High Sensitive DNA Detection Amplified by Enlarging Au-Nanoparticles in situ

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A new approach to amplify the micogravimetric quartz crystal microbalance (QCM) assay of DNA by enlarging Au-nanoparticles in situ has been reported. To avoid the interference of the non-specific enlargement effect mercaptoethanol was served as blocking reagent. The results show that the sensitivity of detecting DNA is remarkably increased to ca. 1×10^{-15} M.

The development of DNA-sensor has recently attracted considerable attention in connection with research efforts directed at gene analysis, identification of genetic disorders, tissue matching, and forensic applications.[1] QCM is a very sensitive mass-measuring device in gas phase and in aqueous phase. Free of using specific indicators, QCM is a promising candidate in biosensor applications. Its potential for the detection of DNA hybridization has recently been demonstrated.[2,3] The functionalized gold nanoparticles used as amplification tags to increase the detecting sensitivity have been attracted special attention[4] due to its special properties including ease of preparation, high density, large dielectric constant, and biocompatibility.[5]

For QCM detection, theoretically, the heavier the mass of the immobilized Au-nanoparticles on the surface of QCM plate, the more sensitive the detection of DNA. However, the Au-nanoparticles can easily aggregate during the preparation of Au-nanoparticles probe if its diameter is more than 30 nm,[6] that is a serious obstacle to further increase the sensitivity of DNA detection by QCM.

In the present work, we report a new approach to improve this situation. Based upon the colloidal Au surface-catalyzed reduction of Au^{3+} by NH_2OH,[7] the thiolated Au-nanoparticles immobilized on QCM plate are enlarged in situ by the seeding method.[8] The sensitivity of this method to detect DNA is significantly increased to ca. 1×10^{-15} M. The sequences of oligonucleotides used in the work are as follows:

S1: 5′-TCTATCCTACGCT-$(CH_2)_6$-SH-3′

S2: 5′-AGCGTAGGATAGATATACGGTTCGCGC-3′

S3: 5′-HS-$(CH_2)_6$-GCGCGAACCGTATA-3′

S2m: 5′-AGATTTGGATAGATATACGGTTCGCGC-3′

The principle of the method used in this study was shown in Figure 1. The Au-electrode surface of the QCM plate is functionalized by surface-assembly of the thiolated oligonucleotide S1 (surface coverage ca. 1.6×10^{-9} mol/cm^2). Then the target DNA S2 hybridizes with the functionalized surface. The Au nanoparticles were immobilized on the surface of QCM through the specific binding of the S3-functionalized Au-nanoparticles to the complementary target DNA S2. These immobilized Au

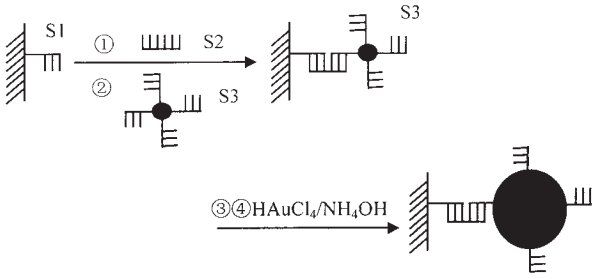

Figure 1. Schematic diagram of the amplified QCM assay of DNA by enlarging Au-nanoparticles in situ.

nanoparticles were then enlarged in situ by 0.01%$HAuCl_4$/0.04 mM NH_2OH (HN) twice. The preparation of the S3-functionalized Au-nanoparticles is according to the literature[9] by the reduction of citrate-stabilized Au-nanoparticles with the thiolated oligonucleotide S3.

While NH_2OH is thermodynamically capable of reducing Au^{3+} to bulk metal,[10] the reaction is dramatically accelerated by Au surfaces.[7] As a result, no new particle nucleation occurs in solution and all added Au^{3+} goes into production of larger particles. We choose 30 s for the following enlargement step one time since the enlarging reaction will be complete within 2 min.[8]

To avoid the nonspecific enlargement, therefore, an appropriate blocking reagent must be used to block the uncovered surface region of the Au-electrode of QCM prior to the hybridization. Herein, mercaptoethanol was served as blocking reagent (surface coverage 9.6×10^{-9} mol/cm^2). If the S1-functionalized surface was not treated with the blocking reagent, and was directly reacted with HN once, a frequency change of QCM was more than −800 Hz. In contrast, the frequency changes were only ca. -20 ± 5 Hz and ca. -55 ± 5 Hz after enlarging once and twice respectively, after blocking with mercaptoethanol. These results imply that there is uncovered surface region after treating the surface with S1 alone, which may induce the nonspecific enlargement to occur directly on the uncovered Au-electrode, and that mercaptoethanol can be used as blocking reagent to prevent effectively from the nonspecific enlargement effect.

Figure 2 shows the QCM frequency changes versus the concentration of the target DNA S2. The curve a in Figure 2 was obtained just after the hybridizing with 15 nm-Au-nanopaticle functionalized with S3 without treatment with HN. The frequency changes are ca. −50, −18, −13, and −3 Hz corresponding to the concentration of the target DNA S2 1×10^{-8}, 1×10^{-9}, 1×10^{-10}, and 1×10^{-11} M, respectively. After treatment once with HN, the frequency changes were ca. −242, −200, −124, −84, −30, and −22 Hz versus the concentration of the target DNA S2

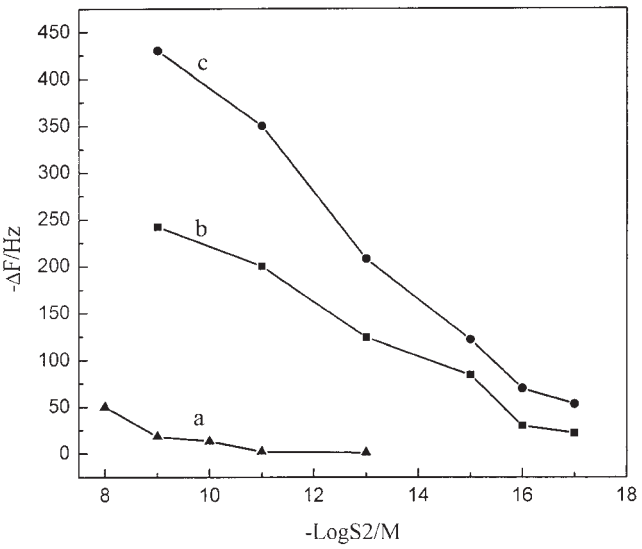

Figure 2. The frequency change of crystal of QCM. (a) after treatment of the assembled S1 sensing interface with S3-functionalized Au-nanoparticles prior to enlargement; (b) and (c) after enlarging the Au-nanoparticles immobilized on the surface of QCM plate by 0.01% $HAuCl_4$ and 0.04 mM NH_2OH for once and twice, respectively.

1×10^{-9}, 1×10^{-11}, 1×10^{-13}, 1×10^{-15}, 1×10^{-16}, and 1×10^{-17} M, respectively (Figure 2 curve b). The frequency changes are less than or comparable with the noise level (ca. -20 ± 5 Hz) when the concentration of S2 is less than 1×10^{-15} M, indicating the sensitivity to be 1×10^{-15} M. After treatment twice with HN, the corresponding frequency changes are further decreased to ca. -430, -350, -208, -122, -70, and -53 Hz (Figure 2 curve c). Comparing the frequency change with that of the noise level (ca. -55 ± 5 Hz), the sensitivity reaches ca. 1×10^{-16} M.

To address the nonspecific adsorption, the S1-functionalized surface was treated with the non-complementary target S2m at high concentration 1×10^{-7} M. Upon interaction with Sm2 and subsequently with S3-functionalized Au-nanoparticles, the QCM frequency was nearly unchanged within ± 2 Hz. The frequency change was about -25 Hz and -58 Hz after treating once and twice with HN, which is within the region of the noise level (ca. -20 ± 5 Hz and ca. -55 ± 5 Hz). These results demonstrate the specific and selective sensing of the functionalized interface to the target DNA S2.

In summary a novel method for the specific and high sensitive detection of DNA through the measuring the mass increase of Au-nanoparticles by enlarging them in situ by 0.01%$HAuCl_4$/0.04 mM NH_2OH was reported. This method can remarkably increase the sensitivity for QCM detecting DNA at least four orders (ca. 1×10^{-15} M). Detail investigation of the method is under investigation.

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