Biosensors Fabricated through Electrostatic Assembly of Enzymes/Polyelectrolyte Hybrid Layers on Carbon Nanotubes

Yuehe Lin*, Guodong Liu and Jun Wang

Pacific Northwest National Laboratory, 902 Battelle Blvd, P. O. Box 999
Richland, WA 99352. Tel:509-376-0529; Email: Yuehe.lin@pnl.gov

ABSTRACT

Carbon nanotubes (CNTs) have emerged as new class of nanomaterials that is receiving considerable interest because of their unique structure, mechanical, and electronic properties. One promising application of CNTs is to fabricate highly sensitive chemo/biosensors.[1-4] For construction of these CNT-based sensors, the CNTs first have to be modified with some molecules specific to the interests. Generally, covalent binding, affinity, and electrostatic interaction have been utilized for the modification of CNTs. Among them, the electrostatic method is attractive due to its simplicity and high efficiency. In present work, we have developed highly sensitively amperometric biosensors for glucose, choline, organophosphate pesticide (OPP) and nerve agents (NAs) based on electrostatically assembling enzymes on the surface of CNTs. All these biosensors were fabricated by immobilization of enzymes on the negatively charged CNTs surface through alternately assembling a cationic poly(diallydimethylammonium chloride) (PDDA) layer and an enzyme layer. Using this layer-by-layer (LBL) technique, a bioactive nanocomposite film was fabricated on the electrode surface. Owing to the electrocatalytic effect of CNTs, an amplified electrochemical signal was achieved, which leads to low detections limits for glucose, choline, and OPP and NAs.

Keywords: carbon nanotubes, electrochemistry, enzymes, layer-by-layer, biosensors.

1. INTRODUCTION

Layer-by-layer(LBL) assembly of opposite charged species is a simple and powerful method for the construction of composite that are self-assembled on a nanometer scale. In the past decade, the LBL method for ultrathin film assembly via alternate adsorption of oppositely charged polyions and proteins on variety of charged substrate materials(clay nanoparticles, polystyrene latex beads and pyrolytic graphite electrodes) have been developed. Recently, this technique has been used to build polyelectrolyte/CNT multilayer composite to improve solubility and mechanical properties. As far as we know, there is no report on the interaction between CNT and protein by LBL. Unlike covalent bonding, electrostatic modifications of CNT, preserves the native conformation of biomolecuar and the unique electronic properties of the CNTs. Additionally, the LBL film can provide a suitable microenvironment to retain the biomolecular activity. Herein we report on the preparation of multiple protein-polyion films comprising of enzymes, alternately assembled with poly (diallydimethylammonium) chloride polymer (PDDA) on carbon nanotubes template, which is coated on the surface of a glassy carbon electrode, and explore possible applications to enzyme electrochemistry and biosensing.

1 GLUCOSE BIOSESOR

Figure 1 illustrates the procedure for preparation of Gox on carbon nanotubes.

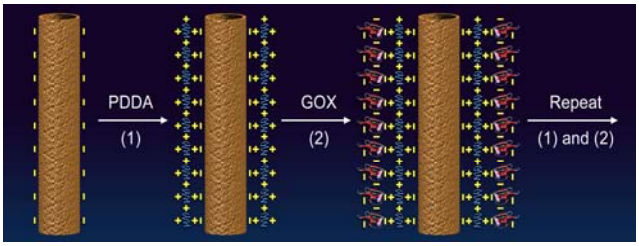

Figure 1 schematics of layer-by-layer electrostatic self-assembly of GOx/polyelectrolyte hybrid layers on carbon nanotubes

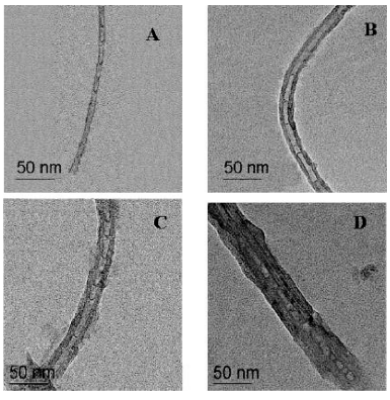

Figure 2 TEM omages of the multiple enzyme/ PDDA layers on carbon nanotubes surface. (A) =0; (B) n=1; (C) n=2; (D) n=6.

Figure 2 shows TEM images of the multiple enzyme/ PDDA layers on carbon nanotubes. It can be seen from this figure that a series of bamboo-like closed graphite shell

along the tubes axis was constructed, which is due to the fact that the graphitic layers are not perfectly parallel to the tube axis and the thickness of the layers increases with the number of layers.

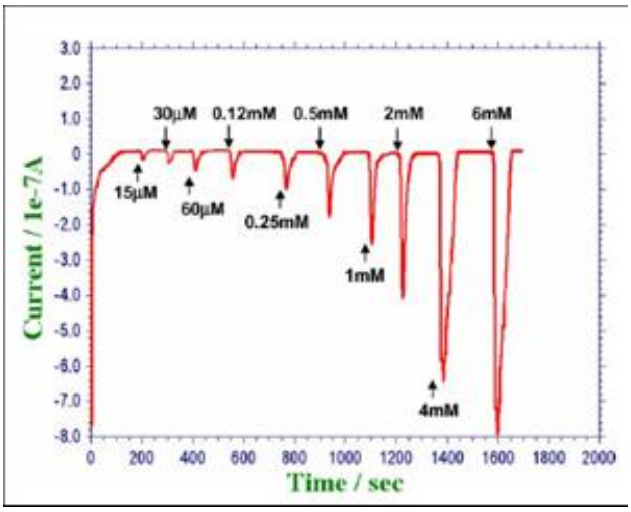

Figure 3 Amperometric response of the PDDA/GOx/PDDA/CNT/GC electrode to various concentration of glucose in the flow injection system. Working potential:-0.1 M; flow rate: 250 μl/ml.

The typical amperometric response of the GOx modified CNT/GC electrode for the successive injection of different concentration of glucose at an applied potential of -0.1 V in a flow injection system. Fast and well defined current response were obtained for different concentration levels of glucose and the peak current increase with the increase of the concentration of glucose. It was found that the detection limit for this glucose sensor is down to 7.0 μM based on the signal-to noise characteristics of these data(S/N=3).

2 CHOLINE BIOSESOR

Self-assembling a bienzyme of Choline oxidase(ChOx) and a peroxide (HRP) on carbon nanotubes for the detection of choline was studied. Figure 4 schematically illustration of the procedure for the attachment of ChO and HRP on carbon nanotubes

The advantage for the construction of mixed enzymes on CNT surface for the choline biosensor is that HRP can catalytically reduce the hydrogen peroxide generated by ChO on the electrode surface, which will greatly enhance the amperometric response and improve the sensitivity for this choline sensor. The catalytic reduction of H_2O_2 by HRP is due to carbon nanotubes because of its excellent electronic properties. Figure 5 illustrates the reaction scheme of ChO/HRP/CNT modified electrode for the detection of choline. From this figure, it can be seen that the reduction of current from HRP could be detected on the carbon nanotubes at low potential only if efficient direct electron transfer reaction between HRP and carbon nanotubes s takes place. However, it cannot exclude the direct reduction of H_2O_2 on carbon nanotubes.

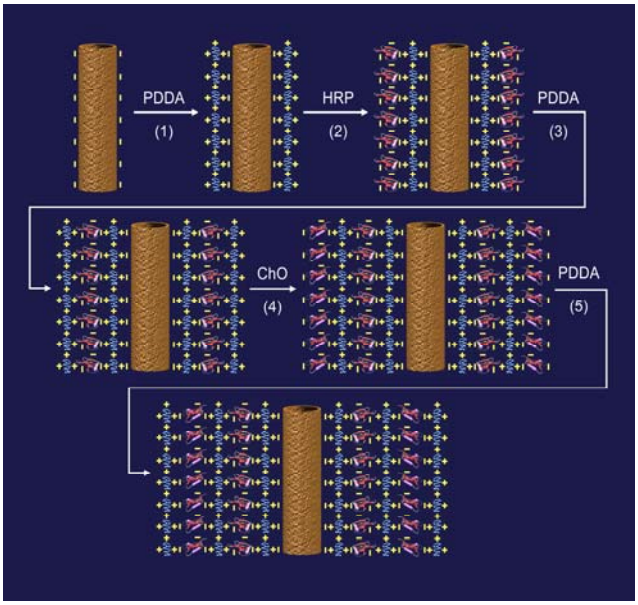

Figure 4 schematically illustration of the procedure for the attachment of ChO and HRP on carbon nanotubes.

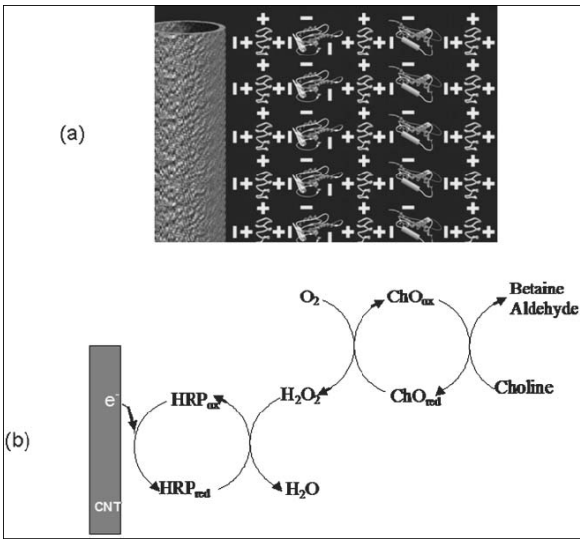

Figure 5 (a) schematics of the multilayers of bienzymes on CNT; (b) the reaction scheme of the ChO/ HRP/ CNT/ GC electrode for the detection of choline in solution.

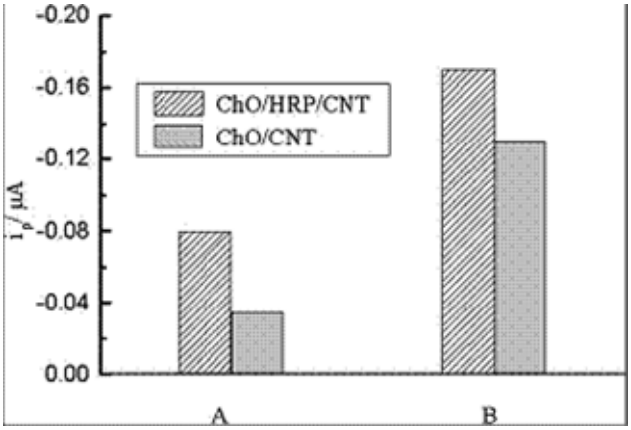

Figure 6. Amperometric response of two types of enzymes modified GC electrode for choline detection in 0.1 M Tris buffer solution containing 2.0mM (A) and 9.0 mM (B) choline (pH8).

Figure 6 shows the histogram comparing the peak current response form the CHO modified electrode and the bi-enzyme of ChO and HRP modified electrode in solution containing different concentration of choline at a potential of -0.1V. As can be observed that the magnitude of peak current for Cho/HRP /CNT electrode is higher than that for the ChO CNT electrode at the same concentration of choline. It is reasonable because HRP catalyzes the reduction of hydrogen peroxide. It indicated that direct electron transfer do take place between HRP and carbon nanotubes. Here, carbon nanotubes can facilitate the electron transfer between HRP and the GC electrode due to their excellent electronic properties. We also found the response time for the choline biosensor is fast, usually few seconds.

3 OP PESTICIDES AND NERVE AGENT BIOSENSOR

The organophosphate pesticides and nerve Agents biosensors have been developed based on following principle:

Acetylcholine + H_2O → choline acetate (1)

Choline +O_2 → Bataine aldehyde + H_2O_2 (2)

Acetylthiocholine + H_2O → thiocholine + acetate acide (3)

Reaction (1), (3) involves enzyme of AChE and (2) with ChO.

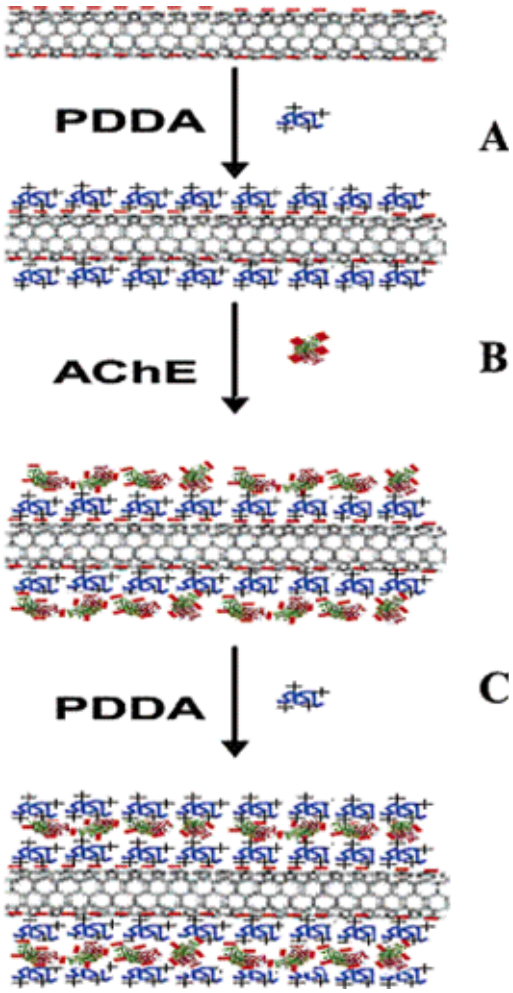

Figure 7. Schematics of layer-by-layer electrostatic self-assembly of AChE on carbon nanotubes: (A) assembling positively charged PDDA on negatively charged CNT; (B) assembling negatively charged AChE; (C) assembling the second PDDA layer.

Figure 7 illustrate layer-by-layer electrostatic self-assembly of AChE on carbon nanotubes: (A) assembling positively charged PDDA on negatively charged CNT; (B) assembling negatively charged AChE; (C) assembling the second PDDA layer. Figure 8 presents a typical current versus ATCh concentration plot obtained during amperometric detection with the PDDA/AChE/PDDA/CNT/GC biosensor; the resulting calibration curve is shown in inset. One notices that the current increase rapidly with the increase of the concentration of AChE.

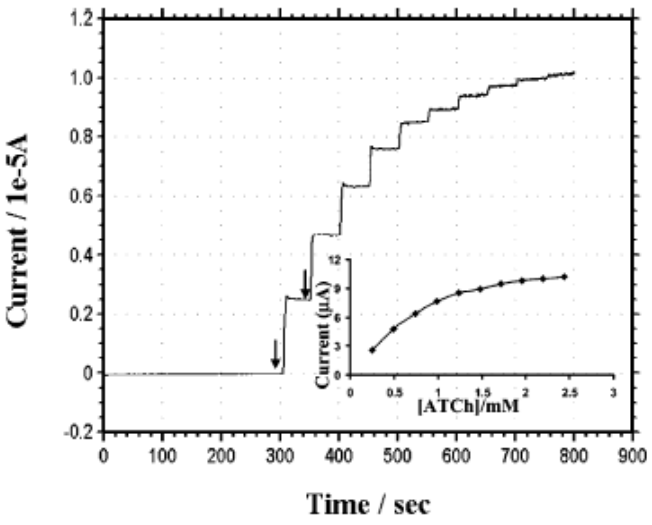

Figure 8. Amperometric response of AChE /PDDA/CNT/GC biosensor on the stepwise addition of 0.25 mM ATCh solution under batch condition in pH7.4 PBS buffer with an potential of 150mV vs Ag/AgCl. Inset: corresponding calibration curve.

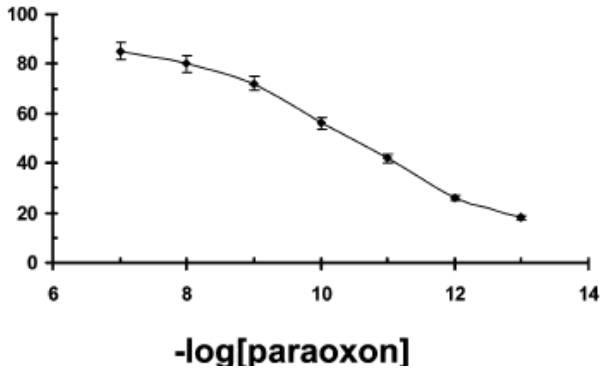

Figure 9. Inhibition curve of the PDDDA/AChE/PDDA/GC biosensor to different concentration of paradoxon.

Using the optimum conditions established in the above studies, calibration plots were generated for paraoxon (Figure 9). As shown, the relative inhibition of AChE activity is increasing with the concentration of paraoxon ranging from 10^{-13} to 10^{-7} and is linearly with -log [paraoxon] at the concentration range 1×10^{-12}-1×10^{-8} M with a detection limit of 4×10^{-13} M (calculated for 20% inhibition). This limit of detection is 2.5 times better than that achieved with a nanoporous carbon matrix 15 and 3 orders of magnitude lower than the covalent binding or adsorbing AChE on the CNT-modified screen-printed carbon electrode under batch conditions. A further improvement of the lower limit can be achieved by increasing the inhibition time or reducing the AChE loading during the preparation of the PDDA/AChE/PDDA/CNT/GC electrode. The reproducibility of the PDDA/AChE/PDDA/CNT/GC biosensor for paraoxon detection was examined. Based on the percent inhibition observed for the concentrations of paraoxon in the range 1×10^{-12}-1×10^{-8} M, a relative standard deviation of <5.6 (n= 6) was obtained.

Acknowledgements

This work is supported by a laboratory directed research and development program at Pacific Northwest National Laboratory (PNNL). The research described in this paper was performed at the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the U.S. Department of Energy's (DOE's) Office of Biological and Environmental Research and located at PNNL. PNNL is operated by Battelle for DOE under Contract DE-AC05-76RL01830.

References

[1] Lin Y*, F Lu, and J Wang. 2004. *Electroanalysis* 16(1-2):145-149

[2] Lin Y*, W Yantasee, F Lu, J Wang, M Musameh, Y Tu, and Z Ren. 2004. "Biosensors Based on Carbon Nanotubes." In *Dekker Encyclopedia of Nanoscience and Nanotechnology*, ed. J. A. Schwarz, C. Contescu, K. Putye, pp. 361-374. Marcel Dekker, New York.

[3] J. Wang, M. Musameh and Y. Lin, J. Am. Chem. Soc., 125,2408, 2003.

[4] Y. Lin, F. Lu, Y. Tu and Z. Ren, Nano Lett, 4, 191, 2004.