Journal of Physics and Chemistry of Solids 116 (2018) 313–323

Contents lists available at ScienceDirect

Journal of Physics and Chemistry of Solids

journal homepage: www.elsevier.com/locate/jpcs

Study of electron transport in the functionalized nanotubes and their impact on the electron transfer in the active site of horseradish peroxidase

Mina Feizabadi [a], Davood Ajloo [a,*], Ahmad Soleymanpour [a], Hassan Faridnouri [b]

[a] School of Chemistry, Damghan University, Damghan 36715-364, Iran
[b] School of Biology, Damghan University, Damghan 36715-364, Iran

ARTICLE INFO

Keywords:
Non-equilibrium Green's function
Density functional theory
Carbon nanotube
Transmission
Horseradish peroxidase
Differential pulse voltammetry

ABSTRACT

Electrochemical characterization of functionalized carbon nanotubes (f-CNT) including carboxyl (CNT-COOH), amine (CNT-NH_2) and hydroxyl (CNT-OH) functional groups were studied using differential pulse voltammetry (DPV). The current-voltage (I-V) curves were obtained from each system and the effect of f-CNT on redox interaction of horseradish peroxidase (HRP) immobilized on the electrode surface was investigated. The non-equilibrium Green's function (NEGF) combined with density functional theory (DFT) were used to study the transport properties of f-CNT. Additionally, the effect of the number of functional groups on transport properties of CNT, I-V characteristics, electronic transmission coefficients and spatial distribution of f-CNTs have been calculated and analyzed. The results showed that the carboxyl derivative has larger transmission coefficients and current value than other f-CNTs. Then, the effect of functional groups on the electron transport in heme group of HRP is discussed. Finally, the effect of a covalent bond between active site amino acids and amine functional group of CNT was investigated and discussed.

1. Introduction

Carbon nanotube is one of the most promising structures in nanotechnology. Due to its structural stability, flexibility and high electronic properties, several applications can be reported [1]. Because of their exceptional physical, chemical, and electrical properties, namely, a high surface-to-volume ratio, their enhanced electron transfer properties and their high thermal conductivity, CNTs can be employed effectively as electrochemical sensors. Recently, researchers have focused great attention on nanomaterials including metal nanoparticles and nanotubes and they are being studied in nearly every field of science [2,3], medicine [4,5] and engineering [6].

CNT is a popular allotrope of carbon material that becomes much attention as one of the main parts for making molecular electronic devices [7]. They have at least two properties of high sensitivity biosensors: size compatibility with many biomolecules and living cells, and charge transport that is entirely on the surface [8]. Carbon nanotubes can have a semiconducting property, and, thus, its electrical conductivities may be altered by external electrical fields. CNTs with semiconducting properties can be utilized as a sensor transducer device.

Abadir et al. [9] used carbon nanotubes as biomolecular sensors through a sequence of simulations using isoleucine and asparagine amino acids as model analytes. The results demonstrated that local density of states (LDOS) is significantly reduced for both molecules. Therefore, nanotubes can act as sensors for these molecules.

In another study, Thiruvadigal et al. [10] focused their attention on the improvement of CNTs based sensor for the detection of NH_3 and NO_2 molecules which are important environmental pollutants. By changing the charge transfer after absorbing NO_2 and NH_3 molecules on the CNT, one can determine the mechanism of sensing.

By a suitable choice of type of modification, the characteristics of electron transfer of CNT can be greatly increased [11]. According to what was mentioned, increasing the transmission characteristics of CNTs is one of the concerns of nowadays studies. Ewels and coworkers [12] investigated nitrogen doping on CNT and then Shah and et al. [11] studied the effect of doping four atoms: tellurium, arsenic, chromium and antimony in the structure of CNT on transport electronic properties and significant changes were observed in the current.

At the nanoscale, absorbing a small amount of material on the nanotube surface causes considerable changes in current and other transport properties of the system [13–15]. Therefore, the study of the effects of defects, impurities, vacancies, and functionalizations in CNTs is a significant aspect to be considered in the engineering of the device.

Over the past decades, CNTs were interesting electrode materials that

* Corresponding author.
E-mail address: ajloo@du.ac.ir (D. Ajloo).

https://doi.org/10.1016/j.jpcs.2018.01.050
Received 8 August 2017; Received in revised form 24 January 2018; Accepted 27 January 2018

have been considerably investigated due to attractive features such as fast electron transfer, great conductivity and high surface area [16]. They also have the unique ability to direct electron transfer between the enzyme and the electrode [17]. A nanotube is a substance that acts as a molecular wire between the underlying electrode and the enzyme redox center. Functionalized CNTs have been used as electrochemical sensors and many review papers concerning CNTs-electrochemical sensors have been investigated [18–20].

Due to the aforementioned properties, especially, functionalized CNT modified surfaces have been extensively used for improving the direct electron transfer of different proteins [21,22]. Actually, CNT has operated as a channel between the proteins and the electrode surface [23].

On the other hand, the determination of hydrogen peroxide has instrumental importance in chemical, biological, clinical and many other fields. Vast techniques have been developed for this purpose amongst which peroxidase modified electrodes are frequently applied in recent decades, particularly HRP enzyme [24]. The active site of HRP enzyme contains a group of heme. Heme is a cofactor consisting of a Fe^{2+} ion contained in the center of a large heterocyclic organic ring called a porphyrin [25]. Porphyrins are the main components of the electron transfer into living organisms, and act as redox mediators and electron transfer regulators [26,27]. Porphyrins have a common macrocyclic skeleton. The main core of porphyrin consists of four pyrrolic rings (tetrapyrrole). Different metals can be placed inside the porphyrin cavity (particularly, Fe) and thus create different and modern structures with various performance. This structure causes a strong electron delocalization and reduction of the band gap and provides easy and reversible charging [28].

Carbon nanotubes either can act as carriers for enzyme stabilization immobilization to provide a suitable micro-environment to retain the enzyme activity or as a transducer to augment the electrochemical signal for the product of the enzymatic reaction [29–31]. Enzymes are used in different industrial processes, and today they have developed in new fields such as medications, biosensors and biofuel cells [32]. Generally, enzymes are immobilized on the solids to improve their stability [33]. Modified carbon nanotube electrodes are better materials for support, compared to other materials such as silica and zirconia; therefore, they are widely used to immobilize the enzymes. Modified CNTs have characteristics such as stability in hard conditions, the ability to load more enzymes and increase the catalytic activity of the enzyme up to 10 times [34]. On the other hand, CNTs via hydrophobic and π-π electronic interactions can interact with the atomic compounds. To make nanotubes more suitable for electrochemical measurements, they must be precisely functionalized. Functionalization of the CNTs creates a unique electron transfer property and a form of surface design and makes them an ideal candidate for electrochemical processes. Toppare et al. [35] developed a biosensor for detection of organophosphorus pesticides via acetylcholinesterase based on a conducting polymer and multi-walled carbon nanotube. The biosensor demonstrated low detection limit in a good linear range with good stability. Brett and co-worker [36] improved transport electronic properties of CNT by using gold nanoparticles for quantitative detection of choline by Choline oxidase enzyme. Erden et al. [37] also used carboxylated carbon nanotube composited by metal oxide to improve electron transfer in the surface of glass carbon electrode at detection of xanthine by xanthine oxidase enzyme. Biosensor demonstrated good long stability, higher sensitivity, lower detection limit.

In this work, we have focused novelty attention on the phenomenon of electron transfer in the CNTs and compared this phenomenon in three functionalized carbon nanotubes. Then experimental electrochemical data was used to confirm theoretical results. In the next part, the effect of the number of these functional groups on the current through the nanotube was evaluated. Finally, each of these nanotubes as a conduit for the current crossing from the group of heme HRP enzyme was placed and the effect of functional groups was investigated on electron transfer in HRP redox active site I-V curves and transmission spectra were obtained for them. I-V curves and transmission spectra were obtained for each system by NEGF combined with DFT.

2. Experimental and computation

2.1. Reagents

The CNT was purchased from Merck and used without further purification. Horseradish peroxidase (E.C. 1.11.1.7 type-VI, essentially salt-free, lyophilized powder, ≥250 units/mg solid), hydrogen peroxide (30% (w/w) in H_2O), Sodium tetrahydridoborate ($NaBH_4$) and Isophorone diamine were purchased from Sigma-Aldrich. Sulfuric acid (H_2SO_4), Nitric acid (HNO_3) and Sodium nitrite ($NaNO_2$) were purchased from Merck. Doubly distilled water was applied in the experiments. All measurements were carried out in 0.1 M phosphate buffer solution (PBS) (pH = 7.0) used as a supporting-electrolyte solution. The PBS was prepared by mixing standard stock solutions of NaH_2PO_4 and Na_2HPO_4 and then adjusted the pH with NaOH or HCl.

2.2. Apparatus

The electrochemical experiments were conducted using a potentiostat/galvanostat (AUTOLAB PGSTAT-30) electrochemical system (Eco-Chemie, Utrecht, Netherlands) equipped with GPES software. DPV measurements were performed by a three electrode system, including a glassy carbon electrode (GCE) (Azar Electrode Co, Iran) as a working electrode, a platinum wire as the auxiliary electrode, and Ag/AgCl/KCl (Metrohm, Switzerland) as a reference electrode, at 27 °C.

2.3. Preparation of functionalized CNT

2.3.1. Functionalization of CNTs by carboxyl group

CNTs were functionalized according to the literature [8,10,26] as follows: At first, 1 mg of purified CNT was dispersed in a glass tube containing 5 ml mixture of concentrated sulfuric acid and nitric acid (3:1, v/v) in order to introduce carboxyl groups on their surface. This slurry was sonicated in an ultrasonic bath (160HT, Soniclean Pty Ltd, Seoul, Korea) for 2.5 h. The product was then centrifuged three times consecutively for 10 min at 3000 rpm and the pellet was washed with 5 mL of 0.1 M phosphate buffer solution (pH = 7.0) between each centrifugation [38,39].

2.3.2. Functionalization of CNTs by amine group

Zhao et al.'s amino functionalization method was used to functionalize carbon nanotubes [40]. 20 mg CNT-COOH was mixed with 58 mg $NaNO_2$ and 0.05 ml isophorone diamine. 0.036 ml concentrated H_2SO_4 and 1 ml DMF were added. The mixture was stirred for 1 h at 60 °C and cooled to room temperature and then DMF was added to wash the solution. The mixture was centrifuged at 3000 rpm for 15 min and the supernatant was discarded, then the sediment was washed by DMF and centrifuged again, and this process was repeated until the DMF was colorless after centrifuge. This process removed any unreacted substances from the product. The sample was then dried at 60 °C for overnight under vacuum to receive CNT-NH_2.

2.3.3. Functionalization of CNTs by hydroxyl group

The reduction of the oxidized CNTs was obtained by using the method of Kim and co-worker [41]. Typically, 10.0 mg of CNT-COOH was dispersed in toluene by ultrasonication for 30 min, and then 2.5 mg of $NaBH_4$ was added gradually. The solution was stirred for 90 min at room temperature. Then, the solution was placed in a centrifuge at 3000 rpm for 5 min. The supernatant was discarded and the sediment was washed by toluene. Likewise, hydroxyl carbon nanotubes were washed by absolute ethanol and acetone three times and then dried in a vacuum oven at 80 °C overnight.

2.3.4. Fabrication of modified electrode

GCE was polished using 0.3 alumina slurry on a piece of chamois