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Edge-to-edge Interaction between Carbon Nanotube-Pyrene Complexes and Electrodes for Biosensing and Electrocatalytic Applications

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We demonstrate here that the edge-to-edge interaction between carbon nanotubes (CNTs) and edge plane electrodes plays an important role in exposing a large proportion of the basal planes of the CNTs to allow enhanced π-π stacking of a pyrenyl compound and subsequent high density protein immobilization yielding large electrocatalytic currents.

Carbon nanotube-mediated enhanced biocatalytic reactions of enzymes is an emerging research area. Applications of CNTs in the globally important areas of biocatalysis, biodevices, biosensing, and sustainable energy have been detailed in the literature.[1-3] In our lab, we have focused on the π-π stacked nanotube-pyrene-based surface chemistry that allows covalent immobilization of enzyme catalysts to study direct electrochemical and biocatalytic properties.[4]

Pyrolytic graphite material consists of basal (parallel to the surface) and edge planes (perpendicular to the surface). Dai et al. reported that the basal plane of sidewalls of CNTs could undergo π-π interaction with functionalized pyrene compounds, which would allow facile covalent protein immobilization.[5] Compton et al. unraveled the contribution of CNT edge planes to the electrocatalytic properties of CNTs.[6] They also illustrated the greater electroactivity of edge plane over basal plane electrodes for a dissolved redox species in solution. In another study, Lisdat et al. investigated the effects of different aromatic compounds stacked with CNT-modified electrodes on the oxygen reduction efficiency of immobilized bilirubin oxidase enzyme.[7]

Krishnan and Armstrong developed a membraneless hydrogen fuel cell with enhanced power output and stability by covalently immobilizing *E. coli* hydrogenase-1 as the anode catalyst and bilirubin oxidase as the cathode catalyst on CNT-pyrenebutyric acid (CNT/Py)-modified electrodes.[8] Katz et al. designed an implantable glucose biofuel cell that utilized CNT-pyrene surface chemistry.[9] Direct and efficient reduction of oxygen by laccase immobilized on different pyrene compounds-modified CNT electrodes has also been shown.[10,11] These studies show that nanotube-pyrene-based electrode surface modification for biocatalytic applications is receiving immense attention at present.

We recently demonstrated the differential immobilization of a heme protein via Lys or Glu residues onto different pyrene derivatives bound to CNTs on the surface of high purity graphite (HPG) electrodes.[4] Since HPG surface consists of a mixture of basal and edge planes to varying extents, the selective interaction of CNT with either basal plane or edge plane in affecting the pyrene compound stacking and electrocatalytic properties of immobilized redox enzyme cannot be understood. We propose that understanding this fundamental feature is crucial for designing efficient electrochemical enzyme biosensors and bioreactors.

Therefore, in this study, we demonstrate for the first time the interaction of purely basal plane (BP) or purely edge plane (EP) pyrolytic graphite electrodes with multi-walled carbon nanotubes (MWNT), π-π stacked with pyrenyl compounds in influencing the electrocatalytic properties of immobilized myoglobin (Mb), used here as a redox protein model. For comparison, we chose two non-graphitic electrodes (with no EP or BP surface) such as glassy carbon (GC) and gold (Au) electrodes. The MWNT/Py modification was performed on each electrode surface, and this step was followed by the covalent immobilization of Mb (denoted as MWNT/Py-Mb). The procedure to covalently attach myoglobin to MWNT/Py modified electrodes to obtain MWNT/Py-Mb films is similar to that described in our prior report (details in Supporting Information).[4]

In this short communication, the results of electrocatalytic currents, electroactive enzyme coverage, and spectroscopic studies are compared to understand the influences of the underlying edge versus basal plane surface on MWNT/Py modification and subsequent peroxidase activity of the attached Mb film. The electrochemical and electrocatalytic peroxide reduction properties of immobilized Mb on the designed various electrode materials were investigated by cyclic and rotating disk voltammetry (RDV) methods, respectively.

Figure 1 represents the background subtracted anaerobic (in nitrogen) cyclic voltammograms (CVs) of the designed Mb films on EP, BP, GC, and Au electrodes. The observed reversible CVs confirm the direct electronic communication between the modified electrodes and the Mb-heme redox center.[12]

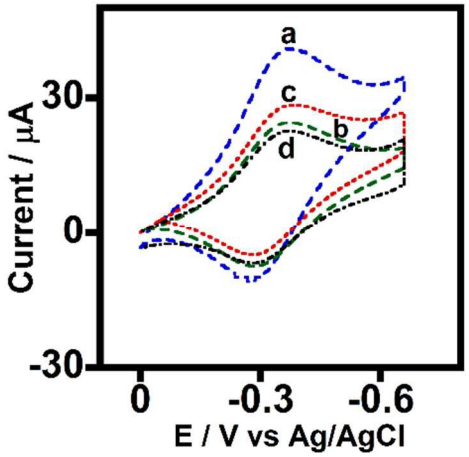

Figure 1. Background subtracted cyclic voltammograms of MWNT/Py-Mb films on **a.** EP, **b.** BP, **c.** GC, and **d.** Au electrodes at 25 ^{o}C in anaerobic nitrogen purged phosphate buffer containing 0.15 M NaCl (pH 7.0), scan rate 0.5 Vs^{-1}.

Figure 2 shows the *tert*-Butyl hydrogenperoxide (t-BuOOH) reduction currents catalyzed by the covalent Mb films on various electrode materials modified with MWNT/Py units (curves a-d). These catalytic currents were subtracted for the small background currents (due to direct oxygen reduction) from the respective MWNT/Py films on each electrode with no attached Mb (Figure 2, e-h). The Tafel analysis of the catalytic voltammograms yielded similar slope values for all Mb films on MWNT/Py-modified electrodes (Figure S1). This indicates that similar peroxide reduction mechanism is followed by the designed Mb films.[13] The decrease in Tafel slope values at low over-potentials can be attributed to the presence of adsorbed peroxide molecules on the modified protein electrode surfaces to rapidly undergo reduction (Figure S1).[14]

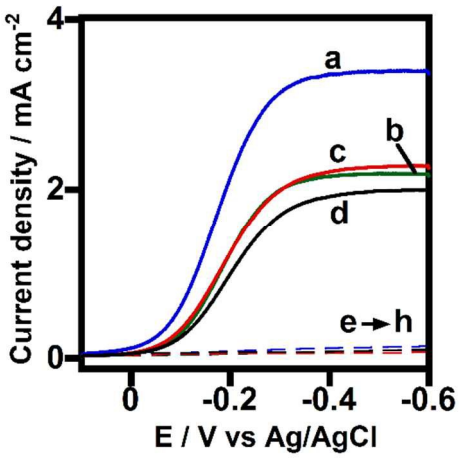

Figure 2. Peroxide reduction currents for modified MWNT/Py-Mb films on **a**. EP, **b.** BP, **c.** GC, and **d.** Au electrodes at 2.0 mM t-BuOOH. The small background currents from MWNT/Py films in the absence of immobilized Mb on each electrode are also shown (curves e-h). Experimental conditions: anaerobic pH 7.0 phosphate buffer, 0.15 M NaCl, 25 ^{o}C, scan rate 0.1 V s^{-1}, rotation rate 1000 rpm.

The average reduction current densities among the BP/MWNT/Py-Mb and non-graphitic GC/MWNT/Py-Mb and Au/MWNT/Py-Mb electrodes were comparable to each other. In contrast, the EP/MWNT/Py-Mb electrode showed a significantly greater reduction current density than the BP/MWNT/Py-Mb electrode (Figure 2). In addition, the EP/MWNT/Py-Mb film required relatively smaller onset peroxide reduction potential compared to the Mb films on the BP/MWNT/Py and other electrodes (Figure 2a versus 2b-d). This suggests the possibility for the existence of a catalytically superior edge-edge interaction between MWNT and edge plane electrodes to expose a large surface area of MWNT-basal planes for high density stacking of Py and the protein attachment. In order to examine this, our further investigation was specifically focused on understanding the interaction of purely edge and purely basal plane characteristics of electrodes towards the MWNT/Py modification and subsequently immobilized heme protein properties as detailed below.

When EP was used as the substrate electrode, the possible interaction of the edge plane sides of the MWNT with the electrode can expose a large portion of the MWNT sidewalls. As a result, the basal plane nature of MWNT sidewalls can thus allow highly dense π-π stacking of Py molecules with exposed –COOH end groups. In contrast, in the case of the BP electrode, the MWNT would interact mainly via the sidewalls basal planes, and thereby expose only a small proportion of MWNT-basal plane sites to undergo π-π stacking with Py molecules. The described edge versus basal plane surface effects was experimentally verified by quantifying the immobilized Py molecules via fluorescence spectrometry. The difference in the fluorescence intensities of free Py solutions before and after stacking onto EP/MWNT or BP/MWNT electrode were measured (Figure 3). From this, we estimated that about 3.6 nmol of Py molecules were bound to the EP/MWNT electrode and 2.7 nmol of Py molecules were bound to the BP/MWNT electrode per unit electrode geometric area (in cm^2). Figure 3 (inset) shows the calibration plot of standard Py solutions used in the estimation of MWNT/Py-bound Py molecules.

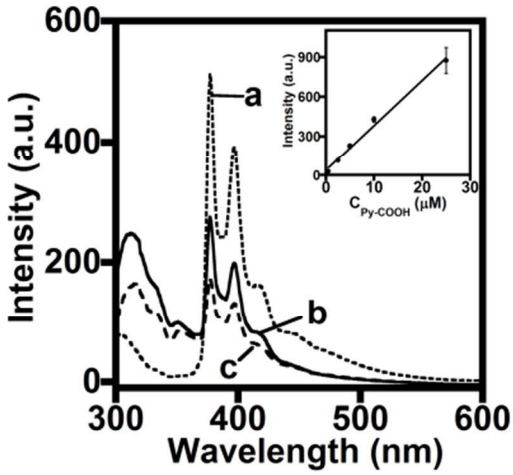

Figure 3. Fluorescence emission spectra of Py solutions: **a.** before adding to electrodes; and those after π-π stacking with **b.** BP/MWNT and **c.** EP/MWNT electrodes. Inset shows the calibration plot of Py standards used for estimating the electrode-bound Py molecules based on the emission peak at 377 nm.

The availability of more π-π stacked Py-linkers on EP/MWNT electrode compared to the BP/MWNT electrode can be further expected to facilitate the covalent immobilization of larger number of Mb molecules on the EP/MWNT/Py electrode. This premise is indeed supported by the observed larger electroactive protein coverage of the Mb film on EP/MWNT/Py electrode compared to the other electrodes (Table 1, Figure 1). The area under the anaerobic cyclic voltammetry peak provided the electric charge (Q

in coulombs) from which the electroactive protein amount was calculated using the equation Q = nFAΓ, where, n is the number of electrons transferred in the redox reaction (n = 1 for Mb-heme), F is the Faraday constant (96485 C), A is the electrode area (0.2 cm^2), and Γ is the electroactive protein coverage in mol cm^{-2}.[15] Table 1 also lists the measured formal potentials and peroxide reduction current densities (reduction current divided by electrode geometric area) of MWNT/Py-Mb films on different electrodes. All modified electrodes exhibited similar formal potentials that corresponded to the Mb-heme redox center at pH 7.0.[4,16]

The estimated Γ of Mb film attached to MWNT/Py units on the EP electrode was ~ 2-fold greater than that of the analogous films on BP and GC electrodes and ~3-fold better than that on the Au electrode. Taken together, the BP, and the smooth and relatively defect free GC and Au electrodes with no edge plane feature do not appear to provide any better enzyme coverage and catalytic properties than the EP electrode for MWNT/Py modification and protein immobilization.

Table 1. Formal potentials, electroactive surface coverage, and reduction current densities of MWNT/Py-Mb films assembled on various electrodes.

Electrode	$E^{o'}$ (V)	Γ (pmol cm^{-2})	Reduction current density[a] (mA cm^{-2})
EP	–0.32 ± 0.01	237 ± 11	3.5 ± 0.2
BP	–0.32 ± 0.01	122 ± 17	2.0 ± 0.1
GC	–0.33 ± 0.01	117 ± 5	2.2 ± 0.1
Au	–0.32 ± 0.01	84 ± 5	1.8 ± 0.1

[a] at –0.5 V vs. Ag/AgCl, 2 mM t-BuOOH, 1000 rpm, pH 7.0.

We also characterized the Mb films on EP/MWNT/Py-Mb and BP/MWNT/Py-Mb electrodes by X-ray photoelectron spectroscopy (XPS) under high vacuum conditions (details in Supporting Information). The XPS data showed the presence of higher quantity of nitrogen element (arising from the Mb polypeptide) in the EP/MWNT/Py-Mb film compared to the BP/MWNT/Py-Mb film (Figure 4). Thus, the fluorescence and XPS results support the observed electrochemical properties and ascertain the favorable edge-edge interaction of EP surface with MWNT/Py modification to allow high density electroactive protein immobilization via the exposed Py-linkers from MWNT-basal planes.

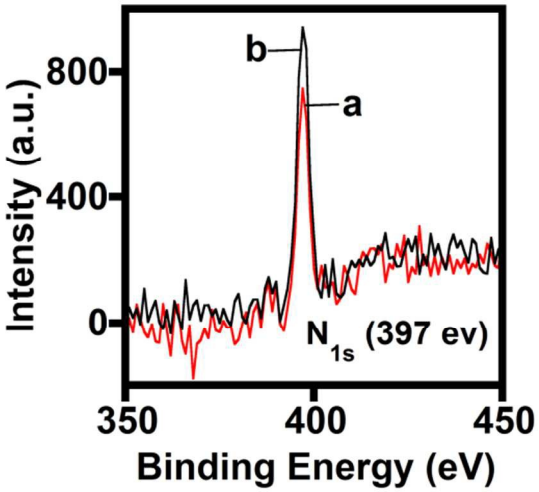

Figure 4. XPS spectra of **a.** BP/MWNT/Py-Mb and **b.** EP/MWNT/Py-Mb electrodes.

In summary, we demonstrated here the edge plane effect in the design of large, conductive MWNT/Py framework for protein immobilization and electrocatalytic applications. The observed catalytic currents suggest the role of MWNT arrangement in controlling the Py stacking and subsequent covalent protein immobilization more favorably on EP electrodes in comparison with BP and non-graphitic GC and Au electrodes. To our knowledge, this is the first report that details the interaction of nanotube-pyrene surface chemistry with various carbon and non-graphitic electrode materials. Exposure of the basal plane of nanotubes for efficient pyrene compound stacking and facilitating high density electroactive protein immobilization on EP electrode is suggested to be crucial in providing high catalytic currents. Detailed kinetic studies of the designed protein electrodes are our next objectives. Overall, the outcome of this communication is expected to be significant in the development of efficient electrocatalytic systems for synthesis, sensing, and renewable energy applications, as the nanotube-pyrene arrangements can be suitably controlled by the choice of underlying electrode substrate.

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Electronic Supplementary Information (ESI) available: Experimental section is provided in detail. See DOI: 10.1039/c000000x/

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TOC:

Edge-to-edge interaction between carbon nanotubes and edge plane electrodes is suggested to favor enhanced π-π stacking of a pyrenyl compound and subsequent high density redox active protein immobilization.