Biosensors and Bioelectronics 34 (2012) 227–231

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Label-free colorimetric sensor for ultrasensitive detection of heparin based on color quenching of gold nanorods by graphene oxide

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ARTICLE INFO

Article history:
Received 1 December 2011
Received in revised form 5 February 2012
Accepted 6 February 2012
Available online 15 February 2012

Keywords:
Heparin
Gold nanorods
Graphene oxide
Colorimetric detection

ABSTRACT

A novel label-free colorimetric strategy was developed for ultrasensitive detection of heparin by using the super color quenching capacity of graphene oxide (GO). Hexadecyltrimethylammonium bromide (CTAB)-stabilized gold nanorods (AuNRs) could easily self-assembly onto the surface of GO through electrostatic interaction, resulting in decrease of the surface plasmon resonance (SPR) absorption and consequent color quenching change of the AuNRs from deep to light. Polycationic protamine was used as a medium for disturbing the electrostatic interaction between AuNRs and GO. The AuNRs were prevented from being adsorbed onto the surface of GO because of the stronger interaction between protamine and GO, showing a native color of the AuNRs. On the contrary, in the presence of heparin, which was more easily to combine with protamine, the AuNRs could self-assembly onto the surface of GO, resulting in the native color disappearing of AuNRs. As the concentration of heparin increased, the color of AuNRs would gradually fade until almost colorless. The amounts of self-assembly AuNRs were proportional to the concentration of heparin, and thereby the changes in the SPR absorption and color had been used to monitor heparin levels. Under optimized conditions, good linearity was obtained in a range of 0.02–0.28 μg/mL (R = 0.9957), and a limit of detection was 5 ng/mL. The simultaneous possession of high sensitivity and selectivity, simplicity, rapidity, and visualization enabled this sensor to be potentially applicable for ultrasensitive and rapid on-site detection toward trace heparin.

1. Introduction

Heparin is a highly negatively charged polysaccharide due to the sulphate and carboxylate residues (Casu, 1985; Rabenstein, 2002). It has been widely used as the anticoagulant during numerous surgical procedures involving extracorporeal blood circulation such as cardiopulmonary bypass surgery (Jaques, 1979; Mulloy and Linhardt, 2001). However, the overdose of heparin often causes potentially fatal bleeding complication (Hirsh, 1984). Hence, close monitoring of heparin levels is extremely important to avoid the risk such as hemorrhage and thrombocytopenia, especially for pediatric patients.

Recently, various techniques and methods have been established for the determination of heparin. For examples, a chromo-fluorogenic sensing platform based on silica nanoparticles was developed, for the detection of heparin (Climent et al., 2009). A multicolor biosensor for heparin detection and quantification was developed on the basis of the fluorescence change of a water-soluble 2,1,3-benzothiadiazole-containing cationic conjugated polymer (Pu and Liu, 2008, 2009). Determination of heparin was completed based on heparin coated poly(methyl methacrylate) (PMMA) intraocular lenses by ion chromatography (Ander et al., 2001). And a reversed-phase ion pair high-performance liquid chromatography (RPIP-HPLC) was developed for the separation of heparins using a C_{18} column (Patel et al., 2009). However, these methods are usually restricted to complex instruments, time-consuming procedure, or low sensitivity. Therefore, it is urgently required to develop simple, fast, sensitive and cost-effective methods.

Colorimetric methods are extremely attractive because they offer advantages of low-cost portable instruments and simple operation process; moreover, they can be easily read out with naked eyes (Carey et al., 2011; Laurieri et al., 2010; Li et al., 2011; Lou et al., 2011). Noble metal nanomaterials, in particular, gold nanorods (AuNRs), have attracted much attention in colorimetric detection due to their interesting physical and chemical properties (Castellana et al., 2011; Kuo et al., 2010; Liu et al., 2011). AuNRs show higher absorption cross sections and stronger light scattering properties than those of spherical gold nanoparticles owing to their stronger surface plasmon resonance (SPR) characteristics (Liao and

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doi:10.1016/j.bios.2012.02.008

Hafner, 2005; Parab et al., 2010). The major advantage of the AuNRs-based colorimetric assay is that it exhibits two SPR bands: one is the transverse band, corresponding to electron oscillation along the short axis of AuNRs; the other is the longitudinal band, corresponding to electron oscillation along the long axis of AuNRs (Parab et al., 2010). The longitudinal SPR band can be tuned from the visible to the near-infrared region of the electromagnetic spectrum along with the changes of molar extinction coefficients, by adjusting the aspect ratio of the AuNRs (Orendorff and Murphy, 2006). Moreover, the longitudinal SPR band of AuNRs has been found to be very sensitive to changes in the local environment, including solvent, substrate, adsorbate and interparticle distances (Parab et al., 2010). Therefore, the AuNRs-based colorimetric assay has extremely high sensitivity, and has been widely applied for the detection of various analytes including DNA (He et al., 2008), peptides (Sudeep et al., 2005), cancer cells (Jiang et al., 2011; Park et al., 2009), etc. Recently, we have used mesoporous silica-coated AuNRs to attain high sensitive and selective colorimetric detection of Hg^{2+}, S^{2-} and ascorbic acid (Wang et al., 2011a, 2011b).

Graphene oxide (GO) is a monolayer of two dimensional carbon-based materials containing multi-functional groups such as carboxyl, epoxy, ketone and hydroxyl groups in its basal and edge planes (Dreyer et al., 2010). GO has shown great potential for biological applications because of its good water dispersibility, high mechanical strength, versatile surface modification, and photoluminescence (Geim, 2009; Stankovich et al., 2006). Due to the ability of quenching fluorescence (Lu et al., 2009; Wen et al., 2010), GO has been widely applied to DNA analysis (Liu et al., 2010), pathogen detection (Jung et al., 2010) and protein assay (Chang et al., 2010). Lately, GO has also been used for the effective adsorption of cationic dyes based on the large negative charge density (Ramesha et al., 2011). However, to the best of our knowledge, to date there are few reports about the use of GO to quench the color of nanoparticles for efficient detection of biomolecules based on the self-assembly through electrostatic interaction.

Herein, we proposed a new colorimetric strategy for ultrasensitive detection of heparin based on GO quenching the color of self-assembly AuNRs. Hexadecyltrimethylammonium bromide (CTAB)-stabilized AuNRs were chosen for monitoring changes in the color and the SPR absorptions to investigate the concentrations of heparin. The specificity of this system was assured through the strong affinity of protamine for heparin. Thus, a rapid, simple, low-cost, visual and label-free colorimetric assay was developed for ultrasensitive and selective detection of heparin.

2. Experimental

2.1. Chemicals and materials

Graphene oxide was purchased from Nanjing XFNano Materials Technology Company. Protamine sulfate salt was obtained from Sigma–Aldrich. Heparin sodium salt from bovine intestinal mucosa was purchased from Aladdin Chemistry Co. Ltd (185 U/mg, Shanghai, China). Hyaluronic acid (HA) salt was obtained from Streptococcus equi (BioChemika). Chondroitin sulfate (Chs) was purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). DNA was provided by Shanghai Shenggong Co. (Shanghai, China). Glucose, cysteine, glutamic acid, aspartic acid, hexadecyltrimethylammonium bromide (CTAB), gold chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), silver nitrate, sodium borohydride ($NaBH_4$), ascorbic acid and other affiliated reagents were all obtained from Sinopharm Chemical Reagent (Shanghai, China). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution was used for all the experiments, adjusting with 1 M NaOH to reach pH 7.4. All chemicals and reagents were of analytical grade or better. Aqueous solutions were prepared with freshly deionized water (18.2 MΩ specific resistance) obtained with a Pall Cascada laboratory water system.

2.2. Synthesis of gold nanorods

AuNRs were synthesized following the seed-mediated and CTAB surfactant-directed method according to that reported (Nikoobakht and El-Sayed, 2003) with necessary modification. Briefly, the seed solution was prepared by mixing 2.5 mL of CTAB (0.2 M) and 2.5 mL of $HAuCl_4$ (0.6 mM) aqueous solutions. Subsequently, to the stirred solution, 0.30 mL of freshly prepared ice-cold $NaBH_4$ solution (0.01 M) was added, resulting in the color of the solution changing from yellow to brown. The obtained solution was stirred for another 2 min and stored as the seed solution for the synthesis of AuNRs.

80 mL of CTAB solution (0.10 M), 1.2 mL of $HAuCl_4$ (50 mM) solution and 0.055–0.33 mL of $AgNO_3$ solution (50 mM) were mixed at room temperature. Then 1 mL of 0.080 M ascorbic acid was added with gentle stirring. Ascorbic acid as a mild reducing agent, only for the reduction of gold ions, changed the growth solution from dark yellow to colorless. It should be noted that solutions were identical except for their Ag^+ content. AuNRs with different longitudinal plasmon bands could be acquired by controlling the aspect ratios via adjusting the addition amounts of $AgNO_3$ (Jiang et al., 2011; Nikoobakht and El-Sayed, 2003).

Finally, 0.16 mL of the seed solution was added to the growth solution. The mixed solution was left undisturbed for at least 20 h at 28 °C. Excess CTAB was removed by centrifuging twice at 8000 rpm. The supernatant solution was discarded and the resultant particles were re-dispersed in pure water.

2.3. Instrumentation

Absorption spectra were recorded at room temperature on a Thermo Scientific NanoDrop 2000/2000C spectrophotometer (USA) using a 1 cm quartz cell. Atomic force microscopy (AFM) images of GO were recorded on a freshly cleaved mica surface by using a Nanoscope V multimode atomic force microscope (Veeco Instruments, USA) in tapping mode. Transmission electron microscopy (TEM) images were obtained by a JEM-1230 electron microscope (JEOL, Ltd., Japan) operating under 100 kV accelerating voltage.

2.4. Sample preparation

0.05 μg/mL protamine solution was prepared using HEPES buffer (pH 7.4, 10 mM). Various contents of heparin (0–160 μL, 0.002 mg/mL) were mixed with protamine solution, maintaining a gentle stirring for 5 min. The mixture was incubated for another 5 min with the addition of 60 μL of GO solution (0.01 mg/mL), allowing for the left protamine to be completely adsorbed onto the GO surface. Subsequently, 200 μL of AuNRs were added into the above solutions. After incubation for 5 min, the UV–visible (UV–vis) absorption spectra were recorded.

3. Results and discussion

3.1. Sensing principle for heparin

As GO has large numbers of negative charges and CTAB-stabilized AuNRs contain numerous positive charges, the AuNRs can easily self-assembly on the surface of GO through electrostatic interaction. The AuNRs which we used not only could be attached to the surface of GO, but also possessed interesting optical properties arising from localized surface plasmon resonances (Quintana et al., 2010). Protamine, a kind of polycation with ~20 positive charges