Electrostatic Layer-by-Layer Assembly of Polycation and DNA Multilayer Films by Real-time Surface Plasmon Resonance Technique

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The assembly of alternating DNA and positively charged poly(dimethyldiallylammonium chloride) (PDDA) multilayer films by electrostatic layer-by-layer adsorption has been studied. Real time surface plasmon resonance (BIAcore) technique was used to characterize and monitor the formation of multilayer films in solution in real time continuously. The results indicate that the uniform multilayer can be obtained on the poly(ethylenimine) (PEI) coated substrate surface. The kinetics of the adsorption of DNA on PDDA surface was also studied by real-time BIAcore technique, and the observed rate constant was calculated using a Langmuir model ($k_{obs} = (1.28 \pm 0.08) \times 10^{-2}\ s^{-1}$).

Keywords electrostatic layer-by-layer adsorption, assembly, multilayer films, DNA, surface plasmon resonance, real-time

Introduction

The construction of functional and organized ultrathin multilayer biomolecule-containing films in predetermined ways is an important area of research in biochemistry and biotechnology. This kind of films will be the potential biomaterials for the fabrication of biomolecule devices.[1,2] The alternate layer-by-layer electrostatic deposition of oppositely charged polyelectrolytes or biomolecules has been developed for ultrathin film assembly.[3] Recently, this layer-by-layer electrostatic adsorption method has been applied to DNA assembly.[4,5] It is of crucial importance to control the adsorption and assembly of DNA to oppositely charged polymers, since cationic polymers have been tested for possible application as genetic support materials in gene transfection.[6,7] Conventionally, the multilayer film was characterized by small-angle X-ray reflectivity, UV-vis spectroscopy, FT-IR, ellipsometry, AFM, SEM *etc*. However, these methods could only provide single information on the film growth, and they could not monitor the formation of multilayer in solution in real-time. Real-time BIAcore technique, based on surface plasmon resonance (SPR) principle, has developed as a very versatile method for the study of biomolecular interaction at surfaces.[8] BIAcore uses continuous flow technology to monitor interactions in real time. This allows direct observation of the whole procedure of an investigation. It may be a useful tool to investigate the formation of layer-by-layer assembly of multilayer films. In this article, we use real-time BIAcore technique to follow the whole procedure of the mutilayer formation on Au surface in solution continuously.

Experimental

Materials

Sodium salt of DNA from Calf Thymus (Sigma), poly(ethylenimine) (PEI) (average $MW = 6 \times 10^4$, 50 wt%, Sigma), poly(dimethyldiallylammonium chloride)

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Received November 17, 2000; revised and accepted January 12, 2001.
Project supported by the National Natural Science Foundation of China (Nos. 29835120 & 20075027).

(PDDA, Aldrich Co.) were used without further purification. All of the other chemicals were of reagent grade and used as received.

Surface plasmon resonance (SPR) *measurements*

The BIAcore 1000 system and commercially available pioneer sensor chip C1 (a flat COOH layer on the surface) obtained from Pharmacia Biosensor AB (Uppsala, Sweden) were used to monitor the whole procedure of multilayer assembly in real time. A flow rate of 5 μL/min was used throughout the assembly procedure. The whole procedure is described as follows. The surface of sensor chip C1 was first equilibrated with PBS buffer (10 mmol/L, pH 7.0) for about 20 minutes to obtain a stable baseline. Then 10 μL of PEI (1 mg/mL aqueous solution) was injected. After a few minutes when the baseline is stable, 10 μL of 0.2 mg/mL aqueous DNA solution (pH 7.0, 10 mmol/L PBS buffer) was injected, then 5 μL of 1mg/mL PDDA aqueous solution was injected. This DNA-PDDA procedure was repeated for several times.

Results and discussion

SPR measures the intensity of reflection of monochromatic p-polarized light incident on the backside of a gold-coated glass slide. Changes in the angle θ_{spr} at which the intensity of reflected light reaches a minimum value correlate linearly with the mass per unit area of protein adsorbed.[9] Commercially available sensor chip C1 has a flat carboxyl surface, which is a negatively charged surface. We chose PEI to adsorb on C1 surface by electrostatic adsorption for PEI is positively charged polyion and can be adsorbed strongly with the negatively charged protein or to other surface. This process made a change of the surface charge from negative to positive. It also assured the sufficient charge on the surface for strong electrostatic adsorption, compared to a flat carboxyl group. The PEI coated surface was used for further study of assembly multilayer. 10 μL of 0.2 mg/mL aqueous DNA solution (pH 7.0, 10 mmol/L PBS buffer) was injected. Then 5 μL of 1 mg/mL PDDA aqueous solution was injected. This DNA-PDDA procedure was repeated for several times just as described at experimental section.

The monitoring of SPR response as a function of time could be used for direct visual observation of binding and dissociation events during the procedure of reaction. Real-time analysis is an ideal way to obtain kinetic data. In addition, following the interactions often gives the valuable dynamic information. It may be a useful tool to investigate the formation of layer-by-layer assembly of multilayer films. In this study, we used real-time BIA technique to monitor the whole process of multilayer films. The sensorgram of layer-by-layer adsorption of DNA-PDDA mutilayer was shown in Fig. 1. After the C1 chip surface was coated with PEI and the baseline was stable, DNA and PDDA solutions were injected across the surface as described in experimental section. The results showed that each injection of DNA and PDDA caused the SPR response (RU) increasing for a certain value. For DNA, the response increased about 2,963 ± 467 RU (0.2963°) for each injection. For PDDA, the response increased about 1,914 ± 255 RU (0.1914°). By plotting the response of the total DNA or PDDA on surface after each DNA or PDDA injection against the number of layers, a good linear relationship can be obtained, as shown in the Fig. 2. As the increase of SPR response means the increase of mass of DNA and PDDA on the sensor chip surface, we can conclude that the uniform multilayer of DNA-PDDA has been constructed on the PEI coated surface.

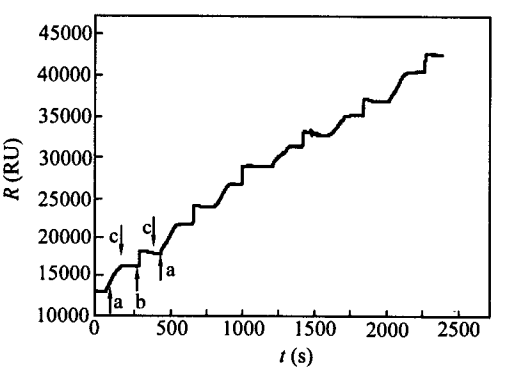

Fig. 1 Real-time sensorgram of alternating assembly of DNA and PDDA. A flow rate of 5 μL/min was used during the whole procedure. (a), 0.2 mg/mL DNA 10 μL, (b), 1 mg/mL PDDA 5 μL. At the end of each injection, HBS buffer (c) was flowed over the chip surface.

Kinetics of DNA adsorption was also studied. The kinetic sensorgram (Fig.3) indicates that the adsorption of DNA on PDDA can be modeled using a Langmuir kinetic equation:[10]

$$R_t = (R_{max} - R_0)[1 - \exp(-k_{obs}t)] + R_0 \quad (1)$$

where R_t represents response units (RU) at time t, R_{max} represents maximum RU, R_0 is response units before injection of DNA, and k_{obs} is the observed "rate constant".

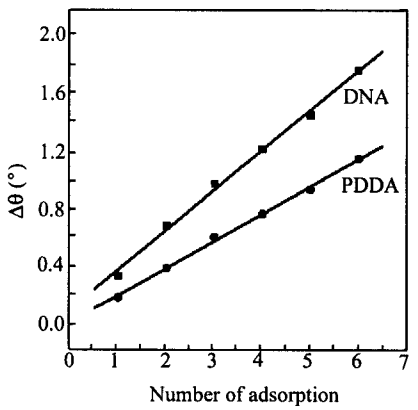

Fig. 2 Plots of the changes of SPR angle ($\Delta\theta$) against number of layers.

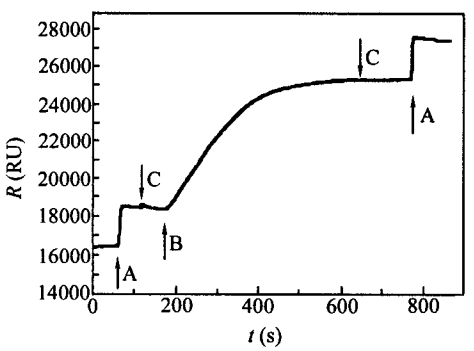

Fig. 3 Real-time sensorgram of adsorption of DNA on PDDA surface. Flow rate: 5 μL/min. (A), 1 mg/mL PDDA 5 μL, (B), 0.2 mg/mL DNA 40 μL. At the end of each injection, HBS buffer (C) was flowed over the chip surface. The section of B-C was used to fit by Eq. (1) to obtain the adsorption rate constants.

By fitting Eq. (1) to the kinetic sensorgrams (B-C in Fig. 3), we can calculate the observed rate constants for the adsorption of DNA on PDDA surface. For eight sensorgrams, $k_{obs} = (1.28 \pm 0.08) \times 10^{-2}\ s^{-1}$ ($n = 8$). For the adsorption of PDDA on DNA surface, the sensorgram indicates that the adsorption rate is very fast. Since PDDA is a linear polymer, the space hindrance is low in the procedure of PDDA adsorption on DNA surface.

In conclusion, the assembly of alternating PDDA and DNA multilayer films by electrostatic layer-by-layer adsorption has been studied in this article. Real-time surface plasmon resonance (SPR, BIAcore) technique was used to monitor layer-by-layer assembly of multilayer films in real time in solution continuously. The results show that the uniform DNA/PDDA multilayer can be fabricated on PEI coated surface. The kinetics of DNA adsorption on PDDA layer was also studied by BIAcore instrument and fitted by a Langmuir model. The alternating layer-by-layer electrostatic adsorption of DNA and polyion multilayers will be used for the further study about the interaction between DNA containing ultrathin films with dyes, drugs and peptides.

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(E200011246 JIANG, X.H.; LING, J.)