Sensors and Actuators B 206 (2015) 728–734

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A Phos-tag-based photoelectrochemical biosensor for assay of protein kinase activity and inhibitors

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ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form 23 July 2014
Accepted 7 September 2014
Available online 16 September 2014

Keywords:
Photoelectrochemical assay
Biotinylated Phos-tag
Protein kinase A activity
Bismuth sulfide
Phosphorylation

ABSTRACT

A novel, sensitive and label-free photoelectrochemical assay for monitoring the activity and inhibition of protein kinase A (PKA) was presented based on the specific recognition ability of biotinylated Phos-tag for kinase-induced phosphopeptides. When the assembled substrate peptides were phosphorylated by PKA, they could bind specifically to the biotinylated Phos-tag in the presence of Zn^{2+}. Then streptavidins could be further captured on the electrode surface through their specific interaction towards biotin, resulting in a significant decrease on the photocurrent of substrate Bi_2S_3 modified ITO electrode. The decreased photocurrent was proportional to the PKA concentration ranging from 0.05 to 100 unit/mL with a detection limit of 0.017 unit/mL ($S/N = 3$). Moreover, this photoelectrochemical method could be also used to quantitative analysis of kinase inhibition. In the light of the inhibitor concentration dependant photocurrent signal, the IC_{50} value for ellagic acid and HA-1077 was estimated to be 3.23 and 1.77 μM, respectively. With changing the sequence of substrate peptide, this method could also be applied to detect other kinase activity and inhibitors. Therefore, the developed protocol provides a new and promising platform for assay of kinase activity and its inhibitors with low cost and high sensitivity.

1. Introduction

Protein phosphorylation is an important biological metabolism process which is catalyzed by protein kinase. It also plays crucial role in regulation of signal transduction, cellar proliferation, hormone secretion, cell differentiation, gene expression and apoptosis [1]. It has been reported that the aberrant protein kinase activity and abnormal protein phosphorylation have related to many diseases, such as cancer [2], diabetes [3] and Alzheimer's diseases [4]. Protein kinase activity might be a kind of new biomarker for early detection of disease and evaluation of therapeutic efficacy. Thus, it is important for monitoring and detecting protein kinase activity, which is not only benefit to understand the signal transduction mechanism, but also crucial for drug discovery and clinic therapy.

Conventional technique for detecting protein kinase activity is radiometric method, which relies radioactive adenosine triphosphate (ATP) [5]. However, though this method can selectively detect protein kinase activity, it is harmful to human health due to the radioactive reagent. To eliminate the drawback of radioactive effect, many new assay techniques have been developed, such as fluorescence [6], photoluminescence [7], electrochemiluminescence [8], Raman spectroscopy [9], mass spectrometry [10] and resonance light scattering [11]. However, these methods suffer from the disadvantages of complicated and expensive instruments, time-consuming, tedious sample treatment and sophisticated operation procedures. Electrochemical method has been demonstrated to be a simple, sensitive and cost-effective technique for assay of protein kinase activity compared with the above methods [12–14]. Recently, another electrochemistry-based detection technique, photoelectrochemical (PEC) assay, attracted our research interest for its distinctive features of high sensitivity, low background signal, simple operation, and inexpensive instrument. Up to now, many PEC sensors have been developed for detecting DNA [13], microRNA [15,16], DNA methyltransferase [17,18], cell [19], antigen [20], metal ion [21] and organic compound [22]. Though no relative work has been done for the detection of protein kinase activity, we think that PEC biosensor should be an alternative technique for assay of protein kinase activity and screening of inhibitors according to previous reports in the field of bioassay.

For protein kinase activity detection, one of the crucial factors is the specific recognition element for phosphate group generated from phosphorylation reaction catalyzed by protein kinase. For this point, several patterns have been developed in previous reports, such as phosphorylation specific antibody [11], specific interaction between phosphate group Zr^{4+} [8] or TiO_2 [6],

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http://dx.doi.org/10.1016/j.snb.2014.09.017
0925-4005/

phosphorylation protection against carboxypeptidase Y [23]. Recent reports have described the use of a dinuclear metal complex that acts as a phosphate-binding tag molecule, commercially known as Phos-tag [24,25]. In the presence of two equivalents of Zn^{2+} or Mn^{2+}, Phos-tag can form a specific non-covalent complex with the phosphomonoester dianion at neutral pH. It has also been reported that Phos-tag can selectively interact with phosphorylated peptides or proteins containing phospho-serine, phospho-threonine, phospho-tyrosine, and phospho-histidine residues [25,26]. Up to now, Phos-tag and its derivatives have been widely applied in characterizing and separating phosphorylated proteins [26]. Therefore, PEC assay based on Phos-tag as Phosphate group identification unit should be a promising and useful detection technique for protein kinase activity and inhibitor screening.

In this paper, we report our study on the development of a novel photoelectrochemical sensor for the detection of protein kinase activity and inhibitor screening based on two kinds of specific recognition reaction, Phos-tag-biotin/phosphate group and biotin/streptavidin. As shown in Scheme 1, after AuNPs were immobilized on Bi_2S_3 nanorods modified ITO electrode surface, the substrate peptide with cysteine at the C-end was further assembled on the electrode surface through the bonding between the AuNPs and thiol group of cysteine. Then the assembled peptide on ITO electrode was phosphorylated by protein kinase A (PKA), and the phosphate group could be specifically captured by biotinylated Phos-tag. Through the further specific interaction between biotin and streptavidin, the photocurrent would decreased significantly due to the block effect of streptavidin towards electron donor of ascorbic acid (AA) diffused to Bi_2S_3 surface. The photocurrent decrease is related to the phosphorylation level, and the PKA activity can be detected based on the photocurrent change.

2. Experimental

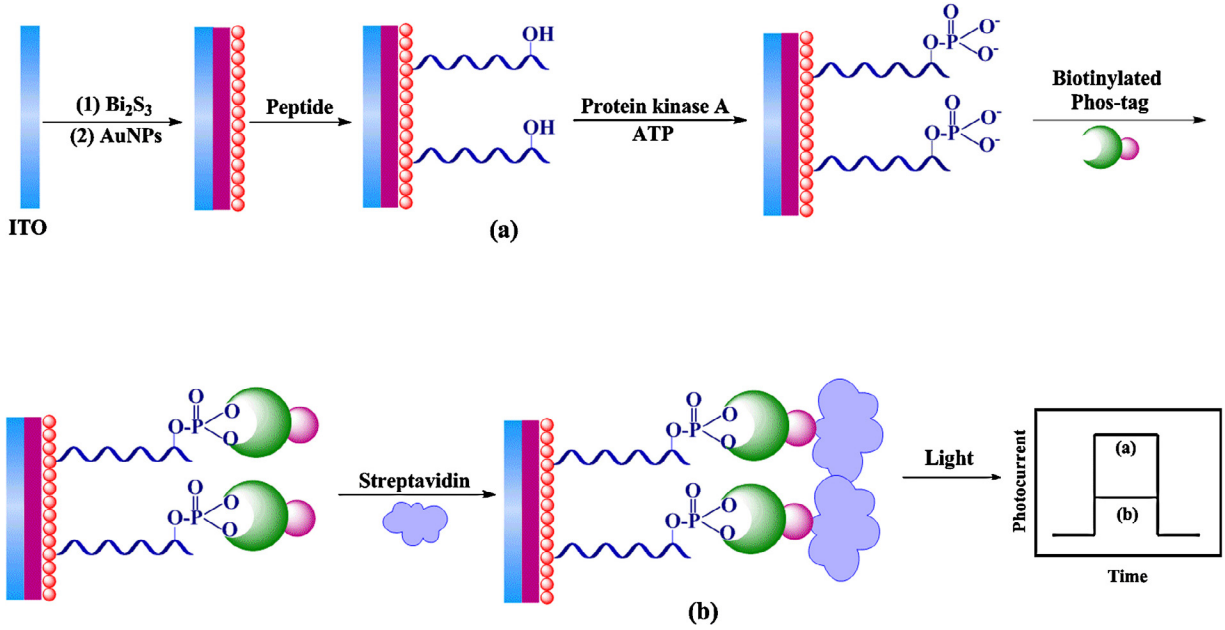
2.1. Reagents and instruments

The substrate peptide (kemptide, H-CGGALRRASLG-NH_2), the control peptide (H-CGGALRRAALG-NH_2), streptavidin and adenosine 5′-triphosphate disodium salt hydrate (ATP) were purchased from Sangon Biotech (Shanghai) Co., Ltd. Mercaptopropionic acid (MPA) was obtained from Fluka. cAMP-dependent protein kinase A (PKA) catalytic subunit, casein kinase I (CK1), casein kinase II (CK2), mitogen-activated protein kinases (MAPK) were supplied by New England Biolabs Ltd. (USA). Biotinylated Phos-tag™ was obtained from Wako Pure Chemical Industries, Ltd. (Japan). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), tris(hydroxymethyl)aminomethane (tris), EDTA, bismuth nitrate ($Bi(NO_3)_3$), ellagic acid, chloroauric acid ($HAuCl_4$) and ascorbic acid (AA) were purchased from Aladdin (shanghai, China). HA-1077 was obtained from EMD Millipore Corporation (Billerica, MA, USA). Gold nanoparticles (AuNPs) [27] was synthesized according to previous reports. Bi_2S_3 nanorods were prepared as described in our previous report [15]. Indium tin oxide (ITO) was purchased from Zhuhai Kaivo Electronic Components Co., Ltd. (Zhuhai, China, ITO coating 180 ± 25 nm, sheet resistance <15 Ω/cm^2). 0.1 M phosphate buffer saline (PBS) was prepared by mixing the stock solution of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4, and the pH was adjusted by 0.1 M HCl or 0.1 M NaOH. Other buffers used in this work, PEC detection system, scanning electron microscopic (SEM) and powder X-ray diffraction (XRD) were listed in Supporting information.

2.2. Biosensor fabrication

The ITO slices were cleaned by immersion in ethanol/NaOH mixed solution (v/v, 1:1) and acetone successively with ultrasonication for 30 min, followed by washing copiously with double distilled deionized water and dried at 60 °C for 2 h. Then, 40 μL of Bi_2S_3 aqueous dispersion (4 mg/mL) was dropped onto a piece of ITO slice. After drying in air, the obtained Bi_2S_3/ITO electrode was ringed with double distilled deionized water for three times. Then, 40 μL of AuNPs was further dropped on the electrode surface and dried under infrared lamp, followed by rinsing with double distilled deionized water for three times (The electrode was noted as AuNPs/Bi_2S_3/ITO).

The AuNPs/Bi_2S_3/ITO was incubated with 40 μL of peptide (0.1 mg/mL, dissolved in 10 mM Tris–HCl containing 10 mM TCEP, pH 7.0) for 12 h in a humidified chamber at ambient temperature. Then, the peptide modified electrode was rinsed with washing buffer for three times to remove the un-immobilized peptide. The peptide-modified electrode (noted as Peptide/AuNPs/Bi_2S_3/ITO) was then immersed in 40 μL of MPA solution (1 mM) for 1 h



Scheme 1. Schematic diagram for the fabrication of biotinylated Phos-tag-based PEC biosensor.