

Detection of guanine at a redox polymer modified indium tin oxide electrode

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Abstract

The electrochemical behavior of guanine at a redox polymer film modified indium tin oxide electrode is examined by voltammetry and amperometry. The results indicate that the modified electrode reduces the overpotential of guanine oxidation by as much as 550 mV. The oxidation of guanine is pH dependent and occurs at 0.61 V (versus Ag/AgCl) under physiological conditions, the lowest oxidation potential of guanine ever reported. Rotating disk electrode (RDE) experiments reveal that the kinetics of the electrocatalysis is very fast, and hence the oxidation current is solely controlled by mass-transport process in solution. Guanine is detected amperometrically at 0.65 V at the modified electrode with a sensitivity enhancement of two orders of magnitude over an unmodified electrode. A calibration curve is linear over the concentration range of 8.0 nM–100 μ M with a correlation coefficient of 0.997 and a detection limit of 5.0 nM. The modified electrode also shows good anti-fouling properties towards the oxidation product of guanine and surfactants.

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1. Introduction

Recently there have been considerable efforts in the development of electrochemical methods for the detection of nucleic acid in biological samples based on guanine oxidation [1–3]. The inherent miniaturization of electrochemical biosensors and their compatibility with advanced semiconductor technologies promise to provide a simple, miniaturized, and affordable platform for nucleic acid assays. It is generally believed that direct oxidation of guanine at bare electrodes is totally irreversible and therefore require a high overpotential [2,3]. The lowest oxidation potential, \sim 0.69 V (versus Ag/AgCl) was obtained at a graphite electrode [4]. And direct oxidation of guanine often suffers from pronounced fouling effect resulting in poor reproducibility. The high overpotential and the poor reproducibility usually limit the use of the bare electrodes for direct detection of guanine in biological matrices [5,6].

There have been numerous attempts to enhance the electrode kinetics using various electrocatalytic approaches. To date, sev-

eral kinds of materials, such as polynuclear transition metal hexacyanometallates [7], transition metal complexes [8–10], redox polymers [11], and carbon nanotubes [12,13] have been explored for their potential utility in the catalytic detection of guanine. For example, it has been demonstrated that the electrochemical oxidation of guanine can be facilitated by ruthenium–bipyridine complexes [8,9]. Such systems have resulted in the decrease in overpotential of guanine oxidation, improvement in the stability of the electrode, and enhancement in sensitivity. Based on these observations, Thorp proposed a few homogeneous electrocatalytic schemes for the detection of nucleic acids using the catalytic nature of Ru(bpy)₃Cl₂ for the oxidation of guanine in nucleic acids [2]. Rusling and co-workers demonstrated that there is a direct correlation between the catalytic current and the degree of DNA damage in a series of reports [14–17]. Another example is the modification of a carbon paste electrode with a cobalt hexacyanoferrate thin film. The resulted electrode shows excellent catalytic activity towards the oxidation of guanine [7]. However, one serious problem associated with the above mentioned modified electrodes is the relatively high operating potentials, \sim 0.90 V for the cobalt hexacyanoferrate modified electrodes and \sim 1.20 V for the Ru(bpy)₃Cl₂ homogeneous electrocatalytic systems [2,8,9], making difficult

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the accurate determination of guanine due to the intrinsically high background current.

Our laboratory is interested in fabricating ultrasensitive nucleic acid biosensors using low redox potential biocatalysts/electrocatalysts as sensitivity enhancers [18,19]. The poor electron-transfer kinetics from nucleic acids to most electrode materials can be addressed using nucleic acid (guanine) oxidation catalysts and sensitive nucleic acid detection systems are expected. In addition, a low potential catalyst is beneficial in enhancing the detectability of guanine because electrochemical detection at a significantly low operating potential minimizes potential interferences and reduces the background signal, yielding an improved signal/noise (S/N) ratio and a low detection limit. In this work, the feasibility of modifying an indium tin oxide (ITO) electrode with a novel low redox potential catalyst in an attempt to develop a sensitive electrochemical procedure for guanine was studied. A remarkable improvement in voltammetric responses of guanine and a noticeable enhancement in amperometric sensitivity were observed at the redox polymer thin film modified ITO electrode. All of these were brought about by the combined catalytic function of the redox polymer film and the minimized background current. Electrocatalytic signal was observed when as little as 5.0 nM of guanine was present in the sample solution.

2. Experimental

2.1. Materials and apparatus

$\text{Ru}(\text{bpy})_2\text{Cl}_2$ (99%) was obtained from Avocado Research Chemicals Ltd. (Leysham, UK). Guanine (>98%) was obtained from Sigma–Aldrich (St. Louis, MO). Oligonucleotides were custom-made by 1st Base Pte Lte (Singapore). The redox polymer, a poly(vinylimidazole-co-acrylamide) (PVIAA) partially imidazole-complexed with $[\text{Ru}(\text{bpy})_2\text{Cl}]^+$ (bpy: 2,2'-bipyridine) (PVIAA–Ru), was synthesized from PVIAA and $\text{Ru}(\text{bpy})_2\text{Cl}_2$, as described elsewhere [20]. ITO coated glass was from Delta Technologies Limited (Stillwater, MN). Conducting epoxy was purchased from Ladd Research (Williston, VT). All other reagents of certified analytical grade were obtained from Sigma–Aldrich and used without further purification. A phosphate-buffer saline (PBS, pH 7.4), consisted of 0.15 M NaCl and 0.020 M phosphate buffer, was used as the supporting electrolyte.

Electrochemical experiments were carried out using a CH Instruments model 660A electrochemical workstation coupled with a low current module (CH Instruments, Austin, TX). A conventional three-electrode system, consisting of the modified electrode ($0.20 \pm 0.02 \text{ cm}^2$), a non-leak Ag/AgCl (3.0 M NaCl) reference electrode (Cypress Systems, Lawrence, KS), and a platinum wire counter electrode, was used in all voltammetric and amperometric measurements. The ITO electrode was etched with an aqueous solution of 20% HCl and 5% HNO_3 except for the area of 0.20 cm^2 used as the working electrode. Electrical contact was made to the ITO electrode using the conducting epoxy and a copper wire. The contact formed had a resistance $<1.0 \Omega$. Rotating disk electrode (RDE) experiments were con-

ducted with an Analytical Rotator (Pine Instrument Co., Grove City, PA). The working electrode was a PVIAA–Ru/poly(T)₃₀ modified gold electrode with thiolated poly(T)₃₀ as the underlayer. All potentials reported in this work were referred to the Ag/AgCl reference electrode unless otherwise stated.

2.2. Fabrication of PVIAA–Ru/poly(T)₃₀ thin film modified ITO electrode

PVIAA–Ru redox polymer thin films were deposited onto the ITO electrode by alternative adsorption from aqueous solutions of the anionic poly(T)₃₀ and the cationic PVIAA–Ru redox polymer through sequential layer-by-layer electrostatic self-assembly. In principle, any guanine-free nucleic acid can be used as the anionic layer. It has been shown that ITO is an excellent substrate for immobilizing nucleic acid or oligonucleotides [21]. As described previously, the ITO electrode was first immersed in a DMF/H₂O mixture (9/1) buffered with 10 mM acetate containing poly(T)₃₀ (1.0 mg/mL) for 10 min. A poly(T)₃₀ monolayer was irreversibly immobilized on the ITO electrode [21]. The high density of negative charges at the ITO surface brought about by the poly(T)₃₀ monolayer provides an excellent vehicle for further electrostatic layer-by-layer self-assembly. After being rinsed with water, the electrode was placed in PBS containing 1.0 mg/mL PVIAA–Ru for 10 min. After a thorough rinsing with PBS, the electrode was then immersed in a 1.0 mg/mL poly(T)₃₀ solution in PBS for 10 min. The last two steps were repeated with a thorough rinsing after each step to make films with multibilayer architecture. The electrode was finally thoroughly rinsed with water and conditioned by cycling the potential for 5 min between 0.0 and 0.80 V at a scan rate of 100 mV/s in PBS. Under optimum experimental conditions, this sequential electrostatic layer-by-layer self-assembly process produced up to 50 bilayers on the ITO electrode.

3. Results and discussion

3.1. Electrochemical behavior of the PVIAA–Ru/poly(T)₃₀ film on ITO electrode

The PVIAA–Ru redox polymer was synthesized using PVIAA and $\text{Ru}(\text{bpy})_2\text{Cl}_2$ as precursors. An appropriate amount of $\text{Ru}(\text{bpy})_2\text{Cl}_2$ was added to ethylene glycol containing 10–20 mg/mL PVIAA, and the mixture was refluxed under nitrogen. The formation of the PVIAA–Ru redox polymer can be conveniently monitored by cyclic voltammetry. During reflux in ethylene glycol, the reaction mixture was sampled and tested by cyclic voltammetry every 5 min. Fig. 1 shows typical voltammograms obtained during the reflux. One pair of minute voltammetric peaks centered at 0.61 V were observed 5 min after mixing $\text{Ru}(\text{bpy})_2\text{Cl}_2$ with PVIAA, indicating the formation of the PVIAA–Ru redox polymer via ligand exchange between chloride and imidazole, grafting $\text{Ru}(\text{bpy})_2\text{Cl}^+$ onto the PVIAA. The other pair of voltammetric peaks at 0.43 V corresponds to the well-known redox process of $\text{Ru}(\text{bpy})_2\text{Cl}_2$ (Fig. 1, trace a). Both electron-transfer processes are clearly resolved and have all the characteristics of reversible processes, except the slightly larger

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