Protein Patterns at the Micro- and Nanoscale by Oxime Bond Formation

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Statement of Purpose: Immobilizing proteins in a specific orientation on surfaces is important in biomaterials for preserving bioactivity. We recently demonstrated a photoacid generator (PAG)-based lithography for micron and submicron protein patterning. This approach employed a pH responsive polymer with acetal side chains that deprotected upon exposure to light to form aldehyde patterns; Streptavidin was then immobilized.[1,2] In this study, we examined another polymer that forms aminooxy-functionalized side chains upon exposure to acid. We investigated whether aminooxy micro- and nanopatterns could be generated on this polymer using a PAG and either ultraviolet (i-line) light or electron beam (e-beam) radiation. We also examined whether this polymer film could be utilized for the oriented attachment of N-terminal α-keto amide and glyoxyl modified proteins.

Methods: Boc-aminooxy modified tetra(ethylene glycol) was synthesized by dicyclohexylcarbodiimide/4-dimethylaminopyridine (DCC/DMAP) coupling of Boc-aminooxy acetic acid and tetra(ethylene glycol) in CH_2Cl_2 for 8 h under argon. Boc-aminooxy tetra(ethylene glycol) methacrylate was synthesized by DCC/DMAP coupling of the above purified product and methacrylic acid in CH_2Cl_2 for 12 hr under argon. Using conventional free radical polymerization with 2,2'-azobisisobutyronitrile (AIBN) as the initiator, this monomer was copolymerized with 2-hydroxyethyl methacrylate (HEMA) using a feed ratio of 95:5. The reaction was allowed to proceed under argon for 1 hr in toluene at 75 °C. Streptavidin (SA) was modified to contain an N-terminal α-keto amide while myoglobin (Myo) was modified to contain an N-terminal glyoxyl group using a previously reported procedure.[3] Modification was confirmed by incubating the proteins with an excess of Alexa Fluor 488 hydroxylamine (Invitrogen, Carlsbad, CA) for 2 hr in the dark, purifying by centrifugal filtration, and then analyzing with a BioMate UV-Vis Spectrophotometer. Polymer films were prepared as follows. A 1 wt% solution of the copolymer in chlorobenzene was spin-coated at 3000 RPM onto freshly cleaned Si chips. After baking at 110 °C for 5 min, a 1 wt% polymer solution in chloroform plus 5 wt% PAG (diphenyliodonium-9,10-dimethoxyanthracene-2-sulfonate for i-line, triarylsulfonium hexafluorophosphate for e-beam) was spin-coated on top of the first layer. The samples were then either exposed to 365 nm light using a GCA 6300 I-Line Wafer Stepper for 4 sec through a mask or to e-beam radiation using a JEOL JSM-5910 Scanning Electron Microscope (accelerating voltage: 30 keV, beam current: 4.4 pA, dose: 10-50 μC/cm^2) with the Nanometer Pattern Generation System software (JC Nabity Lithography Systems, Bozeman, MT). Films were baked at 95 °C for 1 min, rinsed with MeOH and dH_2O, dried with N_2, and then incubated with the modified protein or unmodified protein as a control for 2 hr at room temperature. Bound SA was visualized with rabbit anti-SA (1:100 dilution, 30 min; Sigma, St. Louis, MO) and Alexa Fluor 488 anti-rabbit (1:200 dilution, 30 min; Invitrogen) antibodies. Myo was visualized with goat anti-Myo (1:100 dilution, 30 min; Genetex, San Antonio, TX) and Alexa Fluor 488 anti-goat (1:200 dilution, 30 min; Invitrogen) antibodies. Nanopatterns of attached proteins were visualized with a MultiMode™ atomic force microscope (AFM, Digital Instruments, Santa Barbara, CA) operated in tapping mode.

Results/Discussion: Absorbance spectra of both modified proteins displayed distinct peaks at ~ 488 nm, indicating that the fluorescent hydroxylamine had bound to the newly formed reactive carbonyl at the N-terminus of SA and Myo. Upon exposure to either i-line light or e-beam radiation, the PAGs were activated, resulting in Boc deprotection. Modified SA and Myo then bound specifically to the revealed aminooxy patterns (Figure 1a, b). When unmodified proteins were incubated on the films, no fluorescence was observed. AFM analysis of the e-beam patterned samples revealed nanopatterns of protein immobilized on the polymer film (Figure 1c).

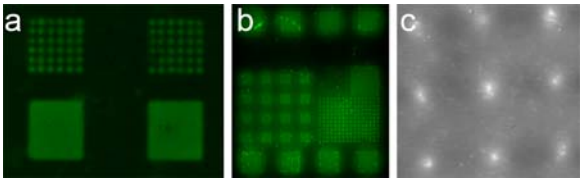

Figure 1. Micro- and Nanopatterns of modified proteins. (a) SA attached to i-line light generated patterns; largest feature is 40 μm. (b,c) Myo attached to e-beam fabricated patterns. Largest feature in (b) is 10 μm. (c) AFM image of ~ 200 nm patterns.

Conclusions: The N-termini of numerous proteins may be transformed to α-keto amides or glyoxyl groups.[3] Thus, poly(Boc-aminooxy tetra(ethylene glycol) methacrylate) films could be used to immobilize many proteins with known orientation through oxime formation between the aminooxy surface and the modified protein. This approach is currently being explored for biomaterials.

References:

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