A Quartz Crystal Microbalance (QCM) Study of Single-Strand DNA Hybridization and Hydrolytic Cleavage

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Abstract—The use of a quartz crystal microbalance (QCM) for monitoring in situ the immobilization of single-strand DNA marked with mercaptol group at the 5’-end on the surface of a gold-filled 7.995 MHz AT-cut quartz crystal by Au–S bond with the self-assembly technique is reported. The hybridization of ssDNA with complementary 10-mer ODN and 8-mer ODN is described. The QCM was also employed to analyze DNA cleavage by cerium(IV) ions under moderate conditions. The results showed that the QCM, which is capable of sensitive measurement, was able to investigate the immobilization, hybridization, and cleavage of ssDNA in situ. The cerium(IV) ions produced no cleavage in double-strand DNA; they were, however, able to hydrolyze single-strand DNA. Thus, the hydrolytic cleavage of ssDNA at a specific site could be ensured by protective hybridization.

INTRODUCTION

The recognition and cleavage of specific sequences of DNA are important experimental techniques in molecular biology, genetics, and medicine [1]. Generally, the methods used for the detection of DNA include radioisotope labels, polymerase chain reactions (PCRs), electrophoresis, and so on. As it does not require the use of specific indicators, the quartz crystal microbalance (QCM) is a promising candidate for biosensor applications. QCM is a new technique for the detection of a microchange on an electrode’s surface. It has become one of the most effective techniques of molecular biology and microchemistry because it offers the advantages of simple, rapid, and real-time monitoring, as well as high sensitivity and specificity [2–6]. The QCM method is based on the Sauerbey equation; the mass change (∆m) is determined by the frequency variety of the QCM. Thus, the immobilization, hybridization, and hydrolytic cleavage of DNA can be monitored in situ from the change in QCM frequency [7–10].

In this work, we used QCM to investigate the immobilization, hybridization, and hydrolytic cleavage of ssDNA in situ.

One sensor configuration is depicted in the following scheme:

EXPERIMENTAL

Materials. A 20-mer oligodeoxynucleotide (20-mer ODN) probe was modified with a mercaptol group at the 5’-phosphate end (HS-(CH₂)₆-GGAGGCGAAC-GATACGCATG; ss-DNA). Unmodified 10-mer oligodeoxynucleotide (10-mer ODN) (5’-GTTCGCTCCGATCCGATCT-3’) and 8-mer oligodeoxynucleotide (8-mer ODN) (5’-CATGCGTA-3’) were both complementary to the probe DNA. (All chemicals were supplied by the Shanghai Sangon Biological Engineering Technology and Service Co., Ltd.) Piranha solution was prepared by mixing one part of 30% H₂O₂ with three parts of 98% H₂SO₄ [6, 7]. All of the reagents were commercially available and of analytical reagent grade. Twice-distilled water was used in all solutions.

QCM apparatus. The quartz crystal resonators were AT-cut with a fundamental frequency of 7.995 MHz. Au (spots 5.1 mm in diameter) was deposited on both sides of the crystals (area, 0.196 cm² × 2). All measurements were carried out in a Model 400 time-resolved electrochemical quartz crystal microbalance (EQCM,
Preparation of QCM surface. The gold QCM surface was firstly ultrasonically cleaned with a 15-min exposure to ethanol. Fresh “piranha” solution was used repeatedly to rinse it for about 30 min; this was followed by rinsing with acetone, ethanol, and twice-distilled water, in that order. The surface was then dried with nitrogen. (Note that piranha solution is volatile and must be handled with extreme care; only small volumes should be prepared at any one time.) In all cases, the QCM crystals were used immediately after preparation.

Immobilization of ssDNA on QCM. The Au-electrode surface of the QCM plate was functionalized by the surface assembly of mercaptol oligonucleotide. The quartz crystal electrode was placed into a Teflon cell immediately after being well cleaned. The surface was modified with a 0.3 mol/L NaCl aqueous solution immediately after being well cleaned. The surface was then dried with nitrogen. (Note that piranha solution is volatile and must be handled with extreme care; only small volumes should be prepared at any one time.) In all cases, the QCM crystals were used immediately after preparation.

Hybridization on QCM. The hybridization buffer solution (45 µL, pH 7.9, 10 mmol/L Tris–HCl buffer solution) was placed on the probe-immobilized electrode inside the Teflon cell. Once the resonant frequency had stabilized, 5 µL 10-mer ODN with the same concentration as the target DNA was injected into the solution, and the frequency change was monitored until a stable value was obtained. The time-dependent frequency changes of the QCM were thus recorded in around 3 h.

The electrode surface was cleaned using the same method as before. The hybridization buffer solution was then put onto the electrode as described for the preceding hybridization. After the frequency became steady, the second hybridization was performed by spiking aliquots of 8-mer ODN solutions into the previous solution and real-time monitoring was carried out by the QCM.

Site-specific cleavage. The same manipulations were completed for the probe-immobilized electrode, which was used for hybridizations with the complementary 10-mer ODN and 8-mer ODN. Finally, the real-time monitoring of the specific cleavage was recorded.

RESULTS AND DISCUSSION

Single-strand DNA immobilization on QCM. The self-assembled membrane (SAM) method was used for single-strand DNA immobilization on the QCM. The probe DNA was covalently bound to the electrode surface via the strong bond of Au–S and formed a steady and ordinal DNA-membrane [11, 12]. We monitored this self-assembly process in our experiment. As is shown in Fig. 1, the frequency fell to ~80 Hz rapidly in the initial 800 s due to the self-assembly of 20-mer ODN on the surface of the QCM gold electrode. Following this, the frequency moved to the fixed value – (80 ± 2) Hz. This suggests that 20-mer ODN had assembled itself on the surface of the gold electrode, and the immobilized amount was (107 ± 3) ng.

DNA hybridization. The curve ABC in Fig. 2 shows the QCM frequency response versus time for the hybridization of 20-mer ODN with the complementary 10-mer ODN. When the 10-mer ODN was injected, the frequency fell with time and saturated at ∆F = −(43 ± 2) Hz within 3000 s. That is, the (57 ± 3) ng 10-mer ODN had bonded with the surface of the QCM gold electrode. The results indicate that the hybridization between the 10-mer ODN and the 20-mer ODN was complete.

According to [13, 14], cerium(IV) cannot hydrolyze double-strand DNA, but it does provide effective cleavage for single-strand DNA. Thus, hybridization