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Measurement of interaction force between nanoarrayed integrin $\alpha_v\beta_3$ and immobilized vitronectin on the cantilever tip

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Abstract

Protein nanoarrays containing integrin $\alpha_v\beta_3$ or BSA were fabricated on ProLinker™-coated Au surface by dip-pen nanolithography (DPN). An atomic force microscope (AFM) tip coated with ProLinker™ was modified by vitronectin. We measured the interaction force between nanoarrayed integrin $\alpha_v\beta_3$ or BSA and immobilized vitronectin on the cantilever tip by employing tethering–unbinding method. The unbinding force between integrin $\alpha_v\beta_3$ and vitronectin (1087 ± 62 pN) was much higher than that of between BSA and vitronectin (643 ± 74 pN). These results demonstrate that one can distinguish a specific protein interaction from non-specific interactions by means of force measurement on the molecular interactions between the nanoarrayed protein and its interacting protein on the AFM tip.
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Keywords: Protein nanoarray; Dip-pen nanolithography; Interaction force; Protein–protein interaction; Integrin $\alpha_v\beta_3$

Arrays of biomolecules immobilized on solid surfaces can serve as a new tool for miniaturized bioanalysis [1–6]. Protein or DNA immobilized microarrays are especially regarded as a useful platform for biological applications such as the development of methods for high-throughput screening, and fabrication of highly packed diagnostic chip [7–13]. 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 [14].

Recently, new techniques including micro-contact printing (MCP) and 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 [15,16]. In recent years, this new technique has been used for fabricating ultraminiaturized biomolecular arrays including DNA, proteins and small organic molecules. In addition, it has contributed to our understanding of molecular interactions in various high-affinity receptor–ligand systems on cell membranes or solid substrates [17–19]. 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 [20]. In this method, one has to

Abbreviations: AFM, atomic force microscope; BSA, bovine serum albumin; DPN, dip-pen nanolithography; PBS, Dulbecco's calcium- and magnesium-free phosphate-buffered saline, pH 7.4; SAM, self-assembled monolayer.

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scan the images of protein nanoarrays several times to get the height information of each spot.

In a previous study, we analysed the molecular interaction between integrin $\alpha_v\beta_3$ and its ligand, vitronectin patterned on ProLinker™-coated Si wafer by DPN, by detecting the height change scanned in non-contact mode [21]. 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.

We report here a new method for probing protein–protein interactions in nanoscale by measuring the interaction force between a nanoarrayed protein and a target protein immobilized on the surface of AFM cantilever.

Materials and methods

Chemicals and reagents. Bovine serum albumin (BSA) and integrin $\alpha_v\beta_3$, used as source materials of protein nanoarray, were purchased from Chemicon (CA, Temecula, USA). Solutions of BSA and integrin $\alpha_v\beta_3$ (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 Si wafer. Gold-coated AFM cantilever (NSC 14/Cr–Au; PSIA, Sungnam, Korea) 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 integrin $\alpha_v\beta_3$ or BSA solutions (100 μg/ml) containing 30% (w/v) glycerol or 20% (w/v) PEG and incubated for 1 h 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 h. 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 h at room temperature at 80% humidity to allow stable interaction between ProLinker™ and protein.

After the incubation, topographic images and height profiles of integrin $\alpha_v\beta_3$ and BSA nanoarrays were obtained in non-contact mode. All processes for the fabrication of protein nanoarrays were performed with the AFM XE-100 (PSIA, Sungnam, Korea) in contact and non-contact mode.

Modification of ProLinker™-coated AFM cantilever tip with proteins. Gold-coated cantilever surface was treated with 1 mM ProLinker™ solution in chloroform for 1 h. After the formation of ProLinker™ self-assembled monolayer (SAM) on cantilever surface, the cantilever was immersed in vitronectin solution (100 μg/ml) to form protein monolayer.

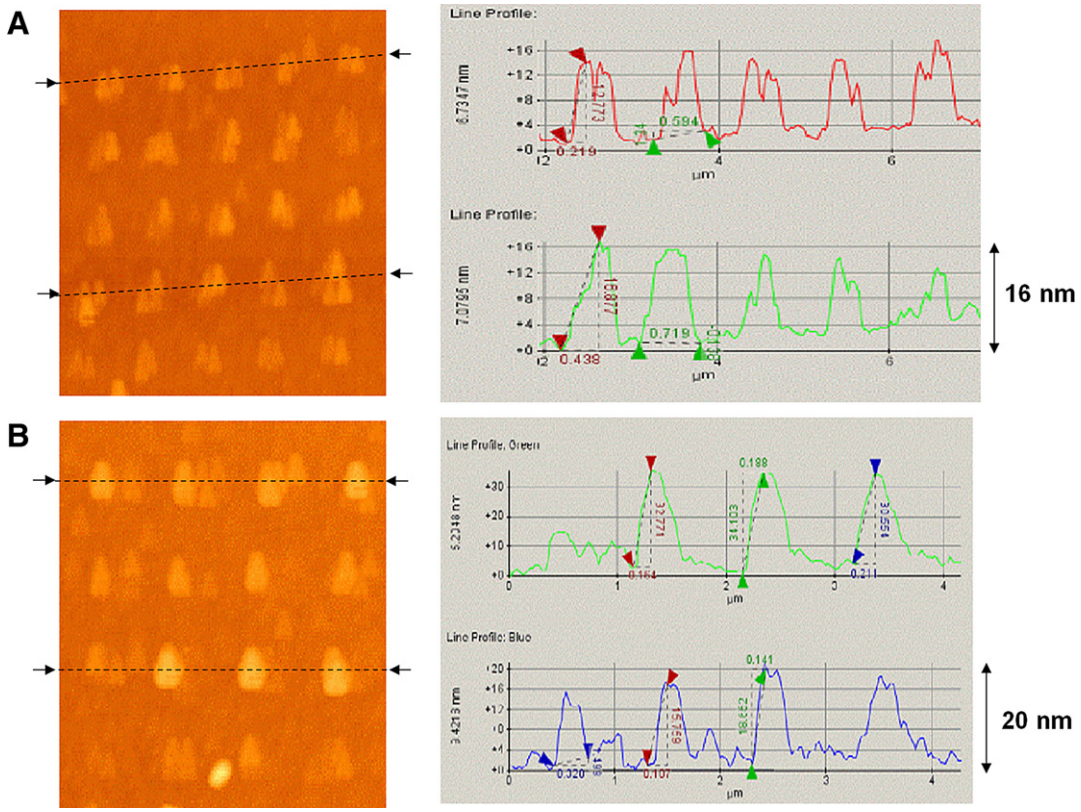

Fig. 1. The topological AFM images and line profiles of integrin $\alpha_v\beta_3$ (A) and BSA (B) nanoarrays spotted on ProLinker™ surface. Nanoarray patterning of proteins adsorbed on cantilever tip in contact mode was carried out and then protein chip was incubated for 3 h at room temperature at 80% humidity to allow stable interaction between ProLinker™ and protein. After the incubation, topographic images and height profiles of integrin $\alpha_v\beta_3$ and BSA nanoarrays were obtained in non-contact mode.

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