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The enhancement effect of gold nanoparticles as a surface modifier on DNA sensor sensitivity

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Abstract

The enhancement of a single strain DNA probe linked to the sensor surface is of crucial importance in DNA molecule recognition. By means of nanogold modification of the sensor surface in addition to the nanogold amplifier, DNA detection sensitivity higher than 10^{-16} mol/L was obtained in a Quartz Crystal microbalance (QCM) system, much higher than the ordinary QCM sensor without surface modification by nanogold.

Keywords: Colloidal Au; DNA sensor; Quartz crystal microbalance

Many DNA devices [1], such as DNA chips, DNA sensors, and DNA computers, are based on the principle of the hybridization of single strain DNA as a probe fixed on a substrate and the target DNA in the detected solution. Many techniques are currently available, such as optical DNA detection by using fluorescence-labeled oligonucleotides [2], application of surface plasmon resonance (SPR) spectroscopy [3], and direct electrochemical assay [4] of double-stranded DNA. Quartz crystal microbalance (QCM) [5] is one of the important techniques developed recently for DNA sensing [6]. The factor that is of utmost importance in all the above-mentioned methods of analysis and detection is the hybridization strength of the DNA probe with the target cDNA, which ensures the accuracy and sensitivity of the DNA devices.

In QCM detection technology, a method known as the "nanogold amplifier method," where DNA-capped gold nanoparticles are used as an amplifier, was developed by Willner [7] and Zhou [8], as well as by our group [9]. This method has shown a great advantage in its improvement of the detection limit. The highest detection sensitivity obtained was 10^{-14} mol/L DNA [7,10]. In our experiment, the QCM plate was dipped into a solution of oligo-1 (5′-CAGGTTCAT-$(CH_2)_6$-SH-3′) DNA to make a DNA probe on the sensor. After 4 h contact for adsorption, the plate was washed successively with phosphate buffer solution (pH 6.83) and water, and then immersed into an oligo-2 (5′-AT GAACCTGAGGCCCAT-3′) solution, the target DNA. One hour later, a few drops of the solution of gold nanoparticles functionalized of oligo-3-(5′-HS-$(CH_2)_6$-ATGGGCCT-3′) were added to the surface terminated with the target DNA molecules. The oligo-1 and oligo-3 were complementary to the 5′ end and the 3′ end of the target oligo-2, respectively. Although the sensitivity of DNA sensors has been greatly improved by this "nanogold amplifier method" and the effect increases as the particle size increases, when the size of gold amplifier further increases, the detection limitation still decreases after a certain size is reached [11]. In order to overcome this difficulty and to understand the nature of this phenomenon, this study investigates nanogold surface modification.

Materials and methods

Reagents. All chemicals were commercially available and used as received. The 3′-(alkanethiol)-oligonucleotide 1 (9 mer, oligo-1), 5′-(Alkanethiol)-oligonucleotide 3 (8 mer, oligo-3), and oligonucleotide 2

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(17 mer, oligo-2) were obtained from Shanghai Sangon Biological Engineering Technology and Services. Oligo-1 and oligo-3 were complementary to the 5′ end and the 3′ end of oligo-2, respectively. The sequences of oligo-1, 2, and 3 are shown in Fig. 2. 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 reagents of analytical grade and were purchased from Beijing Chemical Reagent Company.

Sample preparation. The colloidal gold was prepared by reduction of $HAuCl_4$ (2% by weight) with sodium citrate aqueous solution (2% by weight), while the monodispersed gold nanoparticles of different sizes were obtained by the same procedure with different amounts of citrate. Transmission electron microscopy (TEM) images were obtained with a JEM 100 CX electron microscope at 100 kV.

The amplifier, oligo-3-functionalized gold nanoparticles (DNA: Au conjugate), was prepared by the method that was previously reported by Mirkin et al. [12].

DNA immobilization and hybridization on modified Au QCM. A QCM instrument was used to follow the DNA sensing progress. The 9 MHz gold-quartz crystals purchased from SEIKO EG & G were cleaned with piranha solution (3:1 v/v 98% H_2SO_4/30% H_2O_2) according to the procedure described previously [13]. Then the Au QCM was modified with a 0.5%(v/v) solution of 1,6-hexanedithiol in ethanol for 30 min. Immobilization ofAu-nanopartparticles of different sizes onto the thiol-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 (phosphate buffer solution, pH 6.83) for 1 h, and the 17 mer target DNA 2 was hybridized on the sensing interface 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, which were in different size series.

Results and discussion

In our experiment, a clean gold-coated QCM was treated with a solution of 1,6-hexanedithiol in ethanol. After this treatment, a 1,6-hexanedithiol that could avoid nonspecific adsorption of the amplifier occupied the QCM area. Then, a few drops ofAu-nanopartparticle solution were put onto the thiol-terminated surface to make a nanogold-modified surface, where the HS-DNA oligo-1 was immobilized. The target DNA oligo-2 was hybridized on the sensor surface. The amplification step of the sensing process was subsequently accomplished by the hybridization of the oligo-2-terminated surface with the oligo-3-functionalized Au-nanoparticles, i.e., the amplifier.

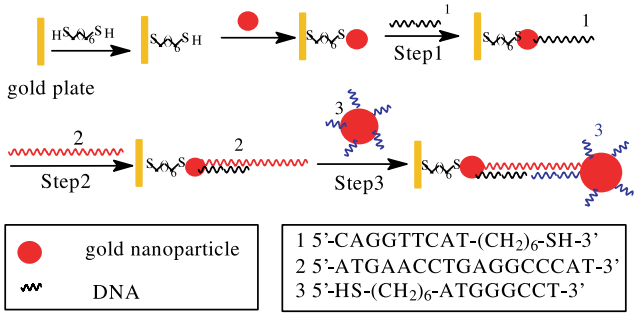

Fig. 1. Schematic illustration of the sensing process of the amplifying system based on Au nanoparticle-covered QCM surface.

The principle of this method is schematically shown in Fig. 1.

The gold particles used in our experiment were prepared by reduction of $HAuCl_4$ with sodium citrate aqueous solution. Monodispersed gold particles could easily be obtained by changing the concentration of sodium citrate. Transmission electron microscopy experiments indicated that the particles obtained in this way had diameters of 5, 12, 20, 30, 42, 50, 60, and 68 nm. The dispersibility is shown in the following table.

d_{real} (nm)[a]	δ[b]	rms (×100)
5	1.01	1.1
12	1.01	1.1
20	1.03	1.5
30	1.05	2.6
42	1.09	2.9
50	1.10	4.5
60	1.12	3.8
68	1.29	4.4

[a] Average of measured diameter.
[b] Aspect ratio.

Fig. 2 shows some pictures of these nanogold particles, indicating that the particles obtained in this way were slightly prolate with an aspect ratio ranging from 1.01 to 1.29.

When the QCM surface was modified by nanogold particles, the maximum amplifier phenomenon, i.e., that hybridization cannot increase further as the amplifier particle size increases, could be overcome. As the amplifier particle size increased, the hybridization could continue to increase with no maximum. Thus, nanogold treatment can enlarge the amplification effect when the amplifier size exceeds the maximum as shown in Fig. 3. The detection limitation was improved to a sensitivity less than 10^{-16} mol/L, implying significant prospects for this effect and method.

Some calculations were made to explain the mechanism of the nanogold surface modification. In our

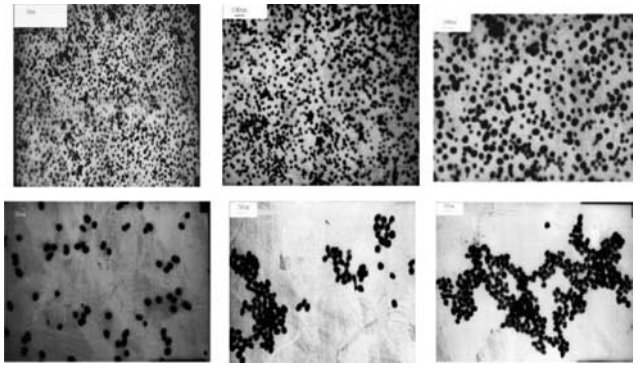

Fig. 2. Electron micrograph of gold nanoparticles of different sizes.

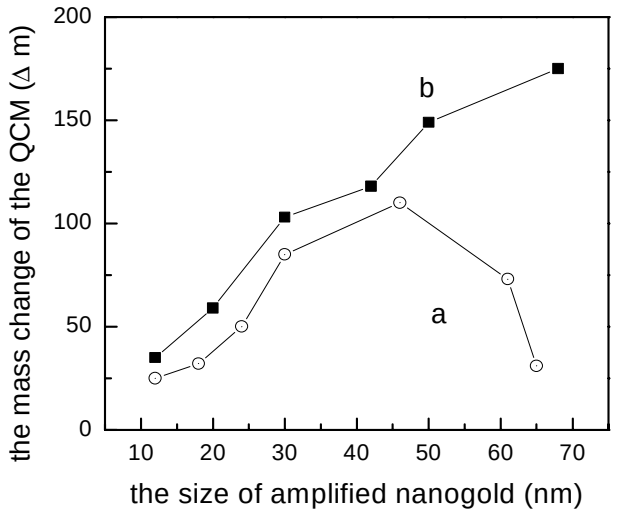

Fig. 3. (a) Sensor without surface modification by nanogold. (b) Sensor with surface modification by nanogold.

experiment, the weight increment of QCM after adsorption of 1,6-hexanedithiol was about 15 ng, while the weight increment of QCM which was surface modified by nanogold after adsorption of 1,6-hexanedithiol was about 55 ng. Since the molecular weight of the DNA strand was 3000 g/mol, the number of DNA molecules on the bare and modified surface is equal to 3.01×10^{12} and 1.1×10^{13}, respectively.

Since the surface area of QCM gold electrode plate was $0.196\,cm^2$. In our calculation, the surface area for one SH group was taken as $0.24\,nm^2$ ($0.24 \times 10^{-14}\,cm^2$) and for 3.01×10^{12} 1,6-hexanedithiol molecules was $0.1288\,cm^2$, Therefore the surface coverage of 1,6-hexanedithiol adsorbed on the QCM bare surface was 0.1288/0.196 = 65.7%, and 34.3% QCM surface remained bare.

When 12 nm gold nanoparticles were adsorbed on the surface of dithiol, about 40 ng mass changing was recorded. Knowing the specific gravity of gold is 19.30 g/cm^3, the number of gold nanoparticles n can be represented as:

$$n = \frac{w}{\frac{4}{3}\pi r^3 \times \rho} = \frac{40 \times 10^{-9}}{\frac{4}{3} \times \pi \times (6 \times 10^{-7})^3 \times 19.30} = 2.29 \times 10^9.$$

The surface area of all the gold nanoparticles was $n4\pi r^2 = 2.29 \times 10^9 \times 4 \times 3.14 \times (6 \times 10^{-7})^2 = 1.035 \times 10^{-2} cm^2$ or $0.01035 cm^2$. Table 1 shows some interesting results.

According to the experiment, the bare QCM surface could fix about 15 ng of DNA probe, while the same one modified by 40 ng nanogold with a diameter of 12 nm could fix 55 ng of DNA probe. The surface density of HS-DNA probe on the surface modified by nanogold increased about three times compared with that on the bare QCM surface. The surface area of the nanoparticles on the surface can be calculated as $1.035 \times 10^{-2}\,cm^2$,

Table 1
The coverage of DNA probe on a bare gold surface and a surface modified by 12 nm nanogold

	Bare QCM electrode	QCM electrode modified by 12 nm nanoparticles
Weight of immobilized HS-DNA probe/ng	15 ± 5	55
Molecules of immobilized HS-DNA probes	3.01×10^{12}	1.1×10^{13}
Calculated surface area	$0.196\,cm^2$	$0.196 + 0.0104\,cm^2$

only about 0.5% of the bare surface. So, the surface increase caused by nanoparticles cannot explain this effect. Since the surface coverage of 1,6-hexanedithiol on the QCM bare surface in our experiment was about 65.7%, and 34.3% of the surface still remained bare, nonspecific interaction between DNA probes and the bare gold surface might still exist. More important, this impressive effect shows the important attribution of the surface multilayer formation after nanogold introduction at the QCM surface. Zhou et al. [8] used oligodeoxynucleoxide-capped gold nanoparticles attached onto a bare gold surface and proved that the nanoparticle nonspecific interaction and network formation should be the reason for the high DNA probe immobilization. Our observation under AFM [11] on a bare mica surface proved the network formation of nanogold was possible, which is in agreement with Zhou's observation.

Besides the fact that a higher population of DNA probe on the nanoparticle surface can firmly make the larger amplifiers stick to the QCM surface, another reason for increasing the sensitivity could be the steric effect of the curled surface, which caused more room for hybridization. As demonstrated in Keating et al. [14] work the low surface coverage and higher DNA probe to c-DNA ratios lead to optimal hybridization. In a work on DNA monolayers on gold by using neutron reflectivity, Tarlov et al. [15] showed that by addition of mercaptohexanol to the HS-DNA, the DNA "stands up" and favors hybridization because space is available more for hybridization. Our results were in agreement with their arguments and this effect is schematically illustrated in Fig. 4. The larger periphery at the external space provides much room for the accommodation of a larger gold-amplifier, improving the extent of DNA hybridization and detection sensitivity, which is of great importance for the explanation of our results in Fig. 3.

Conclusion

In conclusion, by modification of the QCM sensor surface by 12 nm nanogold, the DNA immobilization amount on the nanoparticle surface was much higher than that on the plane surface. This method has also

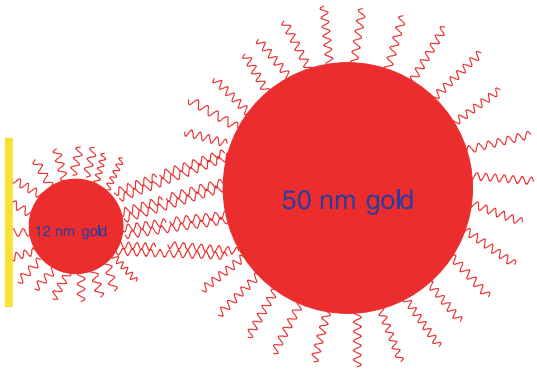

Fig. 4. Schematic representation of the larger space for hybridization by a curled surface.

overcome the limitation on amplifier size in the "amplifier method," so that particles larger than 50 nm can continue working as amplifiers to enhance the DNA detection sensitivity. This effect and calculation reveal the possibility of manufacturing a new QCM DNA sensor having a detection limitation higher than 10^{-16} mol/L. The mechanism of this effect was attributed to the surface population increasing and a larger space at the peripheral area for the accommodation of larger gold-amplifier.

Acknowledgments

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