Self-Powered Enzyme-Based Biosensors

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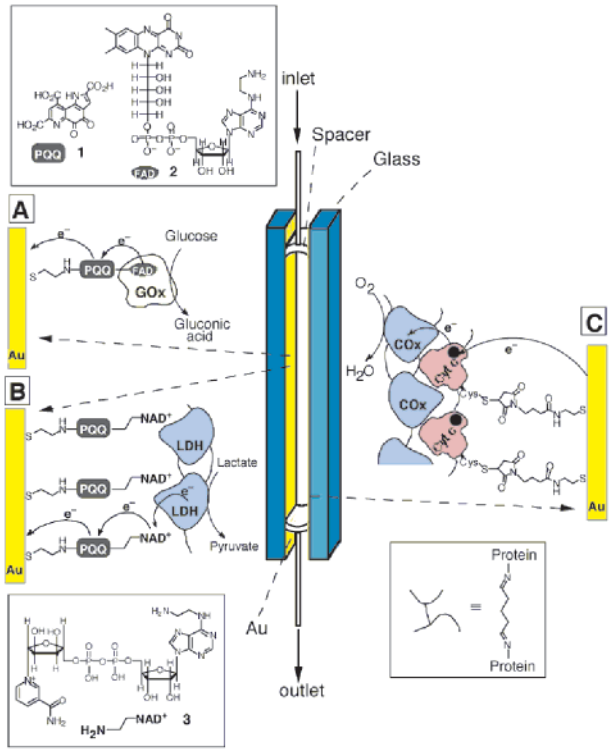
Electrical contacting of redox enzymes with electrode supports attracts substantial research efforts directed to the development of biosensors,[1,2] electrobiocatalyzed chemical transformations,[3] and the development of biofuel cell elements.[4] Tethering of electroactive relays to redox proteins[5] or the immobilization of redox proteins in electroactive polymers[6] are common practices to electrically contact and activate the redox enzymes. Recently, we reported on the effective electrical contacting of flavoenzymes on electrodes by their structural alignment on electrodes through the surface reconstitution of apoflavoenzymes on a relay-FAD monolayer assembly.[7] This concept was further generalized by tailoring integrated, electrically contacted, cofactor-dependent enzyme electrodes by the cross-linking of affinity complexes between NAD^+-dependent enzymes and an electrocatalyst-NAD^+ monolayer associated with electrodes.[8]

One of the major drawbacks in designing amperometric biosensors is the nonspecific oxidation (or reduction) of redox-active interferences upon the application of the potential on the electrode. For example, the electrobiocatalyzed oxidation of glucose is interfered by ascorbic acid or uric acid as contaminants or molecular oxygen. The effective electrical contact of surface-reconstituted glucose oxidase on electrodes led to specific electrodes that are almost insensitive to environmental interferrants or oxygen.[7] The ability to nano-engineer integrated, electrically contacted, enzyme electrodes led to the assembly of a noncompartmentalized glucose-based biofuel cell.[9] The anode and cathode consist of glucose oxidase (GOx) reconstituted on a relay-FAD monolayer electrode and a layered cross-linked cytochrome *c*/cytochrome oxidase (Cyt *c*/COx)-functionalized electrode, respectively. The biocatalyzed oxidation of glucose (the fuel) by

Scheme 1. Configuration of Self-Powered Biofuel Cell-Based Biosensors Composed of: (A) PQQ-FAD/GOx-Functionalized Anode Utilizing Glucose-Analyte as a Fuel or (B) PQQ-NAD^+/LDH-Functionalized Anode Utilizing Lactate-Analyte as a Fuel, and (C) Cyt *c*/COX-Functionalized Cathode Utilizing O_2 as an Oxidizer in Combination with Both Anodes (A or B)



O_2 (the oxidizer) yields electrical power. Although the derived electrical power is low (1 μW at 1 kΩ loading), making the system questionable as a source of energy, we suggest that a biofuel cell configuration consisting of two electrodes may act as a self-powered biosensor device since the open-circuit voltage of the system (V_{oc}) would depend on the fuel concentration. Here we introduce a new and general concept of self-powered biosensors that use a flavoenzyme or a $NAD(P)^+$-dependent enzyme as an electrobiocatalyst. The advantages of such self-powered biosensors are obvious: (i) The sensor consists only of two electrodes, and there is no external voltage applied to the electrodes. (ii) As the system is self-powered by biological fluids, the sensor may function as an implanted invasive sensing device. (iii) As no potential is applied to the electrode, the operation of the biosensor device is specific, and there is no interference by contaminants. (vi) Since the system does not produce voltage in the absence of the substrate, one concentration of the substrate is enough to calibrate the system.

Scheme 1 shows the configurations of two self-powered biosensor devices. The systems consist of two enzyme-functionalized Au electrodes (ca. 0.19 cm^2 active area), acting as anode and cathode and separated by a rubber O-ring (ca. 2 mm thickness). Needles implanted into the rubber ring convert the unit into a flow cell (1 mL·min^{-1} flow rate). In both systems the cathode consists of a glutaric dialdehyde-cross-linked Cyt *c*/COx monolayer assembled on a Au electrode.[9] In one system a glucose oxidase (GOx) monolayer electrode is generated by the reconstitution of apo-GOx on an aminoethyl flavine adenine dinucleotide phosphate (amino-FAD, **2**) covalently linked to a pyrroloquinolino quinone (PQQ, **1**) monolayer.[7] Figure 1A shows the open-circuit voltage, V_{oc}, of the glucose-powered cell upon the

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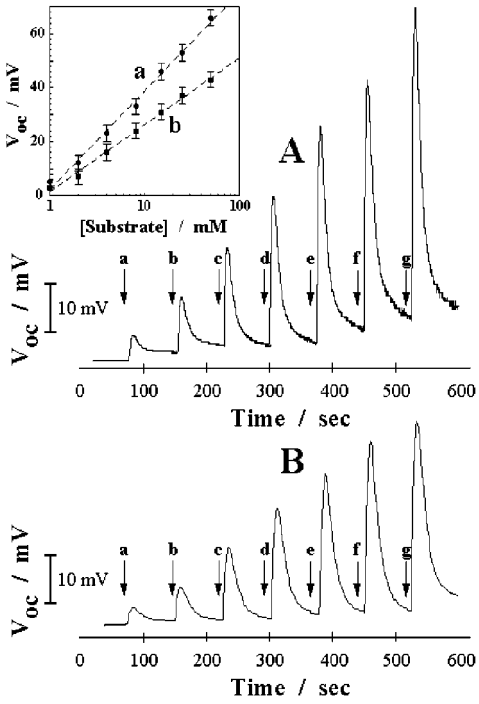

Figure 1. Open-circuit voltage (V_{oc}) at variable concentration of the substrates into the biofuel cell-based sensor devices: (A) upon sensing glucose using the PQQ-FAD/GOx anode; (B) upon sensing lactate using the PQQ-NAD^+/LDH anode. In both systems the Cyt *c*/COx-functionalized electrode was applied as a cathode. The arrows indicate the injections of samples consisting of 1 mL of phosphate buffer, 0.1 M, pH 7.0, that include the substrate at the concentrations (a−g) 1, 2, 4, 8, 15, 25, and 50 mM, respectively. (Inset) Calibration curves (semilogarithmic plot) corresponding to: (a) The analysis of glucose. (b) The analysis of lactate. All data were recorded in air-saturated 0.1 M phosphate buffer, pH 7.0, 30 °C.

injection of variable glucose concentrations into the two-electrode cell under flow conditions. Figure 1, inset (curve a), shows the derived calibration curve. The calibration curve follows a logarithmic relation as expected for a Nernstian-controlled concentration dependence of the electrode potential. Glucose is sensed in the concentration range of 1−80 mM. There is no voltage output in the absence of glucose (Figure 2, injection a). The self-powered cell is stable for 5 h at 30 °C under continuous operating conditions. The anode and cathode are stable for at least 2 months upon storage in the dry state at 0 °C. The voltage cell is not perturbed upon addition of ascorbic acid, 50 mM (Figure 2, injection b). Also, no voltage is developed in the cell upon the addition of glucose 50 mM, under an inert atmosphere of argon (Figure 2, injection d). This later experiment clearly indicates that the sensing of glucose by the cell requires the simultaneous oxidation (of glucose) and reduction (of oxygen to water) by the anode and cathode, respectively.

Scheme 1 also shows the second anode configuration consisting of an integrated lactate dehydrogenase (LDH)-layered electrode. To a pyrroloquinoline quinone (PQQ) monolayer linked to a Au electrode was coupled aminoethyl-functionalized NAD^+ (**3**).[10] The affinity complex[11] formed between LDH and the PQQ-NAD^+

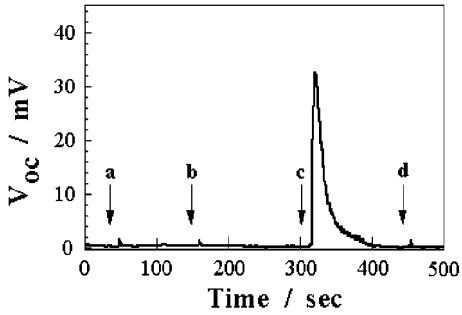

Figure 2. Open-circuit voltage (V_{oc}) of the biofuel cell-based biosensor composed of the PQQ-FAD/GOx- and Cyt c/COX-functionalized electrodes upon injections of: (a) sample of 0.1 M phosphate buffer, (b) 50 mM ascorbic acid, (c) 8 mM glucose, (d) 50 mM glucose without O_2. The arrows show the injection time. Phosphate buffer, 0.1 M, pH = 7.0, equilibrated with air was used as a background electrolyte unless otherwise stated; temperature, ca. 30 °C.

monolayer assembly was cross-linked with glutaric dialdehyde to yield the integrated electrically contacted LDH-functionalized electrode.[8] The LDH-modified electrode and the Cyt *c*/COx-layered electrode were employed as the anode and cathode of a self-powered lactate-sensing cell, respectively. Figure 1B shows the open-circuit voltage of the cell, V_{oc}, upon the injection of variable concentrations of lactate to the cell under flow conditions. Figure 1, inset (curve b), depicts the derived calibration curve, indicating that the lactate is sensed in the concentration range of 1−80 mM. Control experiments reveal that no open-circuit potential is developed in the cell upon injection of ascorbic acid, 50 mM, or glucose, 50 mM, or when lactate, 50 mM, is injected into the cell under an inert atmosphere of argon. These control experiments indicate that the detection of lactate is a result of the simultaneous operation of the anode and cathode as a biofuel cell element. The self-fueled lactate-sensing device is stable for 7 h under continuous operating conditions, and the integrated LDH-functionalized electrode is stable for at least 2 months upon storage in the dry state at 0 °C.

In conclusion, the present study has introduced a novel concept of developing biosensor devices based on chemical-to-electrochemical energy transformations occurring in biofuel cell elements. While these biofuel cells operate at low efficiency and have limited applicability as energy suppliers, the extractable electrical power is sufficient to probe the sensing events. In fact, the low electrical power output of the cells has advantages in the sensing processes, since it eliminates redox transformation of interferrants at the electrode. The sensing devices operate with no external power sources, turning them into attractive invasive sensing elements. The success in tailoring self-powered sensing devices based on integrated NAD^+-dependent enzyme electrodes opens the way to design sensors for other substrates such as alcohol, fructose, or amino acids.

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