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Ultrasensitive cathode photoelectrochemical immunoassay based on TiO_2 photoanode-enhanced 3D Cu_2O nanowire array photocathode and signal amplification by biocatalytic precipitation

Li-Xia Liu [a], Gao-Chao Fan [a, *], Jian-Rong Zhang [a, b, **], Jun-Jie Zhu [a]

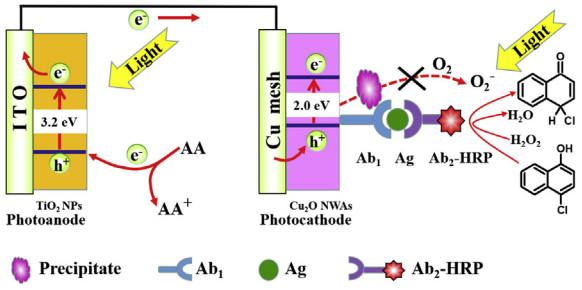
[a] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, PR China

[b] School of Chemistry and Life Science, Nanjing University Jinling College, Nanjing, 210089, PR China

HIGHLIGHTS

- A promising new cathode photoelectrochemical immunoassay was developed.
- TiO_2 photoanode-enhanced 3D Cu_2O nanowire array photocathode could evidently enhance photocurrent response.
- Horseradish peroxidase-induced biocatalytic precipitation could remarkably decrease photocurrent detection signal.
- This cathode photoelectrochemical protocol exhibited both good anti-interference capability and ultrahigh sensitivity.

GRAPHICAL ABSTRACT



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ABSTRACT

Cathode photoelectrochemical immunoassay usually shows better anti-interference capacity toward real samples than anode photoelectrochemical immunoassay. However, its poor photocurrent response has greatly restricted the detection sensitivity. Herein, a promising ultrasensitive cathode photoelectrochemical immunoassay was developed based on TiO_2 photoanode-enhanced 3D Cu_2O nanowire array (NWA) photocathode, and coupled with signal amplification by horseradish peroxidase (HRP)-induced biocatalytic precipitation (BCP). Carcinoembryonic antigen (CEA, Ag) was used as a detection model, TiO_2 nanoparticle-modified indium tin oxide (ITO) electrode served as the photoanode, and Cu_2O NWAs grown *in situ* on Cu mesh was both the photocathode and photoelectrochemical matrix to immobilize the capture CEA antibodies (Ab_1). The signal CEA antibodies (Ab_2) were labeled with HRP to form Ab_2-HRP bioconjugates, and employed as signal amplifiers when the specific immunoreaction occurred. The developed photoanode-enhanced cathode photoelectrochemical immunoassay has good anti-interference capability, outstanding photocurrent response, and high sensitivity for target Ag

Abbreviations: NWA, nanowire array; HRP, horseradish peroxidase; BCP, biocatalytic precipitation; CEA, carcinoembryonic antigen; ITO, indium tin oxide; CS, chitosan; BSA, bovine serum albumin; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; 4-CN, 4-chloro-1-naphthol; AA, ascorbic acid; AFP, α-fetoprotein; CA19-9, carbohydrate antigen 19-9; MMP-2, matrix metalloproteinase-2; PBS, phosphate buffer solution; XRD, X-ray diffraction; FE-SEM, field-emission scanning electron microscopy; TEM, transmission electron microscopy; EIS, electrochemical impedance spectroscopy; GLD, glutaraldyhyde; LOD, limit of detection; RSD, relative standard deviation.

* Corresponding author.
** Corresponding author. State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China.
E-mail addresses: gcfan@qust.edu.cn (G.-C. Fan), jrzhang@nju.edu.cn (J.-R. Zhang).

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detection, which was attributed to the synergistic effects of the 3D nanostructure of Cu_2O NWA photocathode, the introduction of TiO_2 photoanode as counter electrode, and the signal amplification of Ab_2-HRP bioconjugate-induced BCP. The developed cathode photoelectrochemical immunoassay showed a low limit of detection (0.037 pg mL^{-1}) with a wide linear range (from 0.1 pg mL^{-1} to 50 ng mL^{-1}) for CEA detection.

1. Introduction

Photoelectrochemical immunoassay has attracted increasing attention due to its desirable properties and potentials for future protein assays [1]. Because of the reduced background signals originating from the efficient separation of excitation sources and detection signals, photoelectrochemical immunoassay possesses higher sensitivity than electrochemical methods [2,3]. Besides, the instrument is simple and low-cost compared with those used in optical methods such as fluorescence, chemiluminescence, and electrochemiluminescence. Photoelectrochemical immunoassay combines an immune bioprobe and photoactive materials. Under light radiation, the specific immunoreaction between target analyte and the immune bioprobe would change the photocurrent signal of the immunosensor, which is used to detect the target analyte. On the other hand, the photoactive materials play a crucial role in the analytical performance of the photoelectrochemical immunosensor. In particular, a number of semiconductor nanomaterials have been investigated as photoactive materials, based on their exciting features such as ease of modification, wide absorption ranges, adjustable energy band gaps, and high light extinction coefficient.

Presently, various n-type semiconductors such as TiO_2, ZnO, CdS, CdSe, CdTe, and g-C_3N_4 have been widely applied in anode photoelectrochemical immunosensors [4–9], because this type of materials have lower charge recombination rates and therefore higher photocurrent response. However, anode photoelectrochemical bioassay has been reported to be susceptible to interference in real samples, because many reductive species such as ascorbic acid (AA), dopamine, and sulfhydryl compounds coexisting in the complex biological fluids [10–12] could be oxidized by the intrinsic holes produced at the n-type semiconductor/electrolyte interface. Moreover, the existing anode photoelectrochemical immunoassay mostly used highly toxic cadmium compounds to improve the photocurrent response, making it very unfavorable for real biological sample detection.

The newly emerged cathode photoelectrochemical bioassay constructed by p-type semiconductors could avoid the poor anti-interference capability of anode photoelectrochemical bioassay. The reason is that the reductive species in real biological samples cannot react with the intrinsic electrons generated at the p-type semiconductor/electrolyte interface [13–17]. As a result, many works on sensors, solar cells, and artificial photosynthesis have focused on p-type semiconductors such as NiO, ZnTe, PbS, and Co_3O_4 [18–21]. Among various p-type semiconductors, cuprous oxide (Cu_2O) has become the most outstanding performer in the sensor field as a result of its suitable band gap of 2.0 eV that permits efficient light absorption, and low toxicity compared to cadmium compounds [22,23]. However, due to its high charge recombination rate, Cu_2O has a low photocurrent response that would seriously limit the detection sensitivity. On the other hand, in solar-driven applications, engineered nanostructures, especially the three-dimensional (3D) ones, could be used to enhance the photoelectrochemical properties by providing longer optical paths for efficient light capture and shorter carrier diffusion length for rapid electron-hole separation. Therefore, these 3D nanostructures are regarded as perfect building blocks for photoelectrochemical devices [24,25].

Inspired by the advantages of anode and cathode photoelectrochemical immunoassays, a new cathode photoelectrochemical immunoassay was developed on the basis of TiO_2 photoanode-enhanced 3D Cu_2O nanowire array (NWA) photocathode coupled with signal amplification of horseradish peroxidase (HRP)-induced biocatalytic precipitation (BCP). The HRP-induced BCP has been employed as an effective amplification strategy in photoelectrochemical bioassay [26–28]. It functions by producing insoluble precipitate on the electrode surface to efficiently inhibit electron transfer, resulting in an evident decline in the photocurrent signal. In the present photoelectrochemical system, carcinoembryonic antigen (CEA, Ag) was used as a detection model, and TiO_2 nanoparticle-modified indium tin oxide (ITO) electrode served as photoanode and acted as the counter electrode. The construction process of the photocathode-based immunosensing electrode is illustrated in Scheme 1. Firstly, the capture CEA antibodies (Ab_1) were immobilized onto the Cu_2O NWAs-modified Cu mesh electrode by using chitosan (CS) as the linking molecule. The cathode sensing electrode was obtained after using bovine serum albumin (BSA) to block unbound sites on the Ab_1-modified electrode. For target Ag detection, Ag at different concentrations was first bound on the sensing electrode by specific immunoreaction with Ab_1. Then, Ab_2-HRP bioconjugates as the signal amplification element were further bound at a fixed concentration via specific immunoreaction between Ag and Ab_2. For final introduction of BCP, the electrode was immersed in the BCP solution consisting of 4-chloro-1-naphthol (4-CN) and H_2O_2. The cathode photoelectrochemical detection was achieved by an evident decrease of photocurrent produced by the HRP-induced BCP process, when the specific sandwich immunoreaction occurred.

2. Experimental

2.1. Materials and reagents

ITO electrodes (type JH52, ITO coating 30 ± 5 nm, sheet resistance ≤10 Ω cm^{-2}) were obtained from Nanjing Zhongjingkeyi Technology Co., Ltd. (China). TiO_2 powder (P25) was ordered from the Degussa Co. (Germany). The Cu mesh (100 mesh, 0.11 mm wire diameter) was obtained from Alfa Aesar (USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), HRP, BSA, 4-CN, and CS powder (from crab cells, 85% deacetylation) were all obtained from Sigma-Aldrich (USA). Glutaraldehyde (GLD, 25 wt % aqueous solution), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), acetone, ethanol, and AA were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). CEA (Ag), capture CEA antibody (Ab_1), signal CEA antibody (Ab_2), human IL-6, α-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9), and matrix metalloproteinase-2 (MMP-2) were all obtained from Wuhan Uscn Life Science Inc. (China). All other reagents were of analytical grade