DETECTION OF THROMBIN BY APTAMER-BASED SURFACE ENHANCED RESONANCE RAMAN SPECTROSCOPY

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ABSTRACT

This paper reports a selective and sensitive optical detection method using aptamer-based surface enhanced resonance Raman spectroscopy (SERRS). Here we successfully demonstrate thrombin detection down to 100 pM by observing SERRS signal change upon binding. Furthermore, no binding was observed from non-binding protein or non-thrombin binding aptamer. The aptamer is sufficiently stable to detect 1 nM thrombin in the presence of 10% serum. The proposed detection method may be further implemented for multiplexed detection using different aptamer-based Raman probe complexes.

KEYWORDS: aptamer, oligonucleotide, surface-enhanced resonance Raman spectroscopy, thrombin detection

INTRODUCTION

Aptamers are single-stranded (ss) oligonucleotides (<100 nt) that are promoted as potential antibody replacements due to their high specificity and strong affinity against desired targets.[1] Numerous elegant detection methods using aptamers have been proposed.[2] Herein, we report a selective, sensitive, and stable aptamer-based protein detection method employing SERRS.

THEORY

Our detection method combines aptamer-protein interaction and signal detection through SERRS (Fig. 1). The interaction induces the displacement of thrombin binding aptamer (TBA) from gold nanoparticles (GNP)[3] and the displacement is quantitatively monitored by measuring the SERRS signal change of a Raman-probe (methylene blue, MB) covalently attached to TBA.

EXPERIMENTAL

The SERRS-active substrate was fabricated by aggregating citrate-coated colloidal GNPs 80 nm in diameter on Aminopropyltriethoxysilane-coated glass slides. After incubation with MB-conjugated TBA and extensive rinsing, the TBA-coated GNP substrate was measured with SERRS. We subsequently titrated thrombin onto the TBA-GNP substrate, while monitoring the SERRS signal of MB.

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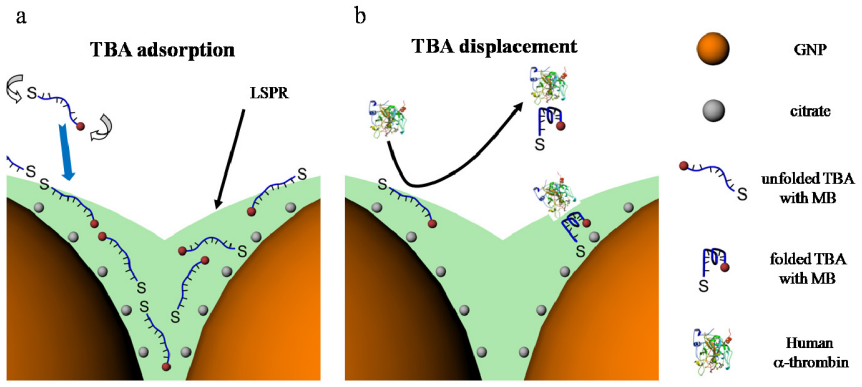

Figure 1. Schematic of the aptamer-based SERRS detection method. a. The unfolded TBA is layered onto the aggregated GNPs with electrostatic interaction. b. TBA undergoes conformation change upon thrombin-binding and is displaced from the GNPs, which cause to decrease the SERRS intensity of MB. Note: LSPR represents local surface plasmon resonance.

RESULTS AND DISCUSSION

The decreased signals of MB SERRS was clearly distinguishable in the presence of thrombin (Fig. 2). We estimated that the limit of detection (LOD) for this method to be 100 pM by comparing the most prominent Raman peak at 1622 cm^{-1} (assigned to ν(CC) ring and ν(CNC) ring modes of methylene blue).

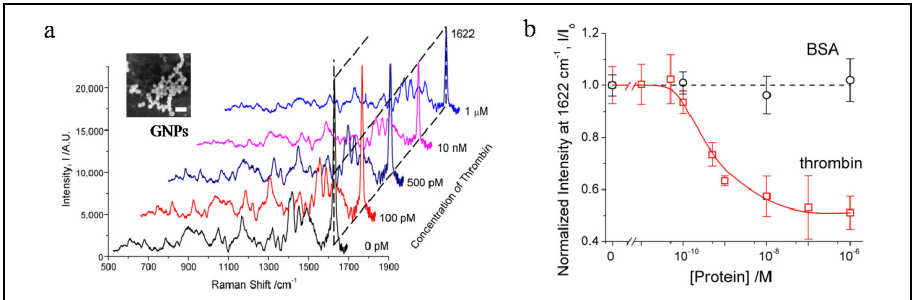

Figure 2. Thrombin detection by observing the SERRS signal change for MB-conjugated TBA. a. SERRS intensity of MB decreased with the increasing thrombin concentration. b. The peak intensity at 1622 cm^{-1} dropped about 10% with 100 pM thrombin and reached the 50% saturating level with 1 μM thrombin. The inset shows a SEM image of aggregated GNPs and the scale bar represents 200 nm.

We further investigated the specificity and the stability of this method (Fig. 3). We did not observe any appreciable decrease in the SERRS signal intensity from TBA in bovine serum albumin (BSA) solution and non-thrombin binding oligonucleotide (mutant TBA) in thrombin solution. We observed a ~20% signal decrease

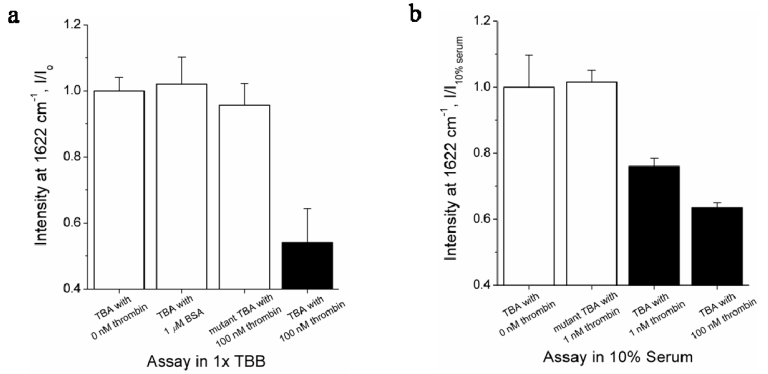

Figure 3. Characterizations of the specificity and the stability of aptamer-based SERRS detection method. a. No binding event was observed from TBA in BSA solution or mutant TBA in thrombin solution. b. With the addition of 1 nM thrombin in 10% serum, the signal drops to ~75%; while the signal drops to a saturating level when the input thrombin was increased to 100 nM.

when the substrate was treated with 10% serum (v/v in binding buffer), indicating that the TBA was minimally displaced from the aggregated GNPs by the serum's constituents. TBA is stable enough to detect 1 nM thrombin in the presence of 10% fetal calf serum.

CONCLUSIONS

In conclusion, we have demonstrated the application of aptamer-based SERRS to create a highly specific and sensitive detection method for thrombin. In addition, this method is sufficiently stable to tolerate target detection in the presence of up to 10% serum. Utility of this method is highly flexible as it can easily accommodate numerous protein specific aptamers, each conjugated to a unique Raman probe, in a single platform, thus enabling multiplex protein detection.

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