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A novel microgravimetric DNA sensor with high sensitivity

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Abstract

A novel method using an amplifier with a cantilever and gold nanoparticles successfully to extend the length of the target for the specific and high sensitive detection of DNA was reported. When the size of gold nanoparticle is 50 nm, a sensitivity of 10^{-15} M for the single base mutation detection has been achieved.

Keywords: Colloidal Au; DNA sensor; Quartz crystal microbalance

In the 21st century, a study on DNA as an important basis of bio-technique will play an important role in preventing and curing diseases [1,2]. Investigation indicates that the appearance of the malignant tumor is highly correlative with the DNA mutation [3,4]. It has become an important topic of the cancer study to detect the gene mutation and look for the relation between mutation and pathological change at the molecular level. Rapid detection for trace gene mutation can provide the basic data for diagnosing disease. Therefore, looking for a rapid and simple method used for detecting trace mutation is important and pressing. Recently, a series of valuable achievements have been acquired by utilizing special color and biological specialty of the nanogold colloid in DNA recognition and detection. Mirkin's group has done a large amount of work in this field [5–10]. This work provided a simple and practical way for DNA detection and recognition through utilization of gold nanoparticles, but what is regretted is that the sensitivity of DNA detection through this simple color change is low, which limits the wide use of this method.

Combining gold nanoparticle with the quartz crystal microbalance technique can resolve this problem of detection sensitivity. QCM is a simple, rapid and real-time measurement of DNA binding and hybridization at the sub-nanogram level [11–13]. The nanogold particle has many special properties, for example, has high density, is of simple operation, and is easily size-controlled. DNA detection sensitivity can be enhanced utilizing nanogold as amplifier. Our group introduced nanogold particle to modify the QCM surface and discovered that nanogold can improve the immobilization property of the DNA probe. This method makes possible the detection of single base mutation less than 10^{-16} mol/L [14–16]. At the same time, Willner's group also used nanogold as amplifier to improve the sensitivity [17,18]. Although these DNA biosensors have high detection sensitivity, the strain length of the target DNA is not longer than 20 bases; otherwise the one base mutation cannot be detected. Therefore it is very far from practical application.

Presently, how to extend more the applied area of the DNA detection method widely is the main subject. In this paper, on the premise of holding higher sensitivity, the hybridization was achieved through three steps. We used a longer single strand DNA as target DNA, another shorter DNA with a cantilever and gold nanoparticles as amplifier to enhance the detection signal. In the presence of the cantilever, the increase in the length of the target DNA could not decrease this sensitivity. Detailed experimental method is shown in Fig. 1.

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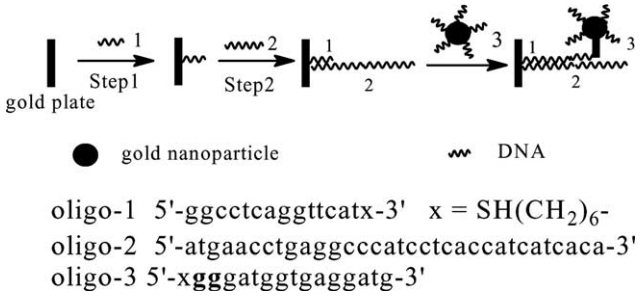

Fig. 1. Schematic illustration of the sensing process.

Materials and methods

Reagents. All chemicals were commercially available and used as received. The 3′-(alkanethiol)oligonucleotide 1 (14-mer, oligo-1), 5′-(alkanethiol)oligonucleotide 3 (15-mer, oligo-3), and the 33-mer oligonucleotide 2 (oligo-2) were all obtained from Shanghai Sangon Biological Engineering Technology and Services. Oligo-1 was complementary to the end of the oligo-2. Mercaptohexanol [$HO(CH_2)_6SH$] was purchased from Fluka. All other chemicals such as sodium citrate and $HAuCl_4 \cdot 3H_2O$ were of analytical regent grade and obtained from Beijing Chemical Reagent. The colloidal gold nanoparticles with an average diameter of 50 nm were prepared according to the literature by reduction of $HAuCl_4$ with sodium citrate aqueous solution [19]. DNA:Au conjugate was prepared by the method that was previously reported by Mirkin and co-workers [20].

DNA immobilization on modified Au QCM and hybridization. AT-cut quartz crystals, with a fundamental frequency of 9 MHz, were received from EG&G (Japan). These crystal wafers were coated with gold electrodes on both sides (area, 0.196 cm^2). Prior to the probe immobilization, the gold QCM surfaces were ultrasonically cleaned by a 2 min exposure to a hot Piranha solution (H_2SO_4:$H_2O_2 = 3:1$). The QCM gold plate was dipped into a PBS solution (pH = 6.83) of 2×10^{-7} M oligo-1 to generate a DNA probe. Then it was modified with a 1 mM solution of mercaptohexanol in water for 30 min to avoid consequent nonspecific adsorption in the following hybridization steps. The QCM surface was washed successively with ethanol solution and distilled water and then immersed into an oligo-2 solution, i.e., the target DNA with the different concentrations at 40 °C. Two hours later a stock of oligo-3-functionalized gold nanoparticle in 50 nm was added to react with the target DNA on the surface. All the experiments were carried out in a 100% humidity environment to prevent water evaporation.

Results and discussion

The sensing process was implemented step by step. The thiol-tagged oligonucleotide 1 was assembled on an Au-quartz crystal microbalance when the Au QCM was immersed in the thiol-oligonucleotide DNA buffer solution of 10^{-7} mol/L for 2 h at room temperature. The modification of the thiol-oligonucleotide caused a mass change of ca. 20 ± 5 ng on the QCM surface.

When the target DNA, oligo-2 with different concentrations was fixed on the chip by hybridization with a part of its tails matched with the probe DNA, i.e., oligo-1, the frequency changes were determined. Fig. 2 shows frequency changes of QCM crystal exposed to different concentrations of the complementary oligo-2. The extent of the crystal frequency decrease is enhanced as the bulk concentration of target DNA increases, consistent with the increased surface coverage of the sensing interface by oligo-2. At a concentration of target DNA corresponding to 10^{-13} mol/L, the crystal frequency decreases by 4 Hz. A gold nanoparticle modified by oligonucleotide with a cantilever, i.e., non-complementary base vs. target DNA, is formed to give an amplified frequency signal.

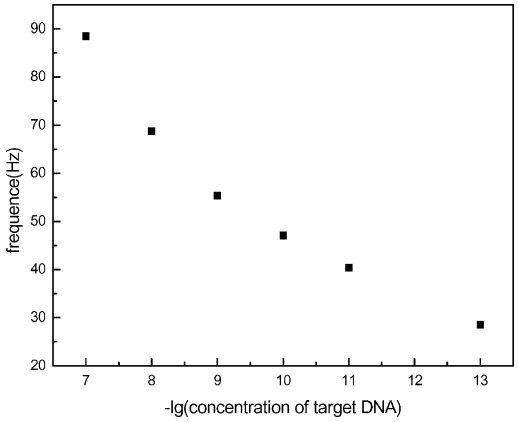

Fig. 2. Frequency change vs. the concentration of the target DNA.

The dot in Fig. 2 was obtained just after hybridizing with 50 nm-Au-nanoparticle functionalized with oligo-3. The frequency changes are ca. -88, -68, -55, -47, -40, and -28 Hz, corresponding to the concentrations of the oligo-2 target DNA 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-13}, respectively. It has a very large enhancement. Nevertheless if oligo-1 was immobilized on the QCM surface and the amplifier oligo-3 directly reacted with the modified surface, QCM has a frequency change of 11 Hz. This indicated that non-specific enlargement exists.

In order to avoid the non-specific enlargement, an appropriate blocking reagent must be used to block the uncovered surface region of the Au-electrode of QCM prior to the hybridization. Herein, mercaptohexanol was served as blocking reagent. Experiments indicated that if the functionalized surface was not treated with the blocking reagent, and was directly hybridized with target DNA or was directly enhanced with gold-nanoparticle, a frequency change of QCM was more than the treated surface with the blocking reagent. Contrarily, after treating the surface with mercaptohexanol, the frequency changes were only ca. 54.2, 18.8, 10.4, and 5.67 Hz, corresponding to the concentrations of the oligo-2 target DNA 10^{-9}, 10^{-11}, 10^{-13}, and 10^{-15}, respectively, after enlarging with gold nanoparticles. These results imply that there is an uncovered surface region after treating the surface with oligo-1 DNA alone, which may induce the non-specific enlargement to occur directly on the uncovered Au-electrode, and that mercaptohexanol can be used as blocking reagent to prevent effectively the non-specific enlargement effect.

After treating the surface with mercaptohexanol, treatment of the oligo-1 functionalized crystal with the buffer solution of oligo-3 modified gold nanopartiles does not yield any significant change in the crystal frequency. Note that the oligonucleotide 3 is essentially complementary to oligo-2. The frequency change observed upon interactions of the 1-modified crystal with 2 originates from specific complementary interactions that generate the ds-assembly. The lack of frequency changes upon interaction of the functionalized crystal with 3 indicates that non-specific oligonucleotide binding interactions are not operative on the interface.

After treatment with mercaptohexanol, when the hybridization signal was enlarged with gold nanoparticles, the frequency changes were ca 5.67 Hz versus the concentration of the target DNA 10^{-15}. Comparing the frequency change with that of the noise level, the sensitivity reaches ca. 10^{-15} M.

To address the non-specific adsorption, the oligo-1-functionalized surface was treated with the non-complementary target oligo-2 at high concentration of 10^{-7} M. Upon interaction with oligo-2 and subsequently with oligo-3-functionalized Au-nanoparticles, the QCM frequency was nearly unchanged within 2 Hz. These results demonstrate the specific and selective sensing of the functionalized interface to the target DNA.

In summary a novel method using an amplifier with a cantilever and gold nanoparticles successfully to extend the length of the target for the specific and high sensitive detection of DNA was reported. When the size of gold nanoparticle is 50 nm, a sensitivity of 10^{-15} mol/L for the single base mutation detection has been achieved. Detailed investigation on the length of the cantilever and gold nanoparticle size for enhancing signal is under investigation.

Acknowledgments

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References

[1] X.H. Xu, A.J. Bard, Immobilization and hybridization of DNA on an aluminum(III) alkanebisphosphonate thin film with electrogenerated chemiluminescent detection, J. Am. Chem. Soc. 117 (1995) 2627–2631.
[2] K. Peterlinz, R. Georgiadis, T. Herne, M. Tarlov, Observation of hybridization and dehybridization of thiol-tethered DNA using two-color surface plasmon resonance spectroscopy, J. Am. Chem. Soc. 119 (1997) 3401–3402.
[3] N. Falette, M.P. Paperin, I. Treilleux, A.C. Gratadour, N. Peloux, H. Mignotte, N. Tooke, E. Lofman, M. Inganas, A. Bremond, M. Ozturk, A. Puisieux, Prognostic value of P53 gene mutations in a large series of node-negative breast cancer patients, Cancer Res. Cancer Res. 58 (1998) 1451–1455.
[4] M. Askari, J.P. Alarie, M. Moreno-Bondi, T. Vo-Dinh, Application of an antibody biochip for p53 detection and cancer diagnosis, Biotechnol. Prog. 17 (2001) 543–552.
[5] K.J. Watson, S.J. Park, J.-H. Im, S.T. Nguyen, C.A. Mirkin, DNA-block copolymer conjugates, J. Am. Chem. Soc. 123 (2001) 5592–5593.
[6] J.M. Nam, S.J. Park, C.A. Mirkin, Bio-barcodes based on oligonucleotide-modified nanoparticles, J. Am. Chem. Soc. 124 (2002) 3820–3821.
[7] R.A. Reynolds, C.A. Mirkin, R.L. Letsinger, Homogeneous nanoparticle-based quantitative colorimetric detection of oligonucleotides, J. Am. Chem. Soc. 122 (2000) 3795–3796.
[8] T.A. Taton, R.C. Mucic, C.A. Mirkin, R.L. Letsinger, The DNA-mediated formation of supramolecular mono- and multilayered nanoparticle structures, J. Am. Chem. Soc. 122 (2000) 6305–6306.
[9] T.A. Taton, G. Lu, C.A. Mirkin, Two-color labeling of oligonucleotide arrays via size-selective scattering of nanoparticle probes, J. Am. Chem. Soc. 123 (2001) 5164–5165.
[10] R. Jin, G. Wu, Z. Li, C.A. Mirkin, G.C. Schatz, What controls the melting properties of DNA-linked gold nanoparticle assemblies?, J. Am. Chem. Soc. 125 (2003) 1643–1654.
[11] F. Patolsky, A. Lichtenstein, I. Willner, Amplified microgravimetric quartz-crystal-microbalance assay of DNA using oligonucleotide-functionalized liposomes or biotinylated liposomes, J. Am. Chem. Soc. 122 (2000) 418–419.
[12] X.C. Zhou, L.Q. Huang, S.F.Y. Li, Microgravimetric DNA sensor based on quartz crystal microbalance: comparison of oligonucleotide immobilization methods and the application in genetic diagnosis, Biosensors Bioelectronics 16 (2001) 85–95.
[13] K. Gotoh, M. Tagawa, Detachment behavior of Langmuir–Blodgett films of arachidic acid from a gold surface studied by the quartz crystal microbalance method, Colloids Surf. A 196 (2002) 145–152.
[14] H.Q. Zhao, L. Lin, J.A. Tang, M.X. Duan, L. Jiang, Enhancement of the immobilization and discrimination of DNA probe on a biosensor using gold nanoparticles, Chin. Sci. Bull. 13 (2001) 1074–1076.
[15] T. Liu, J.A. Tang, H.Q. Zhao, Y.P. Deng, L. Jiang, Particle size effect of the DNA sensor amplified with gold nanoparticles, Langmuir 18 (2002) 5624–5626.
[16] T. Liu, J.A. Tang, L. Jiang, Significant sensitivity enhancement of quartz crystal microbalance based DNA sensors, Biochem. Biophys. Res. Commun. 295 (2002) 14–16.
[17] O. Lioubashevski, F. Patolsky, I. Willner, Probing of DNA and single-base mismatches by chemical force microscopy using peptide nucleic acid-modified sensing tips and functionalized surfaces, Langmuir 17 (2001) 5134–5136.
[18] L. Alfonta, I. Willner, D.J. Throckmorton, A.K. Singh, Electrochemical and quartz crystal microbalance detection of the cholera toxin employing horseradish peroxidase and GM1-functionalized liposomes, Anal. Chem. 73 (2001) 5287–5295.
[19] K.C. Grabar, P.C. Smith, M.D. Musick, J.A. Davis, D.G. Walter, M.A. Jackson, A.P. Guthrie, M.J. Natan, Kinetic control of interparticle spacing in Au colloid-based surfaces: rational nanometer-scale architecture, J. Am. Chem. Soc. 118 (1996) 1148–1153.
[20] C.A. Mirkin, R.L. Lestinger, R.C. Mucic, J.J. Storhoff, A DNA-base method for rationally assembling nanoparticles into macroscopic materials, Nature 382 (1996) 607–609.