Spectrochimica Acta Part A 75 (2010) 1163–1167

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Analysis of immunoreaction with localized surface plasmon resonance biosensor

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ARTICLE INFO

Article history:
Received 25 May 2009
Received in revised form
16 December 2009
Accepted 31 December 2009

Keywords:
Localized surface plasmon resonance (LSPR)
Self-assembled monolayer (SAM)
Gold colloid
Human IgG

ABSTRACT

The localized surface plasmon resonance (LSPR)-based optical biosensor was used as a potential tool for label-free detection of immunoreaction. The glass substrate covered with the self-assembled monolayer (SAM) of gold colloids was used widely in the sensors. Here, the glass substrate was modified by chemical hydroxylation first, and then gold colloids were immobilized on the substrate by electrostatic adsorption. The LSPR spectra were obtained on UV–vis absorption spectrometer. The specificity was examined by extensive nonspecific binding tests. The resonance condition on the local dielectric environment enables a simple form of molecular sensing. The binding of analyte to the biosensor surface causes a change in the absorbance which was responsive to the concentration of human IgG. So, the LSPR sensing yields similar results to the SPR technique, yet with much simpler instrument.

1. Introduction

Surface plasmon resonance (SPR) biosensor was first established in the early 1980s and became increasingly popular as a label-free method for the binding of analytes to functionalized surface and the detection of immobilized biomolecules [1–3]. It was based on the excitation of a surface plasmon at the interface between a dielectric and a thin layer of metal, typically gold [4]. The variation of a basic SPR system with nanostructured metal surfaces typically performed by nanoparticle-based patterning or electrochemical etching is called localized surface plasmon resonance (LSPR). LSPR is currently of considerable interest in the fabrication and optical characterization of gold and silver nanoparticles on solid supports [5,6]. The noble metal nanoparticle can exhibit a strong UV–vis absorption band that is not present in the spectrum of the bulk metal [7]. The absorbance and wavelength of the LSPR band are highly dependent on the size, shape, and local environment of the nanoparticles [8,9]. LSPR-derived properties of metallic nanoparticles are known to depend on the local dielectric environment of the nanoparticles [8,10]. The variations of the local environments experienced by metal nanoparticles are attributed to the changes in the complex dielectric function in the vicinity of the interface between the metal and the media. Therefore, the adsorption of biomolecules on the surface of metal nanoparticles can be detected by the measurement of absorption spectra since the molecular adsorption gives rise to an increase in the dielectric constant near the surface. With a suitable receptor immobilized at the surface of the gold nanoparticles, the resulting LSPR biosensor can detect the corresponding analyte even if the analyte is spectroscopically silent in the UV–vis region. For LSPR allows label-free detection of low concentrations of biomolecules, the use of the LSPR-based phenomena for chemical and biological sensing is of special interest. LSPR nanobiosensors are of great interest in various applications such as environmental protection [11], biotechnology [12,13], food safety [12–14] and so on.

Nanotechnology and nanomaterials are novel and exciting fields of research [15]. Nanoparticles of noble metals such as gold and silver have attracted much interest for their application in biosensing due to their distinctive optical property. Particularly, gold nanoparticles are noteworthy for their biocompatibility [16]. Gold colloid can be used as a robust tool for biosensing based on LSPR. The optical properties of gold colloids are sensitive to the refractive index of the surrounding solvent and the binding events to those functionalized gold colloid. Tamiya and co-workers use gold-capped oxide nanostructures to detect the interactions between aptamer and protein [17]. The novel nanomaterial enabled them monitor the changes in both LSPR and interferometric characteristics simultaneously since the biomolecular interactions occur. Chilkoti and co-workers use gold nanorod sensor to detect the model analyte in serum [18], gold nanorods were chemisorbed onto the glass substrate which was modified by mercaptosilane. The shift of the LSPR peak was monitored by the wavelength in the UV–vis extinction spectrum of the immobilized gold nanorods due to the change in local refractive index at the gold nanorod surface induced by the analyte binding.

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doi:10.1016/j.saa.2009.12.084

Surface-modification technique to nanoparticle system is an important goal in current materials research since the nanoparticles modified on the versatile surface have significant potential applications in biosensing. Recently, many technologies are applied to the immobilization of nanoparticles on the surface, such as nanosphere lithography (NSL) and self-assembled membrane (SAM). In NSL the monodisperse nanosphere template was used as a deposition mask [19]. The conventional process of NSL begins with self-assembly of a nanosphere mask onto a substrate followed by line-of-sight deposition of materials through the mask [20]. The projection of the nanosphere mask interstices on the substrate determined the interparticle spacing and the nanoparticles have an inplane shape. NSL has some limitations on the nanoparticle shape and interparticle spacing, and there is an inherent size limitation due to the increasing polydispersity involved in the synthesis of smaller diameter nanospheres necessary to grow the two-dimension colloidal crystal. SAM is of great interest for the creation of new functional materials. These features are important to many areas of scientific study, such as molecular electronics [21,22], biomimetics [23], lithography [24,25], sensors [26–28], and so on. SAMs have also been used as linker molecules in attaching nanoparticles to bulk surfaces or to other nanoparticles creating new types of macroscopic materials.

In this work, LSPR was developed by measuring the LSPR spectrum using UV–vis absorption spectrometer. The LSPR biosensor was fabricated based on the gold colloids immobilized on the glass substrate by electrostatic adsorption. The proposed biosensor was applied to the detection of human IgG. LSPR-based optical biosensors can detect an immediate increase in the biomolecular monolayer on the sensing surface which is caused by the immunoreactions between the antigen and the receptor layer immobilized on the surface. For such LSPR biosensors, the immobilized gold nanoparticles act as the chromophores, while their interactions with the analytes will result in colorimetric changes.

2. Experimental

2.1. Materials

$HAuCl_4{\cdot}3H_2O$, trisodium citrate dihydrate, poly (diallyldimethylammonium chloride) (PDDA), poly (styrenesulfonate) (PSS), poly(allylamine hydrochloride) (PAH), and 3-mercaptopropionic acid (MPA) were purchased from Sigma, USA. N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma, USA. Ethanolamine, mouse IgG, human IgG, goat anti-human IgG, Hsp 70 and bovine serum albumin (BSA) were purchased from Beijing Ding Guo Biotech. Co. Ltd., China.

All of the chemicals used here were of analytical-reagent grade. All the biological reagents were kept at 4 °C and all solutions were prepared with ultrapure water. Mouse IgG, human IgG, goat anti-human IgG, Hsp 70 and BSA were stored at −20 °C, and all other biological reagents were stored at 4 °C. Phosphate-buffered saline (PBS) buffer (pH 7.4) was used as running buffer. Ethanol and acetone were used for cleaning the glass substrates. Aqua regia solution (3/1 (v/v) HCl/HNO_3) was used for cleaning the glassware. Piranha solution (3/7 (v/v) 30% H_2O_2/H_2SO_4) was used in chemical hydroxylation on the glass substrates. Piranha solution is highly hazardous and reactive that may explode on coming into contact with organic solvents. Extreme precaution should be taken at all times.

2.2. Equipments

Absorption spectra were recorded with an Australian GBC Cintra 10e UV–vis spectrometer within the wavelength range from 400 to 800 nm. SEM micrographs were taken with a JEOL JSM 6700F field emission scanning electron microscope with primary electron energy of 3 kV. A KQ118 ultrasonic clean up device was purchased from Kunshan Ultrasonic Cleaning Instruments Co.

2.3. Gold colloid preparation

All glassware used in these preparations was thoroughly cleaned by immersion in aqua regia solution for 24 h, and rinsed with ultrapure water, then oven-dried prior to use. The standard stock solutions were prepared with ultrapure water that had been filtered through a 0.2 μm membrane. The gold colloid was prepared following the method reported by Wang [29]. The typical gold colloid preparation was described below.

After 100 mL of 0.01% $HAuCl_4$ solution was heated to the boil under continuous stir, 1.5 mL of 1% sodium citrate was added into the solution rapidly. The solution turned blue within 25 s and 70 s later changed to red-violet. Then the solution was kept in boiling and continuously stirred for 10 min. After the heating source was removed, the solution was continuously stirred for additional 15 min and cooled to room temperature. At last, the gold colloid solution prepared was filtered through a 0.2 μm filter membrane and stored at 4 °C in dark bottles for further experiments.

2.4. Preparation of gold colloid-modified glass substrate

Glass substrates were sonicated successively in ultrapure water, ethanol, acetone, ethanol and ultrapure water. Then, the glass substrates were cleaned and chemically hydroxylated by immersing in a piranha solution at 80 °C for 1 h. After cooling to room temperature, the substrates were rinsed repeatedly with ultrapure water and stored in water until used. The substrates should be used as soon as possible or stored in water no longer than one week.

First, the hydroxylated glass substrate was submerged into 0.5% PDDA solution for 40 min. Subsequently, the glass substrate was alternately immersed in PSS (1 mg mL^{-1}) solution and PAH (1 mg mL^{-1}) solution for 20 min. In every step, the glass substrate was rinsed with ultrapure water to remove unbound polyelectrolyte from the surface, and followed with drying under a stream of pure nitrogen (N_2). Then, the substrate was immersed in gold colloid solution for 8–24 h to form the self-assembled gold colloid membrane on the surface. At last, the gold colloid-modified substrate was rinsed with ultrapure water and dried under a stream of pure N_2.

2.5. Immobilization of antibody onto the modified glass substrate

When self-assembling of the gold colloid was performed, the gold colloid-modified glass substrate was immersed in 40 mmol L^{-1} MPA solution for 1 h and MPA monolayer film was first structured on the gold colloid for the strongly binding of S–Au. The MPA binding on the colloid-modified glass substrate is well-ordered to form SAM structure [30]. The MPA modified biosensor was immersed in PBS buffer solution for 20 min to remove the unbound MPA. Then the biosensor was activated by immersion in a solution containing NHS (100 mg mL^{-1}) and EDC (100 mg mL^{-1}) for 20 min. –COOH group reacted with NHS under the catalysis of EDC. The activated biosensor was immersed in goat anti-human IgG solution (30 μg mL^{-1}) for 12 h to assemble the antibody on the activated surface and make the biosensor membrane stable. Then the biosensor was immersed in PBS buffer for 20 min to wash off the unbound antibody. The nonspecific binding sites on the biosensor surface was blocked with 1 mol L^{-1} ethanolamine (pH 8.0) for 30 min.

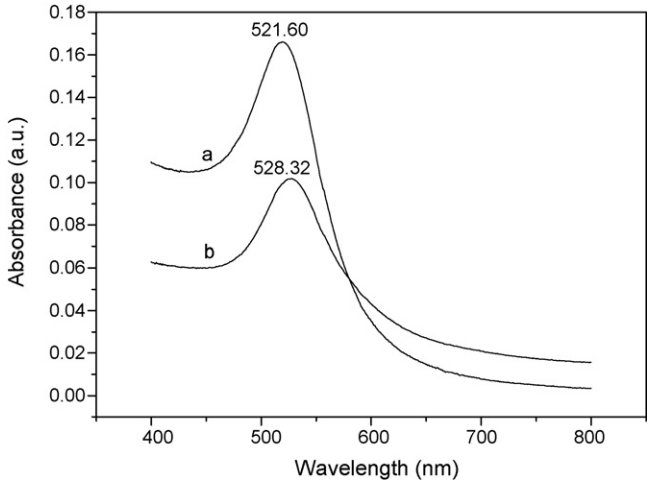

Fig. 1. UV–vis absorption spectra of gold colloid in the solution (a) and a monolayer of gold colloid immobilized on the glass substrate (b).

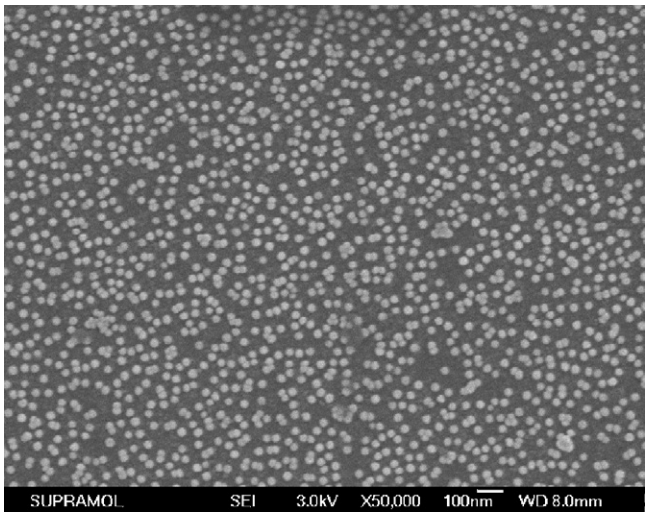

Fig. 2. Scanning electron micrograph of 25 nm diameter gold colloid immobilized on the glass substrate.

2.6. Ultraviolet–visible absorption measurement

At room temperature, before every sample was analyzed, the biosensor was rinsed with PBS buffer solution and then immersed in sample solution containing human IgG for 30 min. Then UV–vis spectrometer was used to measure the absorbance. The glass substrates without gold colloids in PBS buffer solution were used as the reference. After measurement, the biosensor was immersed in PBS buffer solution to dissociate the antigen from antibody for 20 min. Due to a large amount of ions in the PBS buffer solution the antigen could be washed from the sensing film by immersion in PBS buffer [31]. When the antigen dissociated from the antibody surface, the absorbance returned to the baseline and the biosensor can be applied to analyze another sample.

3. Results and discussion

3.1. Modification of gold colloid to the glass substrate

Gold colloids show different colors as a result of the various particle diameters and the absorption peaks of UV–vis absorption spectra range from 500 to 560 nm. The gold colloids with a uniform size of 25 nm were synthesized by a simple method. Fig. 1(a) shows the UV–vis absorption spectrum of gold colloids in the solution. The absorption spectrum of gold colloids has an absorption peak at 521.60 nm, indicating that gold colloid is not congregated in the solution. The narrow peak indicates that the gold colloids synthesized have the same diameter.

The gold colloids used in the study were prepared on the surface of glass substrates using polyelectrolyte PDDA, PSS and PAH. Hydroxyl/oxide groups on the glass substrate surface provide active sites for the attachment of PDDA. Meanwhile, PDDA has strong adsorbability to glass substrates. The PSS can connect with PAH due to electrostatic adsorption. PAH containing functional group $-NH_2$ has a high affinity for gold colloid. The color of the gold colloid-modified glass substrate is red-violet that is similar to gold colloids in solution. From the results mentioned above, it is seen that the gold colloid is immobilized on the glass substrate by self-assembly and not congregated. The UV–vis absorption spectrum is shown in Fig. 1(b). The shape of absorption spectrum of gold colloid-modified glass substrate is very similar to that of gold colloid in the solution (Fig. 1(a)). Glass substrate modified with gold colloid absorbes light strongly due to LSPR effect and has an absorption peak at 528.32 nm. Compared to the absorption peak of gold colloid in the solution, the absorption peak shows a red shift of 6.72 nm.

3.2. The characteristics and the stability of the substrate

The gold colloids are unstable and will cause the aggregation in solution. When the MPA was injected in the solution containing gold colloid, the color of the solution changed from red-violet to blue and the aggregation happened. So, it is difficult to use the gold colloid directly in the immunoassay and store the gold colloid solution. However, when the gold colloid was immobilized on the glass substrate, the stability was improved significantly. Fig. 2 shows a scanning electron micrograph (SEM) of such gold colloids that have been immobilized onto the glass substrate due to the electrostatic adsorption by the polyelectrolyte. The image shows that the adsorbed gold colloid separated from each other in random arrangement. The mean interparticle distance is a few times larger than the particle diameter, which indicates that the interaction between particles could be neglected. After the gold colloid-modified glass substrate was sonicated in ultrapure water for 10 min, the absorbance did not change obviously. The same phenomenon happened in ethanol and PBS solution.

3.3. Preparation of the LSPR biosensor surface

After the activated sensing surface was prepared, the activated substrate was immersed in the solution containing goat anti-human IgG in order to prepare the sensing film. The concentration of the antibody was crucial. If the concentration was too low, there would be many unoccupied sites on the gold colloid-modified film and the change in the absorbance obtained was too little. In this assay, 30 $\mu g\,mL^{-1}$ of goat anti-human IgG was sufficient to produce a monolayer of adsorbed antibodies on the activated sensing film. The antibody has no absorption in the wavelength range of 400–800 nm. The immobilization of goat anti-human IgG on the surface of the gold colloid can cause a change of absorption peak in that range. For the immobilization of antibody, the change of the absorbance for the LSPR sensor is significant and after 12 h, the change of absorbance is 0.008. The results indicated that the antibody was bound steadily on the biosensor surface. After the antibody was immobilized, the biosensor was immersed in ethanolamine solution to block unbound sites on the gold colloid-modified biosensor and no notable change in the spectrum was observed, which indicated that the binding sites of biosensor surface were almost completely occupied by the antibody.

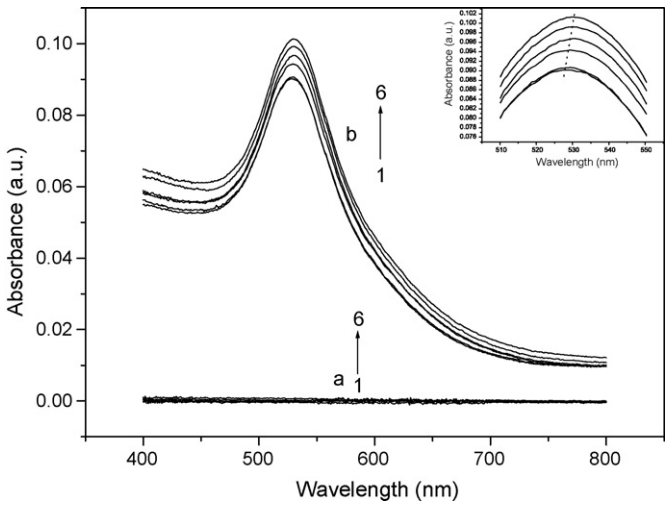

Fig. 3. UV–vis absorption spectra of human IgG solution (a) and the gold colloid-modified substrate in human IgG solution (b). Concentration of IgG: (1) 0 μg mL^{-1}, (2) 1.0 μg mL^{-1}, (3) 2.0 μg mL^{-1}, (4) 5.0 μg mL^{-1}, (5) 10.0 μg mL^{-1} and (6) 20.0 μg mL^{-1}.

3.4. Detection of human IgG

The change of the absorbance induced by binding human IgG to the antibody was measured by UV–vis absorption spectrometry. After the immersion of biosensor in the sample solutions for 30 min, the UV–vis absorption spectra were measured. It is can be seen from Fig. 3, human IgG have no absorption in the wavelength range from 400 to 800 nm. The red shift of LSPR wavelength can be observed from absorption spectra of gold colloids, especially from the spectra shown in inset. However, the change in LSPR wavelength is not obvious. Compared to the wavelength, the change in absorbance is more obvious. Therefore, the absorbance modulation, which is similar to the intensity modulation applied in SPR detection [32], was applied in the work. The change in the absorbance is relative to the concentration of antigen. It is can be seen from Fig. 4 that the lowest concentration of human IgG that can be determined is 1 μg mL^{-1} corresponding to the change of absorbance of 0.001. The maximal change of the absorbance is 0.012 when the concentration of human IgG is 20 μg mL^{-1}. The error bars denote the standard deviation of the absorbance values in the three same assays. When the concentration of human IgG is higher than 20 μg mL^{-1}, the change of the absorbance is very little because the binding sites used for binding antigen may be saturated.

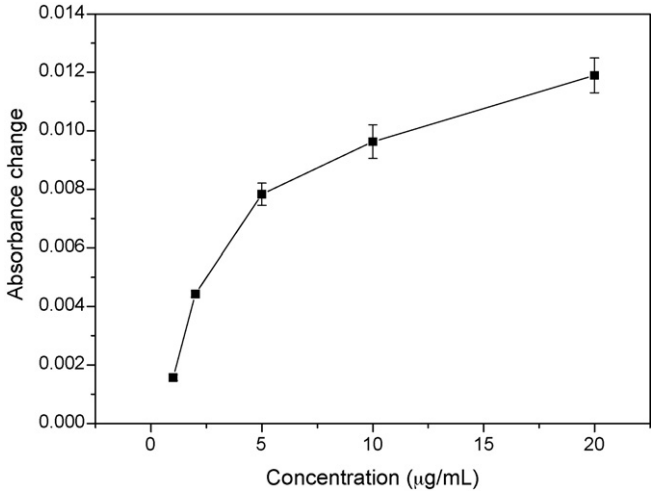

Fig. 4. Dependence of the absorbance on the concentration of human IgG.

3.5. Examination of nonspecific binding

After goat anti-human IgG was immobilized on the biosensor surface, the biosensor was immersed in the mixed solution containing mouse IgG (10 μg mL^{-1}), Hsp 70 (10 μg mL^{-1}), BSA (10 μg mL^{-1}) for 30 min the mixed solution has no absorption in the wavelength range between 400 and 800 nm, and the obtained absorption spectra of the mixed solution and PBS buffer solution are the same, which indicates that there is no nonspecific binding. This result confirmed the high specificity of the LSPR biosensor.

4. Conclusions

Gold colloid-modified LSPR biosensor was fabricated by SAM for the study of biomolecular interactions. The fabrication method of the biosensor based on electrostatic adsorption is very convenient. The LSPR spectra were measured by UV–vis absorption spectrometer which was very easy to be obtained. This study presents a new approach in LSPR biosensor to analyze binding of antibody to the functional surfaces and finally to detect human IgG rapidly. The human IgG has good response in the concentration range of 1–20 μg mL^{-1}, and it is specific binding to the goat anti-human IgG. So, this self-assembly based biosensor is specific and can be used as a good biosensor. The proposed method provides a convenient, low-cost, and label-free detection of biomolecular interactions. The LSPR-based optical biosensor has the potential to be applied as a simplified test kit for biomonitoring.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 20727003) and Science and Technology Developing Foundation of Jilin Province (No. 20070551).

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