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Research Paper

Quenched solid-state electrochemiluminescence of gold nanoclusters and the application in the ultrasensitive detection of concanavalin A

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ABSTRACT

This work investigated the quenching effect of ferrocenecarboxylic acid (Fc-COOH) on the bovine serum albumin-stabilized Au nanoclusters-triethylamine (Au_{25}-Et_3N) electrochemiluminescence (ECL) system. Based on the annihilation between the oxidation product of Fc (Fc^+) and $Et_3N^\bullet$ radical, a quenching-based sandwich-configuration ECL biosensor was established for sensitive and specific detection of concanavalin A (ConA). Au_{25} with strong initial ECL signal were modified onto the glassy carbon electrode (GCE) for immobilizing glucose oxidase (GOD). With the further immobilization of target ConA, the quenching probe of GOD@Fc-COOH-PtPd nanocubes were incubated onto the electrode through a specific carbohydrate-ConA interaction, thus achieving a sandwich structure. The resulting ECL biosensor showed a wide linear range from 0.004 ng/mL to 90 ng/mL and a low detection limit of 0.001 ng/mL (S/N = 3) for ConA detection. The integration of Fc-COOH with Au_{25}-Et_3N system would hold a great promise in bioanalysis.

1. Introduction

Metal nanoclusters such as Au, Ag, Cu, and Pt, containing of several to a hundred of atoms, have a size approaching the Fermi wavelength of the conduction electrons, resulting in unique physical, electrical and optical properties [1]. Among these nanoclusters, Au nanoclusters have drawn vigorous attention in various biochemical assays due to their high quantum yields, quantum confinement, two-photon absorption and catalysis [2]. Generally, the preparation of Au nanoclusters would involve various different capping molecules, e.g., proteins [3], dendrimers [4], mercapto-undecanoic acid [5], amino acids [6], or DNA [7]. Among them, protein-directed synthesis has gained increasing interest because this process provides a simple, green, and effective route for the production of Au nanoclusters. For example, bovine serum albumin (BSA) stabilized Au nanoclusters (Au_{25}), consisting of 25 gold atoms, have been mostly applied as fluorescence probes since they were first reported by Ying et al. [3]. The applications of Au_{25} in analysis field are mainly based on the change of fluorescence property of Au_{25} through the specific interactions between the targets (such as metal ions [8], anions [9], small molecules [10] and even large biomolecules [11]) and either the metallic core or the protecting stabilizer (BSA).

Electrochemiluminescence (ECL) is chemiluminescence triggered by electrochemical processes, which combines the advantages of chemiluminescent analysis with electrochemical analysis [12], and exhibits wide detection range, controlled reaction system, short time consumption, and high sensitivity and signal-to-noise ratio [13,14]. ECL has been used extensively as a detection method in biosensing [14,15]. For example, Stewart et al. reported an ECL biosensor for detecting dopamine from the whole blood without the need of pretreatment or extraction based on the quenching effect of dopamine on the ECL of CdSeTe/ZnS core-shell QDs [15]. Among various luminophors such as luminol, $Ru(bpy)_3^{2+}$ and quantum dots, Au_{25} as burgeoning and promising kind of luminophor have attracted a growing interest to explore the possible applications in ECL on the basis of their strong catalytic properties, easy labeling and low toxicity [16,17]. Up to now, researches about Au_{25}-based ECL in analysis are relatively few [16–18]. Chen et al. fabricated a H_2O_2 sensor based on the quenching effect of H_2O_2 towards the ECL emission of Au_{25} [16]. Fang et al. established a quenching ECL sensor for the heavy metal ions Hg^{2+} detection based on the significant quenching effects of Hg^{2+} on the ECL of Au_{25} [18]. Ferrocenecarboxylic acid (Fc-COOH), as a commonly used quencher in ECL, can efficiently and stably quench the ECL of $Ru(bpy)_3^{2+}$ and quantum dots [19,20]. However, we

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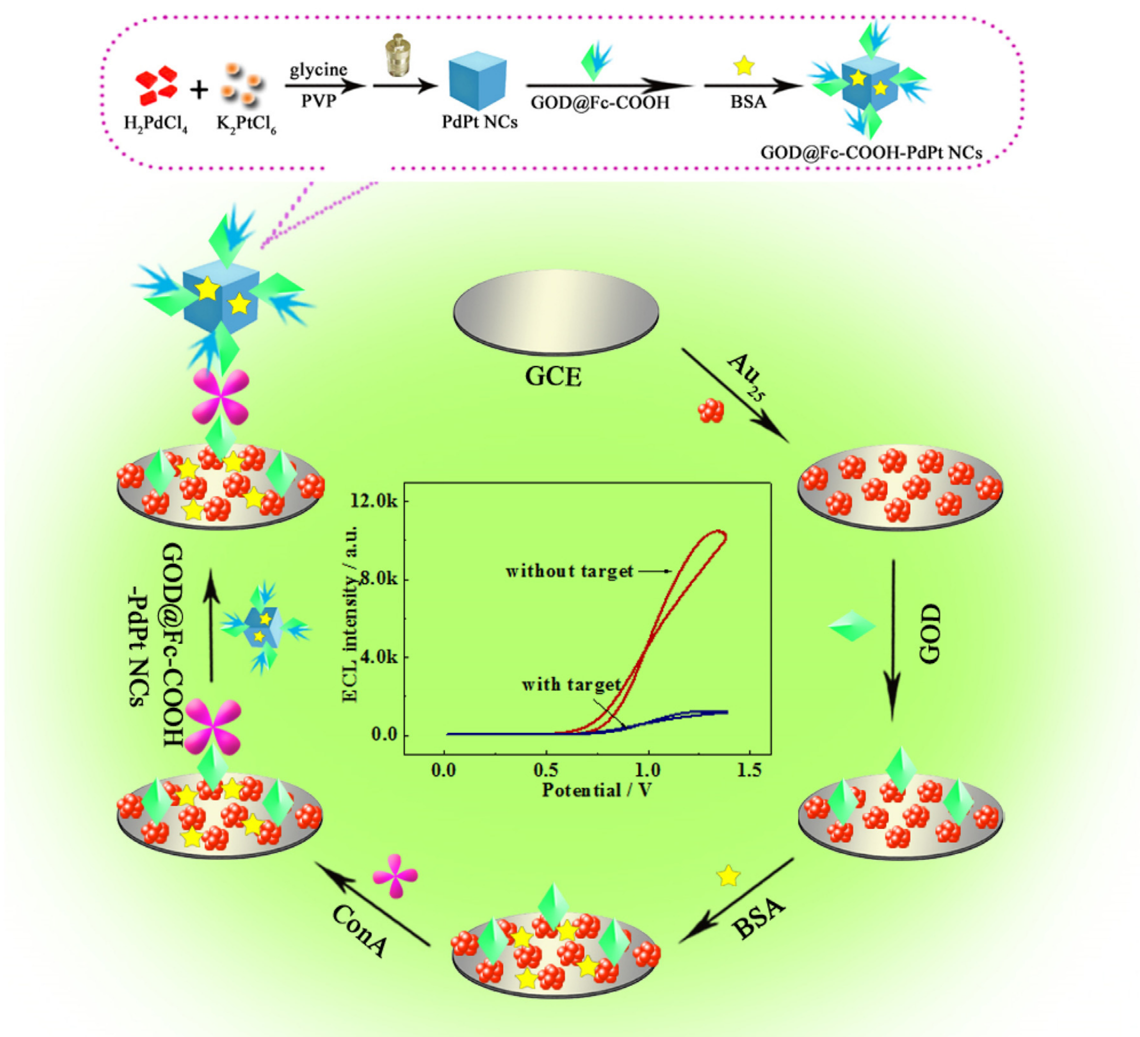
noticed that no report has addressed on the quenching effect of Fc-COOH on the Au_{25}-triethylamine (Et_3N) ECL system, especially investigated the quenching mechanism between Fc-COOH and coreactant Et_3N.

Concanavalin A (ConA), a globulin isolated from jack-bean meal, is one of the most commonly studied lectins with each subunit composed of 235 amino acids. It exists as a dimer below pH 5.5 and a tetramer between pH 5.5 and pH 7.0 (molecular mass, 104 000 for tetramer) [21,22]. Under neutral conditions, each subunit of ConA contains four binding sites [22]: one is for calcium cation and manganese cation to activate the binding site of ConA for carbohydrates; one is for hydrophobic recognition; and another one is specific for R-mannose or D-glucose residues, which belongs to the affinity between lectin and carbohydrate ligand. The interaction between lectins and carbohydrates plays a crucial role in the cell-surface recognition, cell-cell communications, cancer, and host-pathogen infection [23,24]. Hence, it is urgent to quantitate the ConA conveniently and rapidly. Based on the principle of carbohydrate-lectin interaction, many assay methods including electrochemical analysis [25], quartz crystal monitor [26], UV-visible spectroscopy [27], and fluorescence spectroscopy [28], have been reported. However, reports concerning ECL biosensor for the detection of ConA are relatively scarce now [29,30].

Recently, bimetallic materials such as AuPd, PtNi, AgAu, have been widely investigated in organic synthesis, fuel cells and automobile industry based on the bifunctional properties and synergisitic effects [31,32]. The adoption of bimetallic materials is particular importance in efficient catalysis and sensitive detection [33]. Especially, PtPd nanocubes (PtPd NCs), have received much attention due to the large specific surface area, excellent catalytic performance, good chemical stability and excellent biocompatibility [34,35]. In this study, we focus on a facile synthesis of PtPd NCs and make them serve as a promising carrier for high loading of the quencher Fc-COOH, thus improving the sensitivity.

Based on above observation, a sandwich-configuration ECL biosensor was developed with glucose oxidase@Fc-COOH functionalized PtPd NCs (GOD@Fc-COOH-PtPd NCs) as ECL signal quenching probe to detect ConA. Au_{25} with strong initial ECL signal were modified onto the glassy carbon electrode (GCE) for immobilizing GOD. Then, the target ConA was incubated on the modified electrode through a specific carbohydrate-lectin interaction between GOD and ConA. Because the GOD is a glycoprotein with a high mannose-type carbohydrate content [36], thus can specifically bind with ConA through the carbohydrate-ConA interaction. Finally, the GOD@Fc-COOH-PtPd NCs as the quenching probe was bound to the binding sites of Con A, thus achieving a sandwich structure. Based on the annihilation between the



Scheme 1. The illustration of the synthetic process of PtPd NCs and GOD@Fc-COOH-PtPd NCs, and the preparation of the ECL biosensor.