

Application of Positively Charged Carbon Nanotubes to Layer-by-Layer Assemblies of Dehydrogenase Enzymes for Molecular Bioelectronic Devices

Barbara Kowalewska, Maciej Dzwonek, Pawel J. Kulesza

Department of Chemistry, University of Warsaw
Pasteura 1, PL-02-093 Warsaw, Poland

In recent years, carbon nanotubes (CNT) have gained considerable attention, because of their remarkable electronic and mechanical properties, which have made them extremely attractive for a wide range of sensing applications from structural materials to nanoelectronic components. The ability of CNT-modified electrodes to promote electron transfer reactions has been documented in connection with important biomolecules [1]. Our goal is to explore new applications of CNT as an electrode material in facilitating the electron transfer between biomolecules and electrode in bioelectrocatalytic systems for ethanol and glucose oxidation that is of potential utility for bioelectronic devices such as biosensors and biofuel cells.

Recently the ability of poly(diallyldimethylammonium chloride) (PDDA) to solubilize carbon nanotubes provides a useful way for preparing CNT-binder composite modified electrode transducer for a wide range of sensing applications. PDDA is a water soluble, quaternary ammonium cationic polyelectrolyte with adhesion and film-forming properties. It was found that PDDA improved dispersibility of CNT in water and facilitate formation of various functionalized CNT-based films [2].

The layer-by-layer (LbL) assembling approach using CNT and PDDA has been recently adopted to prepare various enzyme biosensor configurations. The electrostatic attraction between positively charged (PDDA) molecules and negatively charged enzymes leads to the formation of stable and useful multiple biocomposites on the electrode surface. The main advantage of multilayer systems is the defined increase in the amount of bio-components. Recognition elements in biosensor applications are one example; here the increase in biomolecule concentration leads to a higher sensitivity of the multilayer sensor.

In our research, we have developed the integrated, structured and multifunctional bioelectrocatalytic systems for effective ethanol and glucose oxidation. The concept is based on the layer-by-layer (LbL) assembly through electrostatic attraction of positively charged multi-walled carbon nanotubes and the controlled combination of biomolecules. More specifically, the LbL technique was employed for sequential immobilization of dehydrogenase enzymes and poly(diallyldimethylammonium chloride)-covered multiwalled carbon nanotubes (CNTs/PDDA) onto glassy carbon electrode substrate [3]. Both monoenzymatic (utilizing a single enzyme, alcohol dehydrogenase, ADH or glucose dehydrogenase, GDH) and bienzymatic (anchoring sequentially both ADH and aldehyde dehydrogenase, AldDH) systems were tested. Multilayers were characterized using scanning electron microscopy (SEM), infrared spectroscopy (FTIR) and cyclic voltammetry. The results are consistent with the view that our approach enables good control of distribution and efficient utilization of dehydrogenase enzymes within the bio-composite film and leads to sizeable enhancement of ethanol and glucose oxidation through significant increase

of bioelectrocatalytic currents and by shifting the oxidation potential or decreasing the overvoltage. These simple bio-composite (with single enzyme or enzyme-cascade) systems permits fabrication of highly sensitive ethanol or glucose biosensors based on nicotinamide adenine dinucleotide (NAD⁺) coenzyme-dependent dehydrogenases. Our biosensors exhibited a good linearity ranging, and it was characterized by high sensitivity as well as a low detection limit.

References

- [1] J.J. Gooding, R. Wibowo, J. Liu, W. Yang, D. Losic, S. Orbons, F.J. Mearns, J.G. Shapter, D.B. Hibbert, *J. Am. Chem. Soc.* 125 (2003) 9006.
- [2] B. Kowalewska, P.J. Kulesza, *Electroanalysis*, 24 (2012) 254.
- [3] B. Kowalewska, P.J. Kulesza, *Analytical Chemistry*, 84 (2012) 9564.