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Highly sensitive photoelectrochemical biosensor for kinase activity detection and inhibition based on the surface defect recognition and multiple signal amplification of metal-organic frameworks

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ABSTRACT

A turn-on photoelectrochemical (PEC) biosensor based on the surface defect recognition and multiple signal amplification of metal-organic frameworks (MOFs) was proposed for highly sensitive protein kinase activity analysis and inhibitor evaluation. In this strategy, based on the phosphorylation reaction in the presence of protein kinase A (PKA), the Zr-based metal-organic frameworks (UiO-66) accommodated with $[Ru(bpy)_3]^{2+}$ photoactive dyes in the pores were linked to the phosphorylated kemptide modified TiO_2/ITO electrode through the chelation between the Zr^{4+} defects on the surface of UiO-66 and the phosphate groups in kemptide. Under visible light irradiation, the excited electrons from $[Ru(bpy)_3]^{2+}$ adsorbed in the pores of UiO-66 injected into the TiO_2 conduction band to generate photocurrent, which could be utilized for protein kinase activities detection. The large surface area and high porosities of UiO-66 facilitated a large number of $[Ru(bpy)_3]^{2+}$ that increased the photocurrent significantly, and afforded a highly sensitive PEC analysis of kinase activity. The detection limit of the as-proposed PEC biosensor was 0.0049 U mL^{-1} (S/N!=!3). The biosensor was also applied for quantitative kinase inhibitor evaluation and PKA activities detection in MCF-7 cell lysates. The developed visible-light PEC biosensor provides a simple detection procedure and a cost-effective manner for PKA activity assays, and shows great potential in clinical diagnosis and drug discoveries.

1. Introduction

Protein phosphorylation regulated by protein kinase and protein phosphatase is important posttranslational modification mechanism and plays cardinal roles in many fundamental cellular processes in eukaryotes, such as signal transduction, cell cycle and transcription (Hunter, 1994). The degree of protein phosphorylation at a certain site depends on the activity of the cognate protein kinase or phosphatase or both (Hunter, 1995). Thus, abnormal protein phosphorylation and aberrant protein kinase activities are closely coupled with many diseases, such as some neurodegenerative diseases, cancers and diabetes (Beristain et al., 2015; Chen et al., 2012; Myeku et al., 2016; Wang et al., 2013). Protein kinase inhibitors can down-regulate the activities of relevant protein kinase and have been emerged as promising drugs for treatment of a series of diseases (Marin et al., 2012; Weickhardt et al., 2012). Therefore, accurate identification of protein kinase activity and their potential inhibitors evaluation are not only essential for the kinase-targeted diagnosis, therapies and drug discoveries, but also helpful for clarifying the molecular signal transduction pathways. Thus, rapid and sensitive assays for protein kinase activity detection and kinase inhibitors screening are highly desirable.

Up to now, various approaches for the protein activity detection have been developed, the traditional one is radiometric methods that use radioactive ^{32}P-labeling (Hastie et al., 2006). However, the inherent drawbacks such as harmful radioactive labels and sophisticated instrumentation limit its further applications. Therefore, alternative techniques have been developed, such as fluorescence (Bai et al., 2013; Shen et al., 2015; Song et al., 2015), chemiluminescence (Wang et al., 2015; Zhao et al., 2015a), mass spectrometry (Deng et al., 2014), and electrochemistry (Shin et al., 2014; Wang et al., 2011), etc. Among these methods above,

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photoelectrochemical (PEC) approach has aroused special attention due to its high sensitivity and hence better analytical performance (Chen et al., 2010; Tang et al., 2013; Wu et al., 2015; Zang et al., 2015; Zhao et al., 2015b, 2014). In the PEC platform, light is utilized to excite PEC active species and the generated photocurrent is utilized as detection signal. Benefiting from the separation of excitation source and detection signal, the PEC method is very sensitive. What's more, it exhibits other merits over the traditional techniques, such as easy readout, inexpensive devices and simple operation (Zhang et al., 2013). Recently, a few of PEC biosensors have been designed for the detection of kinase activity. For example, PEC biosensors for protein kinase A activity determination have been reported based on the specific recognition ability of biotinylated Phos-tag for kinase-induced phosphopeptides combined with the inhibition of electron transfer of streptavidin tag (Zhou et al., 2015) or signal improvement of alkaline phosphatase (Yin et al., 2015). In addition, a sensitive PEC sensing platform for T4 polynucleotide kinase detection based on gold nanoparticles-decorated g-C_3N_4 nanosheets was developed, and DNAzyme-mediated catalytic precipitation signal amplification strategy was applied to improve the sensitivity (Zhuang et al., 2015). However, despite of the improvement of these methods, the synthesizing process of the photoactive species is usually strict and complicated, and generally biotag labeling is needed. Besides, to improve the performances of the PEC sensors, some of the photoactive species are used, such as quantum dots (QDs), noble metal nanoparticles etc. Even though they are effective, most of them are toxic and unstable. For instance, silver nanoparticles are easily oxidized during the light illumination. In addition, QDs have inherent drawbacks such as potential toxicity, high photo bleaching threshold, easy to hydrolysis and oxidation, which limit the applications of the PEC approaches (Zhang et al., 2014).

Metal-organic frameworks (MOFs) are a class of crystalline inorganic-organic hybrids, and they feature large surface area, flexible porosity, easily tailorable compositions and active sites. These remarkable advantages make MOFs attractive in gas storage/separation (Li et al., 2014), catalysis (Liu and Li, 2016), sensing (Zhan et al., 2013), as well as drug delivery (Qin et al., 2012; Tan et al., 2015). UiO series of Zr-cluster-based MOFs are integrated with the merits of high stability in aqueous conditions and less toxicity. Due to the high porosity, they can accommodate a large number of photoactive dye molecules, which can improve the light harvest efficiency. What's more, the metal centers in UiO series of MOFs can serve as "QDs", together with the strong π-π stacking and Van Der Waals interaction between UiO series of MOFs and dye, the electron transport distance can be shorten in the electrode interface. As a result, the electron transfer rate and photo-to-current conversion efficiency can be largely improved (Yuan et al., 2015). In addition, Zr-O clusters in the UiO series of MOFs present high affinity toward phosphoric groups, and they can serve as anchorages for the recognition of phosphoric groups via the formation of Zr-O-P bonds. The high affinity toward phosphoric groups makes Zr-based MOFs being used in phosphates and phosphonates enrichment. For example, UiO-66 nanoparticles with exposed Zr-O clusters and ordered open cavities have been utilized for capture of alendronate, what's more, the Zr-based MOFs also have shown promise in specific enrichment and recognition of phosphopetides (Zhang et al., 2016; Zhu et al., 2015).

Inspired by this, a PEC biosensor for ultrasensitive protein kinase A (PKA) activity detection and inhibitor evaluation was fabricated by utilizing the Zr-cluster-based MOFs (UiO-66 as the model) as anchorages for phosphate groups and carriers for photoactive dyes. In this strategy, when the kemptide modified on TiO_2 fabricated ITO (TiO_2/ITO) electrode was phosphorylated in the presence of protein kinase A (PKA), $[Ru(bpy)_3]^{2+}$ loaded UiO-66 ($[Ru(bpy)_3]^{2+}$@UiO-66) probes were specifically chelated to the phosphorylated kemptide based on the high affinity between surface Zr^{4+} defects on Zr-O clusters and phosphate groups in phosphorylated kemptide. Under visible light irradiation, a sharp photocurrent produced and was applied for PKA activity detection. The simple and cost-effective fabricated PEC biosensor demonstrated good performances with a rapid response and high sensitivity. What's more, the kinase activities analysis in complicated cell lysates samples stimulated by ellagic acid and Forskolin were also carried out. This strategy affords a simple, sensitive and universal platform for kinase activity assays, and presents highly promise in kinases-related life science and medicament field.

2. Material and methods

2.1. Materials and reagents

Cysteine-terminated kemptide (CLRRASLG) was obtained from GL Biochem (Shanghai, China). PKA (catalytic subunit from bovine heart), Adenosin triphosphate (ATP) was obtained from Dingguo Biological Products Company (China). Tris(2,2′-bipyridyl) dichlororuthenium (II) hexahydrate ($Ru(bpy)_3Cl_2·6H_2O$, $[Ru(bpy)_3]^{2+}$), Zirconium chloride ($ZrCl_4$), 1,4-benzenedicarboxylic acid (BDC), and N, N-Dimethylformamide (DMF) were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). 4,4′,5,5′,6,6′-hexahydroxydiphenic acid 2,6,2′,6′-dilactone (ellagic acid), N-(3-chlorophenyl)-6,7-dimethoxy-4-quinazolinamine (Tyrphostin AG1478), Forskolin and 3-isobutyl-1-methylxantine (IBMX) were purchased from Sigma. Other regents of analytical grade were provided by Beijing Chemical Company (China).

2.2. Apparatus and characterization

Photocurrent measurements were carried on a CHI802B electrochemical workstation (Chenhua Instrument Company of Shanghai, China) with 0.1 M PBS containing 0.1 M ascorbic acid (pH 7.4) as electrolyte. The electrochemical cell was in a typical three-electrode configuration: the modified electrode (working electrode), platinum wire counter electrode and Ag/AgCl, saturated KCl (reference electrode). Electrochemical impedance spectroscopy (EIS) was performed on the PARSTAT 2273 (Princeton Applied Research, USA). Ultraviolet-visible (UV–vis) spectra were measured on a UV-3900 spectrophotometer (Hitachi, Japan). Scanning electron microscope (SEM) images were obtained with a Hitachi SU8010 (Japan). Powder X-ray diffraction (XRD) patterns were conducted on a Bruker D8-Advance using Cu Kα radiation (λ=1.5418 Å). Fourier transform infrared spectroscopy (FT-IR) spectra was recorded on a QUINX55 spectrometer (Brucher, Germany). Scanning transmission electron microscopy (STEM), and energy-dispersive X-ray (EDX) elemental mapping images were all recorded on a JEM 2010 (120 kV) high-resolution transmission electron microscope.

2.3. Synthesis of UiO-66 nanoparticles and $[Ru(bpy)_3]^{2+}$@UiO-66 probes

UiO-66 was synthesized following a modified protocol reported before (Wu et al., 2013). Briefly, 240 mg of $ZrCl_4$, 4 mL CH_3COOH and 0.118 mL H_2O were first dissolved in 32 mL of DMF with stirring for 10 min. After that, 94.94 mg of BDC was added into the solution, following the ultrasonic treatment for 30 min in a Pyrex vial. Then, the homogeneous mixture was transferred into a 40 mL Teflon-lined stainless steel autoclave at 120 °C for 24 h. After cooling down to room temperature, the resultant white suspension of UiO-66 particles was collected by centrifugation and then washed with DMF. The ultimately UiO-66 samples were obtained by being activated in high dynamical vacuum at 450 °C for hours. Then the synthetic UiO-66 particles were characterized by SEM, TEM and XRD (Fig. S1), showing them homogeneously dispersed cubic shapes and a mean diameter of 60 nm.

$Ru(bpy)_3]^{2+}$@UiO-66 probes were obtained by adding $[Ru(bpy)_3]^{2+}$ solutions into 0.02g UiO-66 samples and being gently stirred for 36 h, then followed by centrifugation. UV–vis spectra (Fig. S2), STEM and EDX elemental mapping were used to characterize the $[Ru(bpy)_3]^{2+}$@UiO-66 probes. The quantity of $[Ru(bpy)_3]^{2+}$ in UiO-66 was calculated to be $6.5×10^{-6}$ mol g^{-1}, which was measured by Fluorescence spectra.

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