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A simple and sensitive electrochemical immunosensor based on thiol aromatic aldehyde as a substrate for the antibody immobilization

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ABSTRACT

In this work, a novel sulfur-containing compound with aldehyde groups was synthesized, which was used to immobilize antibodies through the covalent bonding of the aldehyde groups with amino groups, in which no additional chemical cross-linker is required. Human IgG was used as a model analyte to fabricate the electrochemical immunosensor. Using the proposed immunosensor, IgG was detected within the range from 0.01 to 25 ng mL^{-1} with a detection limit of 0.003 ng mL^{-1} obtained by 3 S/N. The simple electrochemical immunosensor had a good specificity, stability and reproducibility.

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1. Introduction

Electrochemical immunosensors have received considerable attention due to their high sensitivity, inherent simplicity, rapid detection, miniaturization and low cost [1,2]. One of the most important steps in the development of electrochemical immunosensors with good performance is the antibody immobilization. The immobilization of antibodies on sensing surfaces was mainly based on embedding [3–6] and self-assemble monolayers (SAMs) [7–10]. Embedding method is simple, can improve loading and maintain the activation of antibodies. However, antibodies can leach from sensing surfaces, which results in the decreasing of reproducibility and sensitivity of biosensors. In order to overcome the shortcoming, efforts have been made to immobilize antibodies to transducer surfaces pre-activated with functional films with linkage reagents. Of the functional films available, self-assembled monolayers (SAMs) of sulfur-containing molecules have been widely explored. Unfortunately, sulfur-containing materials with functional groups (carboxylic acid, amine etc.) [11,12] need to be pre-activated with linkage agents (the mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide, glutaraldehyde) [13,14]. This immobilization method works well but the procedures are really trivial and time-consuming in practice. Up to now, there are no sulfur-containing materials which are used to not only modify electrode but also immobilize antibody directly. Thus, it is still important to develop a convenient immobilization method and development new matrices for the fabrication of electrochemical immunosensors.

In this paper, we tried to synthesize a novel sulfur-containing linker with aldehyde groups (thiol aromatic aldehyde, TAA), which was used to immobilizing antibodies through the covalent bonding of the aldehyde groups with amino groups of antibodies, in which no additional chemical cross-linking step is required. Based on the new linker, a typical sandwich-type immunosensor was constructed. It exhibited good selectivity and reproducibility, acceptable sensitivity, which shows potential applications in clinical diagnostics and other fields. The whole process of the immunosensor fabrication is shown in Scheme 1.

2. Experimentals

2.1. Apparatus and reagents

Bovine serum albumin (BSA), human serum albumin (HSA), human IgG (IgG) and goat anti-human IgG were obtained from

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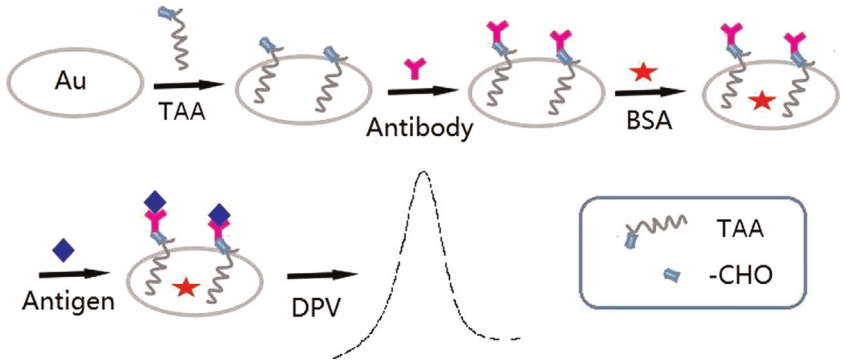
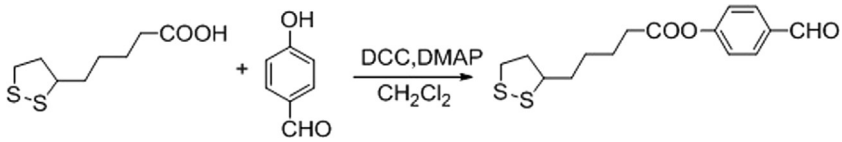


Scheme 1. The whole process of the immunosensor fabrication.



Scheme 2. The synthesis principle of TAA.

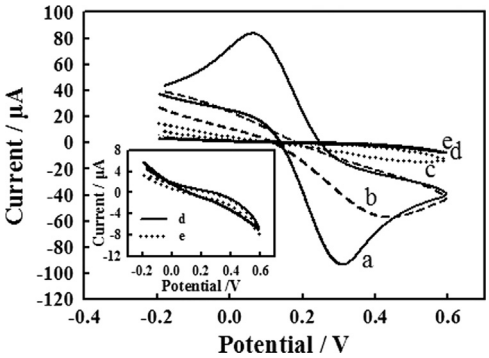


Fig. 1. Cyclic voltammetry profiles of the different modified electrodes: bare Au electrode (curve a), TAA/Au electrode (curve b), anti-IgG/TAA/Au electrode (curve c), IgG/anti-IgG/TAA/Au electrode (curve d), and HRP-anti-IgG/IgG/anti-IgG/TAA/Au electrode (curve e). All measurements were processed in 5 mM $Fe(CN)_6^{4-/3-}$.

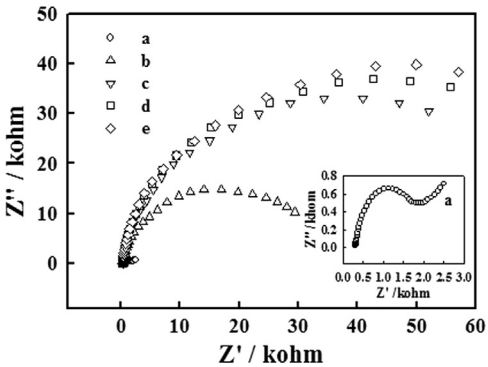


Fig. 2. Electrochemical impedance spectroscopy of the modified electrodes: bare Au electrode (curve a), TAA/Au electrode (curve b), anti-IgG/TAA/Au electrode (curve c), IgG/anti-IgG/TAA/Au electrode (curve d), and HRP-anti-IgG/IgG/anti-IgG/TAA/Au electrode (curve e). All measurements were processed in 5 mM $Fe(CN)_6^{4-/3-}$.

Beijing Dingguo Biotechnology Company (Beijing, China). Phosphate buffered saline (PBS) solution with various pH was prepared using 0.01 M Na_2HPO_4 and 0.01 M KH_2PO_4. (±)-Lipoic acid, 4-hydroxybenzaldehyde, 4-dimethylaminopyridine and dicyclohexylcarbodiimide were purchased from Aladdin Corporation. Unless otherwise specified, all the commercial reagents were of analytical grade and used without further purification.

Cyclic voltammetric measurement and amperometric measurement were carried out on CHI 660B electrochemical workstation (Shanghai, China). A conventional three-electrode system comprised of a Pt electrode as an auxiliary electrode, a saturated calomel electrode (SCE) as reference electrode, and a modified Au electrode as the working electrode. All potentials reported were versus the SCE. NMR spectra were performed with a bruker spectrometer (500 MHz).

2.2. Synthesis of TAA

To a solution of (±)-Lipoic acid (206 mg, 1 mmol), 4-hydroxybenzaldehyde(122 mg, 1 mmol) in dry CH_2Cl_2 (5 mL) was added 4-dimethylaminopyridine (12.2 mg, 0.1 mol) and dicyclohexylcarbodiimide (226 mg, 1.1 mmol). The mixture was stirred over night at room temperature. At the end the dicyclohexylurea that precipitated was removed by filtration and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel with petroleum ether/ethylacetate (V:V=4/1) to afford a yellow solid in 85% yield (264 mg). The synthesis principle of TAA was shown in Scheme 2.

2.3. Fabrication of the immunosensor

The bare Au electrode (3 mm diameter) was first polished with emery paper and alumina slurry, successively rinsed thoroughly with absolute alcohol and distilled water in ultrasonic bath, and dried in air. The polished Au electrode was dipped in thiol aromatic aldehyde (TAA) solution about 2 h at room temperature. Then the electrode was taken out from the solution and rinsed with distilled water to obtain a TAA film-modified Au electrode. After washing with water and being dried in air, 10 μL of 250 μg mL^{-1} antibody was added onto the electrode to be incubated for 1.5 h at 37 °C. Then, the antibody modified electrode was incubated with 10 μL BSA (1.0 wt%) for 1 h at room temperature to eliminate non-specific binding effect and block the remaining active sites. Thereafter, 10 μL of antigen solution at specific concentration was added onto the electrode to be incubated for 1.5 h at 37 °C, followed by washing with distilled water and being dried in air. At last, 10 μL horseradish peroxidase (HRP) labeled goat anti-human IgG was added onto the electrode to be incubated for 1.0 h at 37 °C, followed by washing with distilled water and being dried in air.

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