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An Implantable Biofuel Cell for a Live Insect

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Supporting Information

ABSTRACT: A biofuel cell incorporating a bienzymatic trehalase|glucose oxidase trehalose anode and a bilirubin oxidase dioxygen cathode using Os complexes grafted to a polymeric backbone as electron relays was designed and constructed. The specific power densities of the biofuel cell implanted in a female *Blaberus discoidalis* through incisions into its abdomen yielded maximum values of ca. 55 μW/cm^2 at 0.2 V that decreased by only ca. 5% after ca. 2.5 h of operation.

Methods aimed at converting chemical or mechanical energy either present in or generated by living organisms into electricity are expected to open exciting new prospects for the development of autonomous means to power an array of microdevices such as sensors. Indeed, ingenious devices to accomplish this goal have been described in the literature over the past decade.[1−3] Particularly noteworthy is the pioneering work in the area of biofuel cells by Heller and co-workers,[4,5] who introduced judiciously designed electrode structures incorporating suitable redox mediators to promote electron transfer between enzymes covalently linked to a polymeric substrate and the actual electrode.[4] We describe herein an implantable, enzyme-based biofuel cell that employs the disaccharide trehalose (Tr) present in the hemolymph of a live cockroach (*Blaberus discoidalis*) and oxygen from the air as the anode and cathode fuels, respectively. Efforts to develop an effective trehalase electrode for a fuel cell that could be implanted in an insect have also been made by Heller and his group using a genetically engineered glucose dehydrogenase.[6] As shown schematically in Figure 1, the novel device described here relies on the use of a trehalase (Tre)|glucose oxidase (GOx) bienzymatic anode and a bilirubin oxidase (BOD) cathode,[7] covalently wired in each case through a polymer bearing pendant, redox-active osmium complexes. As detailed elsewhere,[8] this bifunctional Tre|GOx anode dissociates Tr dimers into their constituent glucose (Gl) monomers, which are then oxidized to yield D-glucono-1,5-lactone (DGL). Prior to experiments with the live insect, a few measurements were performed in vitro using the same electrocatalytically active enzyme-based materials.

Electrodes for in vitro experiments were prepared using carbon rods (4 cm in length, 0.071 cm^2 in area) sealed in heat-shrink tubing (RadioShack) with a small length (ca. 0.5 cm) left bare at one end for external electrical connections. The other flat end was then polished and rinsed thoroughly with ultrapure water, and voltammetric curves were collected in phosphate-buffered saline (PBS) to check for cleanliness (see the black curves in Figure S1 in the Supporting Information). Procedures described in the literature were followed for the high-yield

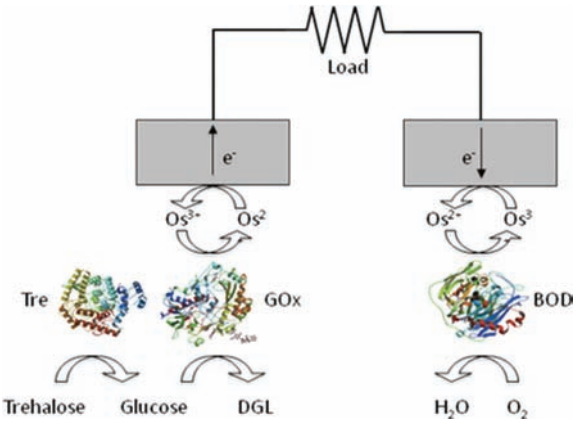

Figure 1. Schematic diagram of the enzyme-based Tr|O_2 biofuel cell examined in this work.

(i.e., ca. 95%) synthesis of bis(2,2′-bipyridine-*N*,*N*′)dichloroosmium(III) chloride dihydrate, $[Os(bpy)_2Cl_2]Cl$, and bis(2,2′-bipyridine-*N*,*N*′)dichloroosmium(II), $[Os(bpy)_2Cl_2]$,[9] using potassium hexachloroosmate(IV) as the metal precursor. Grafting of the Os complexes to poly(1-vinylimidazole) (PVI) was also carried out using methodologies reported elsewhere.[10] Cyclic voltammograms of PVI−$Os(bpy)_2Cl$ and PVI−$Os(dm\text{-}bpy)_2Cl$ (dm-bpy = 4,4′-dimethyl-bpy) (see below) applied as films on the surface of carbon rod electrodes in PBS solution are shown as red curves in the top and bottom panels, respectively, of Figure S1.

The electrocatalytically active cathode material was prepared by mixing 3.8 μL of 5 mg/mL BOD (*Trachyderma tsunodae*, Amano, EC 1.3.3.5, 2.1 units mg^{-1}), 2.1 μL of 10 mg/mL PVI−$Os(bpy)_2Cl$, and 1.2 μL of 2.5 mg/mL poly(ethylene glycol) (400) diglycidyl ether (PEGDGE, Polysciences, Inc.) to yield a final loading of 44 wt % BOD, 49 wt % PVI−$Os(bpy)_2Cl$, and 7 wt % PEGDGE. The corresponding anode material was prepared by mixing 2 μL of 6.7 mg/mL glucose oxidase (GOx) (*Aspergillus niger*, EC 1.1.3.4, 153 units mg^{-1}), 10 μL of 0.25 mg/mL Tre expressed and purified in our laboratory,[8] 1 μL of 2.5 mg/mL PEGDGE, and 4 μL of 10 mg/mL PVI−$Os(dm\text{-}bpy)_2Cl$ to yield a final loading of 23 wt % GOx, 4.3 wt % Tre, 68.5 wt % polymer, and 4.3 wt % PEGDGE. About 17 μL for the anode and 7 μL for the cathode were applied to individual carbon rod electrodes and allowed to dry overnight at ambient temperature in air. The PEGDGE cross-linked the polymer and covalently bonded the enzymes through reaction of the epoxides on the linker with amines on the enzyme surface.

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Measurements involving live insects were performed using as electrodes bundles of thin carbon fibers (ca. 7 μm diameter) sealed in a glass capillary tube, the end of which was polished to expose a disk 0.04−0.05 cm in diameter. A copper wire inserted into the opposite end of the tube was connected to the carbon fibers using nonconductive epoxy to serve as the external lead. About 1 μL of the enzyme/polymer mixture was applied to these smaller electrodes. In the case of the enzymatic O_2 cathode, a small glass frit was affixed to the tip of a glassy pipet (Fisher) filled with PBS, which was then inserted into one of the incisions (see Figure 2).

The electrocatalytic activity of each of the enzyme-modified carbon electrodes was determined in vitro using an all-glass single-compartment cell with a Ag/AgCl (3 M KCl) electrode (Bioanalytical Systems) and a carbon rod (ca. 4 cm^2 cross sectional area) as the reference and counter electrodes, respectively. Polarization curves were recorded with a conventional potentiostat (Bioanalytical Systems CV-50) by applying a series of fixed potentials and monitoring the transient current response until it reached a stable value, generally 60−90 s. Power density curves for the fuel cell, normalized by the cross-sectional area of the Tre electrode, as a function of potential were obtained by applying a fixed voltage (with the potentiostat) across the Tr and O_2 electrodes in N_2-purged 50 mM trehalose in PBS, until a constant value for the current was observed, which took generally ca. 1 min.

For the in vivo measurements, *B. discoidalis* females, which were selected because of their large abdomen in comparison with males, were first sedated using CO_2. Subsequently, the wings were removed, and the body was pinned on its back to a Petri dish with two pins through the pronotum and two more through the posterior region of the abdomen. Finally, wire staples were placed over each leg to prevent movement. None of these procedures had any long lasting deleterious effects on the insects. They represent a typical restraint used by insect neurobiologists for much more extensive surgery followed by video analysis confirming normal behavior. The subjects that were used in this study typically walked away normally after the procedure. Enzymatic electrodes were inserted into two incisions made in the abdomen of the insect, as depicted in Figure 2. The abdomen contained a large low-pressure

Figure 2. Photograph showing the biofuel cell implanted into *B discoidalis*.

blood sinus that could be penetrated by the electrodes with no damage to the insect or any of its critical internal organs.

Shown in the top panel of Figure 3 is a polarization curve collected for the Tre|GOx|Os(dm-bpy)$_2$Cl|carbon rod electrode in a N_2-purged 50 mM solution of Tr in PBS, which yielded an onset potential for Tr oxidation close to 0 V vs Ag/AgCl

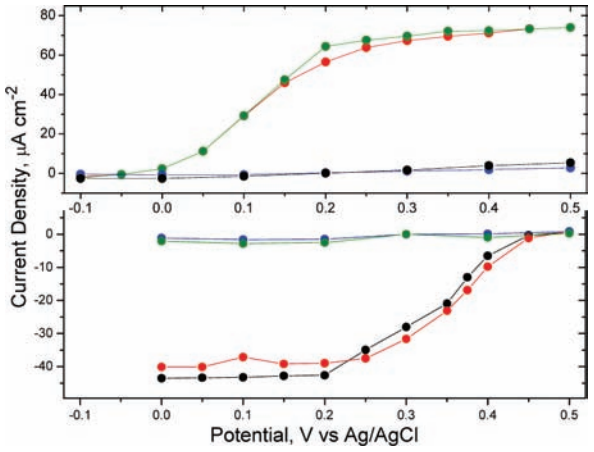

Figure 3. Polarization curves for the Tre|GOx|Os(dm-bpy)$_2$Cl carbon rod anode (top panel) and the BOD|Os(bpy)$_2$Cl|carbon rod cathode (bottom panel) in solutions of PBS exposed to air (black), 50 mM Tr in PBS exposed to air (red), N_2-purged PBS (blue), and 50 mM Tr in N_2-purged PBS (green).

(green curve). Also displayed therein for comparison is a curve recorded in the presence of O_2 from the air (red), which yielded a small but nevertheless measurable decrease in current density over the potential range 0.15−0.4 V. As expected, the current was found to be virtually zero in solutions devoid of Tr (blue and black curves).

In the case of the BOD|Os(bpy)$_2$Cl|carbon rod electrode (Figure 3, bottom panel) in the same medium, the onset potential for O_2 reduction occurred at ca. 0.45 V vs Ag/AgCl. Addition of Tr to this solution led to a small decrease in the current, ca. 3 $\mu A/cm^2$. As expected, negligible currents were observed upon removal of O_2 from the medium.

Experiments aimed at determining the power density of the Tr|O_2 fuel cell in 50 mM Tr in PBS in air were repeated five times, using freshly prepared enzymatic electrodes and Tr solutions for each trial. The results of a statistical analysis of the data (red circles in Figure 4) yielded a maximum power density

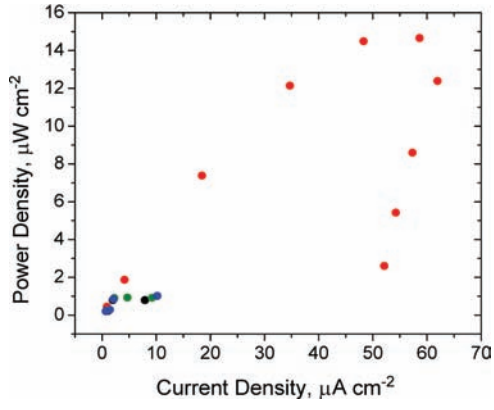

Figure 4. Power density vs current density measured in 50 mM Tr in PBS in air, averaged over five independent trials (red ●). Other symbols are defined in the caption of Figure 3.

of 15 ± 0.6 $\mu W/cm^2$ at 0.25 V and a maximum current density of 65 ± 6 $\mu A/cm^2$. When one or both of the fuels (Tr or O_2) were removed, the power output decreased nearly to zero. Very similar results were obtained in 25 and 100 mM Tr (see Figure S2), indicating that operation of the biofuel cell was not controlled

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by the amount of Tr in the medium within the range of concentrations examined.

Shown in Figure 5 is the average of four totally independent measurements of power density versus applied voltage collected

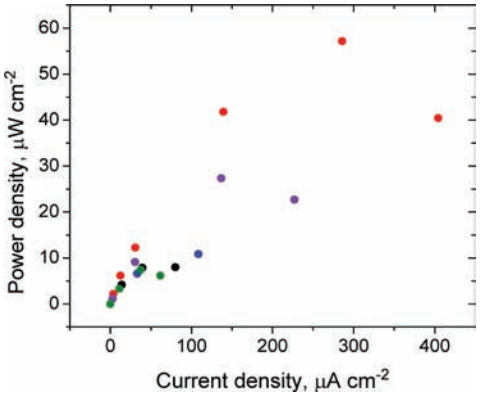

Figure 5. Power density vs current density for the Tr|O_2 fuel cell measured in *B. discoidalis*. The average of four independent trials using different electrodes is shown (red). Results of control experiments are also included: bare carbon (black), osmium polymer only (blue), no GOx in the anode (green points), and no Tre in the anode (purple).

with the biofuel cell implanted in the abdomen of *B. discoidalis* using the same methodology employed for the in vitro experiments (red circles). It is important to note that the Tr concentration in this insect is on the order of 30 mM.[11] As indicated, the peak power density delivered by this device at first reached 55 ± 6 μW/cm^2 at 0.2 V, and the maximum current density was 460 ± 70 μA/cm^2. Over the next 45 min (not shown here) this value increased to ca. 100 μW/cm^2 and then decreased during the next 2 h by 6.5%. Very small responses were observed when the electrodes were replaced with bare carbon (black symbols) and also when either GOx (green) or all enzymes (blue) were excluded from the anode. However, anodes incorporating only GOx (purple) yielded a significant response due to Gl in the hemolymph of this insect, which is present at a concentration of ca. 5 mM.[12]

Further proof of the viability of this biofuel cell in yet a different living organism was obtained by implanting the electrodes into the stipe of a thoroughly hydrated (in PBS) Shiitake mushroom. The peak power density in this case was 1.21 ± 0.07 μW/cm^2 at 0.2 V, and the maximum current density was 8.6 ± 0.7 μA/cm^2 (Figure 6). The use of three Tr electrodes connected together increased the peak power density to 1.9 μW/cm^2 (Figure S3), implying that the power output is primarily controlled by reactions at the anode. As was the case with *B. discoidalis*, exclusion of Tre from the anode yielded a lower albeit significant response. This is not surprising, as the amount of Gl in the mushroom is almost the same as the amount of Tr (i.e., ca. 30 mg/g dry weight).[13]

It may be concluded that the biofuel cell described in this work can indeed convert trehalose contained within the insect and oxygen from the air into electricity that, in principle, could be collected and stored and subsequently used to power a variety of microdevices. It is envisioned that because of the rather low currents this device can generate, any microdevice requiring high power could operate only intermittently. Efforts to design a compact, more versatile Tr|O_2 biofuel cell that could be implanted using a single small incision into the insect body

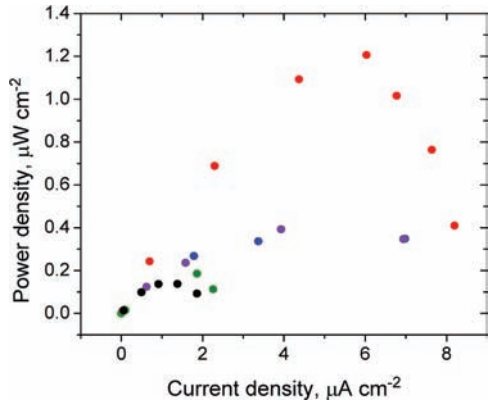

Figure 6. Power density vs current density for the Tr|O_2 fuel cell measured in a Shiitake mushroom averaged over three independent trials using different electrodes (red). Also included are the results of the control experiments: bare carbon (black), osmium polymer only (blue), no GOx in the anode (green), and no Tre in the anode (purple).

are currently underway, and the results will be presented in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Results of additional experiments, including voltammetry of the osmium polymers, biofuel cell tests in solution, and mushroom studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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