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Comparing the performances of electrochemical sensors using p-aminophenol redox cycling by different reductants on gold electrodes modified with self-assembled monolayers

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ABSTRACT

p-Aminophenol (p-AP) redox cycling using chemical reductants is one strategy for developing sensitive electrochemical sensors. However, most of the reported reductants are only used on indium-tin oxide (ITO) electrodes but not gold electrodes due to the high background current caused by the oxidation reaction of the reductants on the highly electrocatalytic gold electrodes. Therefore, new strategies and/or reductants are in demand for expanding the application of p-AP redox cycling on gold electrodes. In this work, we compared the performances of several reductants in p-AP redox cycling on self-assembled monolayers (SAMs)-modified gold electrodes. Among the tested reagents, nicotinamide adenine dinucleotide (NADH), tris(2-carboxyethyl)phosphine (TCEP) and cysteamine were demonstrated to be suitable for p-AP redox cycling on the alkanethiol-modified gold electrodes because of their low background current. The rate of chemical reaction between reductants and *p*-quinone imine (QI, the electrochemically oxidized product of p-AP) increases in the order of NADH < TCEP < cysteamine. The system benefits from the low background current, fast chemical reaction and amenability and sensitivity of the sensor with TCEP or cysteamine as the reductant; these performance enhancements were demonstrated by the detection of microRNA. A detection limit of 2 pmol L^{-1} was achieved. We believe that our work will be valuable for the development of electrochemical sensors using p-AP redox cycling on gold electrodes.

1. Introduction

Electrochemical sensors have been attractive for a broad range of applications in clinical diagnosis, biomedical research, food quality control and environmental monitoring because of their simplicity, rapid response, and compatibility with miniaturization [1]. Currently, the increasing demand for the detection of ultralow amount of analytes with electrochemical techniques is driving the enhancement of detection sensitivity by selecting different signal amplification strategies. Many attempts to develop these features have been focused on the use of various labels, such as functionalized liposomes [2–4], nanoparticles [5–8], enzymes [9–12] and carbon nanotubes [13]. In particular, a more recent method for signal amplification is to employ an approach that entails multiple signal amplification such as enzymatic reaction plus redox cycling reactions [14].

Alkaline phosphatase (ALP) is one of the most widely used reporter molecules for the amplified detection of biomolecules because of its high turnover frequency and high reaction selectivity [15–19]. A standard method is that ALP dephosphorylates p-aminophenyl phosphate (p-APP) enzymatically to produce electroactive species p-aminophenol (p-AP) [20], which is detected by electrochemical oxidation to *p*-quinone imine (QI). The major drawback for this method is the instability of p-AP in air. To overcome this defect, reductants are added to the reaction mixture to protect p-AP from oxidation [8,14,21–24]. Moreover, reductants can regenerate p-AP from electrochemically oxidized p-AP, which greatly enhances the detection sensitivity (Fig. 1). The process of regenerating p-AP using reductants is called p-AP redox cycling. In such cycling, the key is that the reductants can reduce QI rapidly and exhibit poor oxidation signal in the potential scanning range. Currently used reductants for p-AP redox cycling include sodium borohydride ($NaBH_4$), hydrazine, nicotinamide adenine dinucleotide (NADH) and tris(2-carboxyethyl)phosphine (TCEP) (Fig. 1A)

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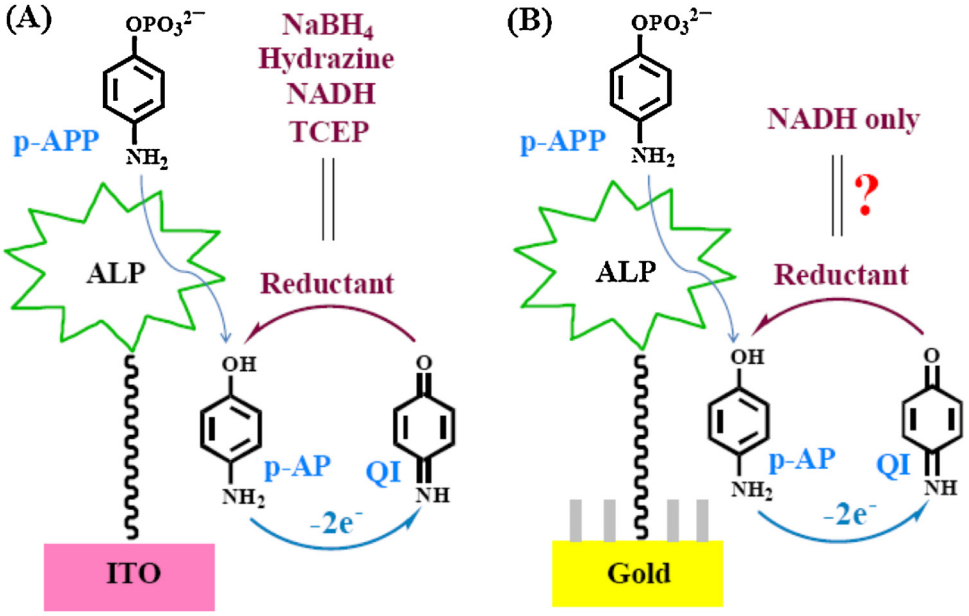

Fig. 1. Description of the process of p-AP production and its electrocatalytic reaction by reductants on ITO (A) and gold electrodes (B).

[8,14,23,24]. However, most of these reagents are only used on indium-tin oxide (ITO) electrodes but not gold electrodes because of their high background currents caused by the oxidation reaction of the reductants [25]. Therefore, there remains significant room to develop strategies and/or find strong reductants with low background current to expand the application of p-AP redox cycling on gold electrodes.

Thiol-linked self-assembled monolayers (SAMs) are a widely used modification method to immobilize biomolecules on gold electrodes through the formation of Au—S bonds. The unreacted gold surfaces are blocked commonly by alkanethiol molecules to eliminate the non-specific interaction. Meanwhile, additional layers of modest SAMs can prevent the absorption of electroactive species on electrode and block direct electron transfer [26,27]. For this reason, we suggested that the direct oxidation of some reductants on SAMs-modified gold electrode would be avoided. Indeed, electrochemical sensors were developed recently for the detection of IgG, cocaine and DNA with p-AP redox recycling by NADH on SAMs-modified gold electrodes [25,28,29] (Fig. 1B). However, there is no report on the systematic evaluation of the performances of the sensors with different reductants. In this study, we compared the background current of different reductants, such as $NaBH_4$, hydrazine, NADH, TCEP, Na_2SO_3 and cysteamine, on SAMs-modified gold electrode. Among these reagents, $NaBH_4$, hydrazine, NADH and TCEP have been used recently in p-AP redox cycling on ITO electrode [8,14,23,24]. We also tested Na_2SO_3 and cysteamine because they are well-known reagents for reducing quinone, and the direct electrochemistry of thiols at solid electrodes often needs large anodic potential to obtain appreciable signal [30]. More intriguingly, quinone-derivatized modified electrodes can be used for the mediated detection of thiols [31–33]. In this process, quinone is reduced chemically by thiols, leading to a significant increase in the oxidation current of quinone-modified electrode. Therefore, p-AP redox cycling is possible with thiol-containing compounds as reductants because of their low background current and fast chemical reaction with quinone. The performances of the sensor using p-AP redox cycling by the reductants with low background current were also addressed.

2. Experimental

2.1. Reagents and materials

6-Mercapto-1-hexanol (MCH), NADH, TCEP, cysteine, cysteamine hydrochloride, glutathione, KH_2PO_4, K_2HPO_4, tris-(hydroxymethyl)aminomethane hydrochloride (Tris–HCl) and streptavidin-conjugated alkaline phosphatase (SA-ALP) were obtained from Sigma–Aldrich. Diethylpyrocarbonate, p-AP, NaCl, Na_2SO_4 and single-stranded DNA (ss-DNA) probe (5′-SH-$(CH_2)_6$-AAC TAT ACA ACC TAC TAC CTC A-3′) was purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). 4-Aminophenylphosphate (p-APP) was purchased from Enzo Life Sciences, Inc. (Farmingdale, USA). The target microRNAs with and without biotin modification (5′-biotin-UGA GGU AGU AGG UUG UAU AGU U-3 and 5′-UGA GGU AGU AGG UUG UAU AGU U-3) were obtained from GenePharma Co., Ltd. (Shanghai, China). The DNA solution was prepared using TE buffer solution (10 mmol L^{-1} Tris–HCl, 1 mmol L^{-1} EDTA, pH 7.4), and kept at −18 °C. The miRNAs stock solutions were prepared daily with diethylpyrocarbonate-treated water in an RNase-free environment. All aqueous solutions were prepared with a Millipore system (Simplicity Plus, Millipore Corp.). The hybridization solution was prepared with TNE buffer (TE + 0.1 mol L^{-1} NaCl). The supporting electrolyte was phosphate-buffered saline solution (PBS buffer, 50 mmol L^{-1}, pH 7.4) containing 50 mmol L^{-1} Na_2SO_4.

2.2. Performances of the p-AP redox cycling using different reductants

The performances of sensor using p-AP redox cycling with different reductants were evaluated at a DNA/MCH-covered electrode. The gold disk electrode with a diameter of 2 mm was polished with diamond paste down to 0.05 μm and alumina pastes down to 0.3 μm, and then sonicated in ethanol and water. The DNA probe-covered electrode was prepared by immersing the cleaned gold electrode in a solution of 1.0 μmol L^{-1} thiolated ssDNA containing 5 μmol L^{-1} TCEP in the dark for 12 h. The amount of probe immobilized on the gold electrode was chosen according to the well-established protocol [34]. This step was followed by washing