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In-situ Measurements of Single-stranded DNA Hybridization and Cleavage by Quartz Crystal Microbalance

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Abstract: The use of quartz crystal microbalance (QCM) for in-situ monitoring the immobilization of single-stranded DNA (oligo-1), marked with mercaptol group at 5′end, on the surface of gold-filled 7.995 MHz AT-cut quartz crystal by Au-S bond with self-assemble technique is reported. And then the hybridization of ssDNA with the complementary 10-mer ODN (oligo-2) and 8-mer ODN (oligo-3) is described. Moreover, the QCM has been employed to analyze the DNA cleavage by cerium (Ⅳ) ions under moderate conditions. The results showed that the QCM, which is capable of sensitive measurement, was able to in-situ investigate immobilization, hybridization and cleavage of ssDNA. And the cerium (Ⅳ) ions had no cleavage for the double-stranded DNA, but it could hydrolyze single-stranded DNA. So the hydrolytic cleavage of ssDNA at the specific site could be realized by the protection of the hybridization.

Key words: Quartz crystal microbalance, Oligodeoxynucleotide, Hybridization, Hydrolytic cleavage
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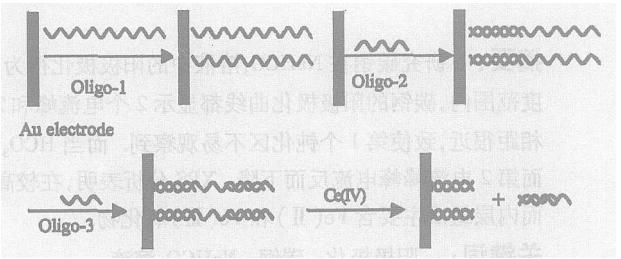
Generally, the methods used for the detection of DNA include radioisotope labels, polymerase chain reaction (PCR), electrophoresis and so on. Free of using specific indicators, the quartz crystal microbalance (QCM) is promising in biosensor applications. QCM is a new technique for the detection of the micro-change on the electrode surface. It has been one of the effective means in the molecular biology and microchemistry fields because of the advantages of simple, rapid and real-time monitoring, as well as high sensitivity and specificity[1~5]. And what′s more, the recognition and cleavage of specific sequence DNA were the important experimental technique for the molecular biology, genetics and medicine[6]. So the immobilization, hybridization and the hydrolytic cleavage of DNA can be monitored in situ from QCM frequency change[6~8]. In this communication, we employed QCM to investigate immobilization, hybridization and the hydrolytic cleavage of ssDNA in situ.

One sensing configuration is depicted in the following scheme.



1 Experimental

1.1 Materials

Oligo-1: HS-(CH_2)$_6$-5′GGAGGCGAACGAT-

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ACGCATG-3′, oligo-2: 5′-GTTCGCCTCC-3′ and oligo-3 5′-CATGCGTA-3′ were supplied by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. All the reagents were commercially available and of analytical reagent grade. Twice distilled water was used in all solutions.

Model400 time-resolved electrochemical quartz crystal microbalance (EQCM, CH Instrument, USA). The gold QCM surface was cleaned with the fresh piranha solution (30% H_2O_2 ∶98% H_2SO_4 = 1∶3[8~9]) and rinsed with twice distilled water several times. After each modified step, the film was rinsed with 10mmol·dm^{-3} Tris-HCl buffer (pH = 7.9) and twice distilled water. The mass change due to DNA modified, hybridization or cleavage on the surface of the QCM plate can be calculated the frequency change. For the QCM in this study, a frequency change of 1Hz corresponds to a mass increase of 1.34 ng. All the experiments were put into practice shielded in an operating box.

1.2 Immobilization of ssDNA on QCM and Hybridization

The Au-electrode surface of the QCM plate is functionalized by surface-assembly of the mercaptol oligonucleotide. The surface was modified with 5×10^{-6} mol·dm^{-3} oligo-1 for 2 h.

The twice hybridization experiments were performed by injected oligo-2 and oligo-3 into the probe-immobilized electrode, respectively. And all the frequency changes were monitored as a function of time.

1.3 Hydrolytic Cleavages on QCM

The spontaneous cleavage was carried out with adding the cleavage reagent (2mmol·dm^{-3} $(NH_4)_2$·$Ce(NO_3)_6$ was dissolved in 10 mmol·dm^{-3} Tris-HCl solution, pH = 7.9) onto the probe-immobilized electrode. The site-specific cleavage was completed by putting the cleavage reagent onto the probe-immobilized electrode which had the twice hybridizations. And the real-time monitoring of the two kinds of cleavage was recorded.

2 Results and Discussion

2.1 Single-stranded DNA Immobilization on QCM

The method of the single-stranded DNA immobilization on the QCM was the self-assemble membrane (SAM). The probe DNA was covalently bound to the electrode surface via the strong bond of Au-S and formed the steady and ordinal DNA-membrane[10,11]. As is shown in Fig. 1, the frequency decreased to -80 Hz rapidly in the initial 800s due to the self-assembly of oligo-1 on the surface of the QCM gold electrode. In the following time the frequency went to the fixed value -(80 ±2) Hz. The phenomenon suggests that oligo-1 has been modified onto the surface of the gold electrode and the immobilized amount was (107 ±3) ng.

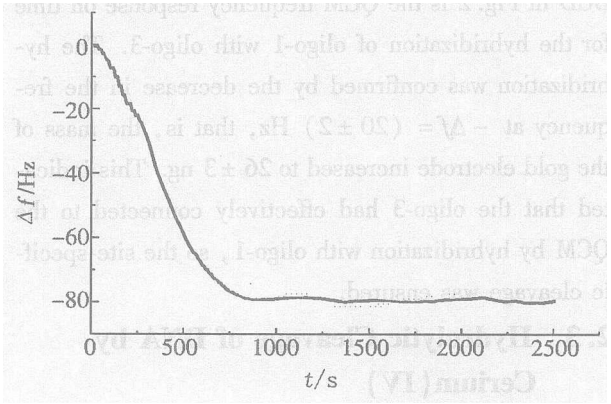

Fig 1　Frequency response on time for ssDNA (5×10^{-6} mol·dm^{-3}) modified on QCM electrode (25 ℃)

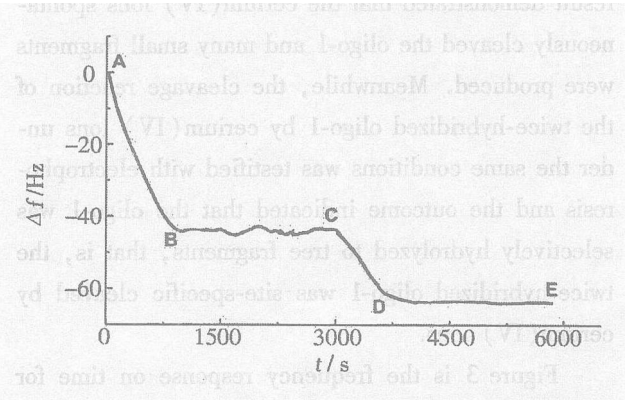

Fig 2　Frequency response on time for the hybridization oligo-2 (Curve ABC) and oligo-3 (Curve BCD) respective with ssDNA modified on QCM electrode (30 ℃)

2.2 DNA Hybridization

The Curve ABC in Fig. 2 shows the QCM frequency response on time for the hybridization of oligo-1 with oligo-2. When the oligo-2 was injected, the frequency decreased with time and saturated at $\Delta f = (43 \pm 2)$ Hz within 3 000 s. That is, the (57 ±3) ng oligo-2 had connected to the surface of the QCM gold electrode. And the result indicates that the hybridization between the oligo-2 and the oligo-1 was completed.

It was reported that cerium (Ⅳ) could not hydrolyze the double-stranded DNA, but it had an effective cleavage for the single-stranded DNA[12, 13]. So the hybridization between the oligo-1 and the oligo-3 was carried out in order to realize the site-specific cleavage of the oligo-1 by cerium (Ⅳ). The Curve BCD in Fig. 2 is the QCM frequency response on time for the hybridization of oligo-1 with oligo-3. The hybridization was confirmed by the decrease in the frequency at $-\Delta f = (20 \pm 2)$ Hz, that is, the mass of the gold electrode increased to 26 ±3 ng. This indicated that the oligo-3 had effectively connected to the QCM by hybridization with oligo-1, so the site-specific cleavage was ensured.

2.3 Hydrolytic Cleavage of DNA by Cerium(Ⅳ)

The electrophoresis was operated for the cleavage of oligo-1 by the cerium (Ⅳ) ions after 24 h and the result demonstrated that the cerium (Ⅳ) ions spontaneously cleaved the oligo-1 and many small fragments were produced. Meanwhile, the cleavage reaction of the twice-hybridized oligo-1 by cerium (Ⅳ) ions under the same conditions was testified with electrophoresis and the outcome indicated that the oligo-1 was selectively hydrolyzed to tree fragments, that is, the twice-hybridized oligo-1 was site-specific cleaved by cerium (Ⅳ) ions.

Figure 3 is the frequency response on time for the cleavage of DNA by cerium (Ⅳ) ions. The curve 1 represents the frequency change with time for the spontaneous cleavage of oligo-1. The curve 2 is the corresponding frequency to time for the site-specific

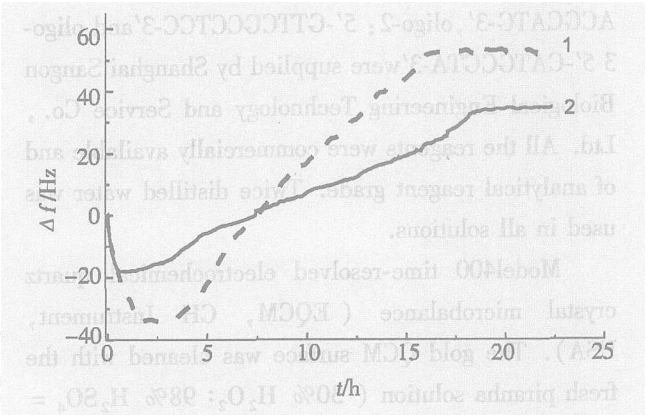

Fig. 3 Frequency response on time for the spontaneous cleavage (curve 1), the site-specific cleavage (curve 2) (30 ℃)

cleavage of oligo-1. It can be seen that the frequency changes of the two curves are accordant. And the frequency decreased rapidly initially, then slowly increased to a stable value within about 24 h. The phenomena were ascribed to the linkage of the cerium (Ⅳ) ions onto the oligo-1 and the hydrolysis of oligo-1 by the cerium (Ⅳ) ions which had been linked to it. The cleavage of DNA by cerium (Ⅳ) ions belonged to the hydrolytic cleavage[13, 14]. The possible mechanism was as follows. At around pH = 7.3, the cerium (Ⅳ) ions were prone to produce the metal hydroxide complex via the hydrolysis, and the hydroxide ion (-OH) attacked the phosphodiester linkages. At the same time, the cerium (Ⅳ) ions formed the coordinator with the phosphodiester linkages. The hydrolysis reaction was accelerated by the acidic catalysis from the water molecule bonded to the cerium (Ⅳ) ions. Furthermore, the cerium (Ⅳ) ions made the negative intermediate which came from the hydrolysis reaction of DNA stable with the electrostatic function. Consequently, the acid-base catalysis and the electrostatic function promoted the hydrolysis of the sequence-stranded DNA under moderate conditions[1, 13, 14].

Comparing curve 1 with curve 2, the larger frequency change in curve1 (-33 Hz) than in curve 2 was charged upon the more cleavable sites (the sites of the phosphodiester linkages) of the spontaneous cleavage than the site-specific cleavage. The more

cleavable sites, the more cerium (Ⅳ) ions were connected to the oligo-1, the decrease of the frequency was bigger and the cleavable function was more quick. So the spontaneous cleavage was almost completed within about 16 h (Δf = 53 Hz) and the site-specific cleavage spent 20 h (Δf = 35 Hz). The efficiencies of the spontaneous cleavage and the site-specific cleavage were 66% and 64%, respectively. And the possible reasons that caused the lower efficiencies than the ideal state (100%) were the mutual steric-block of the DNA chains which made it more difficult for cerium (Ⅳ) ions to combine to the phosphodiester linkages of oligo-1. However, the efficiency of the spontaneous cleavage and the specific cleavage are both high correspondingly.

3 Conclusion

The experimental results demonstrated that single-stranded DNA is immobilized on the surface of the gold electrode of the quartz crystal microbalance (QCM) by the Au-S bond and also hybridized efficiently with the complementary DNA chains. And what's more, the single-stranded DNA can be hydrolyzed at the spontaneous site or specific site by the rare-earth metal cerium (Ⅳ) under moderate conditions.

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杂交及切断过程的 QCM 实时检测研究

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摘要: 采用自组装技术,将 5′端标记有巯基的 20-merODN(oligo-1)以金-硫键形式牢固结合在 7.995MHz的 AT-切石英晶体的镀金表面,然后由石英晶体微天平实时检测了与碱基序列互补的 10-mer ODN (oligo-2)和 8-mer ODN(oligo-3)的杂交,同时还研究了稀土金属铈离子在温和条件下对 DNA 的水解切断作用. 结果表明:应用 QCM 方法可能实时检测 DNA 的固定和杂交, Ce(Ⅳ)能随机切断单链 DNA;但不能切断杂交形成的双链 DNA, 因此可利用杂交保护的方法对单链 DNA 实行定位切断.

关键词: 石英晶体微天平;单链脱氧核糖核酸;杂交;水解切断