J|A|C|S
COMMUNICATIONS

Published on Web 02/22/2003

Electrochemical Coding Technology for Simultaneous Detection of Multiple DNA Targets

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The detection of DNA hybridization is of central importance to the diagnosis and treatment of genetic diseases, for the detection of infectious agents, and for reliable forensic analysis. Recent activity has focused on the development of hybridization assays that permit simultaneous determination of multiple DNA targets.[1−5] These efforts have focused on various optical coding avenues for multi-target detection. Mirkin's group demonstrated that scattered light from different sized particle tags can be used for optical assays of multiple targets,[1] as can Raman-dye labeled gold nanoparticles.[2] Nie's group reported on a multicolor coding based on embedding different quantum dots into microbeads,[3] Keating and Natan employed bar-coded nanorods for the simultaneous analysis of multiple bioanalytes,[4] while Walt's team described a fiber-optic DNA array based on different optically encoded microspheres.[5] Inspired by these novel multicolor optical bioassays, this communication describes an electrochemical coding technology for the simultaneous detection of multiple DNA targets based on nanoparticle tags with diverse redox potentials. Such encoding nanoparticles thus offer a voltammetric signature with distinct electrical hybridization signals for the corresponding DNA targets.

Electrochemical devices have shown great promise for genetic testing and are ideally suited for shrinking DNA diagnostics.[6] However, a limitation of early electrical assays is that they are inherently single-target protocols.[7] The new electrochemical coding bioassay relies on the use of different inorganic-colloid (quantum dots) nanocrystal tracers, whose metal components yield well-resolved highly sensitive stripping voltammetric signals for the corresponding targets. Three encoding nanoparticles (zinc sulfide, cadmium sulfide, and lead sulfide) have thus been used to differentiate the signals of three DNA targets in connection with a sandwich hybridization assay and stripping voltammetry of the corresponding heavy metals (Figure 1). Stripping voltammetry is a powerful electroanalytical technique for trace metal measurements.[8] Because of its effective "built-in" preconcentration (deposition) step, the technique offers remarkably low (picomolar) detection limits. Recent activity has led to highly sensitive nanoparticle-based stripping electrical bioassays based on capturing various colloidal-metal and inorganic-crystal nanoparticle tags.[9] The new strategy thus combines a novel multi-target biodetection with an inherently amplified signal and the high selectivity attribute of magnetic assays.[10]

The new multi-target sandwich hybridization assay involves a dual hybridization event, with probes linked to the tagged inorganic crystals and to magnetic beads (Figure 1). The number of targets that can be readily detected simultaneously (without using high-level multiplexing) is controlled by the number of voltammetrically distinguishable metal markers. Our goal is to carefully select the nanocrystal tracers for creating a pool of nonoverlapping electrical tags. Particularly attractive for such bioassays are ZnS, PbS, CdS, InAs, and GaAs semiconductor particles in view of the attractive stripping behavior of their metal ions. For example, Figure 2A displays a typical stripping voltammogram for a solution obtained by dissolving simultaneously ZnS, CdS, and PbS nanocrystals. The corresponding metal ions yield well-defined and resolved stripping peaks at −1.12 V (Zn), −0.68 V (Cd), and −0.53 V (Pb). The relatively broad potential window (>1.2 V) over which heavy metals are oxidized/stripped, along with the sharp stripping peaks (theoretical peak widths of 75.5/*n* mV;[8] *n* is the number of electrons transferred), imply that up to 5−6 metals can be measured simultaneously (with minimal peak overlap). The inset of Figure 2A shows the stripping response for a solution containing nanomolar (ng L^{-1}) concentrations of five heavy metals (Zn, Ga, Cd, In, and Pb) related to semiconductor nanoparticles. All five metal ions yield well-defined peaks to allow convenient quantitation at this trace level. The peaks are well resolved, with the exception of some overlap for the Cd−In and In−Pb pairs.

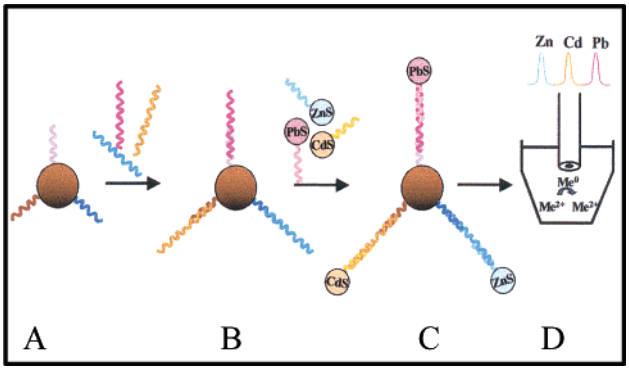

Figure 1. Multi-target electrical DNA detection protocol based on different inorganic colloid nanocrystal tracers. (A) Introduction of probe-modified magnetic beads. (B) Hybridization with the DNA targets. (C) Second hybridization with the QD-labeled probes. (D) Dissolution of QDs and electrochemical detection.

Figure 2A indicates that inorganic crystal tracers could yield characteristic voltammetric hybridization signals whose potentials reflect the identity of corresponding DNA targets. Figure 2B−D displays the hybridization response of the new bioassay to three different 60-mer DNA targets (related to the BRCA1 breast-cancer gene), in connection to their ZnS, CdS, and PbS tracers. As expected (from Figure 2A), the individual DNA targets yield well-defined hybridization peaks (of similar sensitivity) at −1.11 V (T_1,B), −0.67 V (T_2,C), and −0.52 V (T_3,D). A sample mixture containing the three DNA targets thus yielded three distinct and resolved voltammetric peaks for the corresponding targets (Figure 2E). Substantially (40-fold) smaller signals were observed for a large excess (0.54 μM) of a noncomplementary DNA sequence (not shown). Such small signals were similar to those observed in the absence of a DNA target, hence reflecting the minimal nonspecific adsorption of the secondary tagged probe. The similar response for the different targets (Figure 2E) indicates that the nanocrystal tracers have similar sizes and probe-coverages. Sizes of 2.8, 2.7, and 3.1

10.1021/ja029668z CCC: $25.00

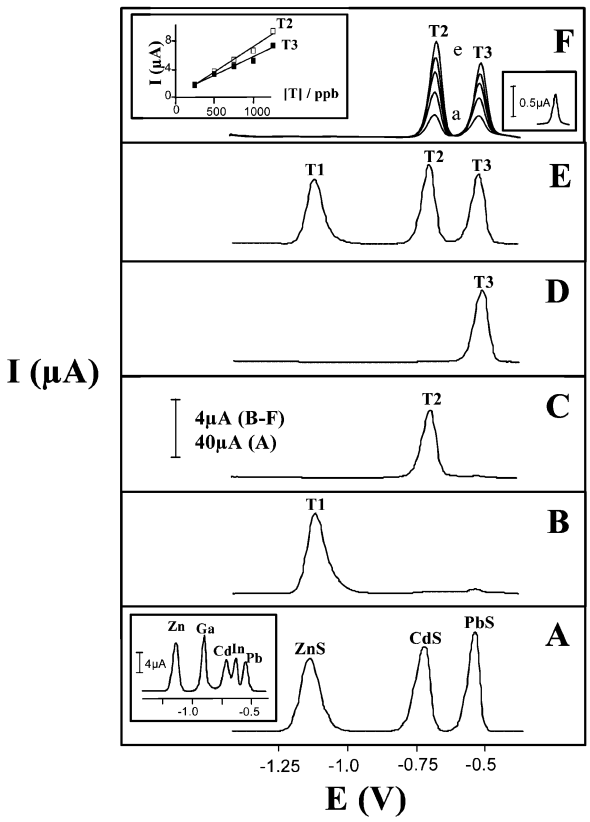

Figure 2. Typical stripping voltammograms for the metal tracers (A) and DNA targets (B−F). (A) Response for a solution containing dissolved ZnS, CdS, and PbS nanoparticles (1 μL of each QD solution dissolved in 20 μL of HNO_3 and transferred into 1 mL of the measuring solution). Also shown (inset) is the response for a mixture containing 20 ng mL^{-1} (ppb) of Zn, Cd, Ga, In, and Pb ions. (B,C,D) Hybridization stripping response for three different DNA targets (T_1, T_2, T_3, respectively), each at the 54 nM (ppm) level. (E) Response for a mixture containing the three DNA targets present at the 54 nM level. (F) Stripping hybridization signals for increasing concentration of the two DNA targets (T_2 and T_3) in 13.5 nM steps (a−e); also shown are the resulting calibration plots (left inset) and the actual signal for a 2.7 nM target T_3 solution (right inset). The measuring solution was a 0.2 M acetate buffer (pH 5.6) containing 10 μg mL^{-1} of Hg^{2+}. Other conditions: in situ plated mercury-coated glassy-carbon electrode, with 1 min pretreatment at 0.6 V; 2 min accumulation at −1.4 V; 15 s rest period (without stirring); square-wave voltammetric scan with a step potential of 50 mV; amplitude, 20 mV; frequency, 25 Hz. (B−F) Amount of magnetic beads, 20 μg; concentration of probes (P_1, P_2, P_3) 200 μg mL^{-1}; concentration of QD-modified probes (P_1', P_2', P_3'), 0.01 mg mL^{-1}; sandwich assay with 20 min for each hybridization step using room temperature and a 750 mM NaCl/150 mM sodium-citrate solution. See Supporting Information for the sequence of all oligonucleotide probes and 60-mer targets. All measurements were carried out with a AutoLab 12 system (Eco Chemie, Netherlands), controlled by the TAP2 software, and a 1.5 mL cell. Background correction was accomplished using the "moving average mode" of the GPES (AutoLab) software.

nm were estimated from TEM imaging of the CdS, ZnS, and PbS crystals, respectively.

The size of each peak reflects the concentration of the corresponding DNA target to allow convenient multi-target quantitation. Figure 2F illustrates that mixtures of two targets can be analyzed in a quantitative fashion. It shows the hybridization response to sample mixtures containing increasing levels of the two breast-cancer related 60-mer oligonucleotides (13.5−67.5 nM, 250−1250 ppb, a−e). The peaks are well defined and proportional to the concentration of corresponding DNA targets, indicating minimal cross interferences. The resulting calibration plots are linear (left inset; correlation coefficients, 0.979(T_1) and 0.975(T_2)). Also shown in Figure 2F (right inset) is the actual signal for a 2.7 nM target T_3 solution. Such response indicates a detection limit of 270 pM (5 ng mL^{-1}) in connection to the 20-min hybridization time. This detection limit corresponds to 250 pg (13 fmol) in the 50 μL samples. Further lowering of the detection limit to 2.7 pM (50 pg mL^{-1}) was obtained by incorporating multiple nanocrystals into a polystyrene-carrier bead (not shown). Hence, the new protocol retains the low detection limits inherent to single-target nanoparticle-based electrical DNA assays.[9] Femtomolar−picomolar detection limits were reported for analogous multicolor optical protocols.[2,4] A series of six repetitive measurements of the 5.4 nM target T_3 solution yielded a reproducible lead peak with a relative standard deviation of 9.4% (20 min hybridization; not shown).

In conclusion, we have demonstrated a multi-target electrochemical DNA detection based on the use of different inorganic-colloid (quantum dots) tags. The new multi-target electrical detection scheme incorporates the high sensitivity and selectivity advantages of nanoparticle-based electrical assays. This approach could be multiplexed and scaled by incorporating additional quantum-dot tracers (including bimetallic ones), by using microtiter plate platforms (with each microwell carrying out multiple measurements), and through particle-based libraries for electrical barcoding. The new electrochemical coding could be adapted to other multianalyte biological assays, particularly immunoassays. The electrochemical coding technology is thus expected to open new opportunities for DNA diagnostics, and for bioanalysis, in general.

Acknowledgment. Financial support from the National Science Foundation (grant number CHE 0209707) is gratefully acknowledged.

Supporting Information Available: Related instrumentation, electrode preparation, reagents, sequence information, particle preparation, and analytical procedure (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA029668Z