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Coupling exonuclease III with DNA metallization for amplified detection of biothiols at picomolar concentration

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ABSTRACT

For early diagnosis of diseases, the need of ultralow detection limit is an ongoing quest. In this work, by taking the uniqueness of Exonuclease III and DNA metallization, we demonstrate a facile turn-on fluorescent method for amplified detection of biothiols at picomolar concentration. This method relies on the amplification process achieved by the recycling of biothiols retrieved target DNA from silver depositions and the specific interactions between quadruplex and NMM. This method is simple in design, economic in operation and exhibits ultralow detection limit and excellent selectivity toward thiol-containing biomolecules among amino acids found in proteins and in serum samples. More importantly, the detection and discrimination process can be seen by the naked eye with the aid of an UV transilluminator. Therefore, this new concept may offer a potential approach for practical applications as an efficient biosensor for early detection of diseases.

1. Introduction

Biothiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (Gsh) play crucial biological roles in human body such as crosslinking proteins, maintaining intracellular redox activities (Stryer, 1995; Yi et al., 2009). Abnormal level of these thiol contained biomolecules in biological fluids is associated with many risk diseases such as cancer, heart disease and AIDS (Herzenberg et al., 1997; Jacobsen, 1998). Accordingly, efficient measurements of mercapto-amino acids, especially under normal physiological conditions, are highly desirable. A variety of methods for detecting biothiols, such as fluorescent assays, (Tanaka et al., 2004; Zhang et al., 2007) high-performance liquid chromatography techniques, (Lu et al., 2007; Tcherkas and Denisenko, 2001) and electrochemical voltammetry (Hignett et al., 2001; Zen et al., 2001) have been developed. However, most of them require complicated instruments, involve cumbersome laboratory procedures and are low throughput, which limits the scope of their practical applications. Thus, the development of rapid, cost-effective, sensitive and specific methods for biothiol detection is vitally important.

DNA has been an extremely favorable tool in establishing biosensors not only for proteins but also low molecular weight substrate (such as ATP, biothiols, etc.) (Wang et al., 2006, Song et al., 2010, Xiao et al., 2005). For example, Lee et al. (2008) developed a colorimetric method for cysteine detection by using DNA functionalized gold nanoparticle probes that contain strategically placed thymidine–thymidine mismatches complexed with Hg^{2+}. Recently, our group has proposed a biothiol detection approach by utilizing silver metallized DNA as probe (Lin et al., 2011). Although promising, for early diagnosis of diseases, the need of ultralow detection is an ongoing quest (Wulfkuhle et al., 2003) and especially, the search for a thiol detection platform with limit below nanomolar still remains a big challenge.

Recently, Exonuclease III (Exo III) has been reported as an efficient biocatalyst for amplified detection of DNAs, proteins, or other small molecules with ultralow detection limit (Freeman et al., 2011; Zuo et al., 2010; Wang et al., 2006; Song et al., 2010; Xiao et al., 2005; Zhao et al., 2003; Liu and Lu, 2003, 2007; Ma et al., 2013; Duan et al., 2013; Wei et al., 2012). Exo III could selectively digest the 3'-end of nucleic acid strands in duplex DNAs, thus enabling the selective cleavage of one of the strands in double-stranded DNA structures (Zhao et al., 2011). This function was used to develop different Exo III-based amplified detection platforms that involved the biocatalytic recycling of the target DNA by Exo III (Peng et al., 2012; Liu et al., 2012; Ju et al., 2012). Nonetheless, up to this stage, Exo III-based amplified platforms that recycle DNA recovered from inorganic depositions at present

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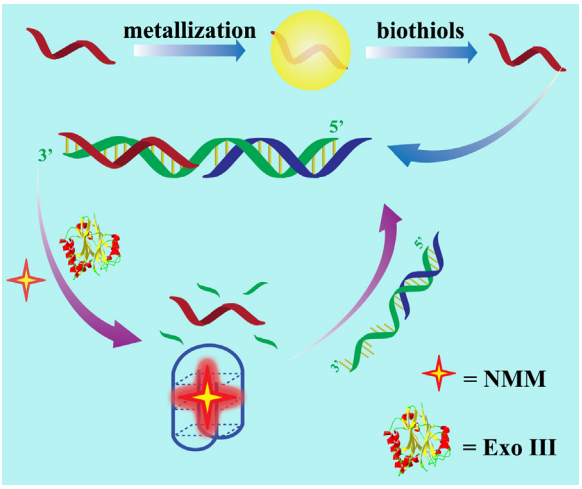

Scheme 1. Schematic illustration of the strategy for amplified detection of biothiols by coupling Exo III and DNA metallization.

are unknown. As a promising deposition technology, DNA metallization, where metal structures deposit following DNA contour, provides a wide range of potential applications in electronics, biosensing, pattern transfer and dynamic nanomaterials (Keren et al., 2002, 2004; Braun et al., 1998; Jin et al., 2013). For example, silver metallization could destroy the recognition properties of the DNA, thus preventing the subsequent biological processes (Keren et al., 2004; Lin et al., 2011). Meanwhile, silver nanoparticles could be mildly etched by thiols, which had been used to fine tune the shape and morphology of metal nanostructures (Cho et al., 2009; Quaroni and Chumanov, 1999). Therefore, bitothiols could be expected to retrieve DNA from biologically blocked state in silver depositions.

Inspired by those phenomena, herein, for the first time, by taking advantages of the uniqueness of Exo III and DNA metallization, we developed a highly sensitive platform for analyzing biothiols at picomolar concentration (Scheme 1). After silver metallization, the target DNA (**T**) is biologically blocked and could not hybridize with its complementary sequence parts (**Tc**) in the probe DNA (**P/G4**), thus avoiding digestion by Exo III. Upon the addition of biothiols, the silver metalized **T** could be readily released due to the etching interaction between thiol and silver. When the duplex probe is challenged with the extracted **T** from silver depositions, the hybridization leads to a blunt 3'-terminus, which can be catalyzed by Exo III. Then, the extracted **T** and a quadruplex-forming oligomer (**G4**) can be released. The intriguing interaction between the activated quadruplex and its specific ligand, N-methyl mesoporphyrin IX (NMM), brings about a great fluorescence enhancement for signal readout (Hu et al., 2010). During the catalysis step, the retrieved **T** is ready to bind to another probe **P/G4** to initiate a new cycle, generating a concomitant increase in fluorescence. The design of each DNA sequence was described in the experiment sections.

2. Experimental section

2.1. Reagents

The DNA sequences used in the study were as follows:

T: 5'-CTGTTGTGACTCGTCGCAATAAC-3',
Tc: 5'-GCGACGAGTCACAACAG-3',
G4: 5'-CTCTTCGAGGGTTTTGGGTTTTGGGTTTTGGGAGCTA-3',
P: 5'-AAAACCCAAAACCCAAAACCCGCGACGAGTCACAACAG-3',

The **G4** DNA was designed partially complementary to **P** DNA with two bases mismatched at 5'-terminus. When the **P/G4** probe was challenged with perfectly matched target DNA, ssDNA at 5' end of the probe hybridized with the perfectly matched target DNA. Then the mismatched part of **G4** would be displaced by the target DNA. Degraded by Exo III, the probe that hybridized with target DNA would release target DNA. Then the mismatched part of **G4** DNA which was displaced by target DNA previously, would hybridize with **P/G4** DNA again to make sure the digestion continue.

The DNA oligomer was synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). NMM was purchased from Porphyrin Products (Logan, UT), and its concentration was measured by using absorbance spectroscopy on a JASCO V-550 and found to be λ=379 nm, assuming an extinction coefficient of $1.45\times10^5\ M^{-1}\ cm^{-1}$. Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Alfa Aesar. Other chemicals including amino acids and biothiols were purchased from Sigma-Aldrich and used without further purification. All the water used to prepare buffer solutions was obtained by using a Milli-Q water system. All measurements were performed in Mg–K buffer (10 mM Tris–HCl, 75 mM KCl, 10 mM $MgCl_2$, pH 8.0). The fluorescence spectra were recorded using a JASCO FP6500 spectrophotometer (JASCO International Co. Ltd., Tokyo, Japan). The concentration of DNA was determined using a JASCO V-550 UV/Vis spectrophotometer, equipped with a temperature-controlled cuvette holder. TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. CD spectra were determined using a Jasco 810 (Jasco International Co., Ltd., Tokyo, Japan)

2.2. Synthesis of DNA–silver nanohybrids

The DNA–silver nanohybrids were synthesized by reduction of $AgNO_3$ with $NaBH_4$. Briefly, DNA was mixed excess $AgNO_3$ (50:1 Ag^+/**T** molar ratio). After 30 min of incubation, freshly prepared $NaBH_4$ solution (Ag^+ and $NaBH_4$ were mixed in a 1:5 molar ratio) was dropped into the above aqueous solution under vigorous stirring. After mixing, the resulting yellow colloidal silver solution was stirred for another 30 min.

2.3. Fluorescence assay for biothiols detection

The fluorescence intensity of all samples was analyzed via a time base scan. The solutions were excited at 399 nm, and emission spectra were collected from 550 to 750 nm. Slit widths for the excitation and emission were set at 10 and 10 nm, respectively. All measurements were performed in Mg–K buffer (10 mM Tris–HCl, 75 mM KCl, 10 mM $MgCl_2$, pH 8.0). In a typical procedure, silver metalized **T** was mixed with different concentrations of biothiols or other analytes. The mixture was equilibrated for 0.5 h at 37 °C. Then, 40 U Exo III and **P**/**G4** were added and the solution was incubated in 37 °C for 0.5 h. Finally, NMM was added and equilibrated for 5 min before spectral measurements. The final concentrations of **T**, **P**/**G4** and NMM were 20 nM, 0.5 μM and 1 μM, respectively. For visual detection of biothiols Exo III catalyzed reactions were kept for 2 h at 37 °C; the final concentrations of **T**, **P**/**G4** and NMM were 200 nM, 1.5 μM and 3 μM, respectively. Photographs of the solutions were taken using a UV transilluminator.

2.4. Preparation of real samples

Fresh human blood samples were collected in tubes containing EDTA, and centrifuged at 5000 rpm for 20 min. The supernatant solution, which contains proteins and amino acids, was used as the