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A new sight for detecting the ADRB1 gene mutation to guide a therapeutic regimen for hypertension based on a CeO_2-doped nanoprobe

Yilin Zhao[1], Junlin He[1], Yazhen Niu, Jun Chen, Jing Wu, Chao Yu*

Institute of Life Science, College of Pharmacy and School of Public Health, Chongqing Medical University, Chongqing 400016, PR China

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ABSTRACT

The β1-adrenergic receptor gene (Entrez Gene:*ADRB1*), as the target of beta-blockers for hypertension, can directly influence the antihypertensive effect of metoprolol in the Chinese population. This therapeutic effect is often hindered by a lack of evidence-based medical information. To address this challenge, we report a novel assay based on graphene oxide and a CeO_2 nanocomposite functionalized by 3-aminopropyltriethoxysilane supported Pt nanoparticles (GO/CeO_2/PtNPs) as a signal probe. Due to the large specific surface area and good adsorption properties of the GO/CeO_2 nanocomposite, large amounts of PtNPs were immobilized, which amplified the electrochemical signal and improved the sensitivity of the biosensor. To further improvement the sensitivity of the biosensor, Streptavidin (SA) was introduced because it can provide more active sites for the immobilization of the biotinylated capture probe (bio-CP). The electrochemical signal was primarily derived from the catalysis of H_2O_2 by GO/CeO_2/PtNPs. Chronoamperometry was applied to record electrochemical signals, which linearly increased with target DNA. Under optimal conditions, the prepared biosensor had a wide linear range from 1 fM to 10 nM and a low detection limit of 0.33 fM in the detecting of ADRB1 gene. Moreover, the proposed method had good stability and recovery, suggesting its potential for use in clinical research.

1. Introduction

The β1-adrenergic receptor gene (Entrez Gene:*ADRB1*) is known to be the target of beta-blockers for hypertension (Thomas et al., 2010). The ADRB1 gene has a common single nucleotide polymorphism (SNP), ADRB1 Arg389Gly (rs1801253,c.1165G > C), which can directly influence the antihypertensive effect of metoprolol in the Chinese population with hypertension (Jiang et al., 2011). Hypertensive patients with Gly389Arg, which is caused by the 1165G > C SNP, need larger doses than who carrying heterozygous Arg389Arg (Jiang et al., 2011). Therefore, it is crucial to prescreen patients to determine their genotypes before using this drug to minimize the risk of the adverse reactions and provide guidance for clinical personalized therapy.

Various techniques for ADRB1 genotyping via prescreening have been reported, such as DNA sequencing (Kang et al., 2015) and real-time quantitative polymerase chain reaction (qPCR) (Lima et al., 2007; Nicoulina et al., 2010). DNA sequencing requires a great deal of preliminary work (Carson and Wanunu, 2015), and qPCR is poorly suited for implementation due to the complicated sample pre-treatment, high cost, relatively long detection time, and likelihood of false or negative results (Jiang et al., 2011). Compared with the above methods, DNA biosensors have received a large amount of attention because of their simplicity, rapidity, low cost, minimal sample preparation, and short response time (Li et al., 2016). Thus, finding a highly sensitive and selective DNA-based electrochemical sensor for ADRB1 mutation detection appears to be a realistic goal.

Cerium oxide (CeO_2) is an interesting metal oxide due to its unique catalysis properties and the high mobility of oxygen vacancies at the surface, leading to extensive applications in the field of catalysis in the form of pure CeO_2 or doped/loaded with transition metal components (Zhang et al., 2012). Although it has certain catalysis properties toward H_2O_2, the stability of CeO_2 needs to be improved to further amplify the signal and improve the sensitivity of the biosensor. For this purpose, graphene oxide (GO) was used here due to its high surface area-to-volume ratio, good electron transfer properties, high mechanical strength and good water dispersibility (Wei et al., 2016). To use catalysis in the form of CeO_2 loaded with transition metal components, platinum nanoparticles (PtNPs) were chosen owing to its high aspect ratio, evident catalysis, and strong adsorption to thiol and amino groups (Zhang et al., 2014). Meanwhile, PtNPs not only improve the ability of the biosensor but are also used to immobilize the capture probe. In general, the combination of GO and CeO_2 enhanced the

* Correspondence to: College of Pharmacy, Chongqing Medical University, No.1, Box 380#, Yi Xue Yuan Road, Chongqing, Yuzhong district 400016, PR China.
E-mail address: yuchaom@163.com (C. Yu).
[1] Authors contributed equally to this work.

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loading capacity of PtNPs and the stability of the biosensor. Furthermore, the PtNPs improved catalysis in the construction of the biosensor (Wei et al., 2016).

Herein, an ultrasensitive DNA biosensor based on biotinylated DNA was used as a capture probe (bio-CP) and GO/CeO_2/PtNPs composites were used as syncatalytic amplifiers to enable the detection of the ADRB1 gene mutation. First, for immobilization of bio-CP, Streptavidin (SA) was introduced during biosensor fabrication because it can provide more active sites for the immobilization of bio-CP. Furthermore, gold nanoparticles (AuNPs) were employed to immobilize SA. The sensitivity of the method was significantly increased with the syncatalytic effect of CeO_2 and PtNPs in the GO/CeO_2/PtNPs nanocomposite. GO/CeO_2/PtNPs and biotin-streptavidin system in our system have the following two major advantages: (1) compared with individual CeO_2 and PtNPs, our synthesized GO/CeO_2/PtNPs have a more efficient catalytic ability because of the syncatalysis of H_2O_2 by CeO_2 and PtNPs, and (2) the biotin-streptavidin system not only provides an active site for the immobilization of bio-CP but also improves the sensitivity of the proposed electrochemical DNA sensor. In order to prove the use of SA is necessary for the construction of the biosensor, the comparison between the biosensor which use SA and that without SA was shown in Scheme 1B. The strategy reported here paves the way for the determination of gene-related mutated nucleotides in biosamples.

2. Experimental

2.1. Materials and chemicals

Graphene Oxide (GO) was provided by Nanjing XFNANO Materials TECH Co., Ltd. (China). Gold (III) chloride trihydrate ($HAuCl_4{\cdot}4H_2O$), Chloroplatinic acid (H_2PtCl_6), streptavidin (SA), cerium (III) nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$) and hexamethylenetetramine (HMTA) were obtained from Sigma-Aldrich (St. Louis, USA, www.sigmaaldrich.com). Bovine Serum Albumin (BSA), potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$) were purchased from the Beijing Chemical Reagents Company (Beijing, China). Clinical serum samples were obtained from the First Affiliated Hospital of Chongqing medical university (Chongqing, China). The DNA oligonucleotides, which are illustrated in Table S1, were synthesized and purified by Sangon Biotechology, Inc. (Shanghai, China).

The buffer solutions involved in this study were as follow: 1×TE buffer (10 mM Trishydroxymethylaminomethane hydrochloride (Tris-HCl), and 1.0 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0), which was used to dissolve all oligonucleotides. The other buffer solutions are listed in Table S2. All other reagents were of analytical reagent grade and used without further purification. Ultrapure water (> 18.2 MΩ) obtained from a Millipore Mill-Q purification system was used throughout the experiment.

2.2. Apparatus and characterization

All electrochemical experiments were carried out on a CHI660E electrochemical workstation (Chenhua Instruments Co., Shanghai, China). Scanning electron microscopy (SEM) images were obtained using a Hitachi S-3000N (Hitachi Limited, Japan). Field emission scanning electron microscopy (FE-SEM) image was conducted using a Hitachi S4800 (Hitachi Limited, Japan). Fourier transform-infrared (FT-IR) spectra were collected using a Nicolet 6700 FT-IR spectrometer with KBr pressed disks (Thermo Nicolet, USA). Energy dispersive X-ray spectroscopy (EDS) was measured using a JEOL JSM-6700F microscope (Japan). A conventional three-electrode system was used for all electrochemical measurements: a platinum wire electrode as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode, and a modified glassy carbon electrode (GCE, 4 mm in diameter) as the working electrode.

2.3. Preparation of GO/CeO_2

GO/CeO_2 composites were synthesized by the method described previously with slight modification (Ujjain et al., 2014). First, 10 mg of GO was dissolved in 5 mL of ultrapure water under ultrasonication for 0.5 h to form a homogenous colloidal suspension of exfoliated GO. Next, 10 mL of 0.025 M $Ce(NO_3)_3{\cdot}6H_2O$ was mixed with 10 mL of 0.025 M of HTMA. Then, both solutions were mixed and kept in a water bath at 80 °C for 5 h and filtered followed by repeated washing with ultrapure water. Finally, the obtained precipitate was dried at 60 °C under reduced pressure.

Subsequently, 20 mg of GO-CeO_2 composites was dispersed in 5 mL of ethanol and ultrasonicated for 15 min to obtain symmetrical dispersions. Then, 0.1 mL of APTES was added and kept in reflux at 70 °C for 1.5 h. After cooling to room temperature, the obtained GO/CeO_2 composites were isolated by centrifugation at 8000 rpm for 5 min and washed three times with ultrapure water. Finally, the prepared products were dried at 50 °C for 12 h before further use.

2.4. Preparation of GO/CeO_2/PtNPs

GO/CeO_2/PtNPs nanocomposites were synthesized using the $NaBH_4$ reduction method. Briefly, 1 mL of H_2PtCl_6 (1%) was added to 1 mL of a (2 mg mL^{-1}) GO-CeO_2 solution and vigorously sonicated for 10 min. Then, 2 mL of a $NaBH_4$ (0.1 M) solution was dropwise added into the mixture with stirring for 30 min, followed by centrifugation and washing with ultrapure water and dissolution in 1 mL of ultrapure water for further use.

2.5. Synthesis of PtNPs, GO/PtNPs and GO/CeO_2/AuNPs

GO/CeO_2/AuNPs were synthesized by the $NaBH_4$ reduction method. Briefly, 1 mL of $HAuCl_4{\cdot}4H_2O$ (1%) was added into 1 mL of a (2 mg mL^{-1}) GO/CeO_2 solution and sonicated for 10 min. Then, 2 mL of a $NaBH_4$ (0.1 M) solution was slowly added into the above mixture with stirring for 30 min, followed by centrifugation and washing with ultrapure water. GO/PtNPs composites were synthesized with a similar method for GO/CeO_2/AuNPs preparation, except that 1 mL of (2 mg mL^{-1}) GO/CeO_2 was displaced by 1 mL of (2 mg mL^{-1}) GO. PtNPs were also synthesized as follows. First, 1 mL of H_2PtCl_6 (1%) was added into 1 mL of ultrapure water. Then, 2 mL of a $NaBH_4$ (0.1 M) solution was slowly added into the above mixture with stirring for 30 min, followed by centrifugation and washing with ultrapure water.

2.6. Preparation of GO/CeO_2/PtNPs/SP, GO/CeO_2/AuNPs/SP, GO/PtNPs/SP and PtNPs/SP

In brief, 200 μL of a 1 μm thiol-modified signal probe (SP) was mixed with 1 mL of as-prepared GO/CeO_2/PtNPs and stirred gently overnight at 4 °C. Afterward, 1 mg of BSA that was suspended in 1 mL of TE buffer was added, and the mixture was allowed to further react for another 4 h at 4 °C for blocking the nonspecific binding sites on the surface of the GO/CeO_2/PtNPs. The product was obtained by centrifuging, washing, and recentrifuging, followed by dispersal in 1 mL of hybridization buffer for further use. The preparation process of GO/CeO_2/PtNPs/SP is shown in Scheme 1A. The GO/CeO_2/AuNPs/SP bioconjugates were synthesized with a similar process for GO/CeO_2/PtNPs/SP, except that GO/CeO_2/PtNPs were displaced by GO/CeO_2/AuNPs. Furthermore, GO/PtNPs/SP and PtNPs/SP bioconjugates were also synthesized with an analogous process for GO/CeO_2/PtNPs/SP, except that GO/CeO_2/PtNPs were displaced by GO/PtNPs and PtNPs, respectively.