

Sensitive dopamine recognition by boronic acid functionalized multi-walled carbon nanotubes†

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Boronic acid functionalized multi-walled carbon nanotubes (MWNTs) have been synthesized and used for sensitive dopamine (DA) detection using electrochemical methods in the presence of excess L-ascorbic acid (L-AA) via specific, reversible formation of a boronate ester between DA and the functionalized MWNTs.

Chemical and physical functionalization endows carbon nanotubes (CNTs) with diverse functions and paves the way for their future applications in different fields.¹ CNTs are generally considered to be ideal electrode materials due to their outstanding characteristics of high conductivity, chemical inertness, large surface area, low mass density and good biocompatibility, and thus have been extensively studied to elucidate their electrochemical properties for nanodevices and biosensors.² As active transducers, CNTs decorated with proteins (such as enzymes and antibodies) were used for detection of glucose,^{2f} proteins,^{2g,2h} and DNA^{2g} with high sensitivity and specificity, but proteins as recognition receptors often suffer from a lack of stability in biosensor measurements. Up to now, few small molecules have been used as recognition receptors with CNTs as relay units.^{2a,2b}

Herein, we report on the specific and reversible binding between boronic acid functionalized MWNTs and DA for DA recognition coupled with sensitive electrochemical detection. DA (**4**, Fig. 1d) is an important neurotransmitter and its deficit induces brain disorders such as Parkinson's disease and schizophrenia.³ In biological matrixes, DA normally coexists with L-AA (**5**, Fig. 1d), which interferes in the electrochemical detection of DA because of their very close redox potentials.⁴ Many attempts have been devoted to overcome this obstacle, including pre-treatment and modification of electrode surfaces, such as laser and heat activation, and modification with polymers, various permselective membranes and carbon materials, *etc.*⁴ However, development of stable and efficient materials for sensitive and selective detection of DA is still a major challenge. By reversibly forming a boronate ester from the reaction between the diol group of DA and boronic acid,⁵ the boronate ester can be electrochemically oxidized at potentials much more positive than those necessary to oxidize DA itself at physiological conditions,^{5b} hence imparting specificity to DA recognition. Further, covalent immobilization of the boronic acid on CNTs could allow highly sensitive, selective, label-free and

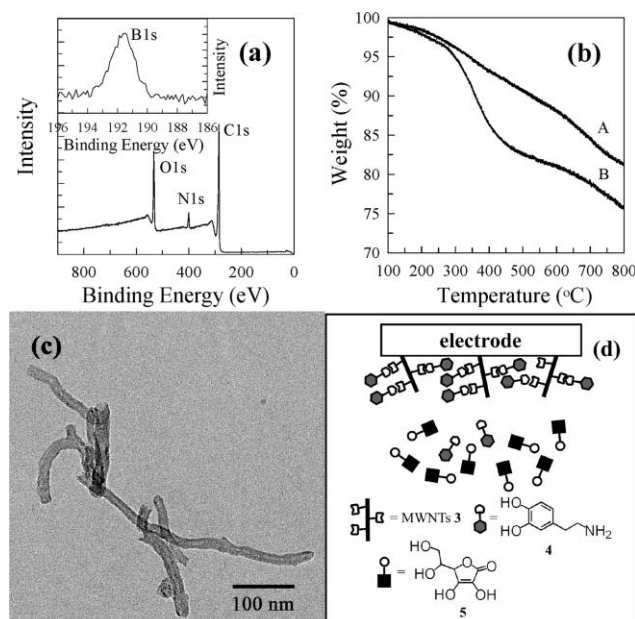


Fig. 1 (a) XPS spectrum of MWNTs **3**. Inset: B 1s spectrum. (b) TGA profiles of MWNTs **1** (A) and MWNTs **3** (B). (c) TEM image of MWNTs **3**. (d) Schematic illustration of recognition binding between MWNTs **3** and DA in the presence of L-AA.

potentially recyclable detection of DA by electrochemical means because the CNTs can effectively short the underlying electrode and the redox-active sites.

Oxidized MWNTs (MWNTs **1**, Scheme 1a) were obtained from treatment of the pristine MWNTs with concentrated HNO₃ at 110 °C for 12 h. Thereafter, the boronic acid functionalized MWNTs (MWNTs **3**) were synthesized *via* a two-step reaction, *i.e.*, amino group introduction⁶ and its coupling to 4-carboxyphenylboronic acid under the activation of *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC) (Scheme 1a; see ESI† for detailed synthetic procedures).

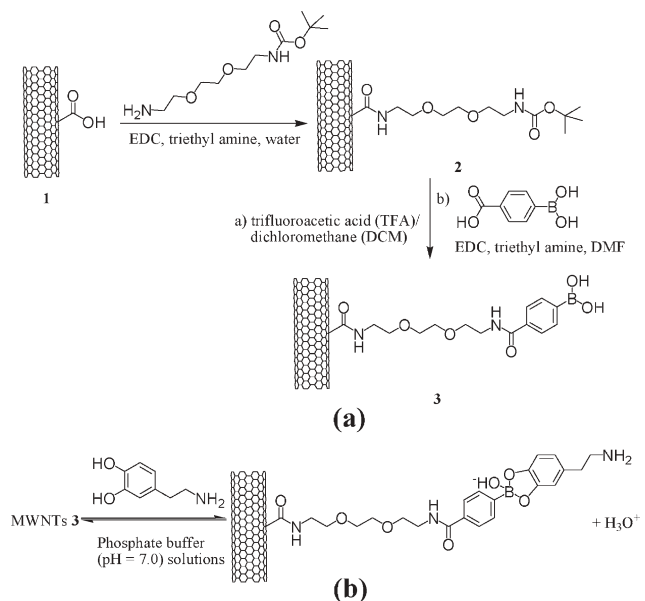
Analysis of MWNTs **3** by X-ray photoelectron spectroscopy (XPS) exhibits a B 1s peak at 191.5 eV, indicating successful attachment of the boronic acid groups to the MWNTs (Fig. 1a). The atomic ratio of N to B, estimated by XPS, suggests that about one third of the CNT-bound amino groups have reacted with the boronic acid. Thermogravimetric analysis (TGA) of MWNTs **3** displays a steep weight loss (~15%) in the temperature range of 270–470 °C (Fig. 1b). This weight loss is attributed to the pyrolysis of the triethylene glycol chains as well as the linked phenylboronic acid groups. On the basis of the XPS and TGA results, we estimate that for every ~300 carbon atoms of MWNTs **3** one boronic acid

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Scheme 1 (a) Functionalization of MWNTs with boronic acid. (b) The reversible condensation reaction between boronic acid functionalized MWNTs and DA.

group is attached. MWNTs **3** were also characterized by transmission electron microscopy (TEM), which shows that the MWNTs have been cut into short nanotubes ($<1 \mu\text{m}$) with open ends (Fig. 1c and ESI†, Fig. S1).

Specific binding between MWNTs **3** and DA (Scheme 1b; Fig. 1d) was revealed from cyclic voltammograms (CVs) and differential pulse voltammograms (DPVs) by using MWNTs **3**-modified glassy carbon (GC) as a working electrode. All of the CVs and DPVs were measured in 0.1 M phosphate buffer (pH = 7.0) solutions at a scan rate of 100 mV s^{-1} for CVs and 20 mV s^{-1} for DPVs unless otherwise stated. The uniform nanostructured morphology of the MWNTs **3**-modified GC electrode can be appreciated from the scanning electron microscopic (SEM) image (Fig. 2a), which was obtained using the same sample preparation procedure as that of the modified electrodes. As a blank experiment, the modified electrode exhibits no significant current peak (see ESI†, Fig. S2), and thus should provide an ideal potential window to probe DA and L-AA. DA has been used extensively to assess electrocatalysis on carbon electrodes because its electrochemical response is very sensitive to the nature of the carbon electrode interface.⁷ In the present case, the redox current of DA on the MWNTs **3**-modified electrode is found to be much higher than that on a bare electrode, which demonstrates the electrocatalytic ability of MWNTs **3** (see ESI†, Fig. S3). Apart from the redox peaks of DA at $\sim 192/106 \text{ mV}$, a new pair of peaks emerged at $380/290 \text{ mV}$ (Fig. 2b). These are assigned to oxidation/reduction of the boronate ester formed between the CNT-bound boronic acid and DA because the MWNTs **3**-modified GC electrode is not electroactive in the potential window examined and the corresponding peaks are not detected in the CV of DA on pristine MWNTs-modified GC electrodes under the same conditions (see ESI†, Fig. S4). The oxidation peak of the boronate ester was also observed at 310 mV in the DPV of DA on the MWNTs **3**-modified GC electrode, together with that of DA at 138 mV (inset, Fig. 2b).

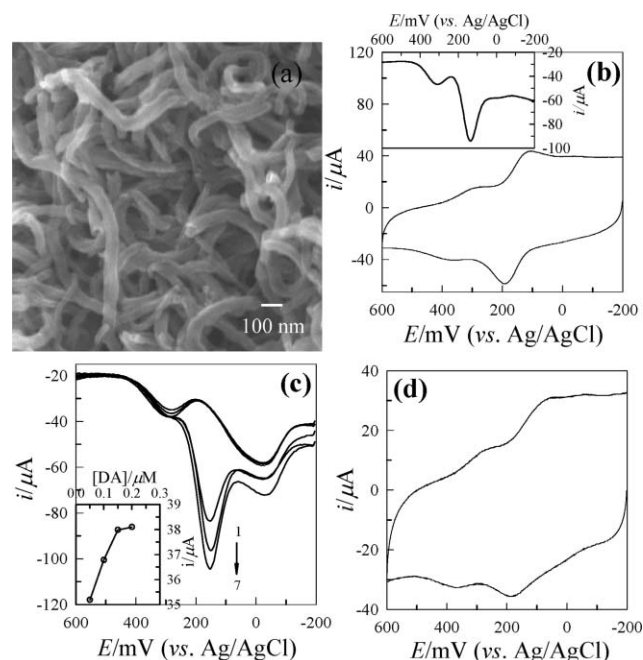


Fig. 2 (a) SEM image of MWNTs **3**. (b) CV of 0.1 mM DA with MWNTs **3**-modified GC electrode. Inset shows the corresponding DPV. (c) DPVs of DA in the presence of 5 mM L-AA. [DA]: (1) 0.05 μM , (2) 0.1 μM , (3) 0.15 μM , (4) 0.2 μM , (5) 100 μM , (6) 300 μM , (7) 500 μM . Inset plots the oxidation current of the boronate ester versus [DA]. (d) CV of the modified electrode after soaking in a solution mixture of DA (0.5 mM) and L-AA (5 mM) in phosphate buffer saline for 5 min and washing with the same solution 10 times.

To confirm the selectivity of MWNTs **3** for DA, the CVs of L-AA were measured on both a bare GC electrode and the MWNTs **3**-modified GC electrode (see ESI†, Fig. S5). The CV of L-AA on a bare electrode presents a broad oxidation peak at 400 mV . On the MWNTs **3**-modified electrode, this is significantly shifted to 75 mV ,⁴ but no new redox peak was detected, ruling out the formation of a detectable boronate ester between the CNT-bound boronic acid and L-AA. Thus, the highly specific DA recognition can be assured from the presence of redox peaks of the boronate ester, which allows selective detection of DA. DPVs of DA in the presence of excess L-AA, fixed at 5 mM, were measured at different concentrations (Fig. 2c). The boronate ester oxidation current (285 mV) displays a linear relationship with the DA concentration below $0.15 \mu\text{M}$, whereas the DA oxidation peak (151 mV) is masked by the strong oxidation peak of L-AA (-28 mV) under these conditions. When the $[\text{DA}] > 0.15 \mu\text{M}$, however, the boronate ester oxidation current levels off due to binding saturation of the recognition sites on the electrode by DA, whereas the DA oxidation current starts to increase linearly with DA concentration. It is fair to conclude that the DA detection limit of the redox marker of boronate ester is far below $0.05 \mu\text{M}$. This low detection limit is attributable to high conductivity and finely-dispersed recognition sites on the MWNTs **3**-modified electrode, as well as the accumulation of DA on the electrode surface through boronate ester formation.^{4d}

Via recognition binding between the boronic acid group and DA, we are able to use MWNTs **3** to pick out DA from solution mixtures of DA and excess L-AA. In a typical experiment,

MWNTs **3**-modified GC electrodes were soaked in a solution of DA (0.5 mM) and L-AA (5 mM) in a 0.1 M phosphate buffer (pH = 7.0) for 5 min. After washing the electrode with the buffer several times, cyclic voltammetry was performed (Fig. 2d). Remarkably, only the redox peaks from DA and its boronate ester with MWNTs **3** were observed, without any signal from L-AA. Presumably, the DA peaks are partially from reversible release *via* hydrolysis of the boronate ester and partially from nonspecific electrostatic binding between CNT-bound carboxylic residues and amino groups of DA. Importantly, the MWNTs **3**-modified GC electrode, after being used, can regain the original DA response by acid treatment in 0.5 M HCl solution with almost no loss of activity, and therefore is potentially recyclable.

In conclusion, we have covalently attached boronic acid onto MWNTs for the first time. Electrochemical detection of DA in the presence of excess L-AA has been demonstrated, with high sensitivity and specificity through the recognition of DA by the functionalized MWNTs (through reversible binding between the boronic acid and the diol group of DA) and the active transduction of MWNTs for shuttling electrons. The recognition binding between the CNT-bound boronic acid and DA can also be used to pick out DA from a solution mixture of DA and excess L-AA in a highly selective fashion. The added molecular recognition capability of the covalently functionalized CNTs, coupled with the inherently good biocompatibility, high conductivity, large surface area, chemical inertness and low mass density of the CNTs, shall provide a strong basis for a new class of materials for chemical and biochemical sensing.

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