Amplified Microgravimetric Quartz-Crystal-Microbalance Assay of DNA Using Oligonucleotide-Functionalized Liposomes or Biotinylated Liposomes

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The development of DNA-sensors attracts recent research efforts directed to gene analysis, detection of genetic disorders, tissue matching, and forensic applications.[1,2] Optical detection of DNA was reported using fluorescence-labeled oligonucleotides[3] or surface plasmon resonance.[4] Electronic transduction of oligonucleotide−DNA interactions enables the quantitative assay of DNA.[5] Electrochemical analysis of DNA was reported by using oligonucleotide-functionalized conductive polymers[6] or by the application of electroactive dyes[7] or transition-metal complexes[8] that intercalate or specifically bind to double-stranded DNA assemblies. Recently, Faradaic impedance spectroscopy was applied to follow the formation of double-stranded oligonucleotide−DNA complexes on electrodes.[9] Two major aspects must be considered upon the development of DNA-sensors: (i) the selectivity of the sensing interface with the optimal goal to identify a single-base mutation and (ii) the sensitivity of the sensing system with the challenge of analyzing a few DNA-strands in the analyte sample. Amplification of the electronic sensing and transduction of oligonucleotide−DNA interactions is mandatory to improve the sensitivity of the devices. In a recent study,[10] we have reported on the amplification of the oligonucleotide−DNA recognition event by the biocatalyzed precipitation of an insoluble product on the electrode and probing the insulation of the electrode interface by Faradaic impedance spectroscopy. Similarly, electrical contact of a redox-enzyme and the electrode, upon the formation of the double-stranded oligonucleotide−DNA complex, enables the amplified amperometric transduction of the DNA sensing event.[11]

Amplification of the oligonucleotide−DNA sensing processes by antibodies and the electronic transduction of the recognition event by microgravimetric quartz-crystal microbalance assay was reported.[12] Here we wish to report on the specific and amplified transduction of oligonucleotide−DNA recognition using functionalized liposomes and a quartz-crystal-microbalance as the amplification and electronic transduction means, respectively. Electronic DNA-sensors of unprecendent sensitivity are assembled.

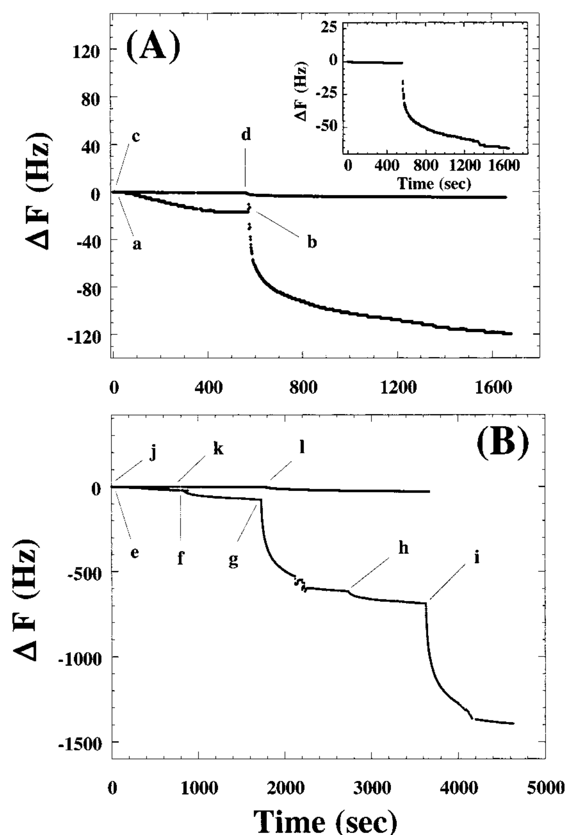

Figure 1. (A) Time-dependent frequency changes of the (**1**)-functionalized crystal upon (a) interaction with **2**, 5×10^{-6} M, (b) after interaction of the resulting electrode with the **3**-functionalized liposomes (lipid concentration 0.2 mM), (c) treatment of the sensing crystal with **2a**, 5×10^{-6} M, and (d) treatment of the resulting crystal with the **3**-labeled liposomes. Inset: Time-dependent frequency changes after treatment of **1**-functionalized crystal with **2**, 5×10^{-9} M, and subsequently with the **3**-tagged liposomes (lipid concentration 0.2 mM). (B) time-dependent frequency changes of the Au-quartz crystal upon (e) interaction of the sensing interface with **2**, 5×10^{-6} M, and **4** complex, (f) as a result of the reaction of the resulting interface with avidin, 2.5 μg·mL^{-1}, (g) upon reacting the resulting assembly with the biotin-labeled liposome, (h) step (f) repeated, (i) step (g) repeated, (j) treatment of the sensing interface with the **2a**/**4** complex, (k) interaction of the resulting interface with avidin, and (l) reacting the resulting interface with biotin-labeled liposomes (lipid concentration 0.25 mM).

One sensing configuration is depicted in Scheme 1A. The thiolated primer (**1**) is assembled on a Au-quartz crystal (AT-cut, 9 MHz) and acts as the sensing interface.[13] Interaction of the functionalized transducer with the 27-mer analyte DNA, **2**, results in the double-stranded (ds) assembly. A liposome functionalized with oligonucleotide **3**, which is complementary to the 3′-terminus of the analyte-DNA, acts as the amplifying label.[14] Association of the oligonucleotide-tagged liposome with the oligonucleotide−DNA recognition complex amplifies the sensing event. Figure 1A shows the QCM transduction of the amplified sensing of the analyte. Interaction of the functionalized crystal with the analyte

(1) Wilson, E. K. *Chem. Eng. News* **1998**, *76*, 47−49. Mikkelsen, S. R. *Electroanalysis* **1996**, *8*, 15−23.
(2) (a) Bard, A. J.; Rodriguez, M. *Anal. Chem.* **1990**, *62*, 2658−2662. (b) Millan, K. M.; Mikkelsen, S. R. *Anal. Chem.* **1993**, *65*, 2317−2323. (c) Yang, M.; McGovern, M. E.; Thompson, M. *Anal. Chim. Acta* **1997**, *346*, 259−275.
(3) Piunno, P. A. E.; Krull, U. J.; Hudson, R. H. E.; Damha, M. J.; Cohen, H. *Anal. Chim. Acta* **1994**, *288*, 205−209.
(4) (a) Lidberg, B.; Nylander, C.; Lundström, I. *Sens. Actuators* **1983**, *4*, 299−302. (b) Jonsson, V. *Biotechniques* **1991**, *11*, 620−624.
(5) (a) Wang, J.; Palecek, E.; Nielson, P. E.; Rivas, G.; Cai, X. H.; Shiraishi, H.; Doutha, N.; Luo, D. B.; Farias, P. A. M. *J. Am. Chem. Soc.* **1996**, *118*, 7667−7670. (b) Ihara, T.; Nakayama, M.; Murata, M.; Nakano, K.; Maeda, M. *Chem. Commun.* **1997**, 1609−1610.
(6) Korri-Youssoufi, H.; Garnier, F.; Srivastava, P.; Godillot, P.; Yassar, A. *J. Am. Chem. Soc.* **1997**, *119*, 7388−7389.
(7) Hashimoto, K.; Ito, K.; Ishimori, Y. *Anal. Chem.* **1994**, *66*, 3830−3833.
(8) Millan, K. M.; Sarullo, A.; Mikkelsen, S. R. *Anal. Chem.* **1994**, *66*, 2943−2948.
(9) Bardea, A.; Patolsky, F.; Dagan, A.; Willner, I. *Chem. Commun.* **1999**, 21−22.
(10) Patolsky, F.; Katz, E.; Bardea, A.; Willner, I. *Langmuir* **1999**, *15*, 3703−3706.
(11) Caruana, D. J.; Heller, A. *J. Am. Chem. Soc.* **1999**, *121*, 769−774.
(12) Bardea, A.; Dagan, A.; Ben-Dov, I.; Amit, B.; Willner, I. *Chem. Commun.* **1998**, 839−840.

(13) A QCM analyzer (Applied Biosensors, Sweden) equipped with a frequency counter (Fluka 164T) and a flow cell (0.48 mL) was used. Oligonucleotides including thiolated oligonucleotides were custom ordered (Genset, France). The surface coverage of the Au-surface was estimated to be 4.6×10^{-11} mol·cm^{-2} by following the frequency changes of the crystal upon modification.

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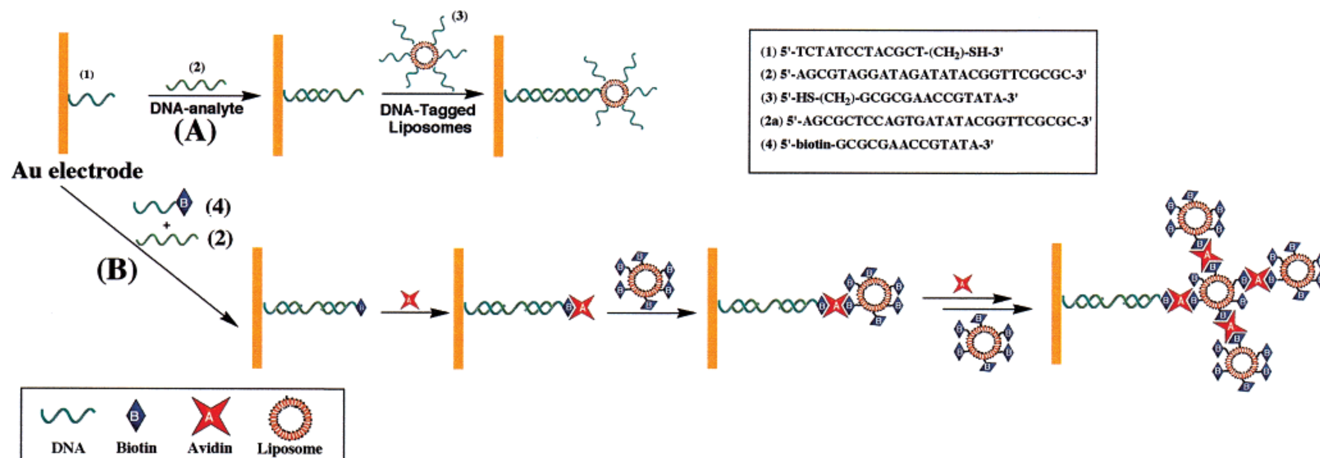
Scheme 1. Microgravimetric Amplified Assay of a Target DNA by an Oligonucleotide-Functionalized Liposome (A) and by an Avidin/Biotin-Functionalized Liposome (B)



(**2**, 5×10^{-6} M (step a)) results in a frequency decrease of $\Delta f = -17$ Hz, implying a surface coverage of the analyte corresponding to 1.2×10^{-11} mol·cm^{-2}. Further reaction of the resulting surface with the **3**-tagged liposome (step b) results in a substantial decrease in the crystal frequency, $\Delta f = -120$ Hz. Figure 1A shows also the control experiment, where the sensing interface was interacted with the noncomplementary DNA, 5×10^{-6} M (**2a**), (step c) and the resulting interface is subsequently treated with the **3**-tagged liposome, step d. The crystal frequency is unchanged, $\Delta f = \pm 2$ Hz, upon interaction with the noncomplementary DNA, **2a**. Interaction of the resulting interface with the tagged liposome slightly alters the crystal frequency, $\Delta f = -5$ Hz. This frequency change may be attributed to minute nonspecific binding of the liposome to the interface. Thus, the results demonstrate the specific and selective sensing of the analyte-DNA and the amplification of the recognition event by the tagged liposome. At a bulk concentration of the analyte DNA (**2**), corresponding to 5×10^{-9} M, hybridization to the serving interface results in a frequency change of $\Delta f = -2$ Hz, which is within the noise level of the instruments. Association of the amplifying **3**-tagged liposome with the interface results in a frequency change of $\Delta f = -70$ Hz (Figure 1A, inset), which allows the easy amplified detection of **2**. The lower sensitivity limit for the detection of **2** by this amplification method was estimated to be 5×10^{-12} M ($\Delta f = -20$ Hz after treatment with the **3**-tagged liposome). The frequency changes of the crystals after treatment with different concentrations of the analyte (**2**) and the **3**-tagged liposomes reveal a nonlinear behavior. While in the concentration range 5×10^{-6} to 5×10^{-8} M, a constant frequency decrease is observed, $\Delta f = -120$ Hz, implying saturation of the sensing surface, the crystal frequency changes from $\Delta f = -10$ Hz at 1×10^{-12} M of **2** to $\Delta f = -110$ Hz at 5×10^{-8} M.

The second configuration of sensing the analyte (**2**) is outlined in Scheme 1B. The **2**-analyte solution is treated with the biotinylated oligonucleotide, **4**, which is complementary to the 3′-end of the analyte. Interaction of the **1**-functionalized crystal with the analyte/**4** complex results in the tri-component biotinylated assembly on the transducer. Subsequent binding of avidin followed by the association of the biotin-tagged liposome amplifies the sensing process. A further amplification step may be designed by the interaction of the resulting assembly with avidin and then with the biotin-tagged liposome.[15] Figure 1B shows the QCM analysis of **2**, 5×10^{-6} M, by this amplification route: Association of the analyte/**4** ds-system to the sensing interface results in a frequency decrease of ca. 25 Hz (step e). Binding of avidin to the biotinylated assembly yields a frequency change of $\Delta f \sim -50$ Hz (step f). Linkage of the biotin-tagged liposome to the system amplifies the primary association of **2**, and a very high frequency change, $\Delta f \sim -500$ Hz, is observed (step g). Additional treatment of the interface with avidin, $\Delta f \sim -50$ Hz (step h), and then with the biotin-labeled liposome (step i) results in a second amplification corresponding to $\Delta f = -690$ Hz. Note that the amplification in the second step is higher than that in the first step, due to the multiligation ability of avidin for the biotinylated liposome. The sensing of **2** is specific, Figure 1B. Treatment of the sensing interface with the noncomplementary DNA, **2a**/**4** complex, does not yield any significant frequency change (step j) and subsequent interaction of the resulting assembly with avidin and the biotin-tagged liposome results in a frequency change of only ca. −30 Hz, (steps k and l, respectively) that is attributed to the nonspecific association of the liposome to the interface. Using the two-step amplification route, the lower sensitivity limit for sensing of **2** is 1×10^{-13} M (or 1×10^{-16} mol·mL^{-1}). Note that by additional binding steps of the avidin−biotinylated liposome the sensitivity of the analysis could be further enhanced.

In conclusion, we have addressed a novel approach for the specific and highly sensitive detection of DNA using oligonucleotide-tagged liposomes and biotin-tagged liposomes as amplifying probes. The sensitivity of the sensing method rests on the fact that the bound liposomes cover a substantial area (footprint ca. 4.2×10^4 nm^2), and thus only a few recognition events of the analyte DNA at the sensing sublayer may lead to a detectable surface coverage by the liposome. Although the reported sensitivity is impressive, a further dilution of analyte samples may lead to unrealistic detection time intervals needed to stimulate the hybridization process. Nonetheless, the availability of small QCM flow cells (5−10 μL) suggests that the sensitivity of the system may be further enhanced.

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(14) The oligonucleotide-functionalized liposome consists of phosphatidic acid, phosphatidyl choline, maleimide-phosphatidylethanolamine, and cholesterol (marked with ^{3}H-cholesterol, 45 Ci·mol^{-1}) at a ratio of 79:20:1:0.1. The liposomes were modified with **3** (4 °C, 20 h) and were purified by gel chromatography (Sephadex G-75). The surface coverage of liposome with **3** (50−60 oligonucleotide units per liposome) was determined by reacting the resulting liposomes with OliGreen (Molecular probes) and following the fluorescence intensity of the resulting liposome assay. The size of the liposomes was determined by dynamic light-scattering to be 220 ± 20 nm.

(15) The biotin-functionalized liposomes are composed of phosphatidyl choline, phosphatidylethanolamine, cholesterol (marked with ^{3}H-cholesterol, 45 Ci·mol^{-1}), and biotinylated phosphatidylethanolamine with a ratio corresponding to 80:20:0.1:0.5. The average coverage of each liposome with biotin units corresponds to 550. The biotin-labeled liposomes were purified by gel chromatography (Sephadex A-25). The size of the liposomes was determined by dynamic light scattering and corresponds to 180 ± 40 nm.