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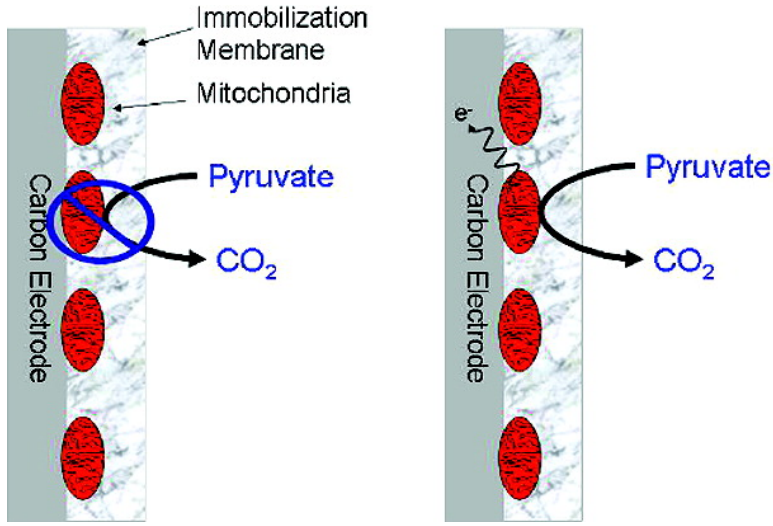

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Nitroaromatic Actuation of Mitochondrial Bioelectrocatalysis for Self-Powered Explosive Sensors

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Ultrasensitive explosive detection has been a popular area of research over the past decade in an effort to better combat terrorism.[1] Common techniques for the detection of explosives have ranged diversely from dogs' acute sense of smell to analytical techniques from ion mobility spectrometry to gas chromatography. Capabilities in explosive detection today are limited by cost, complexity, and bulkiness. Efforts in current research in this area have used selective polymers for more sensitive explosive detection via fluorescent chemosensors.[2−4] This paper focuses on the use of a mitochondria-catalyzed biofuel cell for explosive sensing. The biofuel cell contains two electrodes: a cathode that will reduce oxygen to water and a bioanode that will oxidize pyruvate in a fuel container to carbon dioxide and water. This paper investigates the unique ability of mitochondria on the bioanode to attenuate their ability to oxidize the pyruvate substrate/fuel and produce current in the presence or absence of nitroaromatic explosives. Although bioelectrocatalysis has been considered for a number of biological substrate sensors (i.e glucose,[5] ethanol,[6] hydrogen peroxide[7]), this is the first use of intact and viable mitochondria rather than individual enzymes of the mitochondria or complete microbial cells for bioelectrocatalysis in sensors. The mitochondria bioelectrocatalysis allows for the investigation of unique attenuation mechanisms that are not present with enzyme systems. Bioelectrocatalysis would be beneficial, because it combines the advantages of low cost, simplicity, and ability to detect small quantities.

Mitochondria are the "powerhouse" of the cell and contain enzymes and enzymatic pathways that can completely oxidize biofuel sources, such as pyruvate. Recently, our research group has developed a biofuel cell that employs mitochondria as the anode catalyst which is responsible for oxidizing the fuel.[8] As with any fuel cell, this fuel cell will only produce electrical energy in the presence of fuel, but mitochondria are different than most traditional catalysts in that there are a number of inhibitors (e.g., oligomycin antibiotic) that can stop mitochondria functioning,[9] which in turn will stop the electrical power generation. Oligomycin is a phosphorylation inhibitor that blocks ATP synthesis by the F0/F1 ATPase of the mitochondria. It shuts down the metabolic functioning of the intact mitochondria. However, this mitochondrial function (metabolism of pyruvate fuel) can be restored by the addition of a decoupler.[10] **It is important to note that nitroaromatic compounds are common explosive materials, but are also selective decouplers of mitochondrial inhibition.** Therefore, this paper employs inhibited mitochondria at carbon bioanodes in biofuel cells for nitroaromatic explosive sensing. The sensor will not produce significant power in the absence of the nitroaromatic, but after the nitroaromatic explosive is present, it will decouple the inhibited mitochondria and allow for the mitochondria to catalyze the oxidation of pyruvate to carbon dioxide, as shown in Figure 1. This oxidation at the anode of a biofuel cell in combination with the cathodic reduction of oxygen to water will produce power that can be measured or used to signal the presence of the explosive.

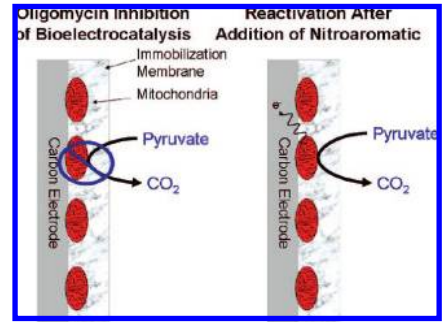

Figure 1. Bioelectrocatalysis mechanism of oligomycin inhibition of pyruvate oxidation at a mitochondria-modified electrode and nitroaromatic decoupling of a mitochondria-modified electrode.

Mitochondria can be isolated from a variety of sources, such as tobacco pollen, beef, and rat liver. We focus here on mitochondria extracted from tubers, since researchers have already shown it is possible to both extract them intact[11] and immobilize them onto a carbon electrode by casting them in a quaternary ammonium bromide-modified Nafion membrane.[8] These mitochondria modified electrodes have shown the ability to completely oxidize pyruvate and to transfer electrons between the mitochondria and the electrode without the addition of external mediators. This has allowed for their use in biofuel cells for power generation from pyruvate fuel via the Kreb's cycle enzymatic pathway.[8]

The mitochondria bioanode (area = 1 cm^2) was fabricated of E-Tek Toray carbon paper. The solution cast on the electrodes consisted of oligomycin treated mitochondria and modified Nafion as per the procedure discussed in the Supporting Information. After the mitochondria bioanode was dried, it was tested in a traditional biofuel cell test cell with an air breathing cathode. The physical test cell is fabricated as described in ref 12. The air-breathing cathode consisted of Nafion 112 fused together with a gas permeable ELAT electrode with 20% Pt on Vulcan XC-72. A 10 mL portion of fuel cell solution, consisting of 0.1 M sodium pyruvate and 6.0 M $NaNO_3$ in pH 7.15 buffer, was placed in the anode compartment.

The open circuit potential was allowed to level off for each biofuel cell before linear sweep techniques were used to obtain a polarization curve and power curve. After this measurement of inhibited performance, various concentrations of nitrobenzene were introduced to the fuel solution. Nitrobenzene was used as a model compound, because it has been employed frequently as a model compound for investigating the effects of explosives and their metabolites on cellular processes.[13]

After concentration optimization, we have inhibited the mitochondria's metabolism of pyruvate with 1 μM oligomycin. Figure 2 shows the power curve response for the pyruvate/air biofuel cells with oligomycin inhibition of the mitochondra and with nitrobenzyme decoupling. It is clear that the nitrobenzene can be used to attenuate the power response from the biofuel cell. However, when

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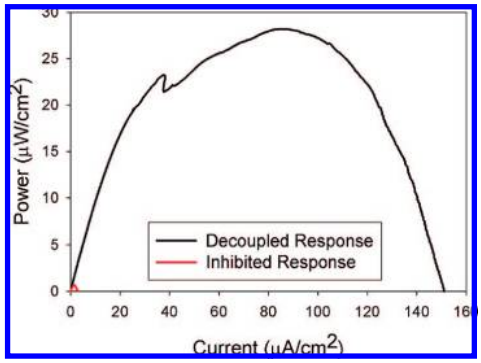

Figure 2. Representative power curves for 1 uM oligomycin inhibition and 1 uM nitrobenzene decoupling of a pyruvate/air biofuel cell in 100 mM pyruvate fuel at room temperature.

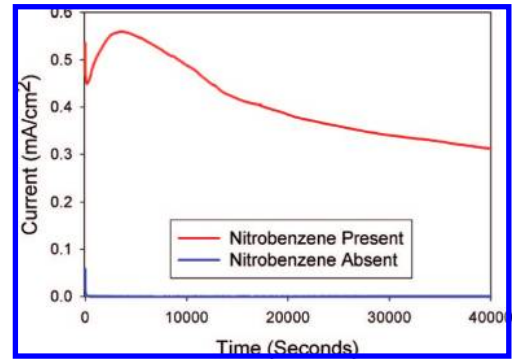

Figure 3. Amperometric curves for an oligomycin-inhibited mitochondrial electrode before and after addition of nitrobenzene to the biofuel cell at 0 V at room temperature.

concentration studies were done, it was found that this type of sensor is not a quantitative sensor, but a threshold sensor which is either on or off, but whose signal does not change with concentration. Concentrations of nitrobenzene from 1 mM to 1fM have been studied, and the power response is not statistically different from the response shown in Figure 2 for concentrations ranging from 1 mM to 1pM nitrobenzene. However, decoupling responses are not observed for concentrations below 1pM (LOD). The power response is a function of surface concentration of active mitochondria (similar to enzyme systems[14]), so future work will focus on improving immobilization and inhibition methods to improve sensitivity.

The inhibited mitochondria have shown a decrease of more than an order of magnitude in power compared to the uninhibited controls, and when reactivated with the nitroaromatic compound show powers not statistically different from the original uninhibited electrodes. The inhibited electrodes have power densities of $(6.43 \pm 5.49) \times 10^{-7}$ W/ cm^2. The decoupled electrodes have power densities of 0.0261 ± 0.0172 mW/cm^2. These experiments have demonstrated that mitochondria-modified electrodes can be used successfully to detect and signal when nitrobenzene is present.

Amperometry was employed on an inhibited electrode to study the time response to reactivation by introduction of nitrobenzene. It is shown in Figure 3 that introduction of nitrobenzene immediately decouples the mitochondria metabolism, but that maximum current density does not occur for over an hour. However, these high current densities sustain for a sufficient period of time to allow for sensor signaling (at least 10 hours).

In conclusion, the unique property of nitroaromatic compounds to selectively decouple the inhibition effects of oligomycin at mitochrondrial membranes was employed to develop a threshold sensor for nitroaromatics employing a mitochondrial-catalyzed pyruvate/air biofuel cell. This sensor could be used as a traditional electrochemical sensor or in the self-powered sensor design described by Katz and Willner.[15]

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Supporting Information Available: Mitochondria and polymer protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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