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Electrochemical detection of protein kinase activity based on carboxypeptidase Y digestion triggered signal amplification

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ABSTRACT

An effective assay method for monitoring protein kinase activity and screening inhibitors is greatly beneficial to kinase-related drug discovery, early diagnosis of diseases, and therapeutic effect evaluation. Herein, we develop a simple electrochemical method for detecting the activity of casein kinase II (CK2) based on phosphorylation against carboxypeptidase Y (CPY) digestion triggered signal amplification, where CK2 catalyzed phosphorylation event protects the substrate peptide from the digestion of CPY, maintains the repulsive force of the substrate peptide towards the redox probe, and results in a weak electrochemical signal. Whereas, without phosphorylation, the substrate peptide is digested by CPY and a strong electrochemical signal is obtained. The detection feasibility is demonstrated for the assay of CK2 activity with low detection limit of 0.047 unit/mL. Moreover, the biosensor was used for the analysis of kinase inhibition. Based on the electrochemical signal dependent inhibitor concentration, the IC_{50} value of ellagic acid was estimated to be 39.77 nM. The proposed method is also successfully applied to analyze CK2 activity in cell lysates, proving the applicability in complex biological samples.

1. Introduction

Protein phosphorylation is a post-translational modification of proteins which was catalyzed by protein kinases, which is one of the most important intracellular biological processes in metabolism and signal transduction (Manning et al., 2002). In phosphorylation process, a serine, a threonine or a tyrosine residue is phosphorylated by the addition of a covalently bound phosphate group. And each protein kinase can recognize the specific amino sequence (see Supplementary materials). The abnormal protein kinase activity has been associated with various diseases such as cancer (Griner and Kazanietz, 2007), or Alzheimer's disease (Flajolet et al., 2007). For instance, the overexpression of CK2β has been found in gastric carcinoma cell (Kai-Yuan et al., 2010). Moreover, it has been proved that many protein kinases are involved in the signal transduction of tumor cells (Wei and Malhotra, 2010). Thus, in order to deeply understand the fundamental biological function of protein kinase and discover drug for protein kinase-triggered diseases, it is significant to develop sensitive and specific detection method for protein kinase activity assay and inhibitor screening. For achieving this aim, many strategies have been investigated including immunoassay with phosphate-specific antibody labeled with fluorophore (Kim et al., 2008; Li et al., 2009; Till et al., 1994), radio-isotope assay with radioactive ^{32}P in the phosphate group donor of ATP (Lehel et al., 1997), electrochemical methods with ferrocene- or thiol-labeled ATP (Kerman and Kraatz, 2007; Kerman et al., 2008; Song et al., 2008). These methods are effective, but require complicated molecule labeling procedure and radioisotope, which might increase the detection step and cost. More importantly, the use of radioisotope can increase the detection risk for human health.

In order to eliminate the deficiency of the additional modification of the substrate peptides, ATP and antibodies, label-free methods are attractive for quantitatively detecting protein kinase activity. For this aim, some strategies based on the identification of phosphate, which is transferred from ATP to the substrate peptides catalyzed by protein kinase, have been developed, such as fluorescence (Bai et al., 2013; Zhou et al., 2013), electrochemiluminescence (Chen et al., 2013), quartz crystal microbalance (Xu et al., 2012), microarray-based resonance light scattering assay (Li et al., 2013b), photoluminescence (Wang et al., 2013), and electrochemistry (Li et al., 2013a; Wang et al., 2010; Xu et al., 2009; Yang et al., 2011). Among them, electrochemical strategy is proved to be a kind of promising way to achieve label-free assay of protein kinase activity due to the advantages of easy operation, simple instrument, low cost and high sensitivity. For instance, Xu et al. used a kind of label-free electrochemical strategy for detecting the activity of protein kinase A (PKA) based on the linkage between the phosphorylated peptide and DNA functionalized Au nanoparticles (DNA-AuNPs) by Zr^{4+} and the

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chronocoulometric response of $[Ru(NH_3)_6]^{3+}$ absorbed on the DNA-AuNPs (Xu et al., 2009). Wang et al. fabricated a simple but ultrasensitive electrochemical biosensor for the identification of phosphorylation and assay of PKA activity, where positively charged electrochemical probe $[Ru(NH_3)_5Cl]^{2+}$ could be hindered to access the peptide modified electrode surface due to the formed compact and positively charged self-assembly monolayer (SAM) of substrate peptide (Wang et al., 2010). After phosphorylation, the SAM became loose and facilitated the permeation of probe due to the electrostatic interaction between the negative phosphate groups and positively charged probe. Based on it, a signal "on" biosensor was fabricated and achieved the aim for kinase activity assay. Yang et al. developed another simple electrochemical method for protein tyrosine kinase activity assay based on the oxidation current change of tyrosine residue before and after phosphorylation (Yang et al., 2011).

In this work, we present a novel and simple electrochemical platform for kinase activity assay and inhibitor screening based on phosphorylation protection against carboxypeptidase Y (CPY) digestion, where the $Fe(CN)_6^{3-/4-}$ were used as the electrochemical probe, and the peptide rich in negative charged aspartic acid was used as substrate peptide. CPY is a kind of proteinase with hydrolytic activity towards peptide linkage from the C-terminal of protein or peptide. However, the activity of carboxypeptidase can be inhibited when the amino residue was modified, such as phosphorylation. In our strategy, without phosphorylation, the electrochemical signal is high due to digestion effect of CPY on the substrate peptide. However, the electrochemical signal would decrease when the amino acid residue is phosphorylated, which inhibits the hydrolysis activity of CPY, remains the peptide fragment with negative charges on the electrode surface and blocks the diffusion of $Fe(CN)_6^{3-/4-}$ to the electrode surface. Based on the variation of the electrochemical response, the activity assay of protein kinase was achieved. Moreover, for evaluating the applicability of the developed method, the protein kinase activity in cell lysates was also detected.

2. Experimental

2.1. Reagents and instruments

The substrate peptide (s-peptide, CDDDDDSDDDA), the control peptide (c-peptide, CDDDDDSDDLA) and adenosine 5′-triphosphate (ATP) disodium salt hydrate were provided by Sangon Biotech (Shanghai) Co., Ltd. Carboxypeptidase Y, 6-mercapto-1-hexanol (MCH), forskolin and 3-isobutyl-1-methylxantine (IBMX) were purchased from Sigma-Aldrich (USA). Casein Kinase I (CK1), casein kinase II (CK2), mitogen-activated protein kinase (MAPK), cAMP-dependent protein kinase A (PKA) catalytic subunit was supplied by New England Biolabs Ltd. (Beverly, MA). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), tris(hydroxymethyl) aminomethane (Tris), EDTA, ellagic acid and chloroauric acid ($HAuCl_4$) were obtained from Aladdin (shanghai, China).

The buffer solutions employed in this study are as follows. Peptide dissolved buffer: 20 mM Tris–HCl, 10 mM $MgCl_2$, 50 mM KCl, pH 7.5. Peptide immobilization buffer: 20 mM Tris–HCl, 10 mM $MgCl_2$, 50 mM KCl, 1 mM TCEP, pH 7.5. CK2 dilution buffer: 20 mM Tris–HCl, 350 mM NaCl, 1 mM Na_2EDTA, 2 mM DTT, 0.1% Triton X-100 (pH 7.5). CK2 reaction buffer: 20 mM Tris–HCl, 80 μM ATP, 50 mM KCl, 10 mM $MgCl_2$, pH 7.5. CPY reaction buffer: 20 mM Tris–HCl, 10 mM $MgCl_2$, 50 mM KCl, pH 7.5. Detection buffer: 10 mM PBS, 5 mM $Fe(CN)_6^{3-/4-}$, 0.1 M KCl, pH 7.4. Electrode washing buffer: 10 mM Tris–HCl, pH 7.5.

2.2. Immobilization of substrate peptide on gold electrode

The gold electrode ($d=2$ mm) was polished with 0.03 μm alumina powder on micro-cloth pad and rinsed thoroughly with redistilled deionized water. Then the polished gold electrode was washed successively with redistilled deionized water, anhydrous ethanol and redistilled deionized water in an ultrasonic bath for 3 min, respectively. Prior to modification, the gold electrode was scanned by cyclic voltammetry from -0.2 to 1.6 V in 0.1 M H_2SO_4 until a voltammogram characteristic of the clean polycrystalline gold electrode was established. Then, the AuNPs were electrodeposited on the pretreated gold electrode surface under constant potential of -0.2 V for 250 s with stirring in 3 mM $HAuCl_4$ solution containing 0.1 M KNO_3. Subsequently, the AuNPs/Au was incubated with 5 μL peptide immobilization buffer containing 1 μM substrate peptide for 16 h at room temperature in a humid cell. The peptide modified electrode (noted as Peptide/AuNPs/Au) was rinsed with washing buffer for three times. Afterwards, the peptide modified electrode was further immersed with 5 μL of 3 mM MCH for 1 h. Finally, the electrode was rinsed with washing buffer for three times and dried under nitrogen blowing. The obtained electrode was noted as MCH/Peptide/AuNPs/Au.

2.3. CK2-catalytic phosphorylation and its inhibition

For CK2-catalytic phosphorylation, the MCH/Peptide/AuNPs/Au was incubated with 5 μL 1X CK2 reaction buffer containing different concentrations of CK2 for 60 min at 37 °C in a humid cell. After that, the reaction was terminated by rinsing the electrode thoroughly with the washing buffer. The obtained electrode was noted as MCH/P-Peptide/AuNPs/Au.

For the inhibition assay, different concentrations of ellagic acid was introduced into the above CK2 reaction buffer and the electrode was then incubated with 5 μL 1 × CK2 reaction buffer containing 20 unit/mL CK2 and different concentrations of inhibitors for 60 min at 37 °C in a humid cell. After that, the electrode was rinsed thoroughly with the washing buffer to terminate the reaction.

2.4. Hydrolyzation of peptide by CPY

The electrode was incubated with CPY reaction buffer containing 100 μg/mL CPY for 50 min at 30 °C in a humid cell. Then, the electrode was rinsed with washing buffer.

2.5. Electrochemical detection

Electrochemical experiments were performed with CHI832D electrochemical workstation (USA) with a conventional three-electrode cell. A planar Au electrode or modified Au electrode was used as working electrode. A saturated calomel electrode (SCE) and a platinum wire were used as the reference electrode and auxiliary electrode, respectively. Differential pulse voltammetry (DPV) was performed in 10 mL of 0.1 M PBS (pH 7.4) containing 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ (1:1) and 0.1 M KCl.

2.6. Cell culture and lysate preparation

Human hepatoma cell line HepG-2 and human hepatic cell line L-02 are cultured in DMEM (Dulbecco's modified Eagle's medium) (Gibco-BRL, USA) supplemented with 10% (v/v) fetal bovine serum (Invitrogen, New Zealand) in a CO_2 (5%) incubator at 37 °C. The cultured cells are washed three times with PBS. Those cells are sonicated for 2 s × 60 times at 4 °C at an interval of 3 s for each time in 0.3 mL lysis buffer containing 80 mM β-glycerophosphate, 20 mM EGTA, 5 mM DTT, 1 μM okadaic acid, 15 mM $MgCl_2$, and

10 μg/ml each of leupeptin, chymostatin, and pepstatin (pH 7.4). The homogenates are centrifuged at 15,000 rpm for 30 min at 4 °C. Then, the obtained supernatants are transferred to a freezing centrifuge tube (Axygen, USA) and stored at −80 °C in a refrigerator.

The total protein concentration in cell lysate is evaluated by modified Bradford protein assay kit according to the manufacturer's recommendation. All cell lysates are diluted to 10 μg/mL total protein concentration for kinase activity assay.

3. Results and discussion

3.1. Detection principle

In this work, CK2 was selected as the model kinase, where the activity of CK2 was detected based on the hindrance effect of phosphorylation towards CPY digestion. The substrate peptide, CDDDDDSDDDA, contains the recognition sites of CK2 (SDDD), in which the serine residue can be phosphorylated by CK2 in the presence of ATP. Moreover, the selected substrate peptide also contains six aspartic acid residues. It is well known that the isoelectric point of aspartic acid is 2.98 (Liu et al., 2004), which indicates that aspartic acid is negatively charged in the detection buffer (10 mM PBS, 5 mM $Fe(CN)_6^{3-/4-}$, 0.1 M KCl, pH 7.4). As shown in Scheme 1, after the self-assembly of the substrate peptide on the gold electrode surface, the negative charges of the substrate peptide will block the penetration of the negative electroactive probes of $Fe(CN)_6^{3-/4-}$ due to the electrostatic repulsion. So the detection system is on the "off" state. However, when the electrode was further incubated with CPY, the substrate peptide will be cleaved from the carboxyl teminal and release the free amino acids into the buffer. Thus, the repulsive force will disappear and the redox probes of $Fe(CN)_6^{3-/4-}$ can generate a strong electrochemical signal. At the present state, the detection system is "on". On the contrary, when the serine residue in substrate peptide is phosphorylated by CK2, the digestion effect of CPY towards the substrate peptide can be hindered at the phosphorylated serine site, and five negatively charged aspartic acid residues are retained. on the electrode surface. In addition, another two negative charges are introduced on the serine residue of the substrate peptide through phosphorylation reaction. Therefore, through the electrostatic repulsion of phosphate group and

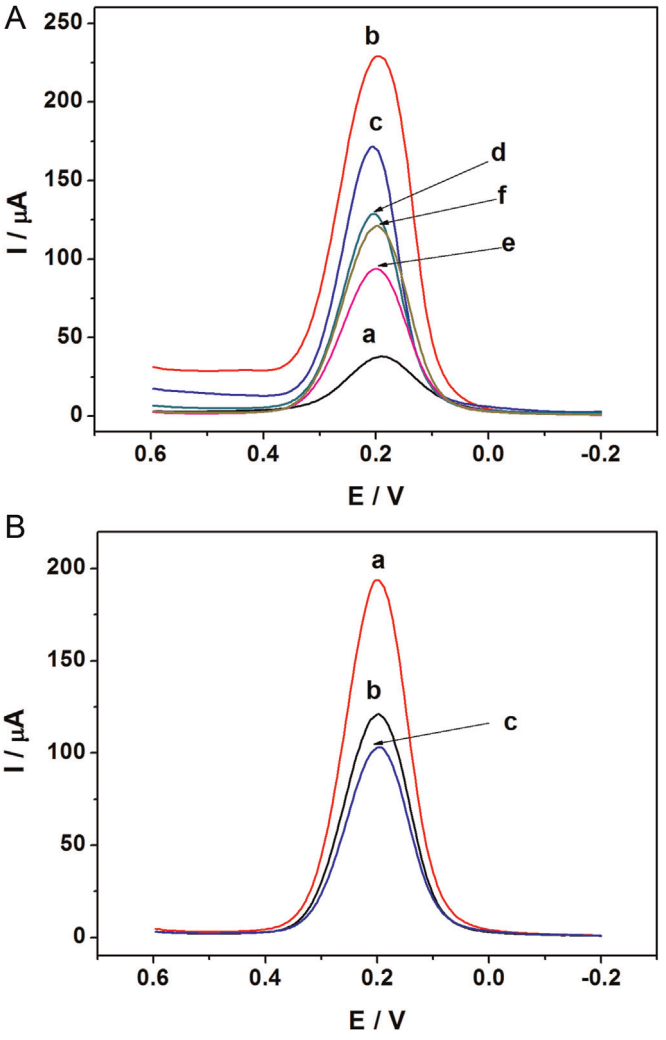
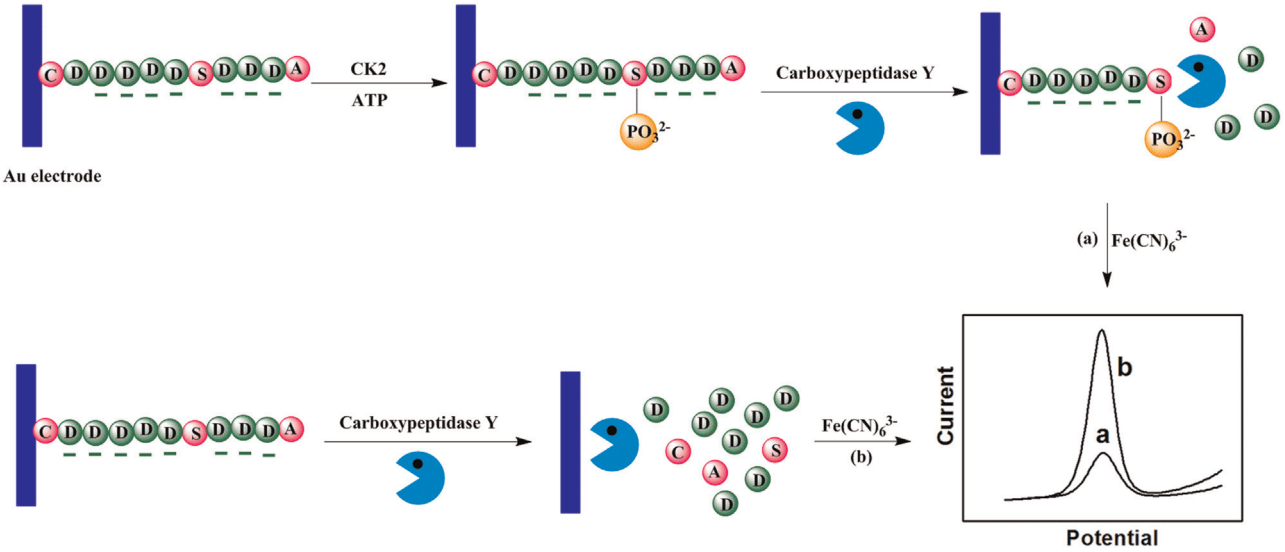


Fig. 1. (A) The difference pulse voltammograms of different electrodes. (a) The bare Au electrode, (b) AuNPs/Au, (c)Peptide/AuNPs/Au, (d) MCH/Peptide/AuNPs/Au, (e) MCH/P-Peptide/AuNPs/Au, aned (f) MCH/P-Peptide/AuNPs/Au digested by CPY. (B) The difference pulse voltammograms of different electrodes digested by CPY. (a) MCH/Peptide/AuNPs/Au, (b) and (c) is MCH/P-Peptide/AuNPs/Au with CK2 concentration of 1 and 10 unit/mL, respectively.



Scheme 1. Schematic representation of electrochemical sensing strategy for CK2 activity detection based on phosphorylation against CPY digestion triggered signal amplification.

aspartic acid residues for $Fe(CN)_6^{3-/4-}$, the detection system is on the "off" state again and the decreased current can be obtained, which is closely related to the phosphorylation level of serine residue. That is to say, the CK2 activity can be detected according to the change of the electrochemical response of $Fe(CN)_6^{3-/4-}$.

3.2. Detection feasibility assay

In order to certify the detection principle, the electrochemical response of $Fe(CN)_6^{3-/4-}$ in detection buffer was investigated and recorded by the DPV technique using peptide modified gold electrode as working electrode after different treatment process. As seen in Fig. 1A, a well-defined reduction peak was obtained for the bare gold electrode (curve *a*). Then, the reduction peak current increased significantly (curve *b*) when AuNPs were electrodeposited onto the bare gold electrode surface due to the good conductivity of AuNPs. After the assembly of the substrate peptide on the AuNPs/Au surface, the reduction peak current decreased greatly (curve *c*). This decrease can be explained as the fact that the negative charge controlled substrate peptides block the penetration of $Fe(CN)_6^{3-/4-}$ to electrode surface due to the electrostatic repulsion. Subsequently, the reduction peak current further decreased when the peptide/AuNPs/Au was sealed with MCH (curve *d*) due to the resistance of MCH towards the diffusion of Fe $(CN)_6^{3-/4-}$. After the MCH/peptide/AuNPs/Au was incubated with 1 unit/mL CK2, the reduction peak current further decreased (curve *e*), which could be ascribed to the phosphorylation reaction and the negative phosphate group inhibited the diffusion of Fe $(CN)_6^{3-/4-}$ to electrode surface. However, the reduction peak current increased inversely when the MCH/P-Peptide/AuNPs/Au was incubated with CPY (curve *f*), which could be explained as two reasons. Initially, the un-phosphorylated substrate peptide is digested by CPY from the carboxyl, which leads to the release of free amino acids from electrode surface and the decrease of the electrostatic repulsion. Additionally, the CPY can also hydrolyze the amino residues ADD in the phosphorylated substrate peptide, and the further hydrolysis is blocked by the phosphorylation event at serine residue site (Zhou et al., 2013, 2014). From these results, we could conclude that the biosensor was successfully fabricated through different modification processes.

To confirm the effect of phosphorylation on the digestion of CPY, a control experiment was also carried out. MCH/Peptide/AuNPs/Au was directly digested by CPY, and the phosphorylation process was ignored. As shown in Fig. 1B, the reduction peak current for MCH/Peptide/AuNPs/Au digested by CPY (curve *a*) was much higher than that obtained at MCH/P-Peptide/AuNPs/Au digested by CPY (curve *b*). After the immobilized substrate peptide was phosphorylated by CK2 at the serine site, the amino acid residues *ADDD* in phosphorylated peptide (NH_2–CDDDDDS$_P$DDDA–COOH) could be digested by CPY and released from electrode surface. However, further digestion was blocked because of the phosphorylation of serine residue. Therefore, five aspartic acid residues with five negative charges were retained on the electrode surface for each substrate peptide. Moreover, owing to the phosphorylation reaction, a phosphate group with two negative charges was transferred to serine residue through covalent linkage. So the total negative charge for each substrate peptide was also seven though two aspartic acid residues were released from peptide due to the digestion of CPY. As a result, after phosphorylation and CPY digestion, the substrate peptide on the electrode surface still controlled by negative charge, which could hinder the penetration of $Fe(CN)_6^{3-/4-}$ towards electrode surface and lead to a weak reduction peak current.

For further proving that the change of the reduction peak current of $Fe(CN)_6^{3-/4-}$ is related with the concentration of CK2 and this change can be used to detect CK2 activity, another control

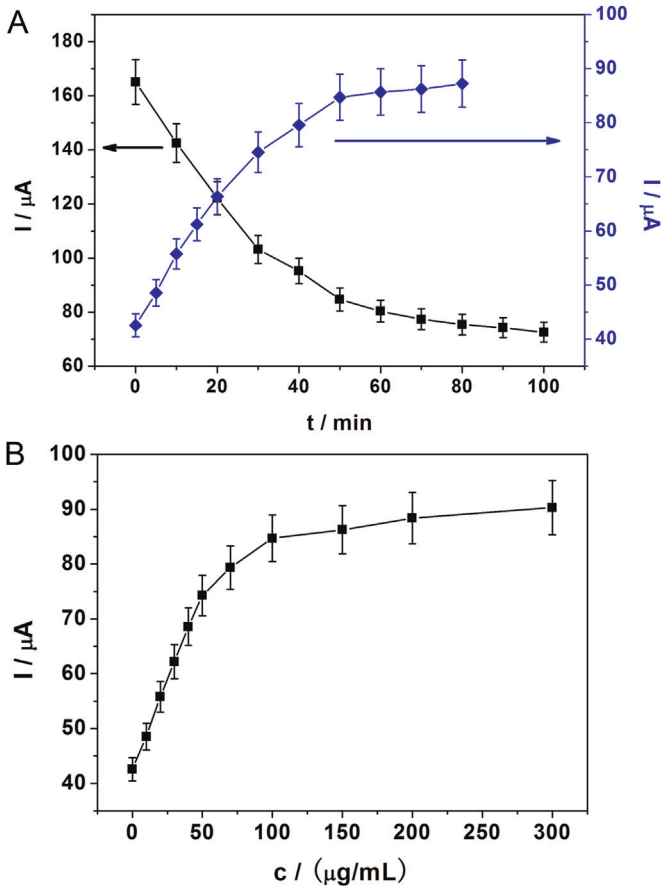


Fig. 2. The effect of CK2 incubation time (A, ■), CPY digestion time (A, ◆) and CPY concentration (B) on the electrochemical response of the biosensor. CK2 concentration is 20 unit/mL.

experiment was performed, and the current was recorded and compared when the MCH/Peptide/AuNPs/Au was firstly incubated with different concentrations of CK2, then digested by CPY. The result was also shown in Fig. 1B. It is clear that the reduction peak current decreased when the CK2 concentration was increased from 1 (curve *b*) to 10 (curve *c*) unit/mL. It also demonstrated that the developed detection strategy can be applied to detect CK2 activity.

3.3. Optimum of detection conditions

In order to increase the detection sensitivity, several experimental parameters were optimized. Fig. 2A illustrated the effect of phosphorylation time on the reduction peak current. With increasing the phosphorylation time from 0 to 60 min, the reduction peak current decreased gradually. When further extending the phosphorylation time, the reduction peak current only decreased a little and tended to level off. Thus 60 min was selected.

The detection strategy of this work was mainly depended on the phosphorylation against CPY digestion, therefore, the effect of CPY concentration and CPY digestion time were investigated. For achieving this aim, the phosphorylated substrate peptide was digested by CPY with different times (0, 5, 10, 15, 20, 30, 40, 50, 60, 70 and 80 min) and different concentrations (0, 10, 20, 30, 40 50, 70, 100, 150, 200, 300 μg/mL), and then DPV was recorded. As also presented in Fig. 2A, with the increasing the digestion time the reduction peak current enhanced gradually and turned to be a platform at the reaction time of 50 min. So 50 min was selected. The effect of CPY concentration on the electrochemical response was illustrated in Fig. 2B. The reduction peak current ascended

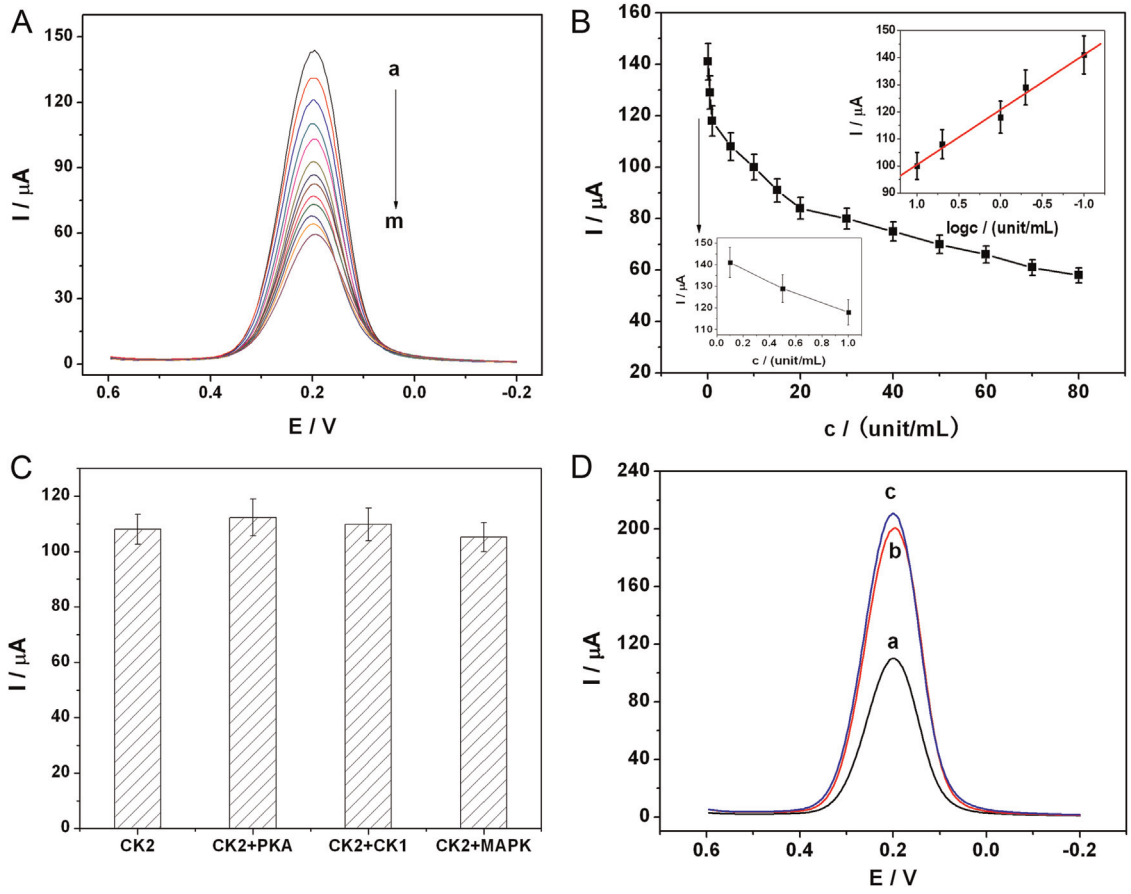


Fig. 3. (A) Differential pulse voltammograms of the biosensor with different CK2 concentrations. a–m: 0.1, 0.5, 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80 unit/mL. (B) The plot for the reduction peak current versus CK2 concentration. Insert: The linear relationship between the reduction peak current and the logarithm of the concentrations of CK2. (C) The histogram for the biosensor incubated with different kinases. (D) Differential pulse voltammograms of the biosensor fabricated with s-peptide (a) and c-peptide (b), respectively; (c) is the electrochemical response of the control peptide modified electrode after directly incubating with CPY.

along with the increase of the concentration of CPY until it reached 100 μg/mL, followed by a plateau at higher concentration. Therefore, 100 μg/mL was selected for the digestive experiments.

3.4. Detection of CK2 activity and inhibitors

Under the optimal conditions, the quantitative assay of CK2 activity using the proposed method was performed. Fig. 3A showed the differential pulse voltammograms of the substrate peptide modified electrode phosphorylated by different concentrations of CK2 and then digested by 100 μg/mL CPY. As shown in Fig. 3B, with increasing the concentration of CK2, the reduction peak current decreased quickly, and this decrease could be explained by exponential decay. Then the decreased tendency of the peak current change was presented when CK2 concentration was higher than 20 unit/mL. The decreased reduction peak current was linearly proportional to the logarithm of CK2 concentration in the range from 0.1 to 10 unit/mL (inset in Fig. 3B). The corresponding linear regressions could be expressed as I=-20.32 log c+120.81 (R=0.9932). The detection limit was estimated to be 0.047 unit/mL (S/N=3), which was lower than those of 10 (Kerman et al., 2007), 0.5 (Miao et al., 2012; Tan et al., 2013), 0.47 (Xu et al., 2011), 0.2 (Ji et al., 2009), 0.15 (Xu et al., 2009), 0.134 (Zhou et al., 2013), 0.1 (Bai et al., 2013), 0.07 (Chen et al., 2010) and 0.061 unit/mL (Xu et al., 2012).

In order to demonstrate the detection specificity of this assay, several control experiments were carried out, in which we selected protein kinase A (PKA), casein kinase I (CK1) and mitogen-activated protein kinase (MAPK) as interferents. As presented in Fig. 3C, the reduction peak current was almost the same after MCH/Peptide/AuNPs/Au was incubated with the mixture of 5 unit/mL of CK2 and 5 unit/mL of other protein kinase, indicating excellent detection specificity of the developed method for CK2 activity assay. Moreover, the reduction peak current change ($\Delta I = I_2 - I_1$, where I_1 is the current of MCH/Peptide/AuNPs/Au after incubated with 10 unit/mL different kinases and 100 μg/mL CPY successively, and I2 is the current of MCH/Peptide/AuNPs/Au after incubated with 100 μg/mL CPY) were also compared. As seen in Fig. S1 (Supplementary materials), the ΔI for the biosensor incubated with CK2 is more higher than that obtained at other three kinases, indicating that PKA, CK1 and MAPK do not influence the detection of CK2. For further verifying the good selectivity of this method, a control peptide (c-peptide, ADDSDDLDDC) without CK2 recognition site was modified on gold electrode surface, and then incubated with 5 unit/mL CK2 and 100 μg/mL CPY successively. Finally the DPV response was recorded. As shown in Fig. 3D, the reduction peak current of the control peptide modified electrode (curve *b*) increased significantly after this electrode was incubated with CK2 and CPY successively when compared with substrate peptide modified electrode (curve *a*). Moreover, this reduction peak current was almost same to that obtained at the control peptide modified electrode directly incubated with CPY (curve *c*), indicated that the control peptide was not phosphorylated by CK2, which benefited to the digestion of CPY. The possible interference of some other bioactive molecules contained by cell lysates were also showed no interfere with the detection of CK2 (Supplementsry materials). This result also demonstrated the good detection selectivity of the developed method.

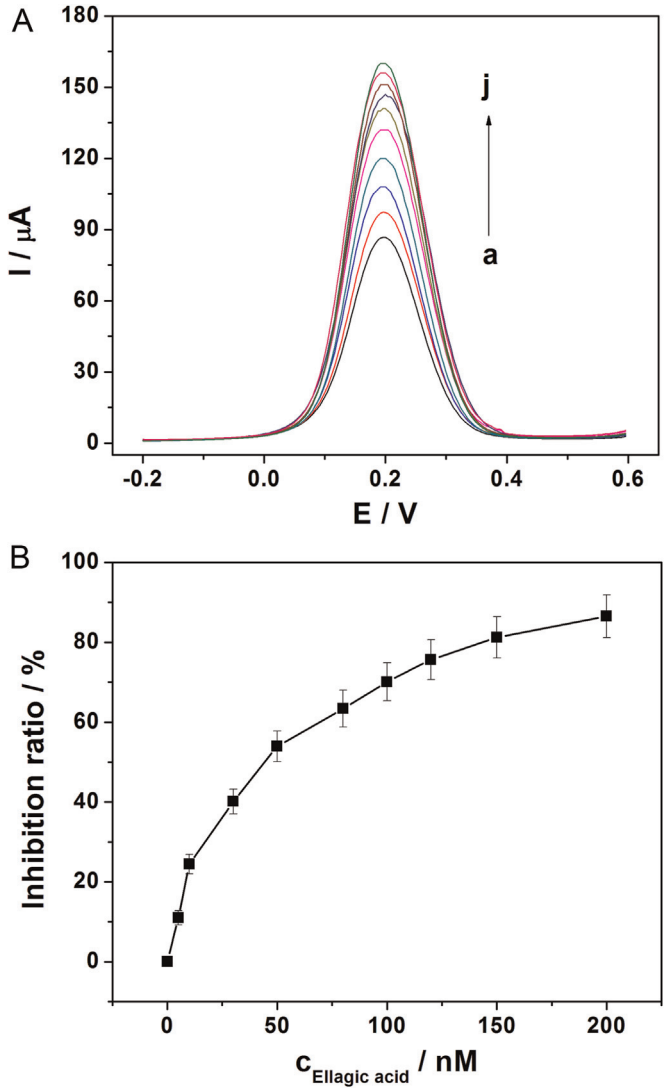


Fig. 4. (A) Differential pulse voltammograms of the biosensor for different concentrations of CK2 inhibitor of ellagic acid. Ellagic acid concentration (a–j): 0, 5, 10, 30, 50, 80, 100, 120, 150, 200 nM. (B) The plot for inhibition ratio versus ellagic acid concentration. CK2 concentration, 20 unit/mL. CPY, 100 μg/mL.

To certify the potential applicability of our strategy in protein kinase inhibitor screening, a potent CK2 inhibitor, ellagic acid, was selected as a model inhibitor, and the inhibition activity of ellagic acid for CK2 was investigated. As can be seen form Fig. 4A, with increasing the concentration of ellagic acid, the reduction peak current of the redox probe increased gradually, indicating the inhibition of CK2 activity and low levels of peptide phosphorylation. The relationship between the inhibition ratio (inhibition ratio= $(I_2-I_1)/I_1$, I_1 is the peak current without inhibition and I_2 is the peak current after inhibition) and ellagic acid concentration was plotted in Fig. 4B, from which the IC_{50} value was estimated to be 39.77 nM, which was consistent with that reported in previous work (41 nM) (Wang et al., 2013). From these results, one can concluded that the developed method could be applied in the field of kinase inhibitor screening.

3.5. CK2 activity detection in cell lysates

On account of the vital regulation effect of protein kinase in cells, it is crucial to detect protein kinase activity in biological systems. Moreover, it has been reported that the aberrant protein kinase activity might be related with cancers (Griner and Kazanietz, 2007). Therefore, we investigated the activity of CK2 in human L-02 hepatic cells, human HepG-2 hepatoma cells, human HeLa cells and human MCF-7 breast carcinoma cells. The four kinds of cell lysates were firstly diluted to be 10 μg/mL, and then 5 μL phosphorylation reaction buffer containing lysate and ATP was dropped on the peptide modified gold electrode surface and incubated for 60 min to complete the phosphorylation reaction. Subsequently, the electrode was further incubated with CPY. Finally the differential pulse voltammograms were recorded. As seen in Fig. 5, the reduction peak current of the MCH/Peptide/AuNPs/Au incubated with hepatoma cell lysate (column b) was obviously lower than that at the electrode incubated with normal hepatic cell lysate (column a), indicating that the activity of CK2 in HepG-2 hepatoma cells was higher than that in L-02 hepatic cells. In addition, the reduction current for HeLa cell lysate (column c) was high than that for HepG-2 hepatoma cell lysate and MCF-7 breast carcinoma cell lysate (column d), indicating low activity of CK2 in HeLa cells. For further evaluating the applicability of this method on evaluation of the drug-therapeutic efficacy for kinase-related diseases, the activity of CK2 in the extract of different cells was detected in the presence of ellagic acid. As presented in Fig. 5, after inhibition by ellagic acid and digestion by CPY, the reduction peak current increased significantly, indicating that more substrate peptide was hydrolyze by CPY, which was resulted from the low phosphorylation level of the substrate peptide. The decrease of the current also demonstrated that the activity of CK2 in cell lysates decreased in the presence of ellagic acid. According to the above results, we can conclude that the developed method can be applied to detect the activity of CK2 and screen kinase inhibitor in complex biological environments. In addition, the proposed method can also be applied to assess the drug-therapeutic efficacy for kinase-related diseases.

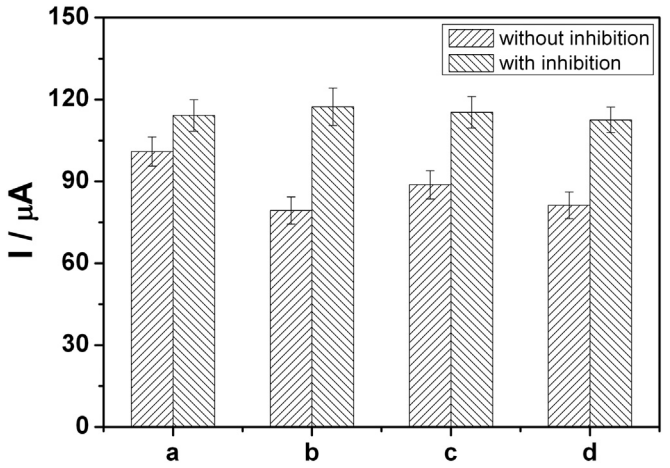


Fig. 5. Histograms for the electrochemical response of MCH/Peptide/AuNPs/Au after incubated with cell lysates (with and without inhibition by 30 nM ellagic acid) and CPY, successively. (a) Human L-02 hepatic cell lysate, (b) human HepG-2 hepatoma cell lysate, (c) human HeLa cell lysate, and (d) human MCF-7 breast carcinoma cell lysate.

4. Conclusion

In conclusion, a novel electrochemical method has been successfully developed for the activity assay of protein kinase CK2 and inhibitor screening based on CK2 catalytic phosphorylation against CPY digestion. Compared with other methods, this phosphorylation against CPY digestion triggered signal amplification method presents the advantages of simple operation, low cost and high sensitivity. The developed method also shows excellent selectivity

due to the phosphorylation-specific inhibition of CPY activity. The inhibition effect of ellagic acid on CK2 activity further confirms the applicability of this method on CK2-related inhibitor screening. Thus, the proposed sensing platform displays great potential for monitoring the activity of other kinases and their inhibitors screening. Furthermore, the developed method also shows potential applicability in the field of kinase-related drug discovery and early diagnosis of some kinase-related diseases.

Acknowledgments

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.11.014.

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