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Sensitivity enhancement of DNA sensors by nanogold surface modification

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Abstract

A novel amplified microgravimetric gene sensing system was developed using quartz crystal microbalance modified by gold nanoparticles anchored on its 1,6-hexanedithiol modified gold electrode surface, and ultrasensitive detection of DNA hybridization was accomplished at the level of at least 2×10^{-16} M.

Keywords: Colloidal Au; DNA sensing; Quartz crystal microbalanace

The microgravimetric quartz crystal microbalance (QCM) allows the real-time measurement of DNA binding and hybridization at the sub-nanogram level, and its potential for DNA hybridization detection has been demonstrated recently [1–3]. In order to broaden the application of this technique, methods for improving the detection limit of the device have been sought [4–6]. The use of DNA-capped gold nanoparticles as amplifier was shown as a promising means for sensitivity enhancement. The systems of a probe/target/probe-nanoparticle sandwich and a dendritic structure based on DNA-capped gold nanoparticles were developed by Zhou et al. [7], Willner and co-workers [8,9], as well as our group [10]. By these inventions, which can be named as "gold amplifier method," such DNA sensors was greatly improved and their sensitivity was approaching that of fluorescent sensors. However, enhancement of their sensitivity still remains as a task for future development. For example, our previous work [11] has proved that by using a 50 nm gold particle amplifier, a detection sensitivity of 10^{-14} mol/L could be obtained. Nevertheless, better result did not occurred when the size of gold nanoparticles further increased, because the weak bonding force could not keep the amplified particles on the sensor surface in the rinse condition when the particle size becomes larger. In addition, we [12] have also studied the immobilization and hybridization of DNA probes onto the gold-coated QCM surface, which was modified by 1,6-hexanedithiol, and approximately 3–5 times of adsorption enhancement of HS-DNA was obtained. In order to overcome the limitation of "amplifier method," these two techniques, i.e., nanoparticle modification of QCM surface and the application of gold nanoparticle amplifier were synchronously utilized here. The preliminary results showed that this approach yielded fairly satisfactory detection sensitivity.

Materials and methods

Reagents. All oligodeoxynucleotides were obtained from Shanghai Sangon Biological Engineering Technology. 1,6-Hexanedithiol [$HS(CH_2)_6SH$] was purchased from Fluka. All other chemicals such as sodium citrate and $HAuCl_4 \cdot 3H_2O$ were analytical regent grade and obtained from Beijing Chemical Reagent Company. The colloidal gold nanoparticles with an average diameter of 20 nm were prepared according to the literature [13] by reduction of $HAuCl_4$ with sodium citrate aqueous solution. DNA:Au conjugate was prepared by the method that was previously reported by Mirkin et al. [14].

DNA immobilization on modified Au QCM and hybridisation. Au QCM was modified with a 0.5%(v/v) solution of 1,6-hexanedithiol in ethanol for 30 min. Immobilization of 20 nm Au nanoparticles onto thio-modified surfaces was performed in aqueous Au colloid for 1 h at room temperature. Then the plate surface was treated with a 2×10^{-6} mol/L solution of HS-DNA (PBS buffer, pH = 6.83) for 1 h, and the 17 mer target DNA 2 was hybridized on the sensing interface

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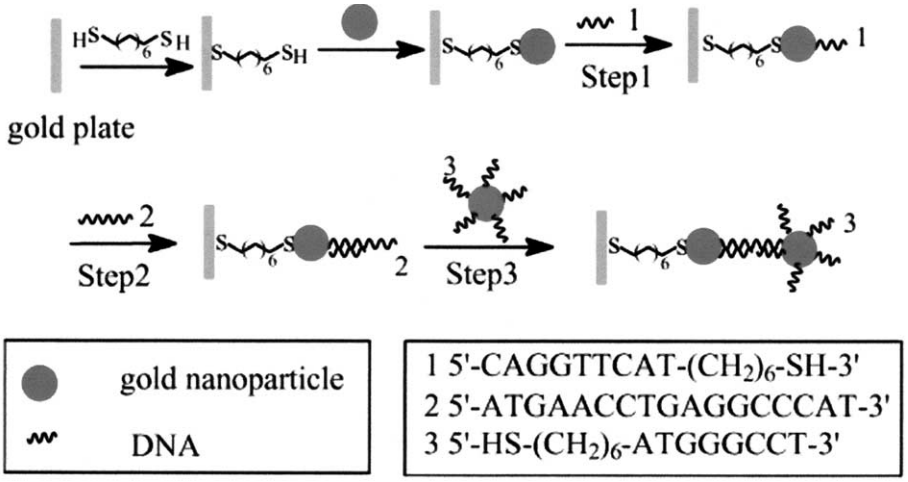

Scheme 1. Schematic illustration of the sensing process of the amplified system based on Au nanoparticle-covered QCM surface.

for 2 h at 40 °C. The amplification step of the sensing process was subsequently accomplished by the interaction of the surface with the Oligo-3 functionalized Au-nanoparticles. The principle of this method is shown in Scheme 1.

Results and discussion

Fig. 1 illustrates the outstanding sensitivity enhancing effect of the sensing system by comparing the mass changes of DNA hybridization on the nanoparticle-covered QCM surface with that of non-modified QCM crystal. After 1,6-hexanedithiol was employed as a medium to assemble to the QCM surface for 30 min, a little mass increase was observed in most cases. Some Au atoms may fall off from the QCM surface due to corrosion effect of the short-chain thiol [15], so that the little mass changes are reasonable. Then (50 ± 3) ng out of the added 20 nm colloidal Au was immobilized because of the adsorption of Au nanoparticles onto the dithiol-modified surface. Curve (i) c in Fig. 1 indicates that HS-DNA adsorption on the coarse nanoparticle-modified surface affords a mass increase of ca. 107 ng, which is much larger than that of direct DNA immobilization on QCM surface (ca. 28 ng, curve (ii) c). Since more HS-DNA can be immobilized, the subsequent processes, i.e., hybridization of target DNA (2×10^{-16} M) with the probe and the signal amplification with Oligo-3-capped gold nanoparticles, afforded mass changes as large as 93 ng in stage d and 164 ng in stage e, respectively. In view of the corresponding steps directly performed on the bare QCM surface only causing mass changes of 4 ng in stage d and 7 ng in stage e, the sensitivity in our experiment was significantly improved.

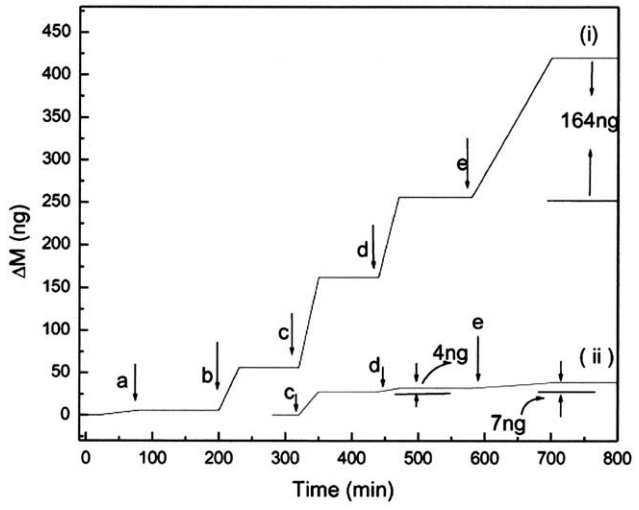

Fig. 1. Time-dependent mass increase (Δm) of the procedure on QCM surface: (i) Immobilization and hybridization of DNA probes onto the Au nanoparticle-covered QCM, which was prepared by anchoring gold nanoparticles on the QCM surface through 1,6-hexanedithiol, and (ii) directly anchoring of oligodeoxynucleotide 1 on the gold surface of QCM. (a) QCM surface modification with 1,6-hexanedithiol according to the reported procedure. (b) Immobilization of 20 nm gold particles on the modified QCM surface. (c) Oligo-1 DNA (2×10^{-6} mol/L) immobilization on the gold surface. (d) Hybridization of Oligo-2 and Oligo-1 DNA. (e) Amplification with Oligo-3-functionalized gold nanoparticles with the size of 20 nm.

Additional experiments [16] were also carried out to ensure that the enhancing result was not affected by the nonspecific adsorption of Oligo-3 on the gold surface. If the Oligo-1 covered nanogold surface was treated with the Oligo-3 without the hybridization step of the target Oligo-2, only negligible mass change less than 10 ng was observed, which was near to the measurement error. Therefore it is believed that the mass change in stage e of Fig. 1 was contributed by the specific amplification rather than the nonspecific adsorption.

Conclusion

In conclusion, we have developed a method to enhance the DNA detection sensitivity based on the

"amplified microgravimetric effect" by modifying the QCM surface with gold nanoparticles. The detection sensitivity of 10^{-16} M was afforded, which is the highest sensitivity to our knowledge. Mediating the gold nanoparticle size both for surface modification and mircogravimetric amplification may further enhance the sensitivity of this kind of sensors.

Acknowledgments

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