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Detection of magnetic-labeled antibody specific recognition events by combined atomic force and magnetic force microscopy

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ABSTRACT

Atomic force (AFM) and magnetic force microscopy (MFM) were developed to detect biomolecular specific interaction. Goat anti-mouse immunoglobulin (anti-IgG) was covalently attached onto gold substrate modified by a self-assembly monolayer of thioctic acid via 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) activation. Magnetic-labeled IgG then specifically adsorbed onto anti-IgG surface. The morphological variation was identified by AFM. MFM was proved to be a fine assistant tool to distinguish the immunorecognized nanocomposites from the impurities by detection of the magnetic signal from magnetic-labeled IgG. It would enhance the understanding of biomolecular recognition process.

1. Introduction

The high specificity of biomolecular interactions, such as antibody/antigen, avidin/biotin interactions, is essential for the immune system to provide the organism with the ability to recognize, respond to, and in most cases successfully defend against a wide variety of infection agents [1]. Given the potential chemical, biological and clinic importance of this high affinity selective observation, significant efforts have been made to investigate the behaviors of the molecular recognition [2].

Atomic force microscopy (AFM) has taken a powerful technique to detect biomolecular recognition, since it can yield nanometer-scale topographical images of biological molecules and probe local surface properties, such as adhesion and elasticity, through either normal or lateral force interactions [3]. A series of successful experiments have been carried out using AFM to visualize the topography of antibody [4] or antibody/antigen complexes [5], and to monitor individual antibody/antigen recognition events [6], biotin/avidin discrete interactions [7] or DNA hybridization [8]. However, several problems hinder the development of AFM into a more widely adopted tool in bioscience and biotechnology. AFM detection is based on height differences, variation in surface roughness or volume distribution. It is liable to be interfered due to the high roughness of the substrate surface, aggregation, or adsorption of impurities, and results in the indistinguishable artificial features. Thus, the confirmation of artifacts becomes more important.

Magnetic force microscopy (MFM) is an extension of non-contact AFM, aimed at imaging the micromagnetic structure of the magnetic materials. It allows imaging magnetization patterns with spatial resolution of less than 10 nm, and the resolution might yet be improved using advanced tip technology [9]. Magnetic contrast is achieved through the magnetostatic interaction between a ferromagnetic tip and the stray micromagnetic fields from the sample. In general, the force acting on the tip can be obtained by integrating the tip–sample force gradient

$$dF/dz = dF_m/dz + dF_v/dz$$

where F_m is the magnetic force between the atoms on the cantilever tip and the sample surface; F_v is the van der Waals force; z is the tip-to-sample spacing. When the tip lifted from the surface by a certain distance, the magnetic force between the tip and the sample is stronger and has a longer range than the attractive van der Waals force. It is possible to image only the magnetic domains on sample, where van der Waals forces are negligible [10]. The possibility of the nonmagnetic artifacts from the topographic change can be excluded in MFM image.

In this paper, the superparamagnetic magnetite/dextran/IgG nanocomposites were used as immunolabels to specifically recognize goat anti-mouse immunoglobulin (anti-IgG), which was covalently attached onto gold substrate. Such magnetic immunolabels have recently attracted broad scientific attention, both fundamental and applied, since they possess high magnetic

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susceptibility, low remanence, low coercivity and high saturation magnetization, and have been widely used in bioseparation [11], targeted drug delivery [12], immunoassays [13] and biosensors [14]. The recognition processes were detected by AFM, MFM and infrared reflection spectra. Moreover, MFM was operated to distinguish impurities from the immunorecognized nanocomposites, which would be helpful for bioassay and biosensor.

2. Experimental section

2.1. Materials

Dextran T40 were obtained from Amersham Pharmacia Biotech. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O > 99\%$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O > 99\%$), thioctic acid and 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) were purchased from Aldrich. IgG (5 mg/ml) and anti-IgG (1 mg/ml) were obtained from the Kunming Monoclonal Antibody Technology Center (China). Anti-IgG was diluted with 20 mM sodium borate buffer (pH 8.5). Milli-Q water with a resistivity of 18.4 $M\Omega\,cm^{-1}$ was used as the solvent throughout the experiment.

2.2. Synthesis of magnetic-labeled antibody

Magnetic nanocomposites were prepared according to our previous work [15]. Briefly, 4 M ammonia water was added into a solution containing dextran (5 g), $FeCl_3 \cdot 6H_2O$ (0.45 g) and $FeCl_2 \cdot 4H_2O$ (0.55 g) with vigorous stirring under a N_2 atmosphere at 60 °C. After 15 min of reaction, the resultant precipitates were separated by a permanent magnet, washed with 0.1 M sodium acetate (pH = 6.5) three times and redispersed by sonication. Then, a 5 mM sodium metaperiodate ($NaIO_4$) solution was added dropwise under a N_2 atmosphere. After vigorous stirring in the dark for 5 h at 10 °C, the magnetic nanocomposites were purified by three magnetic separation/redispersion cycles. For the preparation of magnetic-labeled antibody, IgG (6 μl) was added to 1 ml of magnetic nanocomposite suspension and allowed to bind for 24 h at 4 °C. The residual aldehyde group after IgG attachment was blocked with 10% BSA for 24 h at 4 °C. Magnetic-labeled IgG was purified by three repeated centrifugation (12,000 rpm)/wash/redispersion cycles at 4 °C.

2.3. Preparation of capture anti-antibody substrate

The clean silicon single crystals, which have been cut into $1 \times 1\,cm^2$ sections, were covered with gold by resistive evaporation at a pressure of $< 1 \times 10^{-4}$ Pa. The gold substrate was immersed in a 1 mM ethanolic solution of thioctic acid for 24 h to form a carboxylic acid-terminated monolayer, and was then immersed to 1% (w/w) EDC in anhydrous acetonitrile for 5 h. After three times washes by anhydrous acetonitrile, a 100 μl of anti-IgG dilution was dropped onto the substrate. The reaction was allowed to progress at 4 °C for 24 h. The washing procedure described above was repeated. Then the substrate was incubated for 24 h at 4 °C in a blocking buffer of 10% BSA and soaked three times in sodium borate buffer. Finally, magnetic-labeled antibody (100 μl) was dropped onto anti-IgG-coated substrate for immobilization. After 24 h, the reaction was complete and the substrate was then rinsed with water.

2.4. AFM and MFM imaging

All data presented in this paper were obtained with a Park Scientific Model CP AFM with a combined AFM/MFM head and were performed in laboratory air at a temperature of 23–25 °C. Images were collected using noncontact mode AFM.

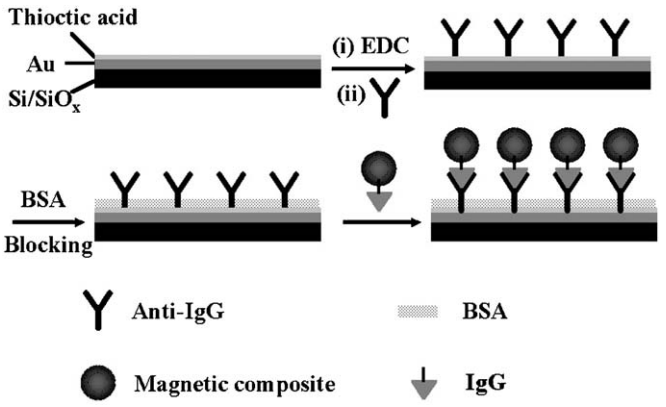
2.5. Infrared measurements

Infrared spectra were taken by reflection of the incident beam at an angle of incidence of 87 °C (3 °C off glancing) using *p*-polaried radiation. A nitrogen-purged Digilab 15-B Fourier transform spectrometer was used in conjunction with modified optics, which permit focusing of the beam outside the instrument, and equipped with stops and apertures to give an ~*f*/15 beam focusing to a ~3 mm spot. A liquid-nitrogen-cooled mercury–cadmium–telluride (MCT) detector, operated in the photovoltaic mode, was used.

3. Results and discussion

The stepwise assembly of magnetic-labeled antibody was described in Scheme 1. To increase the affinity of anti-IgG to surface, thioctic acid was chosen to adsorb on gold-coated Si/SiO_x surface because of its typical self-assembled technique and its feasibility for protein (e.g., anti-IgG) immobilization on a solid support via EDC activating the free carboxyl groups of thioctic acid to form *O*-acylurea intermediate [16]. Higher activating efficiency could be obtained by using acetonitrile as a solvent for EDC due to its potential to prevent the solvolysis of the *O*-acylurea intermediate [17]. After anti-IgG was covalently coupled to the substrate, the remaining bonding sites were blocked with BSA. The blocking step was important to prevent nonspecific absorption of magnetic-labeled IgG onto the substrate. Following this step, the substrate was soaked in magnetic-labeled IgG solution. Once magnetic-labeled IgG molecules recognized any of the anti-IgG on the surface, they would be immobilized tightly. During the repetitious washing procedures, any nonspecifically adsorbed magnetic-labeled IgG could be removed.

The recognition processes were proved by AFM studies, which can provide information concerning the appearance, the homogeneity and the structure of the adsorbed material at nanometer scales. Fig. 1a shows a typical AFM topographic image of the gold deposited on Si/SiO_x substrates. The gold particles are uniform. Their mean diameter is about 38 nm. Here, the gold film was not annealed at high temperature to decrease the surface roughness of the evaporated layer, since we wanted to prove that the surface roughness would not interfere with the final result of specific binding. When anti-IgG was immobilized on activated thioctic acid surface, the



Scheme 1. Diagram of anti-antibody immobilization and the specific recognition between antibody and anti-antibody.