

Sandwich nanohybrid of single-walled carbon nanohorns–TiO₂–porphyrin for electrocatalysis and amperometric biosensing towards chloramphenicol†

Wenwen Tu, Jianping Lei,* Lin Ding and Huangxian Ju*

Received (in Cambridge, UK) 6th April 2009, Accepted 14th May 2009

First published as an Advance Article on the web 9th June 2009

DOI: 10.1039/b906876g

A sandwich nanohybrid of single-walled carbon nanohorn–TiO₂–porphyrin was prepared *via* the dentate binding of TiO₂ nanoparticles to carboxylate groups, which showed an excellent electrocatalytic activity towards the reduction of chloramphenicol in neutral media, leading to a highly sensitive and stable amperometric biosensor for chloramphenicol.

Single-walled carbon nanohorns (SWNHs), as a dahlia-flower-like spherical superstructure of aggregated nanosized graphitic tubes, have attracted considerable attention for hydrogen storage and drug delivery due to the high surface area, excellent porosity, and considerable internal nanospace.¹ The functionalization of SWNHs can improve the solubilization for applications in a variety of fields. Especially, SWNHs functionalized with porphyrins, a mimic of enzymes, is a promising biomaterial for photoinduced electron and energy transfer, and fluorescence properties.²

Meanwhile, the photovoltaic, photophysical, photocatalytic properties of porphyrin bound TiO₂ nanoparticles have been widely studied.³ The porphyrin bound carboxylic groups can spontaneously adsorb onto TiO₂ nanoparticles by bridging, ester-like or bidentate binding.^{3b,c} This work uses hydroxy-ferriprotoporphyrin as a model and TiO₂ nanoparticles as a bridge to prepare a sandwich nanohybrid of SWNHs–TiO₂–porphyrin as shown in Fig. 1. The resulting nanohybrid shows excellent electrocatalysis toward reduction of chloramphenicol (CAP), leading to a sensitive amperometric biosensor for CAP.

CAP is a bacteriostatic antibiotic for the treatment of life threatening infections such as childhood meningitis and typhoid fever. Owing to serious side effects such as aplastic anemia, leucopenia and thrombocytopenia, many countries and organizations have imposed a strict ban on the use of CAP in food-producing animals.⁴ Therefore, effective monitoring of CAP is urgently important. Several methods such as surface plasmon resonance,^{5a} liquid chromatography electrospray ionization tandem mass spectrometry^{5b} and immunochip

processes^{5c} have been developed for determination of CAP. Although the detection limits of some methods reach down to fM or nM levels, they require expensive equipment, time-consuming derivatization and extraction, and professional operation. Particularly, it is difficult to use these methods for *in situ* or online monitoring. This work provides a low-cost method for convenient detection of CAP. Compared with those based on amperometric biosensors⁶ this method shows higher sensitivity and a wider concentration range.

X-Ray photoelectron spectra (XPS) of O 1s were recorded to characterize the formation of the sandwich nanohybrid. The O 1s XPS spectrum of the porphyrin consisted of two peaks assigned to carbon–oxygen double bonding (C=O, 532.3 eV) and single bonding (C–O, 533.7 eV) (Fig. S1a in ESI†). By contrast, the O 1s XPS spectrum of TiO₂–porphyrin exhibited three chemically different peaks corresponding to Ti–O at 531.1 eV, C=O at 532.7 eV and C–O at 533.9 eV (Fig. 2(a)). These results indicated that oxygen of the carboxylate groups of the porphyrin bound to the TiO₂ surface.^{3c} Compared with the O 1s peak at 529.85 eV for Ti–O of TiO₂,^{7a} the Ti–O bond in TiO₂–porphyrin showed higher energy due to the presence of the electron-donating carboxylate groups of the porphyrin.^{7b} The Ti–O bond in SWNHs–TiO₂–porphyrin showed a binding energy of 530.3 eV, lower than that in TiO₂–porphyrin (Fig. 2(b)) due to the relatively lower amount of carboxylate bound to TiO₂ surface. The intensities of C=O at 532.0 eV and C–O at 533.2 eV for SWNHs–TiO₂–porphyrin were slightly larger due to the presence of COO groups on

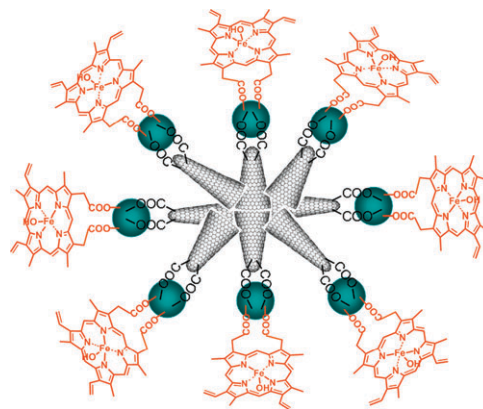


Fig. 1 Schematic illustration of the structure of SWNHs–TiO₂–porphyrin nanohybrids. Other oxygenated species (*i.e.*, hydroxyls, carbonyls, carboxyls, *etc.*) introduced during the oxidation of SWNHs are not shown for simplicity.

Key Laboratory of Analytical Chemistry for Life Science (Education Ministry of China), Department of Chemistry, Nanjing University, Nanjing 210093, P. R. China. E-mail: jpl@nju.edu.cn, hxju@nju.edu.cn; Fax: +86-25-83593593; Tel: +86-25-83593593

† Electronic supplementary information (ESI) available: Related reagents, instrumentation, preparation of nanohybrid and modified electrode, XPS, IR Raman spectra and TEM images, electrochemical behaviors of SWNHs–TiO₂–porphyrin modified GCE, chemical structures, effects of SWNHs and TiO₂ contents on electrocatalysis. See DOI: 10.1039/b906876g

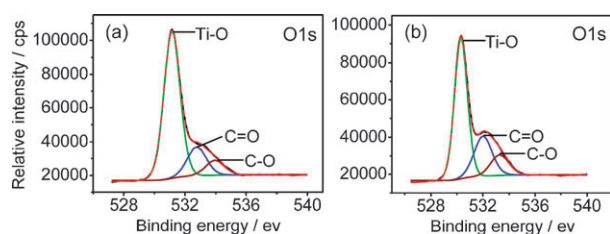


Fig. 2 O 1s XPS spectra of TiO₂-porphyrin (a) and SWNHs-TiO₂-porphyrin (b).

SWNHs produced by the oxidation treatment (Fig. S1b in ESI†).^{1b} These changes identified that a sandwich nanohybrid of SWNHs-TiO₂-porphyrin was formed *via* the dentate binding of TiO₂ nanoparticles to the carboxylate groups of the porphyrin and to SWNHs.

The FTIR spectrum of the porphyrin showed a peak assigned to the C=O stretch mode of carboxylic group at 1722 cm⁻¹ and a peak attributed to the B_{3u} vibration of porphyrin at 1378 cm⁻¹ (Fig. S2A in ESI†).⁸ The appearance of the peaks at 1730, 1581 and 1205 cm⁻¹ for SWNHs indicated the presence of carboxylic and carboxylate oxygen-containing groups on the SWNHs surface (Fig. S2B in ESI†). Compared with SWNHs, the small shifts of carboxylic groups of SWNHs-TiO₂ resulted from the dentate binding between the carboxylic group of SWNHs and TiO₂ (Fig. S2C in ESI†), indicating a good electronic communication between SWNHs and TiO₂. In addition, the fingerprint region of porphyrin appeared from 1200 to 700 cm⁻¹ in SWNHs-TiO₂-porphyrin (Fig. S2D in ESI†), suggesting that porphyrin had successfully assembled on SWNHs-TiO₂.

The Raman spectrum of SWNHs showed a disorder-induced D-band at 1344 cm⁻¹ and a tangential stretch G-band at 1573 cm⁻¹ (Fig. S3B in ESI†). Comparing with SWNHs, these bands of SWNHs-TiO₂ were slightly shifted to 1350 and 1578 cm⁻¹ with remarkably enhanced strength, respectively (Fig. S3C in ESI†), which should contribute to the TiO₂-to-SWNHs charge transfer mechanism related to the surface state energy levels of the TiO₂ semiconductor.⁹ Moreover, the ratios of D- to G-bands for SWNHs and SWNHs-TiO₂ were 1.15 and 1.13, respectively, which indicated the functionalization of SWNHs with TiO₂ did not damage the structure of the SWNHs. TEM images of SWNHs and SWNHs-TiO₂-porphyrin films further demonstrated that the TiO₂ stacked on the surface of SWNHs and the original dahlia-flower-like morphology of the SWNH aggregates was still sustained (Fig. S4 in ESI†). The average diameter of the SWNHs was estimated to be around 80 nm, which was consistent with the previous report.^{2b}

By using the Fe(CN)₆^{3-/4-} redox couple as a probe, Nyquist plots were used to monitor the modification of electrodes (Fig. S5(a) in ESI†). The bare GCE showed an electron transfer resistance of about 360 Ω, while the coating of porphyrin on the electrode increased dramatically the resistance to about 960 Ω, suggesting that porphyrin film blocked the electron exchange between the redox probe and electrode surface. However, the porphyrin-TiO₂ modified GCE showed an electron transfer resistance of 822 Ω, indicating the presence of TiO₂ could improve the electron transfer. Compared with

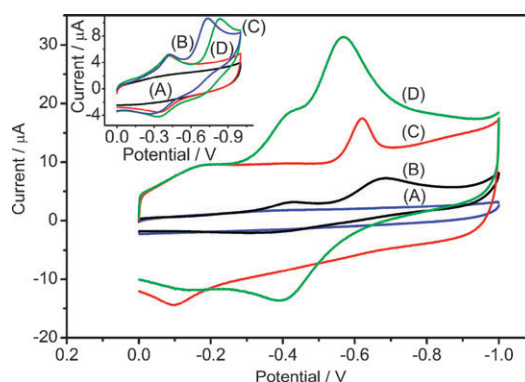


Fig. 3 Cyclic voltammograms of bare GCE (A), TiO₂-porphyrin (B), SWNHs-TiO₂ (C), SWNHs-TiO₂-porphyrin (D) modified GCEs in 0.1 M pH 7.0 PBS with 100 μM CAP at 100 mV s⁻¹. Inset: cyclic voltammograms of IL (A), porphyrin-IL (B) modified GCEs in 0.1 M pH 7.0 PBS containing 100 μM CAP, and porphyrin-IL modified GCE in 0.1 M pH 7.0 PBS in the presence (C) and absence (D) of 100 μM *p*-nitrophenol at 100 mV s⁻¹.

both porphyrin and porphyrin-TiO₂ modified GCEs, the electron transfer resistance of SWNHs-TiO₂-porphyrin was 318 Ω, implying the SWNHs further accelerated the electron transfer due to the excellent electronic conductivity. In addition, SWNHs-TiO₂-porphyrin modified GCEs showed a pair of redox peaks (Fig. S5(b) in ESI†), and the electrode reaction showed a surface controlled electrode process (Fig. S6 in ESI†), which could be attributed to the redox couple of Fe^{III}/Fe^{II} in the immobilized porphyrin.

Upon addition of CAP in PBS the cyclic voltammogram showed another cathodic peak with current of 4.3 and 7.9 μA at -0.68 and -0.62 V at TiO₂-porphyrin and SWNHs-TiO₂ modified GCEs, respectively, while the bare electrode did not show any response (Fig. 3). The presence of SWNHs accelerated the electronic reduction of CAP. At SWNHs-TiO₂-porphyrin modified GCE the reduction peak of CAP further increased to 22.0 μA and the peak potential positively moved to -0.56 V, showing a synergic effect due to the superstructure of the nanohybrid material. Here TiO₂ nanoparticles acted as bridges to form the sandwich nanohybrid, SWNHs increased the electron transfer rate of the sensing layer, and porphyrin acted as a catalyst to reduce the reduction potential of CAP by the 'mediated mechanism'.^{3f} The reduction potential was much more positive than those with amperometric biosensors,⁶ producing an excellent amperometric biosensing application for CAP detection.

In order to confirm the catalytic behavior of porphyrins in electrocatalyzing the reduction of CAP, ionic liquid was used as a solvent to dissolve the porphyrin for its immobilization on an electrode surface. Comparing curves A, B and D in the inset in Fig. 3, the porphyrin undoubtedly acted as a catalyst to electrocatalyze the reduction of CAP. Further, dichloroacetic acid and *p*-nitrophenol (Fig. S7 in ESI†) were used to mimic the dichloroacetic group and nitroaromatic group of CAP, respectively, to identify the reduction site of CAP. Upon addition of 100 μM dichloroacetic acid in PBS, no response was observed at IL-porphyrin modified GCEs, indicating the reduction site was not the dichloroacetic group. In contrast, when adding 100 μM *p*-nitrophenol in PBS, the same

electrocatalytic appearance as the reduction of CAP could be observed (curve C, inset in Fig. 3), thus the electroactive site was the nitroaromatic group. To our best knowledge, this is the first report that porphyrins can be used as catalysts for CAP reduction.

At optimal contents of SWNHs and TiO₂ nanoparticles for preparation of SWNHs–TiO₂–porphyrin nanohybrids (Fig. S8 in ESI†), the current–time curve of SWNHs–TiO₂–porphyrin modified GCE upon successive addition of CAP at an applied potential of –0.56 V clearly illustrated that the modified GCE could respond very rapidly to the change in the CAP concentration within only 5 s (Fig. S9 in ESI†). The linear detection range could be up to 135.7 μM with a detection limit of 0.9 nM at a signal to noise ratio of 3, which was much lower than those of 5.0 nM at IL-gold nanoparticles-SWNTs modified GCE,^{6c} 47 nM at a carbon fibre microelectrode^{6a} and 420 nM at a Nafion coated screen-printed electrode.^{6b} The linear range of five orders of magnitude was much wider than those reported above. Thus the SWNHs–TiO₂–porphyrin based biosensor for CAP showed a better performance.

The CAP biosensor also showed good fabrication reproducibility with a relative standard deviation of only 4.6% as estimated from the slopes of the calibration plots at five freshly prepared SWNHs–TiO₂–porphyrin modified GCEs prepared at different electrodes. The coefficients of variation of the current signals for seven repeated injections of 5 nM and 5 μM CAP examined at the same modified electrode were 4.3 and 3.9%, respectively, indicating a good operational reproducibility. Consecutive cyclic voltammetric sweeps also showed acceptable stability of the sensor (Fig. S10 in ESI†). When the biosensor was not in use, it was stored in the shade at room temperature. No obvious decrease in the amperometric response to CAP was observed after 5 days of storage and it retained 91.7% of initial amperometric response after 25 days. This implied that the structure of the SWNHs/porphyrin–TiO₂ film was very efficient for retaining the activity of porphyrin and preventing it from leaking out of the biosensor.

The effects of common interfering species on the biosensor response were examined in a biological sample. For example, ions (Na⁺, K⁺, Mg²⁺, Cu²⁺, Ca²⁺, Zn²⁺, NH₄⁺, Cl[–], SO₄^{2–}, PO₄^{3–}), saccharide (sucrose, glucose), and antibiotic (chlorotetracycline, streptomycin, penicillin) at 1000 times concentration of CAP did not interfere with the amperometric response of CAP. These results indicated that the amperometric biosensor had an excellent specificity in the detection of CAP.

The concentration of CAP in injection was detected with the proposed biosensor to be 0.40 ± 0.017 M (5 measurements) without any need of sample pretreatment except an appropriate dilution of sample. This value was close to 0.39 M according to the instruction of CAP injection detected by high performance liquid chromatography. Furthermore, recovery testing was carried out to demonstrate the validity of the proposed method. After 15 nM and 5 μM of CAP were added to this sample, the obtained recoveries of CAP were 105.4 ± 4.3% and 96.7 ± 3.9%, respectively, indicating acceptable accuracy.

In summary, a sandwich nanohybrid of SWNHs–TiO₂–porphyrin is prepared *via* the dentate binding of the

carboxylate groups onto TiO₂ nanoparticles as bridges for highly sensitive amperometric biosensing of CAP. The direct electrochemistry corresponding to the redox couple of Fe^{III}/Fe^{II} of the porphyrin is realized. The SWNHs–TiO₂–porphyrin modified electrode shows excellent electrocatalytic activity toward reduction of CAP due to the synergic effect among SWNHs, TiO₂ and porphyrin. The electroactive site of CAP is confirmed to be the nitroaromatic group. The designed biosensor exhibited good analytical performance in the detection of CAP such as rapid response, wide linear range, low detection limit, good fabrication reproducibility, and acceptable accuracy. The sandwich nanostructure of SWNHs–TiO₂–porphyrin provides a functional electrocatalyst to construct a sensitive biosensor and can be extended for the application in the photovoltaic- and photo-catalysis in solar cells.

This work was supported by the fund for Creative Research Groups (20821063), Major Research Plan (90713015), Key (20535010, 20835006) and General (20875044, 20745003, 20705012) Programs from NSFC, PhD Fund for Young Teachers (20070284052) and Science Foundation of Jiangsu (BK2007570).

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