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An Electron-Conducting Cross-Linked Polyaniline-Based Redox Hydrogel, Formed in One Step at pH 7.2, Wires Glucose Oxidase

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Depending on its hydration, polyaniline (PANI) conducts charge carriers by two different mechanisms. When not hydrated, it conducts through one-dimensional (1D) bands. Such conduction requires at least 1D semicrystalline PANI.[1,2] Hydration disrupts the parallel alignment of the chains and lowers the conduction. When dissolved, PANI behaves as a polymeric redox couple,[3,4] and if cross-linked and hydrated, it can form an electron-conducting redox hydrogel.[5] In redox hydrogels, electrons diffuse through electron-transferring collisions between hydrated reducible and oxidizable polymer segments.[6] Hydration lowers the local viscosity and reduces attractive Coulombic interactions, thereby increasing segmental mobility and electron diffusion. It also increases the permeability of water-soluble ions and molecules.

Here we show that an electrostatic adduct of emeraldine-polyaniline and a polymer acid, which exhibits small band gap semiconductor-kind conductivity in the solid state, forms an electron-conducting redox hydrogel when cross-linked with a water-soluble diepoxide at neutral pH and hydrated. The cross-linked polymer triples its weight upon hydration, and the absorbed water makes it permeable to glucose. When glucose oxidase (GOx) is co-cross-linked within the hydrogel, its reaction centers are electrically wired. As a result, the PANI−GOx hydrogel catalyzes the electrooxidation of glucose at +0.3 V vs Ag/AgCl and at a current density of 225 μA·cm^{-2}.

The PANI−GOx bioelectrocatalyst was prepared at neutral pH in one pot in a single step. Methods reported by other groups were more difficult to practice. For example, a recently described high-rate electrocatalytic glucose oxidation method[7] utilized special polyaniline-containing microrods with dissolved, rather than immobilized, GOx. The preparation of the microrods required pyrene sulfonic acid functionalization of single-walled carbon nanotubes, their embedding in aniline and polystyrene sulfonic acid, electropolymerization of the aniline in porous alumina membranes coated with a conductive gold support, and dissolving the alumina membrane. Although the steady-state glucose electrooxidation current densities were not reported for these microrods, voltammetric wave heights of 500 μA·cm^{-2} at 5 mV·s^{-1} scan rate were observed.[7]

Glucose-permeable poly(ethylene glycol) diglycidyl ether (PEGDGE) cross-linked electron-conducting redox hydrogels of Os$^{2+/3+}$ complex, comprising polymers with poly(*N*-vinylimidazole) and partially N-alkylated poly(4-vinylpyridine) backbones, have been studied extensively.[6] PEGDGE cross-links primary, secondary, and tertiary amines, as well as heterocyclic nitrogens, including those of polyanilines.[8] When GOx is coimmobilized in the Os$^{2+/3+}$ complex-comprising hydrogels, it is electrically wired, and 3D glucose electrooxidation catalysts, operating at −0.1 V vs Ag/AgCl and at >1 mA·cm^{-2}, are formed.[9,10] In contrast, when GOx is physically adsorbed on,[11] entrapped in,[12] or electrodeposited on[13] films of metallic or semiconducting PANI or otherwise integrated on or in PANI films,[14−17] the glucose electrooxidation current densities are lower. Such is even the case when PANI is covalently bound to a gold substrate through a flavoenzyme thiol,[18] or through nucleophilic thiol attack at ortho positions of PANI quinoimine mers.[19]

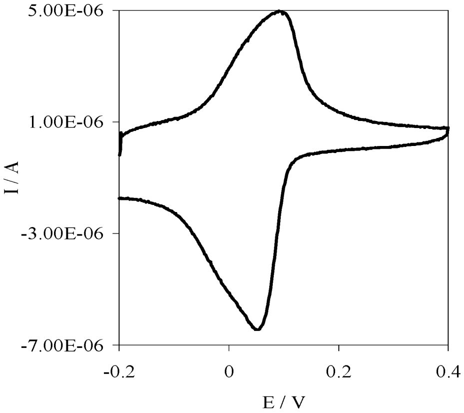

Figure 1. Cyclic voltammogram of PANI/PAAMPSA at 60 μg·cm^{-2} dry-weight loading; 50 mV/s scan rate; pH 7.2, 20 mM PBS.

The emeraldine PANI was made by oxidative template polymerization of aniline on water-dissolved poly(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAAMPSA) with ammonium persulfate.[20,21] The conductivity of the PANI/PAAMPSA salt was 0.430 ± 0.019 S/cm after the purified aqueous polymer/salt dispersion was spin- or drop-cast and dehydrated.[20] The molecular mass of the PAAMPSA was 724 kDa, and solid-state NMR showed that the PANI segments were 8−20 repeat units long. The shorter units were removed by added non-solvent (acetone) precipitation and by filtration and repeated washings. Elemental analysis and X-ray photoelectron spectroscopy of the purified PANI/PAAMPSA indicated an aniline/sulfonic acid molar ratio of about 1:0.9. To show that uncross-linked PANI/PAAMPSA is a typical, diffusionally mobile redox couple, glassy carbon electrodes were coated with 10 μL of a 0.042 wt % aqueous solution of the polymer (60 μg·cm^{-2} initial dry-weight-based loading) and allowed to dry. Figure 1 shows the initial cyclic voltammograms of the PANI/PAAMPSA-modified electrode under argon in a 20 mM, pH 7.2 phosphate buffer (PBS) at 37.5 °C. The voltammogram is characteristic of the doped PANI emeraldine redox couple, peaking at +0.1 V vs Ag/AgCl. Although the film slowly dissolved in the absence of a cross-linker, the voltammetric wave was stable at pH 7.2, and the anodic peak current increased linearly with scan rates up to 400 mV·s^{-1}, as expected for a weakly surface-bound redox couple.

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10.1021/ja071946c CCC: $37.00 © 2007 American Chemical Society

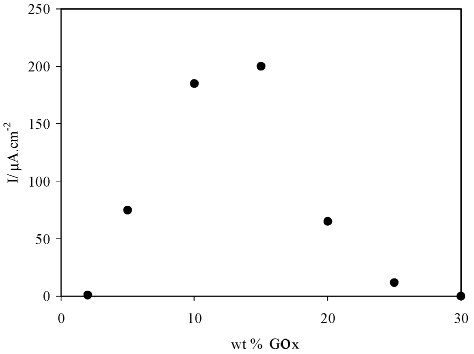

Figure 2. Dependence of the current density on the glucose oxidase weight percentage. The glucose concentration was maintained at 32 mM.

To show that a hydrogel is formed upon cross-linking the PANI/PAAMPSA with a 400 Da PEGDGE, through reaction with PANI nitrogens,[8] we measured the water uptake at 0.1 g·cm^{-2} dry-weight loading of the films (cast from a 5 wt % PANI/PAAMPSA dispersion) in multiple hydration−dehydration cycles. To form the films, aqueous polymer solutions were mixed at 1:0.07 w/w PANI/PAAMPSA solution/PEGDGE ratio, deposited on microscope slides, and cured in ambient air for 48 h. The increases−decreases in mass in cycles of (a) immersion in deionized water for 1 min, (b) tilting to drain the water, (c) contacting the surface with absorbent paper tissue (KimWipe) to remove surface water not bound in the hydrogel, (d) air-drying, and (e) repeat rehydration were measured. In three sets of measurements on different samples, the mass of the water added on swelling and lost upon drying was 2 ± 0.2 times the weight of the dry PANI/PAAMPSA/PEGDGE. To show that the hydrogel is permeable to glucose and is electrically wired to GOx, films were made by mixing droplets of known volume and concentration of aqueous solutions of PANI/PAAMPSA (in pH 7.4, 0.1 M phosphate buffer), GOx (in pH 7.2, 0.1 M phosphate buffer), and PEGDGE (in deionized water) on glassy carbon electrodes. The films were made as earlier described for an Os$^{2+/3+}$ complex comprising redox polymer-wired GOx electrocatalyst,[10] except that the Os$^{2+/3+}$ complex-based polymer was replaced by PANI/PAAMSA, the solution of which was now diluted 10-fold with pH 7.4, 0.1 M phosphate buffer. The films were cured for >18 h at ambient temperature.

Figure 2 shows the dependence of the glucose electrooxidation current density on the GOx weight percentage in the dry films in pH 7.2, 32 mM glucose, 20 mM phosphate buffer at 37 °C at a fixed loading of 0.8 mg·cm^{-2} ($\rho = 1.39$ g·cm^{-3}). In the 2−15 wt % GOx range, the current density increased with the weight percentage of GOx, reaching 200 μA·cm^{-2} at 15 wt %. At higher GOx content the current density declined. The decline is attributed to deswelling when the polyanionic GOx neutralizes the residual net positive charge of PANI/PAAMSA. To confirm that deswelling is caused by charge neutralization, GOx with a high negative surface charge was prepared by periodate oxidation of surface oligosaccharides of GOx.[10] The glucose electrooxidation current density now declined when the oxidized GOx content reached 2 wt %. Figure 3 shows the dependence of the current density on glucose concentration under argon for an electrode that was poised at + 0.3 V vs Ag/AgCl in a PBS buffer. The current density was 225 μA·cm^{-2} at 40 mM glucose. The apparent Michaelis constant, K'_M, obtained from an Edie−Hofstee plot was 16.8 mM glucose.

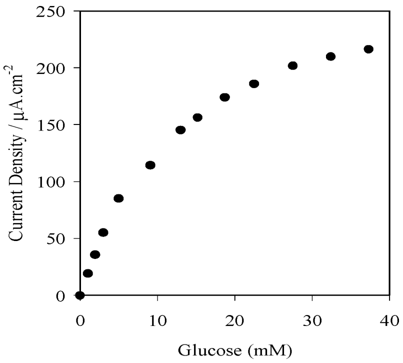

Figure 3. Dependence of the steady-state current density on glucose concentration for an electrode poised at +0.3 V vs Ag/AgCl, rotating at 500 rpm under argon.

In summary a polyaniline-based, electron-conducting, glucose-permeable redox hydrogel was formed in one step at pH 7.2 by cross-linking a polymer acid-templated PANI with water-soluble PEGDGE. Incorporation of glucose oxidase in the hydrogel by co-cross-linking in the same step led to electrical wiring of the enzyme and formation of a glucose electrooxidation catalyst, allowing the electrooxidation of glucose at a current density of 225 μA·cm^{-2} at 0.3 V vs Ag/AgCl.

Acknowledgment. Y.L.L. acknowledges funding from a Beckman Young Investigator Award and a DuPont Young Professor Grant and support from the Keck Foundation. A.H. thanks the Welch Foundation and the Office of Naval Research for financial support. N.M. acknowledges funding from a European Young Investigator Award (EURYI).

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JA071946C