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Iron–sulfur-based single molecular wires for enhancing charge transport in enzyme-based bioelectronic systems

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ABSTRACT

When redox enzymes are wired to electrodes outside a living cell (ex vivo), their ability to produce a sufficiently powerful electrical current diminishes significantly due to the thermodynamic and kinetic limitations associated with the wiring systems. Therefore, we are yet to harness the full potential of redox enzymes for the development of self-powering bioelectronics devices (such as sensors and fuel cells). Interestingly, nature uses iron–sulfur complexes ([Fe–S]), to circumvent these issues in vivo. Yet, we have not been able to utilize [Fe–S]-based chains ex vivo, primarily due to their instability in aqueous media. Here, a simple technique to attach iron (II) sulfide (FeS) to a gold surface in ethanol media and then complete the attachment of the enzyme in aqueous media is reported. Cyclic voltammetry and spectroscopy techniques confirmed the concatenation of FeS and glycerol-dehydrogenase/nicotinamide-adenine-dinucleotide (GlDH–NAD^+) apoenzyme–coenzyme molecular wiring system on the base gold electrode. The resultant FeS-based enzyme electrode reached an open circuit voltage closer to its standard potential under a wide range of glycerol concentrations (0.001–1 M). When probed under constant potential conditions, the FeS-based electrode was able to amplify current by over 10 fold as compared to electrodes fabricated with the conventional pyrroloquinoline quinone-based composite molecular wiring system. These improvements in current/voltage responses open up a wide range of possibilities for fabricating self-powering, bio-electronic devices.

1. Introduction

Redox enzymes are naturally occurring bioelectronic entities that can act as power plants or self-powering sensors due to their ability to generate an electron stream in the presence of an enzyme-specific analyte. However, when these enzymes are wired to electrodes (Heller, 1990) outside a living cell (i.e., ex vivo), their ability to produce a sufficiently powerful electrical current diminishes significantly due to the thermodynamic limitations associated with the coenzymes.

Common coenzymes (also known as cofactors) are typically resistant to cyclic oxidation and reduction ex vivo. Furthermore, they require special molecules, known as electron mediators, to help extract electrons from the coenzyme and transfer them to the final target. The molecular wires that consist of mediators and other interfacing molecules that tether the enzyme to the base electrode (Katz et al., 1997), although help mitigate the thermodynamic issues, create issues associated with electron transport kinetics due to the increased resistance that results from the increased length. This lengthy wiring scheme results due to known electron mediators not having the correct combination of prosthetic groups to anchor the enzyme system (i.e., the apoenzyme–coenzyme complex) from one end and the base electrode from the other. Linker molecules with appropriate functional groups are introduced into the wiring scheme to fulfill the anchoring function. Fig. 1A shows a commonly used, conventional wiring scheme that comprises cystamine, pyrroloquinoline quinone (PQQ), and phenyl boronic acid for tethering the nicotinamide adenine dinucleotide (NAD^+) coenzyme-dependent apoenzyme onto a metal electrode for sensing and biofuel cell applications.

The outcome of such a long wiring scheme is high ohmic resistance, resulting in constrained electron transport (Johnston et al., 2007; Park et al., 1999; Reed et al., 1997) and producing fuel cells with low power density and sensors with low sensitivity (Minteer et al., 2007; Reed et al., 1997; Riklin et al., 1995). The lack of an effective molecular wiring system that can allow unimpeded charge transport is a significant problem that hinders the ability to harness the full potential of redox-enzymes-based, self-powering bioelectronic devices.

Interestingly, nature uses an array of unique molecules, i.e., the iron–sulfur complexes ([Fe–S]), to circumvent the thermodynamic and kinetic electron transport issues associated with redox

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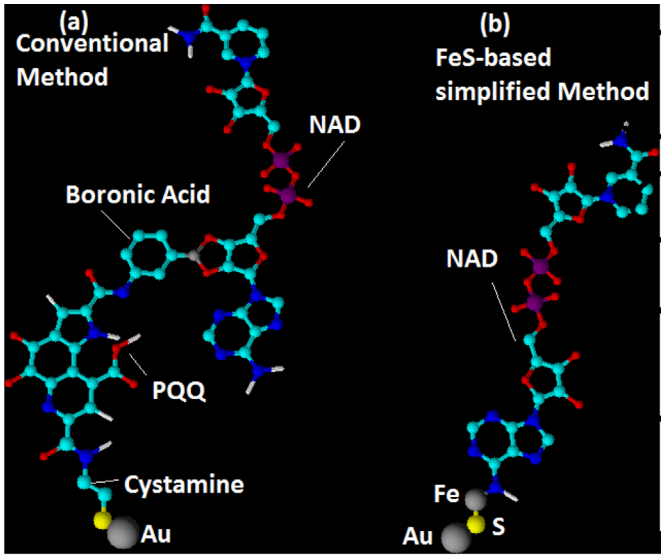

Fig. 1. Schematic of wiring systems. (a) Complex conventional wiring of the coenzyme NAD^+ to a gold electrode using a series of molecules including a cystamine linker, a PQQ mediator, and a phenyl boronic acid linker; (b) Simplified wiring of NAD^+ coenzyme directly onto the gold electrode by the multi-functional mediator, FeS.

enzymes. Iron–sulfur compounds are the key molecules that link enzyme (apoenzyme and coenzyme) complexes to the supporting surfaces in mitochondrial and photosynthetic electron transport chains (Berk et al., 2000). Iron–sulfur groups are ideal linker molecules because they: (1) can mediate electron transport; (2) have correct prosthetic groups to anchor the enzyme complex at one end and provide metal support at the other (sulfur coordinates with metal centers (Graham and Dingman, 2006; Love et al., 2005; Mrksich et al., 1996; Tour et al., 1995; Zhong and Porter, 1994) and onto proteins with cysteinyl residues (Meyer, 2008), while iron coordinates with the heterocyclic nitrogen atoms (Cline et al., 1985; Lavrenova et al., 1986; Marlin et al., 1999) or amine linkages (Bedford et al., 2005; Liu et al., 2013; Zuo et al., 2013) that are present in the coenzymes and cellular peptides); and, quite importantly, (3) are short enough to alleviate charge transport limitations. Even so, to date, we have been unable to duplicate [Fe–S]-based electron transport chains ex vivo, primarily due to the instability of iron–sulfur compounds in aqueous media.

The aim of this work was to determine whether iron–sulfur molecules can be used effectively as linker molecules to attach the redox enzyme complexes to supporting surfaces ex vivo, and, if so, to investigate their performance in the electrical transport function. As a working model, a glycerol-sensitive gold bioanode is described based on direct attachment of the glycerol-dehydrogenase (GlDH)–NAD^+ apoenzyme–coenzyme complex onto the supporting gold surface using iron (II) sulfide (FeS) mediation. A conventional PQQ-based electrode, described previously (Mahadevan et al., 2015), was used as the control. The performances of the two electrode systems were compared using amperometric and potentiometric studies.

2. Materials and methods

2.1. Reagents and apparatus

Iron (II) sulfide (FeS), NAD^+-dependent glycerol dehydrogenase from *Cellulomonas sp.* (EC.1.1.1.6), ß-nicotinamide adenine dinucleotide (NAD^+), glycerol ≥99%, and glutaraldehyde were purchased from Sigma-Aldrich, USA (www.sigmaaldrich.com). 50 mM KOH solution made in 35 wt% H_2O_2 was used to clean the surface of the gold. FeS was suspended in ≥99.5% ethanol, and ß-NAD^+, GlDH, and glutaraldehyde solutions were prepared in a 0.1 M phosphate buffer (pH=7). Molecular-biology-grade water obtained from Sigma-Aldrich was used to prepare all the aqueous-based solutions and for rinsing and cleaning purposes throughout this study. Two-millimeter gold-disk working electrodes, Ag/AgCl reference electrodes, and Pt auxiliary electrodes were purchased from CH Instruments, Inc., Austin, TX. The potentiometric and amperometric tests were performed using a CHI8003D Potentiostat from CH Instruments, Inc. Water containing the enzyme stimulants (Mahadevan et al., 2015) viz. $(NH_4)_2SO_4$ and $MnCl_2 \cdot 4H_2O$ was used as the carrier electrolyte throughout the study.

2.2. Fabrication of the bioanode

Layer-by-layer self-assembly via dip-coating was used to fabricate the FeS-based enzyme electrodes. First, the gold (Au) electrodes were prepared for modification by following the three-step rigorous cleaning procedure as described earlier (Mahadevan et al., 2015). The clean Au electrodes were immersed in a 0.3 M FeS solution suspended in ethanol for 2 h. Then the FeS-modified electrodes were immersed in 1 mM of ß-NAD^+ for 2 h followed by 1 mg mL^{-1} of GlDH for 2 h for formation of NAD^+ and GlDH monolayers respectively. The FeS-NAD^+-GlDH functionalized electrodes were lastly treated with 10% (v/v) glutaraldehyde for 20 min to crosslink and secure the molecular layers. Every step in the dip-coating procedure was followed by rinsing the electrodes with water to eliminate physisorbed molecules, if any. Fig. 1B shows the FeS-based wiring scheme.

Attachment of FeS to the Au surface was verified via a combination of techniques involving cyclic voltammetry (described below), and X-ray photoelectron spectroscopy (XPS) and Fourier Transform Infrared (FTIR) spectroscopy (described in the Supplementary material section).

2.3. Ferricyanide-voltammetry to confirm SAM formation

Electrochemical experiments were performed via the conventional three-electrode system (i.e., enzymatic working electrode, Pt counter electrode, and Ag/AgCl reference electrode) placed in an electrochemical cell containing 5 mL of potassium ferricyanide. A single potential sweep was individually performed for each successive layer of the multi-layer SAMs attached to the Au electrode (with constant surface area 0.031 cm^2). A sweep rate of 50 mV/s was used for all the cyclic voltammetric measurements. A bare Au electrode was used as an ancillary control (or standard) electrode. All experiments were performed at room temperature.

2.4. Potentiometric analysis

Instantaneous open circuit voltage (OCV) measurements of the enzyme electrodes were taken using a potentiostat via the conventional three-electrode system in 5 mL total volume of the carrier electrolyte containing 0.001–1 M glycerol.

2.5. Amperometric analysis

An array of amperometric tests were conducted for the enzyme electrodes via the typical three-electrode system in glycerol (0.001–1 M). First, cyclic voltammetry was conducted in a total volume of 5 mL of carrier electrolyte containing glycerol. Current responses for potential sweeps between 0–1.5 V and 0.665–1.3 V, at a scan rate of 50 mV/s were recorded. The effect of current with respect to time at a constant potential, $E=1.3$ V, was analyzed using constant potential amperometry for 60 s. To evaluate the