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Study of ssDNA immobilization and hybridization on gold substrate with quartz crystal microbalance

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The aim of this study was to apply QCM for the registration of synthetic ssDNA fragments binding to SAM and for DNA hybridization. 5'-amino-alkyl-oligonucleotides were successfully covalently immobilized onto SAM / Au via carboxylate ester linkages, and we observed a reduction in the frequency of 91 Hz. For duplex formation, the oligo-50mer after incubation with 10× ligation buffer was placed on an ssDNA-modified surface, and we observed a reduction in the frequency of 112 Hz. It means that covalently we immobilized 629 ng/cm^2 of 5'-amino-alkyl-oligonucleotides. After the duplex formation, the gold-coated quartz mass increased by 776 ng/cm^2.

Key words: QCM, ssDNA, DNA sensor

INTRODUCTION

In the past few years, the immobilization of DNA strands on electrode surfaces of different types has been the subject of the development of DNA sensors or DNA chips [1, 2]. Different methods for immobilizing DNA onto electrode surfaces have been used, including controlled potential adsorption [3], adsorption of DNA by evaporation of solvent during drying of DNA solution [4], direct covalent binding [5], entrapment in a polymer matrix [6] or indirect binding by the use of intermediate systems [2], through self-assembly of mercapto-modified DNA [7]. Among other sensing devices, piezoelectric quartz crystals are suitable for a direct and label-free real-time monitoring of affinity interaction of biomolecules [8]. The quartz crystal microbalance (QCM) is an ultra-sensitive weighing device which utilizes the mechanical resonance of piezoelectric single-crystalline quartz. QCM is capable of measuring mass changes as small as a fraction of a monolayer or a single layer of atoms. The decrease of the resonance frequency correlates to the mass accumulated on quartz crystal surface [9]. QCM is used for detection of specific antigens [10], for the hybridization between differently immobilized DNA probes and complementary DNA strands [11, 12], for the characterization of specific interactions between proteins and phages [13].

In recent years, the use of self-assembled monolayers (SAMs) in various fields of research is growing rapidly. Many biomedical fields apply SAMs as an interface layer between a metal surface and a solution. Au(III) is most often used for the formation of monolayers, because it is reasonably inert. The aim of this study was to apply QCM for the registration of binding of synthetic 5'-amino-alkyl ssDNA fragments to carboxyl-terminated SAM and for DNA hybridization. The detection and quantification of specific DNA sequences is of great importance in numerous applications, such as medical research and clinical diagnosis, environmental monitoring and food analysis.

MATERIALS AND METHODS

Materials. 11-mercaptoundecanoic acid (MUA) was received from Sigma-Aldrich (Sternhein, Germany), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were received from Alfa Aesar (Heysham, United Kingdom). Absolute methanol, Tris-(hydroxymethyl)-aminomethane, sodium acetate, sodium chloride were received from Carl Roth (Karlsruhe, Germany). Synthetic ssDNA oligonucleotides 5'-H_2N-$(CH_2)_6$-GTA-AAA-CGA-CGG-CCA-GT

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(Mr 6444) (5'-amino-alkyl-oligo), 5'-CCG-TCG-TTT-TAC-$(T)_{26}$-ACT-GGC-CGT-CGT (Mr 15195) (oligo-50mer) purified by HPLC were obtain from MWG-Biotech AG (Ebersberg, Germany). All the chemicals used were of analytical grade. All the solutions were prepared using deionized water purified with a Millipore S.A. water purification system (Molsheim, France). Buffers were filtrated through a 0.22 μm nylon filter (Nalgene, NY, USA).

Aparatus. Microgravimetric measurements were performed by QCM CHI400A (CH Instruments, Austin, USA) using 8 MHz AT-cut quartz crystal disc (13.7 mm in diameter and gold-plated on both faces). The crystal was installed in a cell connected to the oscillation circuit. QCM measurements were carried out using a frequency counter connected to a PC. The measured frequency (counted to an accuracy of ±0.01 Hz) was transferred to the PC every 0.5 s.

SAM monolayer formation and activation on the crystal surface. Quartz crystal was placed in a cell. A monolayer of SAM was formed on the gold surface of the crystals by placing 250 μl of 1 mM solution of the 11-mercaptoundecanoic acid prepared in absolute methanol for 4 h at room temperature. This procedure was used to produce a carboxylic groups-terminated monolayer. After washing with 50 mM sodium acetate, pH 7.4 (washing buffer), activation of carboxyl groups was performed by adding 200 μl of 0.1 M EDC and 0.4 M NHS mixture at a ratio 1:1 for 20 min at room temperature. The most important steps of the immobilization procedure are presented in Fig. 1. These agents enhance the stability of the monolayer and facilitate the formation of an intermediate to condense 5'-amino-alkyl-modified oligonucleotides.

ssDNA immobilization and DNA duplex formation on SAM-modified quartz crystal surface. A SAM-modified QCM crystal was washed with washing

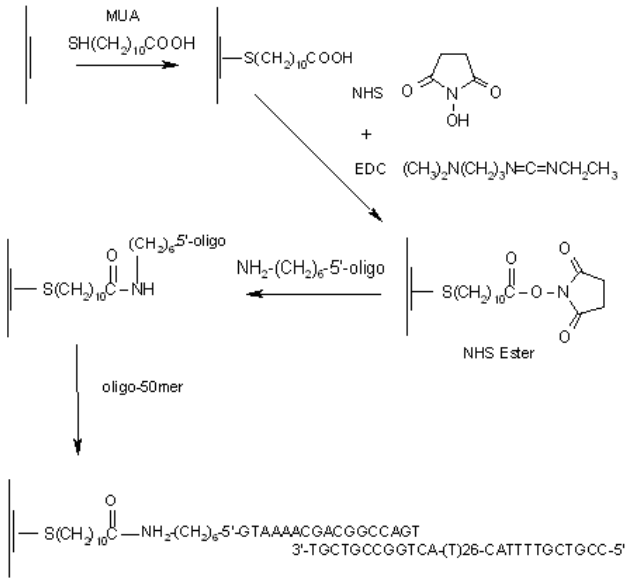

Fig. 1. Schematic diagram showing the procedures performed for prolonged DNA chain formation on a quartz crystal surface

solution. 200 μl of 5'-amino-alkyl-oligonucleotide solution (22 pM) was placed over the activated crystal surface after 100 s. For duplex formation, the oligo-50mer (final concentration 9 pM) was incubated with 10× ligation buffer (1 M sodium chloride, 0.1 M Tris-HCl, pH 7.4) at 70 ˚C for 10 min. After washing, 100 μl of oligo-50mer solution was placed over the crystal surface. In all cases the frequency responses were recorded for 40 min.

RESULTS AND DISCUSSION

SAMs formed by alkanethiols on a gold surface were commonly used as a functionized monolayer to immobilize biological compounds because of the formation of a strong metal–thiolate bond with no oxide formation at the electrode surface [14–16]. The formation of SAM on the gold electrode is based on the chemisorption of the sulfur atom of thiols on the metal through metal-thiolated bonds. Although the hydroxyl-terminated SAM is a better substrate for the covalent immobilization of dsDNA on gold surface [14], carboxyl-terminated SAM was used in our study. Immobilization of 5'-amino-alkyl oligo can be achieved after activating carboxyl acid groups. Because the co-addition of EDC and NHS had been shown to improve the stability of the linker compounds [17], these reagents were used in our work. It is very important to use the linker compounds that are stable, easy to prepare, and form a strong link between the SAM and the ssDNA. A schematic diagram showing the procedures performed for the immobilization of ssDNA onto carboxyl groups--terminated SAM on a gold-plated on the quartz crystal and prolonged chain formation is presented in Fig. 1. The 5'-amino-alkyl-oligonucleotides were successfully covalently immobilized onto SAM / Au via carboxylate ester linkages and the frequency response was recorded for a total of 4 h. A QCM is a very sensitive equipment for mass measurements in aqueous solution, because its resonance frequency decreases linearly upon increasing of the mass on the QCM electrode on the nanogram scale. Figure 2 shows a relationship between the frequency shifts (Hz) and the added oligonucleotide mass. Addition of 5'-amino-alkyl-oligonucleotides to the crystal caused a reduction in frequency of 91 Hz. It means that the mass of the quartz crystal increased and covalently we immobilized 129 ng (629 ng/cm^2) of 5'-amino-alkyl-oligonucleotides. As one can see in the figure, supplementary addition of a long oligonucleotide to the modified crystal surface (crystal/SAM/5'-amino-alkyl-oligonucleotides) caused an additional reduction in frequency of 112 Hz, and after duplex formation the mass of the quartz crystal additionally increased by 159 ng (776 ng/cm^2). Frequency changes (mass increase) as a function of time were registered and analyzed. The most appropriate exponential decay model for binding the affinity reagents [18], well describing the interaction of affinity ligands, was fitted for both interaction

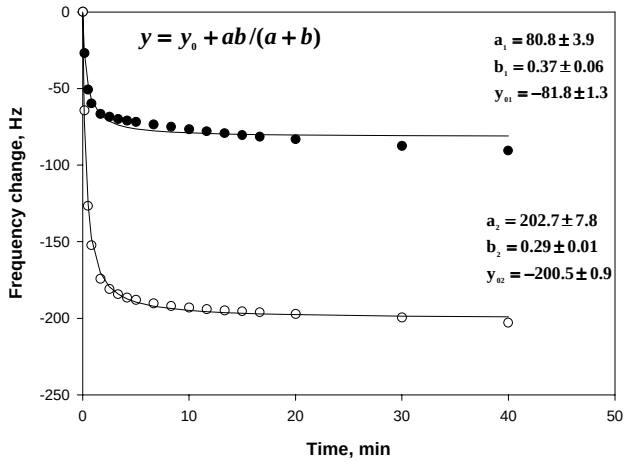

Fig. 2. Binding of synthetic DNA fragments: **A** – 5'-amino-alkyl-oligo; **B** – oligo-50mer in ligation buffer

curves (Fig. 2) which show that 95% of ssDNA binds to monolayer and towards complementary ssDNA within 6.7 min and 7.5 min, respectively.

In conclusion, our results show that QCM can be applied for the registration of hybridization of specific ssDNA fragments with immobilized complementary ssDNA. For this purpose, steady-state QCM signal registration might be applied for the calculation of interacting DNA masses, or the QCM signal change dynamics might be exploited for calculating DNA duplex formation kinetics. Note that the flexibility of the formed ssDNA and DNA duplex was not accounted for, and the calculated QCM crystal mass increase might be slightly shifted towards the higher values, because the flexibility of immobilized ssDNA and formed DNA duplex chains reduces the influence on Δf as compared with the same mass directly (not via flexible chains) deposited on quartz crystal. Both the here reported QCM detection of DNA hybridization and the previously reported electrochemical (pulsed amperometric) detection [6] are suitable for detection of a DNA hybridization event. Our further investigations in this field will involve electrochemical quartz crystal microgravimetry (EQCM), since this method might give simultaneous information on changes in mass and electrochemical properties of ssDNA-modified quartz crystal and thus allow to investigate DNA hybridization in more detail.

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J. Kupčiūnaitė, A. Kaušaitė, A. Ramanavičius, A. Ramanavičienė

PJEZOELEKTRINIO JUTIKLIO TAIKYMAS ĮVERTINANT VIENGRANDĖS DNR IMOBILIZAVIMĄ AUKSO PAVIRŠIUJE BEI DNR HIBRIDIZACIJĄ

S a n t r a u k a

Auksu padengto kvarcinio osciliatoriaus kristalo paviršiuje buvo suformuotas savaime besikaupiantis sluoksnis. Po papildomos šio sluoksnio aktyvacijos buvo sėkmingai imobilizuota viengrandė DNR; vėliau stebėjome dvigrandės DNR susidarymą papildomai pridėję DNR sekas, turinčias komplementarias sritis. Kvarcinio osciliatoriaus kristalo svyravimo dažnių pokyčiai, kurie tiesiogiai priklauso nuo padidėjusios masės kristalo paviršiuje, buvo registruojami pjezoelektriniu jutikliu. Pagal tai buvo apskaičiuota po kiekvieno etapo prisijungusios medžiagos masė ir/arba tankis paviršiaus ploto vienetui. Parodyta galimybė taip modifikuotus sensorius taikyti nustatant specifines DNR sekas.