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Direct DNA Hybridization at Disposable Graphite Electrodes Modified with Carbon Nanotubes

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The performance of glassy carbon (GCE) and graphite pencil electrodes (PGE) modified with multiwalled carbon nanotubes (CNTs) are compared, based on the direct electrochemical detection of nucleic acids. This is accomplished by monitoring the differential pulse voltammetry changes of the guanine signal. CNT-modified PGE compares favorably to that of the commonly used CNT-modified GCE owing to the intrinsic improved performance of the supporting PGE. The better intrinsic characteristics of the PGE are related to its composite structure and higher level of porosity compared to GCE. The performance characteristics of the direct DNA hybridization on the disposable CNT-modified PGE are studied in terms of optimum analytical conditions such as probe concentration, target concentration, hybridization time, and selectivity. The new DNA biosensor described here has shown some important advantages such being inexpensive, sensitive, selective, and able to generate reproducible results using a simple and direct electrochemical protocol.

The detection of DNA sequences is of particular interest in genetics, pathology, criminology, pharmacogenetics, food safety, and many other fields. Extremely sensitive yet inexpensive and robust detection methods that do not require expensive sample pretreatment and derivatization are essential for their efficient use in the above areas. Electrochemical label-free detection techniques have shown great potential in providing viable solutions to this challenge. These types of sensors (genosensors) rely on conversion of the DNA base pair recognition event into a useful electrical signal, eliminating the requirement for an indicator to transduce the hybridization event, thus simplifying the signal readout. Such label-free detection at carbon-based electrodes versus Ag/AgCl can be accomplished by monitoring changes in the oxidation signal of guanine or adenine[1−7] bases at carbon-based electrodes.

In recent years, the modification of carbon-based electrodes with carbon nanotubes (CNTs) has attracted considerable attention in the field of electroanalytical chemistry. The high surface area, hollow geometry, and the useful mechanical properties of CNTs combined with their electronic conductivity and ability to promote electron-transfer reactions provide new exciting nanoelectrodes for the catalysis of biomolecules[7−9] and inorganic compounds.[10] The work of Compton and others[11,12] has recently provided strong evidence that the electrochemistry of carbon nanotubes is dominated by their ends and the presence of carboxylic acid moieties produced during acid purification. Most importantly, the presence of oxygenated groups on nanotubes is necessary for covalently bonding to the amino-terminated ends of the oligonucleotides using standard carbodiimide chemistry.

Direct electrochemistry of DNA guanine and adenine at a multiwalled (MW)NT modified GCE provided significantly enhanced voltammetric signals in comparison to unmodified GCE. Wang et al. [8] detected 100 fmol of breast cancer BRCA1 gene by using enhanced guanine oxidation signal at a MWNT-modified glassy carbon electrode (GCE). A significant increase in the guanine oxidation signal was observed for 5 mg/L guanine solution while comparing a MWNT-modified and MWNT-free GCE. Pedano et al.[13] have recently fabricated a carbon nanotube paste electrode for adsorption and electrochemical oxidation of nucleic acids. Incorporation of MWNT into a carbon paste matrix provided 29- and 61-fold larger current values than the ones obtained from a carbon paste electrode for ss-DNA and 21-mer oligonucleotide.

Improved sensitivity of electrical DNA hybridization has been reported also in connection with the use of daunomycin redox intercalator.[14] A MWCNT-COOH-modified GCE was used for this

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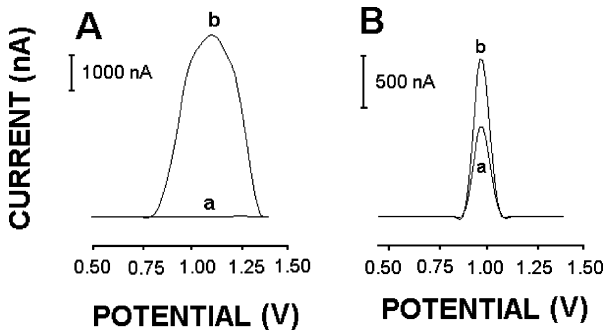

Figure 1. Voltammetric signals of guanine obtained from stDNA immobilized onto bare GCE/PGE and CNT-modified GCE/PGE. (A) The magnitude of guanine oxidation signals obtained with DNA immobilized onto (a) bare GCE or (b) CNT modified GCE (CNT-GCE); (B) the magnitude of guanine oxidation signals obtained with DNA immobilized onto (a) bare PGE or (b) CNT-modified PGE (CNT-PGE). CNT modification onto electrodes: (A) the required amount of CNT was pipetted onto pretreated GCE for 1 h, and (B) the pretreated PGEs were dipped into the vials containing CNT solution for 1 h. DNA immobilization onto electrodes: 2000 μg/mL stDNA was immobilized onto pretreated GCE/CNT-GCE during 3 h and 100 μg/mL stDNA was immobilized onto pretreated PGEs/CNT-PGEs during 1 h. DPV measurement: The guanine oxidation was measured in ABS by scanning the potential range between +0.50 and +1.40 V at 50-mV modulation amplitude, 8-mV step potential.

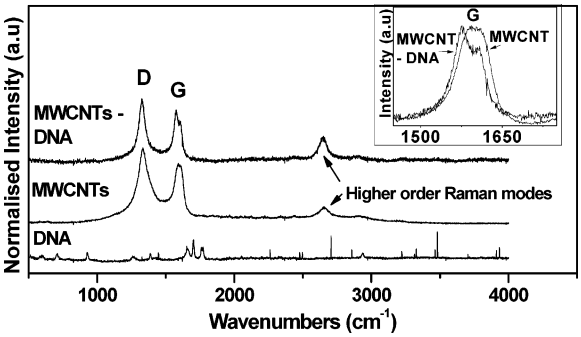

Figure 2. Comparison of Raman spectra of NH_2-probe DNA, CNTs, and NH_2-probe DNA−CNT adducts taken at a laser excitation wavelength of 632 nm. The inset illustrates the G Raman shift between CNTs and NH_2-probe DNA−CNT adducts.

task along with a 5′-amino group functionalized oligonucleotide probe and pulse voltammetric transduction. Wang's group demonstrated recently the use of CNTs for dramatically amplifying enzyme-based bioaffinity electrical sensing of DNA.[15]

In this paper, we report the advantages of using pencil graphite electrode (PGE) modified with carbon nanotubes for the detection of nucleic acids and DNA hybridization based on enhancement of guanine signal using differential pulse voltammetry (DPV). We illustrate that the performance of inexpensive pencil electrode materials modified with CNTs is favorable to the commonly used CNT-modified glassy carbon electrodes, resulting in more sensitive and reproducible DPV measurement of nucleic acids. The overall performance of the CNT-modified PGE sensor is studied and discussed in terms of optimum analytical conditions such as probe concentration, target concentration, hybridization time, and selectivity in the presence of unwanted substituents.

RESULTS AND DISCUSSION

DNA Detection Based on Guanine Oxidation Signal. In Figure 1, DPV voltammograms show the guanine oxidation signals obtained with bare and CNT-modified electrodes, using two electrochemical transducers: GCE and PGE. A signal enhancement of guanine was observed in the presence of CNT modification on both transducers. The magnitude of guanine signal was enhanced by 100% at CNT-modified GCE and by 72.22% at CNT-modified PGE. The optimized conditions, regarding the concentration of stDNA immobilized onto electrodes and the immobilization time were 2000 μg/mL stDNA and 3 h, respectively, for GCE or CNT-modified GCE and 100 μg/mL stDNA and 1 h for PGE or CNT-modified PGE. Apparently the modification of the electrodes by CNTs facilitates higher surface coverage, providing enhanced adsorption of the guanine nucleobases and hence offers enhanced response. Another factor that may contribute to the enhanced response is the high electrical conductivity and electrocatalytic action of CNTs able to promote electron-transfer reactions.

Substantial differences in the DPV response are observed between the bare GCE and PGE electrodes, with the latter one displaying the most favorable signal characteristics. Almost negligible response was observed at GCE electrode even though larger concentrations of stDNA and immobilization times were used. The different response reflects changes in the structure and porosity of the electrodes.[16,17] Glassy carbon is composed of a structure of interwoven ribbons of graphite. which makes it impermeable to liquid or gases.[18] On the other hand, graphite pencil consists of graphite, polymeric binder, and other additives (e.g., clays). The large signal from PGE is attributed to its composite (mixed graphite−insulator) structure. The random assembly of graphite particles inside the rigid nonconducting matrix gives a significant advantage over conventional carbon electrodes such as GCE, in that they display microelectrode array behavior.[19] In addition, the difference in porosity (GCE has a low porosity) can influence both the probe immobilization (i.e., coverage and/or orientation) and the redox activity of the DNA. Although a direct comparison between CNT-modified electrodes cannot be made due to their different surface areas, it is obvious that the PGE CNT-modified electrode compares favorably to that of GCE CNT owing to the intrinsic enhanced performance of the supporting PGE electrode. These results are in agreement with the work carried out by Wang's group,[16] who demonstrated a well-defined guanine response for GPE electrodes in comparison to GCE and carbon paste electrodes.

The binding of DNA with MWCNTs was confirmed through Raman analysis. Figure 2 shows the Raman spectra of NH_2-probe DNA, bare MWCNTs, and NH_2-probe DNA−MWCNT adducts. A downshift of the tangential G band (at about 1600 cm^{-1}) observed in the Raman spectrum of DNA MWCNTs is explained as a consequence of the charge transfer between the oxygen

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groups present on the carbon nanotube surface and the DNA matrix. The value of the shift (5 cm^{-1}) is attributed to the electron-donating groups of the DNA. The nucleic acid bases of DNA have chemical groups (e.g., amines) that can donate electrons to the oxidized nanotube surface. A local strain induced in the nanotubes due to DNA wrapping may be another source responsible for this downshift. In addition, the asymmetric line shape of the G band changed to two higher energy modes, characteristic of heavily functionalized nanotubes.[20]

The immobilization principle of salmon test DNA (stDNA) can be explained by the formation of covalent coupling between the carboxylated ends of MWNTS and the amine groups of the DNA. stDNA as many of the important types of biomolecules (peptides, proteins, antibodies) contain such amine groups.

Our experiments showed that a higher degree of reproducibility could be obtained on a PGE than on a GCE electrode. This higher reproducibility together with other advantages such as commercial availability, easier preparation, and lower cost were the main reasons for choosing the disposable PGE electrode as the preferred electrode to be used in the following experiments of electrochemical sensing of DNA and DNA hybridization.

First, a study was performed to investigate the effect of pretreatment step for a PGE electrode based on the guanine signal. The 100 μg/mL stDNA were immobilized for 1 h onto CNT-modified surfaces of unpretreated/pretreated PGE (see Supporting Information). Based on three repetitive measurements, the RSD ($n = 3$) was calculated as 5.85% and an increase at guanine signal was found to be 58.7% in the presence of CNT modification onto pretreated PGE in comparison to bare pretreated PGE. However, more reproducible results (RSD ($n = 3$) of 3.95%) and also a higher signal enhancement of 89.7% was obtained in the presence of CNT modification onto unpretreated PGE in comparison to bare unpretreated PGE. This higher increase of guanine magnitude in the case of unpretreated PGE is related to the uniform distribution of CNTs resulting in a higher surface area. Our XPS measurements[21] on acid-treated CNTs revealed that approximately 16−20% of oxygen atoms were bound to surface carbon atoms making the surface polar. Pretreatment of PGE also introduces oxygen groups making the surface polar.[18] Hence, when the acid-treated CNTs come in contact with the polar PGE surface there is a repulsion force between them, with the result of a nonuniform distribution across the PGE surface. However, coating of the unpretreated PGE surface results in uniform coverage and hence a larger surface area is been available for adsorption of DNA.

Next, the effect of coating time of CNT onto unpretreated PGEs was studied using different periods such as 5, 15, 30, and 60 min and overnight (not shown). Immersion times up to 1 h increased the signal significantly, reflecting improved surface coverage. However, prolonged overnight immersion times did not cause any further significant improvement. With respect to the reproducibility and signal enhancement, 1 h was chosen as the optimum time for CNT modification onto disposable electrodes.

The above studies demonstrate a very reproducible simple, quick, and sensitive method for DNA analysis by using CNT-modified disposable PGEs (CNT-PGEs). It is worth emphasising that CNT-PGE requires only a small amount of DNA in order to obtain a well-defined guanine signal. Consequently, there is no need to use concentrated DNA solution such as 2000 μg/mL or use any covalent agents or amplification routes as reported in the earlier literature.[8]

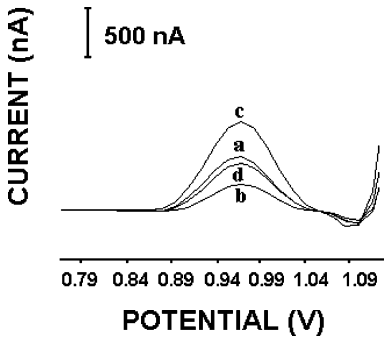

Figure 3. Differential pulse voltammograms for the guanine oxidation signals observed by using CNT-modified unpretreated PGEs in the presence of probe hybridization with 15 μg/mL target (a), hybridization with 15 μg/mL NC (b), hybridization in mixture sample containing target/NC in ratio (1:1) (c), and hybridization in mixture sample containing target/stDNA in ratio (1:1) (d). A 10 μg/mL amino-linked probe was immobilized onto unpretreated CNT-modified PGEs for 20 min and hybridization time was for 20 min. Other conditions are as in Figure 1B.

Detection of DNA Hybridization on Disposable PGEs Modified with CNT. The investigation of DNA hybridization on disposable CNT-PGEs was based on the enhancement of guanine signal. In our assay, we use a guanine-free probe to detect the hybridization through the appearance of the target guanine signal.

A representative DPV voltammogram is shown in Figure 3a for detection of DNA hybridization between probe DNA and its complementary sequence. The selectivity of the DNA probe was tested by using DNA probes to hybrid with noncomplementary (NC) (Figure 3b), a mixture of target and NC in a ratio of 1:1 (Figure 3c), or mixture of target and stDNA in ratio 1:1 (Figure 3d). While a well-defined guanine oxidation signal is observed in the presence of the complementary sequence (Figure 3a), a reduced response is also observed for the noncomplementary sequence (Figure 3b), suggesting a nonperfect selective DNA hybridization process. Note also that there are four guanine bases available in the NC, and therefore, a large signal can arise from nonspecific adsorption of these sequences. Improved rinsing procedures (employing surfactant, SDS in buffer, ABS solution) could eliminate this nonspecific absorption.

The signal from the mixture of target and NC is much higher than the corresponding mixture for stDNA. A possible explanation can be found in the different structures of stDNA and NC sequences. stDNA are naturally in double-stranded form, while NC are short-sequence, single-stranded oligonucleotides. Since the structure of the double helix is more rigid than that of the single helix, it is expected that their hybridization efficiency will be substantially reduced. Helix formation will be much easier between the short, single-stranded sequences than between single- and double-stranded DNA. As a consequence, the interference of stDNA to the hybrid signal will be substantially smaller than the one of short, single-stranded NC oligonucleotides. Another factor that should be taken into account is that the molar concentration of stDNA is lower (due to its high molecular weight) than that of NC in the mixture. Hence, a lower number of stDNA strands is

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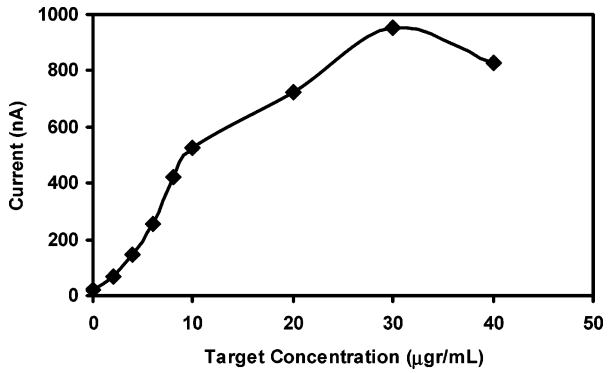

Figure 4. Changes of guanine oxidation signal obtained by using probe immobilized onto CNT-modified PGEs and different concentrations of target from 2 to 40 μg/mL. Other conditions are as Figure 3.

expected to be present compared to those of NC. This could partly explain the smaller hybridization signal observed by stDNA.

A critical step to completely eliminate nonspecific adsorption is to block the free carboxylate groups in the carbon nanotube matrix. A significant portion of the carboxylate groups remains in the matrix after the DNA probe immobilization, which are able to bind with stDNA and NC (through the natural amine groups present in oligonucleotides). The excess of these COOH groups can be blocked with ethanolamine in the presence of EDC.

The effect of probe concentration on the guanine oxidation signal was studied as well (not shown). The concentration of target was kept constant at 15 μg/mL, and the concentration of probe was increased from 10 to 50 μg/mL. The highest hybrid signal was obtained from 10 μg/mL probe with 15 μg/mL target, which practically indicated full surface coverage of the probe-immobilized, CNT-modified PGE. When probe concentration was increased to 20 μg/mL, a lower guanine signal was observed, which remained almost constant up to values of 50 μg/mL. This behavior may reflect the fact that a higher surface coverage results in an unfavorable orientation, i.e., lowest accessibility of the target and reduced hybridization efficiency. Thus, an optimum capture probe concentration of 10 μg/mL was employed.

The effect of hybridization time on the guanine signal obtained from hybridization events between probe and target and between probe and NC was also studied (see Supporting Information). As expected, the probe−target hybridization signal increased linearly with hybridization time between 10 and 40 min after which it levels off. However prolonged exposure caused a simultaneous increase of the probe−NC hybridization signal as well. It was observed that the difference signal based on hybridization between probe−target and probe−NC was maximum at 20-min hybridization time and also of better reproducibility than the ones obtained from 10, 40, and 80 min. Thus, 20 min was chosen as the optimum hybridization time.

The guanine signal changes during hybridization to different target concentrations were also monitored. Typical results are presented in Figure 4. As expected, since probe did not contain any guanine, no guanine signal was obtained at +1.0 V in the absence of target. Well-defined hybridization signals were observed over the range of 2−40 μg/mL target concentration range using a 20-min hybridization time. When the target concentration was increased from 2 to 30 μg/mL, the guanine signal linearly increased. Upon further increase of target concentration to 40 μg/mL and more (not shown), the guanine signal remained almost the same or slightly decreased, indicating the saturation of surface hybridization sites. Probe immobilized on CNT-modified PGE responded to target concentrations even as low as 2 μg/mL. The limit of detection, DL was calculated with the aid of the section of the calibration plot close to the origin, which is linear, utilizing both the regression equation and the definition $y = y_B + 3s_B$ (y_B is the signal of the blank solutions and s_B is the standard deviation of the blank solution) as outlined in ref 22. According to the above procedure, DL was estimated as $1.6\ 10^{-8}$ mol/L (see Supporting Information).

The sensitivity of a technique is defined as the slope of the calibration graph (target concentration vs signal) and, provided that the plot is linear, can be measured at any point on it. A sensitivity of 52.8 nA/(μg/mL) or 0.34 nA L nM^{-1} (after division by the molecular weight) was estimated from the initial slope of the curve (Figure 3 in Supporting Information).

The modification of disposable PGE with CNTs demonstrated here has been very simple and fast. No time-consuming surface chemistry was applied for immobilization of DNA or any bead-based amplification routes.[8] This important advantage brings the possibilities on application of this process based on PCR samples.

CONCLUSION

The direct detection of DNA based on the signal enhancement of guanine oxidation was studied on bare and CNT-modified GCE and PGE electrodes. While both CNT-modified electrodes displayed an attractive DPV performance over their bare counterparts, the modified PGE compared favorably to the commonly used CNT-modified GCE electrode. The improved behavior of the modified PGE has its origin on the composite structure of the PGE behaving as a microelectrode array coupled with its higher porosity. The CNT-modified PGE DNA biosensor tested here offers some important advantages such as being inexpensive; it is based on a simple and direct electrochemical assay able to produce reproducible and sensitive results with a good degree of selectivity regarding to label-free DNA hybridization.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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