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# Enhancement of DNA immobilization and hybridization on gold electrode modified by nanogold aggregates

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**Abstract**

Gold electrodes modified by nanogold aggregates (nanogold electrode) were obtained by the electrodeposition of gold nanoparticles onto planar gold electrode. The Electrochemical response of single-stranded DNA (ssDNA) probe immobilization and hybridization with target DNA was measured by cyclic voltammograms (CV) using methylene blue (MB) as an electroactive indicator. An improving method using long sequence target DNA, which greatly enhanced the response signal during hybridization, was studied. Nanogold electrodes could largely increase the immobilization amount of ssDNA probe. The hybridization amount of target DNA could be increased several times for the manifold nanogold electrodes. The detection limit of nanogold electrode for the complementary 16-mer oligonucleotide (target DNA1) and long sequence 55-mer oligonucleotide (target DNA2) could reach the concentration of $10^{-9}$ mol/L and $10^{-11}$ mol/L, respectively, which are far more sensitive than that of the planar electrode.


*Keywords:* Electrodeposition; Gold nanoparticle aggregates; DNA immobilization; DNA hybridization; Methylene blue; Cyclic voltammetry

## 1. Introduction

The development of DNA sensors has recently attracted substantial attentions in connection with research efforts directed at gene analysis, the detection of genetic disorders, tissue matching, and forensic applications (Heller, 2002; Peterson et al., 2002). Many techniques including fluorescence (Selinger et al., 2000), mass spectrometry (Isola et al., 2001), electrochemical (Park and Hahn, 2004; Fan et al., 2003; Chiorcea Paquim et al., 2004, surface plasmon resonance spectroscopy (Nelson et al., 2001; He et al., 2000) and quartz crystal microbalance (Liu et al., 2002; Patolsky et al., 2000) have been developed for DNA detection. Among them electrochemical techniques offer great advantages because of simple, rapid, low-cost and high-sensitivity. Many protocols have been proposed for electrochemical monitoring of DNA hybridization. For example, metal coordination complexes (Johnston et al., 1995; Napier and Thorp, 1997; Rodoriguez and Bard, 1990) and intercalating organic compounds (Kelley et al., 1999; Hashimoto et al., 1994; Yau et al., 2003) are usually used for DNA hybridization electroactive indicators. The electrochemical response of these labels or indicators changes upon DNA hybridization.

Methylene blue (MB) is an organic dye that exhibits a fast and reversible redox couple according to the reaction (Svetličič et al., 1991).

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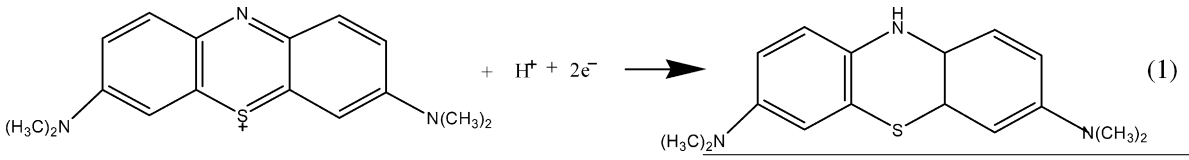


(1)

MB has been received considerable attentions as an electroactive indicator of DNA hybridization (Gu et al., 2002; Zhu et al., 2004; Rohs et al., 2000). The Groups of Ozsoz et al. (Erdem et al., 2000; Kerman et al., 2002) and Barton et al. (Boon et al., 2003; Boon and Barton, 2003) have done many works in this field. Ozsoz et al. observed a decrease in current signal upon hybridization at DNA-modified carbon paste electrode, and concluded that MB had a higher affinity to single-stranded DNA (ssDNA) rather than double-stranded DNA (dsDNA), showing that MB was a promising DNA hybridization indicator.

A key issue with any DNA hybridization biosensor is the immobilization amount and accessibility of probe DNA. Increasing the immobilization amount and controlling over the molecular orientation of probe oligonucleotides would markedly upgrade the detection limit of DNA biosensor (Levicky et al., 1998; Taton et al., 2000; Lin et al., 2000; Liu et al., 2002). It has been reported that gold nanoparticle modification could largely increase the surface area of electrode and enhance the immobilization amount and ability of probe DNA (Lin et al., 2000; Liu et al., 2002; Cai et al., 2001). However, in previous studies gold nanoparticles were mainly modified on DNA biosensor surface through the linkers with two function groups, but often the linkers disturbed the electrochemical signal of DNA biosensor and the mechanism of enhancement need further investigated. In present works, gold nanoparticles were directly electrodeposited onto planar gold electrode surface, the size and morphology of gold nanoparticles could be controlled to some extent through choosing different solution concentrations, deposition time and working potential (Zhang et al., 2004). The immobilization of probe DNA and its hybridization with target DNA on nanogold electrodes were studied using MB as an electroactive indicator. To overcome the shortcoming of small current response during hybridization, an improved method using long sequence target DNA, which greatly enhanced the response signal during hybridization, was proposed.

## 2. Experimental

### 2.1. Reagents

The oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology and Services Ltd. (China); their base sequences are as follows:

Probe ssDNA: HS-$(CH_2)_6$-5′-TTT TTT GGT GAG GAG G-3′;
Target DNA1: 5′-CCT CCT CAC CAA AAA A-3′;
Target DNA2: 5′-CAT CCT CAC CCC CCC CCC CAT GGG CCT CAG GTT CAT CCC CCT CCT CAC CAA AAA A-3′;
Non-complementary DNA: 5′-ATG AAC CTG AGG CCC A-3′.

MB was purchased from Amresco Company (US) and used without further purification. All other chemicals were analytical reagent grade and obtained from Beijing Chemical Reagent Company (China). Solutions were prepared with twice-distilled water.

### 2.2. Instrumentation

Cyclic voltammetry and gold nanoparticle electrochemical deposition were performed with a ZM6E electrochemical analyzer (Zahnek Electric). Electrochemical measurements were performed in a conventional glass-made cell using a three-electrode system consisted of a planar gold electrode with apparent surface area of 20 $mm^2$ or nanogold electrode, a Pt wire as counter electrode, and a saturated calomel electrode (SCE) as reference electrode, respectively. All potentials in the text were referred to the saturated calomel electrode. All experiments were carried out under an inert atmosphere by bubbling nitrogen gas through the working compartment.

Scanning electron microscopy (SEM) (Hitachi S-4300) was used to examine the morphology of nanogold electrodes.

### 2.3. Gold nanoparticle deposition

The planar gold electrodes were successively ultrasonically rinsed with Piranha solution (a mixture with 3:7 (v/v) of 30% $H_2O_2$ and 98% $H_2SO_4$) and ethanol and distilled water for 20 min. Then the gold electrodes were dried with nitrogen gas, and cycled in 1 mol/L $H_2SO_4$ aqueous solution until a stable gold oxide formation/reduction cyclic voltammogram was obtained. The pretreated electrodes were immersed into the $HAuCl_4$ solution containing 0.1 mol/L $KNO_3$ as electrolyte, where electrochemical deposition was conducted at −200 mV by single potential mode.

### 2.4. Immobilization and hybridization

The technique for ssDNA probe immobilization and hybridization with target DNA1 or target DNA2 on planar or nanogold electrodes was illustrated schematically in Fig. 1. The electrodes were immersed into a phosphate-buffered saline (PBS) solution (pH 6.83, 50 mmol/L NaCl) containing 0.01 mmol/L of ssDNA probe for 5 h, and then rinsed successively with PBS solution and distilled water, and dried with nitrogen gas to finish the ssDNA probe immobilization. The