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L-tyrosine polymerization-based ultrasensitive multi-analyte enzymatic biosensor

Lanjunzi Liu, Xiuzhi Kang, Chao Chen*, Heping Zhang, Chenpu Chen, Qingji Xie*

Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China

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ABSTRACT

Novel amperometric bi-enzyme biosensor based on tyrosinase (Tyr)-catalyzed L-tyrosine polymerization to effectively immobilize Tyr and glucose oxidase (GOx) simultaneously has been developed and is demonstrated to be efficient in monitoring multi-analyte (bisphenol A (BPA), phenol, Cr(III), glucose, and Cr(VI)). For the first time, the polymer from L-tyrosine oxidation (PLT) was utilized to in-situ immobilize biomacromolecules and was demonstrated to be effective in entrapping high-load and high-activity enzymes. The prepared bi-enzyme biosensor (PLT-Tyr-GOx/Au electrode) works well in the biosensing of glucose (GOx substrate) and Cr(VI) (GOx inhibitor), and also exhibits excellent performance in Tyr substrates (BPA and phenol) and Tyr inhibitor (Cr(III)) sensing. In addition, the resultant biosensor exhibits excellent stability, precision, high sensitivity and fabrication simplicity, which may find wide applications in diversified fields including biotechnology.

1. Introduction

Discriminative detection of multiple analytes is very challenging and commonly requires bulky and sophisticated instrumentations. Electrochemical biosensors have attracted much attention because of their advantages such as good selectivity, fast response, high sensitivity, simple operation, inexpensive instrument and suitability for field work [1]. Enzyme-based biosensing is one of the most active research areas in analytical chemistry due to its incomparable advantages over other techniques, and enzymatic biosensors have been widely applied in diverse biomedical, environmental and food safety monitoring [2]. Enzyme-based biosensors can be classified into two groups according to the working principles. One group aims at detecting the enzyme substrates based on the catalytic function of enzymes, such as various glucose oxidase (GOx)-based biosensors widely used in blood sugar monitoring [3]; the other group is widely applied in monitoring enzyme inhibitors based on their inhibition effect on enzymes [4], such as acetyl cholinesterase-based biosensors applied in organophosphorus pesticides and anticholinesterase agents sensing [5]. Many immobilized enzymes have been used for catalytic sensing of important substrates (such as H_2O_2, glucose, ethanol, and so on) and inhibitive assays of various environmental pollutants (medicines, herbicides, or heavy metal ions (HMs)) [6–10]. Enzyme inhibition-based biosensors for food safety and environmental monitoring were extensively reviewed by Amine et al. in 2006 [11]. Manju et al. also reviewed the electrochemical biosensors employed in the detection of HMs with various interfaces in 2015 [12]. Tyrosinase (Tyr) derived from the embryonic neural sheath cells is a copper-containing redox enzyme, which plays a key role in the metabolism of melanin in living organisms [13]. Tyr-based electrochemical biosensors [14–16] have been widely utilized in the determination of toxic pollutants (including its substrates such as various phenolic compounds and its inhibitors (herbicides or HMs)) in environmental and biological samples [17,18]. With advantages such as relatively low cost, good stability, and high specific activity, GOx-based amperometric enzyme electrodes plays a leading role in the move to simple easy-to-use blood glucose monitoring, and have also been widely used in the inhibitive assays of HMs because GOx can be inhibited by various HMs (such as Ag^+, Hg^{2+}, Cu^{2+}, and Cr(VI)) [19–21]. However, according to our knowledge, among all the biosensors reported working in enzyme inhibition/catalytic mode, most of them are based on “one analyte-one biorecognition element”. That is to say, most biosensors can only be utilized in the sensing of a single analyte, which is inefficient for real sample analysis and greatly limits the application of resultant biosensors. Thus the development of highly sensitive and discriminative techniques for detection of multi-analyte is important and meaningful for environmental monitoring and has been one of the top research areas for decades. [22]

Environmental pollution is an increasing serious problem which has aroused great attention among scientists around the world. Chromium (Cr) is a common contaminant in surface water and groundwater. Cr

* Corresponding authors.
E-mail addresses: chenchao840103@163.com (C. Chen), xieqj@hunnu.edu.cn (Q. Xie).

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contamination mainly comes from the disposal of Cr-containing wastes and results from numerous industrial and military activities. Cr exists primarily in Cr(III) and Cr(VI) states in environment. Cr(VI), which is widely known to be highly toxic and carcinogenic [23], tends to accumulate in the food chain and thus may cause severe health problems ranging from simple skin irritation to lung carcinoma [24]. Thus Cr(VI) concentration is limited to be a few ppb in groundwater and potable water supplies [25]. Cr(III) is an essential micronutrient which plays important roles in the metabolism of carbohydrates [26], while it exhibits high toxicity under overloading conditions because Cr(III) can bind DNA and affect cellular structures and components [27]. Puja et al. recently outlines the overview of Cr toxicity, conventional analytical techniques along with a particular emphasis on electrochemical based biosensors for Cr detection in potable water [28]. Phenolic pollutants have toxic effects on the nervous, urinary and digestive system and bring a great threat to human health [29]. Bisphenol A (BPA, 2,2′-bis(4-hydroxyphenyl) propane) widely used in plastic food containers [30] can lead to endocrine disorders and threat the health of the fetus and children. Due to the widespread existence and the high toxicity of above mentioned pollutants, it is of great significance to identify and quantify these pollutants in environmental monitoring. As well known to us, various pollutants commonly coexist in real environmental samples. The development of bi-enzyme biosensing systems which can provide information on multiple analytes is promising in opening up new possibilities for multiplexed assays of different chemical analytes. Tyr-modified electrodes and GOx electrodes have been widely fabricated by scientists and applied in determining phenolic compounds and HMs. [19,21,31–35] However, according to our knowledge, the report on ultrasensitive biosensing of multi-analyte (including Cr(III), Cr(VI), BPA, and phenol) with bi-enzyme electrodes fabricated via enzyme-catalyzed polymerization has never seen, which is meaningful both in environmental monitoring and enzymatic inhibitive assays.

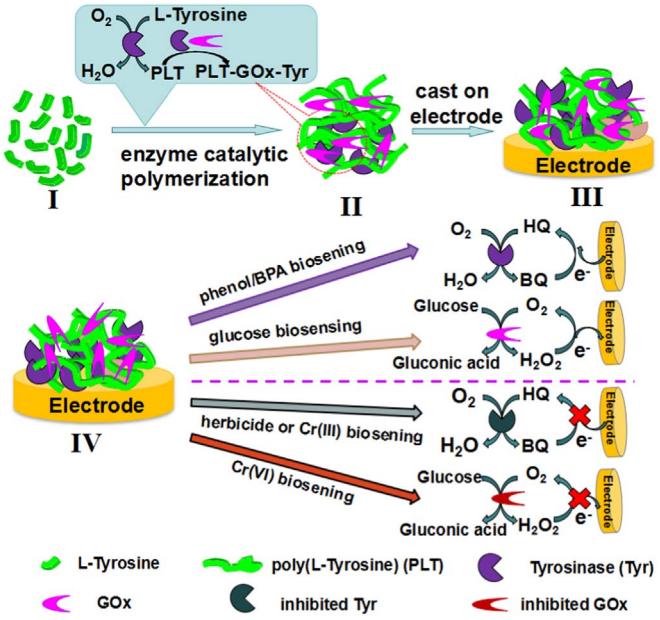
L-tyrosine is unique amino acid which has phenolic functionality in addition to amine and carboxylic units at the α-carbon. It is also one of the most versatile amino acids with the side chain phenolic moiety [36]. L-tyrosine-based polycarbonates have been widely reported in cell adhesion and migration, bone repair, soft tissue fillers because of its excellent biocompatibility, it is also utilized in drug delivery applications [37]. Poly(LPolymer from L-tyrosine oxidation (PLT) is a polypeptide of special properties which is neither hydrophobic nor hydrophilic and retains neutral charge over a wide range of pH. Due to the multiple properties of the phenolic moiety, tyrosine residues are widely used in the molecular recognition site of antibodies. However, as known to us, the utilization of PLT in entrapping enzyme molecules, which is expected to be meaningful and promising, has never been reported.

Herein, we reported a novel high-performance bi-enzyme amperometric biosensor and realized the sensitive and selective determination of a series of analytes including glucose, BPA, phenol, Cr(III) and Cr(VI). The bi-enzyme biosensor was prepared via immobilizing Tyr and GOx simultaneously with PLT resulted from Tyr-catalyzed L-tyrosine polymerization. The thus-prepared biosensors exhibit high sensitivity, rapid response, good reproducibility, long-term stability and freedom from other potentially interfering species.

2. Experimental

2.1. Instrumentation and chemicals

All electrochemical experiments were conducted on a CHI660C electrochemical workstation (CH Instrument Co.). The Au electrode of 3.0 mm diameter served as the working electrode, a KCl saturated calomel electrode (SCE) as the reference electrode, and a carbon rod as the counter electrode. All potentials are cited versus SCE. Scanning electron microscopy (SEM) studies were performed on a Hitachi S4800 scanning electron microscope with a field emission electron gun.



Scheme 1. Schematic representation of procedures for preparing PLT-Tyr-GOx/Au electrode and its application in detection of phenolic/glucose and inhibitive-based detection of Cr(III)/Cr(VI).

Tyr (EC 1.14.18.1; 4.80 kU mg^{-1} of solid, from mushroom) was purchased from Sigma. GOx (EC 1.1.3.4; type II from Aspergillus niger, 150 U mg^{-1}), and L-tyrosine were obtained from Sigma-Aldrich. Glucose, phenol, and BPA were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1.00 M glucose stock solution was allowed to mutarotate overnight at room temperature before use. Cr (III) and Cr(VI) solution were daily prepared from $Cr(NO_3)_3{\cdot}9H_2O$ or $K_2Cr_2O_7$, respectively. Other reagents were of analytical grade or better quality and used as received. Milli-Q ultrapure water (Millipore, ≥18 MΩ cm) was used throughout. All experiments were performed at room temperature around 25 °C.

2.2. Procedures

As shown in Scheme 1, a PLT-Tyr-GOx/Au electrode was prepared by casting 5.0 μL 0.10 M PBS (pH 7.0) containing 3.0 mM L-tyrosine + 4.0 mg mL^{-1} Tyr + 6.0 mg mL^{-1} GOx on Au electrode, keeping the biocomposites modified on electrode by incubating at 37 °C for 1 h in a water bath and then taking it out till air-dried. During catalytic biosensing experiments, the steady-state current response of PLT-Tyr-GOx/Au electrode for successive additions of glucose into 10 mL stirred PBS (pH 7.0) was recorded in air at 0.70 V to anodically detect the enzymatically generated H_2O_2. The steady-state current responses to successively additions of BPA or phenol into 10 mL stirred PBS (pH 7.0) were recorded at −0.10 V to cathodically detect the enzymatically generated *o*-benzoquinone (BQ).

For inhibitive assays of Cr(VI), 10.0 mM glucose was added into stirred pH 3.6 acetate buffer solution to reach a steady-state current at 0.70 V (I_0), the steady-state current after additions of Cr(VI) (I) was recorded, and the inhibition percentage for GOx, $In_{GOx} = (I_0 - I)/I_0$, was used as the quantitative signal for Cr(VI). In Cr(III) sensing, 5.0 μM phenol was added into 10 mL stirred PBS (pH = 5.0) to reach a steady-state current at −0.10 V (I_0'), and the current response after adding Cr (III) (I') was recorded, and the inhibition percentage for Tyr, $In_{Tyr} = (I_0' - I')/I_0'$, was used as the quantitative signal for Cr(III).

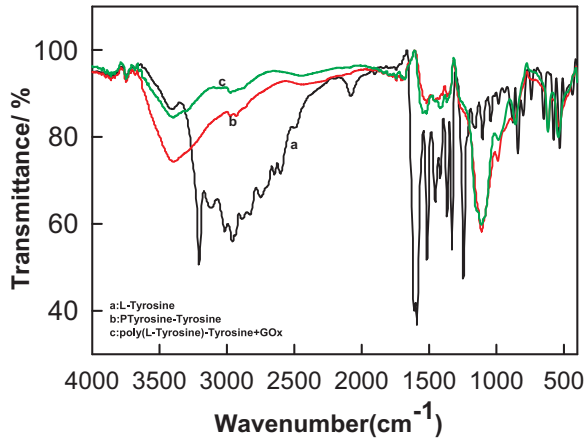


Fig. 1. FT-IR spectra of L-Tyrosine (a), PLT prepared under Tyr catalyzed oxidation (b), PLT-Tyr-GOx composites (c).

3. Results and discussion

3.1. Characterization of PLT biomaterials

As shown in Fig. 1, the FTIR spectra of L-Tyrosine (a), PLT-Tyr (b) and PLT-Tyr-GOx composites (c) were shown for comparison. Significant bands of 3490 ~ 3400 cm^{-1} in the spectrum of L-Tyrosine is the stretching vibration of N-H in free primary amines and 3500 ~ 3200 cm^{-1} should be assigned to stretching or deformation vibrations of phenolic O–H bonds; the significant and long bands of 3000 ~ 2500 cm^{-1} is the stretching vibration of –COOH, and the long bands of 1500 ~ 600 cm^{-1} in L-Tyrosine spectra can also be attributed to significant bands of the phenolic O–H bonds, which contains the surface deformation vibration peak and the out-of-plane deformation vibration peak of O–H bonds; the new bands in PLT (b and c) from 3500 ~ 3300 cm^{-1} can be attributed to the newly formed secondary amine result from the polymerization of primary amines; bands around 1650 cm^{-1} in PLT can be attributed to the stretching of aromatic C–C bonds and the C = O in newly formed benzoquinone; bands from 950 to 650 cm^{-1} are attributed to out-of-plane deformation of aromatic C–H bonds. Therefore, we may conclude that the polymer from L-Tyrosine enzyme oxidation can be most likely speculated as poly(5,6-indolequinone), and many oxygen atoms therein may have developed C–O–C chains during the poly(indole)-like polymerization [38]. This also demonstrated that L-Tyrosine was effectively oxidized under the function of Tyr, and the addition of GOx having little effect on the enzymatic catalytic process.

The SEM images of PLT modified electrode prepared via enzyme oxidation (PLT_E) (A) and chemical oxidation (PLT_C) (B) were collected and shown in Fig. 2. Comparing PLT_C prepared under $KMnO_4$ oxidation (B) with PLT_E (A), we can find that the PLT_E prepared under the oxidation function of Tyr was more uniformly distributed, which may

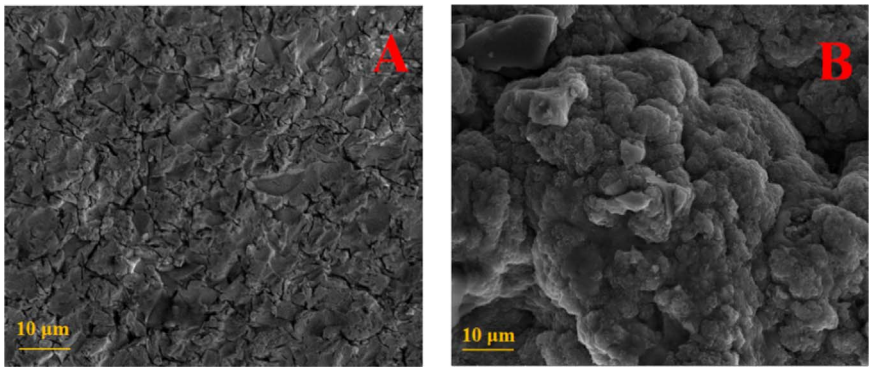


Fig. 2. SEM images of PLT prepared via enzyme oxidation (PLT_E, A) and chemical oxidation (PLT_C, B).

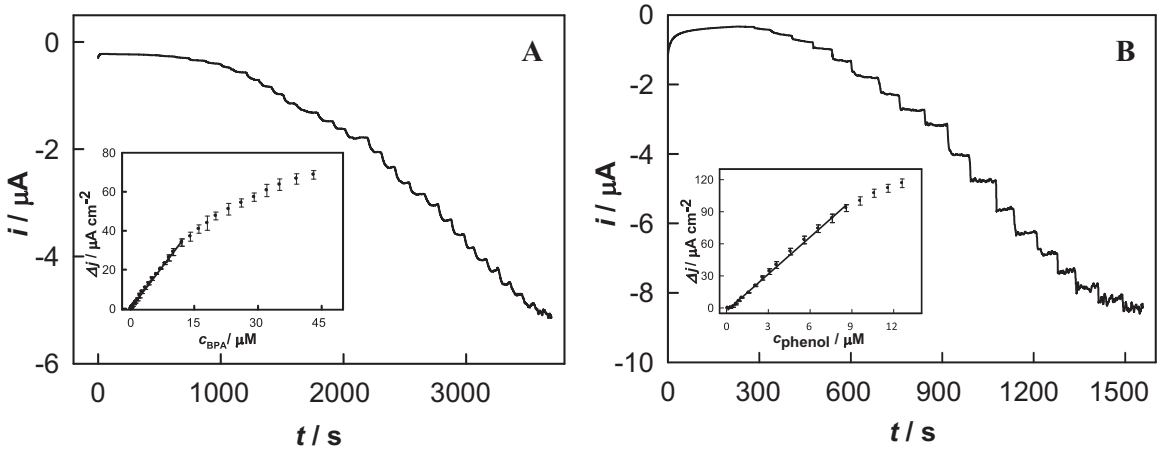


Fig. 3. Typical current-time curve of the PLT-Tyr-GOx/Au electrode for BPA (A), phenol (B) in 0.10 mol L^{-1} PBS (pH 7.0). Applied potential: −0.10 V vs. SCE. Insets: relationship between the catalytic current and the concentration of BPA/phenol for PLT-Tyr-GOx/Au.

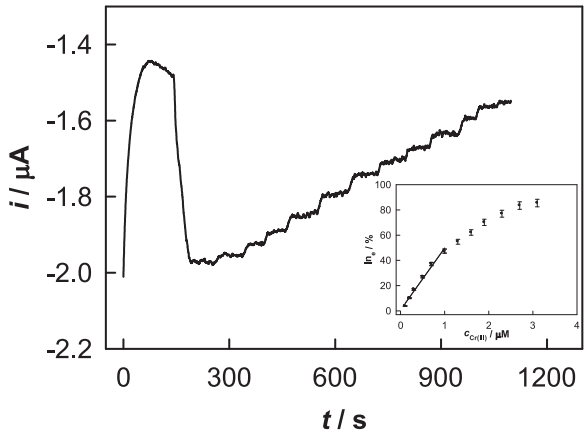


Fig. 4. The inhibition curves of PLT-Tyr-GOx/Au for Cr(III) in PBS (pH 5.0) with continuous additions of Cr(III) solution at -0.10 V. Insert: calibration curve for Cr(III).

demonstrate enzyme oxidation is more uniformly and friendly, this may be just the fact that the resultant PLT_E is more effective in entrapping high load and high activity Tyr.

3.2. Analytical performance of PLT-Tyr-GOx/Au electrode

3.2.1. Performance of resultant biosensor in BPA, phenol and Cr(III) sensing

PLT-Tyr-GOx/Au electrode was utilized for the analysis of Tyr substrates (BPA and phenol) and its inhibitor (Cr(III)). Fig. 3 shows the typical steady state chronoamperometric response of the PLT-Tyr-GOx/Au electrode to successive additions of BPA (A) and phenol (B). In BPA/phenol biosensing experiments, the steady-state current responses of the Tyr-based electrodes to successive injections of a certain concentrated BPA/phenol solution in 10 mL of stirred PBS (pH 7.0) were recorded at −0.10 V by detecting the electro-reduction of the enzymatically generated *o*-benzoquinone (BQ). The response current was marked with the change value between the steady-state current and the back ground current (Δi). Fig. 4 illustrates the time-dependent response of the PLT-Tyr-GOx/Au electrode to additions of Cr(III). In Cr(III) biosensing, the prepared PLT-Tyr-GOx/Au electrode potentiostated at −0.10 V was immersed in PBS (pH 5.0), 5.0 μM phenol (substrate) was added and a steady-state current response (I_0) was reached. Then, the concentration of the added Cr(III) increased stepwisely, with the corresponding current after adding certain concentrations of inhibitor (I_i) recorded. The percentage of inhibition (In(%)) aroused by Cr(III) was evaluated according to the equation: $In(\%) = (I_0 - I_i)/I_0$, which was proportional to the final concentrations of Cr(III) in solution. The biosensing performance of the resultant biosensor towards BPA, phenol and Cr(III) including the corresponding sensitivity (*S*), limit of detection (LOD), and linear range (LR) are presented in Table 1. As comparing with many reported results shown in Table 1, PLT-Tyr-GOx/Au electrode exhibits excellent performance in BPA, phenol and Cr(III) sensing.

3.2.2. Performance of PLT-Tyr-GOx/Au for the determination of glucose and Cr(VI)

We also studied the performance of the resultant bi-enzyme biosensor (PLT-Tyr-GOx/Au) in the biosensing of GOx substrate and inhibitor. Fig. 5(A) shows the steady-state current response of the PLT-Tyr-GOx/Au electrode to successive additions of glucose into PBS at 0.70 V. The bienzyme biosensor displays excellent performance in glucose biosensing. The chronoamperometric determination of Cr(VI) using the developed PLT-Tyr-GOx/Au electrodewas also carried out by taking use of the inhibition effect of Cr(VI) on GOx activity. Fig. 5(B) presented the time-dependent response of the PLT-Tyr-GOx/Au electrode for continuous additions of different concentrations of Cr(VI) in acetate buffer solution (pH 3.6) at +0.70 V. The performance of the biosensors in glucose and Cr(VI) sensing (includes the *S*, LOD, and LR) were presented in Table 2. As comparing with many reported results shown in Table 2, our PLT-Tyr-GOx/Au electrode exhibits higher sensitivity and lower LOD in glucose and Cr(VI) sensing. These results indicate that we have developed a bi-enzyme biosensor which can detect a variety of substances at the same time.

In order to examine the cross-reactivity between Tyr and GOx, we also prepared a PLT-Tyr/Au electrode by casting 5.0 μL 0.10 M PBS (pH 7.0) containing 3.0 mM L-tyrosine + 4.0 mg mL^{-1} Tyr on Au electrode (with other conditions the same as preparing PLT-Tyr/Au electrode) and investigated the performance of the prepared PLT-Tyr/Au electrode in phenol/BPA/Cr(III) sensing. As found in the experiments, under optimized conditions, PLT-Tyr/Au electrode exhibits similar performance with PLT-Tyr-GOx/Au electrode on Tyr-based biosensing, which demonstrated the co-immobilized GOx does not decrease the catalytic

Table 1
Comparison of performances of proposed biosensor for phenolic pollutants and Cr(III) with other reports.

Immobilization method	Detection substrate	LOD (μM)	Sensitivity (μA mM^{-1})	Liner Range (mM)
Tyr-silica sol film [39]	Phenol	0.1	23.1	/
Tyr-AuNPs-graphite–Teflon composite [40]	Phenol	0.06	746	$4.0 \times 10^{-4} \sim 7.0 \times 10^{-2}$
Tyr-copolymerpoly(N−3-aminopropyl pyrrole-*co*-pyrrole) film [41]	Phenol	1.2	3.46	$1.6 \times 10^{-2} \sim 0.12$
Tyr-MWCNTs-Nafion nanobiocomposite matrix [42]	Phenol	0.22	346	$1.0 \times 10^{-3} \sim 2.3 \times 10^{-2}$
Tyr-Fe_3O_4-CNTs/GCE [43]	Phenol	0.005	516	1.0×10^{-5}~3.9×10^{-2}
*PLT-Tyr-GOx/Au (This article)	Phenol	0.017	815	$5.0 \times 10^{-5} \sim 1.0 \times 10^{-2}$
Tyr-SF-MWNTs-CoPc/GCE [30]	BPA	0.03	/	5.0 × 10–5 ~ 3.0 × 10−3
Tyr-MWCN paste electrode [44]	BPA	1	138	$1.0 \times 10^{-3} \sim 1.6 \times 10^{-2}$
Tyr-NGP-Chi/GC [45]	BPA	0.033	220	$1.0 \times 10^{-4} \sim 2.0 \times 10^{-2}$
CuMOF-Tyr-Chit/GCE [46]	BPA	0.013	224	5.0 × 10–5 ~ 3.0 × 10−3
Tyr/TiO_2-MWCNTs-PDDA- Nafion/graphite [47]	BPA	0.066	229	2.8 × 10–4 ~ 4.5 × 10–2
*PLT-Tyr-GOx/Au (This article)	BPA	0.016	221	$5.0 \times 10^{-5} \sim 1.0 \times 10^{-3}$
Tyr/$SPC_{TTF}E$ [18]	Cr(III)	2.0	/	$1.8 \times 10^{-3} \sim 1.58 \times 10^{-2}$
Tyr/PPy [17]	Cr(III)	0.50	/	$5.0 \times 1\,0^{-4} \sim 5.0 \times 10^{-3}$
HRP/PNR/CFE [1]	Cr(III)	0.27	/	$2.0 \times 10^{-4} \sim 5.1 \times 10^{-3}$
*PLT-Tyr-GOx/Au (This article)	Cr(III)	0.08	/	$5.0 \times 10^{-5} \sim 1.0 \times 10^{-3}$

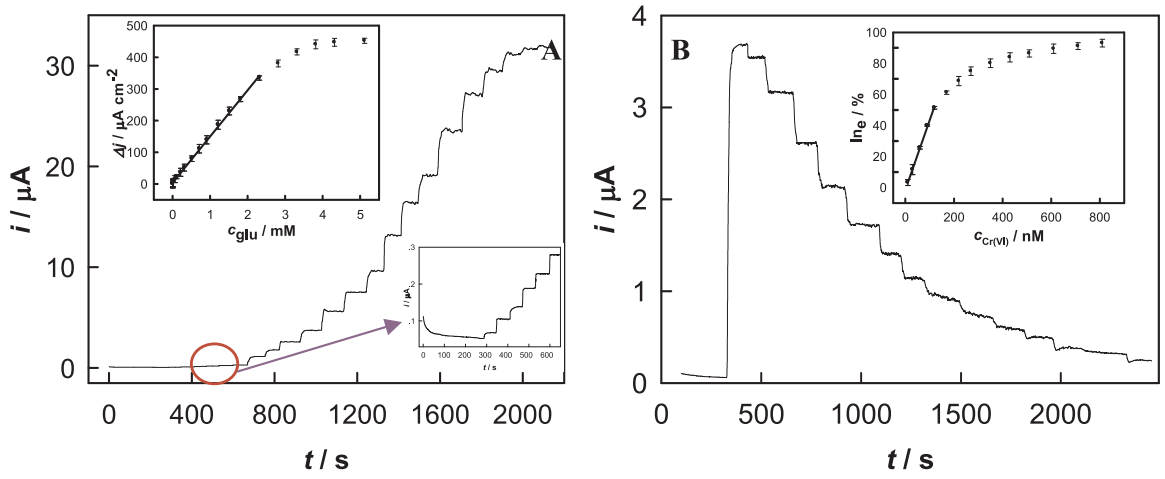


Fig. 5. Time-dependent current responses to successive additions of glucose into stirred 0.10 M PBS (pH 7.0) and the corresponding calibration curves at PLT-Tyr-GOx/Au electrode (A) and the inhibition current responses of PLT-Tyr-GOx/Au electrode in acetate buffer solution (pH 3.6) for continuous additions of different concentrations of Cr(VI) and the corresponding calibration curves (B). Applied potential: +0.70 V vs. SCE.

Table 2
Comparison of biosensing performances of the proposed biosensor with other reports.

Immobilization method	Detection substrate	LOD (μM)	Sensitivity (μA mM^{-1})	Liner Range (mM)
PD-GOx-Tyr/Pt [48]	Glucose	0.1	5.55	$2.0 \times 10^{-3} \sim 5.7$
PtPd-MWCNTs-GOx/GCE [49]	Glucose	0.031	7.91	$0.062 \sim 14$
Pt-N-GSS-GOx/GCE [50]	Glucose	1	6.36	$1.0 \times 10^{-2} \sim 12.55$
*PLT-Tyr-GOx/Au (This article)	Glucose	0.22	10.34	$1.0 \times 10^{-3} \sim 2.3$
$GOx/SPC_{Pt}E$ [18]	Cr(VI)	9×10^{-2}	/	$9.0 \times 10^{-5} \sim 7.96 \times 10^{-4}$
HRP/CFE [1]	Cr(VI)	9×10^{-2}	/	$5.0 \times 10^{-5} \sim 1.5 \times 10^{-3}$
Pt/PPD/GOx [20]	Cr(VI)	48	2.2	$0.048 \sim 4.8$
*PLT-Tyr-GOx/Au (This article)	Cr(VI)	5×10^{-3}	/	$1.0 \times 10^{-5} \sim 1.2 \times 10^{-4}$

activity of Tyr. In order to investigate the impact of Tyr on GOx, we detected the enzymatic activity of GOx with or without the presence of Tyr in electrochemical method, as widely reported in our pervious reports. [19] The experimental results reveal that the cross-reactivity of Tyr and GOx is little in our experiments.

3.2.3. Selectivity and stability of the resultant biosensor

Selectivity is an important parameter in the performance of a biosensor. In order to confirm the selectivity of the resultant biosensor, we studied the common interferences in phenol, glucose, and Cr(VI) biosensing. As shown in Fig. 6, for the measurement of 4.0 μM phenol, 100 μM Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, SO_4^{2-}, HCO_3^- Cl^-, NO_3^- have no obvious interference; for the measurement of 0.3 mM glucose, 0.1 M NaCl, 1.0 mM galactose, 0.1 mM acetaminophen, 0.05 mM uric acid (UA), 0.05 mM ascorbic acid (AA), 0.1 M KCl, and 1.0 mM sodium acetate have little response on PTy-Tyr-GOx/Au electrode; the responses of PTy-Tyr-GOx/Au electrode to the sequential additions of 30 nM Cr(VI) solution, 20 μM Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{3+}, Hg^{2+}, Pb^{2+}, Cr^{3+}, As^{3+}, Ag^+ and 30 nM Cr(VI) solution into acetate buffer solution (pH 3.6) were shown in Fig. 6(C), which demonstrated the excellent anti-interference ability of the resultant PTy-Tyr-GOx/Au electrode.

The long term stability to Cr(VI) sensing was evaluated by monitoring the amperometric responses to additions of 0.1 μM Cr(VI) in acetate buffer solution (pH 3.6) at a period of 6 weeks, with the results shown in Fig. 6(D). Since the inhibition behavior of Cr(VI) on GOx is reversible inhibition, the inhibition sensor exhibits good stability. It can be used to determine of Cr(VI) for 6 weeks, and a decrease of 50% from the initial response was observed after 6 weeks.

4. Conclusions

In summary, we have successfully synthesized PLT-Tyr-GOx bionanocomposites and realized the high sensitive biosensing of glucose, bisphenol A, phenol, Cr(III) and Cr(VI), respectively. The thus-prepared bi-enzyme biosensor exhibit high sensitivity, low LOD, and good storage stability for the monitoring of a variety of analytes, which can be used for the simultaneous determination of glucose, several phenolic compounds or some heavy metals and there will be a broad space for development in the future.

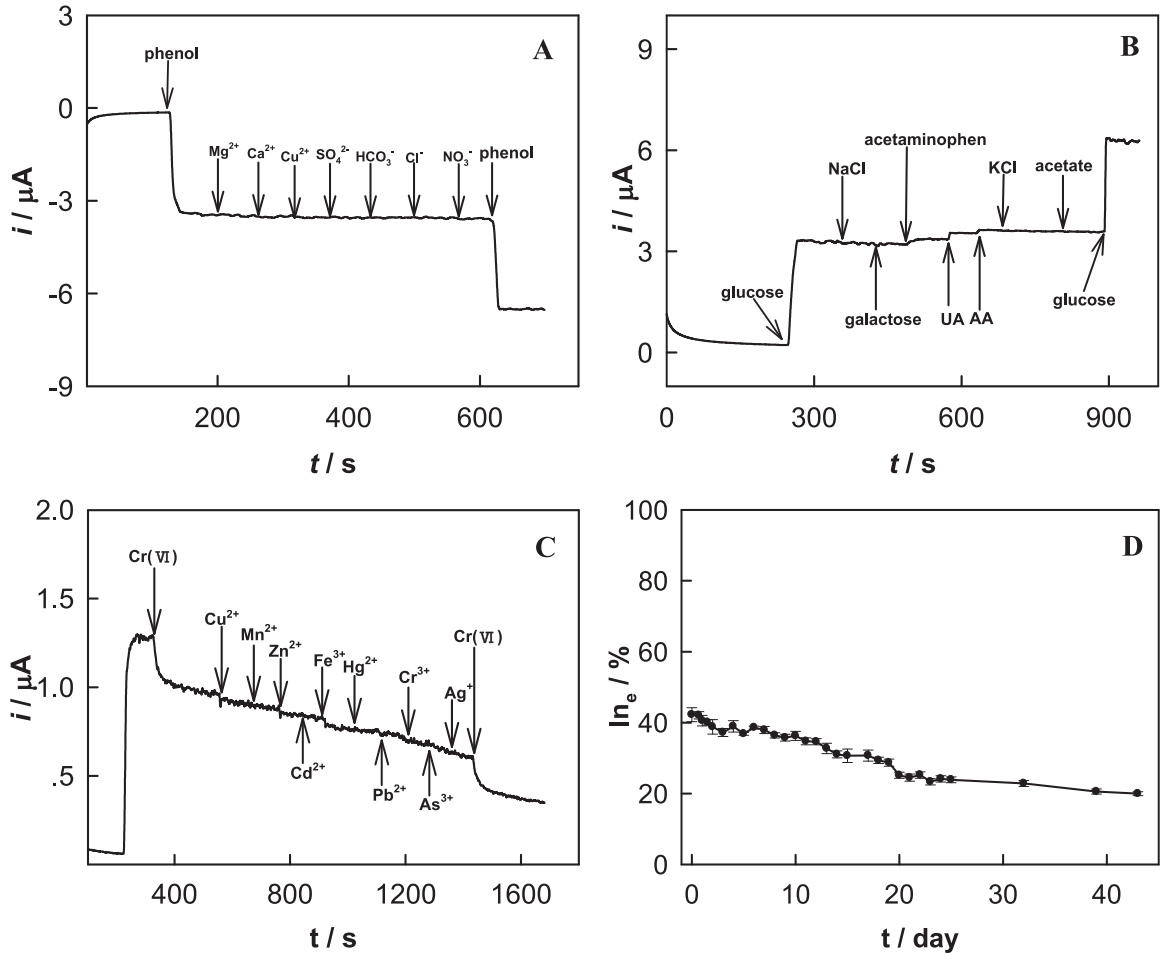


Fig. 6. (A) Current responses at the PLT-Tyr-GOx/Au electrode to the sequential additions of 4.0 μM phenol, 0.10 mM Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, SO_4^{2-}, HCO_3^- Cl^-, NO_3^-, and 4.0 μM phenol into 0.10 M PBS (pH 7.0). (B) Effect of additions of 0.3 mM glucose, 0.1 M NaCl, 1.0 mM galactose, 0.1 mM acetaminophen, 0.05 mM uric acid (UA), 0.05 mM ascorbic acid (AA), 0.1 M KCl, and 1.0 mM sodium acetate on the response of PTy-Tyr-GOx/Au electrode. (C) Current responses at the PTy-Tyr-GOx/Au electrode to the sequential additions of 30 nM Cr(VI) solution, 20 μM Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{3+}, Hg^{2+}, Pb^{2+}, Cr^{3+}, As^{3+}, Ag^+ and 30 nM Cr(VI) solution into acetate buffer solution (pH 3.6). The measurements were conducted at +0.70 V vs. SCE. (D) Storage stability of the constructed PTy-Tyr-GOx/Au electrode under storage conditions in 0.10 M PBS solution of pH 7.0 at 4 °C).

Acknowledgments

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