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Ultrasensitive Electrical Biosensing of Proteins and DNA: Carbon-Nanotube Derived Amplification of the Recognition and Transduction Events

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The detection of DNA and proteins is of central importance to the diagnosis and treatment of genetic diseases, to the detection of infectious agents, drug discovery, or warning against bio-warfare agents.[1−4] Such biodetection commonly relies on hybridization or antigen−antibody (Ag−Ab) interactions, and requires proper attention to the achievement of ultrasensitive measurements. Electrochemical transducers are very attractive for such bioassays, owing to their high sensitivity, inherent simplicity and miniaturization, and low cost and power requirements. The use of enzyme labels to generate electrical signals has been extremely useful for ultrasensitive electrochemical bioaffinity assays of proteins and DNA. Heller's group[5,6] demonstrated that a highly sensitive amperometric monitoring of DNA hybridization (down to 5 zmol) could be achieved in connection with a horseradish-peroxidase (HRP)-labeled target and an electron-conducting redox polymer. HRP label has been combined by Willner's group[7,8] with a biocatalytic precipitative accumulation of the enzyme-generating product to achieve multiple amplifications and very low (25 amol) detection limits. Efforts to amplify enzyme-linked electrical protein assays included dual-enzyme substrate recycling[9] or ion-exchange accumulation of the product.[10] Yet, amplified transduction of biological recognition events remains a major challenge to electrical bioassays. New schemes based on coupling the biocatalytic amplification of enzyme tags with additional amplification units and processes are highly desired for meeting the high sensitivity demands of electrochemical detection of proteins and nucleic acids.

Here we demonstrate the use of carbon nanotubes (CNTs) for dramatically amplifying enzyme-based bioaffinity electrical sensing of proteins and DNA. The unique electronic, chemical, and mechanical properties of CNTs make them extremely attractive for electrochemical sensors.[11,12] Most CNT-sensing work has focused on the ability of surface-confined CNTs to promote electron-transfer reactions involved in biocatalytic devices.[13,14] In our new bioaffinity assays (Figure 1), CNTs play a dual amplification role in both the recognition and transduction events, namely as carriers for numerous enzyme tags and for accumulating the product of the enzymatic reaction. These novel support and preconcentration functions of CNTs reflect their large specific surface area[15] and are illustrated using the alkaline phosphatase (ALP) enzyme tracer. Such coupling of several CNT-derived amplification processes leads to the lowest detection limit reported thus far for electrical DNA detection.

The new CNT-based amplified bioelectronic protocol (Figure 1) involves the sandwich hybridization (a) or antigen−antibody (b) binding along with magnetic separation of the analyte-linked magnetic-bead/CNT assembly (A), followed by enzymatic amplification (B), and chronopotentiometric stripping detection of the product at the CNT-modified electrode (C). Our TEM observations (e.g., Figure 2) indicate that the hybridization event leads to cross linking of the ALP-loaded CNTs and the magnetic beads (with the DNA duplex acting as "glue"). To our knowledge, this is the first example of using DNA for linking particles to CNTs. No such aggregation was observed in the presence of noncomplementary oligonucleotides (B). Apparently, without the recognition event, the ALP-tagged CNTs are removed by the magnetic separation, leaving the magnetic beads behind. ALP was immobilized on CNTs using a 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide linker (see Figure 1 in Supporting Information). A coverage of around 9600 enzyme molecules per a CNT (i.e., binding event) was estimated from a separate electrochemical experiment comparing the α-naphthol response of known amounts of ALP-loaded CNTs and ALP (assuming similar activities for the free and bound ALP).

The dramatic signal enhancement associated with the CNT-based dual amplification route is demonstrated in Figure 3 for DNA-hybridization (A) and Ag−Ab (B) bioassays. The conventional protocols, based on the single-enzyme tag and a glassy-carbon transducer, are not responding to either 10 pg mL^{-1} DNA target (A,a) or 80 pg mL^{-1} IgG (B,a). The first amplification step based on the ALP-loaded CNTs (b) offers convenient detection of these low analyte concentrations. The single-ALP protocols displayed a

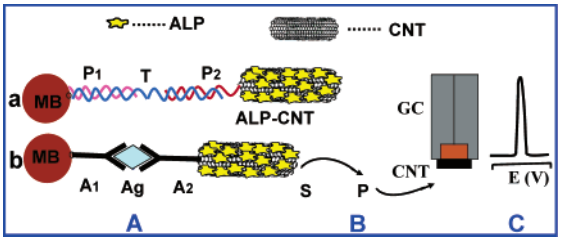

Figure 1. Schematic representation of the analytical protocol: (A) Capture of the ALP-loaded CNT tags to the streptavidin-modified magnetic beads by a sandwich DNA hybridization (a) or Ab−Ag−Ab interaction (b). (B) Enzymatic reaction. (C) Electrochemical detection of the product of the enzymatic reaction at the CNT-modified glassy carbon electrode; MB, Magnetic beads; P, DNA probe 1; T, DNA target; P_2, DNA probe 2; Ab_1, first antibody; Ag, antigen; Ab_2, secondary antibody; S and P, substrate and product, respectively, of the enzymatic reaction; GC, glassy carbon electrode; CNT, carbon nanotube layer.

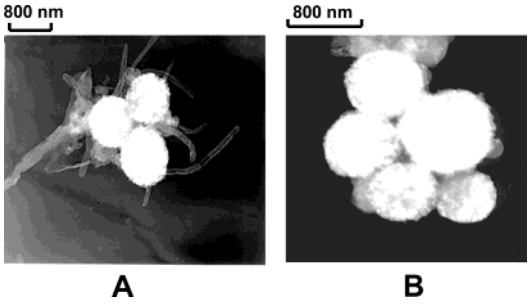

Figure 2. TEM images of the magnetic beads−DNA−CNT assembly produced following a 20-min hybridization with the 10 (A) and 0 (B) pg mL^{-1} target sample. The micrographs were taken with a Hitachi H7000 instrument operated at 75 kV.

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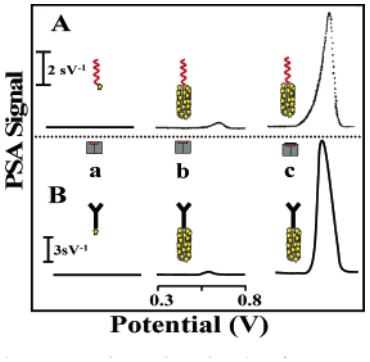

Figure 3. Chronopotentiometric signals for 10 pg mL^{-1} target oligonucleotide (A) and 80 pg mL^{-1} IgG (B) using the glassy carbon (GC) transducer and (a) a single ALP tag and (b) CNT-loaded with multiple ALP tags; (c) same as (b) but using the CNT-modified GC electrode. Amount of magnetic beads, 50 μg; sandwich assay with 20 and 30 min for each hybridization event and Ag/Ab association, respectively; sample volume, 50 μL. Detection, addition of 50 μL α-naphthyl phosphate (50 mM) solution with a 20-min enzymatic reaction. Measurements of the α-naphthol product were performed at the bare or modified GC electrodes, using a 2-min accumulation at +0.2 V in a stirred phosphate buffer solution (0.05 M, pH 7.4; 1 mL), followed by a 10-s rest period (without stirring) and application of an anodic current of +5.0 μA. See Supporting Information for the concentrations of the oligonucleotide probes and antibody, and sequence of oligonucleotide probes, levels and preparation of the ALP−DNA−CNT and ALP−streptavidin−CNT conjugates.

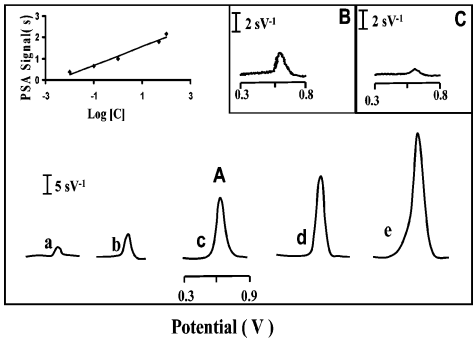

Figure 4. Chronopotentiometric signals for increasing levels of the DNA target: (a) 0.01, (b) 0.1, (c) 1, (d) 50, (e) 100 pg mL^{-1}. Also shown (inset) is the resulting calibration plot (A), and the response for 5 fg mL^{-1} target DNA (B) and 10 ng mL^{-1} noncomplementary (NC) oligonucleotide (C). Sample volume, 25 μL (B) and 50 μL (C). Other conditions, as in Figure 3 (A,c) based on protocol of Figure 1a (A−C).

lower signal for a significantly (1000-fold) higher target concentration (not shown). The nearly 10^4 improvement in the sensitivity is in good agreement with the estimated ALP loading per CNT. Only ~50-fold sensitivity enhancement was observed by using a streptavidin-coated polystyrene carrier bead instead of the CNT support. Further enhancements of the DNA and protein signals (by ~30-fold) are observed in the second amplification path, employing the CNT-modified transducer (c). The latter reflect the strong adsorptive accumulation of the liberated α-naphthol on the CNT layer. The preconcentration feature of the CNT layer was indicated from the use of different accumulation times that led to a sharp increase in the α-naphthol signal (compared to the time-independent signal observed at the bare electrode; see Figure 2 in Supporting Information).

Figure 4A displays typical chronopotentiograms for extremely low target DNA concentrations (0.01−100 pg mL^{-1}; a−e). Well-defined α-naphthol signals are observed for these low DNA concentrations in connection with 20-min hybridization. The resulting plot of response vs log[Target] (shown as inset) is linear and suitable for quantitative work. The favorable response of the 5 fg mL^{-1} DNA target (B) indicates a remarkably low detection limit of around 1 fg mL^{-1} (54 aM), i.e., 820 copies or 1.3 zmol in the 25 μL sample. Such a low detection limit compares favorably with the lowest values of 5 zmol (3000 copies) and 25 amol reported for electrical DNA detection.[6,8] Similarly, IgG was determined with a detection limit of 500 fg mL^{-1} (160 zmol in 25 μL samples) and exhibits a well-defined logarithmic concentration dependence. The smaller signal observed in a control experiment for a huge (~10^6) excess of a noncomplementary oligonucleotide (Figure 4, C vs B) reflects the high selectivity associated with the effective magnetic separation. The amplified electrical signal is coupled to a good reproducibility. Two series of six repetitive measurements of 1 pg mL^{-1} DNA target or 0.8 ng mL^{-1} IgG yielded reproducible signals with relative standard deviations of 5.6 and 8.9%, respectively.

In conclusion, we have demonstrated a CNT-based dual amplification route for ultrasensitive electrical bioassays of proteins and DNA. The use of CNT amplifiers (loaded with numerous ALP tags) has been combined with the preconcentration feature of CNT transducers to yield a dramatic enhancement of the sensitivity. Such coupling of several CNT-derived amplification processes results in highly sensitive detection of proteins and DNA and hence indicates great promise for PCR-free DNA assays. Further improvements in the sensitivity are expected either through reducing the electrode size and sample volume[6] or by substrate recycling.[9] The new CNT-derived amplification bioassays are expected to open new opportunities for medical diagnostics and protein analysis. The finding that DNA hybridization can be used for linking CNTs to particles holds promise for assembling controllable nanoscale systems.

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Supporting Information Available: Related experimental conditions (instrumentation, reagents, sequences, and procedures) along with additional data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Palecek, E.; Fojta, M. *Anal. Chem.* **2001**, *73*, 75A.
(2) Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2003**, *21*, 1192.
(3) Wang, J. *Chem. Eur. J.* **1999**, *5*, 1681.
(4) Gooding, J. J. *Electroanalysis* **2002**, *14*, 1149.
(5) Caruana, D. J.; Heller, A. *J. Am. Chem. Soc.* **1999**, *121*, 769.
(6) Zhang, Y.; Kim, H.; Heller, A. *Anal. Chem.* **2003**, *75*, 3267.
(7) Patolsky, F.; Katz, E.; Bardea, A.; Willner, I. *Langmuir* **1996**, *12*, 3703.
(8) Patolsky, F.; Litchenstein, A.; Willner, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 940.
(9) Bauer, C.; Eremenko, A.; Ehrentreich-Foster, E.; Bier, F.; Makower, A.; Halsall, H. B.; Heineman, W. R.; Scheller, F. W. *Anal. Chem.* **1996**, *68*, 2453.
(10) Limoges, B.; Degrand, C. *Anal. Chem.* **1996**, *68*, 4141.
(11) Baughman, R. H.; Zakhidov, A.; de Heer, W. A. *Science* **2002**, *297*, 787.
(12) Zhao, Q.; Gan, Z.; Zhuang, Q. *Electroanalysis* **2002**, *14*, 1609.
(13) Wang, J.; Musameh, M.; Lin, Y. *J. Am. Chem. Soc.* **2003**, *125*, 2408.
(14) Rubianes, M. D.; Rivas, G. A. *Electrochem. Commun.* **2003**, *5*, 689.
(15) Peigney, A.; Laurent, C.; Flahaut, E.; Basca, R.; Rousset, A. *Carbon* **2001**, *39*, 507.

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