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Oxidative bioelectrocatalysis: From natural metabolic pathways to synthetic metabolons and minimal enzyme cascades☆

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ABSTRACT

Anodic bioelectrodes for biofuel cells are more complex than cathodic bioelectrodes for biofuel cells, because laccase and bilirubin oxidase can individually catalyze four electron reduction of oxygen to water, whereas most anodic enzymes only do a single two electron oxidation of a complex fuel (i.e. glucose oxidase oxidizing glucose to gluconolactone while generating 2 electrons of the total 24 electrons), so enzyme cascades are typically needed for complete oxidation of the fuel. This review article will discuss the lessons learned from natural metabolic pathways about multi-step oxidation and how those lessons have been applied to minimal or artificial enzyme cascades. This article is part of a Special Issue entitled Biodesign for Bioenergetics — the design and engineering of electronic transfer cofactors, proteins and protein networks, edited by Ronald L. Koder and J.L. Ross Anderson.

1. Introduction

Enzymatic fuel cells are a type of bioelectronic device focused on bioenergetic conversion of chemical energy to electrical energy via the utilization of enzymes as electrocatalysts on the anode and/or the cathode. They were first discovered in the 1960s when a glucose oxidase anode was combined with a platinum cathode to produce a positive open circuit potential [1]. That early work invigorated a field that was primarily focused on harnessing electrical energy from biofuels present in living organisms (i.e. glucose in the blood stream) [2–5]. However, as the field of enzymatic fuel cells (often called biofuel cells) advanced, it became clear that there were alternative applications for enzymatic fuel cells. As stability and power density/current density performance increased, there became an interest in biofuel cells for portable power applications [6]. This interest spurred engineering of anodes from the original single oxidoreductase enzyme systems to cascades of enzymes responsible for catalyzing sequential reactions. This started with early work by Palmore and Whitesides, which incorporated the three enzyme cascade (NAD-dependent alcohol dehydrogenase, NAD-dependent aldehyde dehydrogenase, and NAD-dependent formate dehydrogenase) into the bioanode compartment of a methanol/oxygen biofuel cell [7]. This work showed the increase in performance observed with deeper degrees of oxidation of the fuel and postulated an increase in efficiency that would lead to increased energy density of biofuel cells/biobatteries. This early inspiration in enzyme cascades has led to a variety of research efforts in enzyme cascades that will be discussed below. They include natural metabolic pathways, natural metabolons, synthetic metabolons, and minimal enzyme cascades.

2. Natural metabolic pathways

Metabolic pathways are cascades of enzymes responsible for energy conversion in the living organism (oxidation of food/fuel or production of energetic molecules in the cell). These pathways include the glycolytic pathway, the Krebs cycle, the pentose phosphate pathways, fatty acid metabolism, amino sugar metabolism, purine biosynthesis, amino acid synthesis, and sucrose/starch metabolism. These pathways are responsible for catalyzing sequential reactions and therefore have been of interest to the field of bioelectrochemistry, since Suzuki and coworkers first used invertase and glucose oxidase to electrochemically monitor sucrose concentrations [8]. This novel experiment was not a bioanode for a biofuel cell, but it showed the possibility of utilizing sequential enzymes of natural pathways in a bioelectrochemical application. Over the last decade, there has been a wealth of evaluation of natural metabolic pathways for fuel oxidation.

The most common natural metabolic pathway evaluated for bioelectrocatalysis is the methanol metabolic pathway utilizing alcohol dehydrogenase to oxidize methanol to formaldehyde, aldehyde dehydrogenase to oxidize formaldehyde to formate, and formate dehydrogenase to oxidize formate to carbon dioxide. This enzyme cascade has been used for oxidation of methanol in biofuel cells [7,9–11], as well as reduction of carbon dioxide to methanol in bioelectrosynthetic processes [12]. This pathway has been interesting for study, because it contains only three enzymes, all three enzymes are oxidoreductase enzymes capable of generating electrons, and the three enzymes have

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quite different specific activities. The results of this work have shown that the enzyme cascade functions to completely oxidize methanol or to produce methanol from carbon dioxide and that degree of oxidation alters biofuel cell/biobattery performance (current density and power density). It also provided the system for the first work at modeling an enzyme cascade at an electrode for bioelectrocatalysis [9]. This model showed that there are many engineering parameters to consider when designing an enzyme cascade-based bioanode, including transport of fuel and cofactor, individual enzyme kinetics, and cofactor electrocatalysis.

The Krebs cycle (commonly called the citric acid cycle) has been evaluated as a classic example of a natural metabolic pathway. Sokic-Lazic et al. evaluated the fabrication of NAD(P)-dependent dehydrogenase-based bioanodes containing the Krebs cycle enzymes in ethanol, pyruvate, and lactate biofuel cells [13–15]. Since oxidoreductase enzymes in the Krebs cycle are NAD(P)-dependent, then a mediated bioanode is necessary to transfer the electrons from the oxidoreductase enzyme to the electrode surface. The diffusional cofactor NAD(P) is used at the mediator, but it has poor electrochemistry on carbon current collectors (i.e. carbon cloth, carbon papers, and carbon felt). Therefore, an additional electrocatalyst is needed to decrease the overpotential of NAD(P)H oxidation. The electrocatalyst used in this study was methylene green, which was electropolymerized into a conducting polymer layer on the carbon current collector. As shown in Fig. 1, these studies show that the degree of oxidation improves the performance (current density and power density) of a biofuel cell. Theoretically, we would expect as we add additional oxidoreductase enzymes we would see an increase in performance that is a characteristic of the increase degree of oxidation (i.e. one oxidoreductase enzymes gives 2 electrons, but three oxidoreductase enzymes give 6 electrons, so we would expect a 3 fold increase in current and power density). However, due to the metabolic control within the Krebs cycle, performance is quite low until the entire cascade/cycle is immobilized on the electrode surface and then large enhancements (26 fold) in performance are observed due to venting of carbon dioxide and elimination of the build-up of individual byproducts of the cycle that can inhibit different enzymes of the cycle. Therefore, this study showed that natural enzyme cascades can improve efficiency of transformation or in the case of a biofuel cell efficiency of current generation, but due to the metabolic control present in many of these natural pathways, then a complete pathway is needed for optimal performance.

Beilke et al. also evaluated the natural glycolytic process in-vitro [16, 17]. However, the glycolytic pathway is not particularly interesting from a bioelectrochemical perspective, because it only contains one oxidoreductase (electron producing) enzyme, whereas the Krebs cycle contains 5 oxidoreductase enzymes. Therefore, it showed the importance of high oxidoreductase enzyme to total enzyme ratios to ensure high performance (i.e. at least 50% of the enzyme on the electrode needs to be an oxidoreductase enzyme). Beilke et al. had an interest in combining the glycolytic pathway cascade and the Krebs cycle cascade together in a bioanode, but due to the incompatibility of two enzyme systems, no single immobilization material was discovered that could immobilize both sets of enzymes at a single bioanode.

Beyond these three metabolic pathways, there are several other examples of subsets of metabolic pathways used to study enzyme cascades for bioelectrocatalysis. For instance, Moehlenbrock et al. evaluated the cascade of two enzymes of the pentose phosphate pathway to understand the effects of sequential enzyme proximity on bioelectrocatalytic performance [18], while Nguyen et al. studied the two enzyme sucrose metabolic system (invertase and glucose oxidase) while also evaluating the effect of sequential enzyme proximity [19]. Hickey et al. evaluated a slightly different sucrose sub-metabolic system utilizing invertase, glucose oxidase, and fructose dehydrogenase [20] to get 4 electrons per molecule of sucrose. All of these natural metabolic pathways (either as complete pathways or subsets of metabolic pathways) led researchers to realize the importance of enzyme cascades for deep or complete oxidation of biofuels.

3. Metabolons

Paul Srere et al. postulated that most metabolic pathway enzymes exist in supercomplexes that provide proximity between sequential enzyme active sites and may even channel substrate between active sites [21–23]. He termed these supercomplexes as metabolons. After evaluating the performance of Krebs cycle enzyme cascades in biofuel cells and comparing them to mitochondrial bioanodes in biofuel cells, it was clear that for improved performance there was a need for structural proximity between neighboring enzymes. Fig. 2 shows a representative set of power curves for pyruvate/air biofuel cells where the bioanode is either an intact mitochondrial electrode or a lysed mitochondrial electrode, where the loading and therefore the volumetric catalytic activity are the same on both bioanodes [24]. This data makes it quite clear that the structure in the mitochondria is different than the structure in the lysed mitochondria and that the structure of the Krebs cycle enzymes in the mitochondria is critical to high metabolic flux.

This work on mitochondrial bioelectrocatalysis led to an attempt to isolate and purify intact metabolons for bioelectrocatalysis. Moehlenbrock et al. developed a technique for crosslinking metabolons in-vivo in isolated mitochondria with glutaraldehyde and dimethyl suberimidate, lysing

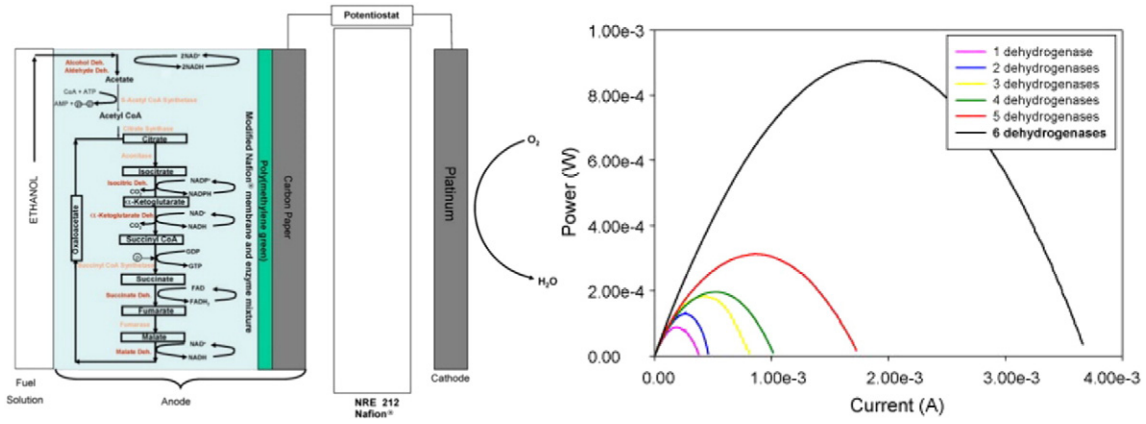

Fig. 1. (Left) Schematic of the complete biofuel cell. Ethanol is oxidized serving as the fuel source at the anode (dark red lettering represents dehydrogenase enzymes, whereas the light red/pink lettering represents other non-energy producing enzymes). Oxygen is reduced to water at the 20% Pt on carbon GDE cathode. Potentiostat is used to measure open circuit potential and linear sweep polarization curves. (Right) Representative power curves of ethanol/air biofuel cells with different enzymatic cascades at the bioanode. All solutions are 100 mM ethanol and 1 mM NAD^+ in pH 7.5 phosphate buffer and all measurements were made at room temperature. Anode electrode area is 1 cm^2. Reproduced with permission from Elsevier [13].