Diels–Alder Reaction for the Selective Immobilization of Protein to Electroactive Self-Assembled Monolayers

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The immobilization of biologically active molecules is important for preparing substrates used in diagnostic assays, high-throughput drug discovery, and attached cell culture.1 A wide variety of methods are available, and the choice for a particular application depends on the relative importance of many factors, including selectivity and efficiency of the coupling reaction, the stability of the resulting complex, and the suitability for preparing patterned arrays of ligands. No single method meets all of these criteria. The complex of streptavidin with biotin is overall the best available method, but it has the limitations that the complex is large and intrusive, and nonspecific adsorption of the protein can be problematic.2 Many chemical methods, including the condensation of amines with activated carboxylic acids or with aldehydes, are convenient and applicable to most molecules but are limited by a lack of selectivity.3a,b Others, including the coordination of Ni(II) complexes with oligo(histidine) motifs, have excellent selectivity but often lack long-term stability.3b In this paper we use self-assembled monolayers (SAMs) that present a quinone group to demonstrate that the Diels–Alder (D–A) reaction of this group with cyclopentadiene (cp) is an excellent method for immobilization. This design also permits the reactivity of the quinone to be modulated either chemically or electrochemically, by way of reduction to the hydroquinone which does not participate in the D–A reaction.

We first investigated the kinetic behavior for the D–A reaction of cp with quinone attached to a monolayer (Figure 1). Cyclic voltammetry of a mixed SAM presenting hydroquinone (HQ) and hydroxyl groups (\(\gamma_{HQ} = 0.25\)) showed that the HQ undergoes oxidation at 220 mV to give the quinone (Q) and reduction at −150 mV.3c Voltammograms over 50 consecutive cycles were indistinguishable and showed the oxidation was reversible. When cp was added to the electrolyte, consecutive voltammograms showed a decrease in the peak current for both reduction and oxidation (Figure 2). Several observations confirm that the loss in current was due to the D–A reaction of cp with Q. The addition of other dienes (including cyclohexadiene and 1-cyclopentadienyl methyl acetate) to the electrolyte gave similar losses in current over consecutive cycles (although with different rates), but the structurally related cyclopentane and cyclopentene had no effect over the same number of scans. Immersion of a monolayer presenting HQ groups in electrolyte containing cp (15 mM) for 20 min had no effect on the voltammograms, demonstrating that only the Q underwent reaction with cp. Grazing angle FTIR spectroscopy further supported the reaction of Q with cp. A monolayer presenting Q groups showed a carbonyl stretching mode at 1663 cm\(^{-1}\). After reaction with cp, this band was absent, and a band at 1653 cm\(^{-1}\) corresponding to the carbonyl stretching mode of the D–A adduct was observed. Figure 3 shows a plot for the loss in peak current for the reduction of Q versus time for the data shown in Figure 2. Because the concentration of cp was much greater than that of immobilized Q, the data could be fit to an exponential decay to obtain a pseudo-first-order rate constant, \(k\):
protein to the surface was prevented and demonstrated that the association was biospecific. Furthermore, streptavidin did not immobilize to monolayers that presented either Q or HQ groups mixed with glycol groups.14

The D–A reaction described here provides an attractive and flexible method for bio-immobilization and is especially well-suited for tailoring monolayers with peptides, carbohydrates, and other low-molecular weight ligands.15 Because the reaction is kinetically well-behaved, this method can be used to quantitatively attach groups in low densities (<1%) where the direct determination of density is not straightforward. This method also allows for the sequential immobilization of several ligands to a common substrate, with independent control over the density of each ligand. This chemistry further makes possible a class of dynamic substrates for attached cell culture, wherein the immobilization of biologically active ligands can be turned on at discrete times. This and related electroactive substrates16 will provide an exciting range of tailored substrates for studies in cell biology and applications in biotechnology.

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(14) The magnitude of change in $\theta$ when streptavidin is flowed over the monolayer presenting quinone and glycol groups is greater than that observed with monolayers presenting only glycol groups and shows that the protein adsorbs to the mixed monolayer. This adsorption does not result in immobilization of streptavidin, since it is weak and rapidly reversible when the protein-containing solution is replaced with buffer.
