Detection of DNA hybridization by use of a lanthanide fluorescent intercalator that specifically binds to double stranded DNA

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ABSTRACT
Toward development of a DNA microarray system in which neither labeling nor amplification of the nucleic acids from living cell is required, we have developed a new method for the detection and quantification of target DNA hybridized with probe DNA fixed on a solid surface. This method utilizes a fluorescent intercalator: naphthalene diimide derivative carrying two fluorescent tetradentate β-diketone-Eu³⁺ chelates. This compound selectively binds to double stranded DNA (dsDNA) fixed on a plastic assay plate. The amount of the compound bound to single stranded DNA (ssDNA) is negligible. The fluorescent intensity of Eu³⁺ was in proportion to the amount of the fixed DNA, showing that the compound quantitatively binds to dsDNA. Therefore, this method can be used not only to detect dsDNA, but also to measure the amount of DNA on a solid surface.

INTRODUCTION
DNA hybridization is one of the powerful methods for the detection of specific nucleic acids sequences, which is required for the analysis of gene expression profile. Traditional methods used for the detection of DNA hybridization required the labeling of target DNA, and both chemical and enzymatic methods are available for the introduction of labeling reagents. Polymerase chain reaction (PCR) (1) is one of the widely used methods in which deoxyribonucleoside triphosphate labeled with dye or radioisotope is enzymatically incorporated to the target DNA during the amplification. However, the amplification may change the population of the target sample. To avoid the problem, nick translation seems to be suitable, however, it is associated with a problem that uniform labeling is difficult. Therefore, detection method in which neither labeling nor amplification of the target sample is required has been desired. In this paper, we tried the detection of double stranded DNA (dsDNA) immobilized on a solid surface using a fluorescent intercalator consists of naphthalene diimide derivative moiety and two fluorescent tetradentate β-diketone-Eu³⁺ chelates. This compound selectively binds to dsDNA, and the binding to single stranded DNA (ssDNA) is negligible.

Figure 1. Scheme for detecting the DNA hybridization using fluorescent intercalator. NDI-(BHHCT-Eu³⁺)₂ is naphthalene diimide derivative carrying two fluorescent tetradentate β-diketone-Eu³⁺ chelates.
RESULTS AND DISCUSSION

Outline of hybridization assay is illustrated in Fig. 1. Structure of naphthalenediimide derivative carrying two tetradeutate β-diketonate europium chelates, 4,4'-bis(1",1",2",2",3",3":heptafluoro-4":6":hexanedion-6":yl)-chlorosulfo-o-terphenyl-Eu$^{3+}$, [NDI-(BHHCT-Eu$^{3+}$)]$_2$ is illustrated in Fig. 2. Double stranded DNA having a chain length of 150 bp was prepared by means of PCR with 5'-biotin and 5'-FITC labeled-oligodeoxyribonucleotides as primers. The obtained dsDNA was dissolved in 1xSSC with various concentrations and spotted on a streptavidin-coated plastic plate at ambient temperature for 2 h, followed by twice washing with 1xSSC. The immobilized DNA was quantified as FITC fluorescence counts. Excess amount of NDI-(BHHCT-Eu$^{3+}$)$_2$ in 1xSSC (150 mM sodium chloride and 15 mM sodium citrate, pH 7.6) was added, and after twice washing with 1xSSC, the Eu$^{3+}$ fluorescence counts were measured (Fig. 3). The fluorescence intensity of Eu$^{3+}$ was in proportion to the amount of the immobilized DNA, showing that the compound quantitatively binds to the double stranded DNA.

Detection of DNA hybridization using intercalators is advantageous for a highly sensitive fluorometry since, according to a previous study (3), one naphthalenediimide molecule intercalates every two basepairs whereas in the traditional enzymatic incorporation of labeled nucleotide, every several ten basepairs. The intercalator gives high density labeling of DNA. Furthermore, lanthanide complexes are advantageous for a sensitive fluorometry because of their properties such as large Stokes shift (>250 nm) and long fluorescence lifetime (a few hundreds μs). Such enables a microarray system characterized with high S/N. Further investigation on the use of this complex for the DNA microarray is currently ongoing.

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