Electrochemically Induced Release of DNA from Gold Ultramicroelectrodes

Joseph Wang,* Gustavo Rivas, Mian Jiang, and Xueji Zhang

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003

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Cathodic desorption is used for inducing the release of single and double-stranded DNA layers from gold microelectrodes (12.5 μm radius). The desorption behavior has been characterized by electrochemical quartz microbalance (EQCM), X-ray photoelectron spectroscopy (XPS), and cyclic voltammetry (CV) "blocking" experiments. These studies indicate an effective removal of the assembled nucleic acids from the surface to solutions of physiological pH and shed useful insights into the confinement of relatively long (350bp) segments of dsDNA on gold surfaces. The effect of various parameters influencing the release of the attached DNA, including the desorption potential and time, has been examined, with 2−5 min at −1.30 V (vs Ag/AgCl) sufficient for a complete removal. The time scale for the desorption of nucleic acids is shorter than that of alkanethiol monolayers. Such an on-demand electrochemical release of DNA holds great promise for delivering small amounts of the genetic material to specific locations at appropriate times.

Introduction

Gene therapy offers tremendous opportunities for the treatment of both acquired and inherited diseases and will almost certainly revolutionize the practice of medicine in the first part of the 21st century.[1,2] Despite these enormous therapeutic prospects, progress in developing effective clinical protocols has been slow.[1,2] The major obstacle for such progress has been the lack of efficient gene delivery vehicles. Although past efforts have focused on the use of viral vectors, there are urgent needs for nonviral delivery systems. Such nonviral routes hold an attractive, cost-effective, safe alternative for efficient gene delivery.[3] Advanced protocols commonly used for drug delivery, including the use of intelligent polymer systems (with thermal-, magnetic- or ultrasound triggered release), electroporation, gene guns, or loaded liposomes, have thus been suggested for delivering DNA.[3]

The main goal of this paper is to demonstrate that DNA-modified ultramicroelectrodes can respond to various electrochemical parameters, inducing the release of the nucleic acid into the solution. Modified electrodes, based on the doping/undoping of conducting polymers, have been proposed earlier for the controlled release of neurotransmitters.[4,5] The present protocol relies on the reductive desorption of thiolated DNA layers from gold microelectrodes (Figure 1). Earlier studies from Porter's laboratory demonstrated that self-assembled monolayers (SAMs) of short-chain alkanethiols can be electrochemically desorbed from gold surfaces through the reductive cleavage of the sulfur−gold bond.[6,7] Assemblies of DNA layers on gold electrodes have been used previously for sequence-specific hybridization detection.[8] In the following sections, we will demonstrate that electrochemistry can be used to trigger on demand the release of assembled 350bp dsDNA and 25-mer ssDNA layers from gold ultramicroelectrodes to pH 7.4 solutions and will discuss the implications of these findings on the nonviral controlled delivery of nucleic acids to specific sites.

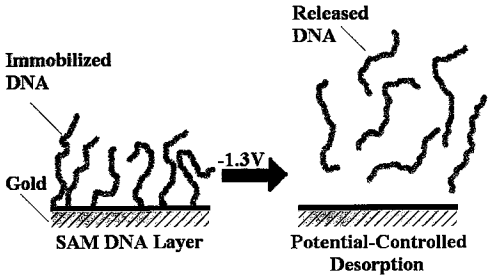

Figure 1. Schematic drawing showing the electrochemical (reductive) removal of DNA layers from gold electrodes.

Experimental Section

Chemicals. The sequence of the 25-mer thiolated single stranded DNA (HS-ssDNA) was the following: 5′-CAG GAT ATG TGG CGG ATG AGC GGC A-3′ (with a mercaptohexyl group at the 5′-phosphate end); it was purchased from Integrated DNA Technologies, Inc. (Coralville, IA). Agarose, calf thymus dsDNA and *N*-morpholinoethane-sulfonic buffer (MES) (Sigma); 2-hydroxyethyl disulfide, 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate, and 1-octadecanethiol (Aldrich); ethanol (Quantum Chemical); and potassium ferricyanide (Fisher) were used as received. All water and pipet tips were sterilized by autoclaving for 30 min. Stock solutions of the 25-mer thiolated ssDNA (around 1000 μg/mL) were prepared with sterilized water and kept in the freezer until use.

Apparatus. Electrochemical quartz microbalance (EQCM) experiments employed a Maxtek Plating Monitor (Model PM-740, Maxtek Inc., Torrance, CA) interfaced to an IBM personal computer and to the CH Instruments 620 electrochemical analyzer (CH Instruments, Cordova, TN). The monitor drives

* Corresponding author. Fax: 505-646-6033; e-mail: joewang@nmsu.edu

(1) Verma, I. M.; Somia, N. *Nature* **1997**, *389*, 239.
(2) Anderson, W. F. *Nature* **1998**, *392* (Supp), 25.
(3) Langer, R. *Nature* **1998**, *392* (Supp), 5.
(4) Lau, A. N.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, *105*, 5271.
(5) Zinger, B.; Miller, L. L. *J. Am. Chem. Soc.* **1984**, *106*, 6861.
(6) Widrig, C. A.; Chung, C.; Porter, M. D. *J. Electroanal. Chem.* **1991**, *310*, 335.
(7) Zhong, C. J.; Porter, M. D. *J. Electroanal. Chem.* **1997**, *425*, 147.
(8) Hashimoto, K.; Ito, K.; Ishimori, Y. *Anal. Chem.* **1994**, *66*, 3830. (b) Okahata, Y.; Matsunobo, Y.; Ijiro, K.; Mukae, M.; Makino, K. *J. Am. Chem. Soc.* **1992**, *114*, 8299. (c) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *70*, 4670. (d) Wang, J.; Nielsen, P.; Jiang, M.; Cai, X.; Fernandes, J.; Grant, D.; Ozsoz, M.; Begleiter, A.; Mowat, M. *Anal. Chem.* **1997**, *69*, 5200.

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the quartz crystal at its resonance frequency while serving to display and record the value of this frequency. The QCM cell, supplied by Universal Sensors Inc. (Metairie, LA), was set up in the static operation mode. It was connected to the frequency monitor as well as to the electrochemical analyzer, providing the in situ frequency monitoring under potential control. AT-cut quartz crystals with a fundamental resonance frequency (F_0) of 5 MHz were provided by the International Crystal Manufacturing Co. (Oklahoma City, OK). The crystal wafers were loaded with gold-coated electrodes (area: 41 $mm^2 \times 2$; average gold thickness, 100 nm) formed by thermal evaporation of gold onto a predeposited chromium underlayer on a quartz matrix. An HC-48 holder (ICM, Oklahoma City, OK) provided the contacts of the oscillating crystals to both an oscillating circuit and an electrochemical closed circuit. A platinum wire counter electrode and a Ag/AgCl (3 M NaCl) reference electrode (Model RE-1, BAS) were used for the three-electrode electrochemical setup. Experiments were conducted by fully immersing the bare/modified crystal into a phosphate buffer solution (0.05M, pH 7.40).

High-resolution X-ray photoelectron spectroscopy (XPS) experiments were performed with an AKIS−HSi XPS Spectrometer (Kratos Analytical Co., U.K.). The unit was equipped with a hemispherical analyzer (with a pass energy of 40 eV and a resolution of 0.1 eV), toroidal monochromator, and multichannel detector. A monochromated Al anode (Al Kα radiation line: 1486.6 eV) was used as the X-ray source. Photoelectrons were collected at a 90° take off angle, with acquisition times shorter than 10 min. The sampling area was 500 μm × 500 μm. The base pressure of the XPS chamber (during the measurements) was below 1×10^{-10} torr.

Voltammetric and chronoamperometric experiments were performed using the Autolab modular electrochemical system (Eco Chemie, Utrecht, The Netherlands) equipped with PGSTAT 10 and driven by GPES software (Eco Chemie). A gold disk microelectrode (of 12.5 or 50 μm radius) was used as the working electrode. The reference and counter electrodes were the same as described above.

The gold ultramicroelectrodes were prepared in the following manner. A 1-cm long individual gold fiber [radius, 12.5 μm (Goodfellow, UK) or 50μm (Aldrich)] was placed at the end of a glass pipet tip (1.5 mm O. D., 1 mm I. D., Fisher). A coil of resistively heated Nichrome wire on a vertical pipet puller (Model 700C, David Kopf Instruments) was used to melt glass around the wire. High-purity silver-conducting paint (SPI suppliers, West Chester, PA) was employed to make electrical contact to a copper wire that was used as the electrical connection. The electrodes were polished first with a 600-grit abrasive paper, followed by polishing with 1 μm and 0.05 μm alumina slurries (on napless nylon cloths) to a mirror finish. The surface of the electrodes was cleaned by sonication in both ethanol and water for 10 min and then with a Piranha (30% hydrogen peroxide/70% concentrated sulfuric acid) solution for an additional 2 min (Caution: Piranha solution reacts violently with organics and must be handled with extreme care.) Subsequently, the electrodes were carefully rinsed with water and sonicated for an additional 10 min in water. The electrodes were repolished with a 0.05 μm alumina slurry and washed (as was described above) prior to each experiment. Some experiments were conducted without exposing the surface to the Piranha solution. Optical characterization of the resulting electrodes (at 4000X magnification using the Olympus BH2−UMA microscope) revealed defined disk shapes and smoothed surfaces. Cyclic voltammetric evaluation of the newly prepared microelectrodes (using ferricyanide) revealed a very good agreement with the theoretical current value.

Preparation of Thiolated dsDNA. Synthesis of the thiolated dsDNA was based on the protocol of Maeda et al.[9] Five miligrams of calf thymus dsDNA were dissolved in 5.00 mL of a 0.015 M NaCl/0.0015 M sodium citrate solution (pH 6.60) and sonicated (with a Branson sonicator) in an ice bath several times (using different powers and durations) until the desired length of the dsDNA was obtained. After every sonication step, the sample was examined by gel electrophoresis. The sonicated DNA was precipitated by adding 10.00 mL of ice-cold 95% ethanol, centrifuged at 14000 rpm for 10 min and redissolved in 0.2 mL *N*-morpholinoethane-sulfonic (MES) buffer (0.04 M, pH 6.0). After electrophoresis in 0.5% agarose gel in TBE buffer (1×) (10 × TBE buffer: 0.89M Tris-HCl + 0.89 M boric acid and 0.02 M EDTA), the sonicated DNA showed a broad band centered around 350bp.

Sonicated calf thymus dsDNA (around 6000 mg/L) was allowed to react with 2-hydroxyethyl disulfide (HEDS, 2.3 mg) in the presence of 1-cyclohexyl-3-(2-morpholinethyl)-carbodiimidemethop-toluenesulfonate (0.2 g) in 0.2 mL of 0.04 M MES buffer (pH 6.0) for 24 h at 25 °C. After reacting, a phosphodiester linkage between the terminal monophosphate ends of the DNA and the hydroxyl group of HEDS was obtained.[9]

The reaction mixture was separated by using a NAP 10 column (Sephadex G-25 DNA grade) from Pharmacia Biotech. (Uppsala, Sweden). The sample was eluted with MES buffer (0.04M, pH 6.0) according to the directions of the supplier. All the collected aliquots were checked by UV spectroscopy. Those displaying the characteristic peak at 260 nm were checked by gel electrophoresis in 0.5% agarose in TBE buffer. The fraction that showed a broad band around 350 bp was used for further work. The final concentration of the thiolated dsDNA in this fraction was 850 mg/L.

Immobilization of DNA on the Gold Disk Microelectrodes. The thiolated DNA or alkanethiol-modified microelectrodes were prepared by self-assembly of the corresponding thiolated nucleic acids (single-stranded or double-stranded) and alkanethiol (1-octadecanethiol). The surface was modified by immersing the electrodes in 50 mM NaCl/5 mM phosphate buffer (pH 7.0) quiescent solutions containing 500 μg/mL of the 25-mer thiolated ssDNA or 425 mg/L of the thiolated dsDNA for a 48h period (at 4 °C). A 1 mM 1-octadecanethiol/ethanol solution was used for preparing the alkanethiol modified microelectrodes. The modified surfaces were then washed with a phosphate buffer solution (0.05M, pH 7.4) and water and were subsequently allowed to dry.

Immobilization of DNA on the Crystals. Prior to use, the gold QCM wafers were ultrasonically cleaned by a 20-min exposure to a fresh Piranha solution followed by a 10-min sonication in water and drying with nitrogen. The surface was modified by casting 20 μL of a solution containing 500 mg/L of the 25-mer thiolated ssDNA or 425 mg/L of the thiolated dsDNA in 50 mM NaCl/5 mM phosphate buffer (pH 7.0) (for thiolated DNA-modified crystals) or the same volume of a 1 mM 1-octadecanethiol/ethanol solution (for alkanethiol-modified crystals) and incubating for 48h at 4 °C. The surface was then washed with a 0.05M phosphate buffer solution and water and was allowed to dry.

Procedure. (a) EQCM Measurements. The resulting crystals were introduced into the piezocell, which was subsequently filled with a 0.05 M phosphate buffer solution. Once the resonance frequency reached the steady-state (usually within 10 min), an initial potential of 0.0V was applied, and the frequency signal was allowed to decay to a stable background. A final potential of −1.30V was then applied to desorb the DNA (or alkanethiol) from the surface while monitoring the removal in real-time. The crystals were reused after a new exposure to the Piranha solution. All experiments were performed at room temperature.

(b) XPS Characterization. XPS spectra were obtained using DNA-coated gold-deposited silicon wafers (discussed in the EQCM section) before and after the electrochemically induced desorption. (See earlier sections for the conditions of DNA immobilization on such crystals and of electrochemical removal.) Before mounting and inserting the samples into the vacuum chamber, they were thoroughly rinsed with water and then dried. All values of binding energies reported are referenced to the Au(4f 7/2) emission line at 84.0 eV.

(c) Electrochemical Experiments. Cyclic voltammetry (CV) "blocking experiments" were performed by scanning the potential at a 100 mV/s or 10 mV/s rate over the −0.10 to +0.50 V range, using a quiescent solution of the ferricyanide marker (1.0×10^{-3} M) in 0.10 M potassium chloride electrolyte. Voltammograms were recorded (in a homemade Faradaic cage) before and after immobilization of the thiolated nucleic acids.

The desorption of the surface-confined thiolated layer was carried out subsequently by applying a potential of −1.30 V for

(9) Maeda, M.; Mitsuhashi, Y.; Nakano, K.; Tagaki, M. *Anal. Sci.* **1992**, *8*, 83.

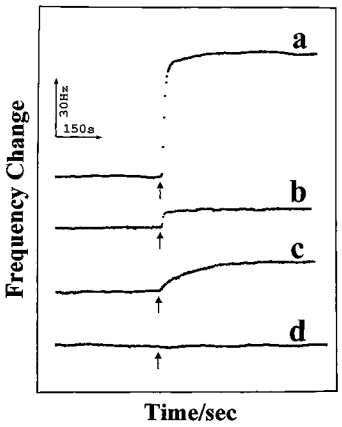

Figure 2. Time course of frequency change of coated (a−c) and uncoated (d) crystals upon switching the applied potential from 0.0 V to −1.30 V (arrow). Surface layers: (a) dsDNA; (b) ssDNA; (c) 1-octadecanethiol. Medium, phosphate buffer (0.05 M, pH 7.4). The crystals were coated by immersion in 0.05 M NaCl/0.005 M phosphate-buffer (pH 7.0) solutions containing 425 mg/L dsDNA (a) or 500 mg/L ssDNA (b) or in a 1 mM 1-octadecanethiol/ethanol solution (c) for 48 h at 4 °C. See Experimental Section for details.

periods ranging from 2 to 10 min, using a phosphate buffer solution (0.05 M, pH 7.40). A new CV, recorded in the ferricyanide solution, was used to evaluate the desorption efficiency.

Results and Discussion

There are several challenges associated with the controlled electrochemical release of self-assembled nucleic acids. Although the reductive desorption of alkanethiol monolayers from gold electrodes has been commonly carried out in alkaline (pH > 11)[7] or nonaqueous[10] media, we examined the use of DNA-modified gold microelectrodes and of a physiological buffer (pH 7.4) for meeting future gene-therapy requirements. Unlike earlier nucleic-acid SAMs, which were prepared for diagnostics/biosensing applications[8] and thus relied on short (20−30 mer) thiolated DNA probes, we employed relatively long (350 bp) dsDNA segments to reflect our long-term goal of gene therapy. Similarly, we used a 12.5μm-radius gold ultramicroelectrode (as compared to conventional-sized electrodes used in the hybridization studies). To investigate the desorption process of the assembled thiolated DNA layers, we have employed several characterization tools, including EQCM, XPS and CV.

EQCM Probing. EQCM is particularly suitable for studying the electrochemically triggered release of nucleic acids, as it offers in situ monitoring of the mass changes associated with the reductive desorption of the surface layer. An EQCM study described the desorption of SAMs of alkanethiols in nonaqueous media.[10] Figure 2 (a−c) displays typical time courses of the frequency change of different DNA- and alkanethiol-coated crystals (at pH 7.4) upon stepping the potential from 0.0 V to −1.30 V (vs Ag/AgCl). Also shown (d) is the corresponding frequency-time response of the uncoated (control) crystal. All coated crystals display an increase of their resonance frequency upon the potential step, reflecting the decreased mass associated with the reductive desorption. Both the ssDNA- and 350bp dsDNA-modified crystals respond very rapidly to the potential step, attaining steady-states within 115 and 170 s, respectively. A slower change (260 s for steady-state) is observed at the 1-octadecanethiol-coated crystal.

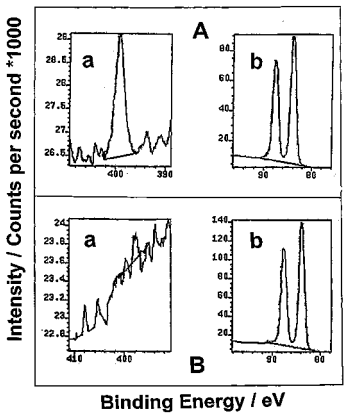

Figure 3. XPS spectra in the N 1s region (a) and Au 4f region (b) of a ssDNA-coated gold surface before (A) and after (B) applying the desorption potential (−1.30 V) for 10 min. See the text for detailed conditions.

As expected, no frequency change is observed in the control experiment using the unmodified crystal. Steady-state frequency changes of 14.7, 7.1, and 64.4 Hz are observed for the desorbed alkanethiol, ssDNA, and dsDNA, respectively. Such frequency changes correspond to the removal of 106 ng alkanethiol, 51 ng 25-mer ssDNA, and 466 ng of the 350-bp dsDNA (and to surface densities of 9.1×10^{-10}, 1.3×10^{-11}, and 4.8×10^{-12} mol/cm^2, respectively). The latter is in agreement with the value (4.4×10^{-12} mol/cm^2) reported for the dsDNA/QCM experiments of Bardea et al.[11] The former value is in full agreement with the theoretical value predicted for a saturated organothiol surface layer,[6,10] indicating a desorption of the entire monolayer. The steady-state frequency response, and the fact that the frequency did not change further when the experiments of Figure 2 (a and b) were followed by a second potential step from 0.0 V to −1.30 V (not shown), indicate a complete removal of the surface-confined nucleic acids. The faster desorption of the DNA layers (vs the alkanethiol SAM) is attributed to their different surface conformation and charge. One would expect a strong electrostatic repulsion between the negatively charged nucleic acids and the surface held at −1.30 V. Self-assembled DNA layers are known[12] for their reduced packing and different organization associated with their multitude adsorption sites (through both the sulfur atom of the thiol group and the nitrogen-containing bases).

X-ray Photoelectron Spectroscopy. X-ray photoelectron spectroscopy (XPS) was also employed for probing the electrochemical removal of the surface-confined nucleic acids. The technique was used recently for characterizing the immobilization of DNA hybridization probes on gold surfaces.[12] Figures 3 and 4 display XPS spectra of ssDNA- and dsDNA-coated gold surfaces, respectively, before (A) and after (B) applying a potential of −1.30 V for 10 min. Both coated electrodes display a distinct N 1s peak (at ca. 400 eV; A(a)), characteristic to the nitrogen-containing bases of adsorbed DNA.[12] Essentially no nitrogen peak is observed after holding the electrode at −1.30 V (B(a)), reflecting the removal of the DNA from the surface. A similar XPS profile was obtained for the unmodified (bare) surface, used as a control (not shown). Changes in the intensity of the Au 4f XPS signals have also been used for assessing the reduction desorption of the nucleic acids

(10) Schneider, T. W.; Buttry, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 12391.

(11) Bardea, A.; Dagen, A.; Ben Dov, I.; Amit, B.; Willner, I. *Chem. Commun.* **1998**, 839.

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

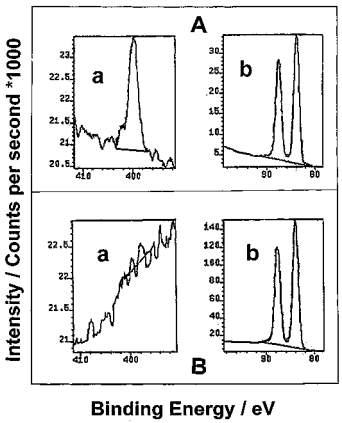

Figure 4. XPS spectra in the N 1s region (a) and Au 4f region (b) of a dsDNA-coated gold surface before (A) and after (B) applying the desorption potential (−1.30 V for 10 min). See the text for detailed conditions.

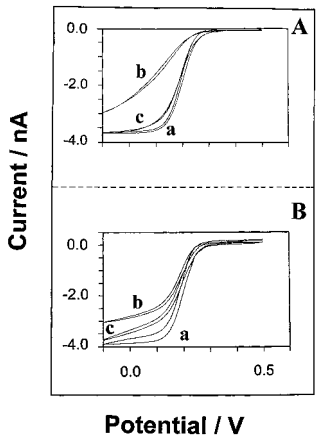

Figure 5. Cyclic voltammograms for 1×10^{-3} M ferricyanide at the bare (a) and nucleic-acid coated (b) gold microelectrodes (12.5 μm radius) and following the reductive desorption of the nucleic acid (c). Confined nucleic acid: (A) ssDNA; (B) dsDNA. Desorption for 2 min at −1.30 V. Scan rate, 10 mV/s; electrolyte, 0.1 M KCl. The electrodes were coated by immersing in a 0.05 M NaCl/0.005 M phosphate-buffer (pH 7.0) solution containing 500 mg/L ssDNA (A) or 425 mg/L dsDNA (B) for 48 h at 4 °C.

(Figures 3(b) and 4(b)). The areas of these Au 4f peaks at the ssDNA and dsDNA-coated electrodes are approximately 55% and 25%, respectively, of those observed after 10 min at −1.30 V (compare b(A vs B). Note the similarity of the XPS Au peaks following the desorption of ssDNA and dsDNA (B(b); Figure 3 vs 4). Such peaks are also similar to those obtained with the uncoated control surface (not shown). An incomplete removal of the sulfur was indicated from the 80% attenuation of the S 2p (at ca. 162 eV) after the desorption (not shown).

Cyclic Voltammetry "Blocking" Experiments. Another set of experiments confirming the controlled release of the genetic material relied on cyclic voltammetry (CV) probing of the barrier properties. Cyclic voltammetry of the ferricyanide redox marker has been widely used for evaluating the "barrier properties" of monolayer-coated electrodes.[9,13] Figure 5 shows typical CVs at 12.5-μm radius microelectrodes before (a) and after (b) the assembly of a thiolated 25-mer oligonucleotide (A) or 350bp dsDNA (B) and following a 2 min reductive desorption of the immobilized nucleic acids (c). The bare electrodes display sigmoidal-shaped voltammograms, with "plateau" currents, characteristic of ultramicroelectrode behavior. Significant suppressions of the marker response (34% (A) and 26% (B) current diminutions at 0.0 V), coupled to distorted voltammograms (with steadily increasing currents), are observed following the adsorption of the ss-oligonucleotide and dsDNA, respectively. Incomplete suppressions of the marker response and a drawn-out response are common at DNA-coated gold electrodes[9] and reflect the partial surface coverage and different organization (discussed above). After holding the electrodes at −1.3 V for 2 min, the ferricyanide signals were restored back to values approaching those of the bare microelectrode (c vs a), reflecting the removal of the nucleic-acid layer into the solution. Note, however, that the resulting voltammograms are drawn out, with the slower electron-transfer kinetics indicative of an incomplete removal (possibly due to the remaining sulfur layer; see XPS data). The greater restoration of the current and shape in the case of ssDNA reflect (in agreement to the EQCM data) its faster desorption kinetics. A control CV experiment, using the same potential and time sequences, but with the blank − rather than the nucleic acid − solution, resulted in no change in the ferricyanide signal (not shown).

We examined the effect of different desorption potentials upon the ferricyanide CV response and the QCM frequency change. Both experiments yielded an excellent agreement, with no current or frequency change for potentials more positive than −1.20 V (not shown). Increased currents and frequencies were observed between −1.20 V and −1.30 V, with the use of more negative potentials suffering from increasing background and noise levels associated with the hydrogen evolution reaction. A potential of −1.30 V was thus selected for all subsequent work. The large hydrogen evolution current, associated with the use of physiological pH, also influenced the direct cyclic voltammetric monitoring of the desorption peak (that appeared only as a shoulder, around −1.1 V, on the rising background; not shown). It also prevented reliable chronocoulometric assessment of the charge involved in the desorption process.

CV ferricyanide probing of the nucleic-acid adsorption/desorption processes at a 50μm-radius gold microelectrode (at 100 mV/s) yielded peak-shaped voltammograms that were partially suppressed and restored following the adsorption and desorption, respectively (not shown; conditions, as in Figure 5A). The shape of the curves following the desorption was nearly identical to those of the bare electrode, with minimal (5−10 mV) shifts of peak potentials. Changes in the ferricyanide peak current with the desorption time were used for monitoring the removal of the immobilized nucleic acid (Figure 6). The reduction peak current increased rapidly up to one minute desorption, then increased more slowly and approached steady-state above 2 min. Such a profile is in excellent agreement with the EQCM desorption behavior (of Figure 2b) where a steady-state was attained within 115 s. Although the profile of Figure 6 indicates the ability to deliver small amounts of the genetic material at specific times, a slower (or repeated) release − over extended periods − may be desired for many practical applications. This may be accomplished through the use of different desorption potentials, the passage of small cathodic currents, or the use of different interfacial environments. These possibilities are currently being elucidated in our laboratory.

(13) (a) Miller, C.; Cuendet, P.; Gratzel, M. *J. Phys. Chem.* **1991**, *95*, 877. (b) Finkela, H. O.; Snider, D. A.; Fedyk, J.; Sabatani, E.; Fafni, Y.; Rubinstein, I. *Langmuir* **1993**, *9*, 3660. (c) Wang, J.; Paz, J. L. L.; Jiang, M. *Langmuir* **1999**, *15*, 1884.

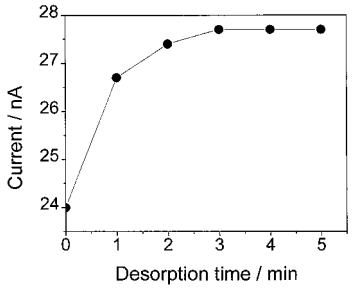

Figure 6. Effect of desorption time on the ferricyanide peak current at the ssDNA-coated gold microelectrode (50 μm radius). Each run involved transfer of the electrode from the phosphate-buffer "desorption" solution to the ferricyanide/potassium chloride test medium. Other conditions as in Figure 5.

Conclusions

The above experiments have demonstrated the ability to trigger electrochemically the release of DNA layers from gold surfaces under physiological pH. This constitutes the first time that a microelectrode is designed to release DNA on demand. Such an electrochemical approach represents a potentially attractive nonviral alternative for carrying therapeutic genes into patients. Other electrochemical release schemes, in addition to different electrode designs or dimensions, are currently being investigated in our laboratory in connection with the controlled delivery of nucleic acids. Future attention will be given to the challenge of crossing the cell membrane (in connection with simultaneous high-voltage electroporation) and to the controlled release of gene-targeting peptide nucleic acids (PNA).

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