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Electrochemical biosensor based on enzyme substrate as a linker: Application for aldolase activity with pectin-thionine complex as recognization element and signal amplification probe

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ABSTRACT

A new strategy to fabricate electrochemical biosensor is reported based on the linkage of enzyme substrate, thereby an electrochemical method to detect aldolase activity is established using pectin-thionine complex (PTC) as recognization element and signal probe. The linkage effect of fructose-1,6-bisphosphate (FBP), the substrate of aldolase, can be achieved via its strong binding to magnetic nanoparticles (MNPs)/aminophenylboronic acid (APBA) and the formation of phosphoramidate bond derived from its reaction with *p*-phenylenediamine (PDA) on the surface of electrode. Aldolase can reversibly catalyze the substrates into the products which have no binding capacity with MNPs/APBA, resulting in the exposure of the corresponding binding sites and its subsequent recognization on signal probe. Meanwhile, signal amplification can be accomplished by using the firstly prepared PTC which can bind with MNPs/APBA, and accuracy can be strengthened through magnetic separation. With good precision and accuracy, the established sensor may be extended to other proteins with reversible catalyzed ability.

1. Introduction

For the establishment of electrochemical sensors, the linkers are essential and they can be classified into the several categories: (a) nanoparticles (Wu et al., 2013), (b) organic molecules (Ghanem et al., 2008), (c) DNA (Wang et al., 2015), and (d) polymers (Wang et al., 2011). Compared with these linkers, enzyme substrate has the following advantages for the construction of electrochemical sensors: (1) the usage of enzyme substrates can be avoided of the addition of other molecules, making the detection system simple, so as to ensure the analytical accuracy; (2) the number of enzyme substrates is directly related to the enzyme activity. In this paper, taking aldolase as an example, the biosensor has been fabricated based on enzyme substrate as a linker.

Aldolase is encoded by three different genes and highly expressed in the developing embryo and in adult muscle. High aldolase expression was found significantly associated with lymph node involvement (Chang, 2014) and a variety of malignant cancers including human lung squamous (Poschmann et al., 2009; Rho et al., 2009), renal cell, and hepatocellular carcinomas. Up to now, aldolase activity has been widely evaluated by testing the amount of nicotinamide adenine dinucleotide hydrogen (Flechner et al., 1999) via colorimetric method (Malcolm and Shepherd, 1972; Michelis and Gepstein, 2000; Roe et al., 1949). It is known that absorbance or optical density is easily influenced by various factors such as pH values of reaction solution and some colored substances. Therefore, it is of great importance to develop accurate and inexpensive method to assay the enzyme activity. As a simple and cost-effective technique (Swisher et al., 2013; Yixia et al., 2012), electrochemistry are accurate and sensitive. However, electrochemical method has not been established to determine aldolase activity.

Aldolase is responsible for catalyzing the reversible conversion of fructose-1,6-bisphosphate (FBP) to glyceraldehydes-3-phosphate and dihydroxyacetone phosphate (Du et al., 2014). As a specific substrate, FBP has greatly attracted our attention due to its interesting molecular structure containing 1,3-*cis*-diol and phosphate group. It is well known that the compounds like saccharides, having prearranged *cis*-diols with multiple chiral centers, can reversibly react with boronic acids to produce typically five- or six-

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membered esters (Shoji and Freund, 2002; Yum et al., 2011). Moreover, phosphate group can react with amino group to form phosphoramidate bond (Suginta et al., 2013). So the two groups, *cis*-diol and phosphate acid, provide the basis for FBP as a linker with the double recognition. Different from FBP, there is no *cis*-diol group in the molecular structures of two products, glyceraldehydes-3-phosphate and dihydroxyacetone phosphate. These structural characteristics contribute to the separation of substrate from the reaction mixture. Some interfering substances such as enzyme itself, enzyme buffer, and hydrolyzed product, may have great influence on the analytical accuracy, so it will be a good manner to separate the substrate after enzyme-catalyzed reversible reaction.

Magnetic nanoparticles (MNPs), with unique magnetic separation ability, can simplify the isolation procedure and speed up the assay process (Ma et al., 2013; Salamon et al., 2015). Meanwhile, MNPs with high surface to volume ratio and good biocompatibility, are capable of trapping a great deal of signal probes with *cis*-diol groups, so as to improve the analytical sensitivity (Zhao et al., 2006). As one kind of polysaccharides, pectin containing a great amount of *cis*-diol (Zhang et al., 2014a), can be loaded onto the surface of the functionalized MNPs (Wang et al., 2013; Yum et al., 2011; Zhang et al., 2015). Meanwhile, pectin containing carboxylic group, can covalently links with numerous electroactive compounds, to form the complex through the amide bonds.

In this work, enzyme substrate, FBP, has been utilized as a linker for the construction of electrochemical sensor for the analysis of aldolase activity with pectin/thionine complex (PTC) as recognization element and signal amplification probes. The sensitivity of the sensor can be enhanced by PTC and MNPs. Furthermore, magnetic separation can well improve the anti-interference ability.

2. Experimental

2.1. Materials and reagents

Trisodium citrate, sodium nitrate, *p*-phenylenediamine (PDA), 3-aminophenylboronic acid (APBA), thionine, pectin, 1-(3-dimethlaminopropyl)-3-ethylcarbodimide hydrochloride, and N-hydroxy succinimidepurum (NHS) were purchased from Sigma (Shanghai, China). FBP was obtained from Aladdin (Shanghai, China). Aldolase (EC 4.1.2.13, 10 U/mg, from rabbit muscle) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). $FeSO_4$ and $FeCl_3$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All buffers and aqueous solutions were prepared with ultrapure water which was purified with a Millipore Mill-Q water Purification system (Barnstead, USA) to a specific resistance of 18 MΩ cm.

2.2. Preparation of the functionalized magnetic nanoparticles

Firstly, Fe_3O_4 magnetic nanoparticles (MNPs) were synthesized by a co-precipitation method. In brief, a 5 mL iron solution containing $FeCl_2$ (40 mM), $FeCl_3$ (80 mM), and 0.4 M HCl was prepared. A 50 mL solution of 0.5 M NaOH was purged with nitrogen to remove oxygen and then heated to 80 °C. Under rapid stirring, the iron solution was added drop by drop, followed by the addition of sodium citrate (0.2 M) after 10 min later. The three carboxylate groups of sodium citrate have strong coordination affinity to iron ions, which favors the attachment of citrate groups on the surface of the MNPs and prevents them from aggregating (Liu et al., 2009; Nigam et al., 2011). After 30 min with continuous stirring, the reaction mixture was washed with water three times and isolated by magnetic decantation. The residue was finally made up to 50 mL with doubly distilled water to give MNPs.

For the immobilization of APBA on the surface of MNPs, EDC (0.22 M) and NHS (0.22 M) were added into 20 mL MNPs and ultrasonicated for 30 min. After magnetic separation to remove EDC and NHS, MNPs were dispersed in water followed by the addition of 2 mL ethanol containing 450 mg APBA. After continuous stirring for 3 h, the resulting mixture was separated by magnet and washed three times with ultrapure water and finally dispersed in 20 mL water to obtain APBA modified MNPs (MNPs/APBA).

2.3. Preparation of pectin/thionine complex (PTC)

1 mg/mL of pectin dispersion was prepared by ultrasonication for 10 min at room temperature. After that, 10 mM EDC and 10 mM NHS were added into the pectin dispersion for 30 min. Then, 1 mM thionine was added into the above solution, and the resulting mixture was stirred vigorously for 12 h. Subsequently, 5 mL NaOH (5%) was added into the mixture for 5 min. After centrifugation at 12,000 rpm for 20 min three times, the deposit was redissolved into 18 mL double-distilled water to give pectin/thionine complex (PTC) which was stored in a refrigerator at 4 °C.

2.4. Modification of gold electrode

Gold electrode was sequentially polished with emery paper (No. 3000 and 5000) and alumina slurry (1.0 and 0.3 μm), followed by ultrasonic cleaning in ethanol and ultrapure water. Subsequently, the electrode was cleaned in piranha solution (98% H_2SO_4: 30% H_2O_2=3:1) for 3 min. Afterward, the electrode was washed thoroughly with ultrapure water and dried under nitrogen gas. After that, the electrode was cleaned electrochemically to remove any remaining impurities with 0.5 M H_2SO_4 (Zhang et al., 2014b). Finally, the electrode was dried by purging with nitrogen.

The treated electrodes were firstly modified with PDA by *in situ* diazonium reduction experiments (Lyskawa and Bélanger, 2006; Zhang et al., 2014b). 5 mM PDA was diazotated in an aqueous solution of 500 mM HCl and 5 mM $NaNO_2$. Then the electrode was dipped in the diazonium solution, and cyclic voltammetric experiment was conducted from 0 to −600 mV at a scan rate of 100 mV/s. After the formation of phenyl ring layer, the electrode was fully rinsed with ultrapure water to remove unbound molecules to give PDA modified gold electrode (PDA/AuE).

2.5. Aldolase activity assay

Aldolase activity assay was performed using the following procedure. The aldolase was firstly dissolved in buffer solution (2.5 M $(NH_4)_2SO_4$, 0.01 M Tris, 0.001 M EDTA, pH 7.5) at different concentrations (0.1–1.25 U/L). After that, 100 μL enzyme solution was added into 100 μL FBP solution (0.3 mM) and the resulting mixture was allowed to stand at 25 °C for 30 min. Then, 200 μL MNPs/APBA was added to the mixture at room temperature, followed by magnetic separation after 3 h later, to give MNPs/APBA/FBP. Subsequently, 0.4 M EDC and 0.1 M NHS was added into the mixture to activate phosphate group and then separated by using magnet after 30 min. Finally, the PDA/AuE was dipped in the mixture containing 100 μL MNPs/APBA/FBP and 100 μL PTC for 4 h. After washing thoroughly using ultrapure water, the electrode was used for electrochemical measurements.

2.6. Electrochemical measurements

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a conventional three electrode cell at the room temperature using a CHI 660C electrochemical

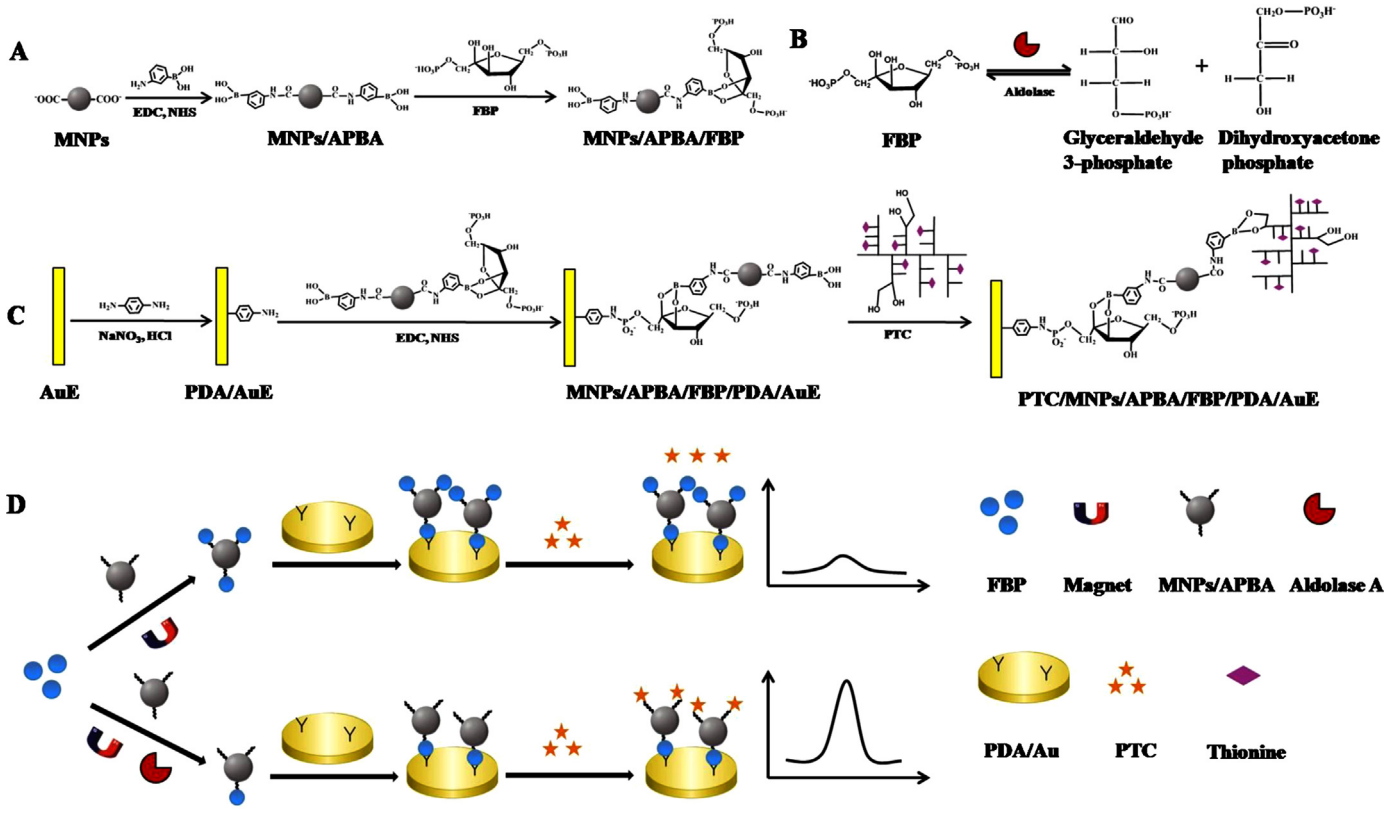
workstation (CH Instruments, Shanghai, China), A modified gold electrode was served as the working electrode, while saturated calomel electrode (SCE) and platinum wire served as the reference and counter electrode, respectively. Cyclic voltammograms were obtained over the potential scan range from −0.5 to 0.1 at a scan rate of 100 mV/s. Differential pulse voltammograms were recorded in the potential scan range from −0.35 to 0.05 with a pulse amplitude of 50 mV and a pulse width of 50 ms in 0.1 M PBS (pH 7.4) at a scan rate of 100 mV/s.

3. Results and discussions

3.1. Mechanism investigation for aldolase activity assay

The principles of the established sensor in this work have been illustrated in Scheme 1. As enzyme substrate, FBP owns the interesting molecular structure containing both 1,2-*cis*-diols and phosphate group. It is well known that boronic acid can react with the molecule containing 1,2-*cis*-diols to form a stable ring esters (Wang et al., 2013; Yum et al., 2011; Zhang et al., 2015). So FBP can be recognized by boronic acid group at the outer layer of MNPs/APBA through the formation of a stable five-member ring ester (Scheme 1(A)). Meanwhile, PDA can be immobilized onto gold electrode surface via electrochemical reduction of *in situ* generated aminophenyl diazoniumcations, and the resultant amino group on the surface of PDA/AuE can react with the phosphate group in the molecular structure of FBP to form phosphoramidate bond (Scheme 1(C)). Therefore, as a linker, FBP can immobilize MNPs/APBA onto the surface of PDA/AuE through the double recognition. Subsequently, the boronic acid groups exposed on the surface of MNPs/APBA/FBP/PDA/AuE can bind with PTC, a signal amplification probe (Scheme 1(C)). MNPs/APBA with high surface to volume ratio and PTC containing numerous thionin molecules, a kind of electroactive substance, can enhance the sensitivity of the electrochemical sensor. As exhibited in Fig. S1, an evidently increasing current response can be observed with MNPs/APBA instead of phenylenediboronic acid. As a key enzyme in glycolysis, aldolase can catalyze the reversible conversion of FBP to glyceraldehydes-3-phosphate and dihydroxyacetone phosphate (Scheme 1(B)) (Du et al., 2014). The two products cannot coordinate with boronic acid groups at the outer layer of MNPs/APBA due to no *cis*-diols species in their molecular structures. Hence after magnetic separation, some boronic acid groups remain on the surface of MNPs/APBA. With the immobilization of MNPs/APBA on the surface of PDA/AuE using FBP as a linker, these boronic acid groups exposed on the surface of MNPs/APBA/FBP/PDA/AuE can coordinate with PTC and the strong signal of peak current can be detected (Scheme 1(D)). Nevertheless, in absence of the enzyme, FBP can avoid to be converted and it can bind with a large proportion of boronic acid groups on the surface of MNPs/APBA. After immobilization of MNPs/APBA on the modified electrode, nearly no remaining boronic groups appear on the surface of MNPs/APBA/FBP/PDA/AuE and PTC cannot bind to the modified electrode surface, leading to the appearance of weak electric signal (Scheme 1(D)). Since the concentration of the enzyme is related to the binding extent of signal probe PTC on the surface of the electrode, electrochemical sensor for monitoring aldolase activity can be developed.

By using enzyme substrate as a linker, the mobilization of MNPs/APBA on the surface of PDA/AuE has been analyzed using cyclic voltammetry. As depicted in Fig. 1(A), no clear redox peaks can be observed in the cyclic voltammograms of PDA/AuE (Fig. 1 (A), curve a). MNPs/APBA/FBP/PDA/AuE also exhibits no redox



Scheme 1. (A) Preparation process of APBA modified MNPs (MNPs/APBA) and recognition of MNPs/APBA on FBP. (B) Aldolase catalyzed hydrolysis of FBP. (C) Mechanism illustration for modification of gold electrode using PDA (PDA/AuE) and the immobilization of MNPs/APBA on the surface of PDA/AuE using FBP as a linker, and binding of signal amplified probe, PTC, to MNPs/APBA/FBP/PDA/AuE. (D) Schematic illustration for the detection of aldolase activity.

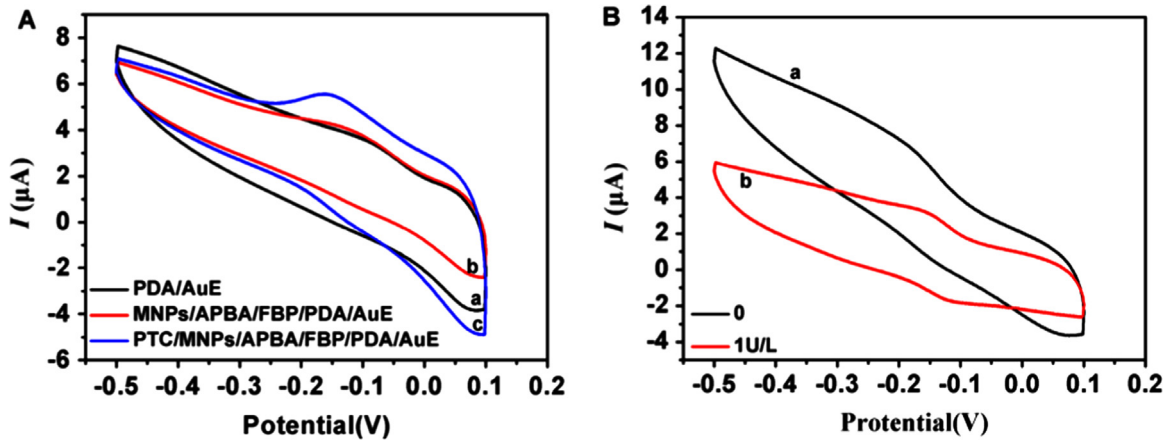


Fig. 1. Cyclic voltammograms (A) obtained using (a) PDA/AuE, (b) MNPs/APBA/FBP/PDA/AuE, and (c) PTC/MNPs/APBA/FBP/PDA/AuE and (B) with different aldolase concentrations at (a) 0 and (b) 1 U/L in 0.1 M PBS (pH 7.4) at a scan rate of 100 mV/s.

peak, owing to inexistence of PTC, i.e., signal probe, on the modified electrode surface (Fig. 1(A), curve b). However, after incubation with PTC, a well-defined redox peak appears in the cyclic voltammogram of PTC/MNPs/APBA/FBP/PDA/AuE (Fig. 1(A), curve c). It demonstrates that FBP is responsible for the immobilization of MNPs/APBA on the surface of PDA/AuE through the formation of a five-member ring ester and phosphoramidate bond (Scheme 1 (C)). Meanwhile, it also implies the successful loading of PTC on the modified electrode surface, owing to the formation of polysaccharide boronate ester (Scheme 1(C)). Moreover, the binding of both FBP and PTC on the surface of modified MNPs can be further confirmed by FT-IR spectra (Fig. S2 in the Supporting Information). Compared MNPs/APBA with MNPs/APBA/FBP, the C=O vibration absorption peak shows a slight red shift from 1756 cm^{-1} to 1750 cm^{-1}, due to the effect of electron donor of FBP moieties at the outer layer of MNPs/APBA. After the addition of PTC, a red shift of the C=O absorption peak can also be detected from 1756 cm^{-1} to 1601 cm^{-1}, suggesting the successful linkage of PTC with MNPs/APBA.

The influences of aldolase activity on current values have been further investigated and the corresponding results are shown in Fig. 1(B). In absence of enzyme, almost no redox peak current can be detected (Fig. 1(B), curve a), implying that the number of signal probe, PTC, is rare on the surface of the modified electrode. It can be explained that almost all boronic acid groups on the surface of MNPs/APBA bind with FBP so that PTC cannot be loaded onto the modified electrode surface. In contrast, a well-defined redox peak appears in the presence of the enzyme (Fig. 1(B), curve b). Aldolase can catalyze the reversible conversion of FBP, leading to the decreasing number of FBP in the reaction system and free boronic acid group remaining at the surface of MNPs/APBA. So PTC can be linked onto the modified electrode surface so as to give the obvious peak current. These are in accordance with our prospective results.

In a word, the experimental data described provide evidence that all necessary conditions for successful implementation of the design of electrochemical sensor are met and the fabricated biosensor can be used for the detection of aldolase activity.

3.2. Electrochemical characterization of modified electrode surface

The gold electrode is directly modified with aminophenyl groups through one-step electrochemical reduction of *in situ* generated aminophenyl diazoniumcations (Baranton and Bélanger, 2005; Lyskawa and Bélanger, 2006). As exhibited in Fig. 2 (A), a well-defined reduction peak (the first scan) can be assigned to the formation of the aryl radical (Lyskawa and Bélanger, 2006). The second and third cycles show lower currents, in accordance with the passivation of the modified electrode owing to the formation of a grafted layer (Fig. 2 (B), curve b).

The changes of the modified electrode surface are further investigated using electrochemical impedance spectroscopy (EIS) which allows an insight into chemical transformation and processes associated with the electrode surface. In the Nyquist

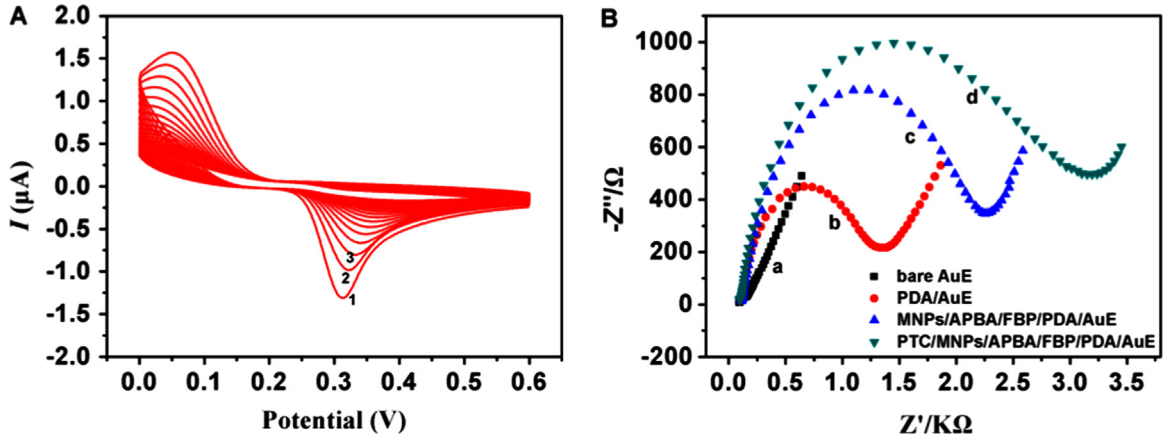


Fig. 2. (A) Cyclic voltammograms of 5 mM PDA in an aqueous solution of 500 mM HCl and 5 mM $NaNO_2$. Scanning rate: 100 mV/s. (B) Nyquist diagrams for the electrochemical impedance measurements of the AuE at different modification stages: (a) bare AuE, (b) PDA/AuE, (c) MNPs/APBA/FBP/PDA/AuE, and (d) PTC/MNPs/APBA/FBP/PDA/AuE. Electrochemical species: 5.0 mM $[Fe(CN)_6]^{3-/4-}$, Biasing potential: 0.224 V, Amplitude: 5 mV, and Frequency range: 0.1 Hz – 10 kHz.

diagram of EIS, the increasing diameter of the semicircle portion equals the increasing electron-transfer resistance (R_{et}). As exhibited in Fig. 2(B), nearly no semicircle can be observed in the EIS of the bare gold electrode (Fig. 2(B), curve a). After electrochemical modification of PDA, a semicircle can be detected (Fig. 2(B), curve b), suggesting the increase of the electron-transfer resistance. It can be explained for the insulating nature of PDA. With the further modification of MNPs/APBA/FBP on the electrode surface through phosphoramidate bond, a further increase of R_{et} can be induced (Fig. 2(B), curve c). On the one hand, it can be attributed to the repulsive interaction between the negatively charged probe and the negatively polarized phosphoric acid group of FBP. On the other hand, the insulating nature of APBA and FBP contributes to the blocking effect on electron transfer. When the signal probes are loaded on the electrode surface, a largest semicircle can be detected (Fig. 2(B), curve d), due to the reason that PTC with high molecular weight forms big barrier to prevent the redox probe from getting access to the electrode surface.

3.3. Characterization of MNPs/APBA and PTC

MNPs/APBA and PTC are identified using FT-IR spectroscopy and the corresponding results are shown in Fig. S3. In comparison with MNPs, MNPs/APBA shows a new absorption peak at 1757 cm^{-1} and a new set of absorption peaks from 1600 cm^{-1} to 1400 cm^{-1}, belonging to C=O stretching vibration and aromatic skeletal vibration (Fig. S3(A)). Moreover, as exhibited in Fig. S3(B), the spectrum of PTC also exhibits two peaks at 1702 and 1566 cm^{-1}, which can also be attributed to the C=O stretching and the aromatic skeletal vibration, respectively. These suggest the successful preparation of MNPs/APBA and PTC.

3.4. Optimization of experimental conditions

As a linker, FBP concentration and its binding pH value and time with MNPs/APBA play key roles on the efficiency of the established electrochemical sensor. A small amount of FBP can lead to the linkage of a small quantity of MNPs/APBA on the surface of modified electrode and the declined current values. Inversely, a great deal of FBP can hinder the recognition between MNPs/APBA and signal probe, resulting in weak current response. As exhibited in Fig. S4(A), (B), and (C), 0.3 mM, 9.2 and 3 h have been separately chosen as the optimal FBP concentration and pH value and time of binding between MNPs/APBA and FBP, in terms of sensitivity and reproducibility (*RSD*=0.75%, 2.1%, and 0.35%, respectively).

Meanwhile, the time of phosphoramidate bond formation influences the loading amount of MNPs/APBA onto the electrode surface. As shown in Fig. S4(D), the current values maximize when 4 h is used with good reproducibility (*RSD*=1.8%). Therefore, for the established electrochemical sensor, the modified electrode is dipped in MNPs/APBA/FBP dispersion liquid for 4 h.

3.5. Selective assay of electrochemical sensor

Different samples are used to investigate the specificity of the current electrochemical sensor. As shown in Fig. 3, a high signal is obtained only when the specific enzyme (aldolase) is tested, whereas the signal was negligible in the presence of five interfering agents, indicating that the fabricated biosensor owns high specificity.

3.6. Stability of electrochemical sensor

The stability of the biosensor has been evaluated by recording the current values with 1 U/L aldolase. The biosensors have been stored under dry conditions at 4 °C over 14 days. After 7 days, the biosensor retains 97.42 ± 0.95% of its original current. The value further decreases to 96.23 ± 1.02% after 14 days. Good long-term stability of the biosensor can be attributed to the covalent linkage effect of substrate FBP between MNPs/APBA and PDA/AuE.

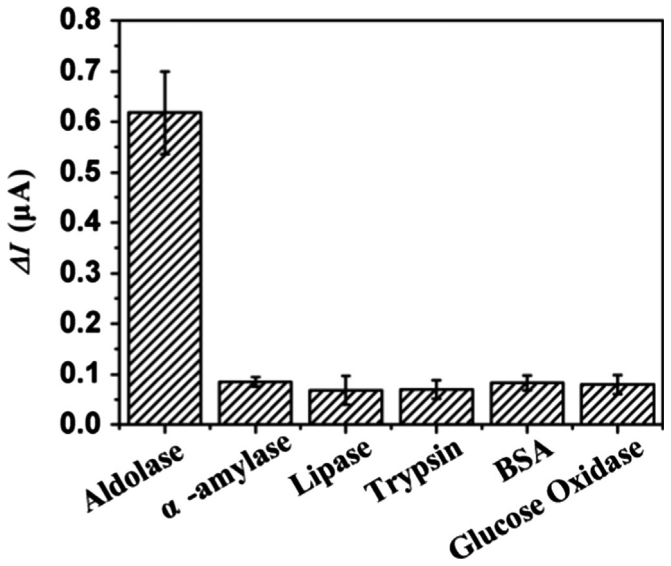


Fig. 3. The specificity investigation of electrochemical sensor against the different samples: α-amylase (1 U/L), lipase (1 U/L), trypsin (1 U/L), BSA (1 U/L), and glucose oxidase (1 U/L). Error bars are obtained based on three independent measurements.

3.7. Electrochemical detection of aldolase activity

Fig. 4(A) shows the differential pulse voltammograms upon analyzing different concentrations of aldolase. It can be well found that the current value increases along with the increase of aldolase concentrations. Aldolase can catalyze the reversible conversion of FBP to glyceraldehydes-3-phosphate and dihydroxyacetone phosphate. Different from FBP, the products cannot bind onto the outer layer of MNPs/APBA due to no *cis*-diol groups in their molecule structure. Hence, the increase of enzymes will result in the increased exposure of boronic acid on the surface of MNPs/APBA, which will undoubtedly benefit the binding of the increasing PTC, so as to give large current response.

The peak current difference (ΔI) between with enzyme and without enzyme has been used for the quantitative detection of aldolase activity, and the values increase linearly with the increase of the aldolase concentrations, corresponding to incremental immobilization of PTC on the surface of the modified electrode (Fig. 4(B)). The values exhibit a linear response towards aldolase within a concentration range from 0.1 to 1.25 U/L, wider than the competitive ELISA method (Shu et al., 1996), and follow the regression equation of $\Delta I = 0.25703 + 0.39145\ C$ (U/L, $R^2 = 0.99865$). The detection limit is calculated to be 0.01 U/L (3 times signal to noise ratio), which is lower than previous reports (Shu et al., 1996). For enzyme concentrations from 0.1 to 1.25 U/L, the electrochemical measurement has been repeated for at least three times independently. The *RSD* of the three slopes is 5.5%, indicating that the precision and reproducibility of the established electrochemical sensor are acceptable.

3.8. Aldolase activity assay in the biological fluids

To further demonstrate the application of the method in biological fluids, a certain amount of aldolase is added into fetal calf serum and the concentration of aldolase in the serum has been measured with our fabricated biosensor. As seen in Table 1, the

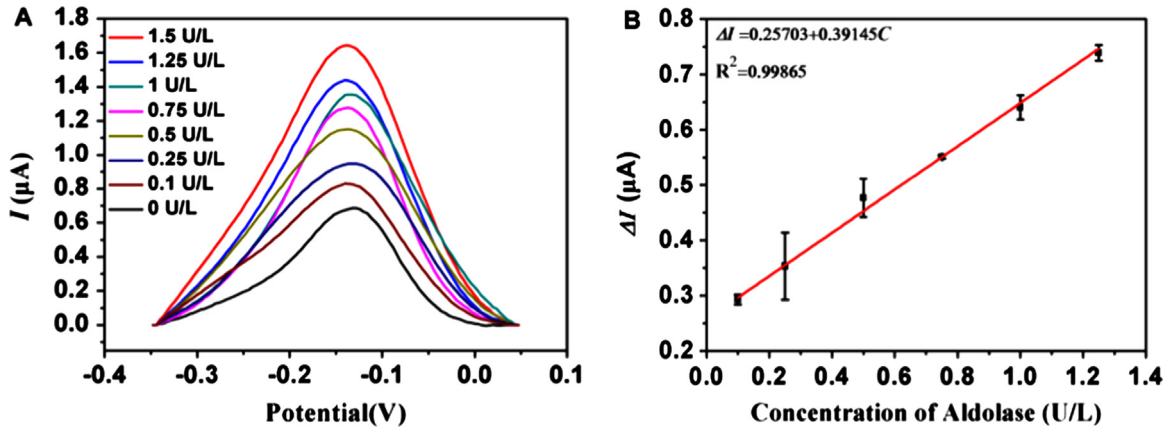


Fig. 4. (A) Differential pulse voltammograms for the aldolase activity at different concentrations: from 0 U/L to 1.5 U/L. (B) The linear relationship between peak current values and the aldolase concentrations.

Table 1
Aldolase concentrations detected by our method and the comparison with given concentrations in serum samples.

Samples	Aldolase concentration detected by our method (U/L)	Standard concentration (U/L)	Relative error (%)
1	0.467 ± 0.029	0.5	7.3
2	0.767 ± 0.048	0.75	2.3
3	0.961 ± 0.053	1.00	3.9
4	1.219 ± 0.061	1.25	2.4

relative errors are basically within 10% (average *RSD*=5.8%), which promises that this sensor might be utilizable for the detection of aldolase in biological fluids such as serum.

4. Conclusions

In summary, this work has introduced a new strategy for the biosensor fabrication based on the linkage of substrate. The established electrochemical sensor has been successful used for the determination of aldolase activity in serum sample. In comparison with the conventional method in which triosephosphate isomerase and glycerol-3-phosphate dehydrogenase are used, the established method is avoid of the usage of expensive enzymes. Moreover, for the conventional method, the NADH dependent absorbance change at 340 nm was determined. It is well known that the absorbance change is always influenced by various factors. In our method, magnetic separation can eliminate the influence of interferences in the mixture after enzyme hydrolysis, so as to improve the analytical accuracy. With the advantages in terms of its low technical and instrumental demands, good selectivity and accuracy, this biosensor has a great potential in the clinical detection of aldolase activity.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.04.009.

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