DOI: 10.1002/cbic.200800767

Optimization of an Electrochemical DNA Assay by Using a 48-Electrode Array and Redox Amplification Studies by Means of Scanning Electrochemical Microscopy

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Due to insufficient detection limits and selectivity, electrochemical DNA sensors are not yet used as everyday tools in diagnostics. Here, we present an electrochemical DNA assay that is based on specific *Salmonella* spp. capture probes. Our optimization strategies and the specific features of related electrochemical DNA sensor arrays, which are comprised of a chip with 48 gold electrodes, are also described. A ssDNA monolayer is formed by chemisorption of the thiol-modified capture strand on the different gold electrodes of the array after spotting with a needle spotter. The assay parameters were optimized for the use of minimum amounts of sample and reagents and short assay times. Scanning electrochemical microscopy (SECM) has been used to visualize the local activity of an enzyme label used for amplified hybridization detection at high lateral resolution. The potential of SECM to further amplify the sensor signal by means of redox cycling is demonstrated by using single-stranded DNA capture probe modified gold microelectrodes as SECM tips. The detection limit of the proposed DNA sensor is shown to be in the femtomolar range without redox cycling amplification.

Introduction

DNA analysis has grown to become a widely established, routine method. The use of DNA microarrays[1,2] allows for a reduction in costs for performing DNA analysis due to their inherently high degree of miniaturization and automation. However, DNA microarray technology is still based on expensive equipment like mass spectrometers[3] or fluorescence microscopes,[4] which must operated by trained personnel. Thus, DNA analysis still has to go a long way until it can be routinely employed in point-of-care diagnostics.

On the other hand, however, the idea of having a DNA-sensing device that is able to perform DNA analysis to easily detect disease markers, or to have a DNA sensor to determine food pathogens seems to be highly attractive. Hence, a lot of effort has been put into the development of easy-to-use and cost-effective DNA assays. In this context, the study of electrochemical DNA-sensing systems has gained increasing attention in recent years.[5,6] In general, electrochemical readout can be performed with less-expensive equipment due to the fact that electrochemical analyzers can be easily miniaturized. Thus, electrochemical DNA assays are interesting options for the design of handheld DNA-sensing devices. In addition, electrochemical DNA sensors can be parallelized in a straightforward way. A large number of individually addressable microelectrodes can be implemented into a single microchip; this results in a large number of different test sites.[7–9] Mass production of such microchips will further increase the cost effectiveness of electrochemical DNA sensors, and microfluidics could be incorporated into the chip format to perform automated assays.

Electrochemical readout of DNA hybridization mainly follows one of three basic concepts: 1) direct detection of DNA electrochemistry or the modulation of direct DNA electrochemistry upon hybridization, 2) change of electrical properties of the capture DNA layer upon hybridization, or 3) electrochemical detection of a label that is attached to a DNA duplex.[5,7–9]

Direct DNA electrochemistry is mainly due to oxidation of the guanine or adenine moieties.[10–12] Changes in the electrical properties or in the conformation of immobilized layers of a nonhybridized capture probe (ssDNA), and hybridization with a complementary DNA strand (dsDNA) can alter the accessibility of the electrode surface for charged redox species; this generates a modulated electrochemical signal.[13–15] DNA is highly negatively charged due to its phosphate backbone, and hybridized dsDNA bears more negative charges than ssDNA. It was demonstrated that the redox conversion of a negatively charged and freely diffusing redox compound is modulated due to charge repelling. Upon hybridization and the concomitant increase in negative charges at the formed dsDNA-modified interface, the charge transfer resistance is increased; this leads to a drop in the measured electrochemical signal.[14–16]

Moreover, long-range electron transfer through dsDNA has been proposed as a means to detect DNA hybridization.

Alternative electrochemical detection schemes for DNA hybridization make use of labels that are exclusively introduced at sites bearing dsDNA. For example, differences in the binding

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constants of ssDNA and dsDNA towards redox-active metal complexes have been exploited.[17,18] Also, nanoparticles[19,20] and enzyme labels[21] have been used as specific markers for dsDNA.

However, there are a number of challenges that have to be addressed when introducing concepts for dsDNA detection and in particular for electrochemical DNA sensors. Highly selective capture probe sequences for a particular complementary target DNA sequence have to be identified, and the supply of these capture probes at the same quality and purity has to be ensured to get reproducible results. Moreover, immobilization of capture probes has to be secure and reproducible without altering the ability of the capture probe to recognize and bind the complementary target strand. Furthermore, the detection limit should be as low as possible while maintaining selectivity to avoid amplification of the target DNA by polymerase chain reactions (PCR) during sample preparation.

Here, we introduce an assay principle and strategies for its optimization for the detection of DNA from *Salmonella* spp. It is based on the specific binding of an enzyme label, namely alkaline phosphatase (AP), exclusively to those surface regions that carry dsDNA. The proposed sensor system is based on a printed circuit-board-based microelectrode array with 48 individually addressable electrodes that are integrated into one microchip, immobilization of capture DNA by using thiol self-assembly, and electrochemical readout and amplification by using specific enzyme labeling. Because AP has been successfully used in amplified electrochemical biosensors,[22,23] we have used an avidin–alkaline phosphatase conjugate as a specific label for dsDNA.

Scanning electrochemical microscopy (SECM)[24–27] is an analytical technique that provides insight into the local electrochemical properties of surfaces. As in other scanning probe microscopies (SPM),[28,29] a tip is scanned over a surface in close proximity, and the signal that is detected at the tip is modulated by the specific local properties of the surface. In SECM, a Faraday current at a disk-shaped microelectrode is the signal that is modulated by the sample properties during scanning. This leads to the visualization of the local distribution of electrochemical activity of the sample surface. SECM has been successfully used as a tool to visualize locally immobilized AP activity[30] and concomitantly the formation of dsDNA at individual electrodes of the array. Furthermore, SECM has been used to determine the amplification of the sensor signal both by using the enzymatic conversation as well as by redox cycling.

Results and Discussion

Assay strategy

The assay strategy proposed here consists only of incubation steps and subsequent washing of the electrode surface. For proof-of-principle and optimization experiments, electrode arrays containing 48 disk-shaped gold electrodes with 100 μm diameter were used. Thiol-modified DNA capture probes that are specific for *Salmonella* spp. (for sequences see the Experimental Section) were spotted on individual gold electrodes and allowed to immobilize by thiol chemisorption. To maintain the flexibility of the immobilized ssDNA capture probes and to ensure efficient hybridization, a thiol anchor consisting of four thiol groups was attached to the 3′-end of the capture probe through a seven-base-long adenine spacer. The target strand was biotin labeled. This allows for a later complementary binding of an avidin–AP conjugate after successful formation of dsDNA. Biotinylated targets from real samples can be obtained by PCR amplification by using a standard "Biotin PCR Labeling Kit", which contains all of the reagents for direct enzyme labeling, including biotin-11-dUTP (biotin-ε-aminocaproyl-[5-{3-aminoallyl}-2′-deoxyuridine-5′-triphosphate]) for incorporation into DNA by using Taq polymerase. AP is able to cleave its nonelectroactive substrate *p*-aminophenyl phosphate (*p*-APP) under formation of the electroactive product *p*-aminophenol (*p*-AP).[31,32] *p*-AP can be oxidized to form the corresponding *p*-(benzo)quinone imine (*p*-QI) at a potential of +400 mV (vs. Ag | AgCl). At this potential, no oxidative damage of dsDNA nor oxidative desorption of the thiol anchors is expected. If a significant oxidation current is detected, binding of the avidin–enzyme conjugate through the target-strand-bound biotin label and hence hybridization under formation of dsDNA is unequivocally indicated.

The proposed assay principle is depicted in Figure 1 along with the typical response of a DNA-modified electrode after hybridization and conjugation of AP upon addition of *p*-APP. An increase in current is observed due to oxidation of the *p*-AP that is formed in the enzyme-catalyzed hydrolysis reaction.

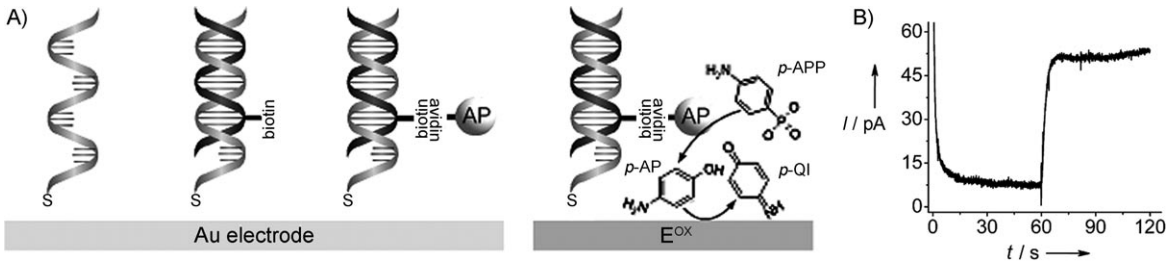

Figure 1. A) Assay strategy. The chemisorbed capture probe is hybridized with a biotin-modified target strand. Subsequently, an avidin–AP conjugate is bound to the dsDNA through the biotin label. Hybridization is detected by oxidation of *p*-AP, which is formed in the enzyme-catalyzed hydrolysis of *p*-APP. B) Typical current response of a DNA-modified electrode after hybridization and conjugation of AP upon addition of 200 μM *p*-APP after 60 s. Recorded in 500 mM $Na_2SO_4/NaCO_3$ (pH 9.8), potential +400 mV versus Ag | AgCl/3 M KCl.

No current is observed upon *p*-APP addition when the immobilized capture probes were not hybridized (data not shown).

Assay parameters

After the principle feasibility of the proposed assay strategy was demonstrated, the assay parameters were further varied to minimize the amount of reagents and the overall assay time. For maximum sensitivity and strongest signal, the enzyme should work at substrate saturation. Figure 2A shows

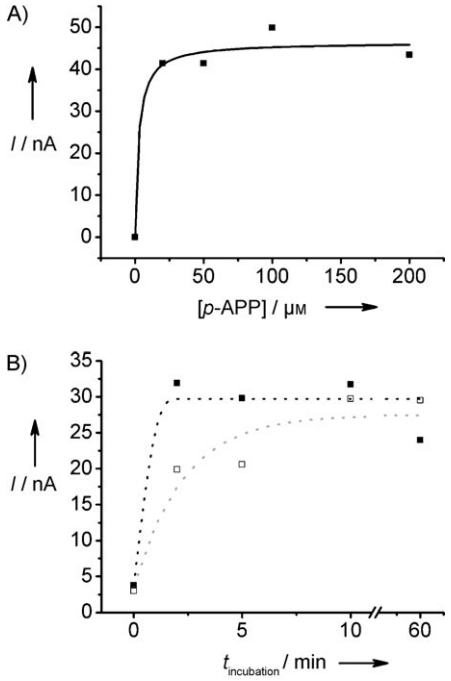

Figure 2. A) Response of DNA-modified electrodes after hybridization and complementary binding of the avidin–AP conjugate at increasing concentrations of *p*-APP (experimental parameters as in Figure 1). B) Response of DNA-modified electrodes after hybridization and complementary binding of avidin–AP conjugate in the presence of 200 μM of *p*-APP after different incubation times with dilutions of the avidin–AP conjugate (■: 1:500, □: 1:1000).

current values for the oxidation of enzymatically generated *p*-AP that were obtained for increasing concentrations of *p*-APP by using a dsDNA-modified electrode surface and binding of AP via biotin–avidin recognition. Enzyme saturation is achieved at concentrations higher than 50 μM of *p*-APP.

Because the binding constant between avidin and biotin is very strong, affinity binding of the avidin–AP conjugate to the biotinylated dsDNA should be very fast and efficient. Indeed, after a 2 min incubation of the dsDNA-modified surface with a diluted avidin–AP conjugate (1:500), the maximum signal was already achieved (Figure 2B). Further dilution of the avidin–AP conjugate (1:1000) yields maximum signal after a 10 min incubation time.

Based on these results, the parameters for the electrochemical readout of the assay have been set to a concentration of 200 μM *p*-APP after incubation with a diluted solution of avidin–AP conjugate (1:1000) for at least 10 min.

Scanning electrochemical microscopy

SECM in the sample generation–tip collection mode (SG-TC) has been employed in this study. In this mode, domains on the sample surface, at which an electrochemically active species is produced, which can be oxidized or reduced at the SECM tip, are visualized by an increase in tip current (Figure 3). Thus, SECM is capable of monitoring the functional molecular assembly of the assay during the immobilization process with a lateral resolution that is determined by the size of the used SECM tip. Furthermore, it is able to confirm that the immobilized DNA maintains its ability to bind its complementary target strand.

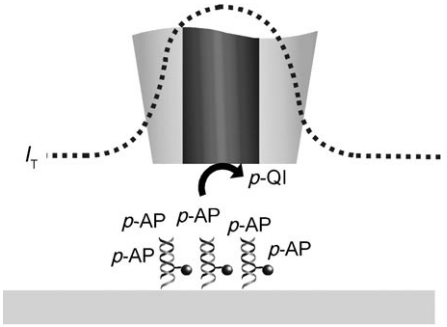

Figure 3. Schematic representation of the sample generation–tip collection mode of SECM for the local visualization of surface-bound AP activity. Over surface areas where AP was bound through a biotin–avidin interaction, the product of the enzymatic reaction, *p*-AP, is formed upon addition of sufficiently high concentrations of *p*-APP in a diffusion-limited process. *p*-APP diffuses within the gap between sample surface and SECM tip to the tip where it is oxidized under formation of *p*-QI. Thus, the tip current increases if the tip is scanned over an area of successful DNA hybridization.

At first, a model system was studied by SECM that consisted of a biotin-modified 25-mer oligonucleotide that simultaneously carried the 3′-thiol units. This ssDNA strand is chemisorbed at the 100 μm Au electrodes of the array as described above, and the avidin–AP conjugate is directly bound to this DNA strand through its biotin label. It is assumed that the current signal at the SECM tip would be maximal at the saturation concentration of *p*-APP because each accessible biotin would be modified with the avidin–AP conjugate.

Due to the high molecular weight and thus large steric demand of the avidin–AP conjugate, it can be assumed that already-bound avidin–AP conjugate might prevent further binding of avidin–AP conjugates at nearby biotin residues. Thus, maximum current signal might be obtained even with lower concentrations of the capture probe at the surface. Moreover, SECM images representing the locally immobilized AP bound to either biotin-labeled 25-mer single-stranded oligonucleotides (Figure 4A) or to biotin-modified target strand after hybridization (Figure 4B) show uniformly distributed enzymatic activity. SECM line scans through the center of each of the spots (not shown) reveal that the maximum current measured for the biotin-labeled 25-mer single-stranded oligonucleotides (40 pA) is higher than the signal for the full assay (20 pA). In

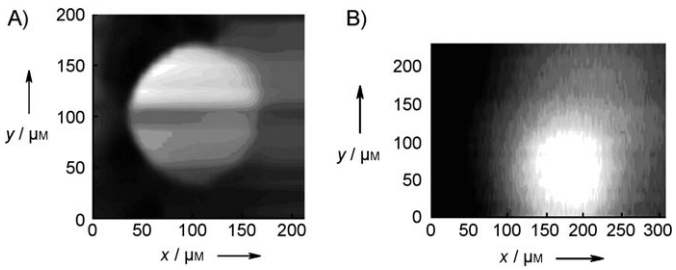

Figure 4. SECM images of A) a spot of thiol and biotin-labeled 25 mer single-strand oligonucleotides after complementary binding of avidin–AP conjugates via biotin, B) a spot of thiol-modified ssDNA capture probes after hybridization with the complementary biotin-labeled target strand and further binding of the avidin–AP conjugate. All measurements were done in presence of 10 mM *p*-APP in carbonate buffer (pH 9.4).

the case of the full assay, either not all of the accessible capture probes are hybridized or the increased rigidity of the formed dsDNA prevents the sterically demanding avidin–AP conjugate from successfully binding to the biotin labels. Both effects will lead to a decreased immobilized AP activity and consequently to a decreased current at the SECM tip.

Limit of detection

To evaluate the sensitivity and limit of detection of the proposed DNA assay, electrodes with the same amount of capture probes bound to their surfaces were hybridized with decreasing concentrations of biotin-modified complementary target DNA and further incubated with the avidin–AP conjugate. Figure 5 shows the current increase upon addition of *p*-APP.

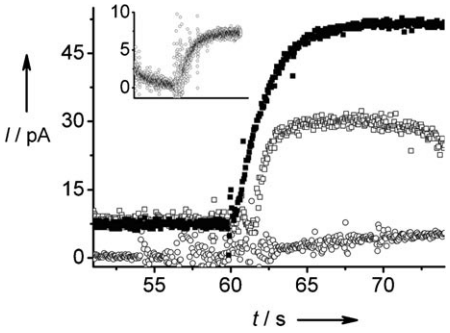

Figure 5. Current response of DNA-modified electrodes after hybridization with decreasing concentrations of biotin-labeled target DNA. Filled squares: 100 pM target strand, open squares: 10 pM target strand, open circles: 100 fM target strand. Inset: magnification of the current response after hybridization with a 100 fM concentration of target DNA.

Even for target strand concentrations as low as 100 fM, an increase in current of about 7 pA is detected; this is clearly above the noise level of the set-up of about 2 pA (Figure 5, insert).

Miniaturization

Further miniaturization by using the same DNA assay strategy was achieved by using 10 μm and 25 μm diameter Au disk electrodes that were sealed in glass as sensor surfaces. Immobilization of thiol-modified single-stranded capture probe DNA onto 25 μm Au electrodes was carried out by using a slightly changed immobilization protocol, by omitting postmodification of the DNA-modified sensor surface with the short-chain thiol that was used to avoid unspecific adsorption of target DNA and proteins. Successful binding of the capture DNA and subsequent hybridization and binding of avidin–AP conjugate was proven by cyclic voltammetry (Figure 6).

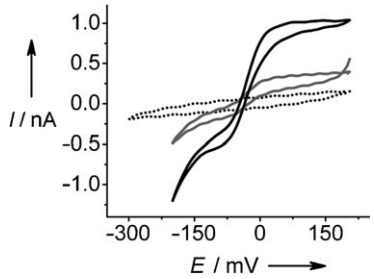

Figure 6. Cyclic voltammograms were recorded in 5 mM *p*-APP at 25 mV s^{-1}; ····: control experiment; ——: DNA assay on 10 μm Au electrode; ——: DNA assay on 25 μm Au electrode.

As expected, the reversible electrochemistry of *p*-AP/*p*-QI couple could be observed at both 10 and 25 μm electrodes. In a control experiment, a pure capacitive cyclic voltammogram was obtained at an electrode that was not modified with the capture probe but incubated with the complementary target strand and the avidin–AP conjugate.

Electrochemical amplification of the assay signal by means of SECM

DNA-modified 25 μm diameter Au microelectrodes were used to investigate the possibility of amplifying the electrochemical readout of the proposed DNA sensors by using SECM. This is considered to be a possibility to evaluate the potential benefits of redox amplification without the need to fabricate interdigitated array electrodes beforehand. For this, the 25 μm diameter SECM tip surface was modified through thiol chemisorption with the specific *Salmonella* capture probe, hybridized with its biotin-labeled target strand, and further incubated with the avidin–AP conjugate. By using the *z*-positioning system of the SECM, we allowed the DNA sensor electrode, which was polarized to a potential to oxidize enzymatically formed *p*-AP, to approach a bare gold surface in the presence of *p*-APP. The perpendicular gold surface was polarized to a potential that was sufficiently low so as to invoke re-reduction of the *p*-QI that was formed at the DNA-modified tip as a consequence of the enzymatic conversion of *p*-APP to *p*-AP. If the DNA-modified tip was positioned within feedback distance of the gold surface, *p*-QI generated at the tip diffused through the gap to the gold surface where it was re-reduced to *p*-AP. Thus, the local concentration of *p*-AP increased within the gap between the positioned DNA-modified tip and gold surface. Redox cycling and an amplification of the tip current can be observed in

analogy to the *positive feedback* effect of SECM (Figure 7). Two parameters influence the level of current amplification that is detected at the miniaturized DNA sensor: the applied potential

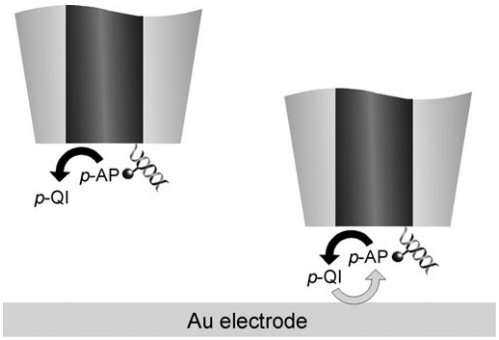

Figure 7. Amplification of the current signal at a DNA-modified Au microelectrode by using SECM feedback experiments. If the DNA sensor approaches the gold surface, which is capable of driving the re-reduction of *p*-QI, the redox couple *p*-AP/*p*-QI is cycled between the sensor and the gold surface, and this invokes multiple redox reactions and thus enhances the current at the DNA tip.

at both the DNA-modified tip and the gold electrode (recycling electrode) and the distance between tip and gold surface. Approach curves of the DNA-modified gold tip towards the gold surface (Figure 8) exemplify the crucial influence of the mentioned parameters on the current amplification at the DNA-modified tip. The DNA-modified SECM tip was polarized to a potential of +250 mV versus Ag|AgCl while decreasing potentials of −150 mV, −250 mV, and −350 mV were applied to the gold surface.

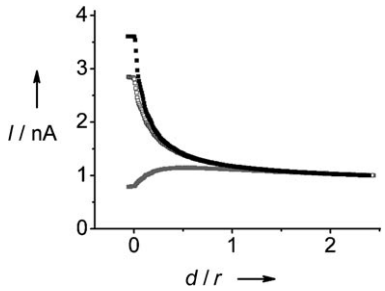

Figure 8. Approach curves of DNA sensor towards gold substrate. Capture probes were hybridized and AP was conjugated to the DNA duplex. Potential at the DNA sensor was +250 mV; potential at the gold substrate was −150 mV (■), −250 mV (□), or −350 mV (■). All approaches were recorded in the presence of 10 mM *p*-APP in carbonate buffer (pH 9.3); *d*/*r* is the distance between tip and sample normalized by the active radius of the SECM tip.

As expected, the highest current amplification is obtained at the closest approach of the tip to the Au surface, and the current becomes constant when the insulating glass sheath surrounding the Au disk electrode is touching the gold surface (Figure 8). At an applied potential to the gold surface of −150 mV, a decrease in the normalized current signal is observed. At this potential the driving force for the re-reduction of *p*-QI is obviously insufficient. The decrease in current is due to the blocked diffusion of the substrate *p*-APP into the gap between tip and gold surface; this leads to depletion of *p*-APP at the tip in (*negative feedback* of SECM). A potential of −250 mV at the gold surface leads to the expected amplification of the current signal at the DNA-modified sensor tip with decreasing *z* distance. The amplification is slightly higher at a further decreased potential of −350 mV and is 3.6-fold.

The SECM-based amplification can be advantageously used to determine the crucial parameters for redox amplifications, and this avoids the need to fabricate interdigitated electrodes.

Conclusions

An amplified electrochemical DNA sensor for *Salmonella* spp. was established based on hybridization detection by means of AP as enzyme label. Immobilization of thiol-modified capture probes was achieved by chemisorption on 100 μm gold disk electrodes that are arranged in an array of 48 electrodes. Assay parameters were optimized for a model system by using a biotinylated capture probe. Incubation time for the binding of the avidin–AP conjugate to the biotin tag at the surface-immobilized DNA for attaining maximum signal was found to be 10 min by using a 1:1000 dilution of a commercially available avidin–AP conjugate. Saturation of enzyme turnover was achieved with *p*-APP concentrations higher than 50 μM. Successful immobilization could be visualized by using SECM. Images of the spatial distribution of surface-bound AP were obtained for both a model system by using a biotinylated capture probe and the full DNA assay.

The detection limit was determined to be as low as 100 fM of the complementary target DNA by using the optimized assay parameters. Further miniaturization of the DNA assay on 25 and 10 μm gold disk electrodes was successful. By using redox amplification in feedback-mode SECM, a current amplification of 3.6-fold could be achieved.

Future work will be directed in establishing the DNA assay in electrode structures that intrinsically allow an amplification by redox cycling, for example, interdigitated electrode arrays[33,34] or more complex nanopore structures.[35] The results that are obtained from the SECM amplification studies will guide the way to an optimal design of the array structures and the optimal concentrations of the assay components. The detection limit is expected to be reduced when the assay is carried out by using this type of electrodes.

Experimental Section

Reagents: Capture probe DNA for *Salmonella* spp. and complementary DNA were provided by FRIZ Biochem (Neuried, Germany) The sequence of the specific capture probe was: 5′-GGT ATT AAC CAC AAC ACC-3′-(A)7-(SS)2. The sequence of the biotinylated target probe was: 5′-GGT GTT GTG GTT AAT ACC CGC AGC-3′-biotin. Ethanol and $NaH_2PO_4{\cdot}2H_2O$ were from Riedel–de Haën (Seelze, Germany), H_2SO_4, $Na_2PO_4{\cdot}2H_2O$, NaCl, and Na_2CO_3 were from J. T. Baker (Deventer, The Netherlands), $NaHCO_3$ was from

Merck (Darmstadt, Germany), $[Ru(NH_3)_6]Cl_3$ was from ABCR (Karlsruhe, Germany), *p*-aminophenyl phosphate (*p*-APP) was from Universal Sensors (Kinsale-Sandycove, Ireland), and avidin–alkaline phosphatase in Tris buffer (0.05 M, pH 8.0) containing bovine serum albumin (1%), $MgCl_2$ (1 mM) and sodium azide (15 mM) was from Sigma (Steinheim, Germany).

Electrode fabrication: Electrode arrays contained 48 gold electrodes with 100 μm diameter on a single microchip. Fabrication and design was described elsewhere.[36] Gold electrodes with active diameters of 10 and 25 μm were made by following an established protocol.[37] Gold wires with the corresponding diameters (Goodfellow, Huntington, UK) were vacuum-sealed into pulled soda lime glass capillaries (o.d. = 1.0 mm, i.d. = 0.5 mm; Hilgenberg, Malsfeld, Germany) by using a homemade heating stage equipped with a tungsten heating coil (Heka, Lambrecht, Germany).

Electrode preparation: Electrode arrays were cleaned in argon plasma (Diener, Nagold, Germany; plasma system Femto; for 3.5 min at 30 W, 40 kHz) and by cyclic voltammetry in 1 M H_2SO_4 (50–1550 mV vs. Ag|AgCl/3 M KCl, three scans at 100 mV s^{-1}). Electrode arrays were rinsed with H_2O and EtOH and dried in an Ar flow. The 10 and 25 μm electrodes were polished prior to use by using alumina polishing pastes with decreasing particle sizes of 0.3 and 0.05 μm (Leco, St. Joseph, USA) on a polishing cloth (Heraeus, Wehrheim, Germany). Further electrochemical cleaning was carried out by cyclic voltammetry by using a potential scan range between −300 and +1600 mV in 0.5 M H_2SO_4 at a scan rate of 100 mV s^{-1}. Typically, 10 or 20 cycles were applied.

Immobilization of DNA capture probes: To immobilize capture probe DNA onto electrode arrays, a 50 μM solution of the capture probe in phosphate buffer (200 pL; pH 7) that contained 0.05 vol% *N*-laurylsarcosin as a detergent was spotted on each electrode of the array by using a needle spotter (Cartesian Technologies, Irvine, USA) and left to dry for 3 h. To prevent unspecific adsorption, the electrodes were immersed in a 1 mM solution of 6-mercaptohexan-1-ol in phosphate buffer/10% EtOH for 16 h. After formation of the self-assembled monolayer, the electrodes were rinsed with H_2O, EtOH, and again H_2O, and dried in an Ar flow; 10 and 25 μm electrodes were modified by dipping into 20 mM phosphate buffer/20 mM NaCl (pH 7.4) containing 2 or 5 μM of the capture probe DNA for 1 to 3 h. After incubation, electrodes were thoroughly rinsed with phosphate buffer and water.

Hybridization with complementary DNA and binding of enzyme conjugation: Array electrodes were incubated in a solution of 500 mM Na_2SO_4 in phosphate buffer (0.05% *N*-laurylsarcosin, pH 7) that contained 1 μM of the synthetic complementary DNA strand for 2 h. The avidin–AP conjugate was diluted 1:500 or 1:1000 from the commercially available solution with acetate buffer (500 mM; pH 9). The electrodes of the array were incubated in this solution for 10 min and then thoroughly rinsed with carbonate buffer; 10 and 25 μm needle-type microelectrodes were incubated in 20 mM phosphate buffer/20 mM NaCl containing 10 μM of the complementary DNA strand for 2 h and then rinsed with phosphate buffer. Subsequently, electrodes were rinsed with 100 mM carbonate buffer (pH 9.8) and incubated with the avidin–AP conjugate (diluted 1:500 in 100 mM carbonate buffer) for 10 min. Afterward the electrodes were rinsed with carbonate buffer.

Electrochemical measurements: Electrochemical measurements with the array electrodes were performed in 500 mM Na_2SO_4/$NaCO_3$ buffer (pH 9.8) by using an AUTOLAB Potentiostat (Eco Chemie, Utrecht, The Netherlands). Electrodes were polarized to +400 mV versus Ag|AgCl (3 M KCl), and *p*-APP in 500 mM Na_2SO_4/$NaCO_3$ buffer (pH 9.8) was added after a stable background current was established. Electrochemical measurements with 10 and 25 μm electrodes were performed by using a Palmsens Bipotentiostat (Palm Instruments, Houten, The Netherlands) in carbonate buffer (100 mM Na_2CO_3/$NaHCO_3$ adjusted to pH 9.8). Electrodes were polarized to +250 mV versus a homemade Ag|AgCl (3 M KCl) microreference electrode, and aliquots of *p*-APP in carbonate buffer were added after a stable background current was established.

Scanning electrochemical microscopy: For SECM experiments, a Sensolytics Base SECM (Sensolytics GmbH, Bochum, Germany) in constant-height mode equipped with a Palmsens bipotentiostat (Palm Instruments, Houten, The Netherlands) was used.

For the visualization of enzymatic activity on the array electrodes after immobilization of capture probe, hybridization and binding of the avidin–AP conjugate, 10 μm gold electrodes were used as SECM tips. Electrodes were polished and cycled as described above prior to use. The SECM tip was positioned close to an array electrode by using a video microscope. A droplet of an aqueous solution containing 5 mM $[Ru(NH_3)_6]^{3+}$ and 100 mM KCl was applied to cover the electrode array. The tip and an Ag|AgCl microreference electrode were inserted into the droplet. A potential of −400 mV was applied to the SECM tip to reduce Ru^{III}. A diffusion-limited steady state tip current (I_∞) was detected. The tip was then approached towards the insulating layer around the array electrode (*z*-approach curve) until a drop in current of about 70% due to the negative feedback effect was detected. The droplet of solution was removed, and the electrode was rinsed with carbonate buffer. A droplet of carbonate buffer containing 10 mM *p*-APP was then applied to cover the electrode, tip, and reference electrode as before. The tip was polarized to a potential of +250 mV and scanned over the surface of the electrode array to detect *p*-AP that was formed in the enzymatic reaction of AP.

In the second type of experiment, 25 μm electrodes modified with the DNA assay architecture as described above were used as the SECM tip. After immobilization of capture probe DNA, hybridization and binding of the avidin–AP conjugate to the biotin label the microelectrode was approached to a 2 mm diameter gold electrode (CH Instruments, Austin, USA) as the recycling electrode. A silicon tube surrounding the 2 mm gold electrode served as electrochemical cell. The DNA-modified SECM tip and a microreference electrode were inserted into the silicon tube, which was subsequently filled with carbonate buffer containing 10 mM *p*-APP. The gold electrode was connected as the working electrode 1 to the bipotentiostat and polarized to the respective potentials and the SECM tip was connected as working electrode 2 and polarized to +250 mV. The *z*-approach curves of the DNA-modified tip towards the gold macroelectrode were performed with a scan speed of 1 μm s^{-1}.

Acknowledgements

The authors are grateful to the German Ministry of Education and Research (BMBF) for funding within the project NanoBioPore (FKZ 13N8607).

Keywords: DNA amplification · DNA assay · electrochemistry · scanning electrochemical microscopy (SECM) · sensors

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Received: November 20, 2008
Published online on April 7, 2009

