Biosensors and Bioelectronics 49 (2013) 525–530

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Replacement of cetyltrimethylammoniumbromide bilayer on gold nanorod by alkanethiol crosslinker for enhanced plasmon resonance sensitivity

Justin Casas, Meenakshi Venkataramasubramani, Yanyan Wang, Liang Tang*

Department of Biomedical Engineering, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA

ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form
29 May 2013
Accepted 30 May 2013
Available online 6 June 2013

Keywords:
Gold nanorod
Surface modification
Functionalization
Biosensing
Surface plasmon resonance

ABSTRACT

Surface modification of gold nanorods (GNRs) is often problematic due to tightly packed cetyltrimethylammoniumbromide (CTAB) bilayer. Herein, we performed a double phase transfer ligand exchange to achieve displacement of CTAB on nanorods. During the removal, 11-mercaptoundecanoic acid (MUDA) crosslinker is simultaneously assembled on nanorod surfaces to prevent aggregation. The resulting MUDA–GNRs retain the shape and position of plasmon peaks similar to CTAB-capped GNRs. The introduction of carboxyl groups allows covalent conjugation of biological receptors in a facile fashion to construct a robust, label-free biosensor based on localized surface plasmon resonance (LSPR) transduction of biomolecular interaction. More importantly, smaller MUDA layer on the GNRs reduces the distance of target binding to the plasmonic nanostructure interface, leading to a significant enhancement in LSPR assay sensitivity and specificity. Compared to modification using conventional electropolymer adsorption, MUDA-coated gold nanosensor exhibits five times lower detection limit for cardiac troponin I assay with a high selectivity.

1. Introduction

In sensor applications, the optical transduction by gold nanoparticles is based upon the phenomenon of localized surface plasmon resonance, i.e. LSPR (Lee and El-Sayed, 2006). According to the plasmon hybridization mode (Prodan et al., 2003), the plasmon resonance frequency is largely determined by the geometry of the colloidal metal structure, as a result of electrostatic interactions between confined electrons distributed over the surfaces of the metal conductor. LSPR offers the advantage of the direct label-free detection method that relies on the measurement of refractive index changes accompanied with the binding of target analyte. Gold nanostructures including shell, ring, prism, star, cage and rod have been fabricated to exploit the full potential of the geometry dependent LSPR for a variety of applications from photonics to biological investigations (Aizpurua et al., 2003; Prodan and Nordlander, 2004; Radloff et al., 2005; Wang and Halas, 2006; Wang et al., 2012). From the viewpoint of plasmon sensitivity and tunability, gold nanorod (GNR) has been at the center of attention (Chen et al., 2013; Kim et al., 2012).

There are several methods which have been widely reported for the GNR fabrication, such as the seed-mediated growth method (Jana et al., 2001; Nikoobakht and El-Sayed, 2003), electrochemical synthesis (Yu et al., 1997), and nano-lithography (Billot et al., 2006). Among these, seed mediated growth is the most common strategy because of simplicity and availability of LSPR wavelength from visible to infrared red region. Although cetyltrimethylammoniumbromide (CTAB) is an essential capping agent to prevent particle aggregation with improved solubility, the resulting bilayer tightly packed onto the nanorod surface usually blocks access for surface modification with bioconjugates. CTAB prefers to bind with the longitudinal side surface of the nanorod rather than the end surface (Gao et al., 2003). Spontaneous reaction of thiolated molecules with GNRs only occurs at the two end faces for partial activation. These factors are problematic owing to nonspecific adsorption, cytotoxicity, and instability. The drawbacks have impaired the use of CTAB-capped nanorods in biological applications, especially compared to nanospheres. As such, it is imperative to find effective methods for the surface modification of GNRs.

To date, a variety of methods have been investigated to improve GNR surface modification (Huang et al., 2013; Liao and Hafner, 2005; Mitamura et al., 2009; Wijaya and Hamad-Schifferli, 2008; Yu et al., 2007). For example, additional layer(s) of silica or polyelectrolyte coating via physical adsorption was used to passivate the CTAB bilayer to allow their functionalization for specific applications (Gole and Murphy, 2005; Sendroiu et al., 2009). However, there is a negative effect on LSPR sensitivity as additional

* Corresponding author. Tel.: +1 210 458 6557; fax: +1 210 458 7007.
E-mail addresses: liang.tang@utsa.edu, fancy5857@gmail.com (L. Tang).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.05.057

coatings will increase the distance between the GNR surface and target molecules (Tian et al., 2012). As such, effective removal of CTAB bilayer is desirable for sensing purposes. Due to the high binding affinity to gold, alkanethiols such as 11-mercaptoundecaonic acid (MUDA) are normally used to replace CTAB molecules (Cao et al., 2012; Yu et al., 2007). Compared to other alkanethiols, MUDA is smaller and its carboxyl terminals can be further functionalized to conjugate with biological receptors. Although shorter chain thiolated carboxylic agents such as cysteamine are more favorable to bring binding event closer to the nanorod surface, they tend to cause nanoparticle aggregation due to instability. Additionally, most of the current work is focused on the improvement in efficient surface modification of CTAB-capped GNRs. There is a lack of elucidation of how these biofunctionalization improvements can affect the biosensing performance in pursuit of low sensitivity and high specificity. These study would be highly meaningful and desirable to improve clinical diagnosis by biomarker detection.

In this paper, we explore a facile process of MUDA displacement of CTAB molecules bound on nanorod surface by double phase transfer ligand exchange. Further immobilization of antibody onto the fully activated MUDA-coated GNR surfaces was achieved with robust covalent bindings. More importantly, the effect of the MUDA modified GNRs on distance-dependent LSPR sensitivity was studied as compared to electrostatic adsorption of polymeric coating over CTAB–GNRs. We attribute the sensing performance enhancement to fully activated nanorods using ligand exchange to remove CTAB bilayer, leading to a dramatically decreased sensing distance from the LSPR surface. To demonstrate the practical application of the functional GNR biosensor with MUDA crosslinker, we performed a model study for human cTnI quantification, whose level is indicative of myocardial infarction in clinical diagnostics (Venge et al., 2009). The sensing performance in terms of sensitivity and specificity was systematically evaluated.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4$; 99%), sodium borohydride ($NaBH_4$; 99%), cetyltrimethylammoniumbromide (CTAB), L-ascorbic acid (AA), silver nitrate ($AgNO_3$; 99%), dodecanthiol (DDT), 11-mercaptoundecanoic acid (MUDA; >95%), N-hydroxysulfosuccinimide (Sulfo-NHS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC), regular human plasma, fibrinogen, bovine serum albumin and poly(sodium-p-styrenesulfonate) (PSS; MW: 70,000) were obtained from Sigma-Aldrich (St. Louis, MO). Highly purified human cardiac troponin I (cTnI) and specific murine monoclonal antibody were from Fitzgerald Industries (Acton, MA).

2.2. Fabrication and characterization of Au nanorods

Gold nanorods were chemically synthesized using seed mediated growth method as described previously with modifications (Sau and Murphy, 2004). Typically, a seed solution of gold nanospheres (5–10 nm) was prepared by mixing gold chloride (5 mL, 0.5 mM) with 5 mL (0.2 M) CTAB for 1 min and adding 0.6 mL (10 mM) fresh, ice cold sodium borohydride under vigorous stirring. After 2 min of mixing, the solution was incubated in water bath at 25–27 °C for at least 2 h. The seed solutions were then added to growth media ($HAuCl_4$, 1 mM; CTAB, 0.2 M; $AgNO_3$, 4 mM; and AA, 78.8 mM) and incubated at 27 °C overnight to allow complete rod growth.

Absorption spectra of the synthesized GNRs were collected with a UV–vis spectrophotometer (Beckman Colter). Electron microscopy images of the nanorods were taken using Hitachi scanning electron microscope (SEM) and elemental analysis was carried out on a Joel SEM equipped with X-ray energy dispersive analysis (EDAX). For each sample, the size of 200 particles was measured to obtain the average nanoparticle dimension, aspect ratio, and yield.

2.3. Surface modification and biofunctionalization of Au nanorods

To construct a specific nanorod probe for biological detection, surface modification is required to functionalize with biological receptors such as antibody. Herein, we investigated two methods as the following.

2.3.1. Double phase transfer ligand exchange

Seed mediated growth method results in the nanorod surfaces covered by a CTAB bilayer. A phase transfer ligand exchange using DDT and subsequently MUDA as reported earlier with modifications were performed to efficiently replace CTAB molecules bound on the Au surfaces (Wijaya and Hamad-Schifferli, 2008). Briefly, 4 mL dodecanthiol and 8 mL acetone were sequentially added to 2 mL highly concentrated nanorod solution and gently swirled. A clear separation of the aqueous and organic layers was present with the DDT coated gold nanorods in the top organic layer. After separation, 2 mL toluene and 9 mL methanol were added to the top layer. The solution was then centrifuged (5000 rpm for 15 min) into a pellet and resuspended in 2 mL toluene via sonication (20 min). This suspension was then added under vigorous stirring to 9 mL MUDA (0.01 M in toluene) at 70 °C. After 15 min the solution was allowed to cool down to room temperature as the nanoparticles settled to the bottom. The toluene solution was decanted and the rods were resuspended in 3 mL toluene and allowed to settle, followed by a second wash with 3 ml isopropyl alcohol to deprotonate the MUDA. The resulting MUDA become negatively charged at physiological pH, thereby minimizing electrical repulsion to little nonspecific binding. The aggregates are finally redispersed in 1 ml of Tris-borate EDTA buffer. After the CTAB was replaced by the MUDA, the carboxylic groups (–COOH) at the nanorods surface were activated by EDC/sulfo-NHS (2:1 mg/mL) to form a stable NHS ester. The NHS ester-activated crosslinker then reacted with the amine groups to yield NH–CO bonds to covalently immobilize anti-cTnI molecules on the surfaces of GNRs.

2.3.2. Electrostatic coating method

Physical adsorption of negatively charged PSS polymers to wrap over positively charged CTAB bilayer on the nanorod surfaces was achieved by mixing GNRs with PSS solution (0.5–20 mg/mL) in the presence of 1 mM NaCl. After vigorous stirring, the solution was incubated at room temperature for 1 h. Afterwards, the solution was centrifuged at 8500 rpm for 12 min to remove excessive PSS molecules. The PSS-coated nanorods were then redispersed in 10 mL of PBS buffer, followed by addition of 5 mL of monoclonal antibody against cardiac troponin I (50 μg/mL) with sonication for 1 h.

2.4. Label free LSPR assay based on gold nanorod probes

Once the specific antibody is immobilized on the rod surface, the absorption spectrum of the functional GNR sensor was taken as a baseline reading. To carry out the immuno-reaction and the resulting spectral shift measurement, biological samples spiked with various cTnI concentrations (0.5–1 mL) were incubated with equal volume of functional GNR probes prepared by ligand exchange and PSS coating, respectively, for 1 h under mild