Electrochemical Deprotection for Site-Selective Immobilization of Biomolecules

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We describe an electrochemical deprotection method as a novel access for the site-selective immobilization of biomolecules using cyclic voltammetry, electrochemical quartz crystal microbalance, and grazing angle Fourier transform infrared spectroscopy. The hydroquinone monoester of thioctic acid and 12,12′-dithiobis-(dodecanoic acid hydroquinone monoester) were introduced as electrochemically removable protecting groups for ω-carboxylic acids of self-assembled monolayers (SAMs) on gold. The electrochemical deprotection method was found to provide very quantitative, rapid, and mild generation of specific binding sites. We additionally show that the resulting acid SAMs provide effective and selective surfaces for immobilizing oligodeoxynucleotides using radioimaging experiments and surface plasmon resonance spectroscopy.

Recently, there has been a great deal of research interest in DNA or protein microarrays for parallel and mass analysis.[1] Developments in the micropatterning of biomolecules or biologically active ligands onto solid supports have been crucial for facilitating the fabrication of spatially defined arrays.[2] These include electrochemical approaches for immobilization of different molecules on particular electrodes.[3] One strategy imposing the site selectivity to immobilization of biomolecules is to use protecting groups for specific binding sites. There have been a lot of reports for micropatterning using photodeprotectable[4a] and acid- or base-labile[4b] protecting groups tethered covalently to organic layers on the substrates. The limitations of those methods are that the quantification of the deprotection processes is not easy and the treatments for complete and selective removal of the protecting groups require severe physical and chemical conditions that may cause a degradation of biomolecules and ligands. Herein, we describe an electrochemical deprotection method for the site-selective immobilization of biomolecules. We introduced the monocarboxylic ester of hydroquinone (HQ) as the electrochemically removable protecting group for ω-carboxylic acids of self-assembled monolayers (SAMs) on gold because it can serve mild deprotection conditions due to its low electrochemical potential for the deprotection. The ester has been used in electrochemical[5a] or chemical[5b] transacylation to various functional groups such as acids, alcohols, and amines. The mechanistic rationale of our approach is the formation of a good leaving group by the electrochemical oxidation of HQ to quinone (Q), followed by chemical nucleophilic acyl substitution of Q by H_2O. From this substitution reaction, Q is removed and then ω-carboxylic acid is produced (see Figure 1). The carboxylic acid terminals can be used as specific binding sites for amine-containing molecules via subsequent covalent coupling that is the widely used technique for anchoring molecules due to its simplicity, rapidity, and diversity.[6] We additionally show that the resulting acid SAMs provide effective and selective surfaces for immobilizing oligodeoxynucleotides (ODNs).

We synthesized the HQ monoester of thioctic acid (**1**) and 12,12′-dithiobis(dodecanoic acid HQ monoester) (**2**).[7] Pure SAMs were prepared by immersion of gold surfaces in 1 mM THF solution of either disulfide for at least 12 h. To monitor the release of Q from the surface by electrochemical deprotection, cyclic voltammetry (CV) and electrochemical quartz crystal microbalance (EQCM) for the **1** SAM were performed.[8] CV (Figure 2a) shows an

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(7) They were checked by TLC and ^{1}H NMR spectroscopy.

(8) Unless noted, all electrochemistry was performed with the Au/SAM working electrode, Pt wire counter electrode, and Hg/Hg_2SO_4 (MSE) reference electrode in a 0.1 M phosphate buffer (pH 7.2). Measuring charge in the CVs informed us that **1** and **2** SAMs are stable in pH 7.2 phosphate buffer and 0.1 M $HClO_4$ solution, at least during 24 h.

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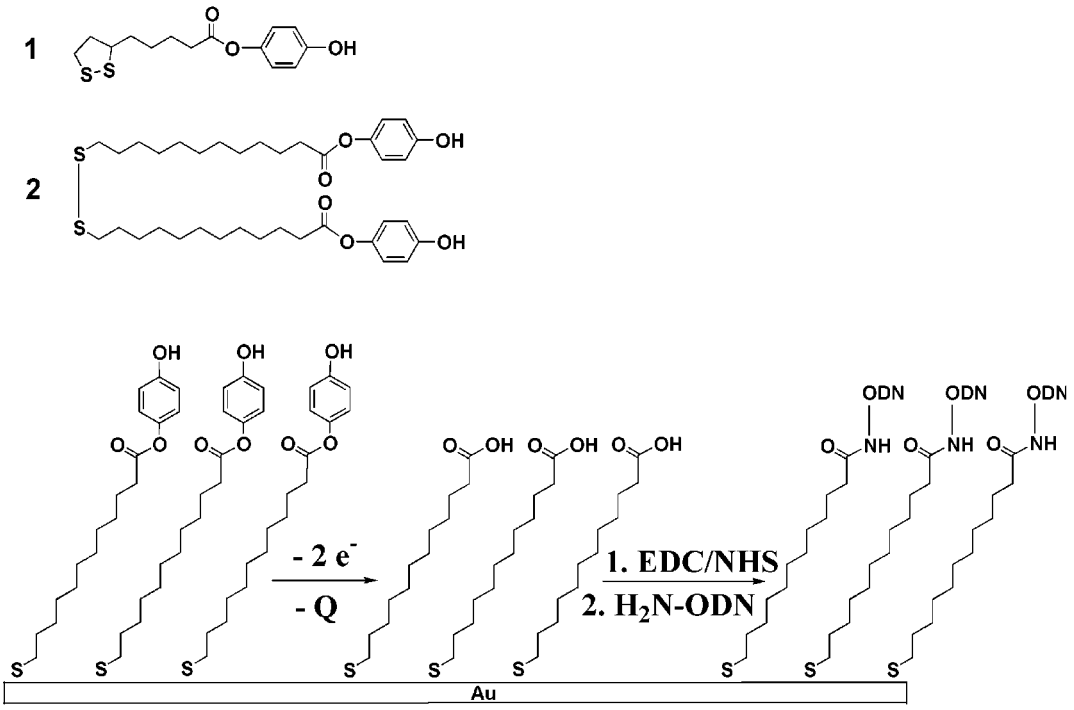

Figure 1. Schematic representation of the generation of ω-carboxylic acids by electrochemical deprotection of a SAM containing monocarboxylic esters of HQ and the subsequent covalent coupling of the acids with amine-derivatized oligodeoxynucleotides (H_2N-ODNs).

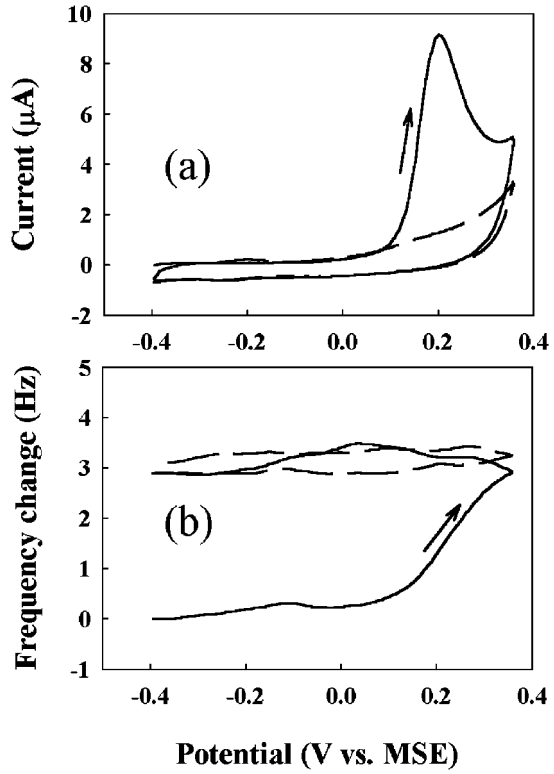

Figure 2. (a) Cyclic voltammogram and (b) frequency–potential curve of a **1**-modified quartz crystal (AT-cut 6 MHz; sensitivity, 4.2 ng/Hz) gold electrode in 0.1 M phosphate buffer (pH 7.2) at a scan rate of 50 mV s^{-1}. The solid line is the first scan, and the dashed line is the second scan.

irreversible anodic peak at 0.2 V on the first scan and a dramatic disappearance of the peak on the second cycle, representing not only fast and nearly complete release of Q but also no readsorption on the resultant surface. The EQCM frequency response for these CV scans, as shown in Figure 2b, clearly supports the CV result. The increase of frequency at 0.1 V on the first anodic scan shows a mass decrease of the SAM surface due to the release of Q. The second cycle gave no remarkable mass changes. The frequency change of 2.5 Hz agrees closely to 2.3 Hz, which is the amount predicted for the release of Q accompanied with addition of H_2O.[9]

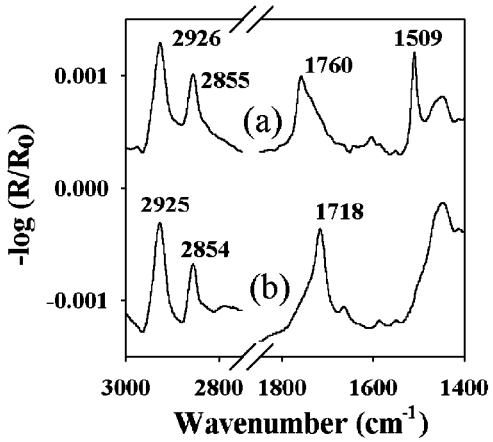

Figure 3. Grazing angle FTIR spectra obtained for (a) a freshly prepared **2** SAM and (b) the SAM after electrochemical deprotection.

Grazing angle Fourier transform infrared (FTIR) spectra confirmed the electrochemical reaction proposed in Figure 1. Figure 3a shows the spectrum of a freshly prepared **2** SAM. We first note two absorption bands at 1760 and 1509 cm^{-1} arising from the C=O stretching vibration of the ester group and aromatic C=C stretching

(9) Considering that the reaction proceeds as a two-electron mechanism, the quantity of released Q estimated from the integration of anodic peak current on the first scan is 2.9×10^{-10} mol cm^{-2}. The low density may be due to the nonlinear structure of compound **1**. The surface coverage of the compound **2** SAM is 4.0×10^{-10} mol cm^{-2}, which was also estimated by CV at pH 7.2 in phosphate buffer solution.

vibration of the HQ moiety. The C−H stretching bands of the methylene groups of the alkyl chain appear at 2926 and 2855 cm^{-1}. Figure 3b is the spectrum obtained from the monolayer subjected to electrochemical deprotection at a postpeak potential of 580 mV for 2 min in a 0.1 M $HClO_4$ solution.[10] While the positions and intensities of the C−H vibrations reveal no significant change, the C=C stretch of 1509 cm^{-1} has disappeared and the C=O stretch of 1760 cm^{-1} is replaced by a band at 1718 cm^{-1} due to laterally hydrogen-bonded COOH groups as compared to Figure 3a.[11] Moreover, unreduced intensities of the C−H stretches imply that the deprotection reaction proceeds under mild conditions due to the low oxidation potential of the monoester of HQ. Further evidence for the conversion to a carboxylic acid group was obtained by measuring pH-dependent behavior of the deprotected SAM for blocking the redox reactions of $Fe(CN)_6^{3-}$.[12]

The site selectivity via our strategy has been examined. The primary amine-derivatized single-stranded DNA of 15 bases with the sequence 5′-CCG ACC GGA ATA AAT−NH_2-3′ (H_2N−ODN) was introduced for subsequent covalent binding on the acid-terminated surface.[13] The acid activation was completed by immersing deprotected **2** SAM surfaces in a mixed solution of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 0.2 M) and *N*-hydroxysuccinimide (NHS, 0.05 M) during 40 min. Immobilization of the H_2N−ODNs was conducted by spotting of H_2N−ODN solution (10 μM) on the NHS-activated SAM surfaces for 20 min. The density of surface-bound H_2N−ODNs estimated by using the ^{32}P radiolabeling method was 1.1 ($\pm$ 0.02) $\times$ 10^{12} molecules cm^{-2}.[14,15] In a control, the density for the HQ-protected surface was 1.9 ($\pm$ 0.1) $\times$ 10^{11} molecules cm^{-2}.

Further evaluation was done by in situ monitoring of adsorption of the H_2N−ODNs to the activated surfaces using surface plasmon resonance (SPR) spectroscopy.[16,17] Figure 4 shows surface-dependent SPR sensorgrams. Although the effect of the nonspecific adsorption cannot be ruled out,[18] it is remarkable that the deprotected **2** SAM, with only ca. half of the value of surface coverage for the mercaptododecanoic acid (MDA) SAM, has a similar binding capacity to the MDA SAM. This result indicates that the deprotection of HQ serves an effective surface for the covalent coupling of DNA molecules.[19] Both control experiments of the radiolabeling and SPR reveal a good signal-to-noise ratio that is at least 4 in the site selectivity.

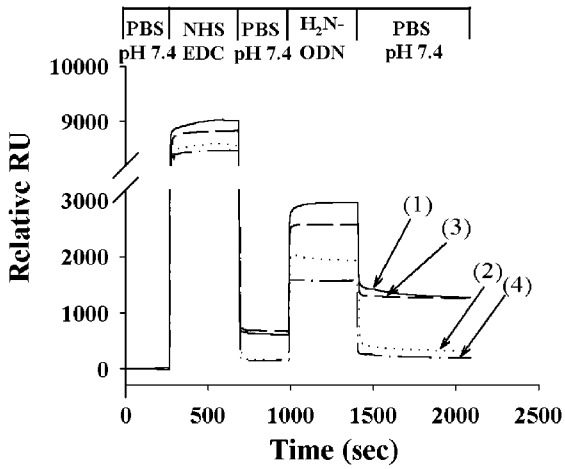

Figure 4. SPR sensorgrams for the covalent immobilization of H_2N−ODN onto **2** SAMs: (1) after and (2) before the electrochemical deprotection. These were compared with (3) a pure mercaptododecanoic acid SAM and (4) a pure mercaptoundecanol SAM. Phosphate-buffered saline has 20 mM phosphate, 28 mM NaCl, and 0.54 mM KCl in water. The concentrations of NHS/EDC and H_2N−ODN solutions were the same as in the radiolabeling experiment. The flow rate was 4 μL/min. An increase of 1000 RU corresponds to a DNA density of 1 ng mm^{-2}.

The electrochemical deprotection method was found to provide very quantitative, rapid, and mild generation of specific binding sites for H_2N−ODNs. A potential utility of the HQ carboxylate SAM in an aqueous system might be the sequential immobilization of amine-containing biomolecules. Under an aprotic solvent condition, electrochemically directed acylation to the biologically active ligands would present another strategy for improving the site selectivity. Furthermore, SAMs of the monoester of ring-substituted HQ with biocompatible molecules are anticipated to be useful for not only the site-selective immobilization of biomolecules but also the site-selective removal.[20]

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Supporting Information Available: Detailed synthetic procedures of HQ monoesters **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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