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A simple colorimetric assay for the detection of metal ions based on the peroxidase-like activity of magnetic nanoparticles

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ABSTRACT

We developed a simple colorimetric assay for the detection of metal ions based on the peroxidase-like activity of magnetic nanoparticles (MNPs) and their interactioin with target specific single-stranded DNA (ssDNA). It was already known that MNPs easily oxidize substrates in the presence of hydrogen peroxide same as a peroxidase. In this study, the target specific ssDNA was adsorbed on the positively charged surface of MNP, then the catalytic activity of MNPs was reduced by the screening effect of adsorbed ssDNA on the surface of MNPs to substrates. The peroxidase like activity of MNPs was recovered by the addition of targets because the target specific ssDNA were displaced from MNPs by the interaction between targets and their specific ssDNA. The degree of color change of mixture was proportional to the concentration of mercury ion in a range of 5–75 μM. In control experiment, other metal ions did not induce the color change.

1. Introduction

Specific and sensitive detection of targets in biosensors essentially depends on target recognizing biological elements such as enzymes or antibodies. Especially, the detection of small molecules including metal ions is not avoidable because a number of concerned substances for the medical diagnosis, food safety, or environmental monitoring are small molecules [1–4]. The high specificity and sensitivity of biosensors based on enzymes or antibodies cannot be easily obtained for the detection of small molecules, mainly due to the affinity of antibodies and the limited number of enzymes available for small organic compounds or metal ions. In addition, the methods for signal generation are limited for small molecules because of their small molecular weight or difficulty on sandwich assay. Therefore, ssDNAs or RNAs with target-binding affinities, called aptamers, have been emerged as attractive bioreceptors due to their characteristics such as relatively high and specific affinities to small target molecules, easy synthesis and modification, high flexibility and thermal stability, compared to the antibodies or enzymes [5–9]. These properties of target specific ssDNA or RNA enable to develop new types of biosensors or signal enhancing methods, such as target-induced conformational change-based detection [10–13]. Therefore, aptamer-based sensors should be not only cost-effective but also able to sensing small target molecules or even ions.

A simple and rapid on-site detection of hazardous molecules is primarily concerned for food safety or environmental monitoring. For this aspect, a colorimetric assay is an attractive method since they enable a rapid visual recognition and do not require sophisticated or expensive instruments. Recently, therefore, many colorimetric methods have been developed using polymer like polydiacetylene or metal nanoparticles [14–18]. Especially, the functionalized gold nanoparticles (AuNPs) have been extensively adopted in the development of colorimetric sensors based-on its strong inter-particle dependent optical property, in which the color change can be observed with the naked eye. Various targets, such as nucleic acids, proteins, enzymes, saccharides, metal ions, and even cells, were reported to cause visual changes of its color from red to purple or vice versa, if the analytes interact with cross-linked oligonucleotides immobilized on AuNPs [19–23]. However, the AuNP-based colorimetric assay has some defects including the fact that the assay is sensitive to salt concentrations, which may lead to low reproducibility and accuracy of detection. The aggregation of AuNPs is mainly triggered by the screening effect of salt (e.g. NaCl) and significantly affected by the salt concentration. Therefore, the salt concentration should be optimized depending on assay conditions or samples in AuNP-crosslinking based colorimetric assay [22]. This might be critical for the test on the real samples which often contain many salt ions.

Recently, Yan et al. identified that Fe_3O_4 magnetic nanoparticles (MNPs) have an intrinsic peroxidase-like activity [24]. In this report, the colorimetric substrates were oxidized in the presence of hydrogen peroxide by the enzyme-mimetic activity of MNPs and the color of solutions was changed. This enzyme-mimetic activity

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of MNPs is more stable against denaturation than nature enzyme, and its preparation or storage is more simple and cost-effective. Based-on this knowledge, most recent, some colorimetric methods using MNPs as a signal producing material have been developed for the detection of various biological interests such as H_2O_2, glucose, proteins, carbohydrates and DNA [25,26,27,28,29]. However, these reports were depending on the interaction of targets with hydrogen peroxide or the shielding effect of MNPs' activity by amplified double stranded DNA.

In this study, we have introduced a simple colorimetric assay for the detection of metal ions based-on the catalytic activity of MNPs which is varied depending on the interactions among MNPs, targets, and their specific ssDNA.

2. Experimental

2.1. Materials

Iron(II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium hydroxide, *o*-phenylenediamine (colorimetric substrate used for MNP's catalytic reaction), and $HgCl_2$ as an analyte were purchased from Sigma–Aldrich (Korea). All chemicals used in this study were of analytical grade. All DNA oligonucleotides were obtained from GenoTech Co. (Daejeon, Korea). The sequences of ssDNAs were followed; Mercury ion specific T-rich ssDNA (receptor for Hg^{2+}): 5′-TTCTTTCTTCCCCTTGTTTGTT-3′, complementary ssDNA with Hg^{2+} specific T-rich ssDNA (used for the forming dsDNA with Hg^{2+} specific T-rich ssDNA): 5′-AAGAAAGAAGGGGAACAAACAA-3′.

2.2. Synthesis of magnetic nanoparticles

Magnetic nanoparticles were synthesized according to the procedure previously reported [30]. Briefly, 1 M sodium hydroxide solution was added to the mixture containing 2 M of $FeCl_3$ and 1 M $FeCl_2$ solution dissolved in distilled water, followed by stirring at 80 °C for 40 min (pH 10). The synthesized MNPs were washed several times with water and ethanol using magnet and dried at 70 °C under a vacuum. The analysis on the size and surface charge of MNPs was performed by transmission electron microscopy (CM-30, Philips) and a Zeta-potential analyzer (Zetasizer 2000, Malvern Instruments Ltd., UK). The MNPs were dispersed in 0.2 M acetate buffer (pH 4) to make a 1 mg mL^{-1} stock solution and stored at 4 °C until use.

2.3. Assay procedure

At first, 25 μl of MNPs (1 mg ml^{-1}) was mixed with 10 μl of ssDNA aptamer (100 μM) and incubated at 25 °C for 30 min. Then, 400 μl of colorimetric substrate (10 mM), 100 μl of H_2O_2, and 475 μl of acetate buffer (0.2 M, pH 4) were added into the mixture and incubated at 25 °C for 10 min. After reaction, the MNPs were removed from the mixture using magnet, the color of mixture was observed with the naked eyes and the absorbance spectra of them was analyzed using UV/Vis spectrophotometer (Ultraspec 6300 pro, Amersham Biosciences).

3. Results and discussion

3.1. Colorimetric assay for the detection of metal ions based-on MNPs' catalytic activity

Recently, the peroxidase-like activity of various nanomaterials including magnetic nanoparticles have been well demonstrated and widely applied for the detection of various analytes. The colorimetric substrates were oxidized with H_2O_2 by the enzyme-mimetic activity of nanomaterials, consequently the color of solutions was changed. In this study, the phenomenon of intrinsic catalytic activity of MNPs was coupled with the specific interaction of functional ssDNA to target for metal ion detection. Scheme 1 showed the basic principle of MNPs based colorimetric aptasensor. In the procedure, the MNPs are firstly mixed with target specific ssDNA and incubated at room temperature. ssDNA having a negative charge at phosphate backbone is well adsorbed on the surface of positively charged MNPs via electrostatic interaction. Then, the addition of colorimetric substrate and hydrogen peroxide (H_2O_2) in acetate buffer (pH 4) is followed. The peroxidase-like activity of MNPs is inhibited by the ssDNA adsorbed on the surface of MNPs because the access of colorimetric substrates to MNPs' surface is screened by the adsorbed ssDNA. If the target molecule is added into the solution, ssDNA is displaced from MNPs by the interaction between targets and their specific ssDNA, consequently the catalytic activity of MNPs is recovered due to the decreasing of screening effect of ssDNA on MNPs. As a result, samples containing target molecules lead the color change of solution by the oxidation of colorimetric substrates, which is easily recognized by naked eyes. Whereas the sample has no target molecules, the reduction of peroxidase-mimetic activity of MNPs is sustained by the screening of access of substrates to MNPs, consequently the color of the solution is not changed.

To verify this sensing principle, mercury ion (Hg^{2+}) and T-rich ssDNA was examined as a model target and its receptor. A T-rich ssDNA can interact strongly with mercury ions through T–Hg^{2+}–T coordination [31,32]. As shown in Fig. 1, the MNPs quickly oxidized the transparent substrate (*o*-phenylenediamine, OPD) to the intensely colored diimine derivatives in the presence of H_2O_2 at acidic condition (pH 4). As a result the transparent OPD solution was changed to red color. However, the color change of solution was not severe in the presence of mercury ions specific T-rich ssDNA compared to only MNPs solution. This indicated that T-rich ssDNA inhibited the catalytic activity of MNPs mainly caused by the adsorption on MNPs' surface. The reduced peroxidase-mimetic activity of ssDNA adsorbed MNPs was recovered by the addition of mercury ions (Hg^{2+}), consequently the color of the solution was changed to red. As another control experiment, the effect of mercury ion on the catalytic activity of only MNPs was examined in the absence of Hg^{2+} specific ssDNA. As a result, the Hg^{2+} enhanced the catalytic activity of MNPs at high concentration (more than 1 mM) in the absence of ssDNA. But, the micro-molar level Hg^{2+} (below 100 μM) did not change the catalytic activity of MNPs significantly.

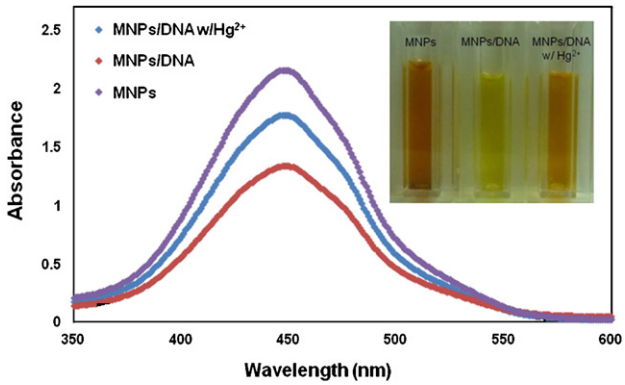

Fig. 1. Absorption spectrum of OPD (colorimetric substrate of MNPs) solution after catalytic reaction with only MNPs (violet), ssDNA adsrobed MNPs (red), and ssDNA adsrobed MNPs containing 100 μM of Hg^{2+}(blue) (inset: the photographs of each samples). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)