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Nucleobase Mapping of Self-Assembled Monolayers by Chemical Force Microscopy

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In order to measure complementary hydrogen bonding between nucleobases, disulfide derivatives of thymine and adenine were newly synthesized. Surface Plasmon Resonance; SPR confirmed the formation of SAMs on Au. SPR shows that SAMs of nucleobase derivatives were completely formed within 60 minutes. Thickness of the adenine SAM and the thymine SAM is nearly the same. AFM tip was modified with the thymine SAM. The adenine SAM-modified Au electrode was clearly observed in an adhesion force map. The adhesion force between complementary nucleobases is larger than that of the non-complementary combination.

Keywords: chemical force microscopy; molecular recognition; force mapping; self-assembled monolayers; hydrogen bond; DNA

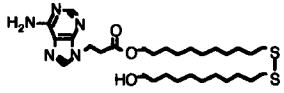
INTRODUCTION

One of the targets of DNA analysis is sequencing nucleobases

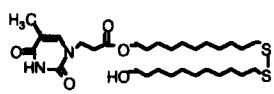
of single DNA molecule. Chemical force mapping by a prove-tip modified with nucleobases is a strong analytical method for sequencing of the single DNA molecule. It is known that the hydrogen bonding of a nucleobase to its complement is enhanced in hydrophobic environment at the at an air-water interface[1-3]. Monolayer of single-alkylated cytosine derivative at the air-water interface shows strong selectivity towards the complementary nucleobases dissolved in the aqueous subphase[4]. Here we propose a method for the binding and detection of nucleobases or monomeric/oligomeric DNA fragments by using a disulfide-modified gold surface as the interface. We herein demonstrate base-pair mapping by using chemical force microscopy.

RESULTS AND DISCUSSION

In order to measure complementary hydrogen bonding between nucleobases, disulfide derivatives of thymine and adenine were synthesized(Figure. 1). Surface Plasmon Resonance; SPR was used to confirm the formation of SAMs on Au. SPR shows that SAMs of nucleobase derivatives were completely formed within 60 minutes when Au substrates were soaked in ethanol solutions of disulfide derivatives (10μM) (Figure. 2). The thickness of the adenine SAM and the thymine SAM are nearly the same.



1: $AdeC_{11}SSC_{11}OH$



2: $ThyC_{11}SSC_{11}OH$

Figure. 1 Chemical formulae of nucleobase disulfide derivatives.

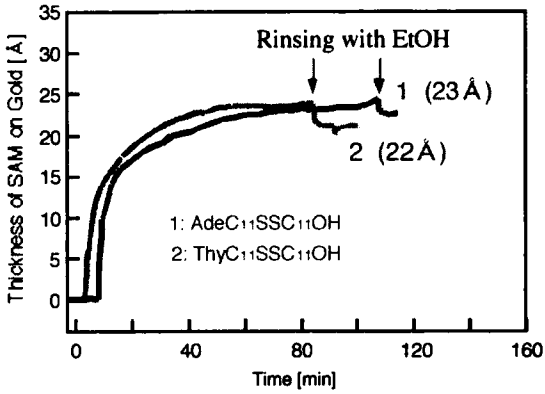
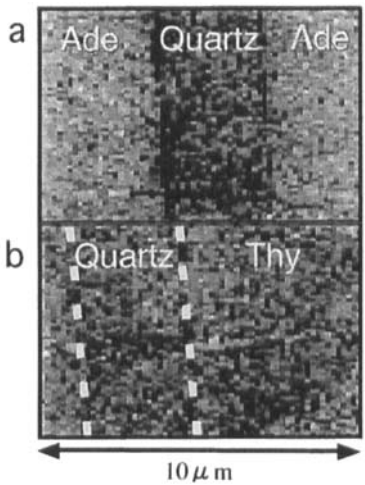


Figure. 2 Time courses of SPR due to adsorption of nucleobase disulfide derivatives in EtOH solution on gold at room temperature.

Gold-coated AFM tips and Au electrodes were modified with the adenine or thymine SAM, respectively. Comb-shape patterned Au electrode (width; 10μm, interval; 5μm, length; 2mm, number; 65) deposited on quartz surface was purchased from NTT advanced technology. Tips and electrodes were soaked in 1mM ethanol solution for 24h at room temperature. Adhesion force curves were measured in pure water at 20℃ by using OLYMPUS-NV2500 AFM (spring constant; 0.18N/m). The adhesion forces were mapped as 64×64 dots image. The adhesion force maps of the thymine- or adenine-modified patterned Au surfaces by the thymine-modified AFM tip are shown in Figure. 3. Alarge contrast between the quartz surface and the Ade-modified gold surface is clearly observed in the force map. While, in the Thy-modified electrode, homogeneous image was observed. Histograms of adhesion forces were obtained from the force-distance curves of 1024 measurement points (Figure. 4). The adhesion force (mean value; 2.01nN) of the complementary adenine-thymine

combination is larger than the non-complementary combination (mean value; 1.02nN). Similar results were observed in the adhesion force mapping of patterned Au electrodes when the AFM tip was modified with the adenine SAM.



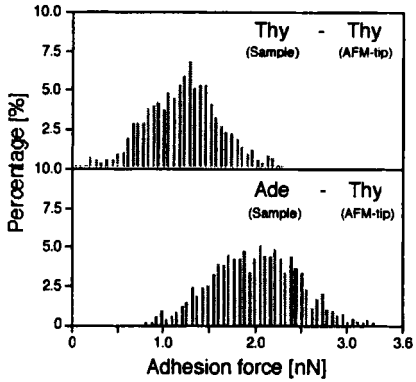


Figure. 4 Histograms for adhesion force obtained from the force-distance curves.

Figure. 3 Mapping of adhesion force between a thymine modified tip and adenine(a) or thymine modified gold micro electrode(b), respectively. White dots lines indicate the electrode edges observed by the topological imaging of AFM.

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