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A label-free electrochemical aptasensor based on the catalysis of manganese porphyrins for detection of thrombin

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ABSTRACT

A novel manganese porphyrin (MnPP)-catalyzed aerobic oxidation of L-cysteine to disulfides (RSSR) was firstly found and applied into electrochemical aptasensor with a label-free technique for signal amplification. The possible catalytic mechanism of the catalytic reaction where MnPP catalyzed L-cysteine with thiol (RSH) structure to RSSR was discussed in detail. For fabrication of the aptasensor, thionine (Thi), which served as an electron mediator, was mixed with MnPP and immobilized on the nafion coated carbon electrode through ion exchange adsorption. Gold nanoparticle (nano-Au) was assembled on the Thi for immobilizing thrombin binding aptamer (TBA). In the presence of thrombin (TB), TBA will capture TB and form TBA–TB composite thus perturbed electron transfer, leading to decrease of the current for quantitatively detecting TB. Under optimal condition, the electrochemical aptasensor exhibited a linear range of 0.1-25 nM with a detection limit of 0.02 nM. This work opens a novel way for signal amplification study about porphyrins that served as mimetic enzyme to thiol in electrochemical aptasensor.

1. Introduction

Mimetic enzymes are classes of non-protein catalyst made by organic chemistry methods (Chen and Corn, 2011). Compared to traditional natural enzymes, mimetic enzymes with their unique advantages including simple structure, stable chemical properties, simple synthesis and high efficiency of catalysis, have attracted substantial research efforts (Wang et al., 2011; Xiao et al., 2007; Sando et al., 2003). So far, various kinds of mimetic enzymes have been synthesized, such as crown ether and cyclodextrin mimetic enzymes, imprinting polymer mimetic enzymes and porphyrin mimetic enzymes. Among these enzymes, porphyrin mimetic enzymes with their superior biocompatibility, high catalyst efficiency and low production cost have been wildly applied for fabricating enzyme-amplified bioaffinity electrodes (Chen et al., 2009; Collman et al., 2004; Xu et al., 2011; Cong et al., 2012).

Manganese porphyrin (MnPP) is a kind of porphyrin derivatives composed by manganese (Bakker and Qin, 2006; Bakker et al., 1994; Groves, 2000). In view of its excellent properties, such as superior biocompatibility and high catalytic efficiency, MnPP has been wildly used as mimetic enzyme for constructing electrochemical aptasensor (Motsenbocker et al., 1993). In the catalytic reaction where MnPP was a mimetic enzyme, the most frequently used catalytic substrate is H_2O_2 (Jie et al., 2013; Yuan et al., 2013; Saeed and Elaheh, 2014; Agnieszka et al., 2013; Wen et al., 2014). However, the unstable chemical property of H_2O_2 restricts its application (Salimi et al., 2007; Sang et al., 2010; Jing et al., 2013a,b). So, the urgent need is to find a stable catalytic substrate. L-cysteine is a non-essential amino acid with stable chemical property and superior biocompatibility (Yaotong et al., 2010; Raoof et al., 2007). Herein, we for the first time tried to utilize L-cysteine as a catalytic substrate for MnPP in electrochemical aptasensor for signal amplification. Through repeated experiments, we found and confirmed that MnPP possess superior electrocatalytic activity for L-cysteine. The novel catalytic reaction exhibits excellent catalytic efficiency and operability. Furthermore, the suggested mechanism that MnPP catalyzed L-cysteine with thiol structure to disulfides was also discussed in detail. The fascinating catalytic reaction system that provided novel research direction for subsequent study about porphyrins served as mimetic enzymes for thiol in enzyme-amplified bioaffinity electrodes.

Herein, we combined the novel electrochemical catalyst with the label-free technique for constructing an electrochemical aptasensor for detection of thrombin (TB). Nafion, a perfluorisalfonated cation-exchange polymer (Hui-Bog et al., 2014), was modified on the electrode surface for immobilizing the electron mediator thionine (Thi) and catalyst MnPP. Through the help of gold nanoparticle (nano-Au), thrombin binding aptamer (TBA) was assembled on the electrode. Thrombin (TB), an extracellular

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serine protease involved in the blood coagulation cascade, was served as a target model. The formation of TBA–TB composite could perturb the interfacial electron transference which can decrease the current. Based on the linearity between the current and TB concentration, we could quantitatively detect TB.

2. Experimental

2.1. Chemicals and materials

Thrombin (TB), nafion, thionine (Thi), gold chloride ($HAuCl_4$), sodium citrate, bovine serum albumin (BSA) and hemoglobin (Hb) were purchased from Sigma Chemical Co. (St. Louis. Mo, USA). L-cysteine was purchased from Kangan Amino Acid Company (Shanghai, China). Manganese prophyrin (MnPP) purchased from J&K Chemical Ltd. Gold nanoparticles (nano-Au) with 16 nm diameter were prepared according to the literature (Jing et al., 2013a,b; Enustun and Turkevich, 1963). Thrombin binding aptamer (TBA): 5′-SH–$(CH_2)_6$–GGT TGG TGT GGT TGG-3′ was obtained from Sangon Biotech (Chongqin, China). 20 mM Tris–HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ was used to prepare aptamer solutions. 0.1 M phosphate buffer solutions (PBS) (pH 7.0) containing 10 mM KCl, 2 mM $MgCl_2$ was used throughout the experiment. $[Fe(CN)_6]^{3-/4-}$ solution (pH 7.4, 5.0 mM) was obtained from dissolving potassium ferricyanide and potassium ferrocyanide with PBS (pH 7.4). Ultrapure water was used throughout the experiment. All other chemicals were of reagent grade and used as received.

2.2. Instrumentation

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out with a CHI 660D electrochemical workstation (Shanghai Chenhua Instrument, China). The pH measurements were made by pH meter (MP 230, Mettler Toledo, Switzerland). The electrochemical system consisted of a three-electrode system: a modified glassy carbon electrode (GCE, ϕ=4 mm) as a working electrode, a platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode.

2.3. Fabrication of the modified electrode

The fabrication procedure for the proposed aptasensor was depicted in Scheme 1. Prior to use, the GCE was polished by alumina powder, followed by rinsing thoroughly by ultrapure water after each polishing step. Then the GCE was sonicated in ethanol and ultrapure water. Then the GCE was dried at room temperature to obtain a mirror-like surface. 4 μL nafion ethanol solution (v/v 2%) was dropped onto the electron surface and dried in air. Then 20 μL mixture solution which contained Thi and MnPP (6 μM) with volume ratio of 1:3 was dropped on the modified electrode surface for 20 min. Next, 20 μL nano-Au was dropped on the electrode surface for 6 h to form a nano-Au monolayer. Subsequently, 20 μL TBA with 2.5 μM was dropped on the electrode for 16 h at room temperature. Then 20 μL BSA (1 wt%) was dropped on the modified electrode for 40 min to eliminate the nonspecific binding effects and block the remaining active groups.

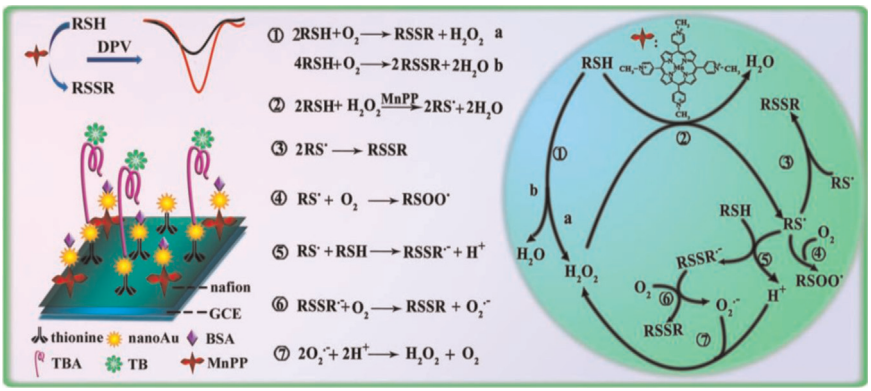
2.4. Measurement procedure

The CV measurements were performed under the condition that the potential ranged from −0.2 V to 0.6 V in 0.10 M PBS (pH 7.0) with the scan rate of 100 mV/s. The DPV measurement conditions were: potential was ranged from −0.5 V to 0 V, modulation amplitude was 0.05 V, pulse width was 0.06 s and the sample was 0.02 s.

3. Results and discussion

3.1. The suggested mechanism of the novel catalytic procedure

As described above, the amplification electrochemical signal was based on the catalysis of MnPP to L-cysteine. Herein, suggested mechanism of this novel catalytic procedure should be discussed. As shown in Scheme 1, for primary step of the reaction (eq. ①), under inefficient noncatalyzed oxidation reaction, L-cysteine with thiol structure (RSH) was converted to disulfide (RSSR) by O_2, accompanied with formation of trace amount of H_2O_2 (eq. a) or H_2O (eq. b). Then, with the help of the generated H_2O_2, MnPP-catalyzed oxidation of thiol could be promoted. Such reaction could produce thiol radical (RS.) with high reaction activity (eq. ②). There were three reaction pathways for thiol radical: (1) two thiol radicals could combine to produce RSSR (eq. ③); (2) thiol radical can react with O_2 to produce RSOO. (eq. ④); (3) however, the major and extremely important reaction is that thiol radical can serve as intermediator that autocatalyze the formation of H_2O_2 (eq. ⑤, ⑥, ⑦). The autonomously regenerated H_2O_2 not only provided a feedback cycle for continuously producing H_2O_2, but also made it possible for continuously catalyzed formation of RSSH. In conclusion, the noncatalyzed formation procedure leads to generation of trace amount of H_2O_2 which activated the MnPP-catalyzed formation of thiol radicals that ultimately initiate the amplification cycle to induce the continuous formation of H_2O_2 and RSSR.



Scheme 1. Schematic diagram for fabrication of electrochemical aptasensor and suggested reaction mechanism of the catalytic reaction.