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Investigation of a Deoxyribozyme As a Biofuel Cell Catalyst

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Supporting Information

ABSTRACT: We examined the ability of a previously identified peroxidase deoxyribozyme to be employed as a catalyst in biofuel cells, as a possible replacement for oxidoreductase proteins. We constructed a biocathode using a covalently linked version of the peroxidase deoxyribozyme-hemin complex and successfully paired it with a glucose dehydrogenase-modified bioanode for power production.

Current microbial and enzymatic biofuel cells suffer from limitations directly related to the choice of catalyst. Microbial fuel cells completely oxidize fuels and have long operational lifetimes, but suffer from low power densities and transport problems due to the cellular membrane.[1−3] Enzymatic fuel cells eliminate the problems caused by the cellular membrane by using isolated enzymes. The resulting fuel cells have higher power densities, but the isolated enzymes can be unstable, limiting the active lifetime of the cell.[4] Despite the advances made in biofuel cells, challenges still remain and new approaches are needed.[1,5−8]

Another class of biocatalysts has been identified and studied in recent years. Ribozymes and deoxyribozymes (DNAzymes) are nucleic acid sequences, RNA and DNA, respectively, with catalytic abilities.[9−11] DNA's stability makes the identification of deoxyribozymes particularly interesting for a variety of applications and an iterative process known as in vitro selection has led to the discovery of deoxyribozymes capable of catalyzing a wide range of reactions.[12] Some identified ribozymes and deoxyribozymes catalyze oxidation and reduction reactions.[13−15] The reaction conditions used for an in vitro selection experiment can be varied from physiological conditions, allowing for the identification of customized catalysts for specific applications. Thus, it would be possible to identify deoxyribozymes that mimic oxidoreductase protein enzymes and operate under biofuel cell conditions. Such oxidoreductase deoxyribozymes could overcome some of the challenges faced when using proteins for electrode construction.

We propose that one identified deoxyribozyme could potentially be useful in biofuel cells. A G-quadruplex-forming DNA sequence was shown to bind hemin and, in doing so, enhanced the peroxidase activity of hemin.[15,16] While this peroxidase deoxyribozyme has been used successfully in sensors,[17,18] its use as a catalyst in a biofuel cell has not been investigated. The peroxidase deoxyribozyme could replace an enzyme previously used in biofuel cells, horseradish peroxidase,[19,20] at a biocathode and would demonstrate the ability of deoxyribozymes to serve as a biofuel cell catalyst. The smaller size of the peroxidase deoxyribozyme−hemin complex (molecular weight ∼11 000) compared to horseradish peroxidase (molecular weight ∼44 000) could also lead to an increased amount of catalyst on the electrode surface.

Previous work with this peroxidase deoxyribozyme on electrodes has typically taken one of two approaches for immobilization. The peroxidase deoxyribozyme has been immobilized on a gold electrode via a thiol modification on the DNA oligonucleotide.[21,22] While this thiol−gold interaction allows for convenient attachment to gold surfaces,[17,23] these interactions have also been shown to undergo oxidation when exposed to air, specifically ozone in the air.[24−27] In other reports, no specific attachment chemistry is used to immobilize the complex and a solution of the peroxidase deoxyribozyme was applied directly to a pyrolytic graphite electrode to create a film.[28,29] While these films are stable for the reported ligand binding studies, we devised a different derivatization strategy to stabilize the peroxidase deoxyribozyme−hemin complex for long-term use in a biofuel cell environment.

A recent study reported the construction of a covalently linked peroxidase deoxyribozyme−hemin complex that showed comparable activity to a noncovalent complex.[30] Covalently attaching hemin to the peroxidase deoxyribozyme would ensure both components of the complex are associated with the electrode and could enhance the stability of the complex by preventing dissociation of the hemin. Thus, we utilized a covalently linked peroxidase deoxyribozyme−hemin complex for electrode construction. As a foundation for the complex, we turned to carboxylate functionalized multiwalled carbon nanotubes (MWCNTs). Carbon nanotubes have become an important tool in the development of new electrode designs[31] and the carboxylic acid functionality allows for the coupling of a short, amino-modified DNA oligonucleotide which will be used to tether the peroxidase deoxyribozyme−hemin complex to the MWCNTs. The complex is joined to the tethering oligonucleotide via splint ligation on the electrode surface (Figure S1).[32−34] To improve the stability of the functionalized electrode, we investigated different polymer coatings, including Nafion,[35] biosilica,[36] and polystyrene sulfonate.[37] We used cyclic voltammetry (CV) to compare the effect of different polymer coatings and to identify which polymer, if any, would not interfere with peroxidase deoxyribozyme activity (Figure 1, top panel).

CV allowed us to study the electrochemical response of the peroxidase deoxyribozyme−hemin complex electrodes to

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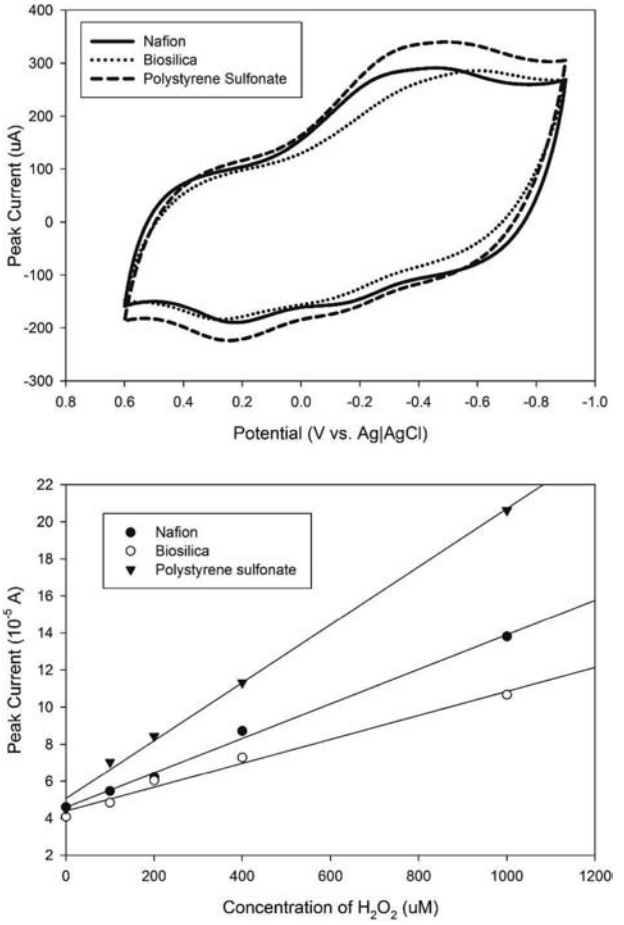

Figure 1. Comparison of polymer-coated, peroxidase deoxyribozyme−hemin complex electrodes. (Top) Representative cyclic voltammograms in the presence of 1 mM H_2O_2. (Bottom) Correlation of peak current to H_2O_2. GCEs were functionalized with the peroxidase deoxyribozyme−hemin complex and coated with Nafion, biosilica, or polystyrene sulfonate. Scans were conducted with increasing concentrations of H_2O_2 in 50 mM HEPES-NH_4OH, pH 7.4, under nitrogen. The current was measured between 0.6 and −0.9 V with a scan rate of 100 mV/s.

increasing concentrations of H_2O_2, as shown in Figure 1. The peroxidase deoxyribozyme-hemin complex is active regardless of which polymer coating was used during electrode preparation. As shown in Figure 1, bottom panel, the deoxyribozyme was active when immobilized in Nafion and polystyrene sulfonate. Activity (slope of current versus concentration plot) was reduced in the presence of biosilica. Biosilica is prepared with sodium phosphate buffer, which has been observed to decrease peroxidase deoxyribozyme activity by ourselves (data not shown) and others.[15] We also found the MWCNT foundation was less stable when coated with biosilica (data not shown), so we did not pursue this polymer further. While Nafion also supported activity, a larger current response was consistently obtained with polystyrene sulfonate coatings and further studies were conducted only with polystyrene sulfonate-coated electrodes.

To ensure that the current response in the presence of H_2O_2 was due to the peroxidase deoxyribozyme−hemin complex and not to the MWCNTs or the tethering DNA, we investigated a series of control electrodes (Figure S3). These controls involved electrodes representing each of the steps required for

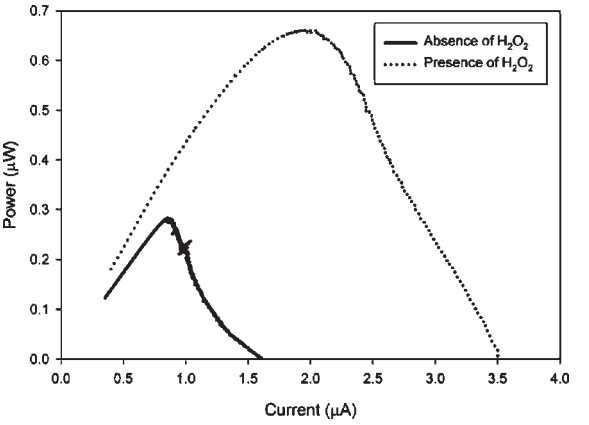

Figure 2. Comparison of the power curves for a biofuel cell using the peroxidase deoxyribozyme−hemin complex as the catalyst on the biocathode in the presence and absence of 1 mM H_2O_2.

attaching the peroxidase deoxyribozyme−hemin complex to the electrode. We also investigated an electrode functionalized with a hemin-linked, unstructured DNA sequence. This "tethered" hemin electrode allowed us to determine the activity enhancement for the peroxidase deoxyribozyme−hemin complex relative to hemin alone. The current increase is due to the activity of the peroxidase deoxyribozyme−hemin complex (Figure S3). While our control electrodes with MWCNTs alone and MWCNTs functionalized with the tethering DNA show increased current in the presence of 1 mM H_2O_2 relative to an electrode coated with polystyrene sulfonate only, the current generated is not the same level obtained when the catalyst, the peroxidase deoxyribozyme−hemin complex, is present. The peroxidase deoxyribozyme−hemin complex also continues to enhance the peroxidase activity relative to hemin alone (Figure S3).

Having demonstrated the stability and the production of current with the peroxidase deoxyribozyme−hemin complex, we prepared a biofuel cell coupling a bioanode that consisted of a Toray carbon paper electrode functionalized with glucose dehydrogenase with our deoxyribozyme-functionalized biocathode (Figure S2). The bioanode has greater than an order of magnitude higher surface area to ensure that the system is biocathode limited. The power generated by the peroxidase deoxyribozyme−hemin complex in the presence of 1 mM H_2O_2 is 2.27-fold larger than the power without H_2O_2, as shown in the representative power curves in Figure 2 and in Table 1. The current also increases 2.15-fold in the presence of peroxide showing catalytic activity for peroxide. For biocathodes with the tethered hemin, the current increases in the presence of H_2O_2, but there is no statistical change in the power as shown in Table 1. It is also important to note that there is a statistical increase in open circuit potential with peroxide for the peroxidase deoxyribozyme−hemin complex, but no statistical difference for the unstructured DNA. Overall, this biofuel cell data shows the applicability for the peroxidase deoxyribozyme−hemin complex to be employed as a direct electron transfer bioelectrocatalyst for biofuel cells.

In conclusion, we have demonstrated the ability of the peroxidase deoxyribozyme−hemin complex to serve as a catalyst in a biofuel cell. We have devised a strategy to construct an electrode using MWCNTs as a stable foundation for the

Table 1. Open Current Potentials, Maximum Current Density, and Maximum Power Density for Glucose/Peroxide Biofuel Cells Employing a Glucose Dehydrogenase Bioanode and a Peroxidase Deoxyribozyme−Hemin Complex Biocathode

	peroxidase deoxyribozyme−hemin complex	unstructured DNA
	0 mM H_2O_2	
Open current potential (V)	0.341 ± 0.017	0.391 ± 0.022
Maximum current density ($\mu A/cm^2$)	22.8 ± 0.4	28.1 ± 2.1
Maximum power density ($\mu W/cm^2$)	4.12 ± 0.29	4.87 ± 0.55
	1 mM H_2O_2	
Open current potential (V)	0.437 ± 0.031	0.373 ± 0.009
Maximum current density ($\mu A/cm^2$)	49.2 ± 2.5	52.5 ± 1.8
Maximum power density ($\mu W/cm^2$)	9.37 ± 2.66	5.02 ± 0.44

covalent linkage of the peroxidase deoxyribozyme−hemin complex and have shown that a polymer, polystyrene sulfonate, provides a nondisruptive environment for the deoxyribozyme. The maximum power densities for literature-based protein-based glucose/hydrogen peroxide biofuel cells are 5.12,[38] 3.7,[20] 0.15,[19] and 8 $\mu W/cm^2$,[39] compared to 9.37 ± 2.66 $\mu W/cm^2$ for the deoxyribozyme-based glucose/hydrogen peroxide biofuel cell. More importantly, the open circuit potential of 437 mV for the deoxyribozyme system is higher than any of the literature for protein systems, which range from 220 mV[19] to 366 mV.[38] This proof of concept study shows deoxyribozymes can be employed as catalysts in biofuel cells. The identification of new oxidoreductase deoxyribozymes using in vitro selection conditions that mimic desired fuel cell reaction conditions will provide customized biofuel cell catalysts that are not impeded by nonphysiological conditions. The small size of deoxyribozymes relative to proteins and the ease with which they can be modified for immobilization will overcome the challenges of enzyme immobilization and potentially improve catalyst density.

■ ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures, cyclic voltammeric studies of control electrodes. This material is available free of charge via the Internet at http://pubs.acs.org.

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