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Simple Label-Free Femtomolar DNA Detection Based on a Nanostructure Composite Material: MWNT-Doped Poly(indole-6-carboxylic acid)

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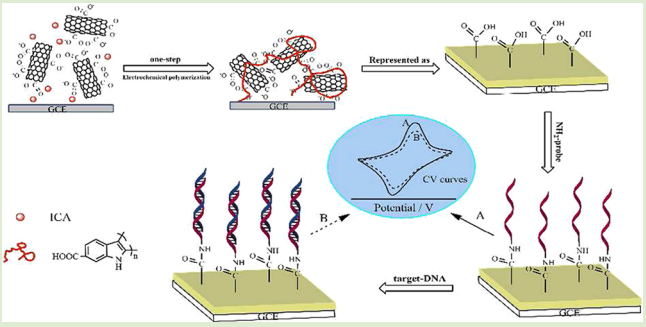
Supporting Information

ABSTRACT: A nanostructure composite material consisting of poly(indole-6-carboxylic acid) (PICA) and carboxylic groups ended multiwall carbon nanotubes (MWNTs) was directly electrosynthesized from indole-6-carboxylic acid (ICA) monomer and MWNTs in one step, in which MWNTs was also used as supporting electrolytes. And a simple electrochemical sensor for recognition of target DNA related to hepatitis B virus (HBV) was directly fabricated by means of this composite material. The corresponding detection limit is 2.0 fmol L^{-1}. This interesting conducting polymer with a very large surface area will provide new insights into how a biosensor is designed.



Electrochemical DNA biosensor is of great significance for the identification of oligonucleotides (ODNs) due to some notable advantages.[1] Electrochemical determination of specific DNA sequences is carried out by means of a hybridization process that is comprised of two main methods: label and label-free. With sophisticated and high-cost assay process, the labeled method tends to be time-consuming, complicated, and expensive.[2] By comparison, label-free DNA sensors can not only eliminate the steps of pre- and posthybridization labeling or incubation with markers, but also offer real-time detection of duplex formation. Thus, the label-free method is regarded as a suitable method for direct and fast detection of DNA because it can transform the hybridization events into direct electrical signals.[3] The primary challenge of label-free electrochemical DNA sensors is to find valid interface between the nucleic acid and the electronic transducer.[4]

Conducting polymers (CPs) or the composites of CPs with nanomaterials (such as nanoparticles and MWNTs), have been successfully employed in electrochemical biosensors.[5−9] For electrochemical DNA sensors based on CPs, the polymer is not only used as an immobilization carrier, but also plays an active role in signal transduction in the presence of a target-analyzed molecule.[10−12] Among CP composite materials, CP−MWNT composites are very useful to facilitate electron transfer and increase efficiency of binding sites for specific sensing interactions without the need of redox mediators.[13] The major advantage of CP−MWNT over CPs is principally owing to the unique properties of MWNT networks and the CP−MWNT interactions,[14−16] which leads to the increase in surface area and the enhancement of the ability to form good electronic contact between composite components and transducers. Usually, the electrodes modified with MWNTs are prepared by dispersing MWNT solution onto the electrode surface. MWNTs are, however, disordered on the electrode, which comes about as a result of this complicated and costly method. The MWNTs will therefore be prone to peel off from the electrode surface, which makes it hard to achieve the detection reproducibility. It is a fact that carboxylic groups of elements can impart negative charges on MWNTs, which becomes beneficial to stabilizing the ensuing dispersion in an aqueous medium and helping anionic MWNTs to act as a strong and conductive dopant or counterion during the electropolymerization.[14,17] Based on these considerations, our group prepared a novel nanostructure composite material: MWNT-doped poly(indole-6-carboxylic acid) (PICA) by direct electrochemical polymerization of ICA and MWNTs in one step. A new simple label-free electrochemical DNA sensor was fabricated based on this composite material (PICA−MWNT) for recognition of target DNA related to hepatitis B virus (HBV; Scheme 1). With larger surface area and quite a few functionalized carboxylic acid groups, PICA−MWNT enhances the detection signal effectively and obviously improves the sensitivity.[5,18]

Morphologically, pure PICA film is compact and resembles ordered arrangements of rods with 50−80 nm diameter in the form of fibrillar network (Figure 1A). By contrast, PICA−MWNT is more homogeneous and presents intact fiber-shaped "multi-nanostructure" (Figure 1B), which is good for the formation of a better membrane on the surface of a glassy

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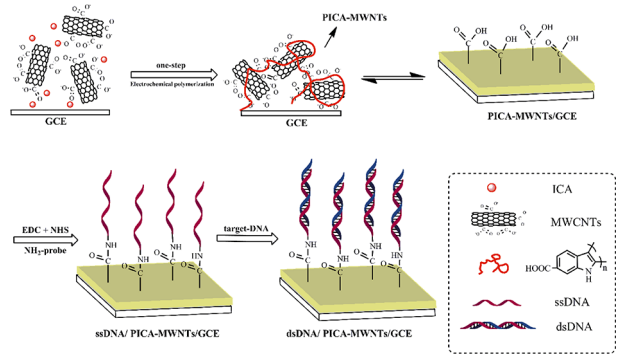
Scheme 1. Preparation of PICA−MWNT Composite Material and the Corresponding Conceptual Scheme for Label-Free DNA Detection



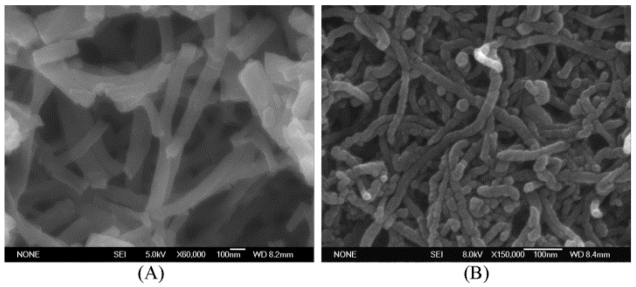


Figure 1. SEM of PICA film (A) and PICA−MWNT composite film (B) deposited on GCE.

carbon electrode (GCE). Moreover, this kind of structure will be conducive to immobilizing amino-substituted ODN probes onto the PICA−MWNT surface by covalent binding due to larger surface area and greater number of functionalized carboxylic acid groups.

The common feature of nanostructure materials is that their electrical properties can be enhanced.[19] To compare the electrochemical properties of PICA−MWNT and pure PICA film, cyclic voltammograms (CVs) of both are investigated. Compared with PICA film (Figure 2A, curve b), the faradaic currents of PICA−MWNT reveal a dramatic increase (Figure 2A, curve a), implying an enhancement of the electrochemical activities due to the so-called "multi-nanostructure". Furthermore, the value of the peak-to-peak split of PICA−MWNT (ΔE_p = 280 mV) is smaller than that of pure PICA film (ΔE_p = 320 mV), which suggests acceleration of electron transfer between the composite material and GCE.[20] A rational interpretation behind such behavior is that the porous "multi-nanostructure" affords adequate channels for the circulation of ions and solvent molecules within the composite film, which leads to excellent electrolyte access with less resistance and accordingly accelerates the electronic transfer process.[21] From a scientific point of view, these excellent properties of PICA−MWNT may be beneficial to investigating the label-free electrochemical DNA sensors by using cyclic voltammeter as a readout method.

Figure 2B shows the CVs of PICA−MWNT (a), ssDNA/PICA−MWNT (b), and dsDNA/PICA−MWNT (c) in NaAc−HAc buffer. The amino-substituted ODN probes were grafted on the PICA−MWNT surface by the formation of covalent bonds between carboxylic groups and amino groups with the catalyst EDC and NHS (Scheme 1). The "multi-nanostructure" increased the surface area as well as active sites

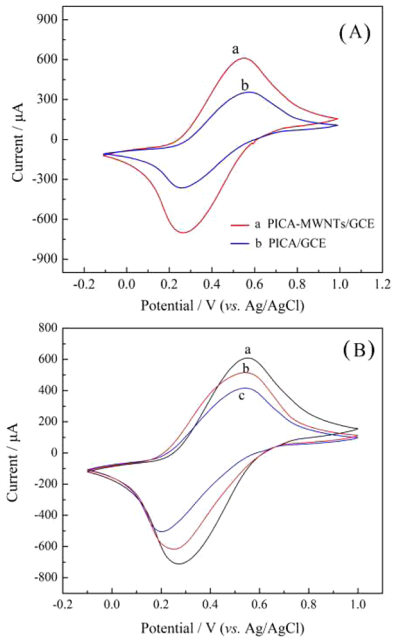


Figure 2. CVs of (A) PICA−MWNT (a) and pure PICA film (b); (B) PICA−MWNT (a), ssDNA/PICA−MWNT (b), and dsDNA/PICA−MWNT (c) in NaAc−HAc buffer (pH = 6.5, 0.1 mol L^{-1} NaCl). Scan rate: 100 mV s^{-1}.

for the loading of ODNs and, thus, improved the hybridization efficiency significantly. Compared with Figure 2B,a, the peak currents of Figure 2B,b decreased because the ODN probes immobilized on PICA−MWNT/GCE prevented the ion exchanges between the electrode and solution species during the redox reaction process.[22] After the incubation with complementary ODNs, the electrode displayed a significant change in CV curves (Figure 2B,c). The peak currents of Figure 2B,c changed to a much lower location than Figure 2B,b, because it was corresponding to the decrease in the electroactivity of PICA−MWNT with the increase in stiffness of polymer structure, partially because of the potential barrier of rotation and bulky conformational modifications along CPs backbone after hybridization.[23] These results also indicate the ODN probes are successfully hybridized.

To investigate the sensitivity of the sensor, the ODN probes were incubated with target ODNs of different concentrations. According to Figure S1 (Supporting Information), the response time for hybridization detection was 10 min, which is lower than several other biosensors reported in literature (25[24] and 30 min[25,26]). As shown in Figure 3A, the CV signals of the sensing interface were decreased with increasing concentration of target ODNs. The relationship between the decreased value of the anodic peak current (ΔI_{pa}) and the negative logarithm of the concentration of complementary target ODNs [−lg(c)] was illustrated in the insert. The dynamic determination range for complementary target ODNs was from 1.0 × 10^{-14} to 5.0 × 10^{-12} mol L^{-1} with the regression equation ΔI_{pa} (μA) = 684.49 − 44.467 lg(c) (mol L^{-1}) and the correlation coefficient γ = 0.9981. Thus, the detection limit is 2.0 fmol L^{-1} using 3σ, which is higher than some reported label-free DNA sensors based on some other CPs (5.0 pmol L^{-1},[27,28] 85 pmol L^{-1},[29] and 50 nmol L^{-1}[30]). This detection limit is close to the value obtained from the more complicated sensor based on poly(pyrrole−nitrilotriacetic acid)/Cu^{2+}.[2]

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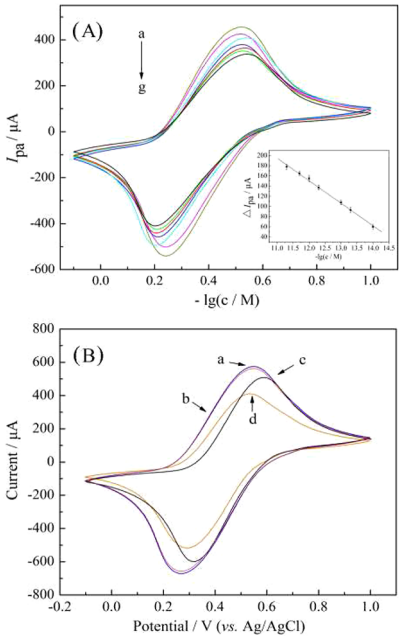

Figure 3. CV responses of (A) the sensor to different concentration of target ODNs; (B) the sensor (a), after the incubation with noncomplementary (b), one-base mismatched (c), and complementary ODNs (d) in NaAc-HAc buffer (pH = 6.5). Insert in (A): plots of the value of ΔI_{pa} ($=I_{pa} - I_0$) vs the negative logarithm of the concentration of complementary target ODNs [$-\lg(c)$]. Error bars were obtained from the three parallel experiments.

The selectivity of this DNA biosensor was investigated by testing the peak current responses of CVs obtained from matched system, one-base mismatched ODN, and noncomplementary ODN sequence (shown in Figure 3B). The recognition layer (Figure 3B,a) showed no obvious change when it was exposed to the noncomplementary ODNs (Figure 3B,b), indicating that the hybridization did not occur. However, when a complementary target ODN (Figure 3B,d) was introduced, there was a notable change of the CV shape indicating hybrids formed on the electrode. When ODN probes were hybridized with one-base mismatched ODNs (Figure 3B,c), the decrease of the peak currents was less than the value obtained from the hybridization with complementary ODNs. Based on the above, it can be concluded that this DNA sensor has an excellent selectivity.

The reproducibility of any biosensor is extremely important for practical applications. To investigate the reproducibility of this DNA sensor, four DNA sensors were prepared following the same procedure. The relative standard deviation was 1.27% (n = 4). This result indicates that a satisfactory reproducibility could be obtained by this treatment. The regeneration of this DNA sensor was studied by a thermal regeneration procedure in which the dsDNA/PICA–MWNT/GCE was immersed into boiling water for 5 min and then cooled rapidly in an ice salt bath (Figure 4). The anodic peak current signal returned to 96% of its original response (magenta solid line, denoted as baseline) after regeneration (see blue curve), implying that the dsDNA was hot denatured and the response of immobilized probe ODNs was not damaged. Subsequently, the regenerated sensor after hybridization with target ODNs produced a similar decrease in the peak of the faradic currents of CVs (see red and green curves), which demonstrated that this DNA sensor was applicable for repeated hybridization reactions. The stability of

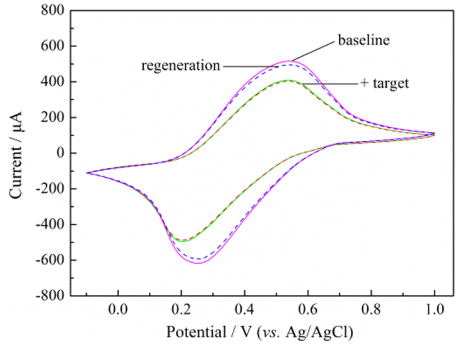

Figure 4. CVs of ssDNA/PICA–MWNT/GCE prior to hybridization (magenta solid line), after the incubation with 1.0 nM complementary ODNs (green solid line); CVs of ssDNA/PICA–MWNT/GCE after regeneration (blue dashed line) and after the incubation with 1.0 nM complementary ODNs again (red dashed line).

this DNA sensor was also discussed. After the storage in a dry state at 4 °C for 48 h, the peak current value of the sensor was 98% of its initial signal and the relative standard deviation for two repetitive measurements was 2.1%, indicating the good stability of the DNA biosensor.

In summary, a novel composite material (PICA–MWNT) was electrodeposited directly in one step and used to construct a simple label-free DNA sensor. The performance of the DNA biosensor system was studied in respect to its sensitivity, selectivity, and reproducibility and regeneration. A larger surface area and a greater number of carboxylic acid groups on the electrode contribute to the improvement of the sensitivity. The detection limit is 2.0 fmol L^{-1}. We anticipate that this novel composite material will provide a unique platform in electrochemical sensors for highly sensitive and sequence-specific detection. Further work is in progress.

■ ASSOCIATED CONTENT

Supporting Information

Materials, instrumentation, experimental conditions, and experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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