Analytica Chimica Acta 934 (2016) 36–43

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

A novel photoelectrochemical biosensor for protein kinase activity assay based on phosphorylated graphite-like carbon nitride

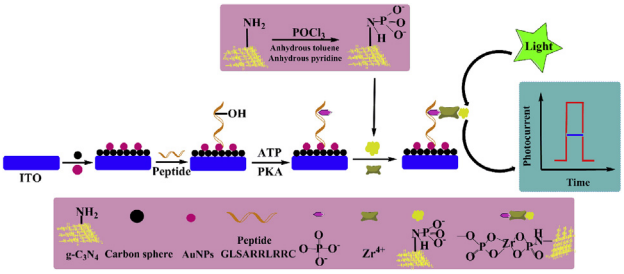
Xue Li, Yunlei Zhou, Yan Xu, Huijie Xu, Minghui Wang, Huanshun Yin**, Shiyun Ai*

College of Chemistry and Material Science, Shandong Agricultural University, Taian, 271018, PR China

HIGHLIGHTS

- A novel photoelectrochemical biosensor is fabricated for kinase activity assay.
- Phosphorylated g-C_3N_4 is used as the photoelectric conversion material with visible-light activity.
- The high affinity between Zr^{4+} and the phosphate group causes the assembly of P-g-C_3N_4 on the substrate peptide.
- The developed method can be applied to detect kinase in real samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form
9 June 2016
Accepted 16 June 2016
Available online 21 June 2016

Keywords:
Photoelectrochemical biosensor
Protein kinase activity
Phosphorylated graphite-like carbon nitride
Inhibitor
Real samples

ABSTRACT

Protein kinases are general and significant regulators in the cell signaling pathway, and it is still greatly desired to achieve simple and quick kinase detection. Herein, we develop a simple and sensitive photoelectrochemical strategy for the detection of protein kinase activity based on the bond between phosphorylated peptide and phosphorylated graphite-like carbon nitride (P-g-C_3N_4) conjugates triggered by Zr^{4+} ion coordination. Under optimal conditions, the increased photocurrent is proportional to the protein kinase A (PKA) concentration ranging from 0.05 to 50 U/mL with a detection limit of 0.077 U/mL. Moreover, this photoelectrochemical assay can be also applied to quantitative analysis of kinase inhibition. The results indicated that the IC_{50} value (inhibitor concentration producing 50% inhibitor) for ellagic acid was 9.1 μM. Moreover, the developed method is further applied to detect PKA activity in real samples, which contains serum from healthy person and gastric cancer patients and breast tissue from healthy person and breast cancer patients. Therefore, the established protocol provides a new and simple tool for assay of kinase activity and its inhibitors with low cost and high sensitivity.

1. Introduction

Photoelectrochemical (PEC) biosensor is a kind of novel and promising detection approach for biological assay with high sensitivity, low background current and simple instrument owing to the separation of electrochemical detection signals and the excitation source [1–3], which has aroused great concern in many aspects such as the detection of enzyme [4], DNA damage [5], subgroup J of avian leukosis virus [1] and small molecule [6]. Thus, we consider that PEC biosensor might be a desired platform for detecting protein kinase activity. As we all know, photoactive material is one of the dominant factors for the performance of PEC biosensor. Photoactive materials utilized in the PEC analysis include ZnS, TiO_2, ZnO and

* Corresponding author.
** Corresponding author.
E-mail addresses: yinhs@sdau.edu.cn (H. Yin), ashy@sdau.edu.cn (S. Ai).

http://dx.doi.org/10.1016/j.aca.2016.06.024
0003-2670/

Bi_2S_3 and so on. For instance, Li et al. reported molecularly imprinted polymer modified TiO_2 nanotube arrays for PEC sensing of perfluorooctane sulfonate [7]. Tu et al. constructed a PEC biosensor of free-base-functionalized ZnO nanoparticles [8]. Sun et al. developed a PEC strategy for subgroup J avian leukosis virus immunoassay based on Bi_2S_3 nanorods [1]. However, it is well known that the wide bandgap (3.2 eV) of TiO_2 impedes intrinsic PEC response under visible light [9], and ZnO suffers from its instability. Also, they are metal-contained, which do not match well with a friendly environment. In consideration of those, it is necessary to develop new photoactive material with environment friendly property.

Recently, as a metal-free semiconductor and the most stable allotrope of carbon nitride material, graphite-like carbon nitride (g-C_3N_4) has become a hot topic. The appealing tri-*s*-triazine ring structure and the medium-gap (2.7 eV) of g-C_3N_4 make it own high stability and an excellent photocatalytic activity with visible light irradiation (λ < 460 nm). Due to its high stability, visible light response and unique electronic band structure, g-C_3N_4 has attracted increasing attention g-C_3N_4 and has been successfully widely applied in many fields, such as photocatalytic water splitting, sensing, photocatalysis and electrocatalysis [10–12]. Recent researches have demonstrated the promising application of g-C_3N_4 in PEC assay. For instance, Li et al. developed a novel multi-amplification PEC immunoassay for NMP-22 detection based on copper (II) enhanced polythiophene sensitized g-C_3N_4 nanosheet [3]. Bu et al. investigated the photo-to-current conversion properties and PEC cathodic protection performance of g-C_3N_4 in 304 stainless steel under visible or white light illumination [13]. Among these PEC assay, g-C_3N_4 and its compounds perform good PEC activity.

Protein phosphorylation catalyzed by protein kinases is a general and significant regulatory mechanism in the cell signaling pathways, which plays an important role in various crucial biological process in metabolism including cellular proliferation, apoptosis, differentiation and gene transcription [14,15]. The over-expression and aberrant expression of protein kinases may lead to severe human diseases such as diabetes, cancer, cardiac diseases and inflammation [16]. Therefore, the simple, rapid and sensitive detection of protein kinase activity is a significant research for pathological process, clinical diagnosis and drug discovery. Traditionally, the detection method of protein kinase activity relied on radioactive adenosine triphosphate (γ-^{32}P-ATP) during the phosphorylation reaction [17], which is general but harmful to the environment and human health. To overcome this defect, various kinase activity assays have been developed as the alternatives of radioactive assay, such as electrochemical [18,19], surface-plasmon resonant [20], fluorophotometry [21] and mass spectroscopic techniques [22,23] etc. In our work, PEC assay is chosen as desired detection method of protein kinase activity due to its high sensitivity, low background current and simple instrument. To achieve sensitive and simple detection, we fabricate a novel PEC method for protein kinase activity assay based on phosphorylated graphite-like carbon nitride (P-g-C_3N_4) nanoparticles as signal transduction probes, where P-g-C_3N_4 nanoparticles are obtained by simple chemical reaction based on amino of g-C_3N_4 according to the previous report with some proper modifications [24–26]. Because of its briefness, convenient and friendly environment advantages and no modification requirement for P-g-C_3N_4 and peptides, this developed strategy represents a potential application in protein kinase analysis.

2. Experimental

2.1. Reagents and instruments

The substrate peptide (s-peptide, CRRLRRASLG) and adenosine 5′-triphosphate (ATP) disodium salt hydrate were obtained from Sangon Biotech Co., Ltd (Shanghai). Protein kinase A (PKA) catalytic subunit, mitogen-activated protein kinase (MAPK), casein kinase I (CK1) and casein kinase II (CK2) were supplied from New England Biolabs Ltd. (Beverly, MA). RIPA Lysis Buffer and phenylmethanesulfonyl fluoride (PMSF) were purchased from Beyotime Biotech (Shanghai) Co., Ltd. Tris (hydroxymethyl) aminomethane (Tris), gold chloride ($HAuCl_4$) and ascorbic acid (AA) were obtained from Aladdin (Shanghai, China). Zirconium oxychloride ($ZrOCl_2$) and phosphorus oxychloride ($POCl_3$) were purchased from Xiya Reagent Co., Ltd. (Chengdu, China). Indium Tin Oxide (ITO, coating 180 ± 25 nm, sheet resistance < 15 Ω/cm^2) slices were acquired from Zhuhai Kaivo Electronic Components Co., Ltd. (Zhuhai, China)). All other reagents are analytically purity graded and used without further purification.

The buffer solutions for this work is shown below. Peptide immobilization buffer, 20 mM Tris-HCl, 2.0 mM EDTA, 2.0 mM NaCl, 2.0 mM TCEP (pH = 7.4). PKA reaction buffer, 0.1 mM EDTA, 80 mM ATP, 2 mM DTT, 50 mM Tris-HCl, 10 mM $MgCl_2$, 0.01% Brij 35 (pH = 7.5). PKA storage buffer, 1 mM Na_2EDTA, 50 mM NaCl, 20 mM Tris-HCl, 2 mM DTT and 50% glycerol. Washing buffer (pH = 7.5), 10 mM Tris-HCl. Detection buffer, 100 mM PBS containing 100 mM AA. All reagents were of analytical purity. All of the aqueous solutions were prepared using redistilled deionized water, which was autoclaved.

Transmission electron microscope (TEM) images were taken with a JEOL-1200EX instrument (Japan). Fourier transformed infrared spectroscopy (FTIR) was obtained as KBr discs on a Nicolet 380 spectrometer. The photocurrent was recorded on a CHI832A electrochemical workstation (CHI instruments, Austin, USA) PEC measurements were carried out with a home-built PEC system with an additional three-electrode system. A modified ITO electrode (0.195 cm^2) was used as the working electrode. A saturated calomel electrode (SCE) and a Pt wire were applied as the reference electrode and the counter electrode. A 500 W Xe lamp equipped with optical filter was employed as the irradiation source to produce the visible light, whose light intensity is 20 mW/cm^2. Electrochemical impedance spectroscopy (EIS) was performed on a CHI660C electrochemical workstation (CHI instruments, Austin, USA).

2.2. Synthesis of carbon microspheres

Carbon microspheres (CS) were prepared according to previous reports [27,28]. Generally, glucose (3.2 g) was first dissolved into deionized water (70 mL) and mixed into a homogeneous solution under vigorous stirring. Then, the mixture was transferred into a Teflon-lined stainless steel antoclave of 100 mL capacity and maintained at 180 °C for 3.5 h. After cooled to room temperature naturally, a dark precipitate was collected by centrifugation and washed successively with ethanol and deionized water several times. Finally, the obtained sample was dried under vacuum at 60 °C.

2.3. Synthesis of gold nanoparticles (AuNPs)

AuNPs were prepared according to the previously reported method [29] with proper modifications. Typically, in a 100 mL two-necked flask, 5 mL of sodium citrate solution (38.8 mM) was rapidly added into 50 mL boiled $HAuCl_4$ solution (1 mM) under magnetic stirring. After a minute of stirring, the liquor's color was observed to change from light yellow to dark red. And then the mixture was maintained further boiling for 20 min. Finally, the obtained gold nanoparticles solution was stored at 4 °C.

2.4. Preparation of g-C_3N_4 and P-g-C_3N_4 nanoparticles

g-C_3N_4 was synthesized according to previous report with some