Biosensors and Bioelectronics 26 (2010) 87–91

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Combination of enzyme catalysis and electrocatalysis for biosensor fabrication: Application to assay the activity of indoleamine 2,3-dioxygensae

Ya Cao [a], Jing Wang [a], Yuanyuan Xu [a], Genxi Li [a,b,*]

[a] Department of Biochemistry and National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, 210093 Nanjing, China
[b] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, 200444 Shanghai, China

ARTICLE INFO

Article history:
Received 27 February 2010
Received in revised form 28 April 2010
Accepted 10 May 2010
Available online 19 May 2010

Keywords:
Indoleamine 2,3-dioxygensae
Platinum nanoparticle
Enzyme catalysis
Electrocatalysis

ABSTRACT

A new strategy to fabricate electrochemical biosensor is reported in this paper based on the selective combination of enzyme catalysis and electorcatalysis, thus an electrochemical method to assay the activity of indoleamine 2,3-dioxygensae (IDO) is proposed. Tryptophan, the substrate of IDO, is firstly covalently immobilized on a gold electrode surface. Oxidation of the tryptophan residue catalyzed by IDO and the subsequent hydrolyzation of the product by acetic acid may yield kynurenine, which may induce the immobilization of dithiobis [succinimidylpropionate] (DSP)-modified platinum nanoparticles (Pt NPs) onto the surface of the gold electrode. Since Pt NPs can electrochemically catalyze the reduction of H_2O_2 to produce electrochemical signals and the electrochemical wave can be correlated with the enzyme activity, electrochemical method to detect IDO activity is thus achieved. Under optimized conditions, IDO activity can be assayed in the range of 20–400 U/mL with a detection limit of 6.84 U/mL. The proposed biosensor shows high sensitivity, acceptable reliability, and can be used for the investigation of the enzymatic inhibition by inhibitors as well as the screen of the enzymatic activity in complex matrix such as serum samples.

1. Introduction

Indoleamine 2,3-dioxygensae (IDO) is an intracellular heme-containing enzyme that catalyzes the cleavage of the pyrrole ring of L-tryptophan (L-Trp) and the insertion of two oxygen atoms into the organic substrate to afford *N*-formylkynurenine (Mellor and Munn, 2004). The importance of IDO is associated with its physiological roles, such as inhibition of T cells proliferation, synthesis of neurontransmitter, regulation of immune responses (Mellor and Munn, 2004; Muller and Scherle, 2006; Munn et al., 2002; Sono et al., 1996; Takikawa, 2005), etc. In particular, recent studies have demonstrated that IDO is overexpressed in a wide variety of human solid tumors (Curti et al., 2009; Katz et al., 2008; Munn and Mellor, 2007; Uyttenhove et al., 2003). It is involved in the induction of tumor tolerance by direct suppression of T cells and enhancement of local T_{reg} (regulatory T cells)-mediated immunosuppression. And, inhibition of IDO may result in a considerable therapeutic effect. So, assay of the activity of the enzyme has received more and more interests.

Up to now, many efforts have been made to determine the activity of IDO. The techniques include spectrophotometry (Takikawa et al., 1988), high-performance liquid chromatography (HPLC) (Ozaki et al., 1991), radioisotope (Ozaki et al., 1991) and fluorescence-based method (Matin et al., 2006), etc. Nevertheless, all of these techniques have some limitations that restrict their further development in clinical applications. For instance, the spectrophotometry method is incompatible with compounds containing certain functional groups; the HPLC and radioisotope-based methods require the use of radiochemical regents and are expensive. The fluorescence-based method may be interfered with compounds whose fluorescence response is similar to kynurenine. Therefore, it has been an urgent demand to develop new IDO activity assay strategies that are selective as well as sensitive.

In this paper, we report a sensitive electrochemical biosensor for the detection of IDO activity. The biosensor is based on the combination of enzyme catalysis and electrocatalysis. As is well known, electrocatalytic readout for protein detection has been mainly used for examples including redox-active proteins which may need the immobilization of proteins onto the electrode surface (Bahshi et al., 2008; de la Escosura-Muniz et al., 2004), or the development of binding-based assays which report on the presence of an analyte rather than its activity (Polsky et al., 2006; Shiddiky et al., 2007, 2008). Here, by using novel functionalized platinum nanoparticles (Pt NPs) with dithiobis [succinimidylpropionate] (DSP) and DSP-based cross-linking reaction, we have developed a new strategy in this work for sensor fabrication based on the combination of enzyme catalysis and electrocatalysis, thus electrocatalytic readout

* Corresponding author at: Department of Biochemistry and National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, 210093 Nanjing, China. Fax: +86 25 83592510.
E-mail address: genxili@nju.edu.cn (G. Li).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.05.019

for the sensitive detection of IDO activity is proposed. Specifically, only when IDO-catalyzed oxidation process has occurred, DSP-modified Pt NPs can be immobilized onto the electrode surfaces, so this electrochemical biosensor may assay the activity of IDO with high sensitivity and selectivity, which may have potential for further clinical applications.

2. Materials and methods

2.1. Chemicals and materials

Trisodium citrate and hydrogen peroxide (H_2O_2) were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Chloroplatinic acid hydrate ($PtCl_6^{2-}$), L-tryptophan, DSP, bovine serum albumin (BSA), and 1-methyl-L-tryptophan were obtained from Sigma–Aldrich. IDO was purchased from Enzo Life Sciences International, Inc. All other reagents were of analytical reagent grade and used as received.

The buffer solutions used in this work are as follows: DSP-reaction buffer for the cross-linking reactions between DSP and primary amine-containing molecules, 0.1 M phosphate buffer saline (pH 7.2) containing 0.15 M NaCl; IDO storage buffer, 50 mM Tris–HCl solutions (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid; IDO-reaction buffer, 0.1 M phosphate buffer saline (pH 6.5); washing buffer, 0.1 M phosphate buffer saline (pH 7.4) containing 0.9% NaCl and 0.05% Tween 20; electrolyte solution for cyclic voltammetry, 0.1 M phosphate buffer saline (pH 7.4); buffer for electrochemical impedance spectroscopy, 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO_3. All the buffers were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of 18 MΩ cm.

2.2. Preparation of DSP-modified Pt NPs

Pt NPs were prepared based on previously reported synthesis methods (Li et al., 2008; Polsky et al., 2006) with some modifications. Briefly, a 100 mL aqueous solution of 1 mM $PtCl_6^{2-}$ was added into a round-bottom flask and heated to boil. Then 10 mL of 38.8 mM aqueous sodium citrate solution was added rapidly into the boiling solution, the color of which became dark brown from pale yellow after boiling for 30 min with vigorous stirring. Finally, the solution was kept stirring for another 30 min at room temperature and then stored at 4 °C. The size distributions of the above-prepared Pt NPs were analyzed, showing that at least 80% of the particles are distributed around 4.0 nm.

Due to the existence of an intramolecular disulfide bond, DSP could adsorb onto gold or platinum surfaces (Durocher et al., 2009; Katz, 1990). Thus, DSP-modified Pt NPs were prepared by adding 2.5 mL of 10 mM DSP solution (dissolved in dimethyl sulfoxide (DMSO)) into 2.5 mL of the above-prepared Pt NPs solution. The mixture was then placed on a shaker. After an overnight duration, it was centrifugated for 30 min at 10,000 rpm. The DSP-modified Pt NPs were resuspended in 1 mL of 10 mM phosphate buffer for the following experiments.

2.3. Preparation of L-tryptophan-modified electrode

The substrate gold electrode (3 mm diameter) was firstly cleaned with piranha solution (70% concentrated sulfuric acid, 30% H_2O_2) for 5 min followed by rinsing with double-distilled water (*Caution: Piranha solution reacts violently with organic solvents and should be handled with great care!*). Then the electrode was polished on microcloth with 1 μm, 0.3 μm, and 0.05 μm alumina slurry in sequence. Residual alumina powder was removed by sonicating the electrode sequentially in both ethanol and double-distilled water. Finally, the electrode was soaked in nitric acid (50%) for 30 min, and electrochemically cleaned with 0.5 M H_2SO_4 to remove any remaining impurities. After being dried with nitrogen, the pretreated electrode was immersed in a 10 mM DSP solution overnight at room temperature, so that DSP self-assembly monolayer could be formed on the electrode surface. Afterward, the electrode was rinsed with DMSO and water respectively to remove any unbound DSP. L-tryptophan was then covalently attached to the electrode by incubating the DSP-modified gold electrode (DSP/Au electrode) with a 10 mM L-tryptophan solution (dissolved in the DSP-reaction buffer) for 2.5 h. After being rinsed with water, it could be used for the following experiments.

2.4. IDO-catalyzed oxidation of tryptophan

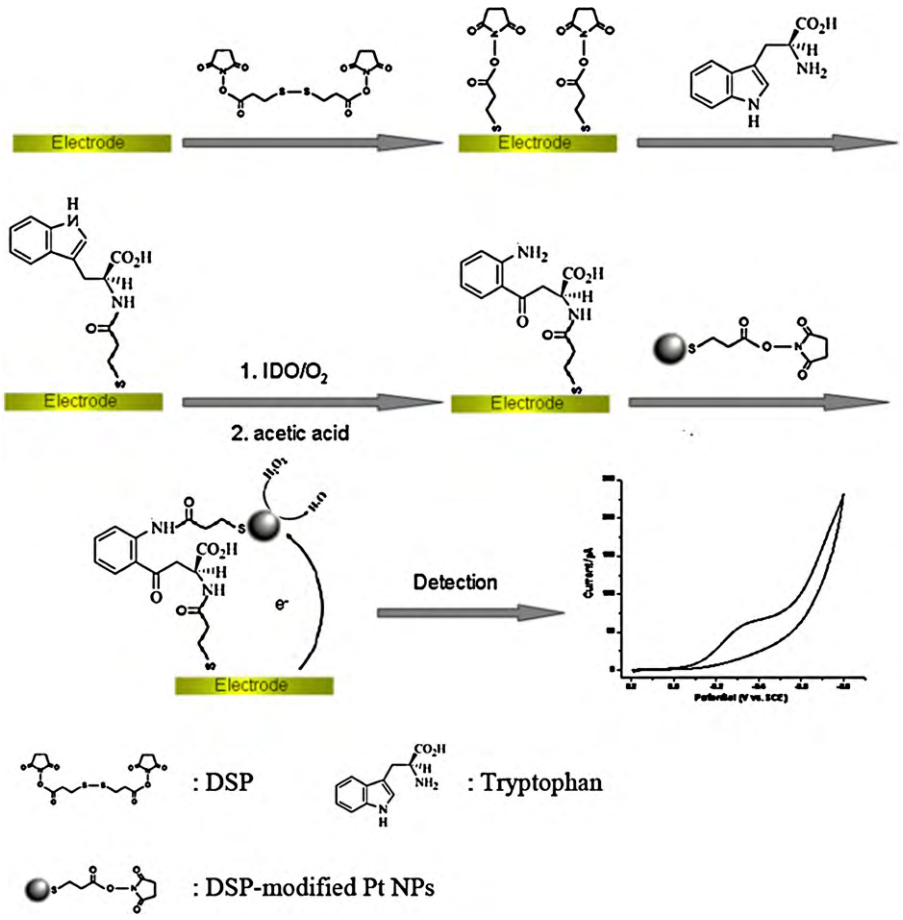
The tryptophan-modified gold electrode (tryptophan/DSP/Au electrode) was firstly incubated with a 400 μL IDO solution with different concentrations at 37 °C for 30 min. Then the electrode was washed thoroughly with the washing buffer to remove non-specific enzymes adsorbed on the electrode surface. Afterward, the electrode was incubated with 1 M acetic acid at 37 °C for 4 h to hydrolyze *N*-formylkynurenine to kynurenine. Finally, the electrode was rinsed and subsequently immersed in a 400 μL solution prepared with 200 μL of the above DSP-modified Pt NPs and 200 μL of DSP-reaction buffers. After 2 h duration, the electrode was rinsed thoroughly with double-distilled water, and could be used for the following electrochemical measurements.

2.5. Electrochemical measurements.

Electrochemical measurements were carried out on a model 660c Electrochemical Analyzer (CH Instruments) with a conventional three-electrode cell at room temperature. The three-electrode system consisted of the modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode. Considering that at pH 7 DSP absorbed on the gold electrode can be easily desorbed at about −0.75 V (Darder et al., 1999), all the cyclic voltammograms were recorded in the first scan in the range of 0.2 to −0.8 V by using 5 mL electrolyte solution with 10 mM H_2O_2, in order to avoid potential interference by the desorption of DSP. The test solution should be thoroughly purged with high purity nitrogen for about 5 min before the addition of H_2O_2, to avoid the interference from the reduction of oxygen. The electrochemical impedance spectra were recorded by applying a bias potential of 0.224 V vs. SCE and 5-mV amplitude in the frequency range of 0.1 Hz–10 kHz.

3. Result and discussion

Scheme 1 may illustrate the principle of this electrochemical biosensor for the detection of IDO activity. Firstly, the gold electrode is covalently modified with DSP, a thiol cleavable cross-linker which contains a primary amine-reactive *N*-hydroxysuccinimide (NHS) ester at each end of an 8-carbon spacer arm. Then, tryptophan, the substrate of the enzyme, is immobilized on the electrode surface by covalent bonding between the primary amine group of tryptophan and the NHS esters of DSP. In the presence of IDO and O_2, the tryptophan residues will be converted to *N*-formylkynurenine, which can be subsequently hydrolyzed with acetic acid to kynurenine, resulting in new primary amine groups. Therefore, if the electrode is exposed to DSP-modified Pt NPs, the affinity between the primary amine group and the NHS esters of DSP will result in the attachment of Pt NPs onto the electrode surface. Since Pt NPs may electrocatalyze the reduction of H_2O_2 to produce electrochemical signal (Karam and Halaoui, 2008; Niazov et al., 2007; Yildiz et al., 2008), redox wave of the electrocatalytic reaction can be correlated



Scheme 1. Schematic illustration of the electrochemical principle for the detection of IDO activity.

with the enzyme reaction, thus detection of IDO activity is possible with electrochemical method.

3.1. Electrochemical characteristics of the electrode surface modification

As is well known to the colleagues, electrochemical impedance spectroscopy (EIS) is sensitive to the changes occurring at the surface of electrode, allowing an insight into the chemical transformation and processes associated with the electrode surface (Arya et al., 2007; Miao et al., 2009). The impedance spectra contain a semicircle portion at higher frequencies and a linear part at lower frequencies. The diameter of the semicircle portion equals the electron-transfer resistance (R_{et}), and the linear part corresponds to diffusion process. Therefore, we have conducted EIS studies to characterize the modification process of the electrode. Fig. 1 shows the Nyquist plots of EIS for the electrode at different stages. It can be observed that the EIS of the bare gold electrode exhibits nearly no semicircle (Fig. 1a). After the electrode is modified with DSP, a semicircle can be observed, indicating the increase of the electron-transfer resistance (Fig. 1b), which is attributed to the insulating nature of DSP. Moreover, when tryptophan is further covalently modified onto the electrode surface, a further increase of the R_{et} will be induced (Fig. 1c). In the last step, DSP-modified Pt NPs are loaded on the electrode surface, so greater increase in diameter can be observed (Fig. 1d), due to the reason that larger amount of DSP will form bigger barrier to prevent the redox probe from getting access to the electrode surface.

Fig. 2 depicts the cyclic voltammograms obtained from the Pt NPs-electrocatalyzed reduction of H_2O_2 upon analyzing the activity of 200 U/mL of IDO, and those obtained in a series of control experiments. As is shown in Fig. 2a, an electrocatalytic peak at around −0.35 V can be observed in the presence of IDO, due to the reason that IDO-catalyzed conversation of tryptophan has induced the immobilization of DSP-modified Pt NPs onto the electrode and the subsequent Pt NPs-electrocatalyzed reduction of H_2O_2. However,

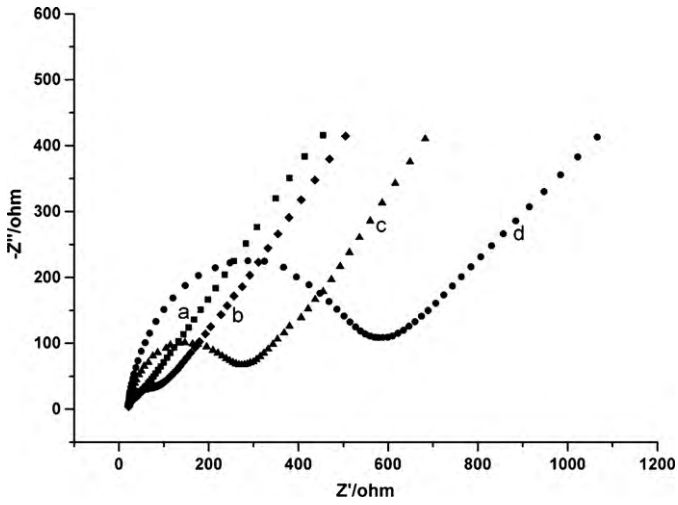


Fig. 1. Nyquist diagrams for the electrochemical impedance measurements of the gold electrode at different modification stages. (a) Bare gold electrode, (b) DSP/Au electrode, (c) tryptophan/DSP/Au electrode, (d) DSP-modified Pt NPs/tryptophan/DSP/Au electrode. Electrochemical species: 5 mM $[Fe(CN)_6]^{3-/4-}$. Biasing potential: 0.224 V. Amplitude: 5 mV. Frequency range: 0.1 Hz–10 kHz.

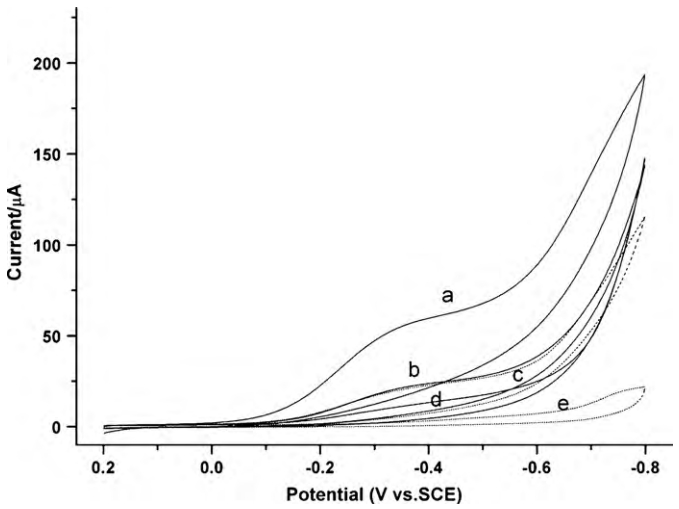


Fig. 2. (a) Cyclic voltammogram obtained upon analyzing 200 U/mL of IDO activity. The curves b–e are separately for the control experiments which are in the absence of (b) IDO, (c) tryptophan, (d) DSP-modified Pt NPs, or (e) H_2O_2. Scan rate: 100 mV/s. Buffer: 0.1 M PBS (pH 7.4) with 10 mM H_2O_2.

in the absence of IDO or tryptophan, only small catalytic peaks are observed (Fig. 2b and c), owing to the nonspecific binding of the Pt NPs to the electrode. Meanwhile, there is nearly no catalytic peak in the absence of Pt NPs or H_2O_2 (Fig. 2d and e), indicating that the catalytic peak comes from the Pt NPs-electrocatalyzed reduction of H_2O_2. These experimental results confirm that IDO and its substrate tryptophan are essential to generate the immobilization of DSP-modified Pt NPs onto the electrode surface, which may make Pt NPs electrocatalyze the reduction of H_2O_2 to give electrochemical response.

3.2. Optimization of electrode modification

The sensitivity of the biosensor should be able to be improved with the increased amount of tryptophan immobilized on the electrode surface and the increased percentage of kynurenine linked to Pt NPs so that more Pt NPs can be loaded onto the surface of the electrode. On the other hand, the attachment of more tryptophan or Pt NPs onto the electrode surface may induce the increase of the electron-transfer resistance. Therefore, EIS has been employed to investigate the effect of the incubation time on the amount of immobilized tryptophan and linked Pt NPs (Fig.S.1 in Supplementary data). Experimental results reveal that the electron-transfer resistance increases with the immobilization time for tryptophan modification, and keeps nearly unchanged after a duration of 2.5 h. Meanwhile, it is found that no significant change in the impedance spectra can be observed after an immobilized time of 2 h for the attachment of Pt NPs onto the electrode surface. Thus, 2.5 and 2 h are separately chosen in this work as the incubation time for the loading of tryptophan and Pt NPs.

3.3. Selective assay of the modified electrode

The selectivity of the modified electrode has been evaluated by H_2O_2 determinations in the presence of some potentially coexisting compounds of H_2O_2 in biological systems. The experimental results reveal that ascorbate, uric acid, or dopamine at concentrations more than two orders higher than expected in biological systems will not cause observable interference with the response of H_2O_2 (Table.S.1 in Supplementary data), indicating that the modified electrode may possess a good selectivity.

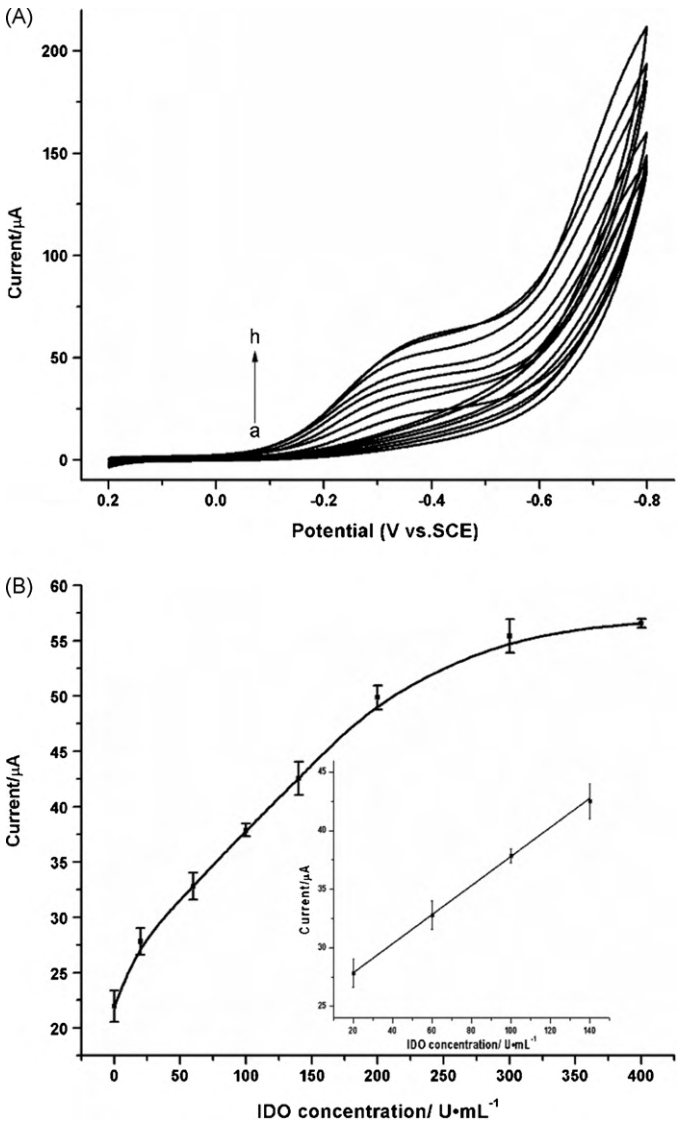


Fig. 3. (A) Cyclic voltammograms corresponding to the analysis of different concentrations of IDO. (a) 0, (b)20, (c) 60, (d) 100, (e) 140, (f) 200, (g) 300, (h) 400 U/mL. Scan rate: 100 mV/s. Buffer: 0.1 M PBS (pH 7.4) with 10 mM H_2O_2. (B) Calibration curve corresponding to the electrocatalytic currents measured at −0.35 V for variable concentration of IDO. Error bars represent standard deviations of measurements ($n = 3$). Inset shows the linear relationship between the reductive peak currents of H_2O_2 and the IDO concentrations.

3.4. Electrochemical detection of IDO activity

Fig. 3A shows the cyclic voltammograms upon analyzing different concentrations of IDO. It can be observed that the electrocatalytic peak increases with the concentration of IDO. This is certainly reasonable, since more enzyme will result in the conversion of increased amount of tryptophan to kynurenine, thus elevated amount of Pt NPs are loaded onto the electrode surface, which make it possible to electrocatalyze more H_2O_2 to give a higher peak. We have also monitored the kinetics of the enzymatic reaction by measuring the electrochemical impedance spectra at different time intervals (Fig.S.2 in Supplementary data). It can be known that the electron-transfer resistance will increase as the reaction time is prolonged, and reach the maximum value after 30 min. So, 30 min is chosen as the optimal reaction time for this study.

The calibration plot obtained for the detection of IDO activity with different concentration has been shown in Fig. 3B. In the range

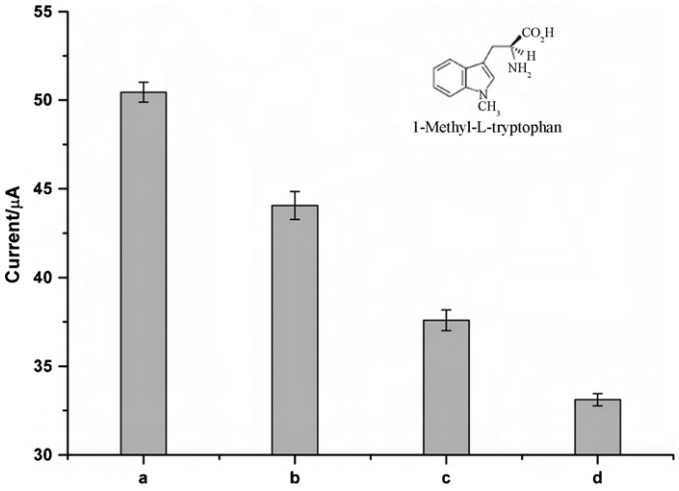


Fig. 4. Plot for the dependence of the electrocatalytic peak currents on the concentration of the inhibitor, 1-methyl-L-tryptophan, (a) 0, (b) 0.25, (c) 0.5, (d) 1 mM. The currents were obtained from the peak at −0.35 V in the corresponding cyclic voltammograms recorded at a scan rate of 100 mV/s.

of 0–400 U/mL, the electrocatalytic peak current increases with the IDO concentration and linear response can be obtained in the range of 20–140 U/mL. For each concentration of IDO, the electrochemical measurement has been repeated for at least three times independently. The average coefficient of variation is 2.59%, indicating that the precision and reproducibility of the proposed biosensor are acceptable.

The detection limit has also been calculated by the interpolation of the mean plus three times the standard deviation of the zero standards (Centi et al., 2007). Since the detection limit can be as low as 6.84 U/mL, which corresponds to 0.077 μg/mL, compared with the previous reports obtained by the other methods (0.36, 0.54, and 0.48 μg/mL for fluorescence, spectrophotometry, and HPLC methods, respectively) (Matin et al., 2006), the detection limit in this work is the lowest.

3.5. Inhibition assay

1-Methyl-L-tryptophan has been reported to be able to abrogate IDO enzymatic activity by competing with the normal tryptophan substrate (Cady and Sono, 1991). The inhibition effect of 1-methyl-L-tryptophan is thus measured by using the proposed biosensor. Mixtures containing 200 U/mL IDO and different concentrations of 1-methyl-L-tryptophan have been included with the tryptophan/DSP/Au electrode, the experiment results of which have been shown in Fig. 4. Gradual decreases of the electrocatalytic peak currents can be observed along with the increasing concentrations of 1-methyl-L-tryptophan. Therefore, the proposed biosensor may provide a promising approach for monitoring the effect of inhibitors and screening new inhibitors of IDO.

3.6. Application in serum samples

The feasibility of the developed biosensor for clinical applications has been also examined by using several serum samples. Different concentrations of IDO were dissolved into human serum to prepare serum sample solutions. The comparison between the assay results and the given concentrations in these serum samples has been shown in Table 1. Although serum is what remains from whole blood after coagulation and contains many kinds of proteins, the relative errors are less than 8%. Therefore, this electrochemical biosensor may be of great value for the detection of IDO activity in clinical applications.

Table 1
Assay results of serum samples by using the proposed biosensor and the comparison of our results with the standard IDO concentrations in serum samples.

Samples	Standard IDO concentration (U/mL)	IDO concentration detected by proposed method (U/mL)	Relative error (%)
1	50.00	46.59	6.82
2	80.00	83.70	4.62
3	100.00	92.63	7.37

4. Conclusions

In summary, this work has introduced a new strategy for biosensor fabrication based on enzyme catalysis combined with electrocatalysis, thus an electrochemical method for the sensitive and selective assay of IDO activity has been proposed. The electrochemical biosensor may give a high sensitivity as well as low detection limit, and the successful determination of IDO activity in serum samples has been achieved. Moreover, study of the inhibition of the enzyme can be also conducted by using the electrochemical biosensor in this work, so the fabricated biosensor may have great potential applications in the future.

Acknowledgements

This work is supported by the National Science Fund for Distinguished Young Scholars (grant no. 20925520) and Shanghai Science and Technology Committee (grant no. 09DZ2271800).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.05.019.

References

Arya, S.K., Pandey, P., Singh, S.P., Datta, M., Malhotra, B.D., 2007. Analyst 132, 1005–1009.
Bahshi, L., Frasconi, M., Tel-Vered, R., Yehezkeli, O., Willner, I., 2008. Anal. Chem. 80, 8253–8259.
Cady, S.G., Sono, M., 1991. Arch. Biochem. Biophys. 291, 326–333.
Centi, S., Tombelli, S., Minunni, M., Mascini, M., 2007. Anal. Chem. 79, 1466–1473.
Curti, A., Trabanelli, S., Salvestrini, V., Baccarani, M., Lemoli, R.M., 2009. Blood 113, 2394–2401.
Darder, M., Takada, K., Pariente, F., Lorenzo, E., Abrua, H.D., 1999. Anal. Chem. 71, 5530–5537.
de la Escosura-Muniz, A., Gonzalez-Garcia, M.B., Costa-Garcia, A., 2004. Anal. Chim. Acta 524, 355–363.
Durocher, S., Rezaee, A., Hamm, C., Rangan, C., Mittler, S., Mutus, B., 2009. J. Am. Chem. Soc. 131, 2475–2477.
Karam, P., Halaoui, L.I., 2008. Anal. Chem. 80, 5441–5448.
Katz, E., 1990. J. Electroanal. Chem. 291, 257–260.
Katz, J.B., Muller, A.J., Prendergast, G.C., 2008. Immunol. Rev. 222, 206–221.
Li, T., Du, Y., Wang, E.K., 2008. Chem. Asian J. 3, 1942–1948.
Matin, A., Streete, I.M., Jamie, I.M., Truscott, R.J.W., Jamie, J.F., 2006. Anal. Biochem. 349, 96–102.
Mellor, A.L., Munn, D.H., 2004. Nat. Rev. Immunol. 4, 762–774.
Miao, P., Liu, L., Nie, Y.J., Li, G.X., 2009. Biosens. Bioelectron. 24, 3347–3351.
Muller, A.J., Scherle, P.A., 2006. Nat. Rev. Cancer 6, 613–625.
Munn, D.H., Mellor, A.L., 2007. J. Clin. Invest. 117, 1147–1154.
Munn, D.H., Sharma, M.D., Lee, J.R., Jhaver, K.G., Johnson, T.S., Keskin, D.B., Marshall, B., Chandler, P., Antonia, S.J., Burgess, R., Slingluff Jr., C.L., Mellor, A.L., 2002. Science 297, 1867–1870.
Niazov, T., Shlyahovsky, B., Willner, I., 2007. J. Am. Chem. Soc. 129, 6374–6375.
Ozaki, Y., Borden, E.C., Smalley, R.V., Brown, P.R., 1991. Adv. Exp. Med. Biol. 294, 547–553.
Polsky, R., Gill, R., Kaganovsky, L., Willner, I., 2006. Anal. Chem. 78, 2268–2271.
Shiddiky, M.J.A., Rahman, M.A., Shim, Y.-B., 2007. Anal. Chem. 79, 6886–6890.
Shiddiky, M.J.A., Rahman, M.A., Cheol, C.S., Shim, Y.-B., 2008. Anal. Biochem. 379, 170–175.
Sono, M., Roach, M.P., Coulter, E.D., Dawson, J.H., 1996. Chem. Rev. 96, 2841–2887.
Takikawa, O., 2005. Biochem. Biophys. Res. Commun. 338, 12–19.
Takikawa, O., Kuroiwa, T., Yamazaki, F., Kido, R., 1988. J. Biol. Chem. 263, 2041–2048.
Uyttenhove, C., Pilotte, L., Theate, I., Stroobant, V., Colau, D., Parmentier, N., Boon, T., Van den Eynde, B.J., 2003. Nat. Med. 9, 1269–1274.
Yildiz, H.B., Freeman, R., Gill, R., Willner, I., 2008. Anal. Chem. 80, 2811–2816.