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Colorimetric immunosensing via protein functionalized gold nanoparticle probe combined with atom transfer radical polymerization

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ABSTRACT

A novel colorimetric immunosensing strategy based on protein-modified gold nanoparticle probes combined with atom transfer radical polymerization (ATRP) technology was proposed. Gold nanoparticles (GNPs, ~15 nm) were functionalized with antibodies through an acylamide-bond between the carboxylic group of 11-mercaptoundecanoic acid that previously self-assembled on the surface of GNPs and the amino group of the protein (here, goat anti-rabbit immunoglobulim G (anti-IgG) used as model). The surface functionalized GNPs were used for IgG capture, which introduced initiator coupled anti-IgG (Ab2*) onto the surface of GNPs through immunoreactions. Subsequently triggered polymer growth resulted in the surface graft of preformed polymer chains onto nanoparticles that altered the optical property of GNPs. A distinct color change occurred. This could be designed for IgG detection. The spectrum absorption and colorimetric detection gave a linear range of 0.5–25 ng mL^{-1} with a detection limit of 0.03 ng mL^{-1} for IgG. The proposed approach showed high sensitivity from both visual and absorbance measurements. In spite of the limitations of available IgG antibodies, this approach could be easily extended to the detection of other biomarkers.

1. Introduction

Development of the gold nanoparticle (GNP) based colorimetric sensors have received considerable attention ever since the first colorimetric polynucleotide sensor (Elghanian et al., 1997). Colloidal gold nanoparticles have a beautiful wine-red color, which is ascribed to the collective oscillation of the conduction band surface electrons on interaction with light of suitable wavelength, the localized surface plasma resonance (LSPR) (Teichroeb et al., 2006). It has been well known that the position of the surface plasmon band of GNPs were sensitive to particle size, the local chemical environment and its surface functionalization. When GNPs were functionalized with polymers, both the position and bandwidth of the surface plasmon band of GNPs were changed. This change was readily observed from the absorption spectra or even by the naked eye. Based on the functionalization of GNPs, a number of colorimetric biosensors have been developed for sensitive detection of diversified analytes, such as nucleic acids (Cao et al., 2002; Nam et al., 2003), proteins (Zhang et al., 2002; Otsuka et al., 2001; Takae et al., 2005), saccharides (Aslan et al., 2005) and cells (Medley et al., 2008; Lu et al., 2010). For example, GNPs functionalized with hybridized oligonucleotides and polyclonal antibodies tend to collect in the presence of its antigen, which led to a color change of the gold colloid solution from a typical burgundy color to blue or purple. Such a color change could be used for sensitive detection of cancer cells by using aptamer-conjugated gold nanoparticle as labels that combined the selectivity and affinity of aptamers and the spectroscopic advantages of gold nanoparticles (Medley et al., 2008). Samples with the target cells present exhibited a distinct color change while nontarget samples did not elicit any change in color. The assay showed excellent sensitivity with both the naked eye and based on absorbance measurements. A simple colorimetric and highly sensitive two-photon scatter assay, for highly selective and sensitive detection of breast cancer SK-BR-3 cell lines, has been developed using a multifunctional (monoclonal anti-HER2/c-erb-2 antibody and S6 RNA aptamer-conjugated) oval-shaped gold-nanoparticle-based nanoconjugate. When these nanoconjugates were mixed with the breast cancer SK-BR-3 cell line, a distinct color change occurred, and the two-photon scatter intensity increased, which was monitored with the colorimetric method (Lu et al., 2010).

There are mainly two synthetic pathways for the functionalization of GNPs with polymer. One pathway is the surface graft of preformed polymer chains onto nanoparticles through thiol-Au interaction, which is the so-called “grafting to” method (Takae et al., 2005; Zhang et al., 2008; Shen et al., 2008; Lowe et al., 2002; Luo et al., 2005). The second pathway is the direct growth of polymer chains from the nanoparticle surface in a “graft-from”

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method (Ohno et al., 2003; Mandal et al., 2002; Matsuura et al., 2007; Pillalamarri et al., 2005; Kotal et al., 2005; Kim et al., 2005; Dong et al., 2008; Holzinger et al., 2006; Li et al., 2007a; Wei et al., 2008). The "graft-from" approaches were most auspicious due to its ease in control of graft densities, thicknesses and versatility of specific chain structures. Among these "graft-from" approaches, the atom transfer radical polymerization (ATRP), a controlled/"living" radical polymerization process, has been widely adapted in polymer functionalized GNPs (Holzinger et al., 2006; Wei et al., 2008; Duan et al., 2005; Nuss et al., 2001; Ohno et al., 2002; Fu et al., 2005; Yoon et al., 2008; Wang et al., 2008). For example, with the help of 2-bromopropionyl bromide as the initiator, thermoresponsive polymer coated GNPs were formed through atom transfer of free radical polymerization of N-isopropylacrylamide (Chakraborty et al., 2010). A direct surface-grafting approach for the formation of DNA-containing polymer shells outside of Au nanoparticles from aqueous atom transfer radical polymerization (ATRP) was reported by He's group (Lou et al., 2007). In their work, DNA molecules were immobilized on Au particles to introduce ATRP initiators on the surface. The immobilized ATRP initiators prompted polymer chain growth to form DNA-polymer hybrids outside of Au nanoparticles, which provided a platform for the DNA sensing. In the present work, a novel colorimetric immunosensing approach for biomarker assay was designed by integration of the direct surface-grafting ATRP polymerization and sandwiched immunoassay. Goat anti-rabbit IgG was immobilized on GNPs to capture polymerization reaction initiator on the surface. Subsequently triggered polymer growth resulted in local accumulation of monomers that altered the optical property of GNPs, which could be detected with the absorption spectra and a color-analysis software (imageworks).

2. Experimental

2.1. Materials

Rabbit immunoglobulin G (IgG) and goat anti-rabbit IgG were obtained from Signalway Antibody (Nanjing, China). 11-Mercaptoundecanoic acid (MUA), 2-bromoisobutyryl bromide (BriBuBr, 98%), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), tetrachloroauric(III) acid trihydrate, sodium citrate dihydrate, 2,2′-bipyridyl were purchased from Sigma–Aldrich (Shanghai, China). Anhydrous diethyl ether, anhydrous ethanol, methanol, triethylamine (TEA), methanesulfonyl chloride, 1,4-dioxane, hydrazine hydrate, sodium hydrogen carbonate, magnesium sulfate, sodium dihydrogen phosphate, and disodium hydrogen phosphate were obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used for investigations were of analytical grade purity. Copper (I) bromide (CuBr, 98%) was purified by stirring in acetic acid, washing with acetone, and drying under vacuum, according to the method described by Wan et al. (Wan et al., 2006). Triethylamine was dried with a 4 Å molecular sieve, and distilled under vacuum. N,N-Dimethylformamide (DMF) was purified by distillation under vacuum. 2-Hydroxyethyl methacrylate (HEMA, 97%) was purified in the Lab, which was passed through a column with activated Al_2O_3 (Aldrich, neutral, Brockmann I, standard grade, ~150 mesh, 58 Å) to remove the inhibitor prior to polymerization. 10 mM pH 7.0 phosphate buffer solution (PB) was prepared by mixture of stock standard solutions of Na_2HPO_4 and NaH_2PO_4. Twice-distilled water was used throughout the whole study.

2.2. Instruments

The initiator NHS–Br active esters were analyzed by ^1H and ^{13}C NMR spectroscopy with a Bruker Avance 400-MHz NMR instrument (Bruker corporation, Germany), with tetramethylsilane as the internal standard in $CDCl_3$, at ambient temperature. An UV1102 spectrophotometer (Tianmei Instruments, Shanghai, China) was used to monitor functionalization of GNPs. The surface plasmon band of GNPs and functionalized GNPs were monitored between 400 and 900 nm to follow the changes in particle sizes and distribution. A Malvern 1000HSa Light-Scattering Instrument (Malvern Instruments, Worcestershire, U.K.) was used to characterize the hydraulic diameter of GNPs and functionalized GNPs. A 5-mW He–Ne laser at 90° was used to measure particle sizes. The size data was collected and averaged from three separate runs. Zeta potential was measured with a Zetasizer Nano Potentiometric Analyzer (Malvern Instruments, Worcestershire, U.K.). The morphology of GNPs and functionalized GNPs were characterized by a transmission electron microscope (TEM) instrument (LEO 1530 VP, Germany) with an acceleration voltage of 10 kV. Fourier transform infrared spectroscopy (FTIR) was performed on a Bruker Tensor 27 instrument (Bruker corporation, Germany) with dry KBr pellets. The data was collected continuously in the 4000–500 cm^{-1} wavenumber range at a resolution of 4 cm^{-1}. All samples were lyophilized overnight, prior to any IR measurements, to reduce the presence of water in the sample.

2.3. Synthesis of NHS–Br active ester initiators

NHS–Br active ester initiators were synthesized according to literatures with some slight modifications (Lou et al., 2005; Lou and He, 2004). In brief, 1.344 mL 2-bromoisobutyryl bromide (10.86 mmol) was dissolved in 50 mL anhydrous diethyl ether, and cooled in an ice bath. Then a mixture solution of 1.25 g N-hydroxysuccinimide (10.86 mmol) and 2.268 mL TEA (16.31 mmol) in 25 mL 1,4-dioxane was added dropwise over a period of 0.5 h. When the addition was completed, the reaction mixture was stirred at room temperature for 3 h, followed by filtration to remove triethylammonium bromide and other precipitates. Then, the solution was extracted with saturated $NaHCO_3$ and twice-distilled water. Finally, the solution was dried over $MgSO_4$. Most of the solvent was removed with a rotary vacuum distillatory. The residues were stored at 4 °C to obtain a white solid product (yield 64%). Note: all solutions must be anhydrous. ^1H NMR (CD_3Cl, 400 MHz): δ 2.88 (s, 4H), 2.09 (s, 6H). ^{13}C NMR (CD_3Cl, 400 MHz): δ 25.6, 30.7, 51.2, 167.5, 168.7.

2.4. Preparation of colloidal gold nanoparticles (GNPs)

Colloidal gold nanoparticles were synthesized by sodium citrate reduction of aqueous $HAuCl_4$ solution, according to the procedure described by Turkevich et al. (Turkevich et al., 1951). All the glassware were cleaned using aqua regia (3:1 HCl/HNO_3), then rinsed with deionized water and dried in an oven for subsequent use. A volume of 25 mL $HAuCl_4$ (1.0 mM) was first heated and refluxed to its boiling point, then 1 mL sodium citrate (38.8 mM) solution was added while being stirred vigorously. The color of the mixture changed quickly from pale grey to bright red during this procedure. After that, the solution was boiled for another 15 min and was kept well stirred and refluxed for another 2 h as it cooled to room temperature. Finally, the resultant solution was filtered through 0.22 μm cellulose nitrate filter and stored in a brown bottle, at 4 °C. The method yielded a bright red solution composed of spherical colloidal gold nanoparticles with an average diameter of about 15 nm. The surface plasmon band of as-prepared colloidal gold was centered at 519 nm (Fig. 1a).