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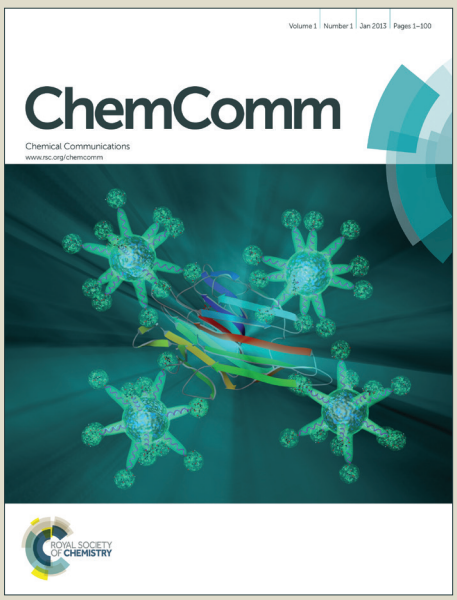

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ARTICLE TYPE

Target protein induced cleavage of a specific peptide for prostate-specific antigen detection with positively charged gold nanoparticles as signal enhancer

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In this work, a new "signal-on" electrochemical biosensor for sensitive detection of prostate-specific antigen (PSA) was developed on the basis of target protein induced cleavage of a specific peptide with positively charged gold nanoparticles (AuNPs) as signal enhancer.

Development of a sensitive, rapid and selective method for the assay of tumor marker is of great significance in early detection and monitoring recurrence of cancers[1]. Therefore, the study of new analytical protocols for tumor marker assay is receiving more and more interests. Prostate-specific antigen (PSA), a kind of serine protease, which is secreted by both normal prostate glandular cells and prostate cancer cells, has been recognized as the most reliable tumor marker for prostate cancer in clinical testing for males[2]. Until now, a variety of methods including surface plasmon resonance[3], fluorescence[4], electrochemical and electrogenerated chemiluminescence (ECL) immunoassay[5-7] have been developed to determine PSA. Among those different techniques, electrochemical immunoassay has received a particular attention for its inherent advantages, including simple instrumentation and fast response time[8,9]. However, current electrochemical immunoassays mainly depend on antibodies as recognition element, which have some fundamental shortcomings for protein analysis, such as the difficulty in site-specific bioconjugation, high cost and high time consumption[10-13].

Recently, specific peptides which are selected using the phage display technique, have been developed as a more compelling choice than antibodies in bioassays due to their advantages, such as being stable, reliable, cost-effective, resistant to harsh environments, and prone to engineering at the molecular level[14]. Denmeade et al. reported that a specific peptide with the amino acid sequence HSSKLQ could be hydrolyzed and cleaved by PSA protease with excellent stability and high specificity[15]. Then several peptide-based electrochemical biosensors had been developed for the PSA detection[16,17]. However, these biosensors usually are "signal-off" based on the cleavage of signal reporter, suffering from the problems of limited signal output and "false positivity". Thus, it is necessary to develop a more sensitive and reliable "signal-on" approach for the determination of PSA.

In order to achieve sensitive detection of proteins, a variety of signal amplification strategies have been developed by employing appropriate labels, including metal nanoparticles[18,19], magnetic particles[20,21], and enzyme label[22,23]. Among these approaches, metal nanoparticles have been widely used as sensitive labels for analytical signal amplification due to their unique properties[24,25], for example, it can overcome some of the problems associated with the inherent instability of biological materials such as enzymes. And gold nanoparticales (AuNPs) is the remarkable one. Nie et al.[26] reported an ultrasensitive impedimetric biosensor based on enlargement of surface-charged AuNPs, which provided a simple and general model for the signal amplification.

Inspired by the above mentioned information, herein, a "signal-on" electrochemical biosensor based on target protein induced cleavage of a specific peptide with positively charged AuNPs as signal enhancer was developed to determine PSA. The peptide served as a molecular recognition element to be firstly immobilized on the dithiobis (succinimidyl propionate) (DSP) modified electrode. AuNPs were subsequently linked on the peptide and further integrated with positively charged surfactant cetyltrimethlammonium bromide (CTAB) to make AuNPs capped with positive charge, which could adsorb the negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$, leading to a low value of electro-transfer resistance. In the presence of PSA, the serine protease would specifically hydrolyse and cleave the peptide modified with positively charged AuNPs, hence the positively charged AuNPs were released from the electrode surface. Subsequently, a high electro-transfer resistance was obtained. And the change of electro-transfer resistance directly relied on the concentration of target PSA. In this work, a "signal-on" approach combinated with a signal amplification strategy by employing positively charged AuNPs as signal enhancer, exhibiting high sensitivity and reliability, which also provided a promising strategy for the detection of other proteins. The schematic diagram of stepwise assembles procedure of the biosensor was shown in Scheme 1 (See ESI for experimental details).

To efficiently characterize the interface properties of the modified electrode, CV experiments were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. As shown in Fig. 1A, a well-defined redox peak of $[Fe(CN)_6]^{3-/4-}$ was observed at the bare electrode (curve a). Curve b showed the electrodeposition of AuNPs (depAu) on the GCE, the peak current was increased, indicating that the depAu could promote the electron transfer. After DSP, BSA and peptide were immobilized onto the depAu layer, a

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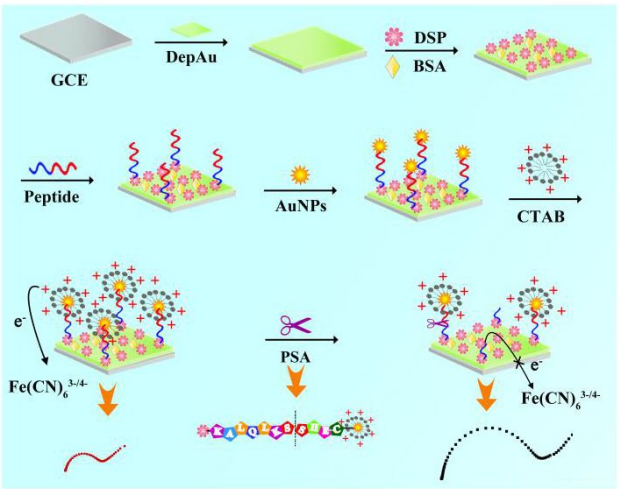
successive decrease of the current (c-e) was detected. This decrease could be attributed to the non-conductive properties of above substances, which obstructed the electron transfer. After incubating with AuNPs, an increased peak current was observed (curve f), indicating that the AuNPs could promote the electron transfer. Following that, the peak current further increased (curve g) after the modified electrode being treated in positively charged CTAB solution, owing to the redox probe $[Fe(CN)_6]^{3-/4-}$ was closer to electrode surface via electrostatic adsorption. Finally, with the presence of PSA antigen, a clearly decrease of peak current was acquired (curve h), indicating the specific peptide was cleaved by PSA successfully. To further confirm the assemble process of the biosensor, electrochemical impedance spectroscopy was employed to monitor the fabrication process of modified electrode (See ESI).



Scheme 1 Schematic diagram of the fabrication process of biosensor for the detection of PSA.

To testify the amplification property of positively charged AuNPs, we conducted a contrast experiment. As shown in Fig. 1B, in the absence of positively charged AuNPs, the electron-transfer resistance (R_{et}) of peptide/BSA/DSP/depAu/GCE was small (R_{et0} = 218.7 Ω , curve a). After incubation of the peptide/BSA/DSP/depAu/GCE with PSA, the PSA cleaved peptide specifically, which resulted in the slight decrease of resistance (R_{et1} = 183.2Ω, curve b). And the response change ΔR_{et} for the electrode was -35.5Ω . Comparatively there appeared a small resistance at the positively charged AuNPs/ peptide/ BSA/DSP/depAu/GCE (R'_{et0} = 34.15Ω, curve c), owing to the electrostatic absorption of positively charged AuNPs towards negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$. Similarly, in the presence of PSA, PSA cleaved peptide with positively charged AuNPs specifically, the resistance thus largely increased (R'_{et1} = 344.9Ω, curve d), and the response change $\Delta R'_{et}$ was 310.75Ω. From the experimental data, we can see that the positively charged AuNPs in this study played a very important role in signal amplification. Moreover, a "signal-on" electrochemical biosensor was successfully constructed to detect PSA with a high sensitivity.

To assess the analytical performance of the proposed biosensor, under the optimal conditions (See ESI), the proposed biosensor was incubated with various concentrations of PSA standard solution and the corresponding resistances were tested in the $[Fe(CN)_6]^{3-/4-}$ solution. Clearly seen from Fig. 2A, the EIS response increased with the increasing concentration of PSA. Fig. 2B showed the corresponding calibration curve of proposed biosensor, where the EIS response was linear with the concentration of PSA ranging from 0.2 pg·mL^{-1} to 45 ng·mL^{-1} The linear regression equation was R_{et} (Ω) = 6.5592 c_{PSA} + 68.996 with a correlation coefficient of 0.9918. And the detection limit was 0.06 pg·mL^{-1} which estimated by 3σ (where σ is the relative standard deviation of a blank solution, n =11). These results demonstrated that the amplification based on positively charged AuNPs as signal enhancer effectively improved the sensitivity, implying that the biosensor provided a promising method to quantitatively detect PSA.

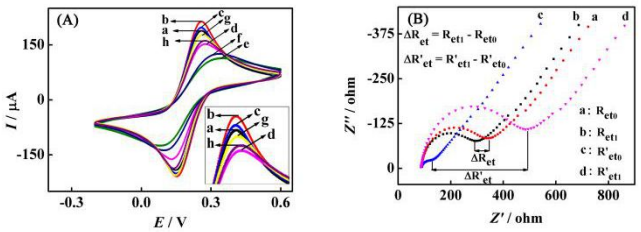


Fig. 1 The electrochemical characterization of the stepwise modified electrodes: (A) CV of bare GCE (a); depAu/GCE (b); DSP/depAu/GCE (c); BSA/DSP/depAu/GCE(d); peptide/BSA/DSP/depAu/GCE (e); AuNPs/peptide/BSA/DSP/depAu/GCE(f); positively charged AuNPs/ peptide/BSA/DSP/depAu/GCE (g); positively charged AuNPs/ peptide/ BSA/DSP/depAu/GCE after being incubated with PSA (1 ng·mL^{-1}) (h) in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. (B) The EIS response of peptide/ BSA/DSP/depAu/GCE (curve a) and electrode after incubation with PSA (30 ng·mL^{-1}) (curve b); the EIS response of positively charged AuNPs/peptide/BSA/DSP/depAu/GCE (curve c) and electrode after incubation with PSA (30 ng·mL^{-1}) (curve d).

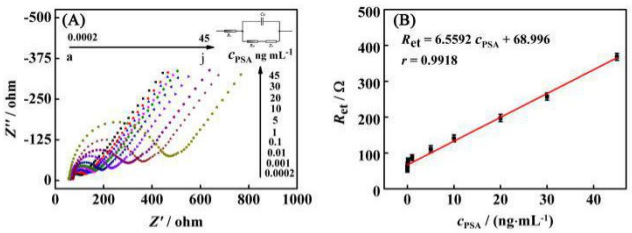


Fig. 2 (A) Nyquist diagrams for the biosensor toward various concentrations of PSA standard solution (from a to j) and (B) the related calibration curves of EIS responses *vs* PSA concentration.

Selectivity is an important criterion for a successful assay system in protein detection. In order to evaluate the selectivity of the developed biosensor for PSA detection, the several interferences: hemoglobin (Hb), bovine serum albumin (BSA), carcinoembryonic antigen (CEA) and human apolipoprotein A-1 (APO-A1) were investigated by detecting the EIS response of the electrode under the same experimental environment respectively. In Fig. 3, a great increase in the EIS response was observed for 1ng·mL^{-1} PSA, while very slight changes in the EIS responses were found for the other tested proteins including Hb (10 ng·mL^{-1}), BSA (10 ng·mL^{-1}) APO-A1 (10 ng·mL^{-1}) and CEA (10 ng·mL^{-1}), respectively. Moreover, the cross-sensitivity of the biosensor in a mixture of the four different cancer biomarkers containing PSA was also examined. The EIS response from the mixture was almost the same as the one obtained from the pure PSA solution. These experimental results indicated that the developed strategy could be used to detect PSA with high

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specificity.

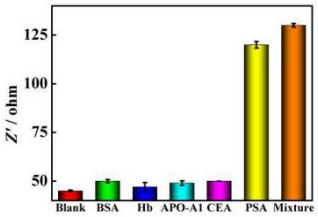

Fig. 3 Specificity study of the proposed biosensor for PSA detection.

In order to test the applicability of the proposed biosensor in real samples, recovery experiments were investigated by standard addition method. Herein, a series of various concentrations of PSA were added into 20-fold-diluted human real serum samples (obtained from the Third Military Medical University of Chongqing, China). As indicated from Table 1 (See ESI), the recoveries were ranging from 94.32% to 102.67% with the relative standard deviation values (RSD) ranging from 2.32% to 5.67%. High recoveries and acceptable RSD indicated the potentiality of this method for PSA detection in clinical applications.

The proposed method was also implemented in the analysis of clinical serum samples. Herein, three clinical serum samples were analyzed by the proposed biosensor for PSA. Prior to the analysis, all of the samples were gently shaken for 5 min at room temperature and then were measured without dilution. The assay results were listed in Table 2 (See ESI). The obtained results showed an acceptable agreement with the data provided by the Third Military Medical University using a standard chemiluminescence (CL) method with an ECLIA-II Chemiluminescence Immunoanalyzer, therefore, indicating a possibility in clinical application.

In this work, a "signal-on" electrochemical biosensor for determination of PSA on the basis of target PSA induced cleavage of a specific peptide was successfully fabricated. Highly sensitive detection of PSA down to 0.2 $pg \cdot mL^{-1}$ was attained based on three advantages of the biosensor: (1) a signal amplification was developed by employing positively charged AuNPs as signal enhancer; (2) a "signal-on" biosensor which overcame the problem of limited signal output and "false positivity", exhibited high sensitivity and reliability; (3) EIS measurement, one of the most powerful tools for interfacial investigation, exhibited high analytical sensitivity. Besides, the method supported in this study can be successfully extended for the determination of other proteins that display enzymatic cleavage activity.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Experimental section; The electrochemical characterization of the stepwise modified electrode; Optimization of the experimental conditions]. See DOI:10.1039/b000000x/

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