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Magnetically-Induced Solid-State Electrochemical Detection of DNA Hybridization

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The enormous information generated in the Human Genome Project has prompted the development of DNA sensors and high-density DNA arrays.[1,2] Nanoparticle-based materials offer excellent prospects for DNA analysis, owing to their many attractive properties.[3−7] Mirkin and co-workers have developed DNA sensors using hybridization-induced changes in (distance-dependent) optical properties of gold-particle-modified oligonucleotides[4] and a scanometric DNA array based on silver amplification of a hybridization event.[5] Analogous nanoparticle-based electrical DNA assays were developed by combining the catalytic enlargement of the metal-particle tags with highly sensitive electrochemical stripping detection of dissolved metal tags.[6−8]

Here we wish to report on a new nanoparticle-based protocol for detecting DNA hybridization based on a magnetically induced solid-state electrochemical stripping detection of metal tags. Magnetic spheres have proven valuable for removing unwanted constituents in nucleic acid assays, [8,9] but not for triggering the transduction of DNA hybridization. The ability of external magnetic fields to control bioelectronic processes, such as biocatalytic transformations of redox enzymes, was recently documented.[10] The present magnetic triggering of the electrical DNA detection is realized through a "magnetic" collection of the magnetic-bead/DNA-hybrid/metal-tracer assembly onto a thick-film electrode transducer to allow direct electrical contact of the silver precipitate (Figure 1). The resulting solid-state electrochemical transduction offers highly sensitive and selective detection of DNA hybridization. The ability to perform direct voltammetric measurements on solid materials, such as microparticles or powders (contacting electrode surfaces) was demonstrated by Scholz's group.[11] Unlike the mechanical immobilization (transfer by rubbing/abrasion) used by Scholz, the present protocol relies on the use of magnetic fields for attracting and confining the particle−DNA aggregates to the surface.

The new bioassay involves the hybridization of a target oligonucleotide to probe-coated magnetic beads, followed by binding of the streptavidin-coated gold nanoparticles to the captured target, catalytic silver precipitation on the gold-particle tags, a magnetic "collection" of the DNA-linked particle assembly and solid-state stripping detection (Figure 1, A−E). Our TEM observations (e.g., Figure 1F) indicate that the DNA hybrid "bridges" the metal nanoparticles to the magnetic beads (with multiple duplex links per particle). Most of the three-dimensional DNA-linked aggregate is covered with silver (following the catalytic precipitation of silver on gold). Such DNA-linked particle assembly can thus be "collected" magnetically (and "anchored") onto the thick-film working electrode. This leads to a direct contact of the silver tag with the surface and enables the solid-state electrochemical transduction (without prior dissolution and subsequent electrodeposition of the

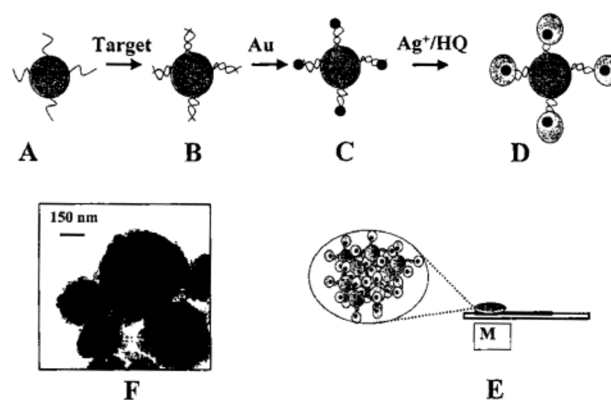

Figure 1. Schematic of the magnetically induced solid-state electrochemical detection of DNA hybridization. (A) Introduction of the probe-coated magnetic beads, (B) the hybridization event (with the biotinylated target), (C) capture of the streptavidin−gold particles, (D) hydroquinone (HQ)-induced catalytic silver deposition on the gold nanoparticle tags, (E) positioning of an external magnet (M) under the electrode to attract the particle−DNA assembly and solid-state chronopotentiometric detection. (F) TEM micrograph of the DNA−particle assembly produced following a 20 min hybridization with the 50 μg mL^{-1} target sample and 10 min silver precipitation. After washing with water a 5 μL drop of the aggregate sample was placed onto a 3-mm-diameter, 200-mesh, carbon-coated copper grid and was allowed to dry; micrographs were taken on an Hitachi H7000 instrument operated at 75 kV.

metal) using oxidative dissolution of the silver tracer in connection to a constant-current chronopotentiometry. No such aggregate and silver metalation were observed in the presence of excess non-complementary DNA, that is, without the linking hybrid and seeding gold nanoparticles (not shown). As a result, the magnetic "collection" and detection of the metal tag are not possible.

The high sensitivity and selectivity of the new protocol was illustrated for the detection of DNA segments related to the BRCA1 breast-cancer gene. Figure 2 depicts the magnetogenoelectronic response to a 200 ng mL^{-1} DNA target (MW = 6331.4) (A), as well as to a large excess of noncomplementary (B) and mismatched (C, D) nucleic acids. A well-defined chronopotentiometric hybridization signal, corresponding to the oxidation of the silver tracer (E_p = 0.28 V), is observed upon positioning of an external magnet under the electrode. In contrast, no response is observed for the 100-fold excess of the noncomplementary nucleic acid (B). Apparently, the magnetic "collection" of the metal label is not possible without the DNA-hybrid linker. Similarly, no target response is observed without positioning the magnet below the electrode, for example, in the absence of direct electrical contact (A, dotted line). A negligible signal is observed in the presence of a 3-fold excess of the three-base mismatched oligonucleotide (C), while the single-base mismatched nucleic acid fragment yields a response which is 12-fold smaller than that of the target (D vs A). A similar degree of discrimination was observed by mixing the target with the

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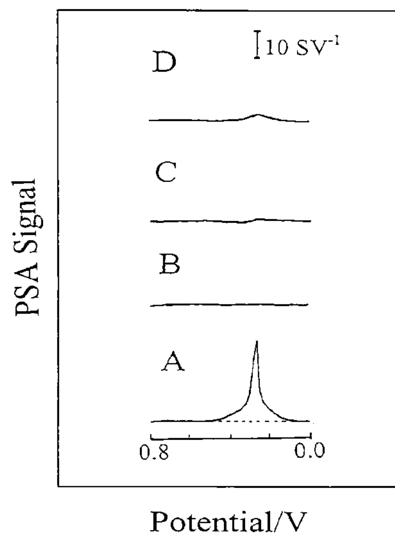

Figure 2. Chronopotentiometric hybridization signals for (A) the 200 ng mL^{-1} target oligonucleotide (with and without an external magnet, solid and dotted lines, respectively), (B) 20 μg mL^{-1} noncomplementary strand, (C) 600 ng mL^{-1} three-base mismatched oligomer, and (D) 200 ng mL^{-1} one-point mutated oligonucleotide. Hybridization time, 20 min; silver enhancement time, 10 min (using the Sigma Silver Enhancement kit). For detection, a 60 μL drop of the silver-enhanced DNA−particle solution was placed onto the thick-film carbon electrode with a magnet placed below the working electrode (solid lines). Measurements were carried out with a Trace Lab PSU20 system (Radiometer), equipped with TAP2 software, using a constant current of 10 μA. Streptavidin-coated gold particles (5 nm) and streptavidin-coated magnetic beads were obtained from Energy Beam Sciences and Bangs Laboratories, respectively. The oligonucleotides (acquired from Sigma-Genosys Ltd.) had the following sequences: *Immobilized probe*: biotin-5′GAT TTT CTT CCT TTT GTT. *Target*: biotin-5′GAA CAA AAG GAA GAA AAT C. *Noncomplementary*: biotin-5′-GGT CAG GTG GGG GGT ACG CCA GG. *Three-base mismatch*: biotin5′-CAA CAA AAG CAA CAA AAT C. *One-point mutation*: biotin-5′GAA CAA AAG GAA **T**AA AAT C.

noncomplementary and three-base mismatched oligomers (not shown). Such negligible contributions of imperfect polynucleotides reflect the minimal linking of the magnetic sphere and metal particle associated with the weak duplex formation. Complete elimination of such mismatch contributions are expected by combining the new genomagnetic protocol with the use of PNA probes.[12]

The quantitative behavior was assessed by monitoring the dependence of the silver hybridization signal upon the concentration of the target oligonucleotide. Well-defined signals, proportional to the target concentration, were observed over the 20−250 ng mL^{-1} range (20 min hybridization and 10 min silver enhancement; (see Supporting Information). The resulting calibration plot was highly linear with a sensitivity of 12.3 s mL ng^{-1} (correlation coefficient, 0.998). A detection limit of around 150 pg mL^{-1} (i.e., 1.2 fmol) was estimated on the basis of the signal-to-noise characteristics of the response to the 2 ng mL^{-1} target DNA [30 min hybridization; other conditions, as in Figure 2 (see Supporting Information)]. Such low detection limit reflects the amplification features of metal-nanoparticle tags and of catalytic metal precipitation/enhancement and compares favorably to values reported for other particle-based DNA assays.[4−7] A series of six repetitive measurements of the 100 ng mL^{-1} breast cancer gene target solution, used for estimating the precision, yielded reproducible signals with a relative standard deviation of 7%. While such magnetically induced precipitation was performed on single-use thick-film electrodes, the process can be reversed by repositioning the magnet.

In conclusion, we have demonstrated a proof-of-concept of a magnetically induced solid-state electrochemical stripping metal analysis for monitoring DNA hybridization. The new method couples the high sensitivity of silver amplified assays with effective discrimination against excess of closely related nucleotide sequences. The magnetic "collection" of the metal label is not possible without the DNA-hybrid linker. A similar "direct-contact" solid-state stripping approach can be carried out for analogous gold-enhanced bioassays. The elimination of the acid dissolution and metal deposition steps greatly simplifies and shortens particle-based electrical bioassays, and eliminates background contributions from electrostatically bound silver ions (which would otherwise be released through the acid dissolution). It also obviates the need for toxic bromine solutions essential for dissolving gold tags. Current efforts are aimed at assessing how the architecture and size of the DNA-linked particle assembly and morphology of the electrode surface affect the ability of silver to contact the electrode surface (and the resulting electrochemistry), and understanding the influence of the DNA-target length on the interparticle separation, aggregate size, and upon the solid-state electrical signal.

The direct electrical detection of DNA/metal-particles assemblies can bring new capabilities to the detection of DNA hybridization. For example, metal-nanoparticle DNA arrays, yielding multiple silver stripping peaks (in connection to microscopic wells and magnetic microelectrode transducers at the individual sites) can be envisioned. The instrumental requirements of such electrical detection could be readily incorporated into a low-cost portable unit. We believe that the methodology presented above opens a new topic of magnetogenoelectronics, and could be applied to different bioaffinity assays (e.g., for magnetic collection and detection of immuno-linked particle networks) and to other solid-state electrochemical applications. Besides their biosensing utility, the DNA-mediated assembly structures should have a profound impact in the fields of nanotechnology and nanoelectronics. Smaller (nanoscopic) functionalized magnetic spheres[13] could be used creating nanoscopic assemblies relevant to nanobiotechnology. A magnetic switching of charge transfer through DNA can also be envisioned.

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Supporting Information Available: Quantitative data and sample preparation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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