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Increasing target hybridization kinetics by immobilizing DNA probes at Au nanoflower ultramicroelectrode

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ABSTRACT

Although different electrochemical DNA sensors have been widely developed for analytical purpose, the effective way to immobilize DNA probes on basic electrode surface for increasing their binding rate with target is rarely reported. Herein, a new scheme for increasing DNA probes-target hybridization kinetics using gold nanoflower ultramicroelectrode (UME) as DNA probe immobilization surface was developed. In details, the ATP aptamer was selected as the model to demonstrate this E-DNA sensor's properties. Our results showed that the DNA aptamer/ATP hybridization rate obtained at gold nanoflower UME was about 10-fold and 4-fold greater than that obtained at the macroelectrode surface, and even in the homogeneous solution, respectively. This increase of DNA aptamer hybridization rate was confirmed to be related with the smaller size of UME and the specific structure of gold nanoflower.

1. Introduction

The electrochemical DNA (E-DNA) sensor have been widely used in the detection of closely related biomolecules, such as DNA, miRNA sequences, protein, enzyme etc due to their advantages of fast response, easy to use, low cost and direct use in complicated samples [1–5]. The E-DNA sensor is comprised of a redox-modified DNA probes that is immobilized on the electrode surface. Upon target hybridization, the binding-induced conformation and/or flexibility change of redox-tagged DNA probe resulted in the measurable change in current. Hybridization reaction is required in these methods. For E-DNA sensors, DNA probe is immobilized on the electrode surface. Target DNA or the analyte in solution diffuses and approaches probe DNA to hybridize with the probe DNA. In general, it always take several hours for hybridization reaction of probe DNA with target DNA at conventional macroelectrode surface due to its slow planar diffusion and high steric hindrance. Even the homogeneous DNA hybridization reaction in solution will take ~40 min.

Ultramicroelectrodes (UMEs) have been widely used in many research areas due to their unique properties, such as high sensitivity and rapid mass transfer [6–8]. Recently, E-DNA sensors on UMEs have been reported and some efforts were made to improve their sensitivity [9]. For example, White's group developed the ATP sensor based on electrochemical deposition of gold nanostructures on gold UME. Their signal-to-noise was increased by increasing the effective electrode surface area and decreasing background currents from the reduction of dissolved oxygen [10]. Wang's group reported a sensitive electrochemical microRNAs sensor on a gold UME based on a new signal amplification mechanism, in which each redox molecule on the detection probe is cyclically oxidized at the electrode and reduced by the reductant in solution [11]. However, in those methods, it still took 2–3 hours for hybridization reaction between probe DNA immobilized on UME surface and target analyte in solution due to the high steric hindrance from the tight rows of DNA sequence.

In this work, the high sensitivity and fast mass transfer of UME and the specificity of aptamer recognition were combined to develop E-DNA sensors with high sensitivity and rapid hybridization reaction. The gold nanoflowers were electrochemically deposited onto Pt UME to form advantaged interface for DNA self-assemble and mass diffusion. The results showed that the sensitivity of this electrochemical ATP sensor was not less than that obtained at macroelectrode because the flower-shaped gold nanostructures provided the enhanced electrode surface area and more active site and consequently sensitivity. More importantly, it takes only several minutes for DNA hybridization reaction at this gold nanoflower UME, which is much more rapid than that occurred at macroelectrode and in solution. When the size of gold nanoflower UME is smaller than 25 μm (the critical dimension of UME), the peak current of ferrocene is linear with the logarithm values of ATP

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concentration in the range of 10 nM-10 μM after 10 min incubation. However, when the size of UME is > 25 μm, the peak current of ferrocene almost remain unchanged because 10 min is not enough for ATP to finish diffusion and hybridization with its aptamer at macroelectrode. Moreover, the sensitivity for the determination of ATP obtained at the gold nanoflower UME was obviously higher than that obtained at Au disk UME with the similar size after 10 min hybridization reaction. Thus, the fast hybridization reaction results from cooperative effect of the enhanced mass transfer and the decreased steric hindrance of the gold nanoflower structure of UME.

2. Experimental

2.1. Materials and instrumentation

All chemicals were not purified additionally before the experiments. Chloroauric acid, ($HAuCl_4$, 99.995%), ferrocene methanol (FcMeOH, 97%), 2-Mercaptoethanol (99%) and 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 99.5%) were purchased from Sigma-Aldrich. DNA oligonucleotides were purchased from Sangon Inc. (Shanghai). The sequences of employed oligonucleotides are as follows:

Anti-ATP aptamer sequence (the first 27-base is the recognition site): 5′-ferrocene-C6-ACCTGGGGGAGTATTGCGGAGGAAGGTTT-C6-SH-3′

Complementary sequence: 5′- ACCTTCC TCCGC AATAC TCCCC CAGGT-3′.

All aqueous solutions were prepared using 18.2 MΩ-cm water with total organic carbon (TOC) ≤5ppb from the Milli-Q Advantage A10 system.

Pt microwires (d = 25 μm, 99.95%, hard) were purchased from Alfa Aesar. Quartz capillaries (o.d. = 1.0 mm, i.d. = 0.30 mm, 15 cm length) were obtained from Sutter Instrument Co.. Colloidal silver liquid (TED PELLA, INC.) was used to contact the Pt wire with a tungsten wire. The UMEs were pulled by a sutter P-2000 Laser Puller (Sutter Instrument Company, USA). Electrochemical experiments (cyclic voltammetry and square-wave votammetry) were performed with an Autolab Model 302 A electrochemical analyzer using two-electrode systems.

2.2. The fabrication of Pt ultramicroelectrode

A Pt disk UME was fabricated by the laser puller P-2000 following the previous method [12,13]. Briefly, the fabrication procedure involved three steps: 1) sealing, 2) pulling, 3) polishing. In the first step, a Pt microwire (d = 25 μm, l = 2.5 cm, 99.95%, hard) was placed in a silica capillary (o.d. = 1.0 mm, i.d. = 0.30 mm, 7.5 cm length) and one end sealed with epoxy resin. The Pt microwire was sealed (sealing parameters: heat = 829, filament = 2, velocity = 60, delay = 140, pull = 225) in silica capillary with laser heating under vacuum, after each heating 45 s cooling 15 s, and repeat for 7 ~ 8 times. The second step, the silica capillary/Pt ensemble was pulled (pulling parameters: heat = 876, filament = 2, velocity = 60, delay = 100, pull = 229) resulting in two sharp quartz tips with the Pt nanowires sealed inside. The final step, the Pt microwire sealed in silica capillary contacted with a tungsten wire by colloidal silver liquid. Polishing the tip of newly pulled UMEs, making Pt nanotip expose, a Pt UME was prepared.

2.3. The electrochemical deposition of Au nanoflower on Pt UME

An Au nanoflower UME was prepared by directly deposition gold on a newly polished Pt UME. Electrodeposition process was accomplished using cyclic voltammetry by scanning from 0.4 V to -0.5 V (*vs.* Ag/AgCl) at a scan rate of 20 mV/s in a solution containing 1.5 wt.% HCl, 1.2 mg/mL $HAuCl_4$ and 0.1 M NaCl [11,14]. The various sizes of Au nanoflower UMEs were prepared by the different number of cycles of deposition gold. The more the number of deposition cycles, the greater the radius of Au UME.

2.4. Aptamer immobilization and ATP detection

Various sizes of Au nanoflower UMEs and Au disk UMEs were electrochemically cleaned in 0.5 M H_2SO_4 to remove any remaining impurities, then rinsed with Milli-Q water for 1 min before DNA immobilization.

DNA duplexes (dsDNA) were prepared as following: mixing two complementary sequences (1 μM each) in 1 M $NaClO_4$ containing 1 mM TCEP (Tris(2-carboxyethyl) phosphine), heating the mixture to 80°C and then slowly cooling it to room temperature. Then the dsDNA was ready for immobilization at electrode surfaces.

To immobilize dsDNA on the surface of Au nanoflower UME, the above cleaned Au nanoflower UME was incubated in thiolated dsDNA solution with appropriate concentration for 16 h, then post-treatment with 0.1 M 2-mercaptoethanol in 1 M $NaClO_4$ for 10 min, and rinsed completely with 10 mM HEPES solution containing 50 mM $NaClO_4$. dsDNA modified gold nanoflower UME was incubated in ATP solutions with various concentrations for 10 min at 37°C. The reaction was carried out in 10 mM HEPES solution containing 50 mM $NaClO_4$ (pH 8.0). Finally, all sensors were investigated using cyclic voltammetry or square wave voltammetry.

3. Results and discussion

3.1. Fabrication and characteristic of Au nanoflower UME

Gold is the most commonly used substrate for E-DNA sensor because DNA can be easy to assemble onto gold electrode via Au-S bond [15]. In this work, Au nanoflower UMEs with different size were fabricated by electrochemical deposition of gold onto the surface of Pt UME and used as DNA probe immobilization surface [14]. In order to investigate the

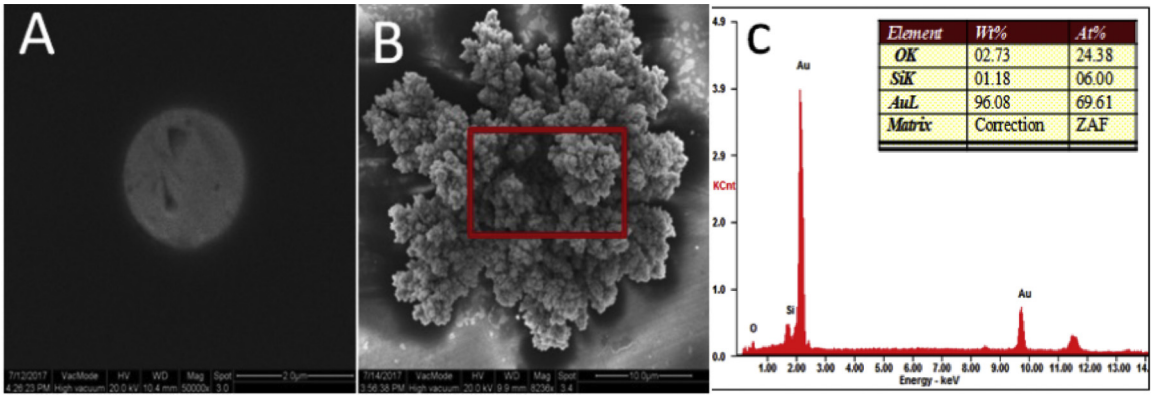

Fig. 1. SEM images of Pt UME before (A) and after (B) deposition of gold. (C) Energy spectrum diagram of Pt UME after deposition of gold.