Sensing and amplification of oligonucleotide-DNA interactions by means of impedance spectroscopy: a route to a Tay–Sachs sensor

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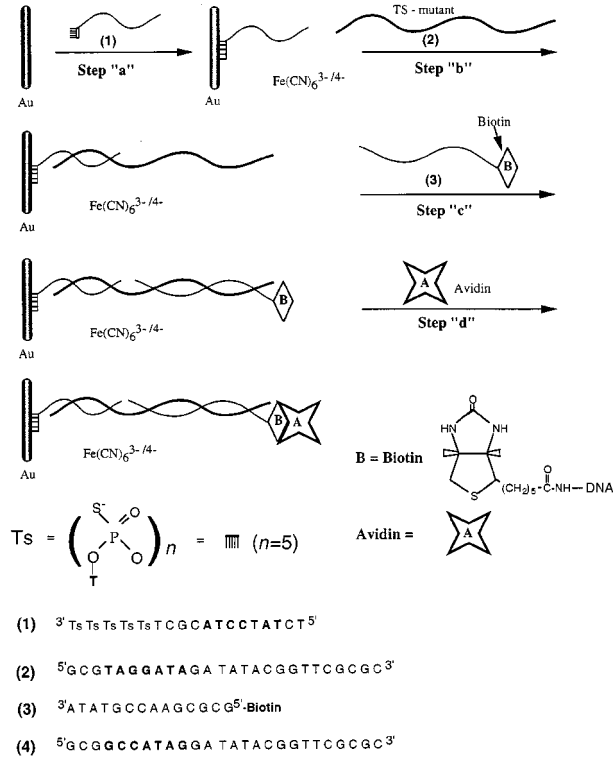
Received (in Cambridge, UK) 27th October 1998, Accepted 19th November 1998

A three-component oligonucleotide/DNA layered assembly on a Au-electrode acts as a specific biosensor for the analysis of the Tay–Sachs mutant by means of Faradaic impedance spectroscopy.

The development of DNA-sensor devices attracts substantial recent research efforts directed to gene analyses, detection of genetic disorders, tissue matching, and forensic applications.[1,2] Significant progress was accomplished recently with the assembly of biochips for the optical detection of DNA.[3] Electronic transduction of the formation of oligonucleotide–DNA complexes could reveal a significant advance in DNA analysis as it could provide quantitative information on the analyte interactions with the sensing interface.[4] The development of a DNA-sensor requires the assembly of the sensing interface on a transducer element for the appropriate electronic transduction of the analyte recognition process, and simultaneously, the tailoring of a specific and selective sensing interface. Electrochemical DNA sensors based on the electrostatic attraction or intercalation of transition metal complexes,[5,6] *e.g.*, $Co(bpy)_3^{3+}$, or dyes such as acridine or Hoechst 33258 were reported. Control of the gate interface potential of field effect transistors, FET, was employed to identify the formation of ds-DNA complexes.[7] Recently, microgravimetric quartz-crystal-microbalance, QCM, analyses were applied to sense oligonucleotide–protein[8] or oligonucleotide–DNA[9] complexes. The sensitivity of these DNA sensors is, however, low, and the specificity of the sensing interfaces needs further studies.

Impedance spectroscopy is an effective method to probe the interfacial properties (capacitance, electron transfer resistance) of modified electrodes.[10] Impedance transduction of the formation of antigen–antibody[11] or biotin–avidin[12] complexes at electrode supports was reported. The assembly of the negatively-charged oligonucleotides and specifically double-stranded (ds) oligonucleotide complexes onto electrode supports is anticipated to control the double-layer potential at the electrode surfaces. As a result, the Faradaic impedance responses of the electrode in the presence of a charged solution-solubilized redox probe, are expected to be controlled upon the formation of a double-stranded DNA oligonucleotide complex at the electrode support. Here we wish to report on the specific, sensitive and confirmative analysis of DNA using Faradaic impedance spectroscopy. As a model system we employ the Tay–Sachs (TS)[13] oligonucleotide mutant that could eventually lead to a TS-sensor device.

The 13-mer oligonucleotide **1** includes a 12-base sequence that is complementary to a part of the TS mutant **2**.[14] In addition, **1** includes a five-base thiophosphate-T tag for its assembly onto a Au-electrode.[15] A Au-electrode was interacted with **1** (50 μM, 10 h) resulting in the assembly of the sensing interface on the gold support, Scheme 1. The resulting **1**-functionalized electrode was interacted with the TS-mutant (30 ng mL^{-1}; 30 min treatment). Fig. 1 shows the impedance features presented as Nyquist plots (Z_{im} *vs.* Z_{re}) of the bare Au-electrode, curve (a), the **1**-functionalized electrode, curve (b), and the functionalized electrode after treatment with the complementary mutant, **2**, curve (c) in the presence of $[Fe(CN)_6]^{3-/4-}$ as the redox probe. Significant differences in the impedance spectra are observed upon the stepwise formation of the ds-oligonucleotide–**2**



Scheme 1 Model system for sensing and amplified impedance analysis of a Tay–Sachs oligonucleotide.

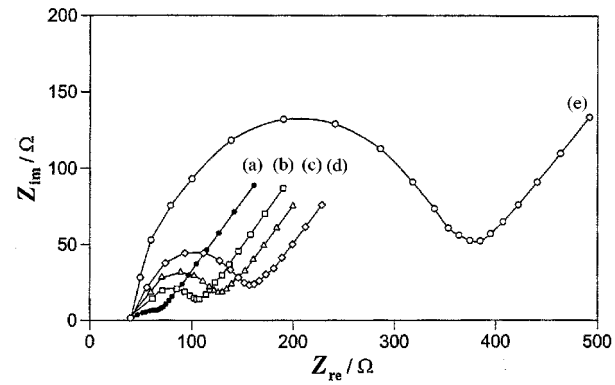


Fig. 1 Nyquist-diagram (Z_{im} *vs.* Z_{re}) for the Faradaic impedance measurements of a Au-electrode in the presence of 10 mM $[Fe(CN)_6]^{3-/4-}$ (1 : 1-mixture): (a) bare electrode, (b) **1**-functionalized electrode, (c) **1**-functionalized electrode after 30 min of incubation with **2**, (30 ng mL^{-1}) in SSC buffer (SSC = 15 mM Na citrate and 150 mM NaCl) , (d) ds-oligonucleotide complex with **2** after 30 min of incubation with **3** (40 ng mL^{-1}) in SSC buffer, (e) after 10 min of incubation with avidin (5 ng mL^{-1}) in phosphate buffer solution, pH = 7.1, 0.1 M. The impedance spectra were recorded within the frequency range 0.1 Hz–50 kHz at the formal potential of the $[Fe(CN)_6]^{3-/4-}$ redox couple. The amplitude of the alternate voltage was 5 mV.

complex. The impedance spectra follow the theoretical shapes and include a semicircle portion, observed at higher frequencies, that corresponds to the electron-transfer limited process, followed by a linear part characteristic of the lower frequency attributable to a diffusionally limited electron transfer. The respective semicircle diameters (obtained by extrapolation of the semicircles to lower frequencies on the Z_{re}-axis) correspond to the electron transfer resistances, R_{et}, at the electrode surface. The electron-transfer resistance, R_{et}, for the bare Au-electrode is *ca.* 60 Ω and it increases to *ca.* 80 Ω and *ca.* 105 Ω upon functionalization of the surface with **1** and formation of the ds-oligonucleotide complex with **2**. This is consistent with the fact that the negatively charged interface, formed upon the assembly of **1** on the electrode, repels the negatively charged redox probe. This introduces a barrier for interfacial electron transfer and an increase in the electron-transfer resistance. Formation of the ds-oligonucleotide complex with **2** enhances the double-layer potential at the electrode interface. This results in a further repulsion of the redox-probe and an increase in the electron transfer resistance at the electrode [*cf.* Fig. 1 (c)].

Although the impedance spectra change upon interaction of **2** with the oligonucleotide sensing interface reflects the hybridization process and the formation of the ds-complex with the analyte DNA, one would like to *confirm* and *amplify* the formation of the ds-complex at the sensor interface. Towards these goals the resulting ds-oligonucleotide interface was interacted with the biotinylated oligonucleotide, **3**, Scheme 1. The latter oligonucleotide is complementary to the residual sequence of the analyte DNA, and hence could be used as an oligonucleotide probe for the primary formation of the ds-DNA complex. Fig. 1(d), shows the impedance spectrum of the ds-DNA-functionalized electrode upon interaction with **3** and using $[Fe(CN)_6]^{3-/4-}$ as redox-probe. An increase in the electron transfer resistance to $R_{et} \approx 134$ Ω is observed, consistent with the formation of an additional negatively charged ds-assembly. Provided the system exhibits specificity, the latter process is observed only if the primary ds-system is formed with the sensing interface. Thus, the interaction of the modified surface with **3** provides a confirmation test for the formation of the primary ds-oligonucleotide assembly. As the secondary oligonucleotide is labeled by biotin, the subsequent reaction of the oligonucleotide-monolayer superstructure with avidin is anticipated to insulate the interfacial electron transfer due to the hydrophobic insulation of the interface. Fig. 1(e), shows the impedance spectrum of the layered ds-oligonucleotide superstructure after interaction with avidin. A substantial increase in the diameter of the semicircle is observed, implying a high electron transfer resistance, $R_{et} \approx 340$ Ω. This originates from the formation of the hydrophobic biotin–avidin complex at the electrode surface, a process that introduces a barrier for the interfacial electron transfer. Through the hydrophobic biotin–avidin complex, the latter process amplifies the initial formation of ds-oligonucleotide superstructure. The increase in the electron transfer resistance upon formation of the initial ds-oligonucleotide complex is controlled by the concentration of the analyte in the sample (and the time of interaction of the sensing interface and the sample). We were able to easily sense oligonucleotide concentrations corresponding to 3.5×10^{-12} mol mL^{-1} using this method (incubation time, 30 min). The incubation time of the sensing interface with the analyte **2** controls the loading of the electrode by the ds-assembly. The content of the ds-oligonucleotide/DNA on the electrode then controls the subsequent interfacial electron transfer resistance upon the association of **3** and avidin. The experimental incubation time, 30 min, represents an optimized parameter. Shorter incubation times perturbed the sensitivity of the system, whereas longer incubation times slightly enhanced the observed interfacial electron transfer resistance.

Several control experiments were performed to reveal the selectivity of the sensing interface. Treatment of the **1**-functionalized electrode with the biotin-labeled oligonucleotide **3** and then with avidin but without interaction of the electrode with **2**, does not lead to any noticeable changes in the impedance spectra. Thus, the sequence of changes in the impedance spectra is only stimulated if the primary analyte **2** is bound to the sensing interface. Also, the impedance responses of the sensing electrode upon interaction with oligonucleotide **4** were examined following the identical sequence of analysis. Oligonucleotide **4** includes the normal gene sequence in which the 7-base mutation shown in **2** leads to the TS-genetic disorder. No noticeable changes occur in the impedance spectrum of the **1**-functionalized electrode upon interaction with **4** or subsequent treatment of the electrode with **3** or avidin. Thus, the **1**-functionalized electrode reveals high selectivity for the detection of the TS-mutant, and the normal gene is effectively discriminated by the sensing interface. The results also indicate that the sensing interface is not affected by the non-specific binding of proteins.

In conclusion, we have demonstrated a novel sensitive method for the electronic transduction of DNA-complexes using Faradaic impedance spectroscopy. The method provides a means to sense the analyte–DNA complex, to confirm the analysis process using a biotin-labeled oligonucleotide probe, and to amplify the sensing process by the biotin–avidin complex at the sensing interface. The method reveals high specificity and selectivity. This originates from the fact that the primary sensing interface consists of an oligonucleotide that includes a limited number of bases capable of forming a single helix with the complementary analyte. Thus, any perturbation will be identified by the sensing interface. Although a detailed stability of the sensing electrode has not yet been performed, it appears that the sensing interface is stable for at least several days upon storage at 4 °C in a dry state. The examination of this biosensor assembly for the analysis of other oligonucleotides, *e.g.* c-DNA or t-RNA, is underway in our laboratory.

This research is supported by the Szold Foundation, The Hebrew University of Jerusalem.

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Communication 8/08319C