ENZYME ENTRAPMENT IN SILICA NANOPARTICLES: MEDIATED BY SINGLE WALLED CARBON NANOTUBES-LYSOZYME FOR BIOSENSOR APPLICATIONS

Saroja Mantha[1], *Madhumati Ramanathan*[1], *Virginia Davis*[2], *Aleksandr Simonian*[1]
[1]Department of Mechanical Engineering, Auburn University, AL 36849
[2]Department of Chemical Engineering, Auburn University, AL 36849

Introduction

Silica compounds are among the most extensively studied materials, especially those prepared by the sol–gel process [1-3]. A wide variety of proteins can catalyze the precipitation of silica and become encapsulated as the silica matrix forms. The reaction provides an efficient method for enzyme immobilization and provides significant mechanical stability to resulting silica matrix. Potential applications of Carbon nanotubes (CNTs) include development of different biosensing platforms such as single-walled carbon nanotubes (SWNT) based electrochemical biosensors [4-6], SWNT-based optical biosensors [7, 8] and SWNT-based electronic biosensors [9, 10]. Unfortunately most processes of (SWNT) syntheses produce large, aggregated bundles of SWNTs. These aggregated bundles often make processing difficult and mask the unique properties of individual tubes. Thus a number of covalent [11, 12] and noncovalent [13-15] methods of protein attachment have been developed to debundle these aggregates into individual tubes. However, covalent coupling normally damages the sidewalls of SWNTs and disrupts their unique electrical and optical properties [16, 17]. In contrast, noncovalent methods can allow for the electronic structure of the SWNTs [18, 19] to be retained but may in some cases lead to a loss of protein function [20]. Organophosphorus hydrolase (OPH) is an enzyme with unique capabilities of hydrolyzing wide spectrum of organophosphates (OPs), into much less toxic products such as *p*-nitrophenol and diethyl phosphate. Recent studies have demonstrated the remarkable versatility of a biomimetic silicification reaction as a means of enzyme immobilization [21]. Biosilicification is a rapid ambient precipitation of silica mediated by a biological catalyst. This work describes the approach to create a bio-nano interface suitable for direct electrochemistry of enzymes. Direct bio-electrocatalysis of paraoxon hydrolysis is demonstrated by entrapping OPH in silica composite obtained through SWNT-LSZ catalyzed synthesis of silica.

Experimental

The bulk gold electrode surfaces were prepared for modification by polishing with 0.05 μM alumina and water slurry on a polishing cloth. The electrodes were then cleaned by cycling between the potentials -0.3 and +1.5 V versus Ag/AgCl in 0.05 M H_2SO_4 solution at a scan rate of 100 mV s^{-1} until reproducible scans were recorded (typically 30 min). The electrodes were rinsed with water before modification. Dispersion of SWNTs in LSZ was achieved by the previously published method [22]. Clean gold electrodes were modified by immersing in SWNT-LSZ solution for 12 hr and excess removed by washing with phosphate buffer (0.1 M, pH 8.1). The silica precipitation mixture consisted of TMOS (30 µl hydrolyzed with 170 µl of 1mM HCl) was mixed with 820 µl potassium phosphate buffer, (of 0.1 M, pH 8.1) containing 100 µl of OPH (~1.5 mg/ml). 10 µl of the above reaction mixture was dropped onto the SWNT-lysozyme (SWNT-LSZ) modified surface and incubated for 1 hr at room temperature to allow silica nanoparticles (SNP) to form. The electrode was washed with buffer and dried with nitrogen. Control experiments were done using pure lysozyme solution (1 mg/ml) instead of SWNT-LSZ. The surface morphology was studied by Scanning Electron Microscopy (SEM, JEOL, model 840). All the electrochemical measurements were performed by CHI 660 (CH Instruments, Austin, TX) at room temperature using three electrode system containing platinum as auxiliary electrode, modified gold as working electrode and a saturated Ag/AgCl as reference electrode. The electrolyte solution was 50 mM PBS (pH 7.54).

Results and Discussion

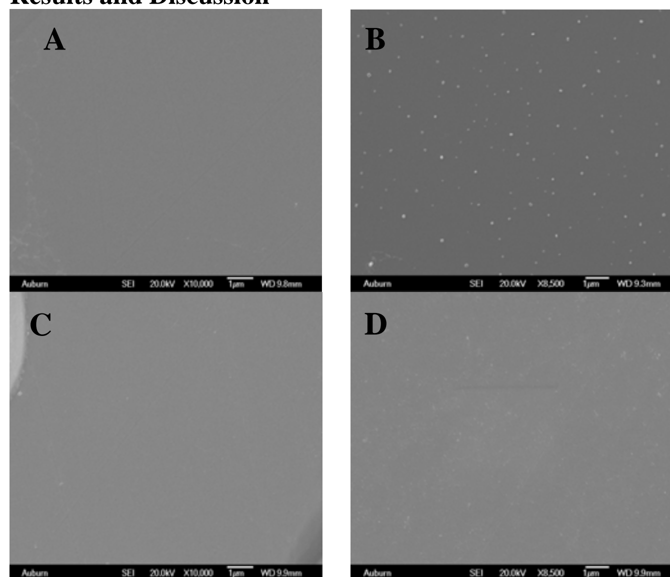

Figure 1. A) Lysozyme, B) SNP using lysozyme, C) SWNT-LSZ, D) SNP using SWNT-LSZ.

Lysozyme and SWNT-LSZ catalyzes the formation of silica from its precursor solutions as seen from Figure 1. B & D. Figure 1. A & C are the reference electrodes. SWNT-LSZ immobilization was achieved by physical adsorption and silica particles were formed *in situ*. OPH is encapsulated as the silica particles are formed. The presence of SWNTs embedded within the silica matrix is also evident. The efficacy of the procedure was evaluated by monitoring the catalytic activity of encapsulated OPH for the hydrolysis of paraoxon. The electrochemical characteristics of the modified electrode were then investigated by batch amperometry. The potential applied

was 0.85 V. The amperometric response of p-nitrophenol was shown in Figure 2A. Calibration data for paraoxon over the concentration ranges 5-50 μM, and shows that current increased proportionally to the concentration in the range from 0 to 50 μM to yield highly linear calibration plot.

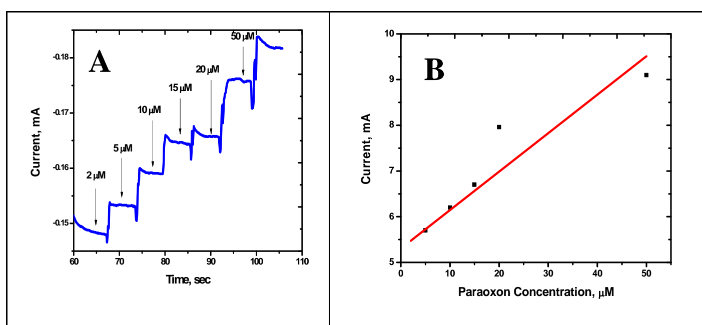

Figure 2. A) Batch amperometry response of modified gold electrode by SWNT-LSZ, B) Calibration plot

Conclusions

In this study, we have demonstrated the development of material scaffolds suitable for wide variety of applications including biosensing. The silica nanoparticles significantly increase the surface area on gold substrate. In addition, encapsulation in silica using this method is rapid with preparation times of a few hours, compared to the several days for covalent immobilization of OPH as reported previously. OPH encapsulation was demonstrated herein as a model enzyme system, but the encapsulation methodology is applicable to a wide variety of biomolecules, providing the potential for multiple enzyme immobilizations and simultaneous analysis of multiple analytes. The entrapped OPH enzyme demonstrated excellent electrochemistry and the presence of SWNTs improved the conductivity of the composite film. This matrix showed a biocompatible microenvironment for retaining the native activity of the entrapped OPH and was in favor of the accessibility of substrate to the active site of OPH, thus the affinity to substrates is improved greatly. This composite film can be extended to immobilize other enzymes and biomolecules, which will greatly facilitate the development of biosensors and other bioelectrochemical devices.

References

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