Multianalyte electrochemical biosensor on a monolith electrode by optically scanning the electrical double layer

Seung-Woo Lee, Ravi F. Saraf*

Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln (UNL), 207 Othmer Hall, Lincoln, NE 68588-0643, USA

ABSTRACT

Redox on an electrode is an interfacial phenomenon that modulates the charge in the electrical double layer (EDL). A novel instrument, the Scanning Electrometer for Electrical Double-layer (SEED) has been developed to measure multiple enzyme reactions on a monolith electrode due to immunospecific binding with a mixture of respective analytes. SEED quantitatively maps the local redox reaction by scanning a laser on the array of enzyme monolayer spots immobilized on the monolith electrode. SEED measures the change in local charge state of the EDL that abruptly changes due to the redox reaction. The measurement spot size defined by the size of the laser beam is $\sim 10\ \mu m$. The SEED signal is linearly proportional to the local redox current density and analyte concentration. The specificity is close to 100%. The SEED readout is compatible with microfluidics platform where the signal degrades less than 2% due to the poly(dimethyl siloxane) (PDMS) body.

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1. Introduction

Amperometric measurement of redox current on immunospecific binding of analyte with immobilized monolayer of enzyme on an electrode has revolutionized chemical sensor industry. The electrochemical detection of analytes at high sensitivity and selectivity using "enzyme electrodes" has had great success as biosensors during the past few decades(Wang, 2008). For example, the glucometer, is arguably the most successful chemical sensor in history, detecting glucose via immunospecific binding to glucose oxidase (GOx) immobilized on an electrode. (Newman and Turner, 2005; Yoo and Lee, 2010). Enzyme electrodes (also referred to as wired enzymes) were first demonstrated over three decades ago, (Eddowes and Hill, 1977; Yeh and Kuwana, 1977) disproving the dogma that a mediator is necessary to electrochemically couple an enzyme to an electrode. Since then, many strategies have been developed to wire the enzyme using a variety of promoters, (Hu, 2001) such as conducting polymer, (McQuade et al., 2000; Ramanavicius et al., 1999) metal cations, (Armstrong et al., 1984) and nanomaterials. (Jacobs et al., 2010; Mano and Heller, 2005; Mao et al., 2003; Willner et al., 2006). Elegant interface chemistries are developed by incorporating the enzyme into conducting polymer media with a redox center tethered to the polymer chain to effectively collect the redox electrons. (Mano and Heller, 2005; Mao

et al., 2003) In a remarkable study, a ~ 2 nm Au nanoparticle was "imbedded" directly into GOx to wire the active site of the enzyme to the electrode. (Xiao et al., 2003) Another highly versatile, scalable method is the layer-by-layer assembly of polyelectrolytes incorporating, moderators and promoters (such as nanoparticles, enzymes, nanomaterials). (Calvo et al., 2000; Zhao et al., 2006) The conduction occurs due to the ionic nature of the polyelectrolytes.

With the success of the single analyte electrochemical biosensors, multiplexing and miniaturization are natural extensions. One of the most pervasive ideas has been to measure a signal in an indirect electrochemical event. The first combinatorial electrochemistry example was to study catalytic action of a combination of five metals to optimize an electrode for fuel cells (Reddington et al., 1998). The level of redox activity was measured as a fluorescent signal from the redox product. For large-scale multiplexing, the most successful approach has been to translate complementary metal-oxide semiconductor (CMOS) technology. In one chip, an antibody-antigen addressable sensor array has been designed using an enzyme-labelled target to generate a redox signal (Dill et al., 2004; Ghindilis et al., 2007). The approach is further modified by incorporating a small redox molecule (i.e., PQQ) at the electrode surface to enhance the signal (Polsky et al., 2008). Recently, in a remarkable approach, an electrochemical DNA sensor was designed where modulation in the pH due to (local) polymerase-based synthesis of DNA on a probe template is measured on each pixel as a change in current (Rothberg et al., 2011). The local pH change modulates the gating potential. However, in multianalyte detection biosensor, array of individual microelectrode for each analyte with corresponding

* Corresponding author. Tel.: +1 402 472 2750; fax: +1 402 472 6989.
E-mail addresses: rsaraf@unlnotes.unl.edu, rsaraf@unlnotes.unl.edu (R.F. Saraf).

(immobilized) enzyme needs to be fabricated (Wilson and Nie, 2006; Yan et al., 2010). The number of sensing electrodes in the array have to be equal to the number of analytes to be tested, with additional counter and reference electrodes for each sensing electrode (depending on the design). The electrical isolation of the individual electrode in the microarray to reduce noise due to coupling adds complexity and cost to the design which remains a challenge (Ebrahimi et al., 2008). Furthermore, the redox current monotonically reduces as the sensor electrode size diminishes.

Here, we describe a novel laser scanning method to quantitatively address multiple redox reactions on a monolith electrode with a microarray of immobilized enzymes. The laser beam measures the local redox reaction on the enzyme spot by measuring the charge state of the electrical double layer (EDL) (Singh and Saraf, 2006). The EDL charge abruptly changes at the redox potential and the change in charge (compared to equilibrium state) is linearly proportional to the redox current density. Thus, the optical signal is linearly proportional to the local redox current density on the $\sim 10\,\mu\mathrm{m}$ laser spot and the analyte concentration. The method is called a Scanning Electrometer for Electrical Double-layer (SEED).

2. Material and methods

In this section, the principle of SEED is briefly described; followed by surface modification and immobilization of enzyme to fabricate the enzyme chip; and lastly the method of SEED measurement is described.

2.1. Scanning electrometer for electrical double-layer (SEED)

The principle of SEED is described in a previous publication (Singh et al., 2009). To facilitate the interpretation of the results and discussion, the basic principle of SEED is briefly outlined. Two orthogonally polarized laser beams, having the same intensity, were incident onto the SU-8 patterned monolith working electrode (WE) that has the immobilized enzymes. The probe beam is on the sensing site while the reference beam is on the WE that is coated with SU-8 (Fig. 1(a) and Fig. A in Supplementary data Information (SI)). The pair of beams is scanned spot-to-spot over various enzyme sites (Fig. 1(a)). Fig. 1(b) shows a photograph of the actual SEED apparatus. In a typical electrochemical experiment, a periodic bias, V_{DC}, at a frequency of 20 mHz from -200 mV to 800 mV is applied between the WE and the reference electrode (RE) by a potentiostat; and the redox current is measured between the CE and the WE. The RE is a standard Ag/AgCl electrode, and CE is Pt wire. Additionally, a 50 mV AC potential ($\omega \sim 2$ kHz) is applied on the WE to produce an optical signal described next. The AC potential will oscillate the ions in the vicinity of the electrode (within 100 nm as described in the next paragraph) to cause a periodic modulation of the ion concentration at ω. The concentration modulation will result in oscillation in the path length. This amplitude of the path length oscillation is measured by differential interferometer as an optical signal, Δ (SI, Fig. A). Because the reference beam (incident on the passivated part of the electrode with no electrochemical activity) and the probe beams are always only $\sim 100\,\mu\mathrm{m}$ apart (see microscope image in Fig. 3(a, inset) to be discussed later), the thermal noise is greatly reduced resulting in highly stable optical signal at pico-meter (pm) sensitivity (Fig. B, SI). To note is that the three electrodes, WE, RE and CE are controlled by a potentiostat to concomitantly acquire both optical (SEED) and cyclic voltammetry (CV) data.

The nature the optical signal, Δ, is considered. As the WE comes into contact with the electrolyte solution, an EDL is spontaneously formed. The thickness of the EDL is $\sim 10\,\zeta$, where ζ is the Debye

length, which is inversely proportional to the square root of ion concentration. Typically, for a 100 mM solution, ζ is ~ 1 nm (Israelachvili, 1992). Due to the ion accumulation in the EDL, the electric field emanating from the WE is screened within $\sim 10\zeta$. (Torrie and Valleau, 1979) Thus, for 100 mM solutions, the ion oscillation due to the AC potential applied on the WE is limited to well within ~ 15 nm. When the V_{DC} ramp cycle is close to the redox potential, $E^{\cdot}$, there is a large amount of electron exchange between the WE and the redox ions in the EDL leading redox current. As the ion diffusion in response to charge imbalance is significantly slower than the rapid electronic exchanges between the redox ion and the WE, the electrostatic screen due to an EDL charge is significantly diminished. As a result, the AC field emanating from the WE will penetrate deeper into the electrolyte solution during redox, resulting in an increase in ion oscillation at the interface. As a result, Δ is maximum, Δ_{max}, close to the redox potential, commensurate with the redox current (Fig. B, SI). From Gauss's law, for a planar electrode (i.e., one-dimensional system), the increase in electric field due to descreening is linearly proportional to the magnitude of charge imbalance (Xiao et al., 2003). As the charge imbalance is proportional to the redox current (for a fixed V_{DC} ramp rate), the increase in the magnitude of the electric field is linearly proportional to the redox current. Thus, the optical signal at redox, Δ_{max}, is linearly proportional to the local redox current density, I_{max}, averaged over the 10 μm laser spot (see inset of Fig. B, SI). Owing to linear proportionality between Δ_{max} and I_{max}, SEED quantitatively measures the local redox current density. Furthermore, because I_{max} is proportional to analyte concentration, Δ_{max} is expected to be linearly proportional to analyte concentration. The linearity is explicitly shown in Fig. 4.

If the solution contains only glucose at the redox potential, a large AC field penetration occurs on the spot with GOx at the redox potential (leading to Δ_{max}), as schematically shown in Fig. 1(a). The oscillation over the other two spots with GalOx and AlOx will not have any significant increase Δ as the V_{DC} is ramped over the redox potentials for all of the enzymes.

2.2. Device fabrication

Three polygonal openings on a gold WE (1 mm(W) $\times$ 10 mm(L)) were fabricated by a UV-lithographic method on 50 μm thick SU-8 film (Fig. 2(a)). The sensing side is at the apex of the isosceles triangle, which is $\sim 600\,\mu\mathrm{m}$ long; and the apex angle is $\sim 45°$. The broad rectangular-like shape facilitated immobilization of the enzyme at the apex by surface tension (i.e., wicking). The SEED measurement was performed at the apex to take advantage of signal enhancement due to (dielectrophoretic) electric field focusing caused by the surrounding SU-8 (Lee et al., 2013). The probing at the apex also demonstrated the feasibility of the possible measurement spot size of $\sim 10\,\mu\mathrm{m}$ by SEED. After O_2 plasma for 1 min., the device was spin-coated with alternating ~ 0.9 nm thick layers of PAH (3 mg/ml, 15,000 Da, Sigma Aldrich) and PSS (3 mg/ml, 70,000 Da, Sigma Aldrich) at 3000 rpm. The GOx, GalOx (galactose oxidase), and AlOx (alcohol oxidase) in 10 mM phosphate buffer saline (PBS, pH.~ 7.4) were locally micro-pipetted at the larger side of the pattern for immobilization (Fig. 2(b)). To immobilize GOx and AlOx, three layers of PAH/PSS/PAH were deposited. For GalOx, only PAH/PSS was deposited such that the top layer was negatively charged (i.e., PSS). The solution was wicked over the opening due to the hydrophilic nature of the surface caused by polyelectrolyte coating. The 50 μm thick SU-8 film confines the enzyme solution droplet over the electrode opening. The device was stored overnight at 4 °C to ensure complete (saturated) immobilization of the enzyme. The device was gently washed with 10 mM PBS, and the other PAH/PSS layers were spin-coated to encapsulate three immobilized enzymes.