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Keywords

Direct, Ascorbic, Acid, detection, ferritin, immobilized, single, walled, carbon, nanotubes

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Direct **Ascorbic Acid Detection with Ferritin Immobilised on Single-Walled Carbon Nanotubes**

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Abstract

Ferritin protein was non-covalently immobilized onto single wall carbon nanotubes (SWNTs). This SWNT/ferritin composite was characterised using cyclic voltammetry, UV-vis spectroscopy, Raman spectroscopy and high resolution transmission electron microscopy. The use of the SWNT/ferritin film as an amperometric biosensor was demonstrated by sensing 1.0 mM ascorbic acid in phosphate buffered saline solution with a sensitivity of 767 (A/mg). It demonstrated that ferritin protein bound to SWNTs enhances the oxidation reaction of ascorbic acid over 11-fold.

Keywords; ferritin, carbon nanotube, ascorbic acid, non-covalent immobilisation

1. Introduction

Ferritin, a spherical iron-storage protein, containing 24 peptide subunits, with an outer diameter of 12 nm and inner diameter of 8 nm can sequester up to 4500 iron atoms in the core, with the iron stored in the Fe (III) oxidation state. Ferritin can control the appropriate level of iron in the body by releasing iron through channels which link the interior to the exterior of the shell [1-3]. The protein is remarkably robust, able to withstand biological extremes of pH (2.0 ~ 10.0) and temperatures up to 70(C. Examples of recent applications of ferritin include their use in nanoparticles [4], nano-bio batteries [5-6], fuel cells and bio sensors [7-10].

Carbon nanotubes (CNTs) have attracted a lot of attention due to their potential in a variety of applications: structural, mechanical, electrical and electromechanical [11]. These applications have considerable use in the area of sensors [12-13], high strength conductive composite materials [14-15], fuel cells and hydrogen-storage media [16-17]. One possible approach to improve the performance of biosensor materials is the interaction of the biomolecule of interest with CNTs. Since ferritin can be dispersed in polar matrices, combining ferritin with SWNTs enhances the nanotube interaction with water and allows for the dispersion of SWNTs in aqueous solution [18]. Indeed, the affinity of CNTs for proteins has been observed and documented in the literature. Many applications of these compounds have been investigated, including the catalysis of ascorbate oxidation [19], and enhanced mechanical properties of polymers by incorporating ferritin-functionalized multi-walled carbon nanotubes (MWNTs) [18].

Ascorbate, a water-soluble vitamin known for its antioxidant properties has more recently emerged as a potentially important modulator of striatal function [20]. The determination of ascorbate is therefore of significant interest because of its functions in physiological and pathological processes. Increasing evidence suggests that, apart from scavenging free radicals, ascorbate influences synaptic function [21] and plays an important role in neuroprotection [20]. Previously, ferritin immobilised on a gold electrode was applied to electrocatalytically detect ascorbic acid [22].

In the present study, ferritin protein was immobilised non-covalently on SWNTs. Here we report the preparation and characterization of a SWNT/ferritin composite electrode and its application in enhancing the oxidation of ascorbic acid.

2. Experimental

Single-wall carbon nanotubes (Carbon Nanotechnologies, Inc, Houston), ferritin (Type I: from Horse Spleen, sterile filtered solution in 0.15 M NaCl, Sigma), phosphate buffer saline (Fluka) and L-ascorbic acid (BioChemika Ultra, (99.5% (RT), Fluka) were used as received. Indium tin oxide (ITO) coated glass was purchased from Delta Technologies Ltd. (USA). The ITO glass was washed with acetone overnight and treated in an UVO-cleaner (Model No. 42-220, Jelight Co. Inc., USA) for 30 minutes before use.

Electrochemical testing of the SWNT/ferritin electrode was performed using an electrochemical hardware system comprising of an EG&G PAR 363 Potentiostat/Galvanostat, a Bioanalytical Systems CV27 Voltammograph, a MacLab 400 with Chart v. 3.5.7/EChem v. 1.3.2 software (AD Instruments). All electrochemical experiments were performed at room temperature in a three-

electrode cell. The ITO substrate was used as the working electrode with ferritin, SWNTs and the SWNT/ferritin composite cast as a film on the cleaned surface. An Ag/AgCl electrode and platinum mesh were used as the reference and the auxiliary electrode, respectively.

UV-visible spectra of the samples were examined over the range of 300-1100 nm (Shimadzu UV1601 spectrophotometer). The zeta-potential and size measurement of all dispersions used was measured using a Zetasizer Nano 3600 (Malvern instruments). The Raman spectra of all compounds were studied using Raman spectroscopy (LabSpec[©] Software, Jobin Yvon, Horiba Group, France). Transmission electron microscopy (TEM) analysis was performed using a Philips CM200 microscope, (200 kV).

The SWNT/ferritin composite was prepared by mixing 10 mg SWNTs with 100 mg ferritin in 10 ml deionized water and sonicating for an hour. Following dispersion, 100 μ l of the stable SWNT/ferritin suspension was cast as a film onto ITO (0.5 x 0.5 cm² test area). As controls, 10 mg SWNTs was dispersed in 10 ml water using the same conditions as the SWNT/ferritin composite and 100 μ l of this suspension and 100 μ l of a concentrated ferritin (85 mg/mL) solution were cast onto separate ITO substrates.

3. Results and discussion

The absorption spectra of films of ferritin, SWNTs and SWNT/ferritin were examined using UV-vis spectroscopy (Figure 1). A clear absorption peak from ferritin was observed around 380 nm, with the well known absorption peaks of the SWNTs van Hove singularities occurring between 500-1000 nm [23]. The composite of ferritin and SWNTs shows a shift in the absorption spectrum compared to ferritin and/or SWNTs alone. The absorption peaks for the ferritin protein in the SWNT/ferritin composite occurred at 360 nm (20 nm red shift) and 470-680 nm for SWNTs. The shift in the absorption peaks of the SWNTs in the composite may be due to selective interaction of the SWNTs with ferritin, as has been previously observed with biomolecules and SWNTs [23].

Raman spectroscopy was performed on films of ferritin, SWNTs and SWNT/ferritin (Figure 2). No Raman peaks are observed on a bare ferritin film due to the strong fluorescence observed. The Raman spectra of the RBM (~ 170 - 310 cm^{-1}), D band ($\sim 1350\text{ cm}^{-1}$) and G band (~ 1550 - 1605 cm^{-1}) of SWNTs film with and without ferritin can provide information on the SWNTs structure [24]. The D and G bands of the SWNTs film were measured at 1311 cm^{-1} (D), 1548 and 1587 cm^{-1} (G), respectively. The D and G Raman bands obtained from SWNT/ferritin occurred at 1324 (D) and 1555 , 1592 cm^{-1} (G), respectively. The shifts in the Raman bands due to the presence of ferritin suggest that the ferritin molecules interact with the SWNTs. To confirm this interaction the peaks in the RBM region in both SWNTs and SWNT/ferritin samples were examined. The RBM band positions of SWNTs differ from the SWNT/ferritin composite film by between 5.0 and 2.5 cm^{-1} respectively [25]. These observations are consistent with de-bundling of the SWNTs as has been previously reported [26-27].

The stability and charge of the dispersions used was investigated using zeta-potential measurements. The average size and charge (3 measurements) of ferritin measured using dynamic light scattering and zeta-potential measurements were 11 nm and -20.13 mV , respectively. This size measurement was confirmed by TEM microscopy. The SWNTs dispersion was observed to be negatively charged (-15.49 mV) presumably due to the carboxy groups on SWNTs that arise from the purification processes used. An overall negative charge was measured for the SWNT/ferritin composite as expected, with a value of -43.09 mV ($>-30\text{ mV}$) which indicates a more stable dispersion than that of the SWNTs alone.

The insert B in Fig.2 shows a TEM micrograph of the SWNT/ferritin composite. Ferritin molecules were observed and attached to SWNTs bundles. Individual SWNTs do not appear to support the ferritin molecules, which is not surprising given the size of the ferritin molecules ($\sim 13\text{ nm}$), compared to the SWNTs ($\sim 1\text{ nm}$). Ferritin disperses the SWNTs well in solution, resulting in a smaller SWNTs bundle size than without the protein. These observations are consistent with the de-bundling observed with Raman spectroscopy.

Figure 3 shows the cyclic voltammograms obtained from a ferritin film and SWNT/ferritin composite film on ITO coated glass, in PBS buffer solution as electrolyte (pH 7.4). No clear redox responses were observed at the ferritin (only) ITO coated glass electrode possibly due to the lack of electronic connection between the ferritin film and ITO coated glass electrode and also the water soluble nature of ferritin (insert in Figure 3). A stable redox couple (labelled peaks A ($+0.05\text{ V}$) & B (-0.38 V)) is observed from the SWNT/ferritin ITO coated glass electrode, which may be assigned to the ferritin moiety in the composite [2]. This suggests that there is an interaction

between the ferritin protein and the SWNTs in the composite which enhances both the electrochemical response and the stability of ferritin. The SWNTs in the composite may act both as an anchor for the ferritin and a 1D-nanochannel for the electron transfer of the protein.

The electrocatalytic oxidation of ascorbic acid using the SWNT/ferritin ITO coated glass electrode was demonstrated via cyclic voltammetry (Figure 4) in PBS buffer solution. Figure 4 shows a well-defined oxidation peak of ascorbic acid at + 0.38 V upon the addition of 1.0 mM ascorbic acid (Figure 4a), while no signal was observed in the absence of ascorbic acid (Figure 4b) at the SWNT/ferritin composite electrode. This suggests that the ferritin moiety in the composite electrocatalysed the oxidation of the ascorbic acid.

Detailed amperometric detection test was performed in a stirred buffer solution (rotating speed 1000 rpm) potentiostatically at +0.30 V (*vs.* Ag/AgCl) using both SWNTs and SWNT/ferritin ITO coated glass electrodes. Ascorbic acid (1.0 mM) was added once the current reached a steady state. Figure 5 shows a typical amperometric response obtained from the SWNT/ferritin ITO coated glass electrode upon the addition of ascorbic acid (1.0 mM). It shows that the anodic current at the SWNT/ferritin electrode increased sharply after the addition of ascorbic acid with a response time of less than 3 seconds, while no response was observed at a SWNTs only electrode (without ferritin) under identical conditions. The presence of SWNTs with ferritin shows an increase in current response from 70 (A/mg (SWNTs itself) to 767 (A/mg (SWNT/ferritin), which is approximately 11 times of that of the pure SWNTs. The increased current clearly demonstrates the beneficial effect of SWNTs on the electrochemical properties of ferritin. This indicates that, for the detection of ascorbic acid, the presence of SWNTs in the SWNT/ferritin composite significantly enhances the oxidation reaction of ascorbic acid. Further investigation of the oxidation of ascorbic acid was carried out by sequential additions of 1.0 mM ascorbic acid into PBS buffer solution using the SWNT/ferritin electrode (the insert in Fig.5). It can be observed that the current increased continuously with sequential additions of ascorbic acid. This suggests that the SWNT/ferritin composite could be used as a potential sensor material for the electrochemical detection of ascorbic acid.

4. Conclusion

In summary, a uniform dispersion of a SWNT/ferritin composite has been successfully prepared. Electrochemical studies on SWNT/ferritin composite films were carried out including amperometric detection of ascorbic acid. The results obtained from UV-Vis and Raman spectroscopy, cyclic voltammetry and TEM imaging provide evidence of the interaction between SWNTs and ferritin. This type of SWNT/ferritin composite displays a stable amperometric response to ascorbic acid; demonstrating its promise as a biosensor material.

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Figure 1: UV-absorption spectra of films coated on ITO glass; (a) ferritin film and (b) SWNT/ferritin (1:10) film. Inset: UV-absorption spectra of SWNTs film.

Figure 2: Raman spectra of (a) SWNTs and (b) SWNT/ferritin (1:10) films coated on ITO glass. Insert A highlights the RBM region; and Insert B shows a TEM image of the SWNT/ferritin composite.

Figure 3: Cyclic voltammograms obtained using (a) ferritin and (b) SWNT/ferritin (1:10) coated on ITO glass as the working electrode in PBS buffer (pH 7.4). Scan rate = 100 mV s^{-1} . The insert is the CV of ferritin itself (coated on ITO).

Figure 4: Cyclic voltammograms of SWNT/ferritin coated ITO glass in PBS buffer (pH 7.4) at 100 mV s^{-1} (a) without ascorbic acid and (b) with ascorbic acid (1 mM).

Figure 5: Amperometric responses obtained for SWNT/ferritin and bare SWNTs, addition of 1.0 mM ascorbic acid. Applied potential = 0.3 V. The insert shows amperometric responses obtained with an applied potential 0.3 V with sequential additions of ascorbic acid at the SWNT/ferritin composite electrode. Each step corresponds to an increase in ascorbic acid concentration of 1.0 mM.

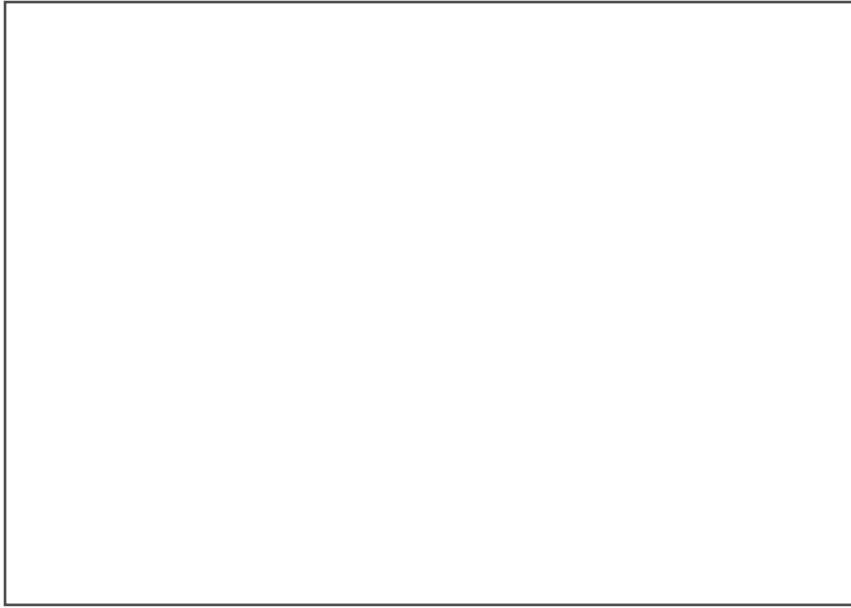


Figure 1

[pic]

Figure 2

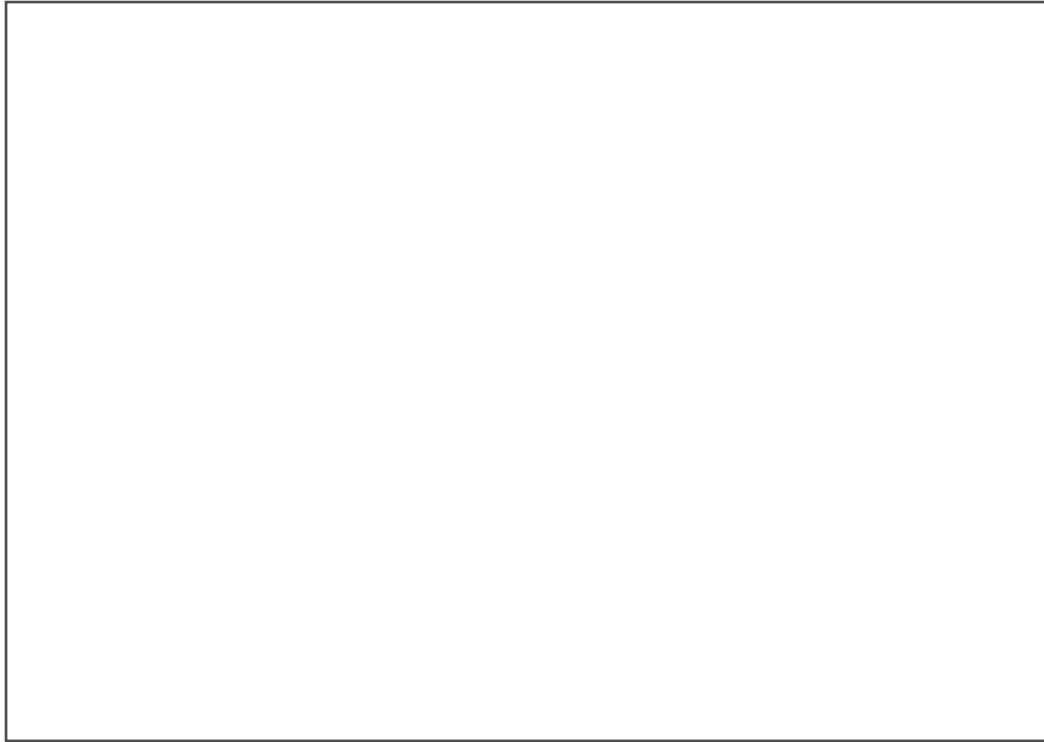


Figure 3

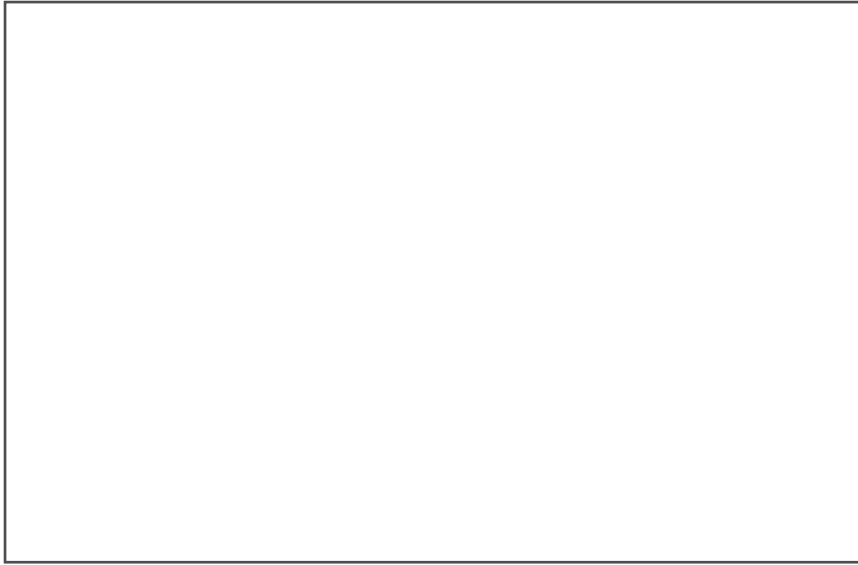


Figure 4

[pic]

Figure 5

A

b

a

E/V

I/mA

a

B