Electrochemical Transduction

Self-Assembled Monolayers That Transduce Enzymatic Activities to Electrical Signals**

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The development of chemical methods to control the interactions of materials with proteins, enzymes, and cells is important to fundamental studies in chemical biology and to many applications in biotechnology.[1] A wide range of strategies for immobilizing ligands to materials now allows excellent control over ligand–receptor interactions at an interface. An emerging theme in this field aims to add dynamic control over ligand–receptor interactions to enable the design of active substrates that can manipulate, in real time, the interactions of proteins and cells with a surface. Okano and co-workers, for example, exploited the thermal-phase transition of poly(*N*-isopropylacrylamide) films to demonstrate substrates that could reversibly modulate the adhesion of cells.[2] Willner and co-workers developed a class of monolayer substrates in which the activities of immobilized ligands can be reversibly switched with light.[3] We have developed electroactive substrates that allow discrete ligands to be switched on or off in response to electrical potentials.[4] These advances have provided an unprecedented ability to study and manipulate the adhesion of cells. In this communication, we extend on these early reports by demonstrating an interface that can specifically translate biological activities to electrical signals.[5] This example now establishes the basis for designing electroactive interfaces that not only manipulate but also respond to cellular activities.

Our approach for transducing a biological activity to an electrical signal relies on the enzymatic conversion of a redox-inactive molecule to generate a redox-active product (Figure 1). By tethering the substrate molecule to an electrode, the redox-active product resulting from enzyme action can be efficiently detected. We implement this strategy with the enzyme cutinase[6] and 4-hydroxyphenyl valerate as the substrate. Cutinase is a 22 kDa serine esterase that efficiently removes the acyl group from this substrate, to produce the redox-active hydroquinone.[7]

We prepared a self-assembled monolayer (SAM) containing an alkanethiolate terminated by a 4-hydroxyphenyl valerate moiety [8], and a tri(ethylene glycol)-terminated alkanethiolate in a ratio of 1:2. We used MALDI-TOF mass spectrometry to establish that the interfacial enzymatic

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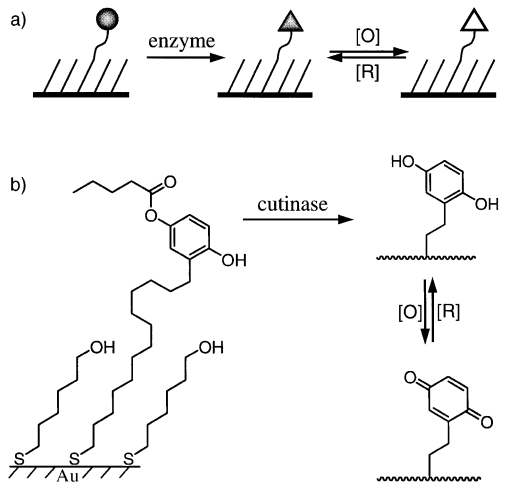

Figure 1. Strategy for designing an interface that transduces a biological activity to an electrical signal. a) A substrate molecule for an enzyme is immobilized to a self-assembled monolayer. The enzymatic reaction converts the substrate into a redox-active product, which can be detected and quantified with cyclic voltammetry; b) this work uses monolayers presenting 4-hydroxyphenyl valerate, which is efficiently hydrolyzed by cutinase, a serine esterase, to afford the hydroquinone. The reversible electrochemical oxidation of the hydroquinone to the corresponding benzoquinone produces an electrical signal.

reaction proceeds to give the intended products. The mass spectrum of the monolayer showed two major peaks at m/z 969.9 and 1246.1 (Figure 2a).[9] The first peak corresponds to the mixed disulfide derived from the two alkanethiolate species, while the second peak results from the symmetric disulfide derived from the 4-hydroxyphenyl valerate terminated alkanethiolate. An identical monolayer was subjected to an enzymatic reaction by applying a solution containing cutinase (10 nM) in phosphate-buffered saline (PBS) at

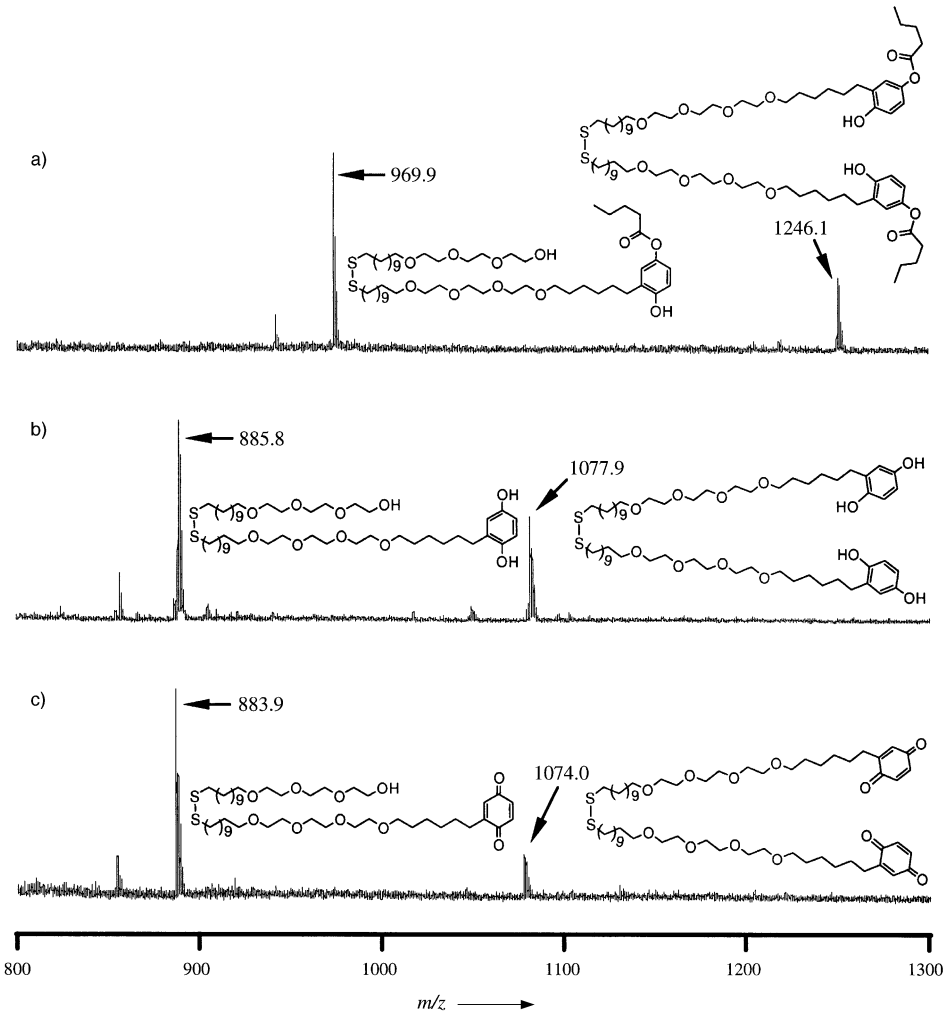

Figure 2. Characterization of the interfacial enzymatic reaction with MALDI-TOF mass spectrometry. All peaks correspond to sodium adducts of disulfides. a) A monolayer containing alkanethiols terminated in 4-hydroxyphenyl valerate and tri(ethylene glycol) groups gave disulfide peaks corresponding to unsymmetric and symmetric disulfides; b) after treatment with cutinase (10 nM in PBS at pH 7.4 for 10 min), the original peaks were absent and gave rise to two new peaks corresponding to the product hydroquinone; c) oxidation of the resulting hydroquinone (10 mM iron(III) chloride in PBS at pH 7.4) afforded the corresponding benzoquinone.

pH 7.4. The reaction was stopped after 10 min and the monolayer was analyzed by MALDI-TOF. Two new peaks corresponding to disulfides containing the product hydroquinone were observed (Figure 2b). Significantly, the peaks for the parent ester are absent, which shows that the enzymatic reaction proceeded in essentially quantitative yield. Finally, treatment of the monolayer with iron(III) chloride (10 mM in PBS) efficiently converted the hydroquinone groups to the corresponding benzoquinone groups (Figure 2c).

Cyclic voltammetry was then employed to monitor the enzymatic reaction in real time.[10] For these experiments, monolayers were prepared from a mixture of the 4-hydroxyphenyl valerate terminated alkanethiol and 6-mercapto-1-hexanol (Figure 1b).[11] Cyclic voltammetry showed that the monolayer was not electroactive, but displayed only a non-faradaic current that remained constant over 50 consecutive cycles. Upon the addition of cutinase to the medium (10 nM), however, voltammetric waves for both oxidation and reduction appeared and increased smoothly with time until they reached a maximum value (Figure 3).[12] These waves are due to the oxidation of the enzymatically generated hydroquinone

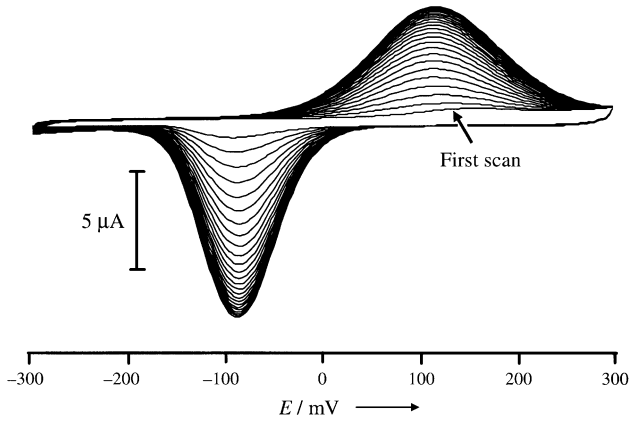

Figure 3. A series of cyclic voltammograms showing the enzymatic cleavage of the 4-hydroxyphenyl valerate group on the monolayer. Prior to cutinase treatment, cyclic voltammetry showed only a non-faradaic current. After cutinase was added to the solution (10 nM), peak currents for both reduction and oxidation appeared and increased with time as the enzymatic reaction generated hydroquinone groups on the monolayer.

group, and the corresponding reduction of the benzoquinone. To provide further evidence that the enzymatic reaction indeed generated the hydroquinone, we treated the resulting monolayer with a tri(ethylene glycol)-substituted cyclopentadiene (5 mM) and found that voltammetric peaks decreased, as expected for a Diels–Alder reaction between the diene and benzoquinone (data not shown).[4a,5] As a final control, we found that cutinase, which was inactivated by a 4-nitrophenyl phosphonate irreversible inhibitor, had no effect on the monolayer.[13]

Finally, we investigated the kinetic profile for cutinase action on the immobilized 4-hydroxyphenyl valerate groups. Figure 4a shows the time-dependent increase in the density of hydroquinone for a series of monolayers that initially present

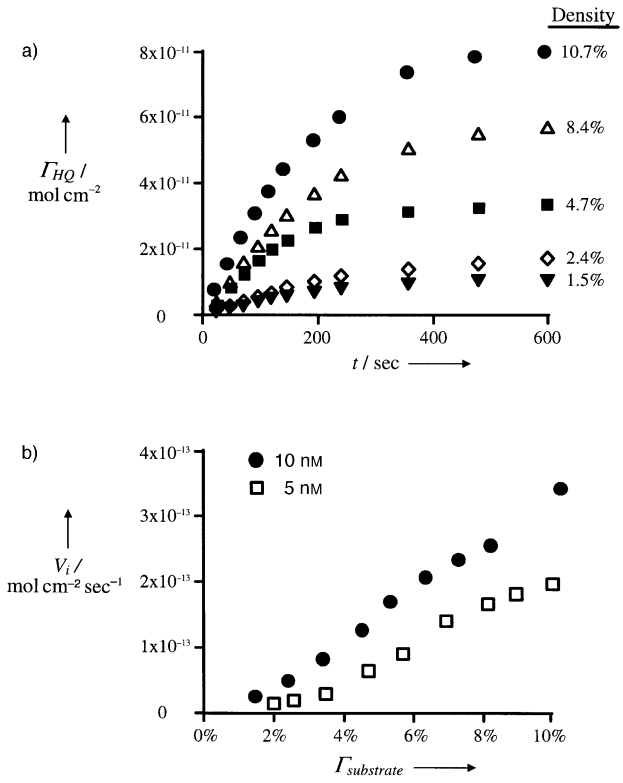

Figure 4. Kinetic profile for the interfacial enzymatic reaction. a) The amount of hydroquinone produced on the monolayer (Γ_{HQ}) was determined by cyclic voltammetry and is plotted versus time for several monolayers presenting 4-hydroxyphenyl valerate at densities ranging from 1.5% to 10.7%; b) the initial rates of the enzymatic reaction (for both 5 nM and 10 nM cutinase) depend linearly on the density of 4-hydroxyphenyl valerate on the monolayers ($\Gamma_{substrate}$).

4-hydroxyphenyl valerate at densities ranging from 1.5% to 10.7%.[14] For each monolayer, the density of hydroquinone initially increased linearly with time, and leveled off at a maximum density that is determined by the initial density of 4-hydroxyphenyl valerate on the monolayer. The initial reaction rates at two different concentrations of cutinase (5 nM and 10 nM) also increased approximately linearly with the density of 4-hydroxyphenyl valerate on the monolayer (Figure 4b). Overall, these results show that this interfacial enzymatic reaction is kinetically well-behaved and that the enzymatic activity can be measured in real time with excellent sensitivity.

This work describes a new strategy for transducing biological activities into electrical signals. The biological and electrical activities are linked by way of a redox-active molecule that results from an enzymatic conversion. This work represents an important advance on previous demonstrations of dynamic substrates that modulate activities of ligands in response to electrical potential. With this new demonstration, it is now possible to design interfaces that transduce biological and electrical signals in both directions across the interface. We believe that this strategy will prove important for fundamental studies of enzymatic activity at interfaces, for mechanistic studies of cellular activity, and for the development of cell-based sensors. For the latter oppor-

tunity, cells can be engineered with enzymes that induce electrical signals—in analogy to the common use of green fluorescent protein (GFP) as a fluorescent reporter—and give a purely electrical interface for recording cellular dynamics. A further attraction of this methodology is that it can take advantage of many enzyme–substrate pairs and therefore realize multiple channels of electrochemical coupling between cells and electrodes.

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[8] The 4-hydroxyphenyl valerate terminated alkanethiol with tri(ethylene glycol) moiety was synthesized in ten steps from commercially available reagents. All intermediates gave satisfactory ^{1}H NMR spectra.

[9] For the mass spectrometry experiments, we used the tri(ethylene glycol)-containing alkanethiols to increase the molecular weight, since ions below m/z 600 are not easily analyzed due to overlap with signals from the organic matrix. The monolayers were analyzed on a Voyager-DE Biospectroscopy mass spectrometer using 2,5-dihydroxyl benzoic acid (1 μL of a 10 mg mL^{-1} solution in acetonitrile) as a matrix. Sodium adducts of disulfides are the major species that are observed in MALDI spectra of the SAMs of alkanethiolates: see J. Su, M. Mrksich, *Angew. Chem. Int. Ed.* **2002**, *41*, 4715, and J. L. Trevor, K. R. Lykke, M. J. Pellin, L. Hanley, *Langmuir* **1998**, *14*, 1664.

[10] Cyclic voltammetry was performed with a Bioanalytical Systems CV-50W potentiostat using PBS as the electrolyte at pH 7.4, scanning from −300 mV to +300 mV at a rate of 100 mV s^{-1}. All experiments used a custom-designed electrochemical cell with the monolayer as the working electrode, a Pt wire as the counter electrode, and an Ag/AgCl reference electrode.

[11] The 4-hydroxyphenyl valerate terminated alkanethiol was synthesized in eight steps from commercially available reagents. All intermediates gave satisfactory ^{1}H NMR spectra. For the synthetic scheme and experimental details, see Supporting Information.

[12] The density of 4-hydroxyphenyl valerate for this monolayer was approximately 9%, as determined by integration of the area under the redox wave after the enzymatic reaction was completed. This analysis assumes that the 4-hydroxyphenyl valerate was quantitatively converted to hydroquinone.

[13] For the protocols used to prepare cutinase and the inhibitor, see; C. D. Hodneland, Y. S. Lee, D. -H. Min, M. Mrksich, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5048.

[14] For each monolayer, the density of hydroquinone on the surface was determined by integration of the areas under the redox waves in the cyclic voltammograms.

