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Organelle-based biofuel cells: Immobilized mitochondria on carbon paper electrodes

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Abstract

This paper details the development of a mitochondria-based biofuel cell. We show that mitochondria can be immobilized at a carbon electrode surface and remain intact and viable. The electrode-bound mitochondria drive complete oxidation of pyruvate as shown by Carbon-13 NMR and serve as the anode of the biofuel cell where they convert the chemical energy in a biofuel (such as pyruvate) into electrical energy. These are the first organelle-based fuel cells. Researchers have previously used isolated enzymes and complete microbes for fuel cells, but this is the first evidence that organelles can support fuel cell-based energy conversion. These biofuel cells provide power densities of $0.203 \pm 0.014\,mW/cm^2$, which is in between the latest immobilized enzyme-based biofuel cells and microbial biofuel cells, while providing the efficiency of microbial biofuel cells.

Keywords: Mitochondria; Biofuel cell; Nafion; Bioanode; Pyruvate

1. Introduction

Mitochondria and fuel cells are both energy conversion matrices. Mitochondria, the power house of the cell, contain the enzymes and coenzymes that drive the Kreb's cycle and electron transport chain of metabolism, ensuring the complete oxidation of biofuels [1,2]. Mitochondria are found in the cytoplasm of most animals, plants, and fungi and are the organelle of a living cell that is responsible for energy conversion. This organelle contains the enzymes and coenzymes of the Kreb's cycle and the electron transfer chain, but unlike a complete cell, it has fewer fuel transport limitations due to smaller diffusion lengths, no biofilm formation, and no need to transport fuel across the cell wall. Therefore, the mitochondria can completely oxidize fuel at a faster rate. Complete oxidation is important in any energy conversion device to ensure that no toxic byproducts are produced as waste in the energy conversion device and also allows for higher energy densities. For instance, the energy density of ethanol is 8010 Wh/kg, but when performing only a single step oxidation of ethanol to acetaldehyde with alcohol dehydrogenase, the maximum theoretical energy density of the fuel cell is 1335 Wh/kg [3].

Biofuel cells are electrochemical devices that convert the chemical energy of a biofuel into electrical energy [4]. Biofuel cells are a type of fuel cell where a biocatalyst is used to convert the chemical energy of a fuel into electrical energy, instead of the metallic catalysts (typically platinum and platinum alloys) of a traditional fuel cell. Biofuel cells are normally divided into two categories: microbial biofuel cells and enzymatic biofuel cells. Microbial biofuel cells employ living cells to catalyze the oxidation of fuels at the anode surface. They have the advantage of being able to catalyze complete oxidation of biofuels and have long lifetimes (up to 3–5 years) [5,6], but are plagued by low power densities ($0.0010–0.09\,mW/cm^2$ [7–10]) due to slow transport of fuel across cellular membranes. Enzymatic biofuel cells employ enzymes to catalyze the oxidation of fuels at the anode surface. They have the advantage of higher power density ($1.65–4.1\,mW/cm^2$ [11] and references within), but are limited by incomplete oxidation of fuel and frequently low lifetimes. This paper details the use of an organelle to catalyze the oxidation of fuel at the electrode surface. These biofuel cells provide power densities between the latest immobilized enzyme-based biofuel cells [12,13,11] and microbial biofuel cells, while providing the lifetime and effi-

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ciency (complete oxidation of the fuel) of a microbial biofuel cell.

2. Experimental

2.1. Mitochondria extraction

Homogenization buffer was prepared and consisted of 100 mM Tris–HCl (pH8), 2.6 M NaCl, 50 mM ethylenediaminetetraacetic acid, 0.4% bovine serum albumin, 0.1% cysteine, and 28 mM dithiothreitol. The solution was prepared with the exception of the cysteine and the dithiothreitol, and then chilled to 4 °C. The cysteine and the dithiothreitol were mixed in immediately before use. Russet potatoes were quartered and immediately juiced in a Green Power GPT-E1303 juice extractor. The juice was collected in an equal amount of chilled homogenization buffer. For the remainder of the experiment, the mitochondrial solution was kept at 4 °C. The juice and buffer were centrifuged at 500 rpm for 10 min and the supernatant was poured into a new centrifuge tube. The precipitate was discarded. The supernatant was centrifuged at 2600 rpm for 10 min and then poured into new centrifuge tubes. This above centrifugation procedure was repeated once again but for 15 min. Then, the supernatant was centrifuged at 15,557 rpm for 15 min and the precipitate was collected. This precipitate contained the isolated mitochondria.

2.2. Preparation of the immobilization membrane

The immobilization membrane was prepared in a two-step process. In the first step, a 5% by wt. Nafion suspension was mixture-cast with 3-fold excess of tetrabutylammonium bromide into a weigh boat. The mixture was allowed to dry overnight in a low humidity environment. Then, the excess salt was extracted by soaking in 18 MΩ water overnight followed by rinsing and air drying. The dry membrane was then re-suspended in lower aliphatic alcohols.

2.3. Preparation of poly(methylene green) coated carbon paper electrodes

E-Tek Toray carbon paper was placed in a solution of 0.4 mM methylene green, 10 mM sodium borate, and 0.1 M sodium nitrate. The poly(methylene green) film was formed by 12 segments of cyclic voltammetry from −0.3 V to 1.2 V vs. Ag/AgCl reference electrode with a platinum mesh counter electrode, using a CH Instruments Model 650 potentiostat interfaced to a PC.

2.4. Immobilization of the mitochondria on Toray paper

The wet mitochondria precipitate was used directly. Wet precipitate (18.7 mg) was suspended in 1 ml of pH 7.15 phosphate buffer with 100 mM NaCl and 1 mg/ml ADP. 100 μl of mitochondria suspension was added to a vial, followed by the addition of 100 μl tetrabutylammonium bromide-modified Nafion suspension and then mixed on a vortex mixer for 15 s. 50 μl of the mitochondria/modified Nafion suspension was then pipetted onto 1 cm^2 pieces of both poly(methylene green) modified Toray paper (E-Tek, Somerset, NJ), and unmodified Toray paper. The electrodes were then allowed to dry in a vacuum desiccator for 15 min to quickly remove the ethanol from the casting solution. Then the electrodes were removed and allowed to finish drying in a refrigerator at 4 °C overnight.

2.5. Determination of NADH leaching

In order to determine if any NADH leached out of the modified Nafion membrane which would indicate that mitochondrial membrane lysis had occurred, an NADH assay was performed. For the assay, 100 μl of TBAB modified Nafion suspension was mixed with 100 μl of mitochondria stock suspension that contained 18 mg/ml mitochondria and 1 mg/ml of ADP in pH 7.40 phosphate buffer that contained 100 mM NaCl. The two solutions were mixed thoroughly on a vortex mixer for 30 s and then cast onto the bottom of cuvettes in 20 μl increments and allowed to dry in a low humidity environment. The cuvettes were then filled a pH 7.40 phosphate buffer solution that contained 100 mM NaCl. An initial absorbance measurement was taken with a UV spectrophotometer at wavelength 340 nm at time zero. Then the cuvettes were allowed to sit for 1 h and the measurement was taken again to determine if NADH had leached into the solution.

2.6. Determination of NADH production

A NADH assay was carried out in order to determine if NADH was being produced by free enzymes that would normally be contained within the mitochondria. If mitochondrial lysis had occurred, NADH should be produced from NAD^+ when pyruvate is present. For the assay, 100 μl of TBAB modified Nafion suspension was mixed with 100 μl of mitochondria stock suspension that contained 18 mg/ml mitochondria and 1 mg/ml of ADP in pH 7.40 phosphate buffer that contained 100 mM NaCl. The two solutions were mixed thoroughly on a vortex mixer for 30 s and then cast onto the bottom of cuvettes in 20 μl increments and allowed to dry in a low humidity environment. The cuvettes were then filled a pH 7.40 phosphate buffer solution that contained 100 mM NaCl, 1 mg/ml of NAD^+, and 50 mM pyruvate. An initial absorbance measurement was taken with a UV spectrophotometer at wavelength 340 nm at time zero. Then the cuvettes were allowed to sit for 18 h and the measurement was taken again to determine, if NADH had been produced.

2.7. Mediatorless bioanode experiment

A two-part experiment was performed to determine if the immobilized mitochondria would still be employed on a bioanode without a mediator. In the first experiment, 5 mg of COOH-modified multiwalled carbon nanotubes (Cheaptubes, Inc.) was dispersed in 200 μl of deionized water by use of 2 mm ceramic mixing beads on a vortex mixer for 5 min. COOH-modified carbon nanotubes were used, because they dispersed in water better than unmodified carbon nanotubes. Then 100 μl of mitochondria stock solution was added and mixed on a vortex