Sensors and Actuators B 230 (2016) 345–352

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

# Bimetallic Pt/Pd encapsulated mesoporous-hollow $CeO_2$ nanospheres for signal amplification toward electrochemical peptide-based biosensing for matrix metalloproteinase 2

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ARTICLE INFO

*Article history:*
Received 12 August 2015
Received in revised form
21 November 2015
Accepted 16 February 2016
Available online 21 February 2016

*Keywords:*
$Pt/Pd/mhCeO_2NS$
Peptide-based biosensor
Matrix metalloproteinase 2
Electrochemical catalysis

ABSTRACT

A facile electrochemical biosensor for matrix metalloproteinase 2 (MMP-2) was developed based on the target induced cleavage of a special designed peptide by using bimetallic Pt and Pd nanoparticles encapsulated mesoporous-hollow ceria nanospheres ($Pt/Pd/mhCeO_2NS$) as nanocarriers and electrocatalysts. Briefly, employing L-lysine as bridge and linker, $Pt/Pd/mhCeO_2NS$ simply synthesized was used as a loading platform to immobilize electroactive thionine (Thi) and streptavidin (SA), resulting in the final formation of $SA/Thi/Pt/Pd/mhCeO_2NS$ nanoprobes. A specific biotin-labeled peptide (biotin-GPLGVRGKGGC, P1) acted as a molecular recognition element was firstly anchored on the Au nanoparticles modified electrode surface. In the presence of MMP-2, the P1 was specifically cleaved into two fragments at a certain site between G and V, while $SA/Thi/Pt/Pd/mhCeO_2NS$ nanoprobes were bonded onto the resulting electrode surface through the inherent interaction between streptavidin and biotin derived from uncleaved P1. In the proposed protocol, the electrochemical signal amplification was achieved by the effectively catalysis of $Pt/Pd/mhCeO_2NS$ to the decomposition of $H_2O_2$. This could result in the significant enhancement of the electrochemical response for determining MMP-2 in the range of 0.1 pg $mL^{-1}$–10 ng $mL^{-1}$ with a detection limit of 0.078 pg $mL^{-1}$. The present work demonstrated that the combination of the direct transduction of peptide cleavage events with the efficient $Pt/Pd/mhCeO_2NS$ catalysis method, providing a promising effective strategy for MMP-2 detection.



## 1. Introduction

Development of simple, rapid, accurate, and sensitive method for the assay of disease-related proteins, especially cancer biomarkers, is of great importance for many aspects of modern clinical and biomedical fields [1]. Currently, immunoassay protocols based on antibody-antigen interaction is a common approach in the quantitative detection of target biomarkers [2–5]. Nevertheless, there are some fundamental shortcomings to these approaches such as the requirement of costly antibodies, long incubation time and easily denaturing of antibodies with temperature change [6,7]. Thus, it is necessary to develop new methods for protein assays. Peptides, with simple and defined structure, which was obtained using phage display technique, exhibit tremendous potential to act as the substitute for bioassay due to their advantages, including versatility, reliability, cost effectiveness, resistance to harsh environments [8,9]. Several biosensors based on target induced cleavage of peptide have been employed to detect some proteases such as matrix metalloproteinases (MMPs) [10], prostate-specific antigen [11], collagenase [12], cathepsin B [13], caspase [14] and thrombin [15].

MMPs, as a class of zinc-dependent endopeptidases, have received extensive attention because their over-expression was observed in many human cancers [16,17]. Among them, MMP-2 has been recognized as a biomarker of breast cancer, protease cancer and ovarian cancer [18–21]. Since a specific peptide with the amino acid sequence PLGVR has been identified as a substrate to measure MMP-2, several peptide-based assay platforms have been explored for the determination of MMP-2, such as fluorescence [22], surface plasmon resonance [23], photoluminescence assays [24]. However, electrochemical methods, which possess such distinct merits as high sensitivity, cheapness, and small size, have been rarely used for detection of MMP-2 in clinical samples [25]. Thus, it is meaningful to search for more sensitive and effective electrochemical platform for the detection of MMP-2 based on specific peptide cleavage induced by target.

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http://dx.doi.org/10.1016/j.snb.2016.02.064
0925-4005/

To improve the sensitivity of the electrochemical method, various nanomaterials with versatile properties have been extensively employed for signal amplification [26]. Particularly, notched mesoporous-hollow ceria nanospheres (mh$CeO_2$NS), with uniform pore distribution, high surface area, and rigid framework, are desirable to be served as supporting materials to immobilize biomolecules [27], even though $CeO_2$ nanoparticles suffer from low electron conductivity [28]. However, this could be greatly improved by forming nanocomposites of $CeO_2$ with bimetallic Pt/PdNPs, which are of unique electronic conductivity and catalytic property [29]. According to previous reports [30], from the chemistry stand point, lanthanide ions such as cerium ($CeO_2$) are hard Lewis acids. And they have high affinity with hard ligands such as phosphate and carboxyl groups. As a result, we attempted in this work to decorate Pt/PdNPs onto the surface of mh$CeO_2$NS by using L-lysine with —$NH_2$ and —COOH as a bridge and linker [30], resulting in the formation of Pt/Pd/mh$CeO_2$NS due to the strong interaction between —$NH_2$ and metal NPs [31,32]. Then, Pt/Pd/mh$CeO_2$NS were served as nanocarriers to anchor streptavidin (SA) and electroactive Thi to obtain the proposed nanoprobes SA/Thi/Pt/Pd/mh$CeO_2$NS. Meanwhile, a specific peptide labeled with biotin (biotin-GPLGVRGKGGC, P1) was firstly oriented onto the electrode surface electrodeposited with Au. Then the introduction of target MMP-2 resulted in the specific recognition and cleavage of P1 at a certain site between G and V. Through the high affinity of SA to biotin in remained peptides not cleaved by MMP-2, SA/Thi/Pt/Pd/mh$CeO_2$NS could be bound to the resultant electrode surface. So the electrochemical response could be detected due to the presence of Thi. Based on our observations, the developed peptide-based detecting platform for MMP-2 exhibited improved analytical performance, which was originated from the desired conductivity and catalytic activity of Pt/Pd/mh$CeO_2$NS as nanocarriers and signal enhancer, indicating the potential application for other proteases detection.

## 2. Experimental

### 2.1. Reagents and material

Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-7 (MMP-7) were bought from Sino Biological Inc., (Beijing, China). Ammonium cerium (IV) nitrate ($(NH_4)_2Ce(NO_3)_6$) and L-lysine were from KeLong Bio. Co., Ltd. (Chengdu, China). Glutaraldehyde (GA) was obtained from Beijing Chemical Reagent Co., (Beijing, China). Palladium potassium chloride ($K_2PdCl_4$), chloroplatinic acid ($H_2PtCl_6$), gold chloride ($HAuCl_4$), streptavidin (SA), bovine serum albumin (BSA), hemoglobin (Hb), L-cysteine (L-cys), thrombin (TB), prostate specific antigen (PSA), human IgG and thionine (Thi) were obtained from Sigma–Aldrich Chemical Co., (St. Louis, MO, USA). Sodium borohydride ($NaBH_4$) and glucose were purchased from Chemical Reagent Co., (Chongqing, China). The MMP-2 specific peptide (P1, biotin-GPLGVRGKGGC) was synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China).

Phosphate buffered solution (PBS) with different pH values consists of 0.1 M $KH_2PO_4$, 0.1 M $Na_2HPO_4$ and 0.1 M KCl, serving as working buffer throughout the experiment. TCNB buffer (0.05% Brij 35, 150 mM NaCl, 50 mM Tris, 10 mM and $CaCl_2$; pH 7.5) was employed in the experiment. All reagents were analytical grade and used without further purification.

### 2.2. Apparatus

Differential pulse voltammograms (DPV) and cyclic voltammetry (CV) were conducted on a CHI-1040C electrochemical workstation (Shanghai Chenhua Instrument, China). Electrochemical impedance spectroscopy (EIS) was conducted using a CHI 660D electrochemical workstation (Shanghai Chenhua Instrument, China). All electrochemical measurements were carried out using a conventional three-electrode system consisting of a platinum wire as counter auxiliary electrode, a glassy carbon electrode (GCE, $\Phi = 4$ mm) as working electrode and a saturated calomel electrode (SCE) as reference electrode. Scanning electron microscopy (SEM) images were taken with a scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). Raman data were obtained using a Renishaw 2400 laser Raman microscope equipped with a 532 nm argon-ion laser as an excitation source (Renishaw, U.K.). The pH measurement was carried out with a pH meter (MP 230, Mettler-Toledo, Switzerland).

### 2.3. Synthesis of Pt/Pd/mh$CeO_2$NS

According to the reference with minor modification [27], mesoporous-hollow ceria nanospheres (mh$CeO_2$NS) were firstly prepared by a facile hydrothermal method. Briefly, 0.840 g $(NH_4)_2Ce(NO_3)_6$ was dissolved thoroughly in 670 μL of double-distilled water under vigorous magnetic stirring. Afterwards, 20 mL ethylene glycol and 670 μL $CH_3COOH$ were mixed with the above solution, following 5 min stirring under ambient temperature. The resulting wine-red solution was maintained at 180 °C for 10 h in a Teflon-lined stainless-steel autoclave. After that, the beige precipitate was collected by centrifugation, washing with ethanol and double-distilled water, and drying at 70 °C in a vacuum drying oven. The final product mh$CeO_2$NS was obtained from the prepared precursor by calcination in a muffle furnace at 300 °C for 2 h.

Lysine, as a friendly amino acid with carboxyl groups and two amino groups [33], was firstly used as bridge and linker to decorate PdNPs onto the surface of mh$CeO_2$NS through the strong interaction between —$NH_2$ and metal NPs [31,32]. Moreover, lysine was further used as bridge and linker to decorate other noble metal nanoparticles on the surface of Pd/mh$CeO_2$NS. And the final double metal nanoparticles decorated mh$CeO_2$NS (Pt/Pd/mh$CeO_2$NS) was obtained. The synthesis produces were as following: 3 mg mh$CeO_2$NS in 2 mL L-lysine (1%, w/w) aqueous solution were sonicated for 1 h, followed by centrifugation and washing. The resulting L-lysine coated mh$CeO_2$NS was redispersed in 2 mL double-distilled water, in which 300 μL of $K_2PdCl_4$ (10 mM) aqueous solution was added dropwise and vigorously stirred for 5 min, followed by the addition of 200 μL freshly prepared $NaBH_4$ (0.1 M) and keeping stirring for 2 h at ambient temperature. After centrifugation and washing, the prepared Pd/mh$CeO_2$NS was again dispersed in 2 mL L-lysine (1%, w/w) aqueous solution by sonication for 1 h. After centrifugation, the L-lysine coated Pd/mh$CeO_2$NS dispersed in 2 mL double-distilled water was mixed with 150 μL $H_2PtCl_6$ (1%, w/w) aqueous solution by successive addition under vigorously stirring. Then, 200 μL freshly prepared $NaBH_4$ (0.1 M) was dropped into the resultant mixture and stirred for 2 h at ambient temperature. The final product of Pt/Pd/mh$CeO_2$NS was obtained after centrifugation and washed with double-distilled water.

### 2.4. Synthesis of SA/Thi/Pt/Pd/mh$CeO_2$NS nanoprobes

After 30 μL Thi (3 mM) was added into 1 mL Pt/Pd/mh$CeO_2$NS solution and stirred at 4 °C for 12 h, the Thi/Pt/Pd/mh$CeO_2$NS particles were collected by centrifugation and washing with double-distilled water. Then, 1 mL Thi/Pt/Pd/mh$CeO_2$NS particles were incubated with 0.1 mg SA for 1 h at 4 °C to form SA/Thi/Pt/Pd/mh$CeO_2$NS nanoprobes. After centrifuged and washed, the nanoprobes were obtained and dispersed again in 1 mL PBS (pH 7.0) for further use.