Enzymatic lithography of biomolecules using AFM

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Recently, applications of atomic force microscopy (AFM) have become widespread in a variety of areas, biotechnology in particular. AFM allows the observation biomolecules on a substrate and investigating the interactions between ligands-receptors or antigens-antibodies. Moreover, attention is currently focused on AFM as a tool which can be used for manipulating biomelecules. AFM has become a useful tool for aligning or modifying biomolecules in a very dense state. In this study, we demonstrated that *Staphylococcal* serine V8 protease, when immobilized to an AFM tip, is able to digest a substrate peptide on a mica surface and that lithographing is possible by scanning the surface with an enzyme-immobilized AFM tip (1).

Staphylococcal serine V8 protease recognizes either glutamic or aspartic acid residues in the peptide and digests the peptides carboxyl acid terminus. The enzyme was covalently immobilized to the tip via amid bonds using N-(6-maleimidocaproyloxy)succinimide (EMCS), a hetero-bifunctional reagent, after the tip was silanized using 3-mercaptoproplytrimethoxysilane. In a similar way, substrate peptide, $A(AEAAKA)_6C$, was covalently immobilized via EMCS to a mica-surface silanized using 3-aminoproplytrimethoxysilane.

First, we investigated activity of the protease immobilized on AFM tip by force curve measurement in 50 mM of phosphate buffer at pH 7.8. During the retracting process, a large force of over 2 nN, expected to rupture enzyme-substrate complex, was sometimes applied to the cantilever in the case of substrate peptide.

Next, to investigate whether the enzyme was able to digest the peptide after forming the intermediate, we tried to lithograph the peptide layer by contact scanning with the enzyme-immobilized tip. The lithograph of the N-terminus-biotinylated substrate peptide layer was carried out by scanning over the surface of the peptide layer to form a square at a scanning rate of 0.05 µm/sec. After lithographing, 1 mg/ml of streptavidin was injected into the AFM cell and was incubated for 20 minutes. Consequently, the lithographed area was clearly observed as a dark area shown in Fig.2 (A). Moreover, a cross section of the sample is shown in Fig. 2 (B) and the height of the dark area was found to be about 4 nm lower than the non-lithographed area. The height corresponds to the theoretical size of the streptavidin molecule.

As a result, we have demonstrated that digestion of a peptide layer on a substrate surface was possible using an enzyme immobilized to an AFM tip in a buffered solution.

Now, we are trying to detect a lithographed area by Fluorescence resonance energy transfer (FRET) using fluorescent labeled peptides.

(1) S. Takeda, C. Nakamura, C. Miyamoto, N. Nakamura, M. Kageshima, H. Tokumoto and J. Miyake
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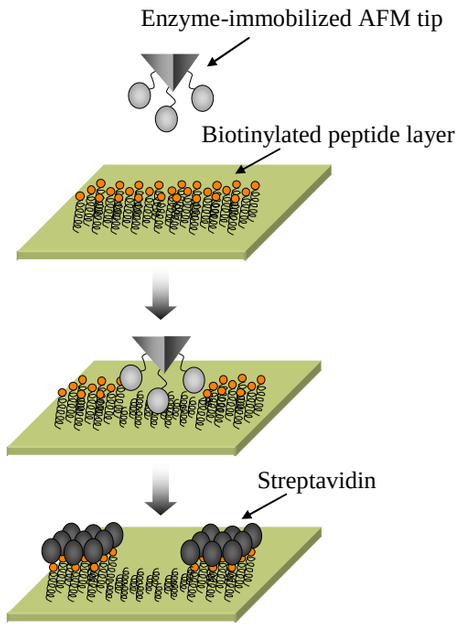

Fig. 1. Experimental schematics of the lithography.

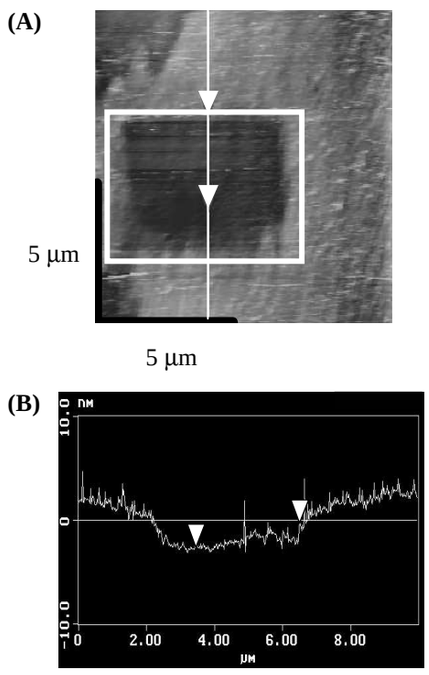

Fig. 2. (A) The surface topography image of the lithographed peptide layer using an enzyme-immobilized AFM tip after addition of streptavidin. A square represents scanned (lithographed) area. (B) Cross-section of image A indicated by a line. Two arrows in image A correspond to arrows in image B.