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An optical immunosensor based on surface plasmon resonance for determination of bFGF

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Abstract

An optical immunosensor based on surface plasmon resonance (SPR) has been developed for immunosensing. The sensor is designed on the basis of fixing incident angle of light and measuring the reflected intensities in the wavelength range of 400–800 nm simultaneously. The SPR spectrum was shown in terms of reflected light intensities versus wavelengths of incident light. The intensity of the reflected light reaches the minimum at the resonant wavelength. Molecular self-assembling in solution is used to form the sensing membrane on gold substrate. The kinetic processes of sensing monolayer formation were studied. The basic fibroblast growth factor, a kind of basic polypeptide, was determined in the concentration range of 0.24–9.6 μg/ml. Under optimum experimental conditions, the sensor has a good repeatability, reversibility and selectivity.
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Keywords: Surface plasmon resonance; Immunosensor; Basic fibroblast growth factor

1. Introduction

The basic fibroblast growth factor (bFGF) is a kind of basic polypeptide and its pI is 9.6. The molecular weight of bFGF with different N group terminals is almost 15.6–18 kDa. One hundred and fifty seven amino acids are included in the bFGF of human placenta. The molecular weight of bFGF with four cysteine residues is 17.5 kDa. Among the four residues, two exist in all bFGF and form intramolecular disulfide bond to maintain the three-dimensional structure of bFGF. The N-terminal and C-terminal are important sites, through which heparin and bFGF receptor can be combined. After the bFGF is combined with the receptor on the surface of a cell, it can assemble in the nucleoli and turned to be stable [1,2].

In recent years, it has been confirmed that the bFGF can hasten blood vessel formation and renovation, induct normal embryo development, have a close concern with oncoma development and immunoregulation. The study of functions of bFGF has become the hotspot in clinical application. To our knowledge, up to now, the bFGF has not been determined by surface plasmon resonance (SPR).

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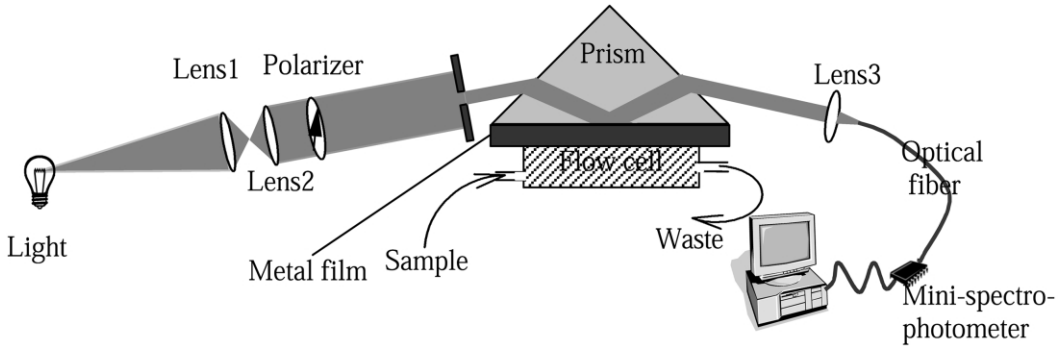

Fig. 1. Schematic diagram of the optical immunosensor based on SPR.

It has also witnessed that increased efforts have been being made in developing SPR sensors, which are very useful tools for macromolecules research, especially for studies of interactions between biomolecules [3–6]. Homola et al. recently reported a newly developed wavelength modulation-based SPR biosensor for the detection of staphylococcal enterotoxin B in milk [7]. The wavelength modulation-based SPR sensors have also been applied to the determination of albumin [8] and B Factor [9] in our laboratory. In this paper, a novel optical immunosensor based on SPR with fixing incident angle of light, and measuring reflected intensities in the wavelength range of 400–800 nm has been developed for immunosensing. The SPR spectrum was shown in terms of reflected light intensity versus wavelength. The intensity of the reflected light is the minimum at the resonant wavelength. The sensing membrane is based on a gold film deposited in vacuum on the bottom of a right angle glass prism and formed by molecular self-assembling in solution.

The mercaptopropionic acid has one functional group that can easily bond with the gold membrane and another carboxyl group, which has the affinity towards protein. The SPR sensor using mercaptopropionic acid as sensing membrane had been reported previously [10]. However, the sensor is difficult to be regenerated. The affinity of staphylococcal protein A (SPA) towards an antigen-bound antibody binding was also assessed by SPR since the SPA molecule has specific affinity to both the Fc terminal of IgG and the gold. It was shown that the sensor was easy to be regenerated but the sensitivity was poor. The SPA could combine with the mercaptopropionic acid and could extend the lifetime of the sensor. The SPR sensor developed in this paper is based on bilaminar sensitive membrane composed by mercaptopropionic acid and SPA. The bFGF was determined in the concentration range 0.24–9.6 μg/ml by using this new sensor. Under the optimum experimental conditions, it was shown the sensor had a good repeatability, sensitivity, reversibility and selectivity.

2. Experimental

2.1. Apparatus

The wavelength modulation SPR instrument used is schematically shown in Fig. 1. The light source is a halogen tungsten lamp powered with a constant voltage source. The light emitted from the lamp is polarized to give TM polarized light, and two lens are employed to make the light parallel. The parallel polychromatic light beam passes through an optical prism with a thin gold film on the bottom and excites surface plasmon at the interface between the gold film and solution. The output light from the prism is guided into the optical fiber and enters the Fullwave Spectrophotometer (Ocean Optics, Inc., USA). The prism was vacuum-deposited with approximately 50 nm gold film. To deposit the gold film, the prism was mounted in an electron-beam evaporator system in

an arrangement so that the flux of evaporated metal was perpendicular to the bottom of the right prism. The deposition process was monitored using a quartz crystal detector. The incident angle is fixed at a suitable angle to ensure the SPR phenomenon to occur. The reflected light intensity is the minimum at the resonant wavelength, a smaller increase of refractive index of the analyte in the proximity of the gold surface would cause a clear shift of the resonant wavelength in SPR reflected spectra towards longer wavelength.

2.2. Reagents and materials

SPA, bFGF, anti-human bFGF, human fibrin, human serum albumin (HSA) and human γ-globulin were purchased from Shanghai Biology Product Research Institute, PRC. Bovine serum albumin (BSA) was purchased from Sino-American Biotechnology Company (SABC). All other chemicals were of analytical reagent grade. All solutions were prepared with ultrapure water (resistivity >18.3 MΩ cm) supplied by an EASYpure RF compact ultrapure water system (Barrnstead Thermdyme Inc., USA).

0.01 mol/l phosphate-buffered saline solution (PBS, pH 7.4) was prepared using 0.2 g KCl, 8.0 g NaCl, 0.24 g KH_2PO_4, and 1.44 g $NaHPO_4$ dissolved in 1000 ml ultrapure water. 0.3 mol/l citrate buffer saline solution (pH 2.7) was prepared by dissolving 21 g $C_6H_8O_7{\cdot}H_2O$ and 11 g Na_2HPO_4 in 350 ml ultrapure water. Ten milligrams per milliliter BSA solution was prepared by dissolving 100 mg BSA in 10 ml 0.01 mol/l PBS buffer (pH 7.4). 100 mg/ml *N*-Hydroxysuccinimide (NHS) was prepared by dissolving 425.2 mg NHS in 4.25 ml ultrapure water. Hundred milligram per milliliter 1-ethyl-3-(3-dimethylaminopropyl)-carbodimide hydrochloride (EDC) was prepared by dissolving 191.7 mg EDC in 1.92 ml ultrapure water.

The prism was cleaned with an ultrasonic clean-up device both before and after it was covered with metallic film using vacuum-deposition. The lens, prisms, and various optical adjusting frames were purchased from the Fifth Precision Optical Instrument Factory of Changchun (PR China).

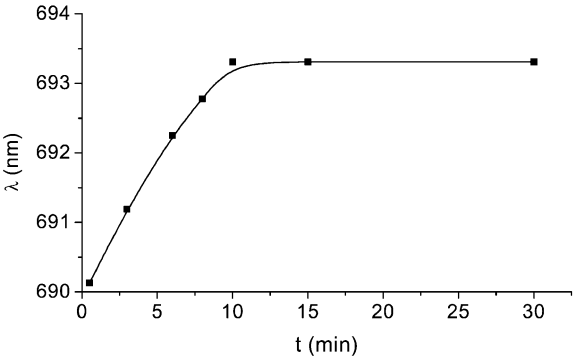

Fig. 2. The adsorption curve of 10 mmol/l mercaptopropionic acid on the gold film.

3. Results and discussion

3.1. Formation of self-assembled layers

The mercaptopropionic acid has short carbon chains and the nonrandom structure formed by molecular self-assembling is not as good as that formed with long carbon chains. The SPR sensor based on the mercaptopropionic acid membrane with active terminal carboxyl is studied in this paper. To monitor the self-assembling process on the gold substrate in real time, the mercaptopropionic acid was injected into the flow cell and the resulting shifts of resonant wavelength as a function of time (adsorption curve) were determined. The adsorption curve of the mercaptopropionic acid at the surface of gold is shown in Fig. 2. The shift of the resonant wavelength ($\Delta\lambda$) reaches 3.18 nm within 10 min in 10 mmol/l mercaptopropionic acid solution. Further increasing the assembling time, the resonant wavelength keeps almost constant. This experimental result implies that the self-assembling has finished and the monolayer is formed. Then a certain amount of NHS and EDC (100 mg/ml, final concentration) solutions were injected into the flow cell, respectively, to block the non - specific binding sites on the sensor surface. The shift of the resonant wavelength reaches 24.74 nm within 40 min. The adsorption curve of the 100 mg/ml NHS at the surface of gold is shown in Fig. 3.

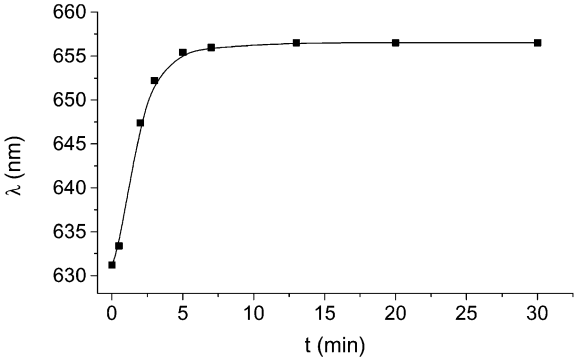

Fig. 3. Dependence of resonant wavelength on the time for adsorption of 100 mg/ml NHS on the surface of the sensor.

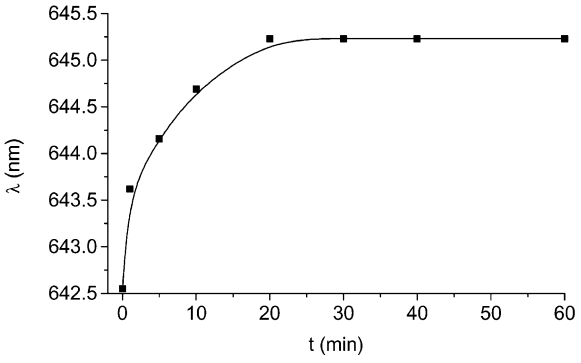

Fig. 4. The kinetic adsorption curve of protein A on mercaptopropionic acid.

SPA is a polypeptide that binds specifically to the Fc region of immunoglobulin molecules without interacting at the antigen binding sites. This property makes it possible to form tertiary complexes consisting of SPA, antibody and antigen. SPA binds the Fc region of antibodies at near neutral pH, leaving the antigen-binding sites free. SPA was injected into the flow cell to monitor its assembling on the mercaptopropionic acid. The adsorption curve of SPA on the surface of mercaptopropionic acid is shown in Fig. 4. The shift of the resonant wavelength reaches approximately 90% of its total shift within 10 min in the 10-mmol/l SPA solution. Further increasing the assembling time, the resonant wavelength keeps almost constant. This indicates the self-assembly has finished and the monolayer formed. The large excess of BSA (10 mg/ml, final concentration) blocked the non - specific binding sites on the sensor surface. Then the antiserum of bFGF (2 μg/ml) was injected into the flow cell. The antibody assembling was monitored again in real time. It was shown that the best dilution titer of bFGF antiserum was 1–20. If the antibody titer is too low, the variation of the resonant wavelength will be imperceptible. The sensor surface is well assembled at this antibody titer. The antiserum was diluted with PBS at pH 7.4. Fig. 5 shows the time dependence of mercaptopropionic acid and SPA layers assembled with antiserum of bFGF at 20 °C. The assembling of the antibody (1:20) was carried out for 1 h to organize the processing antibody molecular on the surface and such sensor membrane was quite stable. The value of adsorption constant (K_a) is calculated to be 4.16×10^6.

3.2. Determination of bFGF

A series of standard bFGF antigen was prepared. Fig. 6 shows the relationship between log C (concentration) of bFGF and log Δλ (resonance wavelength shift). The determination conditions for this sensor are in PBS buffer (pH 7.4) at 20 °C for 30 min. The bFGF is determined in the concentration range from 0.24 to 9.6 μg/ml. Repeating the determination of 4 μg/ml bFGF antigen for 11 times, a relative standard deviation of 1.1% is obtained.

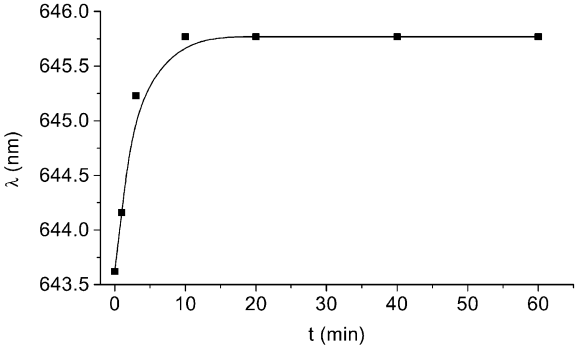

Fig. 5. The adsorption curve of bFGF antibody on mercaptopropionic acid and SPA layers of bFGF antibody concentration: 2 μg/ml, titer 1:20.

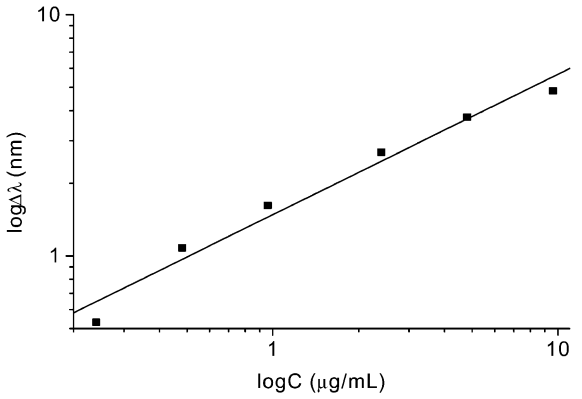

Fig. 6. The relationship between log C (concentration) of bFGF and log Δλ (resonance wavelength shift).

3.3. Selectivity

Under the optimum conditions, 5 mg/ml HSA and 1.0 mg/ml human γ-globulin, 5 μg/ml human fibrin were added to the flow cell. It was found that these components of human serum did not interfere the determination of bFGF. Therefore, the immunosensor has good selectivity.

3.4. Regeneration

The bound antibody can be desorbed with acidic solution [7,8]. After being rinsed for 10 min with 0.3 mol/l citrate buffer (pH 2.7), the sensor can be used repeatedly.

Acknowledgments

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