

Electrochemical Detection of DNA Hybridization Using Biometallization

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We demonstrate the amplified detection of a target DNA based on the enzymatic deposition of silver. In this method, the target DNA and a biotinylated detection DNA probe hybridize to a capture DNA probe tethered onto a gold electrode. Neutraavidin-conjugated alkaline phosphatase binds to the biotin of the detection probe on the electrode surface and converts the nonelectroactive substrate of the enzyme, *p*-aminophenyl phosphate, into the reducing agent, *p*-aminophenol. The latter, in turn, reduces metal ions in solutions leading to deposition of the metal onto the electrode surface and DNA backbone. This process, which we term biometallization, leads to a great enhancement in signal due to the accumulation of metallic silver by a catalytically generated enzyme product and, thus, the electrochemical amplification of a biochemically amplified signal. The anodic stripping current of enzymatically deposited silver provides a measure of the extent of hybridization of the target oligomers. This biometallization process is highly sensitive, detecting as little as 100 aM (10 zmol) of DNA. We also successfully applied this method to the sequence-selective discrimination between perfectly matched and mismatched target oligonucleotides including a single-base mismatched target.

There is an increasing demand for ultrasensitive methods of DNA detection, capable of detecting subfemtomolar concentrations, for various applications including the genetics of disease¹ and medical diagnosis. These methods would allow the detection of the gene products without PCR amplification. Among various detection methods for DNA hybridization, the electrochemical detection of DNA hybridization has attracted particular attention because it provides a simple, inexpensive, accurate, and sensitive platform. Several electrochemical methods with high sensitivity and selectivity have been developed, including an electrochemical DNA sensor based on the oxidation of DNA bases, the redox reaction of reporter molecules and enzyme, DNA-mediated charge transport, and stripping of nanoparticle.²

Several electrochemical DNA sensors using metals have been reported. One such approach is the use of nanoparticle tags. To

date, electrochemical nanoparticle-based DNA assays have commonly relied on anodic stripping³ or conductance measurements⁴ of nanoparticles captured onto hybridized targets. All of these methods have shown high sensitivity to assay target DNA down to the picomolar level. The sensitivity of bioassays based on anodic stripping depends on the size of the metallic nanoparticle. Although the sensitivity in anodic stripping method has been more enhanced by catalytic enlargement using silver enhancement, the enlargement is limited to the preparation of 30-nm particles. Therefore, micrometer-sized tags of indium microrod were used to achieve a lower detection limit of 250 zmol.⁵ Another approach, which does not utilize nanoparticle tags, is DNA-templated metallization by electroless deposition.⁶ The sensitivity of this method is dependent on the length of the DNA, which acts as the binding and nucleation sites of silver ions. Although both approaches lowered the detection limit, they have difficulty achieving the higher sensitivity. In the former approach, the size of the metal tag limits the sensitivity of the assay. The limit of the latter method is the high background signal caused by the capture and deposition of silver even on single-stranded capture DNA as in previous works on DNA-templated metallization.⁷

To overcome these drawbacks, we have developed a new system for the electrochemical detection of DNA hybridization based on stripping voltammetry of enzymatically deposited silver. The target DNA and a biotinylated detection DNA probe hybridize to a capture DNA probe tethered onto a gold electrode. Neutraavidin-conjugated alkaline phosphatase (Av-ALP) binds to the biotin of the detection probe on the electrode surface, converting the nonelectroactive substrate to a reducing agent. The latter, in turn, reduces metal ions in solutions, leading to the deposition of metal onto the electrode surface and DNA backbone. We have called this process, in which metallization is catalyzed by an enzyme, 'biometallization'. In contrast to previously developed methods,

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Table 1. Sequence of Modified Oligonucleotide Stands

oligonucleotide	sequence (5' → 3')
capture probe	GTA AAA CGA CGG CCA G
detection probe	TTA TAA CTA TTC CTA TTT TT
complementary target	TAG GAA TAG TTA TAA CTG GCC GTC GTT TTA C
noncomplementary target	TAG GAA TAG TTA TAA AAA GCT GAC CAG ACA G
single-base mismatched target	TAG GAA TAG TTA TAA CTG GCC GTC GTC TTA C

the sensitivity of this protocol does not depend on the size of the metal marker or the length of the DNA but rather is dependent on the condition of the enzymatic reaction. As a result, the stripping signal of the metal is amplified by the enzymatic reaction without limitation. In contrast, the background signal due to the metallization on the DNA backbone is minimized because the reducing agent is generated only by the enzymatic reaction and hence is not initially present in the solution. Our protocol also resolves problems of bioassays based on enzyme label. In previous enzyme-linked amperometric DNA detection methods, the product of the enzymatic reaction diffuses out and its current is measured.⁸ In contrast, using our method, the electroactive metallic product accumulates on the electrode during the reaction, and the stripping current of the accumulated metal is measured using anodic stripping voltammetry. The introduction of metallization is very advantageous in that there is no need for a conduction layer, such as a conducting polymer and ferrocenyl dendrimer to provide mediated electrical wiring of enzyme enhancing the detection current. These wiring layers are not required because the stripping analysis itself is a very powerful technique that lowers the detection limits by 3–4 orders of magnitude, when compared with pulse voltammetry techniques previously used for monitoring DNA hybridization. Although the electrochemical method, using an insoluble precipitate of organic materials produced by an enzyme,⁹ provides analogous advantages, it requires the expensive and time-consuming impedance measurements.^{9(c)} In contrast, our electroactive metallic product is easily analyzed by the well-established technique of stripping voltammetry, which has a lower detection limit and makes the electrochemical detection of DNA hybridization simple, inexpensive, and sensitive. These advantages of our method allow higher sensitivity on DNA detection compared with the previous works.

We assayed enzymatic silver deposition using a quartz crystal microbalance (QCM) in conjunction with linear sweep voltammetry (LSV) to verify the biometallization of silver. To demonstrate the high sensitivity of this method, we compared the electrochemical results with those obtained by surface plasmon resonance (SPR). We also show that our technique enables not only the detection of sequence-selective discrimination between perfectly matched and mismatched target oligonucleotides including a single-base mismatched target but also the quantification of target oligonucleotides down to 100 aM.

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EXPERIMENTAL SECTION

Chemicals and Reagents. Silver sulfate (99%) and mercaptoethanol (MCE, 98%) were purchased from Aldrich. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and cetyltrimethylammonium bromide (CTAB) were obtained from Sigma. *p*-Aminophenyl phosphate monohydrate (*p*-APP) was purchased from Universal Sensors (Cork, Ireland), and Av-ALP was obtained from Pierce (Rockford, IL). All buffer salts and other inorganic chemicals were obtained from Sigma or Aldrich unless otherwise stated. All chemicals were used as received. Ultrapure water (> 18 M Ω) from a Modulab water system (U.S. Filter Corp.) was used throughout this work. Solutions of *p*-APP and TCEP were prepared daily.

All oligonucleotides employed in this work were synthesized and purified using an HPLC apparatus supplied by GenoTech (Daejeon, Korea). Capture probes were modified with 5'-thiol-terminated 6-carbon spacers $-(\text{CH}_2)_6-$. A 9-atom ethylene glycol linker group $-(\text{C}_2\text{H}_4\text{O})_3-$ was appended on the 5'-end of a capture probe to improve hybridization efficiency and prevent nonspecific binding of proteins. The detection probe was 3'-labeled with biotin group, and a 5-T (thymine) spacer was inserted between the oligomer and the biotin. The targets for sandwich hybridization were designed using complementary and non-complementary oligonucleotides, respectively, as shown in Table 1.

The hybridization buffer (HB) consisted of 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM CTAB, and 1 M NaCl (pH 7.4). The DNA rinsing buffer (DNA-RB) was composed of 10 mM NaCl, 5 mM Tris-HCl (pH 7.4). The binding buffer (BB) for associating with Av-ALP consisted of 50 mM Tris-HCl, 0.5 M NaCl, 0.1% Tween 20, and 1% bovine serum albumin (pH 7.4). The protein rinsing buffer (PRO-RB) was 50 mM Tris, 0.5 M NaCl, and 0.05% Tween 20 (pH 7.4). The enzyme reaction buffer (EB) was 50 mM glycine, 1 mM MgSO₄, and 1 mM ZnClO₄ (pH 9.0).

Electrode Preparation. Gold electrodes were prepared by electron beam evaporation of 40 nm of Ti followed by 150 nm of Au onto Si (100) wafers. The electrode was cleaned in piranha solution (30% H₂O₂/70% H₂SO₄), rinsed with water, and then dried with nitrogen gas. (WARNING: piranha reacts violently with organics) The electrode for the DNA sensor was constructed as following. Mixed monolayer surfaces of thiolated probe DNA and MCE were prepared by immersing the clean gold substrate in a 1 μ M solution of probe oligonucleotide in 1.0 M HEPES buffer containing 5 mM TCEP (pH 7.0) for 1 h, rinsing with DNA-RB for 5 s, immersing in a 1.0 mM MCE aqueous solution for 1 h, rinsing with DNA-RB for 5 s, and drying under a stream of nitrogen. (A scheme of the preparation of mixed monolayer is provided in Supporting Information.) The hybridization step with

the target was performed by incubation for 1 h in 100 μL of HB solution containing each target DNA (complementary, single-base mismatch, and noncomplementary) at 37 $^{\circ}\text{C}$. After annealing at room temperature for 2 min, the electrode was washed with DNA-RB and dried with N_2 gas. Then, electrodes including the target DNA was exposed for 30 min to 100 μL of the 1 μM detection probe solution at room temperature. The electrode was immersed in BB for 10 min to prevent the nonspecific adsorption of protein, and the resulting assembly was soaked in a mixture of 50% BB solution and 50% PRO-RB solution containing 8 $\mu\text{L}/\text{mL}$ Av-ALP for 5 min at room temperature, followed by two washes in PRO-RB. Silver was deposited in EB solution containing 1 mM Ag_2SO_4 and 2 mM *p*-APP in a dark chamber for 30 min. Since the current did not increase substantially when silver was deposited in a solution containing 2 mM Ag_2SO_4 and 4 mM *p*-APP, the composition of solution provides sufficient chemicals for the enzymatic reaction.

Quantification of Immobilized Capture Probe by Autoradiography. To quantify the surface density of capture probes attached on the gold electrode, the 3'-end of the capture probe was labeled with [α - ^{32}P]ddATP (Amersham, Piscataway, NJ) using terminal transferase (Promega, Madison, WI). Quantification was performed from radioimages using a Fuji Bio-Imaging Analyzer Model Bas 2000.

Instruments. Electrochemical experiments were performed using an Autolab potentiostat 10 (Ecochemie). The three-electrode electrochemical cell consisted of the modified Au electrode, a Pt wire counter electrode, and a Hg/Hg $_2$ SO $_4$ (mercury sulfate electrode: MSE, saturated K $_2$ SO $_4$) reference electrode. Linear sweep voltammetry (LSV) was performed in 0.1 M H $_2$ SO $_4$ solution deoxygenated with Ar prior to use. The electroactive area of the electrode is 0.283 cm 2 .

Microgravimetric analysis was performed using a QCM analyzer (Maxtek Inc., RQCM) linked to a personal computer. Quartz crystals (9 MHz AT-cut, Maxtek Inc.) between two Au electrodes (13-mm diameter) were used for QCM and electrochemical QCM (EQCM). The sensitivity of quartz is 7.089 ng/Hz. Before use, the quartz crystals were cleaned by immersion in piranha solution, rinsed with water, and dried in nitrogen gas. During EQCM experiments, the potential was controlled using an Autolab potentiostat 10 with a homemade triggering circuit.

SPR measurements were performed with a BIAcore X instrument. The mixed self-assembled monolayer (SAM) of MCE and the capture probe was achieved on the bare gold substrate of the sensor chip SIA Kit Au (BIAcore AB). SPR experiments were conducted with a constant 5 $\mu\text{L}/\text{min}$ flow of solution over the surfaces. We performed sequential injections of 100 μL of the 1 μM target in HB, 100 μL of the 1 μM detection probe in HB, and 35 μL of the 8 $\mu\text{L}/\text{mL}$ Av-ALP in BB. The surface was washed with PRO-RB after each injection. Adsorption of each component resulted in a shift in the resonance angle that was reported in resonance units (RU; 10 000 RU = 1.0 $^{\circ}$).

RESULTS AND DISCUSSION

Preparation of Capture Probe-Modified Gold Electrodes and Analytical Procedure using Biometallization. Scheme 1 depicts the construction of the sandwich-type DNA sensing electrode based on enzymatic silver deposition. First, mixed monolayer surfaces of capture probe DNA and MCE were

prepared as previously reported.¹⁰ The clean gold substrate was immersed in a 1 μM solution of thiolated capture probe oligonucleotide in 1.0 M HEPES buffer for 1 h, containing 5 mM TCEP to preserve the free sulfhydryl group, followed by its exposure to an aqueous solution of 1.0 mM MCE solution for 1h (Scheme 1a). The surface coverage of the immobilized capture probe, 3.7×10^{12} molecules/cm 2 as determined by autoradiography, was similar to that previously reported¹⁰ and was twice as much as that of the capture probe immobilized in the absence of TCEP (1.8×10^{12} molecules/cm 2). TCEP reduces disulfide bonds as effectively as dithiothreitol (DTT), which is widely used in biochemistry. Unlike DTT and other thiol-containing reducing agents, however, thiol-free TCEP may be suitable as a reducing agent for surface chemistry on gold. Because TCEP reduces the disulfide group of the capture probe in stock solution to the free sulfhydryl group, thiolated capture probe may be immobilized on the electrode with high density and the appropriate spacing, which may lead to efficient hybridization. Mercaptohexanol (MCH) has been frequently used as a spacer to minimize nonspecific binding and maximize hybridization efficiency between capture and target probe.¹¹ We choose MCE, however, because it was reported that MCE-modified gold acts as catalytic surface of electroless deposition¹² and MCE also functions as a spacer well. Although metallization can occur on DNA,⁷ the increased number of nucleation sites on an electrode due to the short chain length and increased defects of MCE would be more effective for the deposition of silver compared with a DNA backbone, and silver may be deposited on both MCE-modified gold and the DNA backbone. In contrast, when we used MCH in mixed monolayer as a spacer, a smaller amount of silver was deposited (result is not shown) because MCH blocks the nucleation sites. This result indicates that an increased number of nucleation sites on an electrode make biometallization of silver more efficient, even in the presence of DNA backbone.

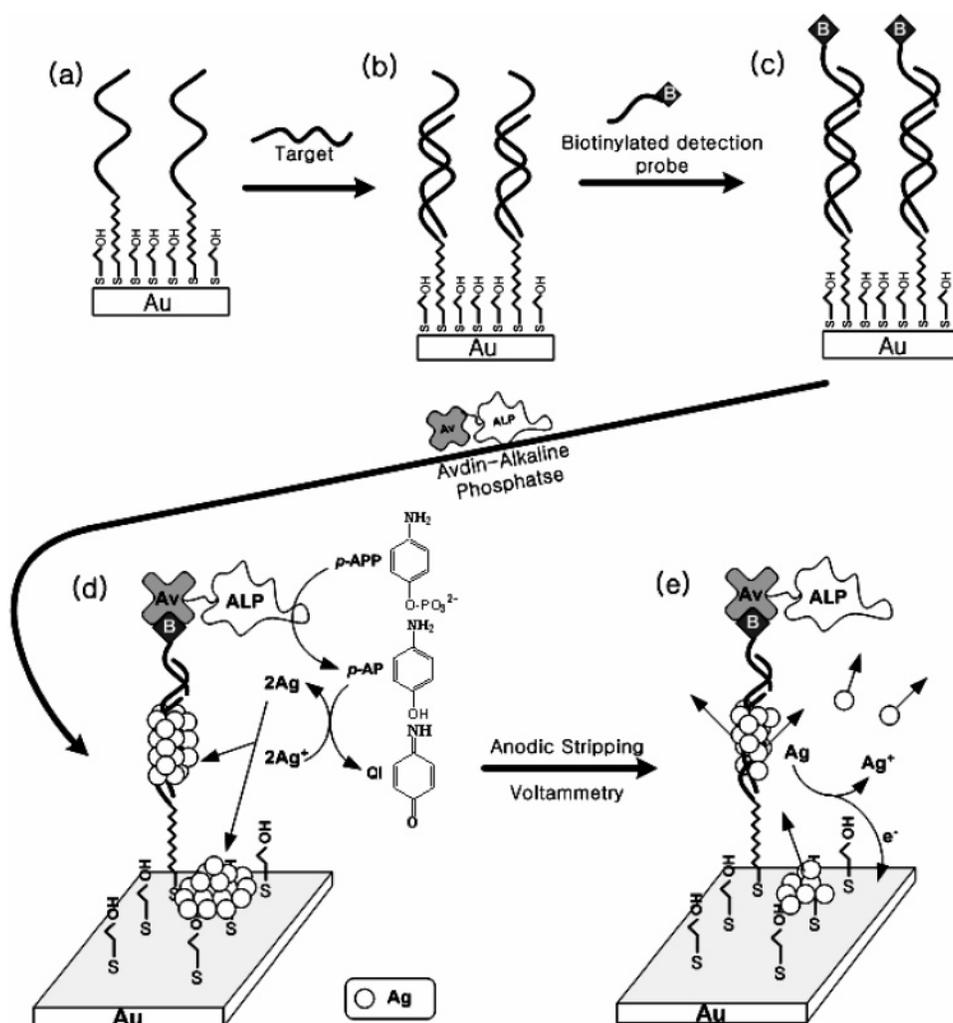
In the next step, the immobilized capture probes were hybridized with target DNA (Scheme 1b) and then sequentially with biotinylated detection probes needed for enzyme binding (Scheme 1c) in hybridization solution containing 1 mM CTAB as a cationic detergent for rapid and specific hybridization.¹³ Subsequently, Av-ALP was added, which binds to the exposed biotin group on the detection probes and converts *p*-APP to a *p*-aminophenol (*p*-AP), a reducing agent that reduces silver ions, forming a metallic silver layer on the DNA backbone and electrode for 30 min of reaction time (Scheme 1d). Since the half-wave potential of *p*-AP is 0.097 V versus NHE,^{8b,14} and that of Ag $^+$ is 0.80 V, *p*-AP spontaneously reduces silver ions in solution. The reduction can be explained by the following reaction:



where QI (quinoimide) is the oxidation product of *p*-AP, caused

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Scheme 1. Stepwise Assembly of a Sandwich-Type DNA Sensing Electrode Based on Enzymatic Silver Deposition^a



^a (a) Formation of mixed SAM on the Au electrode; (b) hybridization with target; (c) hybridization with biotinylated detection probe; (d) association with avidin–alkaline phosphatase and reduction of silver ion by *p*-AP; (e) dissolution of silver during anodic stripping voltammetry.

by the loss of two electrons. Therefore, only the enzymatic reaction can start the reduction of silver ions in solution and the anodic stripping current of deposited silver (Scheme 1e) provides a measure of the extent of hybridization of the target oligomers.

Biometallization of Silver at the Alkaline Phosphatase Tethered Electrode and Anodic Stripping Analysis of Deposited Silver. Figure 1 shows the time-dependent frequency change of the crystal modified with Av-ALP in different target probes. After a stable resonance frequency was achieved in 1 mL of 2 mM aqueous Ag_2SO_4 solution, 1 mL of 4 mM aqueous *p*-APP solution was added to the electrochemical cell for the QCM experiments. We observed a rapid decrease in crystal frequency when the probe was hybridized with 1 μM complementary target, whereas an almost constant resonance frequency was observed on the crystal hybridized with 1 μM noncomplementary target. That is, the association of Av-ALP with complementary DNA duplexes leads to the catalytic generation of reducing agent, which results in an increase in mass due to the silver deposition. These results suggest that the enzymatic deposition of silver effectively detects the selective recognition of target DNA. The crystal frequency

continuously decreases without leveling off to a constant value, but the slope tends to diminish slightly after 25 min. This decrease in frequency changes over time may be attributed to the partial deactivation of the enzyme caused by the formation of a metallic film, as shown previously with horseradish peroxidase enzyme.^{15(a)} The anodic stripping current and frequency change of enzymatically deposited silver of the same electrode used in Figure 1 was observed using EQCM. During the anodic scan, the frequency sharply increased (i.e., the mass decreased) concomitant with the appearance of the anodic stripping peak (Figure 2), which can be attributed to the dissolution of deposited silver. The amount of deposited silver was 43.86 nmol, when calculated from the charge of LSV, and 42.46 nmol, when calculated from frequency change. The solutions used contained 25-fold excesses of silver ions and *p*-APP. Doubling concentrations of these reagents to 2 mM Ag_2SO_4 and 4 mM *p*-APP had no effect on the voltammograms obtained, suggesting that the composition of our solution was appropriate for biometallization. These findings confirm that the use of LSV for stripping analysis is an appropriate approach for discriminating

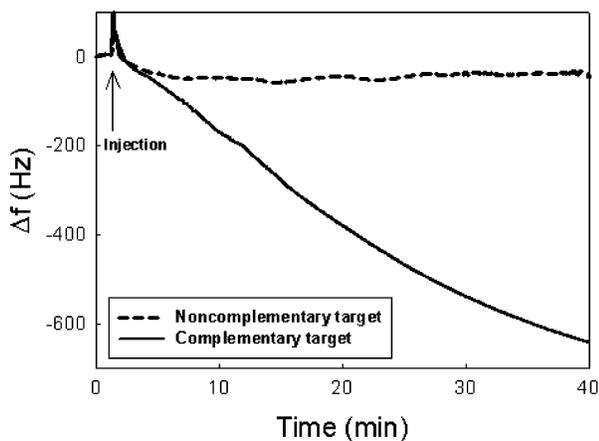


Figure 1. Time-dependent frequency changes of an Au quartz crystal modified by Av-ALP after soaking in $1 \mu\text{M}$ complementary target (solid line) or $1 \mu\text{M}$ noncomplementary target (dashed line). Stable resonance frequency was recorded in 1 mL of 50 mM glycine buffer containing 2 mM Ag_2SO_4 , followed by injection of 1 mL of glycine buffer containing 4 mM $p\text{-APP}$.

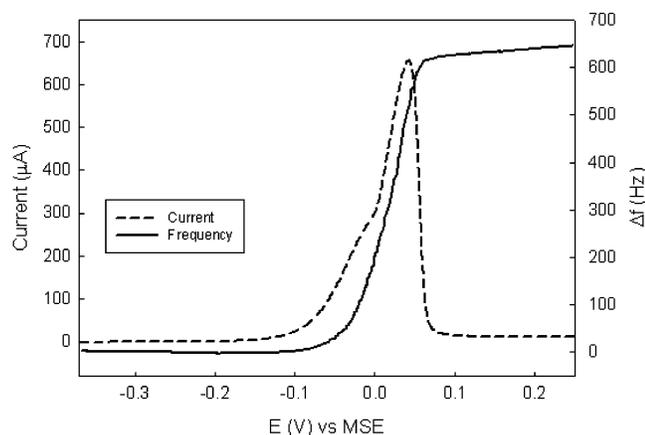


Figure 2. EQCM result on the same electrode after acquisition of Figure 1. The potential was swept to dissolve silver in 0.1 M H_2SO_4 aqueous solution. Scan rate, 0.010 V/s.

between complementary and noncomplementary target DNAs.

Detection of Sequence-Selective Hybridization Using Anodic Stripping Analysis of Biometallized Silver. Figure 3 displays the evident difference in the stripping response of LSV between the complementary and noncomplementary target DNAs after enzyme reaction for 30 min. A well-defined peak corresponding to silver stripping is observed at ~ 0.05 V versus MSE. The current is lower than that in Figure 2 due to the smaller electroactive area of the electrochemical cell and the shorter deposition time. The difference in LSV at ~ -0.17 V may have been caused by changes in surface properties induced by silver deposition. A sharp increase of $212 \mu\text{A}$ in the oxidation peak current was observed for a $1 \mu\text{M}$ complementary target DNA (Figure 3a), whereas a substantially smaller current ($21 \mu\text{A}$) was observed with $1 \mu\text{M}$ noncomplementary target DNA (Figure 3b), compared with $15 \mu\text{A}$ in the absence of hybridization with target and detection probe (Figure 3c). As a result, the signal-to-noise (S/N) ratio in discriminating sequence is 14.1, a value similar to that observed during QCM experiments (Figure 1). When the hybridization of the target and detection probe and the subsequent

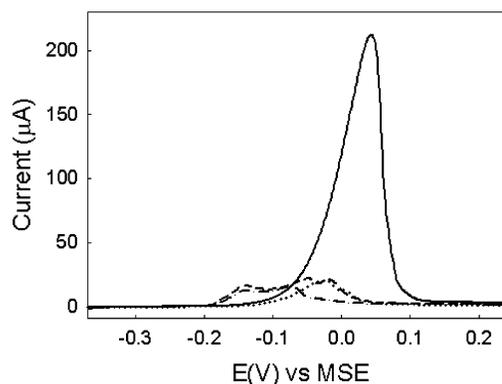


Figure 3. LSVs of enzymatically deposited electrodes in 0.1 M H_2SO_4 solution hybridized (a) with $1 \mu\text{M}$ complementary target (solid line); (b) with $1 \mu\text{M}$ noncomplementary target (dashed line); (c) in the absence of hybridization with target and detection probe (dash-dot line); (d) with $1 \mu\text{M}$ single-base mismatched target (dotted line). Scan rate, 0.010 V/s.

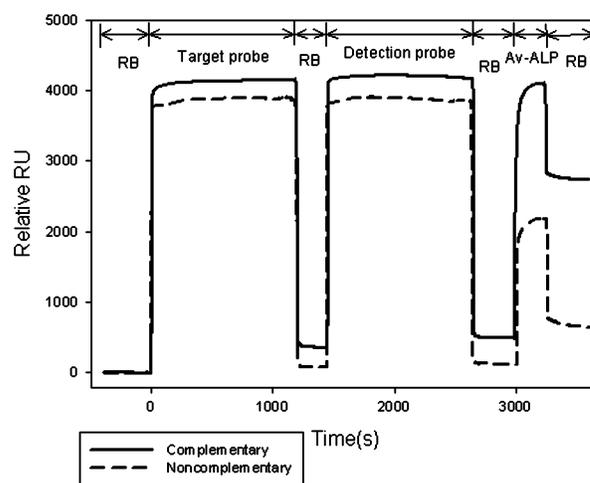


Figure 4. SPR sensograms for the hybridization of target DNAs and detection probe and the subsequent association of Av-ALP onto the capture probe tethered on Au. $1 \mu\text{M}$ complementary target DNA (solid line) or noncomplementary target DNA (dashed line) was hybridized. The flow rate was $5 \mu\text{L}/\text{min}$.

association of Av-ALP were investigated using in situ SPR (Figure 4), the final differential ratio of SPR signal between $1 \mu\text{M}$ complementary and noncomplementary DNA was 3.95 after binding of Av-ALP. Compared with the results obtained by in situ SPR, our method of enzymatic metal deposition gave 3-fold improvement in the hybridization detection. Such high S/N ratio can be attributed to the accumulation of metallic silver, which was reduced by the product of the enzymatic reaction, a result similar to that observed in reports using enzymatic insoluble precipitation.^{9(b)} When the reaction time of biometallization was reduced to 10 min, the peak current for a $1 \mu\text{M}$ complementary target DNA decreased to $75.5 \mu\text{A}$ while the current for a $1 \mu\text{M}$ noncomplementary was similar to that of 30-min deposition. These results show that conditions of enzyme reaction determine the sensitivity of assay as previous reported,¹⁵ and the reaction time of 30 min is regarded as enough time to provide high sensitivity as well as reduce time for analysis.

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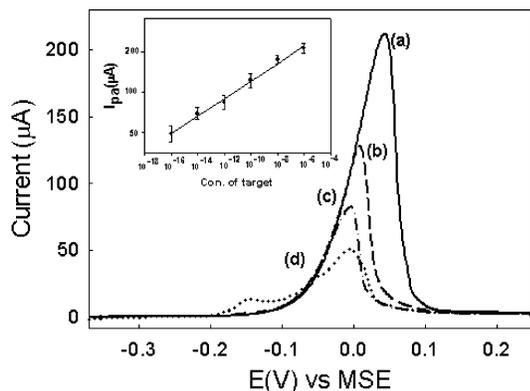


Figure 5. LSVs of enzymatically deposited electrodes in 0.1 M H₂SO₄ solution with complementary target DNA at concentrations of (a) 1 μM, (b) 100 pM, (c) 1 pM, and (d) 100 aM. Scan rate, 0.010 V/s. Inset: plot of peak current as a function of target DNA concentration. Both axes use log scales. Currents are averages for three electrodes.

We also detected an electrochemical signal for a single-base mismatched target DNA, in which a single mismatched base is located in the middle of the section that binds to the capture probe. The voltammogram of the electrode modified with a 1 μM single-base mismatched target is shown in Figure 3d. The current increment was 20 μA, similar to that of 1 μM noncomplementary target, indicating that a single-base mismatched target cannot be readily distinguished from a noncomplementary target but can be easily distinguished from complementary duplexes. These results thus demonstrate that our assay shows high selectivity.

Figure 5 shows the LSVs for sensing electrodes hybridized with different concentrations of the target DNA. The peak of stripping current increased with the target concentration over the range of 100 aM to 1 μM. The positive shift of peak potential dependent on the increase of the target DNA concentration is attributed to an increase in the thickness of the metallic film on an inert electrode, which caused the oxidation peak potential to shift toward more positive values.¹⁶ The calibration curve shown in the inset indicates that peak current versus [target] in log scales is linear over this wide range although the upper limit of detection may be around submicromolar level due to saturation of the DNA-capturing sites on surface. It should be noted that our detection method can cover a wide dynamic range at least 3 orders of magnitude larger than those shown previously.^{8,9,17} Similarly, the stripping charge also increases with target DNA concentration.

When used in Southern blotting, hybridization buffer containing 1 mM CTAB has been found to enhance the renaturation rate of two complementary DNAs \sim 2000-fold when compared with

1 M NaCl and to stabilize duplexes formed between oligonucleotide probes and target sequences selectively even in the presence of as much as a 10⁶-fold excess of noncomplementary DNA. Based on these findings, we utilized CTAB in our system, and we found that it improved reproducibility of experiments at very low concentrations of target DNA (<1 pM).

We found that the lower limit of detection of target DNA was \sim 100 aM (10 zmol) (S/N >3.0), which is 10⁴ lower than those previously observed (\sim 1–20 pM)^{8,9} but similar to the lowest values obtained in bioassays based on carbon nanotube-derived amplification¹⁶ and enzyme-amplified amperometric detection on microelectrodes.¹⁸ This result shows that biometallization successfully overcomes the limitations due to the metallic nanoparticle tag and enzyme label. In addition, the sensitivity of our biometallization method could be adjusted by changing the duration of the enzyme reaction. Although we achieved subfemtomolar sensitivity, further lowering of the detection limit can be expected when the enzyme reaction conditions are optimized, including adjustments in parameters such as temperature, substrate concentration, and reaction time.¹⁵

CONCLUSION

We have demonstrated the recognition of DNA using an enzyme label and the accumulation of metallic silver reduced by the product (*p*-AP) of the Av-ALP reaction. Biometallization, the combination of the biochemical amplification by ALP and anodic stripping voltammetry, results in the selective detection of various targets, including single-base mismatched oligonucleotides, as well as the ultrasensitive detection of target DNA with a lower limit of detection of 100 aM.

To our knowledge, this report is the first demonstrating in vitro enzymatic metallization (biometallization), although there have been sporadic reports on remediation of toxic metals¹⁹ and synthesis of nanomaterial²⁰ by in vivo enzymatic metallization in microbes. The design of our biometallization procedure mimics the indirect reduction by enzymatically produced reducing agents in nature rather than the direct reduction by metal reductase.¹⁸ When applied to a DNA bioassay, our design strategy is very useful for amplifying the signal and minimizing the background. This method can be applied to the recognition of biomolecules such as proteins, as well as various research on biomineralization and biomimetic synthesis of nanometallic structure.²⁰

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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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