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Letters

Electrochemically Induced and Controlled One-Step Covalent Coupling Reaction on Self-Assembled Monolayers

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We report on a novel covalent coupling method using electrochemical activation of hydroquinone monoester self-assembled monolayers. The reaction generates benzoquinone as a good leaving group, followed by nucleophilic acyl substitution with a primary amine to form an amide in high yield. The method allows the site-selective and the reaction-controlled positioning of biotin on the individually addressable microelectrode array and, subsequently, density-differentiated patterning of streptavidin on the biotin surfaces. Because the electrochemical coupling method provides a very rapid, mild, and quantitatively controllable reaction pathway for covalent bond formation on organic surfaces, it will be used as a versatile molecular anchoring tool in fields such as molecular electronics and biochip technology.

Various methods to functionalize surfaces by anchoring molecules have been investigated intensively. As a result of its simplicity and diversity, the most popular scheme is covalent coupling,[1] that is, direct bond formation by the reaction of nucleophile-containing molecules with activated molecular surfaces. In this paper, we report on a novel covalent coupling method using electrochemical activation of hydroquinone (HQ) monoester self-assembled monolayers (SAMs). The reaction generates benzoquinone (BQ) as a good leaving group, followed by nucleophilic acyl substitution with a primary amine to form an amide in high yield (Figure 1).[2] This is a one-step process that does not require chemical activation, for example, with *N*-hydroxysuccinimide. The reaction provides a useful strategy for the selective and controlled positioning of molecules on target sites of a preexisting electrode array.[3] Because the coupling is very rapid (nearly completed within 1 min), it provides favorably resistive conditions for nonspecific binding and for unwelcome coupling at unwanted positions in close proximity. Because the degree of coupling can be easily controlled by the electrochemical conditions, the regulation of the resulting surface density of the target molecules is facile without changing the dilution ratio of the adsorbents in the preparation of SAMs. We also demonstrate that this approach can be applied to the controlled preparation of biotin surfaces, which can

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(2) Johnson et al. experienced a problem with the acylation as a result of competitive oxidation of the amine at the bare electrode while the acylation of alcohol was feasible. Instead of the direct electrode reaction, our system uses an organic insulation SAM on the electrode, which is suspected to efficiently block kinetic access of the amine: Johnson, R. W.; Bednarski, M. D.; O'Leary, B. F.; Grover, E. R. *Tetrahedron Lett.* **1981**, *22*, 3715−3718.

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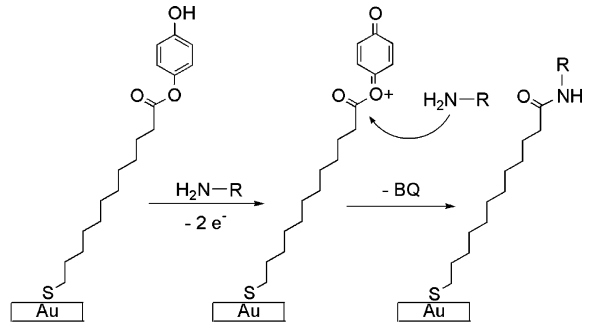

Figure 1. Electrochemically induced amide bond formation on a HQ monoester-terminated SAM.

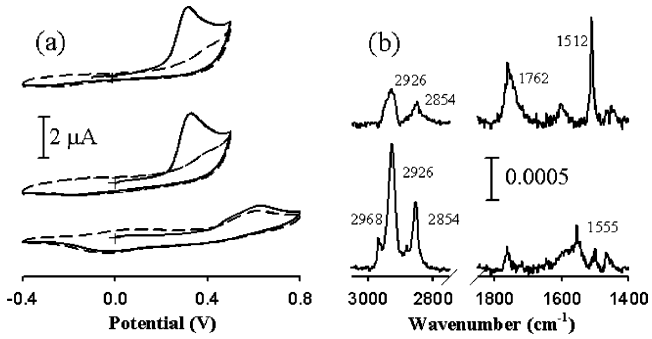

Figure 2. (a) Cyclic voltammograms of SAM I in butylamine (top), DADOO (middle), and pure (bottom) ACN solution (0.1 M tetra-*n*-butyl ammonium perchlorate as supporting electrolyte). The first scan is shown as a solid line, and the second scan is shown as a dashed line. Scan rate is 50 mV/s. Reference electrode is "no-leak" Ag/AgCl (Cypress Systems). (b) Grazing angle FT-IR spectra obtained for a freshly prepared SAM I (top) and the SAM subjected to electrochemical reaction with dodecylamine (bottom).

be used as platforms for density-differentiated micropatterning of streptavidin (SA).

We prepared and used the SAM of 12,12′-dithiobis(dodecanoic acid hydroquinone monoester) (SAM I) as reported previously.[4] To monitor the electrochemical surface reaction, cyclic voltammetry (CV) was performed. The top and middle of Figure 2a show the CVs of the electrochemical oxidation of HQ to BQ on the SAM in 1 M butylamine and 1 M 2,2′-(ethylenedioxy)-bis(ethylamine) (DADOO; diamine) in acetonitrile (ACN), respectively. Both irreversible reactions of HQ in the CVs were nearly completed during the first cycle in a range of 0.2−0.5 V. By integration of the oxidation peak, the reaction density of HQ for DADOO was found to be 4.0×10^{-10} mol/cm^2.[5] This is consistent with 3.8×10^{-10} mol/cm^2 of absolute free-amine density on the surface. The latter was proportional to the absorbance of 4-nitrobenzaldehyde (NB) recovered from hydrolysis of surface imine produced by NB condensation with free DADOO that was coupled to the SAM I with one side of it being amine.[6] Without amine, the peak disappearance during the repeated scan could not be observed even on the further anodic potential scan (bottom of Figure 2a).

The N(1s) peak of X-ray photoelectron spectroscopy (XPS) was not detected for a SAM I surface dipped into the 1 M DADOO solution in ACN under the open circuit potential for 2 min. However, the peak was found at 400.6 eV for the SAM surface treated electrochemically in the same solution for 1 min.[7] This indicates that, at least on a short time scale, only the electrochemical reaction contributes the attachment of amine. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) data support the release of HQ from the SAM surface. After the electrochemical treatment, no noticeable fragment peaks of HQ at 108 ($C_6H_4O_2^-$) and 109 ($C_6H_5O_2^-$) amu were observed. These peaks were very intense in the spectrum of original SAM I.

Grazing angle Fourier transform infrared (FT-IR) spectra (Figure 2b) prove the amide bond formation by the surface reaction. For SAM I subjected to electrochemical treatment in 0.5 M dodecylamine solution, the intensities of both the C═O stretch (1762 cm^{-1}) of the ester group and the C═C stretch (1512 cm^{-1}) of HQ significantly decreased and a strong C—N stretch peak (amide II) appeared at 1555 cm^{-1}. The CH_2 stretches (2854 and 2926 cm^{-1}) became more intense, and a new CH_3 stretch (2968 cm^{-1}) was observed. The water contact angle of 45° for an untreated SAM I increased to 65 and 102° for butylamine- and dodecylamine-reacted surfaces, respectively. All the above characterizations confirmed the covalent coupling (amide bond formation) by electrochemically induced acyl substitution on the SAM I, as shown in Figure 1.

The new coupling concept has been expanded to show the micropatterning of SA on an individually addressable gold electrode array. Initially, we prepared biotin poly(ethylene oxide) amine (BPA; (+)-biotinyl-3,6-dioxaoctanediamine)-reacted SAM I surfaces by using our coupling method and measured biospecific SA binding on the various surfaces with surface plasmon resonance (SPR) spectroscopy. Only the SAM I substrate subjected to the electrochemical reaction with 1 mM BPA for 1 min had significant binding capacity of 230 ng/cm^2 for SA (Figure 3a).[8] Figure 3b supports that the binding shown in Figure 3a is biospecific. The SAM I at the open circuit was resistive to BPA binding (Figure 3c). Nonspecific binding of SA was negligible at a freshly prepared SAM I (Figure 3d). The SPR experiment indicates that the selectivity in discriminating surfaces was at least 25. The selectivity can be tuned by simple manipulation of the electrochemical conditions, such as charge and potential, which permits

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(5) The reaction density calculated from the oxidation peak (probably due to H−e−e mechanism) decreased with decreasing DADOO concentration (4.0 and 1.9×10^{-10} mol/cm^2 at 1 M and 1 mM, respectively). See Supporting Information. In contrast, the small redox peak at near −250 mV in the CV slightly increased. This may be caused by different oxidation mechanisms for HQ depending upon solution basicity. See: Sato, Y.; Fujita, M.; Mizutani, F.; Uosaki, K. *J. Electroanal. Chem.* **1996**, *409*, 145−154.

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(7) Unless noted, all electrochemical treatments were conducted by holding the working electrode at a postpeak potential on each CV for 1 min.

(8) In this case, to enhance the solubility of BPA (1 mM), H_2O (1 M) was added to the ACN solution. The surface reaction density of HQ was 1.9×10^{-10} mol/cm^2. Although the competitive reaction of H_2O cannot be excluded, FT-IR data for the electrochemically treated SAM I exhibited a successful coupling of BPA. See Supporting Information.

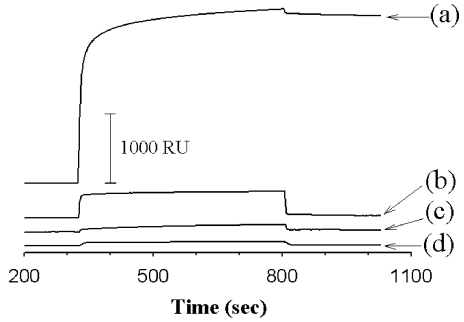

Figure 3. SPR sensorgrams of SAM I surfaces treated by various methods. Interaction between (a) SA and the SAM I surface after electrochemical reaction with 1 mM BPA, (b) SA presaturated with excess free biotin and the BPA-reacted surface, (c) SA and the SAM I surface dipped into the same BPA solution at open circuit potential for 45 min, and (d) SA and a freshly prepared SAM I surface.

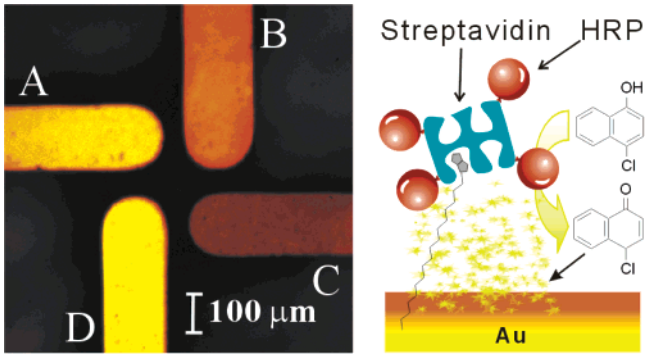

Figure 4. Optical microscope image displaying a density-differentiated SA patterning on the individually addressable 2 × 2 microelectrode array. The pattern was visualized by the precipitation of 4-chloro-1-naphthone produced by HRP-mediated catalysis. For electrodes A and B, the surface reaction densities of SAM I were about 6 and 33% of the maximum, respectively, as calculated from the surface-passed charge during each CV scan. The electrode C was subjected to a postpeak potential for 1 min. The SA density of each biotin-reacted surface was estimated to be (A) 55, (B) 180, and (C) 230 ng/cm^2 in a separate SPR experiment. No potential was applied to the electrode D.

regulation of the protein density (bound ligand-biotin) without preparing mixed SAMs to change the dilution ratio of the ligand on the surface.

To visualize SA surfaces, poly(horseradish peroxidase) (polyHRP)-conjugated SA has been used. The HRP-mediated catalyzed oxidation of 4-chloro-1-naphthol by H_2O_2 produces insoluble products in aqueous solution,[9] which paints the SA-coated region in a dark color and consequently enables the discernment of the region from the uncoated region in close proximity. Figure 4 shows an optical microscope image of density-differentiated painted SA on the reaction-controlled biotin surfaces. To control the SA density, we regulated the surface biotin density by changing the amount of charge passed to the SAM I surface during the electrochemical reaction with 1 mM BPA. Although the amount of immobilized SA is not necessarily proportional to the biotin on the SAM, the gradual increase of the precipitation density from electrode A to electrode C in the array demonstrates the density-controlled patterning of SA by our electrochemical coupling method. In addition, the painting inside each electrode seems to be uniform, indicating that the regular biotin coupling reaction had occurred throughout the electrode. This highly contrasted patterning must be attributed to the biospecific interaction-dominated SA adsorption following the rapid biotin coupling on the surfaces.

Because the electrochemical coupling method provides a very rapid, mild, and quantitatively controllable reaction pathway for covalent bond formation on organic surfaces, it will be used as versatile molecular anchoring tool in fields such as molecular electronics and biochip technology.

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Supporting Information Available: Graph of HQ reaction density versus DADOO concentration. XPS and TOF-SIMS data of DADOO-reacted SAM I. FT-IR spectrum of BPA-reacted SAM I. Detailed experimental procedures for CV, UV, SPR, and micropatterning. This material is available free of charge via the Internet at http://pubs.acs.org.

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