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In situ study of self-assembled nanocomposite films by spectral SPR sensor

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ABSTRACT

Spectral surface plasmon resonance (SPR) sensor with a time-resolved charge-coupled device (CCD) detector is a powerful analytical tool for label-free detection of biomolecular interaction at the liquid/solid interface and for in situ study of molecular adsorption behavior. In this work, the layer-by-layer self-assembly processes for three nanocomposite films were monitored in real time using a broadband spectral SPR sensor with a large dynamic range. Kinetics studies suggest that cytochrome c (Cyt c) and deoxy ribonucleic acid (DNA) adsorptions obey the Langmuir-isotherm theory, while gold nanoparticle (GNP) adsorption follows the Diffusion-controlled model. Using poly(sodium 4-styrenesulfonate) (PSS) and poly(dimethyldiallylammonium chloride) (PDDA) as the positively charged agents, three kinds of multilayer films such as the PSS/Cyt c, GNP/Cyt c and PDDA/DNA binary nanocomposites were fabricated on the SPR chips by the electrostatic attraction based on self-assemble. The SPR response in terms of $\Delta\lambda_R$ was measured to linear increase with increasing the number of layers for a six-bilayer PSS/Cyt c nanocomposite film, indicating that every PSS/Cyt c layer has equal mass coverage. In contrast, the nonlinear dependences of $\Delta\lambda_R$ on the number of bilayers were observed for the GNP/Cyt c and PDDA/DNA nanocomposite multilayer films.

1. Introduction

Layer-by-layer self-assembly is based on alternate immobilization of different chemical and biological species via either electrostatic attraction or covalent binding, and it allows for synthesizing novel thin-film functional materials on a large variety of substrates with different shapes. Owing to its simplicity, flexibility and effectiveness, layer-by-layer self-assembly has been recognized as a powerful technique with great interest in the fields of nanomaterials engineering, and surface modification as well as biochemical sensor fabrication [1–14]. In order to understand the self-assembly mechanism and to obtain the self-assembled parameters for reproducibly controlling the synthetic process and also for optimizing the resultant materials, the adsorption kinetics of precursor species and the relationship between the adsorbed amount of the precursor species and the number of layers have been investigated by using a variety of optical methods, including UV–visible absorption spectroscopy [15,16], surface-enhanced Raman spectroscopy [17–19], fluorescence spectroscopy [20], X-ray reflectometry [21] and angular-interrogated surface plasmon resonance (SPR) spectroscopy [22].

In this work, the layer-by-layer self-assembly of binary nanocomposite films and the monolayer adsorption of different precursor species were in situ studied using a broadband spectral SPR sensor. The precursor species used include cytochrome c (Cyt c), deoxy ribonucleic acid (DNA), gold nanoparticle (GNP), poly (sodium 4-styrenesulfonate) (PSS), and poly (dimethyldiallylammonium chloride) (PDDA). The PSS/Cyt c, GNP/Cyt c and PDDA/DNA binary nanocomposite films were prepared on the SPR chips by layer-by-layer self-assembly based on electrostatic interaction.

SPR is an evanescent wave sensing technique capable of in situ monitoring physical and chemical changes in the near-field region. SPR devices have been widely used for label-free biochemical detection and as a powerful tool for surface and interface analysis. The conventional angular-interrogated SPR sensors need a large-sized, highly-precise goniometer, operating at a single wavelength and having a fixed sensitivity. In contrast, broadband spectral SPR sensors allow one to readily adjust the incident angle to change its sensitivity in a wide range and to broaden the dynamic measurement range. Now sophisticated charge-coupled-device (CCD) spectrometers with a millisecond time resolution are easily available, enabling spectral SPR sensors to in situ study different reaction processes at the solid/liquid interface. Here a broadband time-resolved SPR device operating in the wavelength-interrogation mode was established. By using this apparatus, the layer-by-layer self-assembly process of multilayer films and the adsorption kinetics of precursor species were in situ studied. The novel results useful to the materials engineering were obtained.

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2. Experimental section

2.1. Reagents

Cytochrome c (Cyt c, MW = 12.3 K), deoxy ribonucleic acid sodium salt (DNA, MW = 1.3 M), poly (allylamine hydrochloride) (PAH, MW = 56 K), poly (sodium 4-styrenesulfonate) (PSS, MW = 70 K), poly (dimethyldiallylammonium chloride) (PDDA, MW = 100 K–200 K) and hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$, MW = 393.83) were purchased from Sigma-Aldrich. Sodium chloride (NaCl, MW = 58), sucrose (MW = 342.29) and phosphate buffered saline (PBS, pH = 7.0) were received from Beijing Chemical Reagent company. The above analytical-grade reagents were used without further purification. The two biosamples, 10 μM Cyt c and 10 μM DNA in PBS, were prepared. A stock solution of 0.1 M NaCl in deionized water was prepared and it was then used to make the three different solution samples of 1 mg/ml PAH, 1 mg/ml PSS and 1 mg/ml PDDA. By using hydrogen tetrachloroaurate and sucrose, an aqueous colloidal solution of gold nanoparticles (GNPs) with a 6 nm diameter was prepared according to the synthetic method described in the previous work [20]. The above solution samples, when not in use, were kept at 5 °C. The isoelectric point (pI) of Cyt c is 10 [23–25], thus Cyt c molecules in the pH = 7 PBS solution are positively charged. The PAH and PDDA in their solutions are also positively charged. The GNPs, DNA molecules and PSS in the individual solutions are negatively charged [21,22,26]. The PAH solution was used only for surface modification of SPR chips. The Cyt c, GNP, DNA, PSS and PDDA solutions were used for layer-by-layer self-assembly of different multilayer films.

2.2. SPR instrument

A number of SPR chips were prepared by successive sputtering of a 3-nm-thick chromium layer and a 50-nm-thick gold layer on the glass substrate. A Kretschmann-type SPR sensor operating in the wavelength interrogation mode was established using a tungsten-halogen lamp connected with a fiber collimator, a linear polarizer mounted in front of the collimator, a CCD spectrometer linked with another fiber collimator, a 45°/45°/90° glass prism, and a SPR chip. The back of the SPR chip was attached to the prism with a high-index liquid and its sensing layer was tightly covered with a measuring chamber [27]. Broadband light from the tungsten-halogen lamp is passed through the fiber collimator and the linear polarizer to produce a collimated *p*-polarized beam that is incident upon a side of the prism at a given angle θ with respect to the normal of the side face, leading to the attenuated total reflection (ATR) at the interface between the glass substrate and the gold layer. The ATR is accompanied by an evanescent field that penetrates through the gold layer to excite the SPR mode at the gold-layer/dielectric-medium interface at a specific wavelength at which the phase-matching condition is satisfied. This wavelength is referred to as the resonance wavelength (λ_R). The SPR-enhanced absorption of the gold layer can result in a deep ATR dip in the reflected light intensity spectrum. The minimum of the dip locates at λ_R. Therefore, λ_R and its shift induced by molecular adsorption can be easily determined by using the CCD spectrometer to record the reflected light intensity spectrum of the sensor. The CCD spectrometer used in this work has a time resolution of 1 ms, offering the SPR sensor an ability to monitor the molecular adsorption process in real time.

The unique feature of the wavelength-interrogated SPR sensors is that the larger the incident angle is, the longer the resonance wavelength and the greater the sensitivity is. However, the full width at half maximum (FWHM) of the spectral SPR band is larger at a longer wavelength. As a result, a large incident angle can lead to a poor spectral resolution of the sensor. In all the experiments performed in this work, the incident angle θ was precisely adjusted to make sure that the initial value of λ_R is between 620 nm and 720 nm.

2.3. Layer-by-layer self-assembly of PSS/Cyt c, GNPs/Cyt c and PDDA/DNA nanocomposite films on the SPR chips

The three different multilayer films consisting of PSS/Cyt c, PDDA/DNA and GNPs/Cyt c binary nanocomposites were self-assembled on the SPR chips based on electrostatic attraction. Prior to the self-assembly process, the SPR chips were immersed in the PAH solution for 15 min to immobilize positively charged PAH on the gold layer. After sufficient washing with deionized water, the PAH-modified SPR chip was closely sandwiched between the prism coupler and the measuring chamber. The single-layer adsorption of molecules and the layer-by-layer self-assembly processes were in situ investigated by SPR spectroscopy.

The multilayer film of PSS/Cyt c nanocomposite was first fabricated on the PAH-modified SPR chip by alternative injection of the PSS and Cyt c solutions into the chamber. Each solution was kept for 15 min in the chamber. Before the solutions were injected in turn, the chamber was in situ washed 5 times with deionized water to remove those molecules loosely attached to the surface. After preparing each PSS/Cyt c layer, the reflected light intensity spectrum was recorded with deionized water in the chamber. Fig. 1 shows the schematic diagram of $(PSS/Cyt\ c)_n$ multilayer films assembled on the surface of gold chip. With the use of the resonance wavelength obtained with the first PSS/Cyt c layer as the initial λ_R, dependence of changes in resonance wavelength ($\Delta\lambda_R$) on the number of PSS/Cyt c layer was obtained. In addition, the monolayer adsorption of Cyt c molecules on the PSS-modified surface from the PBS solution was investigated by successively recording the intensity spectrum with the adsorption time. The protein adsorption kinetics was attained by fitting the time course of $\Delta\lambda_R$ with Langmuir isotherm model. The $(GNPs/Cyt\ c)_n$ multilayer films were prepared in the same way, but a little difference of assembled time of GNPs is 1 h. To assemble the $(PDDA/DNA)_n$ multilayer films, we have directly used PDDA instead of PAH to obtain a positively charged gold chip.

3. Results and discussion

3.1. Adsorption kinetics of Cyt c, GNPs and DNA

Adsorption of Cyt c molecules on the PSS-modified SPR chip was monitored in real time using the broadband time-resolved SPR device. To observe the initial adsorption behavior of Cyt c molecules, the reflected light intensity spectrum was successively recorded at intervals of 1 s before injecting the protein solution into the chamber. Fig. 2a displays the intensity spectra measured during the adsorption process of Cyt c molecules on the sixth PSS layer. Each spectrum exhibits a deep ATR dip with the minimum locating at λ_R. As the adsorption time increases from t = 0 (at which the first spectrum with a deep ATR dip was recorded), the dip undergoes a fast redshift and then tends to stabilize. After determining λ_R from Fig. 2a, $\Delta\lambda_R$ as a function of the adsorption time was obtained, as shown in Fig. 2b. A stable value of $\Delta\lambda_R$ = 31.3 nm was observed 30 s after the solution injection, indicating that the time for Cyt c adsorption to reach equilibrium is 30 s. The solid line is the fitting curve. The adsorption behavior of Cyt c was found to obey Langmuir isotherm theory [28–30]. According to the Langmuir isotherm adsorption model, adsorption of molecule M from the bulk solution onto the interface can be expressed by the following reaction:

$$M + S_E \underset{k_d}{\overset{k_a}{\rightleftharpoons}} M{-}S_F \tag{1}$$

where, S_E and S_F represent the empty and filled surface active sites, respectively, k_a and k_d are the adsorption and desorption rate constants. From Eq. (1) the following kinetic equation can be derived:

$$\frac{d\Gamma}{dt} = k_a \frac{c}{55.5}(\Gamma_{max} - \Gamma) - k_d\Gamma \tag{2}$$