



# Electrochemical biosensor based on silver nanoparticles–polydopamine–graphene nanocomposite for sensitive determination of adenine and guanine

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## ABSTRACT

A multifunctional Ag nanoparticles (AgNPs)–polydopamine (Pdp)@graphene (Gr) composite was prepared by a simple and mild procedure. Gr was easily coated with Pdp at room temperature and then AgNPs was deposited by mildly stirring. The nanocomposite was characterized by scanning electron microscope (SEM) and transmission electron microscope (TEM). Guanine and adenine as model molecules were employed to study their electrochemical responses at the Ag–Pdp@Gr composite modified electrode, which showed more favorable electron transfer kinetics than Gr modified glassy carbon and AgNPs modified glassy carbon electrodes. The Ag–Pdp@Gr modified electrode exhibited linear ranges of 0.04–50  $\mu\text{M}$  and 0.02–40  $\mu\text{M}$  with detection limits of 4.0 nM and 2.0 nM for guanine and adenine, respectively. The developed method was applied for simultaneous determination of trace-level adenine and guanine in fish sperm. The results demonstrated that the AgNPs–Pdp@Gr nanocomposite was a promising substrate for the development of high-performance electrocatalysts for biosensing.

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## 1. Introduction

Guanine and adenine are important components found in deoxyribonucleic acid and play fundamental roles in life process. They have widespread effects on coronary and cerebral circulation, prevention of cardiac arrhythmias and inhibition of neurotransmitter release [1]. Therefore, the determination of guanine and adenine has great significance to the bioscience and clinical diagnosis.

Electrochemical method has been widely used in the determination of guanine and adenine [2]. However, the low concentrations in biosamples and the complexity of their matrices make determination of guanine and adenine challenging tasks. It is very important to explore new method for signal amplification in order to increase the sensitivity of the detection. Several methods for signal enhancement have been investigated, such as enzyme labeling [3], rolling circle amplification [4] and nanomaterial introduction [5,6]. Among these methods, application of nanomaterial has gained growing interest due to the intrinsic advantages of nanomaterials, such as low cost, good thermal stability and large surface area.

Graphene (Gr) has stimulated intense research interest because of its unique physical and chemical properties, such as high

surface area, high electrical conductivity, good chemical stability, and strong mechanical strength [7]. These properties make it an attractive candidate for fabricating various functional devices, such as electrodes, sensors, photovoltaics and photodetectors [8,9]. Silver nanoparticles (AgNPs) have good conductivity and high electrochemical catalytic activity. The availability of AgNPs will expand the possibilities for the preparation of Ag-doped nanomaterials and extend its application in biosensor [10]. It has been reported that the integration of carbon-based materials and metal nanoparticles usually shows synergistic effects in electrocatalytic applications [11], so there is a reason to expect the integration of Gr and AgNPs will obtain the similar effect on the electrooxidation of guanine and adenine.

Recently, a thin, surface adherent and multifunctional biopolymer–polydopamine (Pdp) layer was prepared on a wide range of inorganic and organic materials by self-polymerization of dopamine in an aqueous solution [12]. A variety of ad-layers, including self-assembled monolayer through deposition of long-chain molecular building blocks, metal films by electroless metallization and bioactive surfaces via grafting of macromolecules have been prepared by Pdp coating [13]. The polymerization of dopamine offers the advantage of a one-step surface functionalization and allows the introduction of a new paradigm in the field of surface modification. In this work, a new multifunctional Ag nanoparticles (AgNPs)–polydopamine (Pdp)@graphene (Gr) composite was firstly prepared through the oxidation of dopamine on Gr at room temperature and subsequent electroless silver deposition by

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mildly stirring. The AgNPs–Pdop@Gr modified glassy carbon electrodes (GCE) showed enhanced catalytic efficiencies towards guanine and adenine oxidation in acetate buffer solution.

## 2. Experimental

### 2.1. Chemicals and materials

Graphite powder, hydrazine solution (50 wt%) and ammonia solution (28 wt%) were purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Guanine, adenine, AgNO<sub>3</sub>, dopamine hydrochloride (DA) and 2-amino-2-hydroxymethylpropane-1,3-diol (Tris) were obtained from Sigma (Saint Louis, MO, USA). Acetate buffer solutions (ABS) were prepared by mixing of 0.1 M CH<sub>3</sub>COOH and CH<sub>3</sub>COONa and adjusting the pH with NaOH. All chemicals were of analytical grade and doubly distilled water was used throughout.

### 2.2. Apparatus

Electrochemical measurements were performed on a CHI 660D Electrochemical Workstation (Shanghai CH Instruments, China). A conventional three-electrode system was used throughout the experiments. The working electrode was a bare, a pretreated or AgNPs–Pdop–Gr composite modified GCE (3.0 mm in diameter); the auxiliary electrode was a platinum wire and a saturated calomel electrode (SCE) was used as the reference. Electrochemical impedance spectroscopy (EIS) was performed in 5.0 mM K<sub>3</sub>Fe(CN)<sub>6</sub>/K<sub>4</sub>Fe(CN)<sub>6</sub> (1/1) mixture with 0.1 M KCl as supporting electrolyte, using an alternating current voltage of 5 mV, within the frequency range of 0.1–10<sup>5</sup> Hz by Autolab Electrochemistry Instruments (Autolab, Eco Chemie, The Netherlands). The morphologies of the nanocomposite were recorded on a JEM 2100 transmission electron microscope (TEM) and a Hitachi S-4800 scanning electron microscope (SEM).

### 2.3. Preparation of graphene and its functionalized products

Graphene oxide (GO) was prepared from graphite powder by the modified Hummers method [14]. In a typical process, 5 g graphite was slowly added into a mixture of concentrated H<sub>2</sub>SO<sub>4</sub> (87.5 mL) and fuming HNO<sub>3</sub> (45 mL) (warning: concentrated H<sub>2</sub>SO<sub>4</sub> and fuming HNO<sub>3</sub> are strongly oxidizing and should be handled with care!). KClO<sub>3</sub> (55 g) was then added in above mixture, and was kept stirring for 96 h. Then the slurry was poured into water and filtered to obtain graphite oxide. After dried at 80 °C, graphite oxide (0.5 g) was exfoliated in 500 mL water with ultrasonic treatment to form a colloidal graphene oxide suspension (1 mg mL<sup>−1</sup>). To get Gr, chemical reduction of the suspension of GO was carried out with hydrazine monohydrate for 24 h at 80 °C. The final product was isolated by filtration, and rinsed thoroughly with pure water and ethanol. Then the product was dried in vacuum and Gr was obtained.

AgNPs–Pdop–Gr nanocomposite was prepared as following: 100 mg Gr was dispersed in 100 mL water by sonication, then 200 mg DA and 120 mg Tris were added into above mixture and dispersed by 1 min sonication in ice water bath. The mixture was magnetically stirred at room temperature for 20 h. The product were filtered, washed and then dried in vacuum overnight at 60 °C to obtain Pdop@Gr. To mildly deposit AgNPs onto the surface of Pdop@Gr, 25.0 mL AgNO<sub>3</sub> aqueous solution (1.0 mM) was added in 25.0 mg Pdop@Gr. The mixture was mildly stirred for 2 h at room temperature, and then the product was filtered, washed and dried in vacuum overnight at 60 °C to obtain AgNPs–Pdop@Gr nanocomposite.

### 2.4. Preparation of Ag–Pdop@gr modified GCE

For electrode preparation, 1 mg AgNPs–Pdop@Gr was dispersed in 10 mL DMF using an ultrasonic bath to give a black suspension. The GCE (3 mm in diameter) was polished carefully with 0.3 and 0.05 μm alumina slurry, and sonicated in water and ethanol, respectively. Then, 8 μL of the suspension was placed on the GCE surface by micropipette and left to dry at room temperature (30 min) to obtain AgNPs–Pdop@Gr/GCE. The Gr/GCE without AgNPs and AgNPs/GCE without Gr were also prepared according to the similar procedure, using a suspension of Gr and AgNPs in DMF. Before voltammetric measurements, the modified electrode was cycled five times between 0.5 and 1.4 V (scan rate 100 mV s<sup>−1</sup>) in a 0.1 M ABS of pH 4.0. The renewal of the electrode surface was easily accomplished by soaking the modified electrode in ABS and cycling the potential as mentioned above.

### 2.5. Preparation of DNA samples

Thermally denatured dsDNA was prepared according to the previous report [15]. In short, 3 mg of the fish sperm DNA was digested using 1 mL of 1 M HCl in a sealed 10 mL glass tube. After heating in boiling waterbath (100 °C) for 80 min, 1 mL of 1 M NaOH was added. After cooling to room temperature, the solution was diluted to 10.0 mL using 0.1 M PBS (pH 7).

## 3. Results and discussion

### 3.1. Characterization

The morphology of the AgNPs–Pdop@Gr nanocomposite was examined by SEM and TEM. Fig. S1a showed the SEM image of the obtained Gr sheets, illustrating the flake-like shapes of graphene. Fig. S1b showed the SEM image of AgNPs–Pdop@Gr nanocomposite. It was clear that AgNPs distributed well on Pdop@Gr sheets, evidencing the well-behaved assembly process. The Pdop acted as a glue reagent connecting the Gr with AgNPs. Such morphological characteristics might result in high loading of guanine and adenine and fast response to the substrate. Fig. S1c showed the typical TEM image of the Gr nanosheets. Fig. S1d showed TEM image of the obtained Pdop@Gr. The transparent Gr turns into blackish nanosheets, illustrating Pdop was successfully coated on Gr. The TEM image (Fig. S1e) showed the AgNPs were deposited on the Gr surface. However, the size of these nanoparticles was not the same, and the dispersion was heterogeneous. The reason might be attributed to the stirring inhomogeneity of graphene nanosheets since the formation of silver nanoparticles was based on Pdop@Gr nanosheets as substrates. In fact, this was just an advantage over the composite surface for increasing the immobilized amount of the target molecules. These AgNPs were firmly attached to Gr sheets, even after the ultrasonication used to disperse the AgNPs–Pdop@Gr composite for TEM characterization. EDX measurement was performed to validate the presence of AgNPs on graphene (Fig. S1f).

### 3.2. Electrochemical reactivity

The capability of electron transfer of different electrodes was investigated by AC impedance experiments and the results were shown in Fig. S2. The sequence of the values of charge-transfer resistance for different electrodes was bare GC electrode (a, 742.6 Ω) > AgNPs/GCE (b, 627.3 Ω) > Gr/GCE (c, 498.2 Ω) > AgNPs–Pdop@Gr/GCE (d, 245.6 Ω). This result demonstrated the AgNPs–Pdop@Gr/GCE had higher electrochemical activity than other electrodes.

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