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Improving sensitivity of gold nanoparticle based fluorescence quenching and colorimetric aptasensor by using water resuspended gold nanoparticle

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ABSTRACT

Gold nanoparticles (AuNPs) based fluorescence quenching or colorimetric aptasensor have been developed for many analytes recently largely because of the ease of detection, high sensitivity, and potential for high-throughput analysis. However, the effects of remnant non-AuNPs components in the colloid gold solution on these assays performance remain unclear. For the first time, we demonstrated that the remnant sodium citrate and the reaction products of three acids play counteractive roles in AuNPs based fluorescence quenching and colorimetric aptasensor in three ways in this study. First, the remnant sodium citrate in the colloid gold solution could increase the fluorescence intensity of FAM labeled on the aptamer that reduce the efficiency of AuNPs fluorescent quenching. Second, the reaction products of citric acid, HCl and ketoglutaric acid reduce the fluorescence recovery by quenching the fluorescence of FAM labeled on the aptamer dissociated from the surface of AuNPs upon addition of target. Lastly, the reaction products of three acids reduce the pH value of the colloid gold solution that reduce the sensitivity of AuNPs based colorimetric aptasensor by increasing the adsorption of aptamer to surface of AuNPs. With sulfadimethoxine and thrombin as model analytes, we found that water resuspended AuNPs can significantly increase the sensitivity by more than 10-fold for AuNPs based fluorescence quenching aptasensor. In the AuNPs based colorimetric aptasensor for sulfadimethoxine using the water resuspended AuNPs, the sensitivity also was increased by 10-fold compared with that of original AuNPs. The findings in this study provide theoretical guidance for further improving AuNPs based fluorescent quenching and colorimetric aptasensor by adjusting the composition of AuNPs solution.

1. Introduction

Aptamer has received tremendous attention in analytical application as an excellent example of affinity molecules which can bind tightly to a broad range of targets (e.g., proteins, peptides, amino acids, drugs, metal ions and even whole cells) (Ellington and Szostak, 1990; Iliuk et al., 2011; Tuerk and Gold, 1990; Wang et al., 2010; Yuan et al., 2012). Compared with conventional antibodies, nucleic acid based aptamer probes show more flexibility in design various types of electrochemical (Ho et al., 2012; Liu et al., 2012), fluorescence (Kim et al., 2012; Lu et al., 2013; Yuan et al., 2011; Zhang et al., 2011), chemiluminescence (Freeman et al., 2011), or colorimetric (Chang et al., 2012; Zhu et al., 2010) sensing schemes for a broad-spectrum of targets. Among these, Gold nanoparticles (AuNPs) based fluorescence quenching or colorimetric aptasensor has been given prominent attention largely because of the ease of detection, high sensitivity, and potential for high-throughput analysis (Kim and Jurng, 2011; Li and Rothberg, 2004; Peng et al., 2013; Song et al., 2012a; Yang et al., 2011, 2012). Among these assays, the aptasensor where the aptamer are directly adsorbed onto the unmodified AuNPs surface show more simplicity without laboursome modification of AuNPs with thiolated DNA and extensive optimization of distance between the aptamers and AuNPs by various techniques. The unfolded aptamers are postulated to be adsorbed on AuNPs by the interaction between the positively charged bases of ssDNA aptamers and the negatively charged surface of AuNPs (Liu et al., 2008; Wang et al., 2006; Wei et al., 2007). Then the AuNPs are stabilized by ssDNA aptamers against the aggregation at high salt concentration. In fact, the folded aptamers are not

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easily adsorbed on the AuNPs, mainly due to the higher rigidity in the structures with the hidden status of the positively charged bases positioned inside of double stranded. However, in the presence of targets, the conformation of aptamers is transited to folded state and desorbed from the surface of AuNPs. Consequently, AuNPs are aggregated upon adding high concentration salt (Zhang et al., 2008). For colorimetry, the AuNPs was used as extremely sensitive colorimetric indicator due to the extraordinarily high extinction coefficient and strongly distance-dependent optical properties (Ghosh and Pal, 2007; Storhoff et al., 2000). Different aggregation states of AuNPs correspond to distinctive color, which can be appreciably discerned with the naked eye. For AuNPs based fluorescence quenching, AuNPs serve as ultra-efficient quenchers of molecular excitation energy (Dulkeith et al., 2002; Maxwell et al., 2002), because of the strong local surface plasmon resonance (LSPR) absorption in the visible region (Burda et al., 2005; Liaw et al., 2012; Lin et al., 2011). The chromophores conjugated to aptamer or its complementary sequences were introduced to near the AuNPs surface for fluorescence quenching through adsorption, covalently immobilization, or hybridization (Sun et al., 2011; Wang et al., 2008a, 2008b; Xu et al., 2012; Zhang et al., 2010). Upon the target addition, the chromophores were taken away from the AuNPs through aptamer structure-switching resulting in a recovery of fluorescence.

AuNPs are often synthesized through the method of reduction of $HAuCl_4$ via sodium citrate for its simple procedure, yielding stable and reproducible AuNPs of narrow size distribution and different sizes of AuNPs can be generated by simply adjusting the gold-to-citrate concentration ratio (Ferns, 1973; Gross, 2008). Despite the wide application of AuNPs in fluorescent or colorimetric aptasensor and the studies of effects of different sizes of AuNPs or different aptamers on the sensitivity of these methods, (Chavez et al., 2012; Cheng et al., 2011; Kim et al., 2011) systematic and comprehensive characterization of the effects of non-AuNP components in the reaction system on the AuNPs based fluorescence quenching or colorimetric aptasensor performance has not been conducted. It has been reported that more than 75% of citrate originally employed as reactant remained in the synthesized AuNPs solution which may help to stabilize the AuNPs (Balasubramanian et al., 2010; Hazarika et al., 2004). Based on the AuNPs synthesis reaction equation with sodium citrate reduction (Balasubramanian et al., 2010; Kumar et al., 2007), multiple byproducts (e.g. sodium ketoglutarate, sodium chloride, and sodium acetate, HCl, ketoglutaric acid, citric acid) would be expected to remain in the synthesized AuNPs solution (Balasubramanian et al., 2010; Kumar et al., 2007; Schneider and Decher, 2004; Turkevich et al., 1951).

To get most efficient performance of AuNPs based fluorescent quenching or colorimetric aptasensors, for the first time, this study systematic investigated the non-AuNPs components effects on the sensitivity of AuNPs based fluorescent quenching or colorimetric aptasensors. We found that sodium citrate and three acid products play a counteractive role in AuNPs based fluorescent quenching and colorimetric aptasensor respectively. With sulfadimethoxine (SDM) and thrombin as the model analytes/targets for which aptamer based detection methods have been developed, (Chen et al., 2013; Wang et al., 2008b) the sensitivity of AuNPs based fluorescent quenching or colorimetric aptasensors can be improved by 10-folds by using the water resuspended AuNPs.

2. Materials and methods

2.1. Materials

The FAM labeled sulfadimethoxine aptamer sequence is 5′–GAGGGCAACGAGTGTTTATAGA-3′FAM. (Song et al., 2012b) The thrombin aptamer sequence is 5′–GGTTGGTGTGGTTGG-3′FAM. (Bock et al., 1992) The ssDNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and the lyophilized powder was dissolved in ultrapure water and before use it was stored at 4 °C. The concentration of the oligonucleotide was determined by measuring the UV absorbance at 260 nm. Chloroauric acid ($HAuCl_4$), Sodium citrate ($C_6H_5Na_3O_7$), ketoglutaric acid disodium salt were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other normal chemicals, including sodium chloride, ketoglutaric acid, citric acid, sodium acetate, HCl were all purchased from Beijing Chemical Reagent Company (Beijing, China). All of the chemicals were at least analytical grade. A 96 well black polystyrene microplate (12 strips of 8 wells) was purchased from Corning (Corning, NY). The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were analytical reagent grade and used without further purification or treatment unless specified.

2.2. Synthesis of the citrate-protected AuNPs

AuNPs were synthesized using the classical citrate reduction method (Ferns, 1973; Turkevich et al., 1951). Briefly, different volumes of 194 mM of sodium citrate solution were rapidly injecting into a boiling aqueous solution of $HAuCl_4$ (100 mL, 1 mM) with vigorous stirring. An obvious color change of the reaction mixture was observed from trans-parent to dark blue and finally wine red. After boiling for 20 min, the reaction flask was removed from the heat to allow the reaction solution to cool at room temperature. Transmission electron microscopy (TEM) was performed on JEM 2010 Microscope (200 kV). The samples were prepared by casting the nanoparticle solution onto a carbon-coated Cu-grid and followed by evaporation of the solvent. The particle sizes are analyzed statistically using an acquisition and analysis software, DigitalMicorgraph.

2.3. Non-AuNPs components effect on the fluorescence of FAM labeled aptamer

Sodium citrate effect on the FAM labeled aptamer was studied by incubating 100 μL different concentration of sodium citrate solution with 50 μL FAM labeled thrombin or SDM aptamer (1 μM) for 15 min at room temperature and then the fluorescence intensity was recorded using a multifunction microplate reader (Tecan Infinite 200, Tecan Austria GmbH, Austria) with an excitation of 490 nm and emission of 520 nm. Other non-AuNPs components were tested with the same procedure as sodium citrate but with the estimated concentration same as in the original 12 nm AuNPs solution buffer synthesized with 3 mL sodium citrate according to the reaction equation (Balasubramanian et al., 2010).

2.4. Detection procedure of AuNPs based fluorescence quenching aptasensor

The thrombin and SDM detection assays were performed by incubation of 100 μL the original AuNPs solution or the water resuspended AuNPs solution with 50 μL FAM labeled thrombin or SDM aptamer (5 μM) for 15 min at room temperature. Then 50 μL different concentration of thrombin or SDM was added and incubate for another 15 min at room temperature. After that the fluorescence intensity was recorded using a multifunction microplate reader (Tecan Infinite 200, Tecan Austria GmbH, Austria) with an excitation of 490 nm and emission of 520 nm.