

FABRICATION OF A COMPOSITE OF CONDUCTING POLYMERS, NANOPARTICLES, AND AN ENZYME FOR BIO-ELECTROCATALYSIS OF OXYGEN AND HYDROGEN PEROXIDE REDUCTION

James A. Cox¹, Andrzej Z. Ernst², Kamila M. Wiaderek¹ and Pawel J. Kulesza²

¹Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056

²Department of Chemistry, University of Warsaw, ul. Pasteura 1, Warsaw 02093 Poland

Introduction

Hybrid of metal nanoparticles and various organic and inorganic compounds are attracting interest because of promising characteristics such as electrical, electrochemical, and optical properties [1]. By integration of biomolecules with nanoparticles one may obtain hybrid composites that combine the recognition and catalytic properties of selected biological substances with the unique electronic and catalytic functions of nanomaterials. In the present study, a composite combining an inorganic electrochemical catalyst, an enzyme, metal nanoparticles, and conducting polymers is formed using layer-by-layer (LbL) electrostatic assembly [2]. Gold nanoparticles, AuNPs, were protected from aggregation and provided a negative charge by phosphomolybdate, PMo. The PMo strongly adsorbs to glassy carbon (GC) electrodes and catalyzes the reduction of O₂ to H₂O₂. The negatively charged surface serves as the foundation for deposition of monolayers of polyions such as a poly(aniline), poly styrenesulfonate co-polymer (PANI/PSS). Residual negative sites permit assembly of a poly(3,4-ethylenedioxythiophene), PEDOT overlayer.

In combination, the conducting polymers, PANI/PSS and PEDOT, provide a host matrix for horseradish peroxidase, HRP [3], which is further stabilized by intercalation of these polyions [4]. The electrocatalytic properties of AuNP-PMo are enhanced by the conducting polymers [5], and, in turn, they enhance the conductivity of composites thereof.

The goal of the present study is to test the hypothesis that via LbL assembly, the above composite can be fabricated in a manner that does not compromise the activity of the catalytic species. The hypothesis is that stable immobilization of HRP will facilitate the 2-electron reduction to H₂O₂ to water, yielding an overall facile 4-electron reduction of O₂ to H₂O.

Experimental

Electrochemical experiments were carried out with a CH Instruments (Austin, TX) Model 660B electrochemical workstation. All potentials were measured and reported v. a Ag|AgCl reference electrode. Platinum gauze was

the counter electrode, and glassy carbon (area, 0.071 cm²) served as the working electrode and base on which the composite was fabricated.

Hexanethiol-protected AuNPs were prepared in toluene [6]. Using ligand place-exchange [7], the AuNP core was extracted into 0.1 M H₂SO₄ (aqueous) that contained 5.0 mM PMo. The free PMo was removed by dialysis, yielding an aqueous solution of AuNP-PMo with a particle diameter of 4.4 ± 1.8 nm. The AuNP-PMo was adsorbed as a monolayer on GC by immersion for 2 h in aqueous AuNP-PMo.

The GC|AuNP-PMo was modified by electropolymerization in an acidic mixture of 1.0 mM PSS and 8.0 M aniline. The polymerization was performed by the application of 12 potential cycles in the range, -0.1 to 0.9 V, at a scan rate, ν , of 50 mV·s⁻¹. The resulting GC|AuNP-PMo|PANI/PSS electrode was rinsed, and an overlayer of PEDOT was deposited by voltammetry in 4·10⁻⁶ M 3,4-ethylenedioxythiophene solution using five scans between -0.4 V and 1.0 V at 50 mV·s⁻¹. Immobilization of an enzyme was by pipetting 100 μ L of 5 mg·mL⁻¹ HRP (pH 7.4) onto the surface, air-dry, and storing overnight in a refrigerator.

Results and discussion

Initial experiments showed that HRP was denatured immediately by direct contact with AuNP-PMo, precluding design of a composite that did not include conducting polymers to prevent direct contact between the enzyme and AuNP-PMo. Cyclic voltammetry was used to monitor the formation of the proposed composite, AuNP-PMo|PANI/PSS|HRP, and to test the hypothesis with the 4-electron reduction of O₂. Fig. 1 shows the voltammetry of GC|AuNP-PMo. The three redox processes in the potential range from 0.45 to -0.10 V are characteristic of PMo, which provides evidence that the AuNP-PMo synthesis was successful and that adsorption of this species to GC occurred.

The PANI/PSS monolayer was formed by cyclic voltammetry of GC in an aniline, PSS mixture (Fig. 2). The voltammogram shows the current increase with cycle number that is consistent with film growth. Although deposited from acid solution, the system was electrochemically active at pH 7. Coulometric analysis

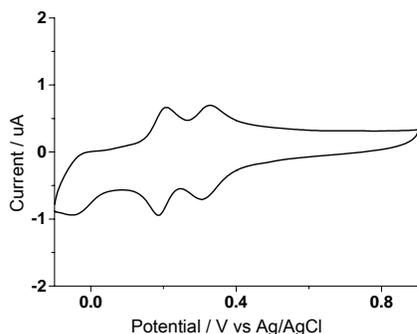


Fig. 1 Cyclic voltammetry (CV) of AuNP-PMo in acid solution. Scan rate, ν , 50 mV s^{-1} .

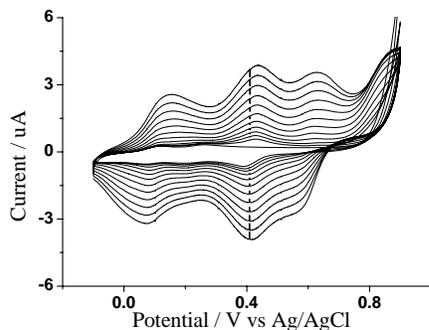


Fig. 2 CV of aniline in acid solution. ν , 50 mV s^{-1}

yielded a surface coverage of $2.2 \cdot 10^{-10} \text{ mol} \cdot \text{cm}^{-2}$, consistent with the electrolysis of a single monolayer.

Conditions for the deposition of PEDOT on GC | AuNP-PMo | PANI/PSS were developed by performing initial experiments on the voltammetry of $4 \cdot 10^{-6} \text{ M}$ 3,4-ethylenedioxythiophene at GC. Five cycles between -0.4 and 1.0 V at $50 \text{ mV} \cdot \text{s}^{-1}$ were sufficient for this purpose.

To demonstrate the formation of GC|AuNP-PMo | PANI/PSS | PEDOT, voltammetry was performed on a GC electrode that was modified sequentially by AuNP-PMo, PANI/PSS, and PEDOT. For each layer, the above-described conditions were employed. The resulting voltammograms that were obtained after transferring the modified electrode (after each deposition step) in $0.10 \text{ M H}_2\text{SO}_4$ at $50 \text{ mV} \cdot \text{s}^{-1}$ are shown in Fig. 3. The systematic increase in current after each layer is deposited and the retention of voltammetric features of the underlying layer(s) demonstrate that each component of the composite retains electroactivity; hence, the composite is a conductive assembly. In addition, the GC | AuNP-PMo | PANI/PSS | PEDOT assembly process was reproducible (voltammetric currents on replicates varied by less than 5%) and was stable for at least two weeks.

A series of experiments demonstrated that the mixed outer layer of PANI/PSS and PEDOT comprises a superior substrate for immobilization of HRP than either polymer alone. The test for activity of HRP was to do

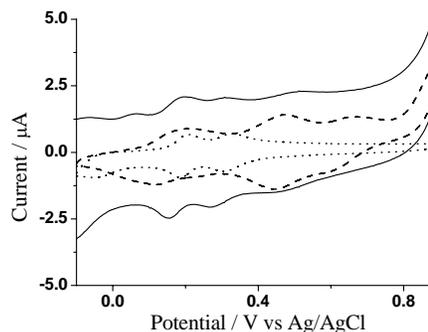


Fig. 3 Comparison of the voltammetry of GC | AuNP-PMo (dotted-line display); GC | AuNP-PMo | PANI/PSS (dashed line); and GC | AuNP-PMo | PANI/PSS | PEDOT (solid line) electrodes in $0.1 \text{ mol} \cdot \text{dm}^{-3} \text{ H}_2\text{SO}_4$. Scan rate, $50 \text{ mV} \cdot \text{s}^{-1}$.

amperometry at 0.20 V in 0.10 M KCl solutions in which the concentration of H_2O_2 was increased in a stepwise manner. When either PEDOT or PEDOT/PSS was alone in the composite, HRP activity was not observed. When PANI/PSS| PEDOT was the surface upon which HRP was deposited, a current increase upon consecutive additions of hydrogen peroxide was obtained. Moreover, inclusion of AuNP-PMo in the composite has a profound influence on the sensitivity. The GC | AuNP-PMo | PANI/PSS | PEDOT| HRP electrode developed currents related to H_2O_2 concentration in less than 5 s. The detection limit was $1.0 \cdot 10^{-5} \text{ M H}_2\text{O}_2$.

References

1. Granot, E., Katz, E., Basnar, B. and Willner, I. Enhanced bioelectrocatalysis using Au-nanoparticle/polyaniline hybrid systems in thin films and microstructured rods assembled on electrodes *Chem. Mater.*, **17** (2005), 4600-4609.
2. Decher, G., Fuzzy nanoassemblies: Toward layered polymeric multicomposites *Science*, **277** (1997), 1232-1237.
3. Ernst, A. Z., Makowski, O., Kowalewska, B., Miecznikowski, K., Kulesza, P. J. Hybrid bioelectrocatalyst for hydrogen peroxide reduction: Immobilization of enzyme within organic-inorganic film of structured Prussian Blue and PEDOT **71** (2007) *Bioelectrochem.*, 23-28.
4. Simon, E., Halliwell, C.M., Toh, C.S., Cass, A. E. G., Bartlett, P. N. Immobilisation of enzymes on poly(aniline)-poly(anion) composite films. Preparation of bioanodes for biofuel cell applications **55** (2002) *Bioelectrochem.*, 13-15.
5. O'Mullane, A. P., Dale, S. E., Macpherson, J. V., Unwin, P. R. Fabrication and electrocatalytic properties of polyaniline/Pt nanoparticle composites (2004) *Chem. Commun.*, 1606-1607.
6. Brust, M., Walker, M., Bethell, D., Schiffrin, D. J., Whyman, R. Synthesis of thiol-derivatized gold nanoparticles in a 2-phase liquid-liquid system (1994) *J. Chem. Soc. Chem. Comm.* 801- 02.
7. Hostetler, M. J., Wingate, J. E., Zhong, C. J., Harris, J. E., Vachet, R. W., Clark, M. R., Londono, J. D., Green, S. J., Stokes, J. J., Wignall, J. D., Glush, G. L., Porter, M. D., Evans, N. D., Murray, R. W. Alkanethiolate gold cluster molecules with core diameters from 1.5 to 5.2 nm: Core and monolayer properties as a function of core size **14** (1998) *Langmuir*, 17-30.