

DNA electrochemical sensor based on an adduct of single-walled carbon nanotubes and ferrocene

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Abstract A novel electrochemical sandwich-type gene sensing system was designed by using a DNA probe (DNA-probe1) immobilized on a gold electrode, the target DNA, and another DNA probe (DNA-probe2) conjugated on a single-walled carbon nanotubes/ferrocene (Fc–SWNT) adduct. In this sandwich-type gene-sensing electrode, the Fc–SWNT adduct could significantly amplify the electrochemical response of the reduction of H₂O₂. The target DNA could be detected selectively and sensitively based on the much enhanced electrochemical catalytic property of the Fc–SWNT adduct toward H₂O₂ reduction.

Keywords DNA · Electrochemistry · Immobilization · Single-walled carbon nanotubes

Introduction

The unique electrical properties of single-wall carbon nanotubes (SWNT) have generated a huge

amount of interest for nanoelectronic devices and nanosensors (Wang et al. 2003a, b; Chen et al. 2003). The excellent conductance, large surface area and high aspect ratio of these materials provide a favorable microenvironment for many electroactive substances and biochemical molecules (Azamian et al. 2002; Wang et al. 2003a, b). Thus it is possible to use this material to achieve a new and reliable method for the bioelectrochemical applications. Recently, SWNT have been used to construct biosensors for DNA detection. For example, Li et al. (2003) selectively functionalized oligonucleotide probes to the open ends of nanotubes and detected the target DNA sequence by combining such electrodes with Ru(bpy)₃²⁺-mediated guanine oxidation. Cai et al. (2003) immobilized probe DNA sequence onto the SWNT-modified glassy carbon electrode and detected the target DNA by using an electroactive intercalator daunomycin as an indicator. SWNT can amplify electrochemical signals by carrying numerous CdS nanoparticles (Wang et al. 2003a, b) and alkaline phosphatase (ALP) enzyme tags (Wang et al. 2004) to detect target DNA sequences. He and Dai (2004) chemically grafted DNA chains onto aligned carbon nanotube electrodes, leading to novel aligned carbon nanotube-DNA sensors of a high sensitivity and selectivity for probing complementary DNA and target DNA chains of specific sequences.

In our previous work (Yang et al. 2006), we found that SWNTs can form a nanoscale, non-covalent

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adduct with Fc and dramatically enhance reduction response of hydrogen peroxide and this nanoscale adduct shows high stability in aqueous solution under sonication due to the strong π - π stacking interaction between Fc and SWNT. This makes it a convenient and excellent electrode material in electrochemical detection. In this paper, using this adduct we designed a novel nanohybrid system for the electrochemical detection of DNA hybridization and successfully detected the target DNA.

Materials and methods

Materials

The SWNTs were prepared by a modified direct current arc-discharge method (Lv et al. 2005). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS), MES, Triton X-100 were purchased from Aldrich. Ferrocene was from Tianjin Guangfu Fine Chemical Research Institute, China. DNA-probe2 (5'-AAA-AAT-GCA-(CH₂)₆-NH₂-3'), DNA-probe1:SH-DNA (5'-SH-(CH₂)₆-TAA-ATA-CCA-3'), target DNA: complementary-DNA (c-DNA: 5'-TGC-ATT-TTT-AAT-GGT-ATT-TA-3') and non-complementary-DNA (n-DNA: 3'-ACG-CTC-CTG-AAA-CGA-CGA-TA-5') were purchased from Shanghai Sangon Biological Engineering Technology & Services CO., Ltd, China.

Preparation of Fc-SWNT adduct

The SWNTs were purified using nitric acid and cut as reported previously (Liu et al. 1998). The Fc-SWNT adduct was processed according to our previous work (Yang et al. 2006). The functionalization reaction was processed by suspending purified SWNT (1 mg) and Fc (20 mM) in a mixture (10 ml) of water and DMF (1:4 v/v). The reaction mixture was alternatively mechanically agitated and immersed in a sonication bath at room temperature for 4 days. The hybridization adduct (Fc-SWNT) was collected with centrifugation and washed thoroughly by several sonication and centrifugation cycles in DMF.

Fc-SWNT adduct conjugated with DNA-probe2 (Fc-SWNT-DNA-probe2)

Fc-SWNT functionalized with DNA-probe2 were accomplished by modifying the procedure of Hazani et al. (2003). Fc-SWNT (0.5 mg) were resuspended in 5 ml Triton X-100 in MES (100 mM) at pH = 6.0, EDC (100 mM) and sulfo-NHS (100 mM) for 12 h at room temperature to form a labile intermediate. The pH was then raised to 8.5 and the amino-modified DNA-probe2 (5 μ M) was added. The mixture was sonicated for 2 h in a bath, then stirred at room temperature for 48 h to form the amide linkage between the primary amine located at the 3' of DNA-probe2 and the carboxylic acid groups on the SWNT. Finally, the reaction mixture was centrifuged at 21,000g for 1 h. The aggregates and bundles of SWNT of the centrifuge tubes were discarded, and the supernatant was collected and underwent an additional centrifugation round and washed with double distilled water.

Electrochemical properties of Fc-SWNT and Fc-SWNT-DNA-probe2

Electrochemical experiments were performed with a microcomputer-based electrochemical analyzer (LANLIKE, LK98B). Fc-SWNT and Fc-SWNT-DNA-probe2 were first suspended at 0.02–0.03 mg/ml in double distilled water. Then 6 μ l of the suspension was cast on the surface of glassy carbon (GC, diam. = 4 mm) electrode. With Ag/AgCl as a reference electrode and platinum electrode as a counter electrode, the electrochemical measurements were carried out in 4 mM H₂O₂. Phosphate buffer (50 mM, pH 7.4) and KCl (100 mM) were used as the supporting electrolyte media. The electrochemical behaviors of the bare GC and SWNT modified electrodes were also performed similarly for comparison.

Electrochemical detection of DNA hybridization

The DNA-probe1 was anchored on the gold electrode via chemisorption in the following manner: Au electrode (diam. = 4 mm) was etched by scanning from -0.2 to 1.15 V in 100 mM H₂SO₄, and sonicated in distilled water for 2 min, then immersed

in 50 μM SH-DNA (containing 100 mM KCl) for 2 h. The electrode was then washed with 100 mM KCl.

Hybridization proceeded by two steps: the first hybridization proceeded by immersing the SH-DNA modified Au electrode in 0.54 μM complementary-DNA or 0.54 μM non-complementary-DNA with hybridization buffer (50 mM PBS buffer pH 7.0, 25 mM NaCl) at 37°C for 4 min, respectively. The electrode was rinsed with hybridization buffer and then Milli-Q water. The second hybridization proceeded by immersing the above electrode in 0.02–0.03 mg Fc–SWNT–DNA–probe2/ml with hybridization buffer at 37°C for 4 min, respectively. The electrode was then rinsed with hybridization buffer and Milli-Q water in turn. Finally, the electrode was dried under an infrared lamp. As a comparison, we also incubated the SH-DNA probe1-modified Au electrode with c-DNA not modified with the SWNT–Fc adduct.

The Au electrode after the above hybridization steps was measured in 10 mM H_2O_2 ; PBS buffer (50 mM, pH 7.4) and KCl (100 mM) were used as the supporting electrolyte media.

Results and discussion

Fabrication of the Fc/SWNT-based gene electrochemical detection electrode

Our new Fc/SWNT-based bioelectronic protocol involves a sandwich-type hybridization system: a DNA-probe1 immobilized on a gold electrode, the target DNA sequence, and a DNA-probe2 conjugated on the Fc–SWNT adduct. The shortened SWNTs were first sonicated with Fc to form Fc/SWNT hybridization adduct, and then used to immobilize DNA-probe2 through an amide covalent linkage. In many biotechnology and nanotechnology application, single-stranded DNA probes with –SH modification immobilized on gold surfaces are a common element (Pirung 2002; Ihara et al. 1997). Here we took a similar strategy to immobilize DNA-probe1 on a gold electrode, followed by two-step hybridization: hybridizing with the target DNA and then with the DNA-probe2 immobilized on Fc/SWNT. This Au electrode, conjugated with, Fc/SWNTs through specifically bonded double-stranded DNA, was used to detect the target DNA via the detection of H_2O_2 . The

target DNA sequence can be detected according to the strong reduction current response based on electrocatalytic effect of Fc/SWNT toward the reduction of H_2O_2 . The schematic representation of the protocol is depicted in Fig. 1.

Electrochemical characterization

We analyzed the detection abilities toward H_2O_2 of the four electrodes: bare GC, SWNT, Fc/SWNT and Fc–SWNT–DNA probe2 modified GC electrodes using cyclic voltammetry (see Fig. 2). The cyclic voltammetry curve of bare electrode shows a typical reduction peak toward H_2O_2 positioned at -0.48 V with a peak current of 7.9 μA and an integral current density of 3.8×10^{-4} C/cm². The integral current density is calculated by integrating the reduction peak of cyclic voltammetry curve, which expresses the total charge per unit area that the electrode captures from H_2O_2 molecules in solution. The SWNT-modified GC electrode had an enhanced reduction current response toward the H_2O_2 with the same reduction peak position of ~ -0.48 V as that of the bare one. The peak current increased to 9.6 μA and the integral current density increased to 4.5×10^{-4} C/cm², which shows the electro-catalytic properties of SWNT towards the reduction of H_2O_2 (Wang et al. 2003a, b).

The Fc–SWNT-modified electrode shows a great increase in the reduction current response of 52.1 μA toward the H_2O_2 and an obvious decrease in the reduction peak potential at -0.26 V, and the integral current density increases to 2.7×10^{-3} C/cm². This suggests that the GC electrode modified with Fc–SWNT shows much better properties than the one with SWNT. Fc–SWNT is prepared through the strong physical interaction between Fc and SWNT based on the structure similarity of cyclopentadienyl anion in Fc and the graphene of SWNT (Yang et al. 2006). The strong interaction between Fc and SWNT dramatically promotes heterogeneous redox reaction through the direct electron transfer between the electrode and redox species in solution, therefore Fc–SWNT modified electrode shows an excellent electrocatalytic performance. After the GC electrode is modified with Fc–SWNT–DNA, the electrochemical properties are almost unchanged, except that the peak position slightly shifts to a lower potential, the reduction peak is a little broadened and the integral

Fig. 1 Schematic illustration of the electrochemical gene sensing system based on the formation of complementary sandwich-type complex

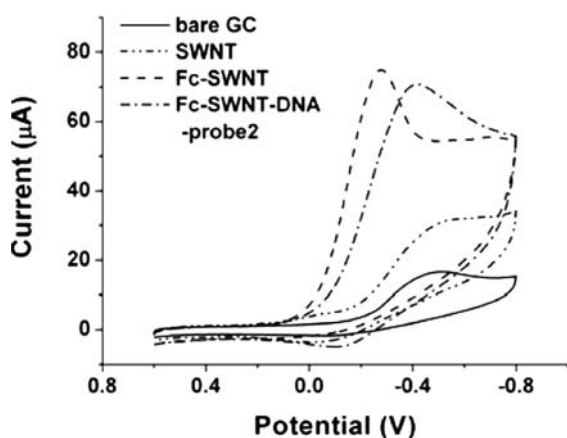
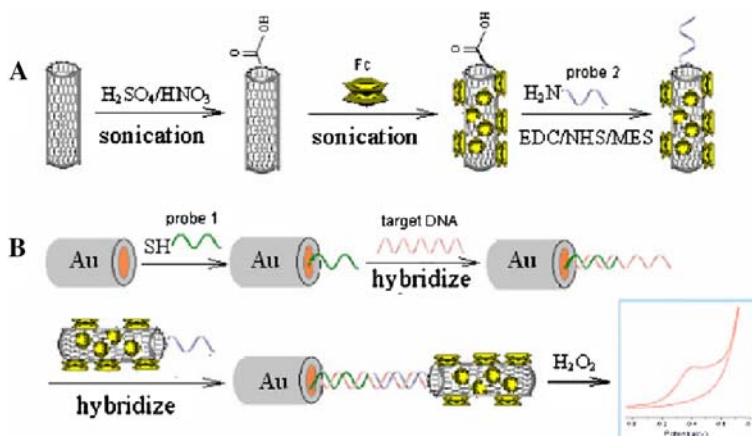


Fig. 2 Cyclic voltammetry curves of bare GC, SWNT, Fc/SWNT and Fc-SWNT-DNA modified GC electrode in 4 mM H_2O_2 , 50 mM phosphate buffer (pH 7.4), 100 mM KCl at a 50 mV/s scan rate, respectively

current density slightly decreases to $2.4 \times 10^{-3} \text{ C/cm}^2$ compared with the Fc-SWNT modified electrode, which may be due to the wrapping of DNA chains on the SWNT.

Electrochemical detection of DNA sequences

As discussed above, Fc-SWNT have good electrocatalytic properties toward the reduction reaction of H_2O_2 , therefore it can be used as an excellent electrode material. In addition, SWNT can be used as matrix of a variety of biochemical molecules through covalent or non-covalent bonding (Chen et al. 2003; Hazani et al. 2003; Lee et al. 2006). After the Fc-SWNT are bonded with DNA, the

electrocatalytic properties toward the reduction reaction of H_2O_2 is not affected, therefore, it can be used as an electromaterial to detect DNA sequences. In the detection of DNA sequences, the sandwich-type gene sensing system is always adapted and proved to be an effective method, in which the target DNA serves as a bridge to link the electroactive species to the surface of the electrode (Ihara et al. 1997). Usually, the target DNA can be detected through the electrochemical response of the electroactive species. Here we use Fc-SWNTs as the electroactive substance to design a novel sandwich-type gene sensing system, and the target DNA can be detected by use of the electrocatalytic properties of the Fc-SWNTs toward H_2O_2 .

Figure 3 shows cyclic voltammetry curves of the DNA-probe1, modified-Au electrode after two hybridization steps with $0.54 \mu\text{M}$ c-DNA and $0.54 \mu\text{M}$ n-DNA, respectively, and then with Fc-SWNTs-DNA-probe2 in 10 mM H_2O_2 . In the detection system of c-DNA, a significant reduction peak at about -0.4 V for H_2O_2 was observed as expected and the integral current density was $3.8 \times 10^{-4} \text{ C/cm}^2$. This indicates that the DNA-probe2 functionalized Fc-SWNT are conjugated onto the surface of Au electrode through specific hybridization with target DNA (c-DNA) and catalyze the reduction of H_2O_2 . For the electrode hybridized with n-DNA under the same conditions, only a very weak peak can be observed at about the same position with an integral current density of only $6.1 \times 10^{-5} \text{ C/cm}^2$, showing that almost no Fc-SWNT are linked to the surface of the Au electrode due to there being no specific DNA pairing interaction with the non-complementary DNA chains. Thus there is no

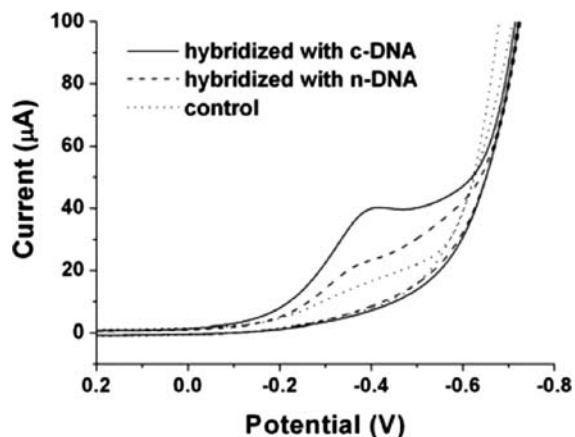


Fig. 3 Cyclic voltammetry curves for Au electrode modified by DNA-probe1 after hybridized with c-DNA and n-DNA and then with Fc-SWNTs-DNA probe2 in 10 mM H₂O₂, 50 mM phosphate buffer (pH 7.4), 100 mM KCl at a 50 mV/s scan rate. The control experiment is Au electrode modified by DNA-probe1 after hybridized with c-DNA only

obvious response toward H₂O₂. To compare incubating probe1-modified Au electrode with c-DNA not modified with the SWNT-Fc adduct, the slight electrochemical response, which might have been caused by the physical adsorption of Fc-SWNT-DNA-probe2, appears to be insignificant in this particular case.

In conclusion, using the electrocatalytic properties of Fc/SWNT toward H₂O₂ we succeeded in designing a novel sandwich-type gene detection system that recognizes target DNA. The different responses toward c-DNA and n-DNA sequences, combined with Fc/SWNT stability, makes it a useful avenue for CNT-based DNA biosensors. SWNT play a dual role in both the recognition and transduction events, namely as the matrix for probe DNA and enhancer to prompt the redox electron transfer. Our work is now directed to further enhance the sensitivity for the detection of DNA for the super-sensitive detecting need in many fields and applications.

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