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Direct electrochemical detection of guanosine-5′-monophosphate at choline monolayer supported and gold nanocages functionalized carbon nanotubes sensing interface

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ABSTRACT

A convenient and rapid system was developed for direct electrochemical detection of guanosine-5′-monophosphate (GMP) based on the construction of choline (Ch) monolayer supported and gold nanocages (Au NCs) functionalized carbon nanotubes sensing interface. Taking advantage of positively charged Ch film with $-N^{+}(CH_3)_3$ polar head groups, Au NCs-carbon nanotubes nanocomposite could be uniformly and compactly assembled onto electrode surface, and Au NCs were favorable for the functionalization of sensing interface because of their hollow interiors and porous walls. The proposed system displayed prominent electrocatalytic activity towards the oxidation of GMP due to the well distribution status, large electroactive area, as well as fast electron transfer kinetics of sensing interface. As a result, GMP was sensitively detected with a low detection limit of 0.1 μM. Moreover, the sensing platform was applied in the assessment of GMP levels in oligonucleotides with satisfactory results. In contrast to the detection performances of other methods, the present work exhibited advantages of rapidity, low cost, and ease of operation, which may make the sensing system promising for potential applications in DNA assay and the fundamental research of interfacial electrochemistry.

1. Introduction

Guanosine-5′-monophosphate (GMP), as a kind of purine nucleotide, plays vital roles in many functions related to normal cellular metabolism and cardiac activities, such as improving the gastrointestinal tract repair after damage [1], protecting brain slices in a model of hypoxia [2], influencing the metabolism of fatty acids [3], enhancing the immune response [4], and stimulating the removal of extracellular glutamate [5]. GMP synthesized by a purine pathway from xanthine monophosphate is the starting material for the preparation of other guanosine monophosphates [6]. Further studies revealed that α-adrenergic receptor activation and blood pressure are associated with the change of GMP level in cells [7]. In order to elucidate the physiological function of GMP in organism, it is necessary to establish reliable and sensitive method for GMP determination. In 2010, Lowery's group explored an efficient enzymatic Transcreener GMP assay based on the recognition of polyclonal antibody to GMP with a far-red fluorescence polarization (FP) readout, which exhibited excellent performances of extremely high selectivity and nanomolar sensitivity [8]. Afterwards, they further developed a high-throughput assay for Rho guanine nucleotide exchange factors based on the Transcreener GDP assay [9]. Interestingly, Parak et al. constructed a photo-electrochemical biosensor for the specific detection of GMP using a sequential enzymatic reaction cascade at a CdS/ZnS quantum dot electrode, which opened up a new functional sensing system [10].

Besides enzyme-based assays, a variety of analytical methods have been developed to detect GMP, such as thin layer chromatography [5], capillary zone electrophoresis [11], high performance liquid chromatography (HPLC) [12], fluorescence spectrum [13], and electrochemical method [14]. Among them, electrochemical approach has gained increasing attention due to its inherent features of low cost, instrument simplicity, fast response, high selectivity, and real-time assay in situ conditions [15–21]. The electrochemical reaction mechanism of GMP was based on the oxidation of electroactive guanine base, and the N7 = C8 bond of guanine was oxidized to form 8-oxoguanine [22–26]. In recent years, different kinds of electrode materials have been designed to investigate the oxidation behavior of guanine in GMP or guanosine, such as nanocarbon film electrode [27], chemically reduced graphene oxide modified electrode [28], boron-doped diamond electrode [29], anodized epitaxial graphene electrode [30], carbon

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ionic liquid electrode [31], and graphene nanowalls modified electrode [22]. Carbon-based electrodes were demonstrated to be efficient sensing platforms for the detection of electroactive biomolecules, while the enhancement of sensitivity is highly required because the amount of analyte is often minimal in many biological samples. It was well accepted that larger surface area and greater number of surface active atoms can promote the electron transfer process involved in electrochemical reaction [32,33]. In order to improve the sensitivity of carbon-based electrode for target analysis, the functionalization of carbonaceous material with noble metal nanomaterial is an encouraging way.

Gold nanocage (Au NC) is a class of interesting nanostructure possessing hollow interiors and porous walls [34,35]. In contrast to solid nanoparticle, both the inner surface and the outer surface of nanocage contributed to catalytic reaction process [35]. Therefore, the hollow interiors of Au NC significantly increased the effective surface area, which provided more active sites for the enhancement of interfacial electron transfer. Choline (Ch) containing $-OH$ and $-N^+(CH_3)_3$ groups is an integrant constructional component for cell membrane in human body. In our previous work, it was revealed that Ch film could be covalently immobilized onto the surface of carbon electrode [36,37]. Further studies demonstrated that Ch monolayer provided a functionalized substrate for the construction of Pt nanoclusters [38], Au nanoflowers [39,40], and graphene nanosheets [17]. In these researches, Ch film with $-N^+(CH_3)_3$ polar head groups not only increased the active site density of electrode surface for electron transfer, but also conferred a favorable local microenvironment with positive charges for the assembly of nanomaterials [36–40], which demonstrated the promising application prospect of Ch film in the research of interfacial electrochemistry.

Herein, based on the outstanding properties of Ch monolayer and Au NCs, an efficient nanocomposite sensing interface was constructed for GMP detection. Benefitted from the distinctive physicochemical characteristics of Au NCs, Ch monolayer, as well as carbon nanotubes, the resulting sensing interface exhibited remarkable catalytic activity towards the direct electrochemical oxidation of GMP, which paved the way for voltammetric detection with high sensitivity. Compared with the detection performances of chromatography-based methods, the advantages of the system were shown to be rapid, convenient and inexpensive. Moreover, the proposed system realized the analysis of GMP contents in different lengths of oligonucleotides with acceptable results.

2. Experimental

2.1. Materials and reagents

Ch was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). GMP and oligonucleotides containing 10, 25, 40 and 80 bases were obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). Their base sequences were 5′-AGG GCA GCT A-3′, 5′−CCG CAG TCA GAT CCT AGC GTC GAG C-3′, 5′-CAG CAT CTT ATC CGA GTG GAA GGA AAT TTG CGT GTG GAG T-3′, and 5′-TCC AGT GGT AAT CTA CTG GGA CGG AAC AGC TTT GAG GTG CGT GTT TGT GCC TGT CCT GGG AGA GAC CGG CGC ACA GAG GA-3′. $HAuCl_4{\cdot}4H_2O$, $AgNO_3$, ethylene glycol, polyvinylpyrrolidone (PVP) and sodium sulfide were obtained from Sigma-Aldrich (USA). The dispersion of multiwalled carbon nanotubes (MWCNTs, OD: 30–50 nm; length: 0.5–2 μm; purity ≥ 95%) was purchased from Nanjing Xianfeng Nano Material Tech Co., Ltd. (Nanjing, China). Nuclease P1 was obtained from Yamasa Shoyu Co., Ltd. (Tokyo, Japan). Phosphate buffer solutions (PBS, 0.1 M) with different pH were prepared by mixing stock solutions of H_3PO_4, KH_2PO_4, Na_2HPO_4 and NaOH. Ch solution was freshly prepared in pH 7.0 PBS containing 10 mM KCl. The deionized water (specific resistance > 18.2 MΩ cm) from a Milli-Q gradient system (Millipore) was used throughout the experiment.

2.2. Apparatus and measurements

Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were carried out with a CHI 660E electrochemical workstation (Chenhua, Shanghai, China). A conventional three-electrode system consisted of a glassy carbon working electrode (GCE), a platinum wire counter electrode, and an Ag/AgCl reference electrode. Electrochemical impedance spectroscopy (EIS) experiments were performed on a PGSTAT30/FRA2 system (Autolab, Nertherlands). Field emission scanning electron microscope (FE-SEM) images were obtained on an S-4800 microanalyzer (Hitachi, Japan). Transmission electron micrograph (TEM) images were acquired on a JEM-2100 transmission electron microscope (JEOL, Japan). Fourier transform infrared (FT-IR) spectra were recorded by an AVATAR-370 Fourier transform infrared spectrometer (Thermo Nicolet, USA). UV–vis absorption spectra were taken with a Shimadzu UV-3600 spectrophotometer (Shimadzu, Japan).

2.3. Preparation of Au NCs-MWCNTs nanocomposite

Au NCs were prepared based on literatures with slight modifications [41,42]. Firstly, silver nanocubes (Ag NCs) were synthesized through the following procedures. Briefly, under the nitrogen flow atmosphere, 30 mL of ethylene glycol was heated upto 150 °C with continual stirring. Then, a fresh solution of Na_2S (3 mM) was slowly injected into the above solution and refluxed for 10 min. After incubating with 7.5 mL of PVP (200 mg/mL) for 10 min, 2.5 mL of silver nitrate solution (48 mg/mL) was added and allowed to react until the formation of metallic greenish brown mixture. The resulting Ag NCs were washed with acetone and water for three times, respectively, to ensure the complete removal of reagents. Finally, the precipitate was stored in water for latter use. For the synthesis of Au NCs, 5.0 mL of the as-stored Ag NCs were added into 45 mL of PVP solution, and the mixture was heated to 90 °C with stirring under nitrogen flow. Subsequently, 2.5 mL of $HAuCl_4$ solution (0.5 mM) was added dropwisely, and the resulting mixture was allowed to react untill the color was stable. After washing with saturated NaCl solution to remove any formed AgCl and water to remove excess PVP, Au NCs were finally dispersed in water for further use.

For the preparation of Au NCs functionalized MWCNTs nanocomposite (Au NCs-MWCNTs), a small amount of cysteamine was added into Au NCs colloid and reacted for 3 h at room temperature under vigorous sitirring. Then, the mixture was allowed to age at 4 °C overnight. The resultant Au NCs were collected by centrifugation and washed throughly with water to remove excess cysteamine, and the purified and cysteamine-modified Au NCs were dispersed in water directly for latter use. Besides, in order to improve the solubility and compatibility of MWCNTs, carboxylic functional groups ($-COOH$) were grafted onto the surface of MWCNTs. Briefly, 0.3 g of raw MWCNTs were treated with 50 mL of 3:1 mixture of concentrated sulfuric and nitric acid at 70 °C with vigorous stirring for 24 h. After cooling to room temperature, the mixture was washed thoroughly with water for six times and then dried in vacuum for 12 h.

Subsequently, Au NCs-MWCNTs nanocomposite was prepared by the following procedures. At the first step, EDC (20 mg/mL) and NHS (10 mg/mL) were added into 300 μL of carboxylated MWCNTs (0.75 mg/mL), and the mixture was incubated for 1 h at 37 °C to activate the carboxyl groups on MWCNTs. After being rinsed with water, cysteamine-modified Au NCs were added into the activated MWCNTs, and the resultant mixture was incubated for 1 h with gentle stirring. After washing, the obtained Au NCs-MWCNTs nanocomposite was dispersed in 1 mL water.

2.4. Fabrication of Au NCs-MWCNTs/Ch/GCE

Firstly, GCE was applied as the substrate for the immobilization of Ch monolayer. The GCE was carefully polished in sequential order with 1.0, 0.3 and 0.05 μm of alumina slurries, and then successively rinsed