Fabrication and characterization of SPR chips with the modified bovine serum albumin*

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(Received 8 December 2015)

A facile surface plasmon resonance (SPR) chip is developed for small molecule determination and analysis. The SPR chip was prepared based on a self assembling principle, in which the modified bovine serum albumin (BSA) was directly self-assembled onto the bare gold surface. The surface morphology of the chip with the modified BSA was investigated by atomic force microscopy (AFM) and its optical properties were characterized. The surface binding capacity of the bare facile SPR chip with a uniform morphology is 8 times of that of the bare control SPR chip. Based on the experiments of immune reaction between cortisol antibody and cortisol derivative, the sensitivity of the facile SPR chip with the modified BSA is much higher than that of the control SPR chip with the un-modified BSA. The facile SPR chip has been successfully used to detect small molecules. The lowest detection limit is 5 ng/mL with a linear range of 5—100 ng/mL for cortisol analysis. The novel facile SPR chip can also be applied to detect other small molecules.

Document code: A **Article ID:** 1673-1905(2016)02-0093-4
DOI 10.1007/s11801-016-5248-6

Surface plasmon resonance (SPR) as a sensing technology can be used to monitor the whole process of molecular interactions[1-4]. However, it is much more difficult to detect small molecule antigens than large molecules by using SPR technology[5]. To obtain optimal assay sensitivity, the antigen is therefore labeled with a high mass label and used in competition with un-labeled sample antigen for binding to the surface in a competitive immunoassay[6], or the small molecule antigen is conjugated to the SPR sensor surface, the primary antibody is mixed with the sample containing free antigen, and the mixture passes over the sensor surface[7]. The second method is an indirect inhibition immunoassay, in which derivatives of small molecules are usually used to immobilize on the SPR chip.

The SPR chip is important for target molecules detection and analysis. Various surface modification procedures, such as simple physical adsorption, self assembly, covalent binding, and complementary DNA binding, have been used for fabricating SPR chips with different functional groups[8-11]. The formation of Au-S bond is the important step to fabricate SPR chips. The thiol end of the molecules forms an Au-S bond with the gold surface, while the other end of the molecule provides a functional group for further attachment chemistries.

In this paper, the modified BSA with 35 free mercapto groups was directly self-assembled onto the bare gold surface with Au-S bond to form a facile SPR chip. At the same time, the un-modified BSA with one free mercapto group and 17 disulfide bonds was also directly assembled onto the bare gold surface to fabricate a control SPR chip. Both of the SPR chips were characterized and their optical properties were investigated.

N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), glycine and bromoacetic acid were purchased from Shanghai Chemical Reagents Corp. (China). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from Saixiefeisier Corp. (China). BSA, tween-20, and phosphate buffer (PBS) tablets were purchased from Xinjingke (China). All other chemical reagents were from Sigma-Aldrich. Cortisol was obtained from Meck (Germany). Monoclonal primary antibody (mAb) to cortisol was obtained from Cloud-Clone Corp. (raised in mouse, CPA462Ge11, China). Cortisol derivative of cortisol-BSA was obtained from Fitzgerald (80-1060, USA).

The facile SPR chip with the modified BSA and the control SPR chip with the un-modified BSA were fab-

* This work has been supported by the National Basic Research Program of China (No.2014CB744600), the National High Technology Research and Development Program of China (No.2014AA022303), and the National Natural Science Foundation of China (Nos.61571420, 31571007, 61201079, 61372055 and 81371711).
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ricated and compared as shown in Fig.1. A cover glass was extremely cleaned. And then Cr (2 nm) and Au (48 nm) were deposited on the glass slide by using a sputtering deposition process, leading to the bare Au SPR chip. 200 μL 2 mg/mL modified BSA or un-modified BSA in PBS was dropped onto the bare gold chip and incubated at room temperature for 30 min. 2 mg/mL BSA in PBS was mixed with 60 μL 0.1 mol/L TECP and incubated at room temperature for 30 min to obtain the modified BSA. After washing, EDC (0.4 mol/L) was mixed with NHS (0.1 mol/L) (volume ratio of 1:1) and added on the above chip to activate the surface for 20 min. After washing, 200 μL bromoacetic acid (2 mol/L, in NaOH) was dropped onto the chip and incubated at room temperature for 16 h. A bare facile SPR chip was ready for on-line and off-line immobilizing the derivative of small molecules. After washing, EDC (0.4 mol/L) was mixed with NHS (0.1 mol/L) (volume ratio of 1:1) and added on the above chip to activate the surface for 20 min. After washing, the derivative of small molcules (100 μg/mL in 0.01 mol/L acetic acid buffer, pH=4.5) was dropped onto the above chip at room temperature for 30 min. Finally, the surface of the chip was then deactivated with ethanolamine (1 mol/L, pH=8.5). Step (e) could also be implemented on line.

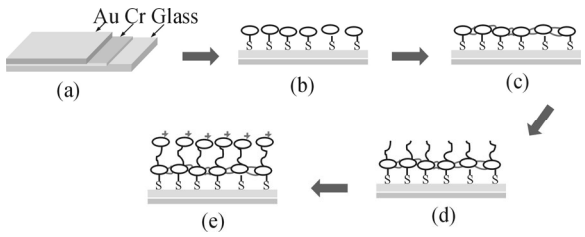


Fig.1 Fabrication procedure of BSA-SPR chips and dBSA-SPR chips: (a) Bare gold SPR chips; (b) SPR chips modified with modified BSA or un-modified BSA; (c) SPR chips with cross-linked modified BSA or un-modified BSA; (d) SPR chips with carboxylated BSA or dBSA; (e) SPR chips with derivatives of small molcules

The surface morphologies of the bare facile SPR chip and bare control SPR chip were characterized by atomic force microscopy (AFM) (Bruker, Multimode 8), respectively.

We use our SPR instrument[7,12] to study the bare Au SPR chip, the bare facile SPR chip and bare control SPR chip. The bare gold SPR chip, the bare facile SPR chip and the bare control SPR chip were mounted on the SPR instrument, respectively, and the resonant absorption peak was measured.

And then both of the bare facile SPR chip and bare control SPR chip were exposed to the flow of cortisol-BSA conjugate, respectively, in order to characterize their surface binding capacities. The experimental procedure is similar to Step (e) above. The difference is that all the reagents and solutions were injected to activate, react, and deactivate the surface by using a peristaltic pump.

The facile SPR chip and the control SPR chip with cortisol derivatives were docked into a home-made SPR instrument, respectively. 200 μL mAb to cortisol was introduced into the SPR chip, leading to immune reaction between the cortisol derivative immobilized on the SPR chips and the mAb dissolved in the PBS solution. Then the surface of the SPR chip was regenerated with 100 mmol/L NaOH (200 μL). An mAb dilution plot was prepared by injecting various concentrations of mAb (1 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, 30 μg/mL, 200 μL). There are 5 replicates of each point with regeneration as above.

The facile SPR chip with cortisol derivatives was docked into a home-made SPR instrument. After 30 min incubation at room temperature, 200 μL mixture of mAb (5 μg/mL) and cortisol with different concentrations in PBS was introduced into the facile SPR chip, leading to an indirect inhibition immunoassay. Then the surface of the facile SPR chip was regenerated with 10 mmol/L glycine (100 μL, pH=2.5). A buffer assay standard curve for cortisol was constructed by the mixture of mAb (5 μg/mL) and cortisol (0 ng/mL, 5 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL) in PBS. There were 5 replicates of each point with regeneration as above.

The surface morphologies of the facile SPR chip and the control SPR chip were investigated by AFM and their optical properties were characterized by using our home-made SPR analytical instrument. The AFM images of the facile SPR chip and the control SPR chip are shown in Fig.2. The surface of the facile SPR chip (Fig.2(a)) is covered with a flat, uniform and dense layer of the modified BSA, while there are some pits on the non-uniform surface of the control SPR chip (Fig.2(b)), resulting in some bare Au particles. Because the disulfide bonds in the un-modified BSA do not break up, it is lack of Au-S bonds formed between the Au and the un-modified BSA.

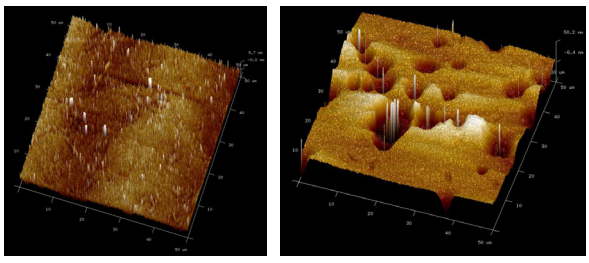


Fig.2 AFM images of (a) the facile SPR chip and (b) the control SPR chip (Scan size is 50 μm.)

A home-made SPR instrument based on the prism coupling mode of the Kretschmann structure was used to detect the SPR absorption peak[12]. The facile SPR

chip and the control SPR chip were mounted on the SPR instrument, respectively. The incident angle of our SPR instrument is capable of changing from 40° to 70°.

The curves of SPR absorption peaks for different SPR chips were detected, which are shown in Fig.3. From Fig.3(a), the SPR absorption peak shifts from low angle (bare gold surface) to high angle (the surface with a self-assembled layer), meanwhile the depth of SPR absorption peak decreases. And the SPR absorption peak of the facile SPR chip shifts more than that of the control SPR chip, which means that the self-assembled modified BSA layer is thicker and denser than the self-assembled un-modified BSA layer on the bare gold surface.

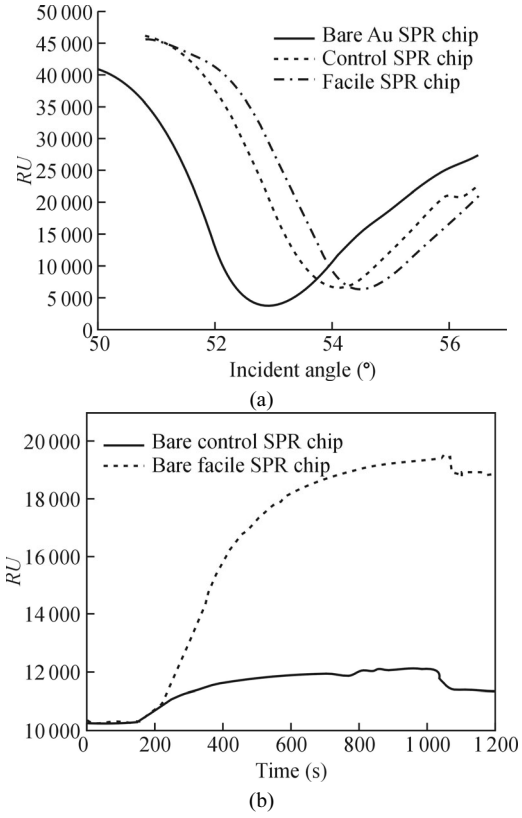


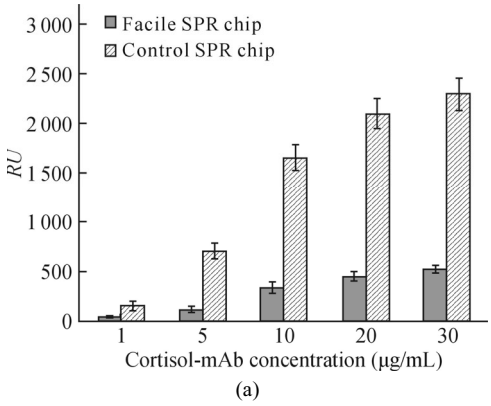
Fig.3 SPR curves: (a) SPR peak curves for the bare Au SPR chip, the control SPR chip and the facile SPR chip; (b) Responses of SPR sensors according to the covalent amide bonding of cortisol-BSA to the bare control SPR chip and the bare facile SPR chip

The covalent amide bonding of cortisol-BSA on the bare facile SPR chip and the bare control SPR chip is investigated. SPR sensorgrams observed for the flow of 100 μg/mL cortisol-BSA over the pre-activated surfaces of the bare facile SPR chip and the bare control SPR chip are shown in Fig.3(b). Both the sensorgrams show a steady and gradual increase in SPR response of intensity, indicating a uniform binding of cortisol-BSA on the surfaces of the SPR chips. However, the SPR response unit change (ΔRU) of intensity obtained for covalent binding of cortisol-BSA on the surface of the bare control SPR chip (ΔRU=1 065) is much smaller than that obtained on the surface of the bare facile SPR chip (ΔRU=8 537). The surface binding capacity of the bare facile SPR chip is almost 8 times of that of the bare control SPR chip.

The immune reactions between the mAb of the small molecule in PBS solution and small molecule derivatives bonded on the facile SPR chip with the modified BSA and the control SPR chip with the un-modified BSA were investigated. In this case, cortisol and cortisol-BSA were used as the small molecule and the small molecule derivative, respectively. The SPR response unit (*RU*) obtained for the flow of the cortisol-mAb is plotted against the concentration of the cortisol-mAb. As shown in Fig.4(a), the *RUs* of the SPR signal for both the facile SPR chip and the control SPR chip increase more or less linearly with the low concentration of the cortisol-mAb, and achieve at a nearly saturated steady state at higher concentrations. From Fig.4(a), the facile SPR chip with the modified BSA has a much better sensitivity than the control SPR chip with the un-modified BSA.

The concentration of antibody appropriate for highly sensitive detection in indirect inhibition immunoassay experiments would be in the region where the antibody binding is diffusion limited rather than reaction limited[13]. So, the appropriate concentration of the cortisol-mAb will fall in the range of 5—10 μg/mL. The increasing speed of *RU* of the SPR signal is greatly reduced, when the concentration of the cortisol-mAb is more than 10 μg/mL, shown in Fig.4(a). Thus, considering both reagent cost and SPR signal, the antibody concentration of 5 μg/mL is optimal for conducting indirect inhibition immunoassay experiments of cortisol detection.

Cortisol is a key steroid hormone required for regulating physiological function. Cortisol concentration increases in response to various stresses[14]. Normal serum concentration of cortisol varies in a diurnal cycle from 30 ng/mL to 140 ng/mL, peaking in early morning[15]. Indirect inhibition immunoassays are used to detect cortisol, which are based on the inhibition of cortisol antibody binding to the immobilized cortisol-BSA by free cortisol in solution. The facile SPR.



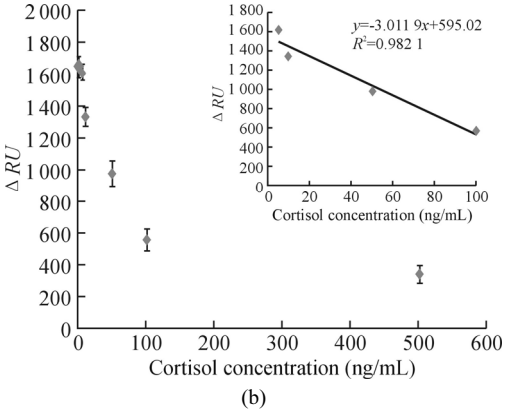


Fig.4 Responses of SPR signals according to different concentrations of (a) cortisol-mAb and (b) cortisol

chip with the modified BSA was used to detect cortisol SPR response unit change (ΔRU) is plotted against the concentration of cortisol, as shown in Fig.4(b). The detection limit of the facile SPR chip is 5 ng/mL, with a linear range of 5—100 ng/mL. It is worth noting that the detection limit of the cortisol changes slightly by using different batches of antibodies.

In this work, a novel facile SPR chip with the modified BSA was fabricated and characterized. The disulfide bonds in BSA were broken up to form free mercapto groups, which were used to bond the bare gold surface with Au-S bonds. The surface morphologies of the SPR chips with the modified BSA and the un-modified BSA were investigated by AFM. And then their optical properties were characterized by using our home-made SPR analytical instrument. The surface binding capacity of the facile SPR chip with the modified BSA layer is much higher than that of the control SPR chip with the un-modified BSA. From the results of immune reaction between antibody and cortisol derivative, the sensitivity of the facile SPR chip with the modified BSA is higher than that of the control SPR chip. The facile SPR chip has been successfully used for cortisol detection, which has a great potential for clinical detection.

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