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Communications to the Editor

The "Wired" Laccase Cathode: High Current Density Electroreduction of O_2 to Water at +0.7 V (NHE) at pH 5

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We report on the four-electron electrocatalytic reduction of oxygen to water at a current density of 5 mA·cm^{-2} and at +0.7 V (NHE) [all reported potentials are vs the potential of the normal hydrogen electrode (NHE)] in pH 5 citrate buffer at 37.5 °C. The electroreduction of O_2 to water near neutral pH and at ambient temperature is one of the longstanding problems of electrochemistry. Until now, only noble metal and activated carbon O_2 cathodes, respectively at pH 0 and pH 14, operated at current densities of >1 mA cm^{-2} below 100 °C.

The novel cathode was made by cross linking on a hydrophilic carbon cloth (∼10 μm diameter fibers) the electrostatic adduct of laccase from *Coriolus hirsutus*, a polyanion above pH 4, and the cationic electron-conducting redox polymer (Figure 1). The redox polymer formed of poly-*N*-vinylimidazole by coordinating one fifth of its rings to [Os(tpy)(dme-bpy)]$^{2+/3+}$ (where tpy is 2,2′:6′,2″-terpyridine and dme-bpy is 4,4′-dimethyl-2,2′-bipyridine) electrically connected ("wired") the laccase reaction centers to the carbon fibers. The electron cascade of the catalytic process is shown in Scheme 1: The half cell potential of the O_2/H_2O electrode at pH 5 is 0.94 V; the open circuit potential of a vitreous carbon electrode with the laccase directly adsorbed was 0.82 V under 1 atm of O_2 in pH 5 citrate buffer; the redox potential of the laccase wiring polymer was 0.78 V; and the mass-transport limited current was reached when the cathode is poised at 0.65 V.

Laccase cathodes on which O_2 is electroreduced to water were introduced by Tarasevich et al., who formed these by directly adsorbing the enzyme on Teflon-bound high-surface area carbon-black particles.[1] Their cathode, poised at 1.10 V at pH 5, operated for days with little degradation at a current density of 175 μA·cm^{-2}. The group later reported a current density of 10 mA·cm^{-2} at pH 3.5 for an electrode poised at 0.8 V, but did not provide sufficient information to allow other investigators to reproduce these cathodes.[2] For this reason, the current densities of the more recently described cathodes made by immobilizing laccases on carbon or gold ranged from <1 to 190 μA·cm^{-2}.[3−6] The current densities for carbon rods immersed in homogeneous laccase solutions were 0.3 to 120 μA·cm^{-2}.[7,8] Trudeau et al. "wired" laccase through a 0.44 V redox potential polymer, forming a pH 4.7 azide sensor, with a current density of ∼100 μA·cm^{-2} below 0.4 V.[9] Tsujimura et al. described a bilirubin oxidase-carbon felt electrode operating at pH 7 at a current density of 0.89 mA·cm^{-2} when poised at 0.22 V.[10]

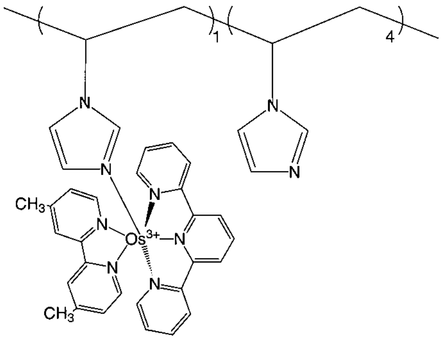
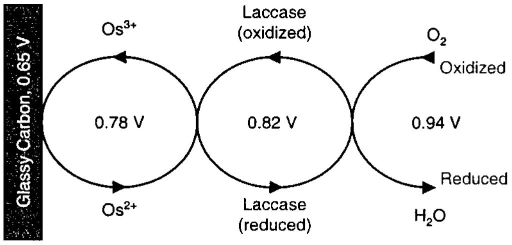


Figure 1. Structure of the laccase-"wiring" redox polymer.

Scheme 1. The Electrocatalytic Cascade



The principles of the design of the novel electrocatalyst were the following:

Independence of orientation and electroreduction of multiple layers of laccase: When a redox enzyme is adsorbed on the surface of an electrode, its electroactivity is orientation-dependent. Only ∼1% of the molecules, oriented with their redox centers proximal to the surface, are electroactive.[11] In contrast, when the enzyme is immobilized in an electron-conducting redox polymer hydrogel, electrons are transferred through colliding mobile polymer-bound redox functions. As a result, all enzyme molecules are electroactive, irrespective of their orientation. Furthermore, because multiple layers of enzyme molecules are "wired" in the film, the current density is ∼10^3 -fold greater than that of an absorbed, randomly oriented, enzyme monolayer directly adsorbed on an electrode.

Formation of an electrostatic complex of laccase and its connecting redox polymer. Because the increase of entropy is small when two macromolecules are mixed, they phase separate unless they are chemically bound together. Electrostatic bonding of the polycationic redox polymer and the polyanionic laccase prevents phase separation.

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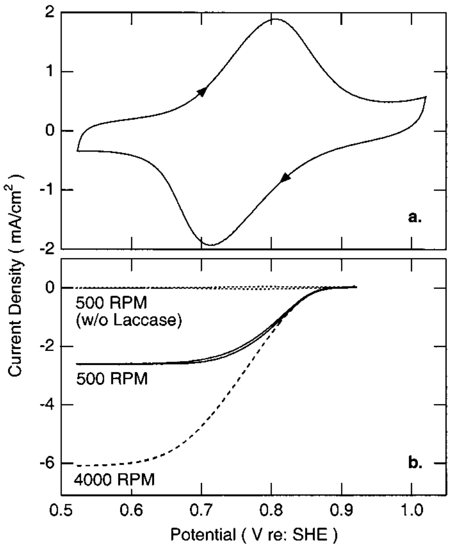

Figure 2. (a) Cyclic voltammogram of the cathode under argon, citrate buffer (200 mM, pH 5, 37.5 ± 1 °C), 500 rpm, 50 mV·s^{-1} scan rate. (b) Polarization of the cathode under 1 atm of O_2, rotating at 500 rpm with and without laccase and at 4000 rpm. Scan rate 1 mV·s^{-1}; current densities were based on a projected area of 4 mm.

Hydrophilic carbon cloth composite. The large ion-accessed area of the hydrophilic carbon fibers and the large open pores of the carbon "paper" provide for rapid diffusion of ions and for prompt neutralization of the base produced in the electroreduction of O_2.

Potential of the redox polymer. Tailoring the redox potential of the laccase-wiring polymer to +0.78 V, just below the potential of the oxidized enzyme, minimizes the overpotential.

The O_2 cathode was formed by cross-linking the redox polymer−enzyme adduct (0.53 mg·cm^{-2} projected area, 42 wt % laccase, 42 wt % redox polymer, and 16 wt % poly(ethylene glycol diglycidyl ether), PEGDGE) on a 4 mm diameter piece of carbon cloth, which was cemented with a carbon-loaded epoxy to a vitreous carbon electrode mounted in a Teflon sleeve. The composite cathode was mechanically tough and withstood the 0.1 N m^{-2} shear stress generated by rotating the cathode at 1000 rpm in the aqueous solution. Its cyclic voltammogram under argon at 50 mV·s^{-1} sweep rate is seen in Figure 2a. The polarization curves of the cathode, rotated at 500 and 4000 rpm in 0.2 M pH 5 citrate buffer at 37.5 °C to vary the mass transport of O_2, are seen in Figure 2b. The current was limited by O_2 mass transfer, not by electrocatalyst kinetics, at angular velocities up to 4000 rpm. Upon increasing the angular velocity 10-fold, the current increased about 3-fold, as expected from the Levich equation. At 4000 rpm, where the current density reached 6.0 ± 0.8 mA·cm^{-2} at 0.6 V (Figure 2b), it became limited by the kinetics of the electrocatalyst. In a 22-h test, a current density of >2 mA·cm^{-2} was maintained when the electrode was poised at +0.7 V and rotated at 1000 rpm.

To illustrate an application of a future compartment-less biofuel cell, we consider a tissue-implanted cell having a 1 mm^2 cathode and a 1 mm^2 glucose-electrooxidizing anode, both operating at 0.5 mA·cm^{-2}, with the anode poised at 0.2 V. The cell would produce 1 μW, more than enough to power an autonomous implanted sensor-transmitter system. Today the size of these systems is defined by their >10-fold larger batteries, the case and the seal of which cannot be miniaturized to ~1 mm^2.

Methods: Laccase (SynectiQ, Dover, NJ, EC 1.10.3.2) from *Coriolus hirsutus* (60 catechol units·mg^{-1} protein) was received as a 3 mg·mL^{-1} solution and was concentrated to 50 mg·mL^{-1} by ultrafiltration. The carbon cloth TGPH-120 was made by Toray, Japan. PEGDGE was purchased from Polysciences, Warrington, PA. The redox polymer was prepared as follows: 2,2′:6′,2″-terpyridine (tpy) and $(NH_4)OsCl_6$ (1:1 molar ratio) were dissolved in ethylene glycol and refluxed under argon for 1 h to form $Os(tpy)Cl_3$, which was precipitated in ethyl ether (yield 90%).[12] $Os(tpy)Cl_3$ and 4,4′-dimethyl-2,2′-bipyridine (dme-bpy) were reacted (1:1 molar ratio) to form [Os(tpy)(dme-bpy)Cl]Cl, also precipitated in ethyl ether (yield 85%).[11] The redox polymer was formed by reacting [Os(tpy)(dme-bpy)Cl]Cl with poly(1-vinyl-imidazole) at 1:5 Os:imidazole molar ratio and purified as described.[13]

Carbon cloth, nominally of 78% void fraction, 350 μm thickness, 10 mm fiber diameter, was cut into 4 mm diameter disks. These were cemented, using conductive carbon paint (SPI, West Chester, PA), to the tips of 3-mm diameter vitreous carbon rotating disk electrodes. The rod-bound cloth was made hydrophilic by exposure to a 1-Torr oxygen plasma for 5 min.[14] A 10 μL sample of the 10 mg·mL^{-1} redox polymer solution was mixed with 2 μL of 200 mM citrate buffer (pH 5), 2 μL of 50 mg·mL^{-1} laccase, and 4 μL of 10 mg·mL^{-1} PEGDGE. A 5 μL aliquot of this mixture was pipetted onto the mounted carbon cloth, which was wetted and penetrated by the solution. The composite mounted disks were cured for >12 h in air at room temperature.

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