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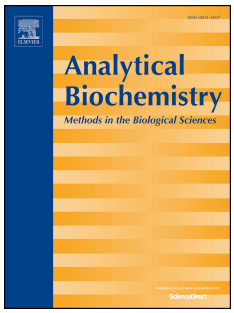

The detection of a mismatched DNA by using hairpin DNA-templated silver nanocluster

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The detection of a mismatched DNA by using hairpin DNA-templated silver nanocluster

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Abstract

Fluorescence assays have been developed to detect biomolecules using DNA-templated silver nanocluster (DNA-AgNCs) utilizing their unique physical and optical properties. This study was designed to detect single-mismatched DNA by the hybridization of target DNA to template DNA either before or after DNA-AgNCs synthesis. The results showed that the detection specificity of a single-mismatched DNA was clearly enhanced when the target DNA (cDNA) was hybridized to template DNA prior to DNA-AgNCs synthesis compared with cDNA hybridization subsequent to DNA-AgNCs synthesis.

Keywords; DNA detection, biosensor, DNA-templated silver nanoclusters (DNA-AgNCs), hairpin DNA.

Fluorescence-based detection assays have been widely used due to their many advantages such as high sensitivity, specificity, and simplicity. However, traditional fluorescence methods require the addition of fluorescence dye or the pre-labeling of the fluorophore which may influence the binding affinity and specificity of the probe [1,2].

Recently, the DNA-templated Ag nanoclusters (DNA-AgNCs) have been developed as the fluorescence probe to measure the biomolecules of interest without fluorescence dye and attached fluorophore. DNA-AgNCs have been utilized to detect metal ions [3,4], proteins [5,6], biomolecules [7,8] and DNA [9] due to the unique optical properties including strong fluorescence, high photo-stability, and long lifetime [10-12].

DNA used in this study contains nine cytosines looped hairpin DNA, favoring silver nanoclusters formation [13]. For the detection assays with DNA-AgNCs, some assays performed the detection reactions after DNA-AgNC synthesis [14,15], but others conducted the reactions before DNA-AgNC synthesis [16-18]. For effective DNA detection, in this study, target DNA (cDNA) was hybridized to template DNA (9C6b DNA) either before or after DNA-AgNCs synthesis.

Scheme 1 shows the schematic representation for effective DNA detection using DNA-AgNCs. 9C6b DNA used in this study contains a single-strand region at the 5’ end, and the 9C loop is followed by a 6 base-paired stem region. In addition, m-cDNA and cDNA oligomers contain the complementary sequences to the stem region and additional 4 (m-cDNA) and 8 (cDNA) complementary bases to the extra arm of 9C6b DNA, respectively. The sequences of oligonucleotides (Bioneer, Chungwon, Korea) are listed in Table 1. In the first method (top), 9C6b-AgNCs was synthesized by adding $AgNO_3$ (Sigma-Aldrich, St. Louis, USA) and $NaBH_4$ (Tokyo Chemical Industry, Tokyo, Japan) to reaction. Then the fluorescence changes of 9C6b-AgNCs were measured in the presence otarget f different

cDNAs which play roles as target DNAs (shown in Table 1). In the second method, 9C6b DNA was hybridized with different cDNA oligomers, and then, these reactions were used for 9C6b-AgNCs synthesis to measure the detection specificity (bottom).

In the first method, 9C6b-AgNCs (5 μM) was incubated in 20 mM sodium phosphate buffer (pH 7.0) and 50 mM $NaNO_3$ in the presence of different cDNAs at room temperature for 1 h. The fluorescence intensity was measured with a Molecular Devices XPS fluorescence microplate reader (Sunnyvale, USA) with excitation and emission wavelengths of 558 nm and 628 nm, respectively.

Figure 1A shows UV and fluorescence spectra of 9C6b-AgNCs either in the presence or absence of cDNA. These spectra indicate that absorption was observed with a sharp peak at a wavelength of 560 nm, and fluorescence emission was measured at 640 nm for the excitation wavelength of 560 nm. Figure 1B was captured using a UV transilluminator in the presence of 9C6b-AgNCs only (tube 2) and 9C6b-AgNCs + cDNA (tube 3). The fluorescence intensity of 9C6b-AgNCs was quenched by the addition of cDNA (tube 3) compared with that of 9C6b-AgNCs (tube 2), indicating that the silver nanocluster formed in the 9C loop were disrupted by the hybridization of cDNA to 9C6b-AgNCs.

To measure the dependence of DNA detection on the length of hybridized cDNA, 3 different cDNAs (cDNA, s-cDNA, and m-cDNA) were used. In Figure 2A, the F/Fo of 9C6b-AgNCs is plotted in the presence of different cDNA oligomers. F and Fo represent the fluorescence intensities either in the presence or absence of cDNA, respectively. The hybridization of cDNA and m-cDNA resulted in a decrease in F/Fo, indicating that cDNA hybridization opened the stem region of 9C6b DNA, and then it caused the disruption of the metal bound nanoclusters (9C6b DNA-AgNCs), However, F/Fo decreased slightly for s-cDNA because of the short DNA length. The results indicated that the specificity of DNA detection was enhanced by the increase in the length of DNA hybridized to the stem and extra

arm regions of 9C6b DNA (specificity of DNA detection was as followed; s-cDNA< m-cDNA < cDNA).

For single mismatched cDNA detection, the addition of cDNA-1 and cDNA-2 to 9C6b DNA-AgNCs decreased F/Fo without a clear discrimination of their values. The values were 0.57 for cDNA-1 and 0.52 for cDNA-2. It indicated that the hybridization of cDNA-1 and cDNA-2 did not differentiate the disruption of DNA-metal nanoclusters owing to the rigid compact structure of DNA-AgNCs [19]. For DNA detection with a molecular beacon containing a fluorophore and quencher at the end of each strand at the end of each strand, the fluorescence intensity is quenched through the disruption of hairpin structure in the molecular beacon by the addition of target DNA to the molecular beacon [20-22].

For the second DNA detection method (as shown in Scheme 1), 9C6b DNA was hybridized with different cDNAs, and then, 9C6b-AgNCs were prepared for the measurement of fluorescence intensity under different conditions. As shown in Fig. 3A, the specificity of DNA detection according to hybridized DNA length showed the same results shown, s-cDNA< m-cDNA < cDNA.

Contrastingly, for single mismatched cDNA, the F/Fo values of cDNA-1 and cDNA-2 were ~0.7 and 0.3 with high specificity, respectively, compared with those (0.57 and 0.52 for cDNA-1 and cDNA-2, respectively) obtained using the first method. The disruption of the hairpin structure by cDNA-1 containing a single-mismatched base at the center of the stem region was distinctly difficult, compared with that by cDNA-2 containing a single-mismatched base at the end of the stem region [23,24]. This study demonstrated that a single-mismatched DNA was detected with high specificity using the second method.

Conclusion

This study showed that DNA detection was effectively enhanced by the length of the cDNA hybridized to 9C6b DNA, allowing the stable duplex formation to open the hairpin structure. In addition, the detection specificity of a single-mismatched DNA was clearly enhanced in cDNA-1 and cDNA-2 when using the second detection method, in which cDNA hybridization to 9C6b DNA was conducted prior to the synthesis of 9C6b-AgNCs.

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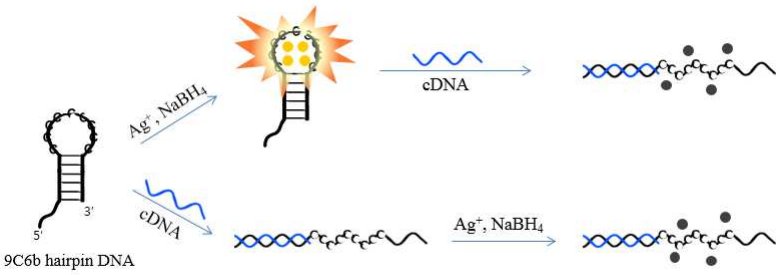
Table 1. Sequences of oligonucleotides used in this study.

Name	Sequence (5'→3')
9C6b DNA	CGATCGTACAGCTGCCCCCCCCCCAGCTG
s-cDNA	CAGCTG
m-cDNA	CAGCTGTACG
cDNA	CAGCTGTACGATCG
cDNA-1	CACCTGTACGATCG
cDNA-2	GAGCTGTACGATCG

-The underlined bases indicates the stem region of 9C6b hairpin DNA.

Scheme 1



Scheme 1. Schematic representation of the proposed strategy for DNA detection assay. In the first method, DNA-AgNCs were synthesized before hybridization of cDNA with 9C6b DNA (top). In the second method, 9C6b DNA was hybridized with cDNA, and then, DNA-AgNCs were synthesized (bottom).

Figure 1.

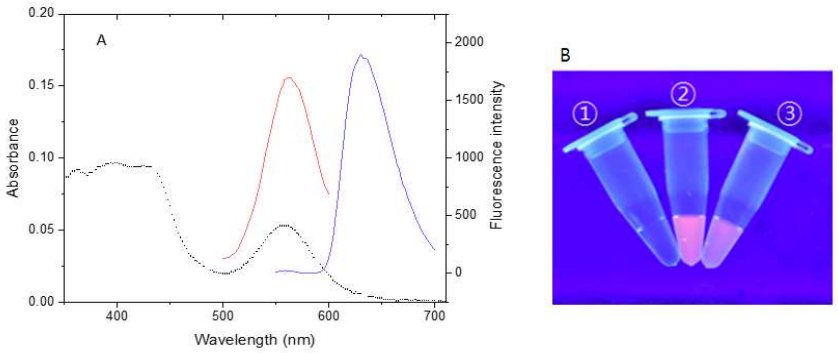


Figure 1. (A) UV-vis absorption spectra (dot) and fluorescence excitation (red) and emission (blue) spectra of 9C6b DNA-AgNCs. (B) Photograph was captured using a UV transilluminator in the presence of distillated water (①), 9C6b DNA-AgNCs (②), and 9C6b DNA-AgNCs hybridized with cDNA (③).

Figure 2

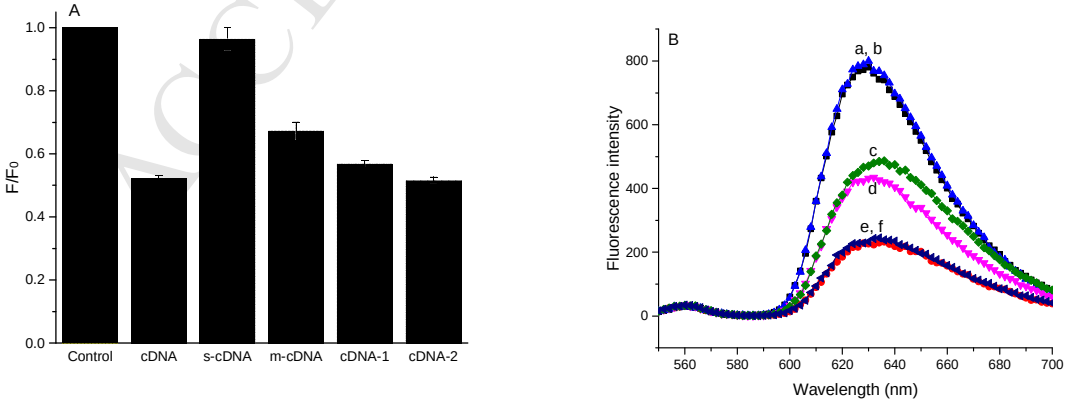


Figure 2. (A) F/Fo was plotted against 9C6b DNA-AgNCs hybridized with different types of cDNA using the first method in scheme 1. The control contains 9C6b DNA-AgNCs only.

Except for the control, all samples contain 9C6b DNA-AgNCs hybridized with different cDNA oligomers. (B) For the samples in Figure 2A, the fluorescence spectra were scanned from 550 to 700 nm at an excitation wavelength of 560 nm. From the top to bottom, the spectra are for control (a), 9C6b DNA-AgNCs with s-cDNA (b), 9C6b DNA-AgNCs with m-cDNA (c), cDNA-1 (d), and 9C6b DNA-AgNCs with cDNA (e) and cDNA-2 (f).

Figure 3.

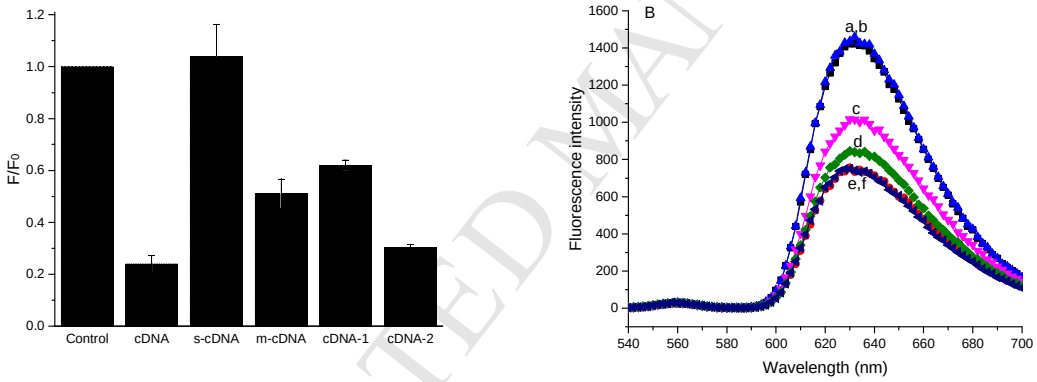


Figure 3. (A) F/Fo was plotted against 9C6b DNA-AgNCs hybridized with different types of cDNA using the second method in scheme 1. The control contains 9C6b DNA-AgNCs only. Except for the control, the samples contain 9C6b DNA-AgNCs hybridized with different cDNA oligomers. (B) From the top to bottom, the spectra are for control (a), 9C6b DNA-AgNCs with s-cDNA (b), 9C6b DNA-AgNCs with cDNA-1 (c), m-cDNA (d), and 9C6b DNA-AgNCs with cDNA (e) and cDNA-2 (f).