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Enzymatic Nanolithography of a Self-Assembled Oligonucleotide Monolayer on Gold

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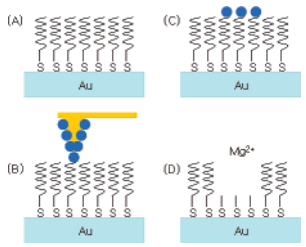
The ability to carry out biochemical reactions catalyzed by enzymes with nanoscale precision at a surface is an important goal in the development of bottom-up nanomanufacturing. This paper describes a small step in the realization of this goal by describing proof-of-principle of an enzyme-catalyzed reaction on an immobilized substrate, a self-assembled monolayer (SAM) presenting a terminal oligonucleotide, without the constraint of using an enzyme that is tethered to an atomic force microscope (AFM) tip.

An ongoing challenge in the development of nanolithography, especially methods that use proximal probes, such as dip-pen nanolithography (DPN),[1,2] nanoshaving,[3,4] and scanning near field optical lithography,[5,6] is the extension of these methods to carry out surface chemistry at the nanoscale. DPN has recently been applied to proteins and DNA,[1,7−9] but its extension to carry out biochemical manipulations at the nanoscale remains unexplored. The use of enzymes to catalyze biochemical reactions at a surface with nanoscale spatial resolution is extremely attractive for nanomanufacturing using proximal probes, because: (1) enzymes are the nanoscale factories of biology, in their ability to catalyze the conversion of myriad substrates into products; (2) a large number of enzymes are readily available as off-the-shelf reagents, so that diverse biochemical manipulations should be possible by enzyme-driven nanolithography; and (3) enzymes are among the most widely studied class of biomolecules, so that their use to catalyze reactions at the nanoscale can benefit from the detailed structure−function studies of these enzymes that are available.

Despite the attractive features of enzymes as biochemical tools for nanomanufacturing, there has been only one report to date that combines enzymatic catalysis and DPN. In that work, Staphylococcal serine V8 protease was tethered to an AFM tip and used to digest a glutamate-rich polypeptide that was adsorbed onto a surface.[10] In this paper, we present an alternative and, we believe, considerably simpler strategy that exploits an enzyme that is directly patterned on a surface by DPN to biochemically manipulate a surface that presents a homogeneous monolayer of a covalently tethered substrate; we refer to this method as enzyme DPN for brevity.

To demonstrate proof-of-principle of enzyme DPN using an adsorbed enzyme, we chose DNase I, a nonspecific endonuclease that digests double-stranded and single-stranded DNA into nucleotide fragments, as the enzyme. We chose as the substrate an oligonucleotide-terminated self-assembled monolayer of an alkanethiol (oligonucleotide SAM) that is tethered to the surface of gold by chemical self-assembly. The scheme that we used for enzyme DPN is shown in Scheme 1. An AFM tip, inked with DNase I, was used to write patterns of DNase I onto a gold substrate that is functionalized with an oligonucleotide SAM. Upon activation

Scheme 1



of the adsorbed enzyme by Mg^{2+} ions in solution, the enzyme locally digests its surface bound substrate, resulting in nanotrenches of DNA that are biochemically carved by the enzyme (vide infra).

Gold substrates were prepared by thermal evaporation of a chromium adhesion layer (100 Å), followed by gold (1000 Å), onto a glass cover slide at 4×10^{-7} Torr. Before deposition, the glass surface was cleaned in a 5:1:1 (v/v) mixture of H_2O, H_2O_2, and NH_3 at 80 °C for 20 min. Next, an oligonucleotide SAM was prepared by immersing a gold substrate into a 1 μM solution of 5′-GGTATACC-$(CH_2)_3$-SH-3′ in PBS, pH 7.0, overnight (Scheme 1A). The DNA-thiol was synthesized using standard automated phosphoramidite chemistry and then deprotected and purified on the bench. The SAM-functionalized gold surface was ultrasonicated in PBS, rinsed with deionized water, and then dried under a stream of N_2 gas. The ellipsometric thickness of the oligonucleotide SAM on gold was ∼4 nm (M-88 spectroscopic ellipsometer, Woollam, Inc.), which is close to the expected molecular length of the oligonucleotide SAM.[3]

To examine the feasibility of creating nanopatterns of DNase I, the enzyme was patterned onto the oligonucleotide SAM on gold by DPN, using a commercial AFM (MultiMode, Digital Instruments) (Scheme 1B and C) as follows. An AFM cantilever (silicon nitride cantilever, 0.05 N/m, Digital Instruments) was incubated in a degassed solution of 0.3 mM DNase I (Promega) in PBS, pH 7.4, for 30 min, and dried with N_2 gas before DPN. The relative humidity during patterning ranged from 35% to 55%. Patterns were generated by DPN with writing speeds up to 0.2 μm/s on the surface. Figure 1A shows adsorbed DNase I molecules patterned by DPN on the oligonucleotide SAM in single line scans and verifies the successful transfer of enzyme molecules onto the substrate by DPN. The width of the lines of DNase I shown in Figure 1A is 116 ± 2 nm (the full-width-at-half-maximum (fwhm) is 102 ± 3), and the height of the DNase I molecules in the pattern is 3.6 ± 0.2 nm, as determined from a line profile of the AFM image shown in Figure 1a. These heights are consistent with the placement of a layer of individual molecules of DNase I within the 100 nm lines on the surface.[11,12]

A nanopatterned surface was then immersed in a buffer solution containing Mg^{2+} to activate the enzyme (40 mM Tris-HCl, 10 mM MgSO, 1 mM $CaCl_2$, pH 8.0) for 1 h (Scheme 1D).

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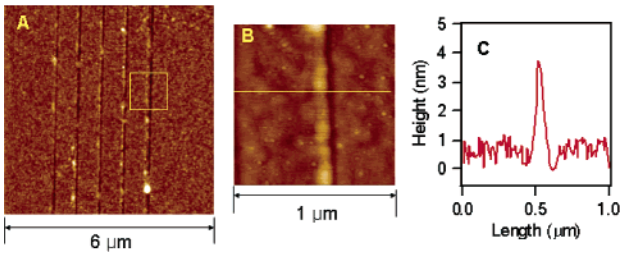

Figure 1. Tapping mode AFM images in air of DNase I nanopatterned on an oligonucleotide SAM on gold by DPN. (A) Height image of 6 μm scan size. (B) Magnified view of a 1 μm square (yellow box) in (A). (C) Line profile of B.

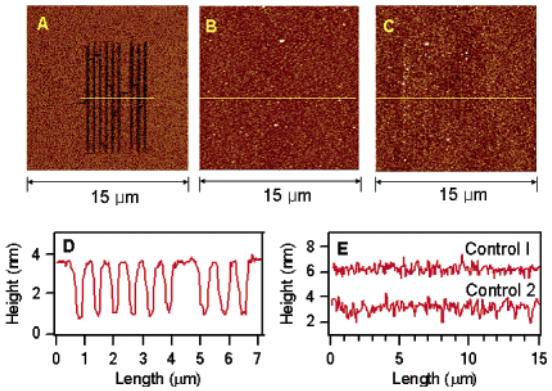

Figure 2. Tapping mode AFM image of the oligonucleotide SAM on gold patterned by enzyme DPN of DNase I. (A) Oligonucleotide SAM digested with DNase I that was nanopatterned on the SAM by DPN. (B) Oligonucleotide SAM without nanopatterns of adsorbed DNase I, processed identically to the sample in (A). (C) Oligonucleotide SAM with nanopatterns of adsorbed DNase I, incubated in the buffer solution without Mg^{2+}. (D) Line profile of (A). The decrease in the height in the trenches is ~3 nm. (E) Line profiles of (B) and (C). Line profiles have been offset for clarity.

The surface was subsequently rinsed with PBS and deionized water to deactivate the enzyme and was then dried under a stream of N_2 gas for AFM imaging. Tapping mode AFM in air was used to image the surface so as to minimize the introduction of any tip-induced artifacts due to tip−surface contact during imaging. Figure 2A shows a topographical image of the oligonucleotide SAM that is locally digested by the DNase I that is patterned on the surface. The line features showed a decrease in height of 3 ± 0.1 nm relative to the background, consistent with the near-complete digestion and release of nucleotides in that region of the oligonucleotide SAM (Figure 2D). The width at the top of the trenches carved by the adsorbed DNase I into the surface was 438 ± 32 nm, and the fwhm of the trenches was 353 ± 29 nm (Figure 2D), which is wider than the ~100 nm line width of the DNase I that was typically obtained in DPN (Figure 1C). The increased line width of the trenches carved by the enzyme likely is indicative of lateral diffusion of the enzyme on the surface without appreciable dissociation from the surface.[13] The apparent lateral diffusion rate coefficient for DNase I is $\sim 10^{-13}$ cm^2/s, somewhat lower than previous measurements of collagenase.[13] The lateral diffusion rate coefficient likely reflects the binding and catalytic rate constants of the enzyme, its adsorption coverage, and the high density of immobilized oligonucleotide on the surface.

Two control experiments were performed to confirm the local digestion of the oligonucleotide by DNase I: (1) DPN was performed on an oligonucleotide SAM without adsorbed DNase I on the AFM tip, and the surface was then incubated in a buffer containing Mg^{2+} ions (Figure 2B); (2) an oligonucleotide SAM with DNase I nanopatterned on its surface was incubated in the buffer solution without Mg^{2+} for 1 h, prior to being dried for AFM imaging (Figure 2C). Neither case showed any significant decrease in height by AFM (Figure 2E), which clearly indicated that activation of the nanopatterned DNase I by Mg^{2+} ions was responsible for the digestion of the immobilized oligonucleotides on the surface and that the decrease in height was not caused by scratching of the surface by the AFM tip during imaging.

An important finding of this study is that an adsorbed nanopattern of an enzyme can be directly used to carry out nanoscale enzymology at the surface without tethering the enzyme to the AFM tip. The patterns of DNA that are digested by the adsorbed enzyme retain the fidelity of the patterned enzyme, although there is some broadening due to lateral diffusion. Nonetheless, the present work for the first time demonstrates the potential of enzyme DPN, and previous reports of catalytically active, adsorbed enzymes[4,13] suggest that the technique has potential generality.

Future studies will optimize the technique by exploring the interrelated effects of reaction time, substrate density, dissociation rate constants, catalytic rate constants, and lateral diffusion coefficients. By understanding these relationships, the variety and versatility of biological reactivity might one day be applied to the digestion, transformation, and construction of a wide range of nanoscale features. Despite the constraint of lateral diffusion, enzyme DPN is an important complement to enzymes tethered to a scanning probe tip, in that it might provide: (1) generality, when covalent tethering would deactivate the enzyme; (2) ease of use, in that one step of covalent surface chemistry is removed; (3) potential for sequential transformations without replacing a covalently modified tip; and (4) greater throughput than would be possible by rastering an AFM tip with tethered enzymes across a surface. In conclusion, the demonstration that an enzyme does not have to be tethered to an AFM tip to catalyze a nanoscale transformation at a surface provides a new, and we believe useful, tool in the nanotechnology toolbox.

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