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Note

Exonuclease III-assisted Rapid Detection of HIV DNA by Using DNA-templated Silver Nanoclusters

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Fluorescence-based methods, including fluorescence resonance energy transfer (FRET) assays[1–4] and signal amplification-based fluorescence assays,[5–10] have been widely used for the detection of biomolecules because of their high sensitivity, simple operation, and diverse fluorophores. However, there have been some drawbacks such as photobleaching, high cost of prelabeling probes, and influence of labeled probes on binding affinity and target specificity.[11,12] Therefore, there is a need for the development of alternate fluorescence methods.

DNA-templated metals (gold, platinum, silver, and copper) nanoclusters have been developed for over a decade for the detection of biomolecules of interest. In particular, DNA-templated silver nanoclusters (DNA-AgNCs) have attracted attention owing to their facile synthesis, tunable fluorescence emission, high luminescence quantum yield, low toxicity, and biocompatibility,[13–15] as well as their increased detection sensitivity by coupling with enzyme-assisted target recycled amplification.

Human immunodeficiency virus (HIV) is a human retrovirus that causes immune deficiency syndrome (AIDS) which has high mortality rate.[16,17] HIV detection is routinely performed using western blotting and enzyme-linked immunosorbent assay (ELISA), which are highly selective and sensitive. However, due to a relatively long time for antibody production or insufficient antibody to be detected during this period, new strategies would be developed for the detection of viral DNA at the earliest time after infection.[18,19] Therefore, we developed in this study a method for the rapid detection of HIV DNA using exonuclease III (Exo III)-assisted target recycled amplification and DNA-AgNCs.

Scheme I shows the detection strategy for HIV DNA using Exo III-assisted target recycled amplification and DNA-AgNCs. In the absence of HIV DNA, single-stranded template DNA (tDNA) was not digested by Exo III. Thus, high fluorescence emission in the presence of an enhancer after the synthesis of DNA-AgNCs was observed. In contrast, fluorescence intensity is lowered in the presence of HIV DNA and Exo III, because HIV DNA is recycled to degrade tDNA by Exo III. The oligomers used in this study are listed in *Table* 1. tDNA composes of two segments: one segment serves as nucleation sequence for DNA-AgNCs formation and another is hybridizing with HIV DNA. In mutant HIV DNA, thymine (T) is substituted for adenine (A) in HIV DNA sequence.

Fig. 1 shows the fluorescence spectra of DNA-AgNCs in the presence or absence of HIV DNA. The fluorescence intensity was monitored at excitation and emission wavelengths of 563 and 625 nm, respectively. It decreased from 101.78 (a) in the presence of tDNA only to 17.44 (b) in the presence of HIV DNA and Exo III. This indicated that HIV DNA detection was sensitive, as mentioned in the detection strategy in *Scheme* 1.

Fig. 2 shows the results of detection sensitivity of HIV DNA in the presence or absence of Exo III. Reactions were carried out at 37 °C by adding 50 U of Exo III to the

***Table* 1.** Sequences of oligonucleotides used in this study

Name	Sequences
Template DNA (tDNA)	5'-CCTTAATCCCCTAATGTGGAAAATCTCTAGCAGT-3'
Wild-type HIV DNA (WT HIV)	5'-ACTGCTAGAGATTTTCCACATTAGGGGTACTGTCA-3'
Mutant HIV DNA (MT HIV)	5'-ACTGCTAGAGTTTTTCCACATTAGGGGTACTGTCA-3'
Enhancer	5'-AGATTTTCCACATTAGGGTGGGGTGGGG-3'

red: nucleation sequences, blue: HIV DNA sequences, underlined base: mutation base

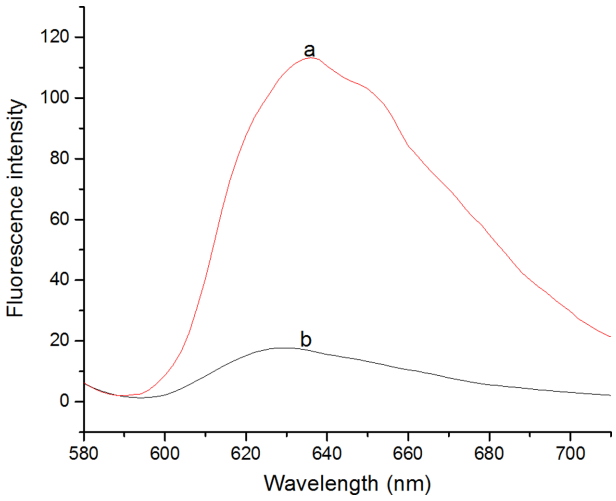
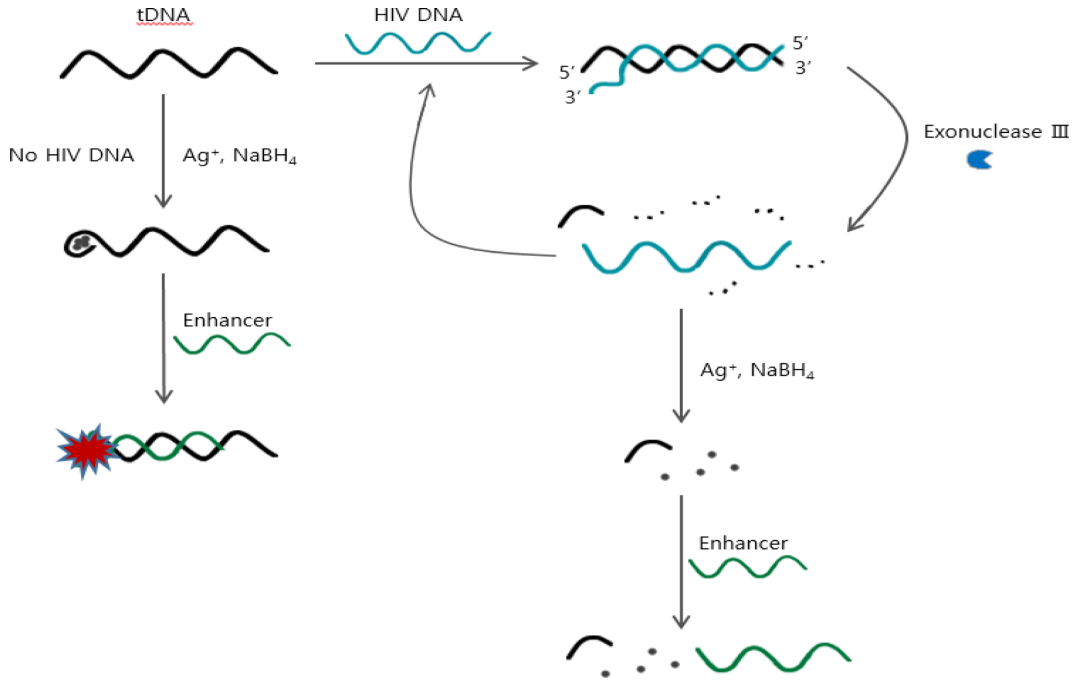


***Figure* 1.** Fluorescence spectra of reactions containing tDNA (2 μM) (a) and tDNA + HIV DNA (0.3 μM) + Exo III (b). Fluorescence spectra were measured from wavelengths of 580 to 710 nm at an excitation wavelength of 563 nm.

reaction mixture containing tDNA and HIV DNA in 50 mM Tris-HNO_3, pH 8.0, 5 mM $Mg(NO_3)_2$. In a recent publication,[6] annealing reaction was conducted for 4 h by incubating X-DNA (hybridizes with one HIV DNA and two F-DNAs) and F-DNA and then amplification reaction was allowed to proceed for 0.5 h by adding p-DNA (hybridizes with F-DNA and serves as template for DNA-AgNCs), HIV DNA, and Exo III to the annealed dsDNA. However, the reactions in this study were conducted for 1 h by incubating tDNA, HIV DNA and Exo III.

F/Fo was observed to measure the enhancement of detection sensitivity by Exo III using this method. Fo indicates the fluorescence emission of the reaction in the presence of tDNA only. F represents fluorescence intensities of reaction containing tDNA and HIV DNA in the presence (HIV + Exo III) or absence (HIV) of Exo III in *Fig.* 2. F/Fo was 0.93, in the presence of HIV DNA. However, F/Fo decreased 5.4-fold in the presence of HIV DNA and Exo III. This was because Exo III recycled HIV DNA by digesting the tDNA for signal amplification. In a recent study,[7] Exo III-assisted DNA detection resulted in a 4.45-fold amplification in the fluorescence intensity. In addition, a sensitive and selective electrochemical method was reported for the detection of HIV gene by using Exo III-assisted target recycling and guanine nanowire amplification.[20]

Fig. 3 shows the fluorescence spectra of DNA-AgNCs in the presence of various HIV DNA concentrations (*Fig.* 3A). The fluorescence intensities decreased from 90.47 to



***Scheme* 1.** Schematic diagram showing the detection strategy for HIV DNA by using Exo III-assisted target recycled amplification and DNA-AgNCs. Reaction (left) is conducted using tDNA only and DNA-AgNCs is synthesized. Reactions (right) are proceeded by annealing, amplification and Exo III digestion in the presence of tDNA and HIV DNA. Then, fluorescence intensity is measured after DNA-AgNCs are synthesized.

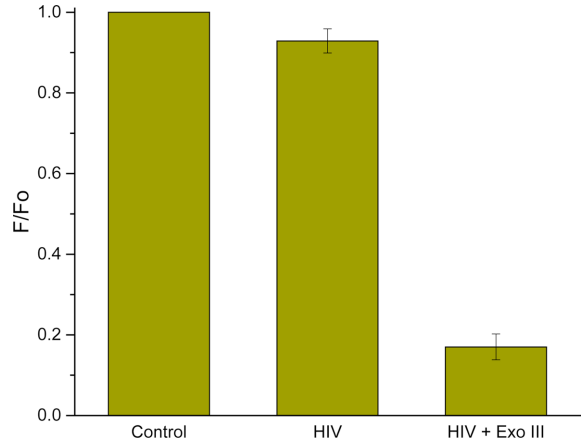


***Figure* 2.** Fluorescence intensities in the presence of tDNA only (Control), tDNA + HIV DNA (HIV), and tDNA + HIV DNA + Exo III (HIV + Exo III). Fluorescence emissions were measured at 625 and 563 nm of excitation and emission wavelengths, respectively.

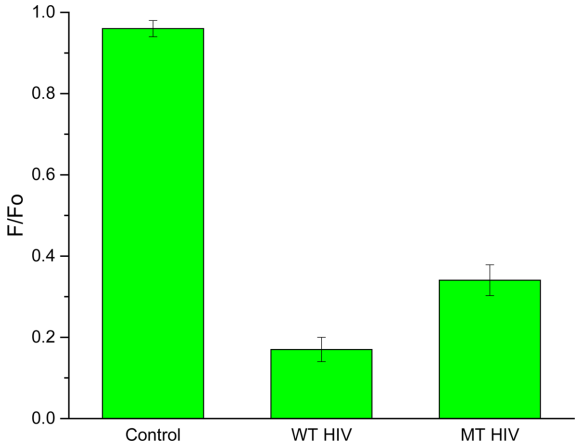


***Figure* 4.** Fluorescence emissions of control, WT HIV (wild-type HIV DNA) and MT HIV (mutant HIV DNA). Control contained tDNA only. In WT HIV and MT HIV, tDNA + Exo III + wild-type HIV DNA (WT HIV) and tDNA + Exo III + mutant HIV DNA (MT HIV) were retained.

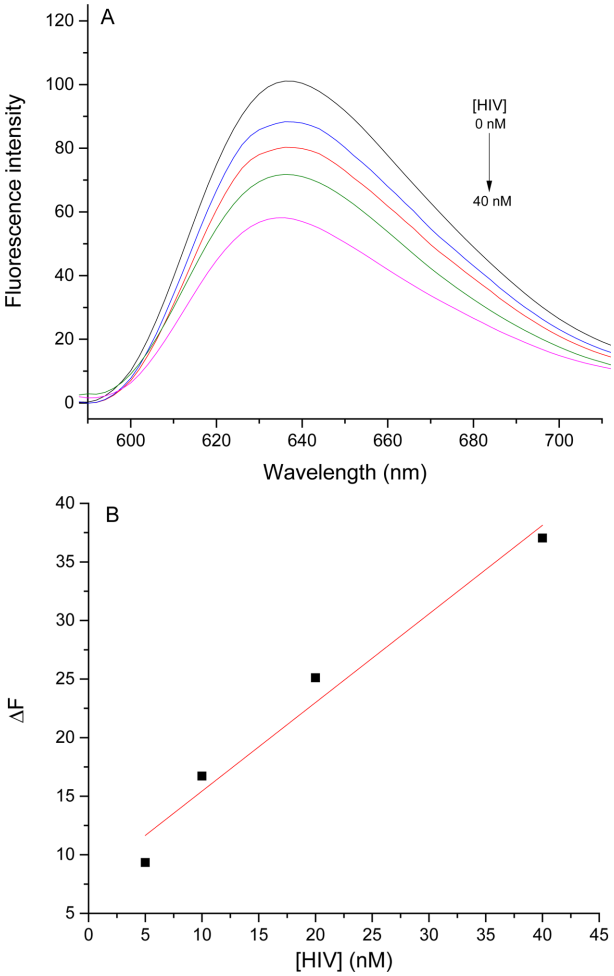


***Figure* 3.** (A) Fluorescence spectra of reactions at different HIV DNA ranging from 0 to 40 nM. (B) Plot of ΔF against different concentration of HIV DNA ($R^2 = 0.96$).

53.43 as HIV DNA concentrations increased from 0 to 40 nM. This indicated that Exo III treatment of HIV DNA:tDNA duplex resulted in the removal of tDNA and thus decreased fluorescence intensities. The limit of detection (LOD) for HIV DNA was 5 nM. In addition, ΔF was linearly correlated with the concentration of HIV DNA ($R^2 = 0.96$). In a recent publication,[10] Exo III-aided fluorescence method was applied to detect DNA sequence, and this method greatly improved sensitivity relative to traditional molecular beacons without any significant restriction in the choice of target sequences.

Fig. 4 shows the F/Fo of wild-type and mutant HIV DNA. F/Fo in mutant HIV DNA was 0.34 ± 0.04, which was higher than that of wild-type HIV DNA (F/Fo = 0.17 ± 0.03). Mutant HIV DNA was not easily released from the HIV:tDNA duplex after Exo III treatment due to Exo III digestion being hindered by the mismatched base, which was 12 bases apart from 3'- end of the tDNA. This method is also applied to detect wild-type and mutant DNA sequence of some organism and protein (enzyme) activity.

In summary, fluorescence emission in the presence of Exo III decreased 540% relative to that in the absence of Exo III. In the mutant DNA, F/Fo was not greatly lowered compared to that of wild-type HIV DNA because of the hindrance of Exo III digestion due to the mismatched base. This study demonstrated that the Exo III-assisted HIV detection method is rapid and sensitive without time consumption and probe labeling.

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