Anal. Chem. **2004,** *76,* 900−906

Electrochemical Sensing of DNA Hybridization Based on Duplex-Specific Charge Compensation

Nokyoung Park and Jong Hoon Hahn*

National Research Laboratory for Advanced Biotechnology and Biomedical Microinstrumentation, Department of Chemistry, Division of Molecular and Life Sciences, Pohang University of Science and Technology, San 31 Hyoja-dong, Pohang, 790-784, South Korea

A nonlabeling voltammetric detection method for DNA hybridization has been developed, in which $[Fe(CN)_6]^{3-}$ in solution can readily approach an electrode surface covered with a charge-compensated DNA duplex layer and thus provides a strong redox-sensing current. Charge compensation for negative charges on the DNA backbone has been specifically accomplished on DNA duplexes by discouraging nonspecific binding of positively charged intercalating molecules with single strands. A pretreatment of DNA-modified electrodes with sodium dodecyl sulfate before the intercalator binding process is essential in preventing the nonspecific binding. Since ferricyanide, the only electrochemically active species, is present in the voltammetric solution, the detection signal can be amplified by increasing its concentration. Combination of the duplex-specific charge compensation with the signal amplification has achieved a remarkable signal difference: in 30 mM $[Fe(CN)_6]^{3-}$, the area ratio between cyclic voltammograms of the hybridized and unhybridized electrodes is ∼200 when 3,6-diaminoacridine is used as the intercalator. High sensitivity of the method has been demonstrated by detecting 10 fM (100 zmol in amount) of a target probe DNA.

Hybridization of nucleic acids to their complementary sequences is the essence of DNA chip technology. Detection of hybridization on a microarrayed surface has been largely based on detecting fluorescence from fluorophore-labeled target probes hybridized with surface-bound capture probes;[1−6] however, target labeling and expensive optical systems associated with the fluorescence detection have encouraged development of nonlabeling and lower-cost detection techniques using nanoparticles,[7,8] mass spectrometry,[9] surface plasmon resonance,[10,11] a quartz crystal microbalance,[12,13] a cantilever,[14] electrochemistry,[15,16] etc. Among them, electrochemical detectors are particularly promising because of their low cost, simple design, small dimensions, and low power requirements.

Thorp and co-workers[17] have developed a nonlabeling voltammetric detection method for DNA hybridization by using unlabeled target probes and an electrocatalyst, $[Ru(bpy)_3]^{3+}$ (bpy = 2,2′-bipyridine) and by substituting electrochemically inactive inosine for guanosine in capture probes, where the catalytic oxidation of guanosines in a target strand provides the signal for hybridization. This approach, however, requires synthesis of the artificial capture probes. Using exogenous redox intercalators, such as daunomycin, acridine orange,[18] and methylene blue,[19] has been suggested as the alternative. But these intercalators have not shown high specificity in binding to DNA duplex-modified electrodes because of the strong, nonspecific electrostatic interaction between the cationic intercalators and the polyanionic phosphate backbones of single-stranded DNA (ssDNA). In particular, Barton's group has developed a DNA mismatch detection method using the electrical conductivity of DNA duplexes and the electrocatalysis of intercalators, such as daunomycin and methylene blue. The group has also attempted to apply this

* To whom correspondence should be addressed. Phone: +82-54-279-2118. Fax: +82-54-279-8365. E-mail: hahn@postech.ac.kr.

(1) Marshall, A.; Hodgson, J. *Nat. Biotechnol.* **1998**, *16*, 27−31.
(2) Ramsay, G. *Nat. Biotechnol.* **1998**, *16*, 40−44.
(3) Lockhart, D. J.; Dong, H. L.; Byrne, M. C.; Follettie, M. T.; Gallo, M. V.; Chee, M. S.; Mittmann, M.; Wang, C. W.; Kobayashi, M.; Horton, H.; Brown, E. L. *Nat. Biotechnol.* **1996**, *14*, 1675−1680.
(4) DeRisi, J. L.; Iyer, V. R.; Brown, P. O. *Science* **1997**, *278*, 680−686.
(5) Lashkari, D. A.; DeRisi, J. L.; McCusker, J. H.; Namath, A. F.; Gentile, C.; Hwang, S. Y.; Brown, P. O.; Davis, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13057−13062.
(6) Selinger, D. W.; Cheung, K. J.; Mei, R.; Johansson, E. M.; Richmond, C. S.; Blattner, F. R.; Lockhart, D. J.; Church, G. M. *Nat. Biotechnol.* **2000**, *18*, 1262−1268.
(7) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078−1081.
(8) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757−1759.
(9) Isola, N. R.; Allman, S. L.; Golovlev, V. V.; Chen, C. H. *Anal. Chem.* **2001**, *73*, 2126−2131.
(10) He, L.; Musick, M. D.; Nicewarner, S. R.; Salinas, F. G.; Benkovic, S. J.; Natan. M. J.; Keating, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 9071−9077.
(11) Nelson, B. P.; Grimsrud, T. E.; Liles, M. R.; Goodman, R. M.; Corn, R. M. *Anal. Chem.* **2001**, *73*, 1−7.
(12) Caruso, F.; Rodda, E.; Furlong, D. N.; Niikura, K.; Okahata, Y. *Anal. Chem.* **1997**, *69*, 2043−2049.
(13) Okahata, Y.; Kawase, M.; Niikura, K.; Ohtake, F.; Furusawa, H.; Ebara, Y. *Anal. Chem.* **1998**, *70*, 1228−1296.
(14) McKendry, R.; Zhang, J. Y.; Arntz, Y.; Strung, T.; Hegner, M.; Lang, H. P.; Baller, M. K.; Certa, U.; Meyer, E.; Guntherodt, H. J.; Gerber, C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9783−9788.
(15) Wang, J.; Xu, D.; Kawde, A.; Polsky, R. *Anal. Chem.* **2001**, *73*, 5576−5581.
(16) Lee, T.; Shim, Y. *Anal. Chem.* **2001**, *73*, 5629−5632.
(17) Napier, M. E.; Loomis, C. R.; Sistare, M. F.; Kim, J.; Eckhardt, A. E.; Thorp, H. H. *Bioconjugate Chem.* **1997**, *8*, 906−913.
(18) Hashimoto, K.; Ito, K.; Ishimori, Y. *Anal. Chim. Acta* **1994**, *286*, 219−224.
(19) Kelly, S. O.; Jackson, N. M.; Barton, J. K.; Hill, M. G. *Bioconjugate Chem.* **1997**, *8*, 31−37.

10.1021/ac026368r CCC: $27.50
Published on Web 01/20/2004

method to hybridization detection,[19−21] but only a <2-fold signal from a hybridized electrode has been obtained, as compared with the signal from unhybridized one. They have explained this small signal difference might be caused by the exposed electrode surface, but nonspecific binding of the intercalators to ssDNA also seems quite significant. In attempts to attain specificity in binding to hybridized electrodes, Millan and Mikkelsen,[22] and Hashimoto et al.[23] employed metal complexes and a groove binder, respectively, but remarkable improvements have not been achieved. Takenaka et al.[24] synthesized for the same purpose a threading intercalator with electrochemically active ferrocenes, but they obtained only an ~4-fold signal difference between the hybridized and unhybridized electrodes. The poor discrimination of hybridized electrodes from unhybirdized ones, due to the nonspecific binding of intercalators or metal complexes, has made the practical application of these electrochemical DNA hybridization detection techniques difficult.

In this work, we propose a method to discourage nonspecific binding of an intercalator to ssDNA, thus to minimize the background signal and attain a large discrimination of hybridized electrodes from unhybridized ones in voltammetric detection of DNA hybridization. A long hydrophobic chain of a negatively charged surfactant, such as sodium dodecyl sulfate (SDS), binds to bases of ssDNA, whereas in a DNA duplex, stacked bases are surrounded with two sugar−phosphate polyanionic backbones, and therefore, binding with the surfactant would not be favorable. Now ssDNA covered with the surfactant molecules can avoid binding with an intercalator, because the surfactant molecules could form a micelle with the approaching intercalator molecule. We have applied this way of preventing nonspecific binding of intercalators to voltammetric detection of DNA hybridization and extended the hybridization detection to using electrochemically inactive intercalators. Here, $[Fe(CN)_6]^{3-}$ in the bulk voltammetric solution is the only electrochemically active species. When an electrode surface is covered with ssDNA, the negative charges of the phosphate backbones repel $[Fe(CN)_6]^{3-}$ from the surface.[21,25] But on the surface, with double-stranded DNA (dsDNA)/intercalator complexes, the negative charges of DNA are largely compensated for by positive charges of intercalating molecules. Therefore, $[Fe(CN)_6]^{3-}$ now rather freely approaches the electrode surface and provides a strong voltammetric signal. Furthermore, the signal can be amplified by increasing the concentration of $[Fe(CN)_6]^{3-}$. Through combination of the discouraging of nonspecific binding of an intercalator, 3,6-diaminoacridine in this work, with the signal amplification, we have achieved an unprecedented signal discrimination between hybridized and unhybridized electrodes (200:1) in voltammetric detection of DNA hybridization.

EXPERIMENTAL SECTION

Materials. Oligonucleotides, including a thiol-linked capture probe (5′-HS$(CH_2)_6$-GTTTTCCCAGTCACGACGGG-3′, hereafter referred to as HS-5′-DNA) and its complementary and a noncomplementary target probes (3′-CAAAAGGGTCAGTGCTGCCC-5′ and 3′-CACCAGTGTCTGTACCGCTC-5′, respectively), were purchased from MWG Biotech. (Ebersberg, Germany). The manufacturer supplied the measured value of optical density, corresponding to the strand amount for each oligonucleotide. Each oligonucleotide was dissolved in an adequate volume of sterilized water to prepare a stock solution of 100 μM strand concentration. The stock solutions of the target probes are diluted to 20 pM or 20 fM before the hybridization process. The dilutions are made stepwise so that no step exceeded 10-fold dilution. Potassium ferricyanide ($K_3Fe(CN)_6$), sodium dodecyl sulfate (SDS), and 3,6-diaminoacridine hydrochloride are obtained from Sigma-Aldrich (St. Louis, MO). Deionized water was prepared with a Milli-Q purifying system (Millipore, Milford, MA). A 99.999% gold rod (1.0-mm diameter) for fabrication of gold electrodes was also purchased from Sigma-Aldrich.

Preparation of ssDNA-Modified Electrodes. Because electrodes with a high surface coverage of DNA are recommended, we tried several kinds of protocols to modify gold surfaces with HS-5′-DNA, and found that the following protocol gives the most reproducible electrodes with high surface coverage of DNA. Before modifying a gold electrode with HS-5′-DNA, we polished its surface with alumina particles (0.05-μm diameter) and cleaned it thoroughly by boiling in saturated potassium hydroxide solution for 1 h and dipping in concentrated sulfuric acid for 24 h. After the electrode was rinsed with deionized water thoroughly, 10 μL, 0.06 mM HS-5′-DNA solution (pH 7.0, 0.2 M $MgCl_2$, 250 mM phosphate buffer) was dropped on the gold electrode surface, and then the electrode was incubated for 8−48 h at room temperature. Finally, the electrode was washed with a rinsing buffer (pH 7.0, 10 mM NaCl, 5 mM phosphate buffer).

Target Probe Hybridization. A 10-μL portion of 10 pM target oligonucleotide (matched or mismatched) solution (mixture of 5 μL of 20 pM oligonucleotide in water, 2 μL of 1.0 M $MgCl_2$, 2.5 μL of pH 7.0, 1.0 M sodium phosphate buffer, and 0.5 μL of water) was dropped on each ssDNA-modified gold electrode surface, and then the electrode was incubated for 2−8 h at 37 °C. After the hybridization, the electrode surfaces were washed thoroughly with the rinsing buffer.

Quartz Crystal Microbalance (QCM) Measurements. A gold-coated, AT-cut quartz crystal and an EG&G-Seiko QCM system (model 917, Tochigi, Japan) were used in the QCM measurements. The crystal had a density of 2.684 g/cm^3, a shear modulus of 2.947×10^{11} g/cm·s^2, and a fundamental resonance frequency of 9 MHz. The area of the gold disk coated on the crystal was 0.196 cm^2. The gold-plated quartz crystal was cleaned and rinsed with piranha solution (70% H_2SO_4/30% H_2O_2) and deionized water. After being dried under a stream of nitrogen, the gold surface was loaded with 100 μL of 0.05 mM HS-5′-DNA solution (pH 7.0, 0.2 M $MgCl_2$, 250 mM phosphate buffer), and the frequency change in the course of DNA immobilization on the gold electrode surface was monitored. The immobilized electrode was then rinsed with the pH 7.0 buffer and dried under a stream of nitrogen. After loading the electrode with 100 μL of 0.01 mM complementary target probe solution (pH 7.0, 0.2 M $MgCl_2$, 250 mM phosphate buffer), we monitored the frequency change during the hybridization process.

(20) Kelly, S. O.; Boon, E. M.; Barton, J. K.; Jackson, M. N.; Hill, M. G. *Nucleic Acids Res.* **1999**, *27*, 4830−4837.
(21) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096−1100.
(22) Millan, K. M.; Mikkelsen, S. R. *Anal. Chem.* **1993**, *65*, 2317−2323.
(23) Hashimoto, K.; Ito, K.; Ishimori, Y. *Anal. Chem.* **1994**, *66*, 3830−3833.
(24) Takenaka, S.; Yamashita, K.; Takagi, M.; Uto, Y.; Kondo, H. *Anal. Chem.* **2000**, *72*, 1334−1341.
(25) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *70*, 4670−4677.

SDS Treatment and 3,6-Diaminoacridine Intercalation. The hybridized electrodes were dipped in 1% SDS solution (1% SDS in pH 7.0, 10 mM NaCl, 5 mM phosphate buffer) for 10 min and washed with the rinsing buffer, then 20 μL of a 3,6-diaminoacridine solution (0.5 mg/mL in pH 7.0, 10 mM NaCl, 5 mM phosphate buffer) was dropped on each hybridized electrode, and the electrodes were incubated for 10 min at 25 °C. The electrodes were washed with the rinsing buffer (pH 7.0, 10 mM NaCl, 5 mM phosphate buffer) and stored in the same solution until used in cyclic voltammetry.

Circular Dichroism (CD) Measurements. CD spectra (220−300 nm) of ssDNA and dsDNA in SDS solutions were obtained under constant nitrogen flush at room temperature using a spectropolarimeter (J-715, JASCO, Tokyo, Japan) and a quartz cell with a 2-mm path length (JASCO, Tokyo, Japan). The signals were averaged over 3 scans to generate results. Two 0.05 mM solutions of the complementary target probe (pH 7.0, 10 mM NaCl, 5 mM phosphate buffer), containing 0 and 1% SDS, respectively, and two 0.025 mM solutions of a double-stranded oligonucleotide (3′-CAAAACGCCGCCAATACGGT-5′ hybridized to its complementary sequence) (pH 7.0, 10 mM NaCl, 5 mM phosphate buffer), containing 0 and 1% SDS, respectively, were prepared for the measurements.

Cyclic Voltammetry. All DNA-modified electrodes used as working electrodes in cyclic voltammetry were made of 99.999% gold rod with 1.0-mm diameter (0.785 mm^2). Cyclic voltammetry of the DNA-modified gold electrodes in pH 7.0, 5 mM phosphate buffer containing 10 mM NaCl was carried out anaerobically (by bubbling nitrogen for 5 min before cyclic voltammetric measurements) using a multichannel potentiostat (VMP, Perkin-Elmer Instruments, Boston, MA) at room temperature. A Ag/AgCl reference electrode (Bioanalytical Systems Inc., West Lafayette, IN) and a platinum wire auxiliary electrode were used for cyclic voltammetric measurements. The voltage scan range was 0.6−0.5V (versus Ag/AgCl reference electrode), and scan rate was 100 mV/sec for hybridization detection.

RESULTS AND DISCUSSION

Negatively Charged Surfaces Modified by DNA. For the platform of our work on the electrochemical detection of DNA hybridization, a self-assembled monolayer of a thiol-linked oligonucleotide (HS-5′-DNA) is formed on a gold electrode, and then hybridized with its complementary sequence, 3′-CAAAAGGGT-CAGTGCTGCCC-5′. We have evaluated the surface coverage of the monolayer and also the hybridization efficiency using QCM method. Figure 1a and b represents the time profiles of frequency decrease due to the increase in mass on the electrode during the immobilization and hybridization processes, respectively. Using the physical properties of the quartz crystal and the Sauerbrey equation,[26] we have estimated that a 1-Hz decrease in frequency corresponds to a 1.068-ng mass increase onto the surface of the crystal. The surface coverage corresponding to the frequency decrease in Figure 1a is 27.5 pmol/cm^2, which equals 1.66×10^{13} molecules/cm^2. With the frequency change in Figure 1b, we have also estimated the hybridization efficiency of the 10 μM complementary target on this layer to be ∼54%. It has been reported that the theoretical maximum surface density of dsDNA is 3.0×10^{13} molecules/cm^2,[27] but the optimum capture probe density for 100% hybridization efficiency is $\sim 5.2 \times 10^{12}$ molecules/cm^2.[28] Our layer is, therefore, somewhat too dense to get a very high efficiency hybridization.

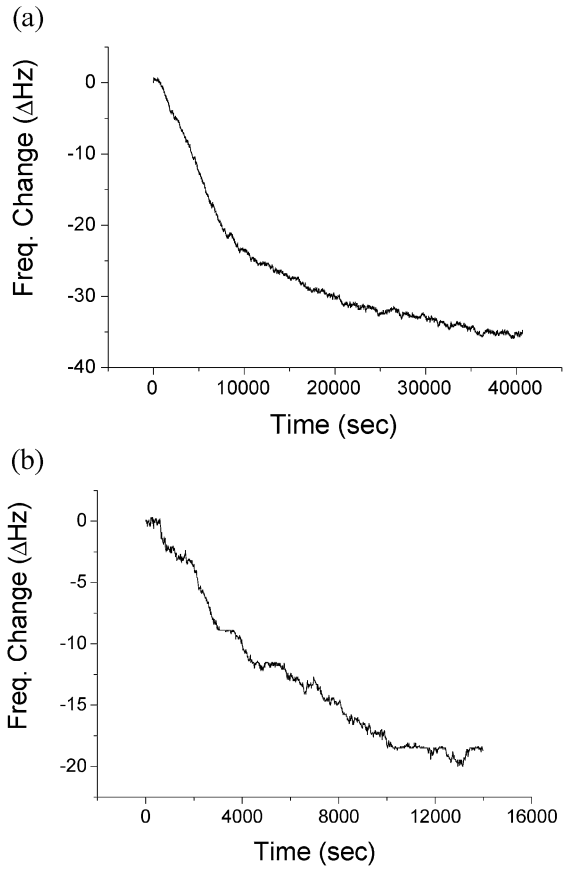

Figure 1. Decrease in the resonance frequency of the QCM sensor during (a) chemisorption process of HS-5′-DNA on a gold electrode, and (b) hybridization process of the complementary target probe on the electrode immobilized with HS-5′-DNA.

HS-5′-DNA was densely immobilized on the gold electrodes by chemisorption. The surface of the electrode was, therefore, covered with negative charges on the phosphate backbone of the oligonucleotide. The single-stranded oligonucleotide immobilized on the gold electrode was readily hybridized with its complementary strand. The electrode covered with the hybridized, double-stranded oligonucleotides also had a layer of negative charges on its surface. Figure 2 confirms the existence of the negatively charged layer on the DNA-modified electrodes. When placing the DNA-modified gold electrodes in an electrolyte solution containing $[Fe(CN)_6]^{3-}$, an electrochemically active, highly anionic species, we expected that repulsive electrostatic interactions would keep $[Fe(CN)_6]^{3-}$ away from the anionic DNA layer, and thus heterogeneous electron transfer from and to the electrodes would be greatly hindered during a redox cycle. A bare gold electrode in 30 mM $[Fe(CN)_6]^{3-}$ provides a reversible cyclic voltammogram with very high peak currents of ∼90 μA (trace 1 in Figure 2). But cyclic voltammograms using electrodes covered with both single-stranded and hybridized HS-5′-DNA show unnoticeable peak

(26) Huang, E.; Zhou, F.; Deng, D. *Langmuir* **2000**, *16*, 3272−3280.

(27) Georgiadis, R.; Peterlinz, K. P.; Peterson, A. W. *J. Am. Chem. Soc.* **2000**, *122*, 3166−3173.

(28) Herne, T. M.; Tarlov, M. J. *J. Am. Chem. Soc.* **1997**, *119*, 8916−8920.

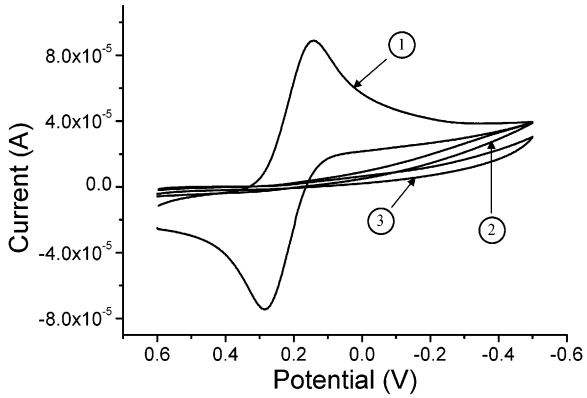

Figure 2. Cyclic voltammetry of 30 mM $[Fe(CN)_6]^{3-}$ obtained by using each of the following variations of a gold electrode as working electrode: (1) a bare gold electrode, (2) a ssDNA-modified electrode, and (3) a dsDNA-modified electrode.

currents (traces 2 and 3 in Figure 2, respectively), indicating a negligible surface concentration of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ on the modified electrodes. This remarkable reduction in redox current after the surface modifications with DNA is ascribed to the electrostatic repulsion between $[Fe(CN)_6]^{3-}$ and the polyanionic DNA layer, which is illustrated in Figure 3a−c.

Our electrode immobilized densely with ssDNA is very advantageous in providing highly dense negative charges on its surface, but it could discourage target probe hybridization. Therefore, it would be desirable that the surface coverage of ssDNA be adjusted by using a density-controlling method, such as mixed SAM methods,[28,29] to achieve an optimum condition.

Charge Compensation for Polyanionic DNA Duplex Using Intercalators. DNA intercalators are known to bind specifically to DNA duplex and in most cases, have one or more positive charges per molecule.[30] Therefore, the negative charges on duplex backbone can be, at least partially, compensated through binding with intercalators. This charge compensation by intercalator binding facilitates approach of $[Fe(CN)_6]^{3-}$ to the surface layer of a DNA duplex and increases the redox current of $[Fe(CN)_6]^{3-}$ (Figure 3d). Cyclic voltammogram 1 in Figure 4a has demonstrated such a current increase. HS-5′-DNA immobilized on a gold electrode is hybridized with its complementary oligonucleotide. The DNA duplex-modified electrode is then treated with 3,6-diaminoacridine (proflavine), a topical antiseptic intercalating agent. The voltammogram is obtained with the treated electrode in 30 mM $[Fe(CN)_6]^{3-}$. Note that proflavine is not electrochemically active within the voltage range of the voltammetric cycle of this work, 0.6 to −0.5 V. The peak cathodic current of the voltammogram, ~50 μA, reaches up to almost 60% of that obtained from a bare electrode (trace 1 in Figure 2), indicating charge compensation using this acridine is very efficient.

When the same treatment with the intercalating agent has been done on an electrode covered with the single-stranded HS-5′-DNA monolayer, we obtain a voltammogram (trace 2 in Figure 4a) that is very similar to that from the duplex-modified electrode. This implies that nonspecific interactions between the positively charged proflavine and the polyanionic ssDNA backbone are strong and cause unwanted charge compensation.

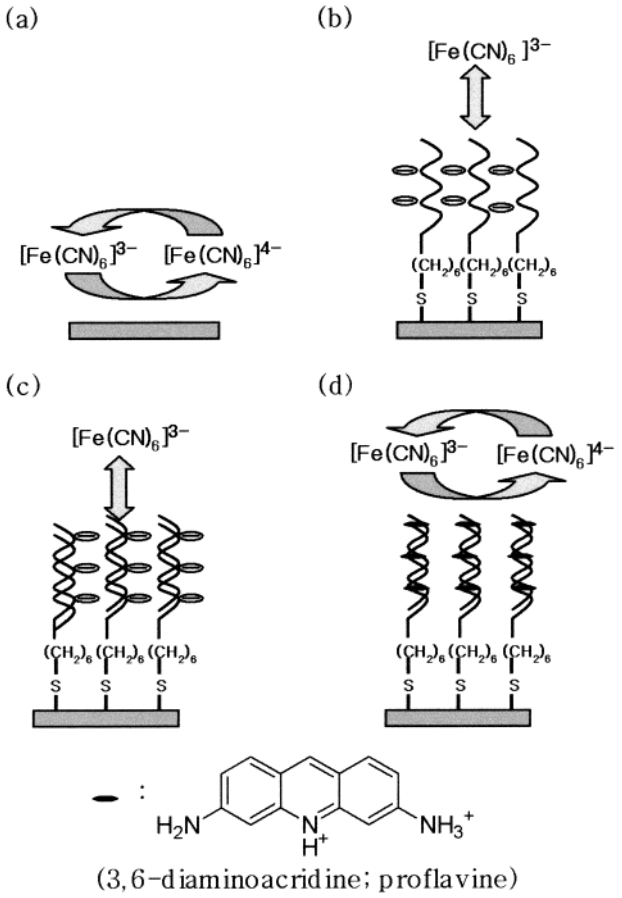

Figure 3. Changes in the surface charge of gold electrodes and the consequent electrochemical interaction between $[Fe(CN)_6]^{3-}$ and the electrode, according to surface modifications. (a) Bare gold electrode; no charge on the electrode allows $[Fe(CN)_6]^{3-}$ to approach the electrode surface and, therefore, the reduction/oxidation of $[Fe(CN)_6]^{3-}$ occurs without difficulty. (b) ssDNA-modified electrode; negative charges on the sugar−phosphate backbone of ssDNA cover the electrode surface, which prevents $[Fe(CN)_6]^{3-}$ from approaching the surface, and the reduction/oxidation demands excessive overvoltages. (c) dsDNA-modified electrode; the situation is almost the same as that in (b). (d) dsDNA-modified electrode treated with an intercalating agent; when bound with an intercalating agent, such as proflavine, the negative charges on the duplex strands are compensated with the positive charges of intercalator molecules, which provides a surface-charge condition close to that of the bare electrode and, thus, facilitates the approach of $[Fe(CN)_6]^{3-}$ to the electrode surface.

We have found that other widely used intercalators, such as YOYO-1 and SYBR Green, also provide voltammetric results very similar to Figure 4a, suggesting the nonspecific binding could be a common problem in the application of intercalators to DNA hybridization detection.

Voltammetric DNA Hybridization Detection by Discriminated Charge Compensation. We have discouraged the charge compensation in the layer of single-stranded HS-5′-DNA by dipping the HS-5′-DNA-modified electrode into 1% SDS solution prior to the proflavine treatment, which generates an almost completely suppressed cyclic voltammogram like trace 2 in Figure 2. To investigate the interactions between ssDNA and SDS, we obtained a CD spectrum of a 20-mer oligonucleotide in 1% SDS solution (spectrum 1 in Figure 5) and compared it with the spectrum from a SDS-free solution (spectrum 2 in Figure 5). There are obvious

(29) Satjapipat, M.; Sanedrin, R.; Zhou, F. *Langmuir* **2001**, *17*, 7637−7644.
(30) Thomas, G. *Medicinal Chemistry: an Introduction*; John Wiley & Sons, Ltd: Chichester, U.K., 2000; Chapter 10.

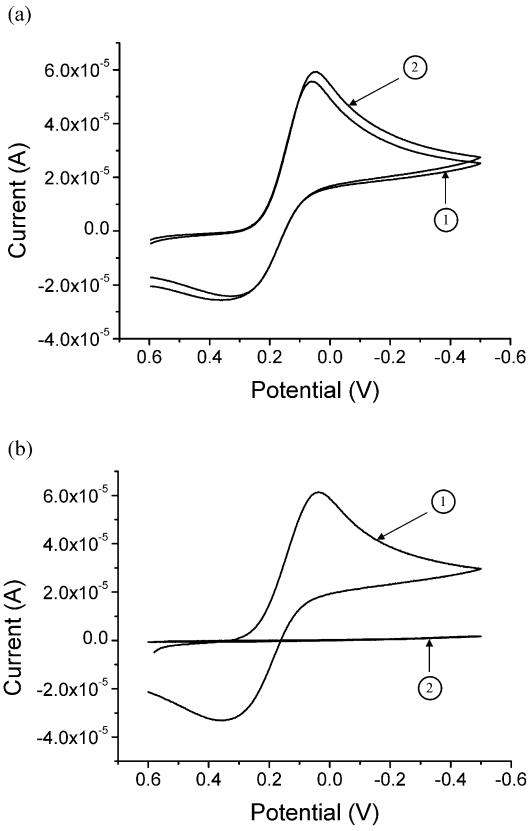

Figure 4. (a) Cyclic voltammograms of 30 mM $[Fe(CN)_6]^{3-}$ obtained from (1) dsDNA- and (2) ssDNA-modified electrodes after treatment with proflavine. (b) Effect of SDS treatment: cyclic voltammograms from (1) hybridized and (2) unhybridized electrodes modified with HS-5′-DNA capture probe. Before the cyclic voltammetric measurements, the modified electrodes are incubated with a complementary and a noncomplementary target probe and treated with SDS and then proflavine.

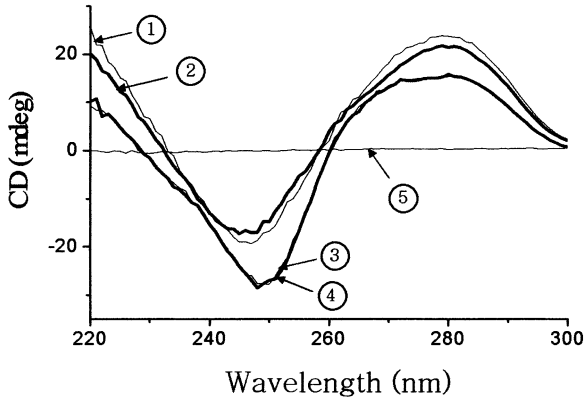

Figure 5. CD spectra of 20-mer single-stranded and double-stranded oligonucleotides in SDS solutions. (1) Thin line, CD spectrum of the complementary target probe in 1% SDS solution; (2) thick line, that in 0% SDS solution. (3) Thin line, CD spectrum of a 20-mer double-stranded oligonucleotide in 1% SDS solution; (4) thick line, that in 0% SDS solution. Spectrum 5 was obtained from a blank solution of 0.5% SDS.

differences between two spectra, both in the magnitude of CD and in the position of peak wavelengths (in spectrum 1, the negative peak around 248 nm is red-shifted, and the positive one around 280 nm blue-shifted, as compared with spectrum 2.), indicating SDS molecules interact significantly with the single-stranded oligonucleotide, which consequently changes its conformation. Strong hydrophobic interactions would be quite possible between the aliphatic chain of SDS and the bases of ssDNA. It has been known that ssDNA generates an active CD spectrum because bases on the single strand are locally staked.[31−33] The hydrophobic interaction by SDS would change the stacking structure and, thus, its CD spectrum. In contrast to ssDNA, the CD spectrum of a double-stranded 20-mer oligonucleotide does not change at all in the presence of 1% SDS (see spectra 3 and 4 in Figure 5).

On the basis of the results from the CD study, it would be very reasonable that the long aliphatic chains of SDS molecules interact attractively with the bases of ssDNA. When proflavine molecules approach the SDS-treated single strands, however, some of the SDS molecules on single strands would interact with proflavines and eventually form micelles that can be washed out in the subsequent rinsing step. Therefore, this sequential process could avoid the strong nonspecific interactions between proflavines and ssDNA. It has been reported that acridines, especially proflavine, tend to be stabilized in the hydrophobic core of SDS micelles.[34,35] However, since a DNA double helix is composed of two intertwined strands arranged such that the sugar−phosphate backbone lies on the outside and the bases on the inside, it would be expected that the hydrophobic chains of SDS molecules now hardly interact with the internally stacked bases. The results from the CD study (spectra 3 and 4 in Figure 5) support this suggestion. Therefore, the positively charged proflavines are relatively freely intercalated into a double helix. We have observed that the SDS treatment does not noticeably change the cyclic voltammograms of the duplex-modified electrodes.

This discriminated charge compensation technique employing the sequential electrode treatments with a surfactant and then a positively charged intercalator has been successfully applied to a voltammetric detection of DNA hybridization in the present work. Target probes with two different sequences were used in demonstrating the voltammetric DNA hybridization detection by discriminated charge compensation. One has a sequence of 3′-CAAAAGGGTCAGTGCTGCCC-5′, complementary to theHS-5′-DNA capture probe, and the other a noncomplementary sequence of 3′-CACCAGTGTCTGTACCGCTC-5′. When using the complementary target probe, we obtained a cyclic voltammogram with high peak currents in 30 mM $[Fe(CN)_6]^{3-}$ (trace 1 in Figure 4b); however, the noncomplementary target probe provides a voltammogram with negligible redox current (trace 2 in Figure 4b), which is even much smaller than the current generated from the untreated electrode modified with HS-5′-DNA (trace 2 in Figure 2), indicating that a significant fraction of SDS molecules remains on the unhybridized capture probe strands after the electrode treatments and washes, and thus, the density of negative charge on the unhybridized electrode surface becomes greater than that on the untreated electrode. Note that the separation of peak

(31) Olsthoorn, C. S. M.; Bostelaar, L. J.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1980**, *112*, 95−110.
(32) Olsthoorn, C. S. M.; Bostelaar, L. J.; Derooij, J. F. M.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1981**, *115*, 309−321.
(33) Jung, K. S.; Kim, M. S.; Lee, G. J.; Cho, T. S.; Kim, S. K.; Yi, S. Y. *Bull. Korean Chem. Soc.* **1997**, *18*, 510−514.
(34) Park, J. W.; Chung, H. *Bull. Korean Chem. Soc.* **1986**, *7*, 113−116.
(35) Bercu, C.; Lupan, L.; Bandula, R.; Vasilescu, M.; Sandu, T.; Mitran, L. *Rev. Roum. Chim.* **1997**, *42*, 693−699.

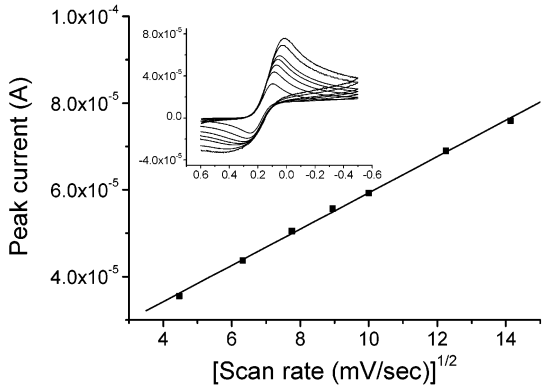

Figure 6. Linearity between cathodic peak current and [scan rate (mV/sec)]$^{1/2}$, (R = 0.99908). The inset represents corresponding voltammograms obtained at various scan rates (20, 40, 60, 80, 100, 150, and 200 mV/sec) by using the charge-compensated hybridized electrode.

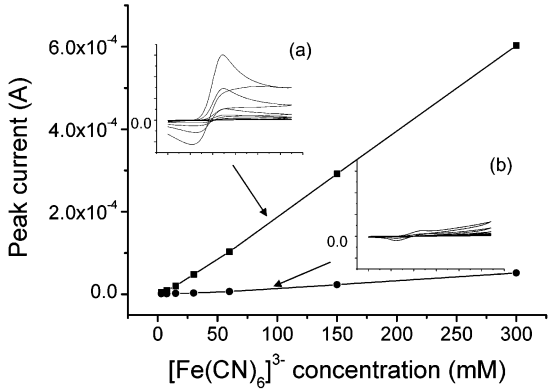

Figure 7. Plot of cathodic peak currents of cyclic voltammograms at various concentrations of $[Fe(CN)_6]^{3-}$, 3, 7.5, 15, 30, 60, 150, and 300 mM, (■) hybridized and (●) unhybridized electrodes modified with HS-5′-DNA capture probe. Insets (a) and (b) represent corresponding voltammograms obtained at the concentrations from the hybridized and unhybridized electrodes, respectively.

potentials of voltammogram 1 in Figure 4b is about 2 times wider than that of the voltammogram of a bare gold electrode (trace 1 in Figure 2). This difference is ascribed to the barrier for electron transfer provided by the surface layer of DNA. When increasing the voltage scan rate of cyclic voltammetry from 20 to 200 mV/s, we have found that the cathodic peak current from the hybridized electrode linearly increases with (scan rate)$^{1/2}$ (Figure 6), implying that the heterogeneous redox process is diffusion-limited, and $[Fe(CN)_6]^{3-}$ is free from adsorption.[36]

The voltammetric detection in this work is based on the $[Fe(CN)_6]^{3-}$ redox reaction induced by the electrical potential applied on a gold electrode. In contrast to other electrochemical DNA hybridization detection techniques using electrochemically active intercalators,[18−21,24] our technique features use of an electrochemically inactive intercalator as a charge-compensating agent for DNA strands. Hybridized electrodes are well-discriminated from unhybridized ones by duplex-specific charge compensation, and this duplex-specific charge compensation effectively minimizes background signal from the unhybridized DNA electrode. At 10 pM concentration of the target probes, the area ratio between cyclic voltammograms of hybridized and unhybridized electrodes (traces 1 and 2 in Figure 4b, respectively) is ∼200. This remarkably large discrimination has not been attained in other electrochemical detection techniques for DNA hybridization.[17−24] It should be also noted that in our case, the electrochemically active species is present in the voltammetric solution, not bound on DNA strands, and therefore, the detection signal can be amplified by simply increasing the concentration of $[Fe(CN)_6]^{3-}$. Figure 7 demonstrates that the peak cathodic current linearly increases with an increase in $[Fe(CN)_6]^{3-}$ concentration at fixed concentrations of the intercalator and target probes. Since the peak current of the unhybridized electrode (solid circles) becomes appreciable above 0.1 M, we have confined the hybridization detection to ∼50 mM of $[Fe(CN)_6]^{3-}$.

Figure 8 demonstrates the highly sensitive detection capability of our DNA hybridization technique. Note that at the target probe concentration of 10 fM, the signal of the hybridized electrode is clearly discriminated from that of the unhybridized one. This

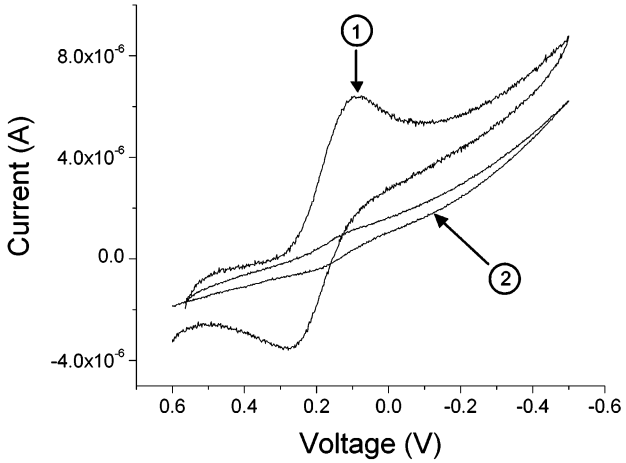

Figure 8. Cyclic voltammograms of 30 mM $[Fe(CN)_6]^{3-}$ obtained from (1) hybridized and (2) unhybridized electrodes. The concentration of the complementary target probe used in hybridization was 10 fM.

femtomolar-level detectability is comparable to the lowest detection limit ever achieved in electrochemical DNA hybridization detection using intercalators.[24] Since the volume of the target probe solution, loaded on an electrode for hybridization, is ∼10 μL, the probe amount in this volume at 10 fM is only ∼100 zmol. This highly sensitive detection would not require PCR amplification in sample processing.

CONCLUSIONS

The highlight of our electrochemical technique for DNA hybridization detection is the large discrimination between hybridized and unhybridized electrodes that has been achieved by minimizing the background signal and amplifying the sensing signal for hybridization. Our technique, therefore, promises to greatly improve the practicability of electrochemical detection approaches because the large signal difference provides a high reliability of hybridization detection in DNA chips. And our simple way to prevent the nonspecific binding of intercalators to ssDNA will open wide applications of intercalating agents in areas in which DNA strands are employed as sensing elements.

However, there remain many things to be done for improving the performance of our method. Above all, capture-probe surface

(36) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; John Wiley & Sons: New York, 2000; p 231.

coverage and hybridization efficiency should be optimized to maximize discrimination by charge compensation. Quantitation of target probe amounts from sensing signals should be accomplished for practical applications. Intercalators with better charge-compensation capability need to be searched. We should also elucidate the mechanism of preventing nonspecific binding of intercalators by SDS, from which better electrochemical sensing techniques for DNA hybridization can be derived. Different kinds of probe DNAs, particularly in length, should be tested with the method so that the most general protocol for sensing can be established.

ACKNOWLEDGMENT

This work has been supported by grants from POSCO through the Biotechnology Center (3PD0102101) of Pohang University of Science and Technology, South Korea, and the National Research Laboratory program (2000-N-NL-01-C-132) of the Ministry of Science and Technology, South Korea. Professor Su-Moon Park and Mr. Jung-Suk Yoo in the Department of Chemistry, Pohang University of Science and Technology, South Korea, have kindly supported the authors by supplying a BASIC program for frequency response monitoring in the QCM measurements. J. H. Hahn is very glad to express thanks and respect to Professor Dong Han Kim in the Department of Chemistry, Pohang University of Science and Technology, South Korea, through dedicating this paper to Professor Kim on the occasion of his retirement.

Received for review November 29, 2002. Accepted December 4, 2003.

AC026368R