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Analysis of Antigen-antibody Interactions Using Combination of Protein Nanoarray and Atomic Force Measurement

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Abstract

Protein nanoarrays containing anti-angiogenin antibody IgG were fabricated on ProLinker™-coated Au surface by dip-pen nanolithography (DPN). An atomic force microscope (AFM) tip coated with ProLinker™ was modified by angiogenin. We measured the interaction force between nanoarrayed anti-angiogenin antibody IgG and immobilized angiogenin on the cantilever tip by employing tethering-unbinding method. The unbinding force between anti-angiogenin antibody IgG and angiogenin was 1029±63 pN. These results demonstrate that combination of protein nanoarray by DPN and detection using direct force measurement can be applied to analyze protein-protein interactions in nanoscale.

Keywords: Protein nanoarray, Dip-pen nanolithography, Interaction force, Protein-protein interaction, Angiogenin, Anti-angiogenin antibody IgG

Introduction

Protein-protein interactions are critical for many biological functions. The ability to probe protein-protein interactions in a high-throughput manner is recognized to be important in developing effective diagnostic techniques, cultivating disease therapies and discovering new drug candidates[1,2]. Protein-immobilized microarrays are especially regarded as a useful platform for biological applications such as the development of methods for high-throughput screening[3-5]. Conventional methods for detecting biomolecular interactions need the additional step of tagging biomolecules with radioisotopes, fluorescence dyes, or chromic dyes. Detection of protein-protein interactions in protein microarrays are commonly carried out employing fluorescence labeling[6].

Dip-pen nanolithography (DPN) have been developed as patterning techniques for ultraminiaturized biomolecular arrays. DPN uses atomic force microscope (AFM) without the need for any additional tagging. DPN also allows fabrication of high-density arrays in nanometer scale[7,8]. In protein nanoarrays patterned by DPN, interactions between probe proteins immobilized on the surface and target proteins in solution can be detected by measuring *the change in the height of each protein spot* after incubation and dry processing[9]. The molecular interaction between integrin $\alpha_v\beta_3$ and its ligand, vitronectin patterned on ProLinker™-coated Au surface by DPN, has been analyzed by detecting the height change scanned in non-contact mode[10]. In spite of the fact that DPN is a new technology to directly detect protein-protein interactions, it took a relatively long time to scan the topological images of a given surface area by AFM and thus it may difficult to analyze protein-protein interactions in high-throughput fashion.

Combination of the protein nanoarray and the use of force spectroscopy would give a new method for measuring protein-protein interactions in a high-throughput manner. In a previous study, we measured the interaction force between nanoarrayed integrin $\alpha_v\beta_3$ and immobilized vitronectin on the cantilever tip by employing tethering-unbinding method[11].

In this study, we report analysis of antigen-antibody interactions using combination of the protein nanoarray by DPN and detection using direct force measurement. Angiogenin and its antibody are used as a model antigen and antibody, respectively, to analyze protein-protein interaction. Angiogenin is a 14-kDa protein implicated in angiogenesis and in tumor growth[12,13].

Results and Discussion

Anti-angiogenin Antibody IgG Nanoarray on ProLinker™-coated Au Surface

We constructed 4×4 anti-angiogenin antibody IgG protein nanoarray on ProLinker™-coated Au surface by dip-pen nanolithography (DPN). The topological AFM images and line profiles of anti-angiogenin antibody IgG was shown in Figure 1. Anti-angiogenin antibody IgG nanospots showed 11-20 nm height values for all the nanospots (Figure 1).

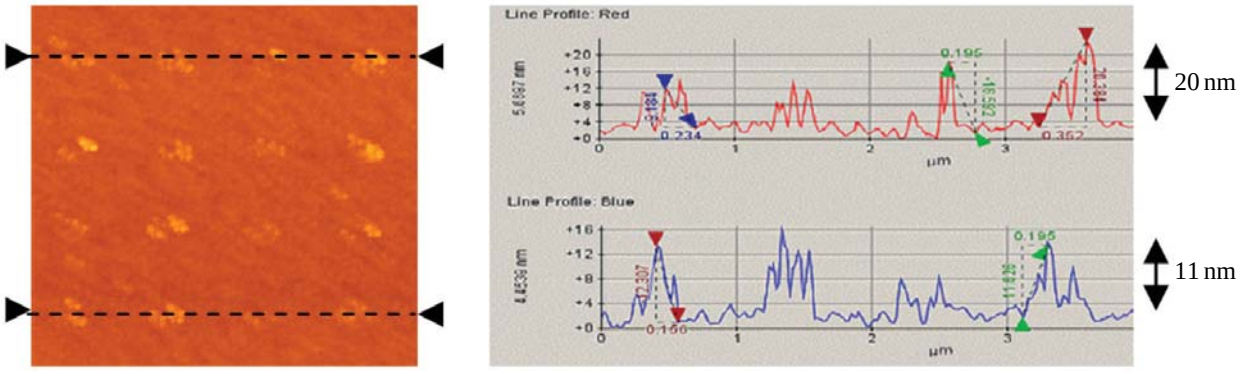

Figure 1. The topological AFM images and line profiles of anti-angiogenin antibody IgG nanoarrays spotted on ProLinker™-coated Au surface.

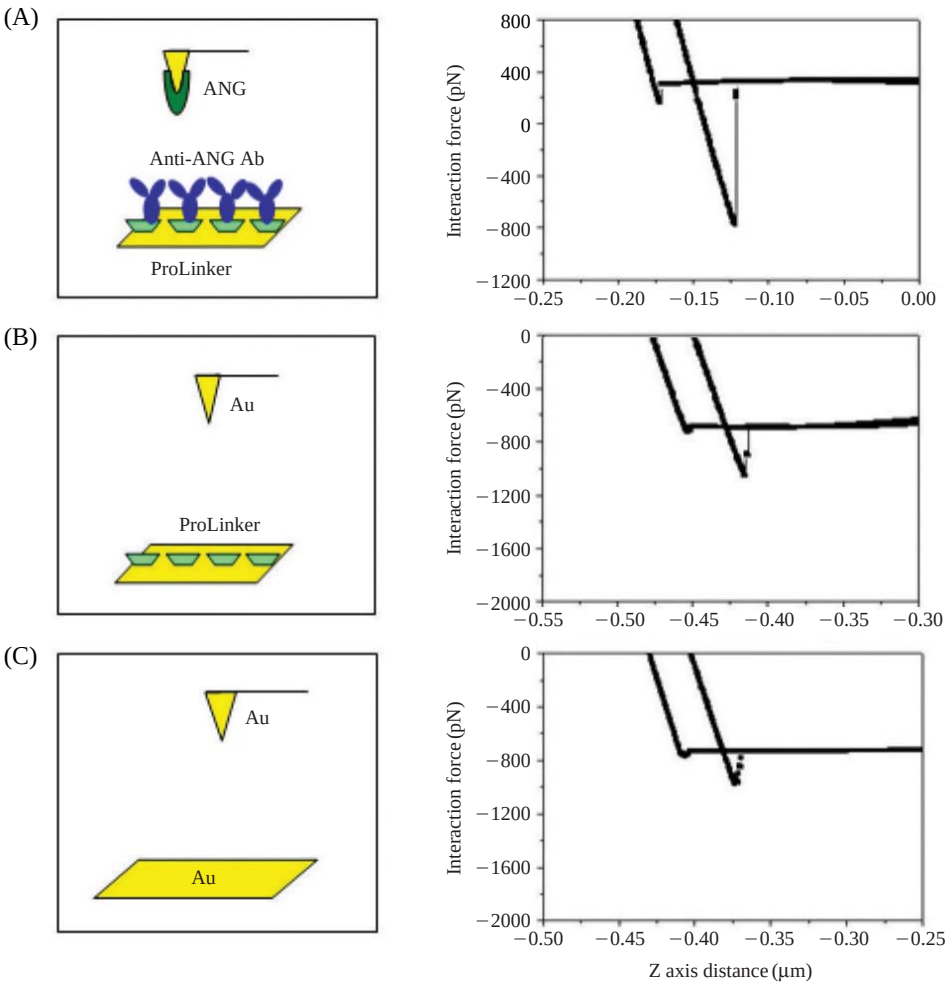

Figure 2. Schematic diagram of molecular interaction and typical interaction force curves for angiogenin/anti-angiogenin antibody IgG (A), Au-coated cantilever (Bare Tip)/ ProLinker™ SAM (B), and Au-coated cantilever (Bare Tip)/Au (C).

Analysis of Interaction between Nanoarrayed Anti-angiogenin Antibody IgG and Immobilized Angiogenin on the Cantilever Tip

In order to detect interaction force between two proteins directly, AFM cantilever tips were modified with the interacting protein corresponding to the capture protein nanoarrayed on ProLinker™-coated Au surface. Sixteen spot (4×4) images were obtained to

measure unbinding force between *capture protein* angiogenin antibody IgG nanoarrayed on ProLinker™-coated Au surface and *interacting protein* angiogenin on cantilever tip. We measured the deflection of the AFM cantilever modified with angiogenin as the fixed end of the cantilever is brought vertically towards and then away from the one of sixteen anti-angiogenin antibody IgG protein nano spots on ProLinker™-coated Au surface. Typical interaction force-versus-distance curves for angiogenin/anti-angiogenin antibody IgG are shown in Figure 2A. In addition, typical interaction force-versus-distance curves for Au-coated cantilever (Bare Tip)/ProLinker™ self-assembled monolayer (SAM) and for Au-coated cantilever (Bare Tip)/Au surface are shown in Figures 2B and 2C, respectively, as negative controls.

Table 1 shows the measured numerical values of interaction force during the detaching event. The unbinding force between angiogenin and anti-angiogenin antibody IgG was calculated to be 1029 ± 63 pN. However, Au-coated cantilever (Bare Tip)/ProLinker™ self-assembled monolayer (SAM) and Au-coated cantilever (Bare Tip)/Au surface interactions that were used as controls showed a relatively low interaction force 415 ± 44 pN and 258 ± 59 pN, respectively. These results demonstrate that the molecular interaction between anti-angiogenin antibody IgG and angiogenin is a strong and specific binding event.

Table 1. Measured values for interaction forces.

	Force (pN) Mean ± SD
Angiogenin/Anti-angiogenin antibody IgG	1028.5 ± 63.4
Au-coated cantilever (Bare Tip)/ProLinker™ SAM	415.4 ± 43.6
Au-coated cantilever (Bare Tip)/Au	258.0 ± 58.9

Note. Data were combined from multiple sets of experiments each using 16 spots of the nanoarrayed anti-angiogenin antibody IgG.

The cantilever surface was modified with ProLinker™ which can serve as a base for the formation of interacting protein monolayer (Figure 3). It is reported that ProLinker™ surface could capture the protein molecules strongly and, serve as a powerful linker system for the immobilization of protein molecules[14]. In Figure 3, a schematic diagram of tethering-unbinding method was illustrated.

Conclusions

In this study, we constructed 4 × 4 anti-angiogenin antibody IgG protein nanoarray on ProLinker™-coated Au surface, and angiogenin was immobilized with ProLinker™-coated AFM cantilever tips. We then measured the interaction forces between sixteen spots of *the nanoarrayed* anti-angiogenin antibody IgG and angiogenin on the cantilever tips, respectively. These results are important since combination of the protein nanoarray and the use of force spectroscopy would give a new method for measuring protein-protein interactions in a high-throughput manner. In summary, we demonstrated that combination of protein nanoarray by DPN and detection using direct force measurement can be applied to analyze protein-protein interactions in nanoscale.

Materials and Methods

Chemicals and Reagents

Bovine serum albumin (BSA), used as source mate-

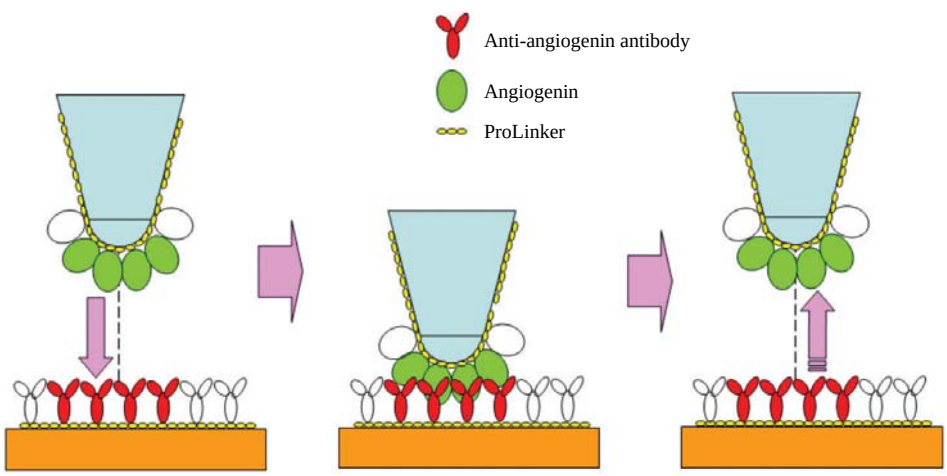

Figure 3. A schematic diagram of tethering-unbinding method. The interaction force between nanoarrayed anti-angiogenin antibody IgG and immobilized angiogenin on the cantilever tip was measured by employing tethering-unbinding method.

rials of protein nanoarray, was purchased from Chemicon (CA, Temecula, USA). Angiogenin (ANG) was purified from cow's milk as previously described[15]. Anti-angiogenin antibody IgG was prepared as previously described[16]. Solutions of BSA and anti-angiogenin antibody IgG (500 μg/mL) were prepared in PBS buffer. Mercapto-undecanoic acid (MUDA), PBS buffer, and other reagents were obtained from Sigma Chemicals (St. Louis, MO). Milli-Q grade ($>$ 18.2 mΩ/cm) water was used for the preparation of sample and buffer solutions. ProLinker™ which is one of calix[4] arene derivatives with crown-ether moiety, was purchased from Proteogen Inc. (Seoul, Korea) and used as a linker system for the immobilization of protein.

Fabrication of Protein Nanoarray on ProLinker™-coated Au Surface

Gold-coated AFM cantilever was used to deliver protein molecules to the surface of gold-coated silicon wafer. To increase the hydrophilicity of cantilever surface, gold-coated cantilever tip was immersed into 1 mM mercapto-undecanoic acid (MUDA) in ethanol for 30 min and then dried under the N_2 gas stream at room temperature. After modification of gold-coated silicon cantilever with MUDA, the cantilever was immersed in anti-angiogenin antibody IgG solutions (100 μg/mL) containing 30% (w/v) glycerol or 20% (w/v) PEG and incubated for 1 hr to adsorb proteins on the cantilever surface for subsequent delivery onto the surface of gold-coated silicon wafer for preparing protein nanoarray.

A silicon wafer with 50 nm Au deposition layer was used as a substrate for protein nanoarray. To make linker layer for stable immobilization of protein, the gold-coated silicon wafer was immersed in the 1 mM ProLinker™ solution in chloroform for 1 hr rinsed with acetone followed by methanol, and then dried under N_2 gas at room temperature. Nanoarray patterning of proteins adsorbed on cantilever tip in contact mode was carried out and then protein chip was incubated for 3 hr at room temperature at 80% humidity to allow stable interaction between ProLinker™ and protein.

After the incubation, topographic images and height profiles of anti-angiogenin antibody IgG nanoarrays were obtained in non-contact mode. All processes for the fabrication of protein nanoarrays were performed with the AFM XE-100 (Park System Corp., Suwon, Korea) in contact and non-contact mode.

Modification of ProLinker™-coated AFM Cantilever Tip with Proteins

Gold-coated AFM cantilever (NSC 14/Cr-Au; Park System Corp., Suwon, Korea) surface was treated with 1 mM ProLinker™ solution in chloroform for 1 hr. After the formation of ProLinker™ self-assembled monolayer (SAM) on cantilever surface, the cantilever was immersed in angiogenin solution (100 μg/mL) to form protein monolayer. Physically absorbed excess protein was removed by rigorously rinsing with PBS buffer.

Interaction Force Measurements

The protein-modified cantilever was used to measure interaction force in non-contact mode. Cantilever tip immobilized with angiogenin was approached to anti-angiogenin antibody IgG protein nanorray spot on ProLinker™-coated Au surface with the approaching speed of 0.0001 μm/min and the spring constant of 0.015 N/m. After the cantilever tip immobilized with angiogenin was moved apart from the anti-angiogenin antibody IgG protein nanorray spot on ProLinker™-coated Au surface, the unbinding force between the proteins was measured. Au-coated cantilever (Bare Tip) was approached to ProLinker™ self-assembled monolayer (SAM) on the Au-coated Si wafer or Au-coated Si wafer as negative controls.

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