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Biofuel cell controlled by enzyme logic network — Approaching physiologically regulated devices

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ABSTRACT

A "smart" biofuel cell switchable ON and OFF upon application of several chemical signals processed by an enzyme logic network was designed. The biocomputing system performing logic operations on the input signals was composed of four enzymes: alcohol dehydrogenase (ADH), amyloglucosidase (AGS), invertase (INV) and glucose dehydrogenase (GDH). These enzymes were activated by different combinations of chemical input signals: NADH, acetaldehyde, maltose and sucrose. The sequence of biochemical reactions catalyzed by the enzymes models a logic network composed of concatenated AND/OR gates. Upon application of specific "successful" patterns of the chemical input signals, the cascade of biochemical reactions resulted in the formation of gluconic acid, thus producing acidic pH in the solution. This resulted in the activation of a pH-sensitive redox-polymer-modified cathode in the biofuel cell, thus, switching ON the entire cell and dramatically increasing its power output. Application of another chemical signal (urea in the presence of urease) resulted in the return to the initial neutral pH value, when the O_2-reducing cathode and the entire cell are in the mute state. The reversible activation–inactivation of the biofuel cell was controlled by the enzymatic reactions logically processing a number of chemical input signals applied in different combinations. The studied biofuel cell exemplifies a new kind of bioelectronic device where the bioelectronic function is controlled by a biocomputing system. Such devices will provide a new dimension in bioelectronics and biocomputing benefiting from the integration of both concepts.

1. Introduction

Biofuel cells based either on enzyme biocatalyzed reactions or on microbial cells are promising future alternative sources of sustainable electrical energy [1–4]. Miniaturized biofuel cells based on enzyme-catalytic electrodes are considered as implantable sources of energy for various biomedical applications [5–7]. Integration of the miniature biofuel cells with various implantable bioelectronic devices requires their regulated operation controlled by physiological/medical needs. The biofuel cells must be switchable and tunable releasing electrical power on demand. This requires a novel approach to design "smart" biofuel cells accepting information from the biological environment and adjusting the electrical power production to the needs of the body. The system should allow collection of biochemical signals, processing of the obtained information, making decision and switching / tuning the biofuel cell activity. This could be achieved by integration of biocomputing/logic systems [8] with switchable/tunable biocatalytic electrodes [9] in a biofuel cell. Recently emerged research area of the enzyme logic systems has already resulted in the design of various logic gates (AND, OR, XOR, etc.) [10–13] and their networks composed of several concatenated logic gates [14,15] processing biochemical information. On the other side, switchable tunable biofuel cells controlled by external electrical [16] or magnetic [17] signals were reported recently. Concerted operation of the information processing units and the biofuel cell producing electrical energy requires interfacing of the biocomputing-enzyme logic systems with biocatalytic electrodes in the cell. Some examples of the interfacing between the enzyme logic gates with biocatalytic electrodes [18,19] and other bioelectronic devices [20] were reported recently. A biofuel cell composed of switchable electrodes controlled by a single AND/OR logic operation performed by an enzyme system was designed upon integration of the enzyme-information processing system and enzyme-power producing electrodes [21]. However, the most important feature of the enzyme logic systems is the ability to scale up their complexity [22] integrating single logic gates into complex logic networks similarly to the natural biochemical pathways. The very first example of an electrochemical interface functionally controlled by an enzyme logic network composed of many logic gates was reported recently [23]. The present paper is aimed one step forward to integrate an enzyme logic network with a biofuel cell illustrating the concept of "smart" biofuel cells logically responding to their biochemical environment.

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2. Experimental section

2.1. Chemicals and materials

The enzymes for the bioelectrocatalytic electrodes and logic operations were obtained from Sigma-Aldrich or Fluka and used without further purification: glucose oxidase (GOx) from *Aspergillus niger*, type X-S (E.C. 1.1.3.4); laccase (Lac) from *Trametes versicolor* (E.C. 1.10.3.2), alcohol dehydrogenase (ADH) from baker's yeast (E.C. 1.1.1.1), glucose dehydrogenase (GDH) from *Pseudomonas sp.* (E.C. 1.1.1.47), amyloglucosidase (AGS) from *Aspergillus niger* (E.C. 3.2.1.3), invertase (INV) from baker's yeast (E.C. 3.2.1.26) and urease (Ur) from jack beans (E.C. 3.5.1.5). Other chemicals purchased were analytical quality and used as supplied. From Sigma-Aldrich: β-nicotinamide adenine dinucleotide, reduced dipotassium salt (NADH), >95% purity; β-D-(+)-glucose, 99.5% GC; D-(+)-maltose monohydrate, SigmaUltra, >99%; sucrose, ACS reagent; urea, ACS reagent 99–100%; 4,4′-dimethoxy-2,2′-bipyridine (dmo-bpy); poly(4-vinyl pyridine) (P4VP, M.W. 160 kDa). Acetaldehyde, technical grade, was purchased from Eastman. Methylene blue was from Riedel-de Haën and $(NH_4)_2OsCl_6$ from Alfa Aesar. Bromomethyldimethylchlorosilane was from Gelest. Ultrapure water (18.2 MΩ cm^{-1}) from NANOpure Diamond (Barnstead) source was used in all of the experiments. ITO conductive glass (20 ± 5 Ω/sq) was purchased from Aldrich and used as an electrode material. Synthesis of $Os(dmo\text{-}bpy)_2Cl_2$ was performed according to the published procedure [24]. P4VP was functionalized with $Os(dmo\text{-}bpy)_2$ pendant groups in a solution and then the redox polymer was grafted onto the ITO electrode surface according to the procedure published in details elsewhere [25]. Briefly, the ITO electrode was reacted in toluene with 0.1% (v/v) bromomethyldimethylchlorosilane for 20 min at 70 °C. Then the silanized ITO glass was washed with toluene and then reacted with Os-complex-functionalized P4VP in toluene (10 mg mL^{-1}) to yield the redox-modified electrode.

2.2. Bioelectrocatalytic electrodes in the biofuel cell

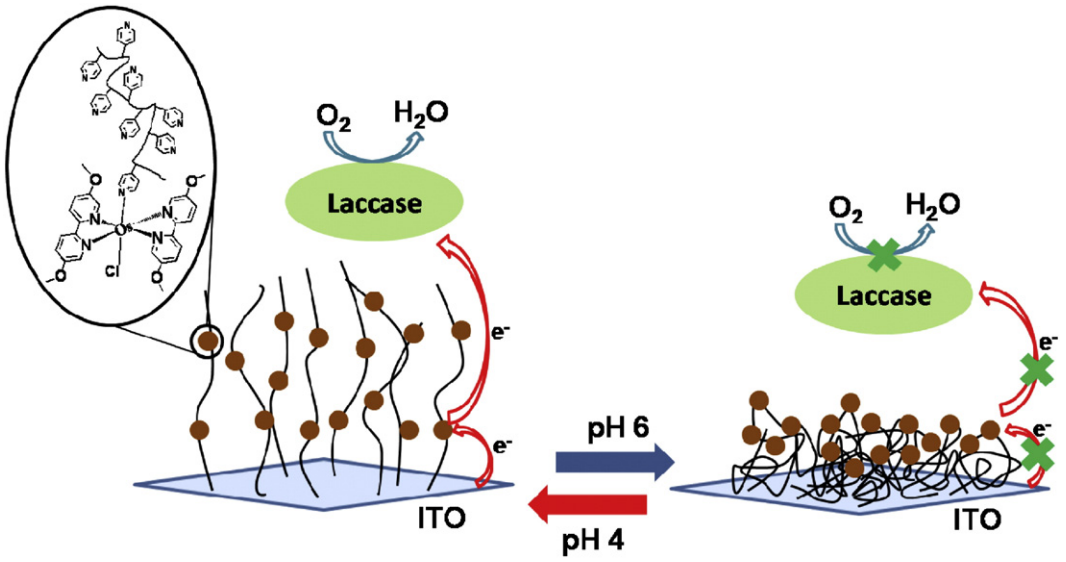
The Os-P4VP-modified ITO electrode (1.2 cm^2 geometrical area) was used to mediate laccase-biocatalyzed O_2 reduction. The enzyme (laccase) was applied in a solution (112 U mL^{-1}) using 0.1 M sodium sulfate as a background electrolyte being under equilibrium with air. Air was bubbled during pH adjustment and while adding different substrates (not during measurements). In addition to the laccase biocatalytic system, the enzymes operating as the logic gates and reset were added to the solution in the cathode compartment (the compositions of the logic gates and the reset system are given below). A bare ITO electrode (1.2 cm^2 geometrical area) for the glucose oxidation was used in 0.1 M phosphate buffer, pH 7, solution containing GOx (250 U mL^{-1}), methylene blue (0.1 mM) and glucose (100 mM). The glucose oxidizing electrode operated under Ar. The biofuel cell was custom made of two curved glass compartments (cathodic and anodic) with clamping ledges and Nafion® membrane (0.09 mm thick, Alfa Aesar, CAS # 66796-30-3) between them with the overall configuration of a "U"-shape. The voltage and current generated by the biofuel cell were measured by a multimeter (Meterman 37XR) using a variable resistance load. The measurements were carried out at ambient temperature (23 ± 2 °C).

2.3. Composition of logic gates and input signals

The enzyme logic "machinery" contained unbuffered sodium sulfate solution (0.1 M) and four different kinds of enzymes, ADH (5 U mL^{-1}), GDH (20 U mL^{-1}), AGS (100 U mL^{-1}), INV (40 U mL^{-1}). The inputs used to activate the gate based on ADH were 0.5 mM NADH and 10 mM acetaldehyde. The inputs used to activate the gate based on AGS and INV were 100 mM maltose and 300 mM sucrose. *In situ* generated glucose (output signal from the AGS-INV-based OR gate) and NAD^+ (output signal from the ADH-based AND gate) served as input signals for the AND gate based on GDH. The reset function was achieved by the addition of urea (30 mM) and urease (5 U mL^{-1}).

3. Results and discussion

Modification of electrodes with signal-responsive materials was used in many different configurations to achieve a switchable/tunable electrochemical activity at interfaces [9]. Recently developed electrode interfaces functionalized with polyelectrolyte brushes allowed the switching ON and OFF electrochemical reactions by swelling/shrinking the polymer layer upon the polyelectrolyte protonation/deprotonation, respectively [25]. The redox units randomly bound to the polyelectrolyte backbone associated with the electrode surface revealed their electrochemical activity only when the polymer layer was in the swollen state allowing flexibility of the polymeric chains. This originated from long distances between the immobilized redox species bound to the polymer chains restricting electron hopping between them. The redox species were able to exchange electrons with the conducting support only upon reaching short distances with



Scheme 1. The pH-switchable bioelectrocatalytic electrode for oxygen reduction biocatalyzed by laccase and mediated by the redox-polymer brush bound to the electrode surface.