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Using personal uric acid meter and enzyme-DNA conjugate for portable and quantitative DNA detection

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ABSTRACT

A simple and distinctive assay for quantitating target DNA was developed based on personal uric acid meter (PUAM) and xanthine oxidase modified DNA (xanthine oxidase-DNA). Capture DNA was assembled on the Au film by the formation of Au—S bond. When target DNA was added, it was complementary to capture DNA at one-half of the segment and complementary to the xanthine oxidase-DNA at the other half-segment, resulting in the formation of a stable duplex complex. As a result, the xanthine oxidase was dragged to the Au film. Since xanthine oxidase could catalyze hydrolysis of xanthine to uric acid, the concentration of target DNA in the sample could be quantified by monitoring uric acid using PUAM. Since the signal was amplified based on the enzymatic turnovers, this enzyme-based assay made it possible that 10 pM p53 DNA or 4 pM HBV DNA could be detected using PUAM. This assay also showed excellent selectivity toward mismatches, and demonstrated its applicability for the target detection in human serum. Furthermore, this protocol showed a new strategy to detect non-uric acid targets using PUAM.

1. Introduction

Since point-of-care (POC) devices have many significant advantages, such as convenient, inexpensive and time-saving, they have aroused people's great interest in the field of biology and medicine [1–6]. Among these devices, personal glucose meter (PGM) and personal uric acid meter (PUAM) have been developed successfully [7]. Generally, PGM or PUAM can only detect a single target, blood glucose or uric acid. However, various kinds of targets (such as small molecules, nucleic acids and proteins) need to be detected in food safety, environmental monitoring and personal healthcare. For keeping pace with expectations in future POC testing, it was essential to develop a new strategy for expanding the range of detectable targets. Recently, it has been reported that some non-glucose targets, such as metal ion [8,9], small molecule [10,11], DNA [12,13] and protein [8,10,14] could be quantified by personal glucose meter (PGM). These researches provided the new key to expand the application of PGM. To date another popular device, i.e. PUAM, has not been applied to the sensing of molecules other than uric acid.

Herein, the use of low-cost and widely accessible PUAM for portable and quantitative DNA detection was demonstrated. Xanthine oxidase-DNA conjugates which could convert xanthine to uric acid were used in this assay, and thus a direct relationship can be established between the concentrations of uric acid and target. Due to the high catalytic efficiency of xanthine oxidase, it was easy to obtain a sufficiently strong signal which could be detected by PUAM. To test the general feasibility of this assay, 24-nucleotide p53 DNA and 35-nucleotide hepatitis B virus DNA were chosen as targets. In this assay (Fig. 1), capture DNA was first immobilized on Au surface, and then 6-mercapto-1-hexanol (MCH) was used to block Au surface. When xanthine oxidase modified DNA (xanthine oxidase-DNA) and target were added, the "sandwich" structure was formed by hybridization. As xanthine was added, it was turned into uric acid by the xanthine oxidase-DNA conjugates. The quantitative detection was achieved through the relation between the concentration of target DNA and the uric acid measured by PUAM. Due to the enzyme-based amplification, sensitive DNA detection method was developed with a detection limit of about picomolar level, which was comparable to or better than that of the previous methods [15–20]. Theoretically, this protocol may have the potential to be applicable to the portable and quantitative detection of various kinds of non-uric acid targets, such as proteins and small molecules, at home or in the field, as long as the appropriate aptamers or antibodies can be found and replaced.

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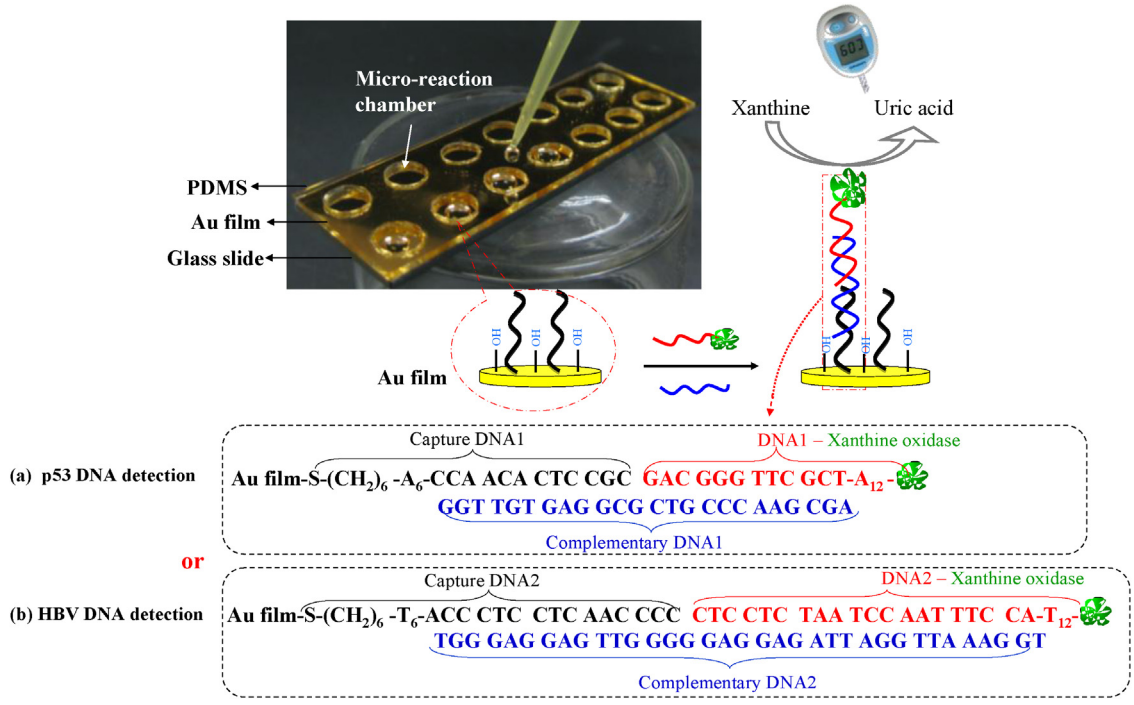

Fig. 1. The mechanism of (a) p53 DNA and (b) HBV DNA detection using PUAM.

2. Materials and methods

2.1. Reagents

Xanthine, tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), and sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) were obtained from Sigma–Aldrich Corp. (USA). Amicon ultra centrifugal filters (3 K, 30 K) were from Millipore Corporation (Billerica, MA, USA). Xanthine oxidase (40 U/mg) was purchased from J&K Scientific Ltd. (Beijing, China). All of the other chemical reagents were of analytical grade or higher. Ultrapure water (18.2 MΩ cm) was used throughout.

Oligonucleotides, which were designed according to the human p53 gene and HBV gene, were synthesized by Sangon. The sequences of DNA oligonucleotides used here were listed in Tables 1 and 2.

2.2. Xanthine oxidase – DNA conjugation and characterization

The synthesis of xanthine oxidase-DNA conjugate was similar to the previous work [8,21]. Detailed description of synthesis of xanthine oxidase-DNA conjugate was presented in the supplementary material. The report DNA was first modified with sulfhydryl at the 5′-end. Then sulfo-SMCC was used as a linker to conjugate xanthine oxidase and report DNA. Next, the xanthine oxidase-DNA conjugate was characterized using an SDS-PAGE experiment. This gel was stained by Coomassie brilliant blue.

2.3. Preparation and modification of micro-reaction chamber

Au film, which was 50 nm thickness of gold deposited on 1 nm Cr adhesive layer coated microscope glass slide (25.4 mm × 76.2 mm × 1 mm), was used here. Then, Au film were first cleaned in piranha solution (H_2SO_4/H_2O_2, 3:1 (v/v)), followed by a thorough rinsing with water. Next, it was dried by N_2 steam for further use. A 1-mm-thick perforated PDMS (2 × 7, 8 mm in diameter) was reversibly bonded onto this substrate (shown in Fig. 1). Thus micro-reaction chamber was formed and many samples could be detected at the same time due to the use of micro-chamber array.

In each chamber, Au film was incubated with 2 μM capture DNA for 2 h at room temperature. After a thorough rinsing with 100 mM phosphate buffer (pH = 7.5), Au film was blocked by 1 mM MCH for 30 min at room temperature. Then Au film was thoroughly rinsed with 100 mM phosphate buffer (pH = 7.5) and dried with N_2 steam.

2.4. DNA hybridization and surface enzyme-catalyzed reaction

Different concentrations of target DNA in 6 × SSC (pH = 7.5) was dropped into each chamber and incubated for 1 h at room temperature. After Au film was washed by 6 × SSC (pH = 7.5), xanthine oxidase-DNA conjugates was added into each chamber and incubated for 1 h at room temperature. For improving the selectivity, each chamber was washed repeatedly under the stringent elution conditions (10 mM Tris–HCl, pH = 7.5, 25 mM NaCl). After the removal of solution, xanthine solution (10 mM phosphate buffer, pH = 8.5) was added into each chamber. After incubated for 90 min at optimal temperature, 3.3 μL of the reaction solution was measured by PUAM. PUAM with a dynamic range of 181–1180 μM was a gift from Sannuo Company, China. A signal of PUAM larger than 181 μM was regarded as signal.

3. Results and discussion

3.1. Characterization of Xanthine oxidase–DNA1 conjugate

Xanthine oxidase-DNA1 conjugate was characterized using SDS-PAGE analysis. As shown in Fig. S2 of the Supplementary Information, the band of xanthine oxidase in the lane 3 was located between 100 kDa and 150 kDa, which was in agreement with the molecular weight of xanthine oxidase (ca. 100–300 kDa). Upon conjugation with report DNA1, the migration of the xanthine oxidase-DNA conjugate band in the lane 1 was less than that of