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DNA Hybridization Detection at Heated Electrodes

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The detection of DNA hybridization is of central importance to the diagnosis and treatment of genetic diseases. Due to cost limitations, small and easy-to-handle testing devices are required. Electrochemical detection is a promising alternative to evaluation of chip data with optical readout. Independent of the actual readout principle, the hybridization process still takes a lot of time, hampering daily use of these techniques, especially in hospitals or doctor's surgery. Here we describe how direct local electrical heating of a DNA-probe-modified gold electrode affects the surface hybridization process dramatically. We obtained a 140-fold increase of alternating current voltammetric signals for 20-base ferrocene-labeled target strands when elevating the electrode temperature during hybridization from 3 to 48 °C while leaving the bulk electrolyte at 3 °C. At optimum conditions, a target concentration of 500 pmol/L could be detected. Electrothermal regeneration of the immobilized DNA-probe strands allowed repetitive use of the same probe-modified electrode. The surface coverage of DNA probes, monitored by chronocoulometry of hexaammineruthenium(III), was almost constant upon heating to 70 °C. However, the hybridization ability of the probe self-assembled monolayer declined irreversibly when using a 70 °C hybridization temperature. Coupling of heated electrodes and highly sensitive electrochemical DNA hybridization detection methods should enhance detection limits of the latter significantly.

Introduction

Electrochemical detection of DNA hybridization is a powerful alternative to optical techniques. Indeed, the instrumentation needed is less sophisticated, and thus cheaper and easier to handle. The whole analytical process is more elegant and directly provides electronic signals which can be more conveniently processed compared to complicated interpretation of optical readouts. Recent activity in this area has been reviewed.[1−4] So far, much work has been conducted to find optimum strategies for the electrochemical detection step itself. One promising approach is the use of redox markers which are covalently attached to the target or reporter strand. Further, the use of gold electrodes with thiol-linked probe self-assembled monolayers (SAMs) is a good way to minimize undesired adsorption of nonspecific DNA or indicator molecules. Herne and Tarlov et al. started with surface plasmon resonance, XPS, and radiolabeling studies of DNA immobilization and hybridization, where the probe strands were tethered onto gold using thiol linkers.[5,6] Applying DNA redox intercalators together with a DNA SAM on gold electrodes, Barton et al. discuss direct charge transport through the DNA double strand. They used this effect for the detection of base pair mismatches. The current flows between the electrode surface and indicator molecules, while it depends on the number, type, and position of the mismatches.[7−10] Hartwich et al. found that irregularly formed DNA strands and such which were lying at the electrode surface can conduct neither electrons nor holes. There are strong requirements for the ternary structure of the double helix to form the conducting π-stack.[11]

Electrochemical detection of DNA hybridization provides high sensitivity, especially if amplifying platforms based on enzyme labels[12,13] or nanoparticle tracers[14] are used. This way, lowest detection limits of 0.5 and 1.5 fM target were obtained using enzyme labels[15] and nanoparticles,[16] respectively. Another amplifying technique applies conducting nanoparticles, which grow when treated with silver developing solution, tend to come into contact with one another, and close an electric contact. The latter is detected by conductivity measurements, yielding detection limits of 500 fM target.[17] A very low

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detection limit of 1.5 fM target is attainable by dissolving the silver followed by its electrochemical detection.[18]

Another key challenge to DNA chip technology is the acceleration of the hybridization process. There are only a few studies about this topic. One approach uses so-called acoustic micromixing, where hybridization can be accelerated 5-fold.[19] Alternatively, both November AG and Nanogen Inc. have used a potential-induced accelerated hybridization. Sosnowski et al. (Nanogen Inc.) applied constant electrical fields on agarose−streptavidin-modified electrode arrays coupled with optical hybridization detection.[20] The adjusted direct current of 100 nA at each positively polarized single electrode (80 μm diameter) allowed attraction of negatively charged DNA molecules, yielding rapid immobilization and 25-fold-accelerated hybridization. Mismatched DNA sequences could be discriminated by pulsed negative polarization (controlled current of up to 1500 nA). A 1 μm agarose layer between the electrode surface and the DNA molecules protected them from undesired reactions. The authors discussed combined effects of weak electric fields, pH alterations, and small temperature changes. Such electric polarization can provoke many unfavorable side effects. The protecting agarose layer would complicate electrochemical detection considerably and is probably penetrated by disturbing substances. Despite such protection and especially at a controlled current, disturbing redox reactions of many substances in real samples would occur. Furthermore, uncontrolled local pH changes due to electrolysis of the solvent at such high current densities would affect most biological substances, e.g., enzyme labels. Hence, independent control of electrode temperature and potential to affect DNA hybridization would be most desirable.

To manipulate DNA hybridization thermally, we proposed a DNA-modified electrode design which can simultaneously perform both direct electrical heating and electrochemical measurements.[21] Caillat et al. invented a heated electrochemical DNA array.[22] So far, no practical results in this field have been published.

In former studies we used our technique of directly heated electrodes for the enhancement of heavy metal stripping analysis[23−26] and DNA adsorptive stripping analysis.[27,28] We found that mass transport (as the limiting parameter for fast reactions such as metal deposition) is intensified typically up to 10-fold.[23,25,26] Furthermore, we could accelerate reaction or adsorption rates of kinetically inhibited processes (reduction of arsenic(V) or adsorption of dsDNA at carbon) 30-fold and more, if we performed the accumulation step at elevated electrode temperature.[24,27]

Here we used a heated gold wire electrode to demonstrate the positive effect of elevated temperatures during the hybridization step and presumed a mass-transfer control, using 20-base oligonucleotides for both the probe and target. Mass transfer is affected by generation of convection streams and a change of the diffusion coefficient, the first playing the more important role. Diffusion is relevant only if the particles are very large or if the viscosity of the medium is high. We illustrate how direct local electrical heating of a DNA-probe-modified gold electrode can dramatically accelerate the hybridization process compared to very low temperatures.

Experimental Section

Materials. All oligonucleotides were purchased from FRIZ Biochem GmbH (Munich, Germany). The probe strands comprised a HO−C_3H_6−S−S−C_3H_6− disulfide linker and the following base sequence: 5′-TGC GGA TAA CAC AGT CAC CT-[C3−S−S−C3−OH]. Probe strands were dissolved in sterilized 250 mM phosphate buffer (PB) solution at pH 7. The full complementary target strand contained an electrochemically active ferrocene group and the following base sequence: FerroAc-NH-C6-AGG TGA CTG TGT TAT CCG CA-3′. A noncomplementary strand (also featuring the ferrocene group) had the following base sequence: FerroAc-NH-C6-GAG GTA TCG GTT ATT GCC CA-3′. Target and noncomplementary strands were dissolved in sterilized distilled water. All nucleotides were stored at −18 °C. Sodium hydrogen phosphate, sodium dihydrogen phosphate, potassium chloride, potassium hexacyanoferrate(II), tris(hydroxymethyl)aminomethane, and potassium hexacyanoferrate(III) were purchased from Fluka. Hexaammineruthenium-(III) chloride was purchased from Alfa Caesar Johnson Mattey GmbH (Karlsruhe, Germany). Water, buffer and electrolyte solutions, and pipet tips were sterilized by autoclaving.

Preparation of DNA-SAM-Modified Gold Electrodes. The gold wire electrode was annealed in air by applying a 0.65 A alternating current (red glowing). The gold disk electrode was cleaned by polishing it with alumina. Then both electrode types were treated electrochemically in 0.5 M sulfuric acid by 25 cyclic voltammograms (100 mV/s) between −0.2 and 1.85 V vs Ag/AgCl (3 M KCl). Next, the electrodes were rinsed with water and then with ethanol. A droplet of 17 μL of probe solution (30 μM probe strands in 250 mM PB at pH 7) was then placed on the plastic stage around the Au wire or on top of the Au disk. This assembly was kept for 16 h in a vapor-saturated atmosphere at 5 °C. In the next steps the electrode was rinsed with 250 mM PB (pH 7) and then with water, and then the electrode was immersed for 30 min in a 1 mM aqueous solution of pentanethiol to close holes in the DNA SAM and to straighten the single strands from the Au surface, according to the literature.[6] Finally, the electrode was rinsed with ethanol and water. The surface coverage with probe strands calculated from chronocoulometric measurements ranged from 3.7 to 5.9 pmol/cm^2 on wire electrodes and from 12 to 18 pmol/cm^2 on disk electrodes, respectively.

Hybridization, Measurements, and Dehybridization. For hybridization, the electrode was immersed for the appropriate time in a 10 mL beaker containing 3 mL of 0.5 M PB (pH 7) and a given concentration of target or noncomplementary strands. This hybridization solution was kept at 3 °C. Through hybridization the electrode was heated to a specified temperature. For comparison, in some experiments, the electrode was heated together with the bulk solution to the desired temperature in a thermostated beaker. Then the measurements were performed at room temperature in a cell containing 20 mL of 0.5 M PB (at pH 7). All alternating current voltammetry (ACV) measurements were conducted phase sensitively using a frequency of 10 Hz, a 25 mV (root-mean-square) amplitude, and a phase shift of 0°. During the measurements the wire electrode was separated from the heating device by means of a double switch. Thereafter the electrode was heated to 41 °C inside a beaker containing 50 mL of distilled water to dehybridize the double strands and get back the single probe strands.

The melting temperature was determined by monitoring hypochromicity at 260 nm by means of a CARY 1E UV/vis spectrophotometer (Varian) during thermal denaturation in a solution containing 80 nM probe−target−duplex in 0.5 M PB (pH 7).

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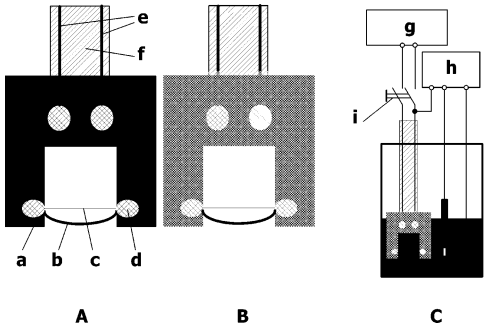

Figure 1. (A) Heated electrode design comprising (a) a printed circuit board with copper tracks, (b) a plastic stage, (c) a 25 μm Au wire, (d) a soldering joint, (e) supply lines, and (f) a glass tube. (B) Heated electrode with paraffin/polyethylene-isolated soldering joints and copper tracks. (C) Measurement arrangement including an electrochemical cell with working, reference, and counter electrodes (from left) and (g) a heating device, (h) a potentiostat, and (i) a double switch.

For the heating method study, hybridization was executed in a heated hybridization solution without electrical electrode heating and in a cooled hybridization solution while the wire electrode was heated using an ac current. Hybridizations and measurements were carried out in the same manner as in the other hybridization experiments.

The chronocoulometric studies for the determination of DNA surface coverage were carried out (according to the method of Steel et al.[33]) in a solution containing 10 mM Tris buffer at pH 7.4 without further electrolytes. To get information about the amount of $[Ru(NH_3)_6]^{3+}$ that was bound to the probe−SAM, we performed a potential step experiment in which we recorded charge vs time. For mathematical interpretation, we plotted charge vs $\sqrt{t}$ to linearize the diffusion component of the registered charge. To get the charge amount that is caused by immobilized species and charge of the double layer, we used the chronocoulometric intercept at $t = 0$. At first, measurements in pure buffer delivered necessary information about the charge taken by the double layer. After the addition of 50 μM $[Ru(NH_3)_6]Cl_3$, the surface coverage could be determined. For this purpose, the double layer charging component was subtracted. This way we could differentiate between charges that were caused by diffusing $[Ru(NH_3)_6]^{3+}$, immobilized $[Ru(NH_3)_6]^{3+}$, and the double layer.

Apparatus. Figure 1 depicts the experimental setup. The heated working electrode consisted of a 25 μm diameter and 5 mm long gold wire, which was soldered onto a printed circuit board. Two contacts provided the connection to the heating device; one of the contacts was connected to the potentiostat. To hold the 17 μL immobilization reagent droplet in position, a plastic stage was placed below the electrode wire. We have to point out that our hot-wire electrode does not provide a perfect uniform temperature along the wire. Both ends are at lower temperature compared to a large middle part due to cooling by the bulky soldering joints.[29] However, at the moment the wire is our best overall solution to realize an electrically heated gold electrode. It can be cleaned easily by glowing on air and provides a very smooth surface. Modifications (e.g., with SAMs) are simple also if the solution volume is limited to a few microliters. Presently we are developing heated electrodes that feature a consistent temperature. ACV, cyclic voltammetry (CV), and chronocoulometry (CC) were performed by means of a μAUTOLAB potentiostat (Eco Chemie, Utrecht, The Netherlands). The instrument was controlled by a PC using the GPES 3.3 DOS software. The chronocoulometric measurements were evaluated using Xact 7 software (SciLab GmbH, Hamburg, Germany). A multimeter (VOLTCRAFT M-4660A) together with a resistor (1 Ω) and an HF Probe GE 7130 (Elditest Electronic GmbH, Germany) was used for heating current measurements. All electrochemical measurements were carried out in a Faraday cage containing a VA Stand glass cell, a Ag/AgCl (3 M KCl) reference electrode, and a glassy carbon counter electrode (all by Metrohm AG, Herisau, Switzerland). The working electrode was heated by means of an MXG-9802 function generator (VOLTCRAFT), connected to a CA2100 automotive power amplifier (Concord Car Audio, Woodbury, NY), an FPS 15A dc power supply (VOLTCRAFT), and a laboratory-made high-frequency power transformer. A sine wave alternating current of 100 kHz was used for electrode heating in all experiments. This device heated the working electrode to the desired temperature within 2 s. The heating current did not exceed 1200 mA, the resistance of the Au wire was 0.5 Ω, and hence, the heating voltage did not exceed 600 mV. The precision of the heating current adjustment was 1%. The high frequency of the applied alternating current prevented polarization. Therefore, undesired faradaic effects at the heated electrode did not occur. The relationship between working electrode temperature and heating current was determined in a separate experiment: We measured the open circuit potential upon adjustment of different heating current values in a cell containing 50 mL of 0.1 M KCl and a 5 mM concentration each of potassium hexacyanoferrate(II) and hexacyanoferrate-(III). The well-known temperature coefficient of 1.6 mV/K was used to calculate the electrode temperature from the electrode open circuit potential. Later on, this verified correlation was used to adjust the desired electrode temperature by tuning the heating current. More details about our heating devices and techniques can be found in refs 23, 30, and 31.

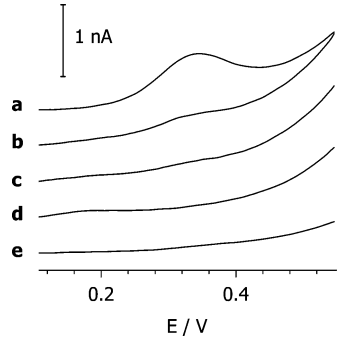

Figure 2. ACV signals following 4 min exposures of probe-SAM-modified Au electrodes to (a) 80 nM target at 42 °C, (b) 80 nM noncomplementary strand at 42 °C, (c) 80 nM noncomplementary strand at 3 °C, (d) bare probe SAM at 3 °C, and (e) 80 nM target at 3 °C electrode temperature, keeping the bulk solution (0.5 M PB solution, pH 7) at 3 °C. Target and noncomplementary strands were modified with a covalently attached ferrocene label at the 5′ position to enable electrochemical hybridization detection. Measurements were conducted at 20 °C in separate electrolyte containing 0.5 M PB at pH 7. All ACV measurements were carried out phase sensitively using a frequency of 10 Hz, a 25 mV (root-mean-square) amplitude, and a phase shift of 0°. All signals were smoothed using Savitzky−Golay filtering at level 4.

Results and Discussion

Hybridization of Target and Noncomplementary DNA at Different Temperatures. Figure 2 displays the ACV response following the hybridization at the gold wire electrode modified with a probe SAM. We chose this electrochemical technique to obtain a good separation between faradaic and charging currents of the low ferrocene signals. No ferrocene response was detected without the target DNA (d). Hybridization in an 80 nM target solution at 3 °C yielded a very small signal (e), which was enhanced 128-fold upon heating the electrode to 42 °C during the hybridization step (a). No change in the noise level was observed. Hybridization in an 80 nM noncomplementary solution gave a very small signal at 3 °C (c) and a slightly higher response at 42 °C (b). We could completely depress this small signal of the non-

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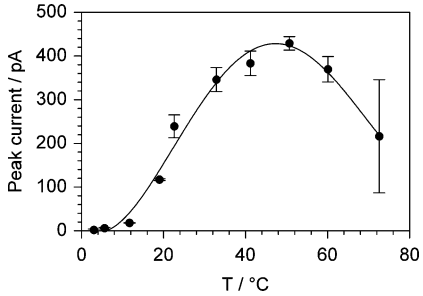

Figure 3. Dependence of the ACV peak current on electrode temperature during a 4 min hybridization period with 80 nM target. The error bars represent the standard deviations of three measurements. Other conditions are as in Figure 2.

complementary sequence by rinsing the electrode thoroughly for 1 min in a 50 °C 0.5 M PB solution after the hybridization step; in comparison, the target signal was not affected by this procedure (data not shown). This demonstrates the very good discrimination between full complementary and noncomplementary sequences by using SAMs for the probe immobilization and the covalently attached ferrocene marker for the electrochemical transduction. Undesired adsorption is depressed effectively.

Effect of Electrode Temperature during Hybridization. The ac voltammetric signals in Figure 3 illustrate the effect of electrode temperature during a 4 min hybridization period, keeping the bulk target solution at a constant 3 °C. This study yielded a 140-fold signal increase when going from 3 to 48 °C electrode temperature. Beyond this point a decrease of signals was observed, which we attribute partially to starting melting curve behavior. We calculated a melting temperature of 74 °C (at 0.75 M Na^+) according to the following equation:[32]

$$T_m = 81.5\ °C + \left[(16.6\ °C)\log\left(\frac{[Na^+]}{mol/L}\right)\right] + (41\ °C)\frac{G+C}{L} - (500\ °C)\frac{1}{L}$$

Here, T_m is the melting temperature, $G + C$ the sum of guanine and cytosine bases, and L the total base number. A photometric control experiment yielded a duplex melting temperature of 73 ± 2 °C. The "hot-wire effect" is 10-fold more pronounced during hybridization detection than during heavy metal stripping analysis. It is not clear at this time which step of the entire hybridization process on our heated electrode is the slowest. While hybridization of short oligonucleotides in homogeneous solution should be fast, the combination of probe and target strands at a surface can include a slow 2-dimensional diffusion step. This means that immobilized probe molecules at a surface need more time to find the optimal fit with the targets. Additionally, the recombination can be sterically and electrostatically hindered due to a high surface coverage of the probe strands. In this study we used a heated wire electrode whose soldering joints on the printed circuit board were isolated with a paraffin/polyethylene mixture. This isolation limits the electrode temperature due to its melting point of 70 °C. Therefore, we had to choose a low bulk solution temperature of 3 °C to achieve a large temperature difference, which is needed for strong thermal convection. Moreover, a low bulk temperature in general prevents a sample from decomposition. Especially RNA is well-known to be a delicate analyte and unstable at elevated temperatures. On the other hand, very short oligonucleotides of only 8−15 base pairs are often used as probes to limit costs. Many of them can have melting temperatures below room temperature. By using heated electrodes, it becomes possible now to keep the bulk sample, e.g., at a conserving 3 °C and still apply the desired stringent hybridization temperature at the probe-modified electrode surface. The practical relevance will become clear especially if natural RNA or long DNA strands of PCR products instead of short oligonucleotides have to be analyzed. We expect that the effect displayed in Figure 3 will be even higher at real samples containing long-chain nucleotides. With increasing temperatures the slope in Figure 3 becomes lower. One reason could be the melting curve behavior, which starts to overlay. Other possible explanations are annealing processes (changes in the structural arrangement) of the probe SAM, desorption of the thiol-linked probe strands, or depurination of the probe strands.

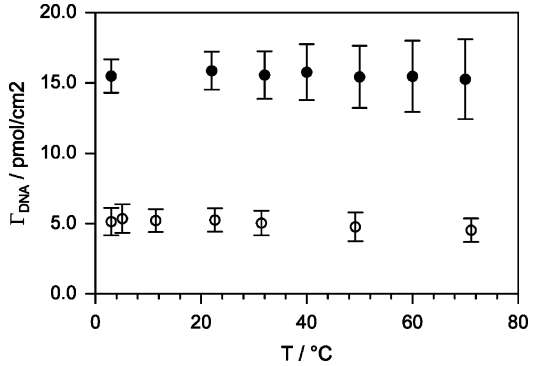

Figure 4. Effect of electrode temperature on DNA coverage at a heated Au wire electrode in cold electrolyte (open circles) and at a Au disk electrode which was heated together with the electrolyte in a thermostated cell (closed circles). The amount of Ru(III), deposited on the probe-SAM-modified electrode surface, was measured by chronocoulometry in a solution containing 50 μM $[Ru(NH_3)_6]^{3+}$ and 10 mM Tris−HCl at pH 7.4. This served as a measure for DNA surface coverage. A potential step from +130 to −370 mV was applied. The values for each point were calculated as the mean of three measurements, and the error bars indicate the standard deviation.

To study the stability of the DNA SAM, we chose the method of Steel et al. to determine the amount of DNA which is present on the electrode surface.[33] Briefly, the amount of $[Ru(NH_3)_6]^{3+}$, electrostatically bound to the probe SAM, was measured by means of chronocoulometry. Figure 4 illustrates that elevating the temperature yields no profound effect on the Ru(III) signals at the heated wire and disk electrode. Therefore, the integrity of the probe SAM is likely to be stable. Only a very small desorption can be observed up to 70 °C. This corresponds with earlier results of Kelley et al., where DNA-SAM-modified gold electrodes showed only a small decrease of hybridization signals even after being heated several times to 90 °C in hot distilled water.[8] The surface coverage at the Au disk electrode was found to be ca. 3 times higher than at the Au wire. This is possibly caused by the different cleaning procedures prior to the immobilization step.

Three consecutive desorption experiments with heated wire (hatched columns) and disk electrodes (gray columns) in PB solution at 24, 70, and again 24 °C using chronocoulometry of electrostatically bound $[Ru(NH_3)_6]^{3+}$ are displayed in Figure 5A. The thermal conditions were the

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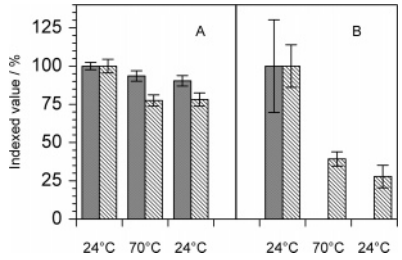

Figure 5. Two series (A and B) of three consecutive experiments using the same probe-modified electrode for (A) chronocoulometric measurements of DNA probe coverage and (B) ac voltammetric measurements of target hybridization at the heated Au wire electrode (gray) and the Au disk electrode (hatched). The *x*-axis represents the temperature of the heated electrode (gray) and the bulk solution containing the Au disk electrode (hatched), respectively. This temperature was applied during (A) the simulated hybridization step and (B) the hybridization step. After each measurement a simulated dehybridization step (A) or a dehybridization step (B) at 48 °C in distilled water was applied. (A) Measurements without target. (B) Measurements following a 4 min hybridization period with 80 nM target. (A) 10 mM Tris buffer at pH 7.4 with 50 μM $[Ru(NH_3)_6]^{3+}$ and potential step from +130 mV to −370 mV. (B) Same conditions as in Figure 2. The values were indexed to the highest value of either experiment A or B. Each value was calculated as the mean of three measurements; the error bars indicate the standard deviation.

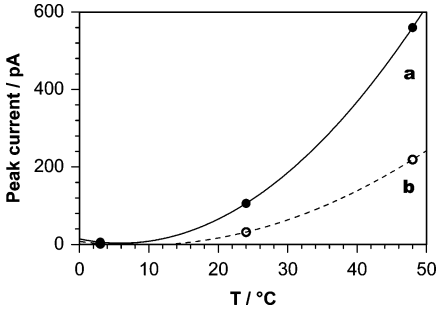

Figure 6. Effect of temperature on ACV hybridization signals while (a) the wire electrode and bulk solution were heated together and (b) only the wire electrode was heated in a 3 °C bulk solution. Other conditions are as in Figure 2.

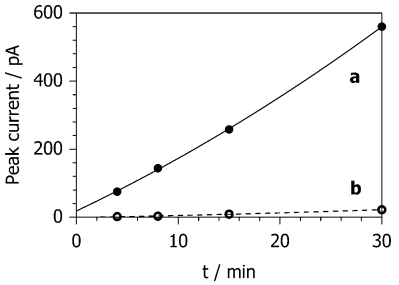

Figure 7. Effect of hybridization time on ACV hybridization signals at (a) 24 °C and (b) 3 °C electrode temperature. Other conditions are as in Figure 2.

same as in the hybridization experiments, with immobilized probe strands, but without any target in the buffer solution; even the dehybridization step was executed as usual for 30 s in distilled water at 42 °C. It can be seen that, after the experiment at 70 °C, DNA coverage decreased by 15−20%. But the third experiment at 24 °C gave no additional loss in DNA coverage. Figure 5B shows that a measurement at the wire electrode after hybridization with the target strand for a period of 4 min at 70 °C yields some signal decrease of about 60%. A measurement after a third hybridization experiment at 24 °C resulted in a signal decrease to 30% of the first value (hatched columns). That means the effect was not reversible. Hybridization at the disk electrodes in the heated bulk solution gave a more dramatic decrease of the ACV signals (gray columns). After hybridization was performed while the bulk solution was kept at 70 °C, the signal was gone completely and irreversibly, because subsequent hybridization at 24 °C also gave no signal. The hybridization solution was tested afterward using a new probe-modified electrode and was found to be unaffected by the heating to 70 °C (data not shown).

We can summarize that we found a slightly decreasing DNA surface coverage probably due to desorption processes. This is not alone responsible for the irreversible loss of signal height after application of 70 °C by direct or bulk solution heating. One reason for this effect could be annealing of the DNA SAM, forming a layer, which is no longer available for target hybridization. At the gold disk, the annealing probably was even more effective due to the 3 times higher surface coverage. This can probably be fixed by modifying the immobilization protocol. The immobilization of probe strands and alkanethiol could be carried out together in one solution, and the immobilization time could be decreased. Steel et al. found that a high surface coverage of more than 4 pmol/cm^2 can constrain hybridization considerably, because of sterical hindrance.[33]

Figure 6 displays the effect of hybridization temperature and a comparison of two heating methods. Hybridization using an electrically heated wire electrode in a cooled target solution is compared with that using a wire electrode that is heated together with the bulk target solution in a thermostated beaker. All the other conditions were the same in both hybridization methods. Direct electrical heating of the wire electrode has almost the same dramatic effect on hybridization as using a thermostated beaker to heat the whole assembly. The difference can probably be attributed to the nonuniform temperature along the wire electrode during electrical heating. More important, the thermal convection which is present around the electrically heated wire electrode but not in the thermostated beaker does not play any role in the hybridization speed. This is a strong indication that hybridization here is limited by slow diffusion or reactions directly at the surface rather than by mass transport toward the surface.

Effect of Hybridization Time at Different Temperatures. Figure 7 demonstrates that the electrode heating during hybridization can greatly reduce the hybridization time. Applying only 24 °C (a), we achieved a 3-fold higher signal at 4 min compared to 30 min at 3 °C (b). Conditions are far from probe saturation due to a low target concentration of 80 nmol/L. Therefore, we found an approximately linear function rather than signal leveling. The thermal stability of the SAM needs special attention in the future, because repeated measurements at the same probe SAM including (hot) hybridization and hot dehybridization lead to slow signal decay. To obtain complete studies of temperature and time effects (Figures 3 and 7), we had to combine the measurements of several series, each carried out with a freshly formed SAM. Ferrocene-labeled targets yielded only very low electrochemical signals in the picoampere range, because every target molecule hybridized with a probe molecule at the surface contributed only one electron to the signal. However, by using ac voltammetry and a carefully grounded Faraday cage, we still obtained a very good signal-to-noise ratio. At 8 min of hybridization and a 42 °C electrode temperature we could detect a target concentration of 500 pmol/L (data not shown).

We coupled successfully hot-wire electrochemistry with in situ DNA hybridization by using electrically heated

DNA-SAM-modified gold electrodes. This novel combination allowed us to perform highly sensitive and selective hybridization detection. Direct electrical heating of the DNA-modified gold electrode enhanced hybridization dramatically and provided considerable advantage over passive, acoustically forced and potential-induced hybridization. Such signal amplification will be useful in analysis of low target concentrations. Most other bioelectronic amplification routes should be compatible with our technique, leading to the lowest detection limits for nucleic acids. Heated electrodes permit independent local temperature control while leaving the bulk solution unaffected. We believe that miniaturized directly heated electrodes could contribute a valuable benefit to future arrangements where DNA sensor arrays are essential.

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