Sensing of nucleic acid sequences using unmodified nucleic acid as a probe

Atsushi Narita, Shinsuke Sando and Yasuhiro Aoyama
Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

ABSTRACT

We present a strategy to generate a light-up fluorophore-aptamer pair. The strategy was based on a modification of a conventional DNA-staining dye to suppress its affinity to the original targets, and subsequent re-selection of aptamers that would bind to the modified dye. Along this line, we prepared an environmental polarity-sensitive Hoechst derivative with low affinity to the usual AT-rich dsDNA targets. DNA aptamers, in vitro selected from a random pool, worked as a trigger to enhance the fluorescence of an otherwise nonfluorescent Hoechst derivative.

INTRODUCTION

There has been much recent attention on sensing of nucleic acid sequences (1). Among the various types of homogeneous sensing methods so far reported, including a representative molecular beacon (2,3), several new strategies have enabled the imaging of a target sequence using an unmodified nucleic acid as a probe. For example, Rao and coworkers have reported on a split ribozyme reporter strategy (4), wherein an activity of trans-acting ribozyme was controlled by the presence of a target nucleic acid sequence as an allosteric effector. They demonstrated that the strategy can be applicable to mRNA sensing, even in mammalian cells (4). We have also reported on a new strategy of a molecular beacon-mRNA for sensitive genotyping (5,6). This was based on the system of naturally occurring (7) or engineered (8) hairpin-shaped RNAs to control the translation frequency, so that the sensing of the nucleic acid sequence, even at a single nucleotide resolution, could be carried out using a genetically encodable unmodified RNA as a probe.

Another choice is to use a light-up fluorophore-aptamer pair. Tsien and coworkers first showed that malachite green (MG) fluorophore dramatically enhances its fluorescence intensity upon binding to specific RNA sequences (aptamers) (9). The MG-aptamer was well engineered to binary probes, which were programmed to restore the aptamer core structure only in the presence of the target sequence. The binary aptamer was applicable to the fluorescence detection of nucleic acids (10). This method is very promising for nucleic acid detection/imaging due to its compatibility with in vitro technologies, and also for in-cell applications. To make this method a general method, we need to establish a strategy to generate new light-up fluorophore-aptamer pairs, hopefully with multicolor-multipaptamer combinations. To this end, Sparano and Koide recently reported on a fluorophore-quencher photoinduced electron transfer (PET) system that exhibits fluorescence enhancement upon binding to an in vitro selected RNA aptamer via perturbing PET (11,12). Here, we present a potential approach for generating a bioorthogonal light-up fluorophore-aptamer pair from a single microenvironment-sensitive fluorophore.

RESULTS AND DISCUSSION

Our approach is based on an improvement of the known fluorescent dyes used as a versatile, and hence less sequence-selective, nucleic acid imager to a structure/sequence-specific one by narrowing the adaptable target sites using the following steps. The first step is the chemical modification of a conventional nucleic acid-binding fluorophore so as to suppress its binding to the original target. The second step is re-selection of a new target (aptamer) for the modified fluorophore using an in vitro selection method. Typical nucleic acid-binding light-up fluorophores are known to fluoresce more strongly in a low dielectric environment. Therefore, we hypothesized that simply in vitro selected aptamers, having a relatively nonpolar pocket for the binding of a hydrophobic dye, would work as a switch to lower the dielectric microenvironment of the bound dye, and the binding could lead to an enhancement of the fluorescence.

We focused on the Hoechst dye as a conventional DNA-imaging probe (13,14). Hoechst dye is a polarity-sensitive fluorophore composed of a bisbenzimidazole chromophore, which preferentially binds to the minor groove of AT-rich dsDNA, and enhances its fluorescence intensity upon binding (dielectric environment of the groove ≈ 20D, similar to propyl alcohol) (13). Also, the high specificity of the Hoechst dye to dsDNAs, among various other biological components, i.e., its practical bioorthogonality, has been confirmed in a number of studies on selective genome staining in live cells (15).

We prepared a set of Hoechst derivatives having a variety of substituents on the terminal phenol ring, which could lead to a loss of a binding affinity to AT-rich dsDNAs, the natural targets of the unmodified Hoechst dye.
Fig. 1 shows the Hoechst derivatives synthesized and used in this study:

1. $R_1 = H$, $R_2 = H$, $R_3 = OH$ (Hoechst 33258)
2. $R_1 = OMe$, $R_2 = H$, $R_3 = OH$
3. $R_1 = CH_2$, $R_2 = H$, $R_3 = OH$
4. $R_1 = H$, $R_2 = CH_3$, $R_3 = OH$
5. $R_1 = H$, $R_2 = Pr$, $R_3 = OH$
6. $R_1 = H$, $R_2 = Bu$, $R_3 = OH$

![Chemical structures of Hoechst derivatives 1-7 used in this study.](image)

The binding affinities of Hoechst derivatives to AT-rich dsDNAs were evaluated by measuring the increase in melting temperature ($\Delta T_m$) of dsDNA(AT), having an AT-rich sequence in the middle position of the duplex, by the presence of these derivatives. Due to its lowest binding affinity to dsDNA(AT), we selected Hoechst derivative 6 as our first candidate for generating a new pair.

We then carried out an *in vitro* selection (16,17) of a DNA aptamer against the Hoechst derivative 6 immobilized on magnetic beads. *In vitro*-selected DNA aptamers showed an affinity to the Hoechst derivative and interestingly worked as a trigger to enhance the fluorescence of Hoechst 6 derivative. In marked contrast, the enhancement in fluorescence was highly suppressed for a hairpin-structured DNA possessing a 5'-AATT-3'5'-TTAA-5' moiety, which caused a remarkable enhancement in fluorescence in the original (unmodified) Hoechst 33258 (18). Thus, the original Hoechst 33258, which was specific to AT-rich dsDNAs, was rendered aptamer-selective during this process. One of the aptamers thus obtained was shortened to 25-mer sequence with retaining the light-up activity. The shortened 25-mer sequence was split into binary-type aptamer probes referring to the design of Kolpashchikov (10), enabling us to detect a target nucleic acid sequence with a single nucleotide resolution using unmodified DNA as a probe.

CONCLUSION

We showed that a microenvironment-sensitive fluorophore can be converted into an aptamer-selective light-up fluorophore in combination with SELEX technology. It is interesting that a simple *in vitro* selection could generate active aptamers, which work as a trigger for lighting up otherwise nonfluorescent fluorophores, without taking into account the molecular conformation or rigidity of the bound dye. We also successfully selected RNA aptamers that could induce an enhancement in fluorescence of the Hoechst derivative via the same approach. This will also be discussed in this presentation.

ACKNOWLEDGEMENTS

This work was supported in part by an Industrial Technology Research Grant Program of the New Energy and Industrial Technology Development Organization (NEDO) of Japan. AN acknowledges a fellowship from JSPS.

REFERENCES


*Corresponding Author. E-mail: ssando@sbchem.kyoto-u.ac.jp (S.S) or aoyamay@sbchem.kyoto-u.ac.jp (Y.A)