasing fluorescence (Figure 2A). The other one is exploited a fluorescence resonance energy transfer (FRET) (Figure 2B).

In the first methodology, pyrene sulfide derivative having an ethyl amide linker was used as the fluorescence group but the elimination of sulfide group did not take place after the addition of the complementary strand.

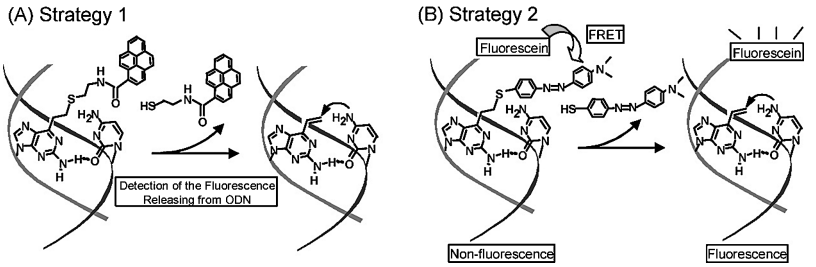

FIGURE 2 Strategy for visualization of the releasing system.

We searched the proper structure of pyrene sulfide derivatives for the easy elimination reactions, but we could not find appropriate structure for the visible detection of the reaction.

For strategy 2 utilizing the FRET system, the 5′-fluorescein labeled ODN (**1**) having 2-amino-6-vinylpurin derivative at 3 bases away from the 5′ end was synthesized.

When 4-dimethylaminoazobenzenethiol as a quencher was added to the vinyl group of 2-aminopurine of **1**, the fluorescence intensity of the resulting double-labelled ODN probe was decreased compared to the 5′-fluorescein single-labelled ODN (**1**). The subsequent treatment of ODN (**2**) with MMPP and aqueous NaOH regenerated ODN (**1**) having strong fluorescence (Figure 3). These results have indicated that the elimination of the sulfide group can be detected by observing the increase of fluorescence intensity of the double-labelled ODN. To test this drug releasing system, both the cross-linking reaction and increase of the fluorescence intensity were measured after mixing of the double-labelled ODN (**2**) and the target ODN (**3**). A cross-linking reaction proceeded selectively with the target ODN (**3 Y = dC**), which suggested that ODN (**2**) was activated within the duplex having cytosine at the target site to liberate the sulfide group. Unlike strategy 1, the elimination reaction occurred using the double-labelled ODN (**2**). These results were in agreement with our previous studies,[4] which the introduction of the electron donating group on the phenyl ring accelerated the elimination reaction. In the meantime, selective increase of the fluorescence intensity of the reaction mixture was observed in the combination of the ODN (**2**) and (**3**) having C at the target site, clearly indicating selective release of the quencher (Figure 4). The target strand with other base, T, G, A instead

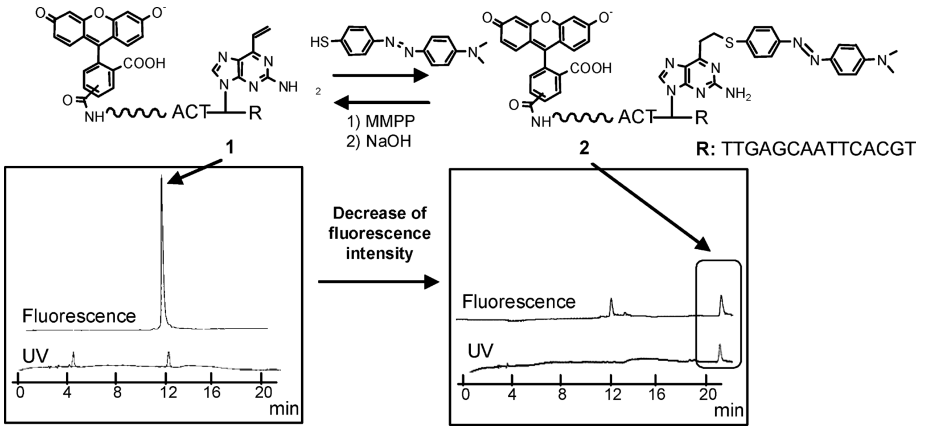

FIGURE 3 Analysis of the changes of fluorescence intensity of double-labelled ODN by HPLC. HPLC chromatograms were monitored by fluorescence with emission at 518 nm and excitation at 494 nm. Column: nacalai tesque COSMOSIL 5C18-MS (4.6 × 250 mm) Solvent: A:0.1 M TEAA Buffer B: CH_3CN, B10%->30%/20 min, 30-> 100%/30 min.

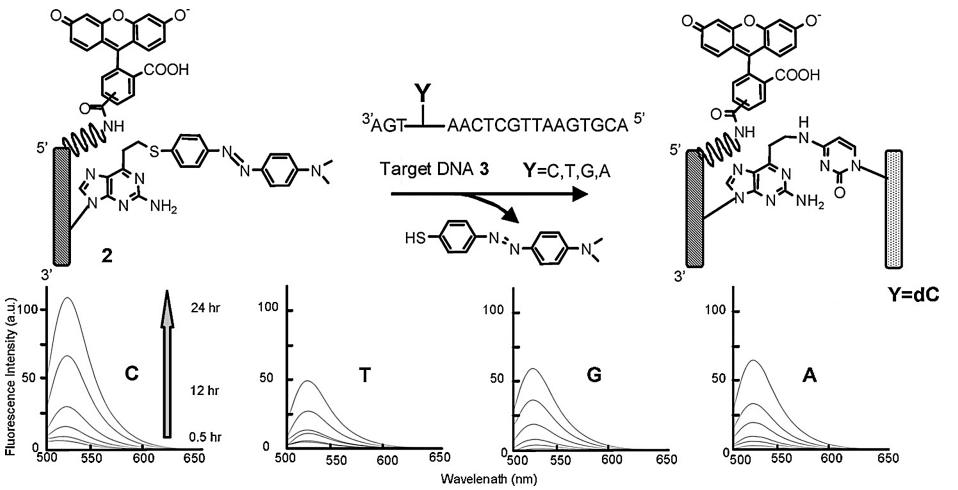

FIGURE 4 Fluorescence spectra of the ODN (2) hybridized with target DNA. The reaction was performed with 50 μM ODN (**2**) and target ODN (**3**) (Y=C, T, G, A) in 0.1 M NaCl, 50 mM MES, pH5.0, 37°C. Fluorescence spectra of the reaction mixture were measured at indicated time.

of C at the target site showed similar behavior at much slower rates. The addition of the non-complementary ODN to the double-labelled ODN (**2**) did not lead to an increase of fluorescence intensity. These results have suggested that thiol-containing drugs are released by hybridization-triggered activation in a highly sequence specific manner, and that the double-labelled ODN probe is useful to monitor the in situ activation process.

CONCLUSION

The in situ activation process, in which the sulfide precursor of the 2-amino-6-vinylpurine nucleoside is selectively activated in the hybrid, has been applied to develop a new drug releasing system triggered by hybridization with the target DNA. A unique benefit of this system is reflected in its high selectivity that may permit discrimination of a single nucleotide difference. As the releasing of the drug is accelerated not by external signal but by intrinsic activation associated hybridization to the target sequence, this releasing system may be applicable to in vivo study. Further study is now ongoing in this line.

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