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A novel and sensitive chemiluminescence immunoassay based on AuNCs@pepsin@luminol for simultaneous detection of tetrabromobisphenol A bis(2-hydroxyethyl) ether and tetrabromobisphenol A mono(hydroxyethyl) ether

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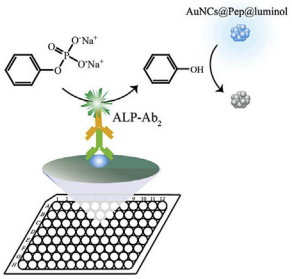
HIGHLIGHTS

- A novel chemiluminescence immunoassay was developed for simultaneous detection of TBBPA-DHEE and TBBPA-MHEE.
- An excellent AuNCs@Pep@luminol was synthesized to improve the performance of the chemiluminescence immunoassay.
- The established method showed good accuracy, reproducibility and satisfactory sensitivity.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel chemiluminescence immunoassay based on luminol-modified gold nanoclusters (AuNCs@Peps@luminol) was developed for simultaneous detection of tetrabromobisphenol A bis(2-hydroxyethyl) ether (TBBPA-DHEE) and tetrabromobisphenol A mono(hydroxyethyl) ether (TBBPA-MHEE), an important derivative and byproduct of tetrabromobisphenol A (TBBPA), respectively. In the system, alkaline phosphatase (ALP) was labeled on the second antibody (Ab_2) for signal amplification. When ALP-Ab_2 was captured by antigen-primary antibody (Ab_1) complex, disodium phenyl phosphate (PPNa) generated massive phenol under the catalysis of ALP, markedly inhibiting the chemiluminescence intensity of AuNCs@Peps@luminol. Under the optimized conditions, the calculated detection of limit (LOD, 90% inhibition) was 0.078 μg/L for TBBPA-DHEE with a linear range of 0.23–9.32 μg/L, which was lower 9 times than that of conventional ELISA with the same antibody. In addition, our method showed satisfactory accuracy and precision (recoveries, 88.00–113.4%; CV, 2.75–8.14%), it can be applied to systematically investigate the concentration of the trace TBBPA-DHEE and TBBPA-MHEE in environmental and food samples.

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1. Introduction

Tetrabromobisphenol A (TBBPA) is a kind of important halogenated flame retardants with the highest production volume worldwide [1–3], and 25% of this chemical is used to manufacture its derivatives, such as TBBPA bis(allyl) ether (TBBPA-DAE), TBBPA bis(2,3-dibromopropyl ether), (TBBPA-DBPE), TBBPA bis(2-hydroxyetyl) ether (TBBPA-DHEE) [4]. During the production of TBBPA and their derivatives, various byproducts are unintentionally produced, like TBBPA mono (2-hydroxyetyl) ether (TBBPA-MHEE). Among them, TBBPA-DHEE was reported that it exhibited potential neurotoxins and the highest cytotoxicity in comparison with TBBPA and other derivatives/byproducts [5]. Given that TBBPA-DHEE may be discharged into the food and environments after its extensive use, it is necessary to investigate its concentrations in these samples for further risk assessment.

Currently, only a few studies reported for determination of TBBPA-DHEE/TBBPA-MHEE using extremely expensive equipments [4,6,7]. For instance, Liu et al. developed a high performance liquid chromatography coupled with inductively coupled plasma tandem mass spectrometry to detect TBBPA and their derivatives after complicated sample pre-treatments [6]. However, the defects of the established instrument methods (high cost, poor throughput, tedious pretreatment procedures) hindered their further application, especially in routine monitoring. As an alternative, antibody-based immunoassays provide a better way to analyze the chemicals due to their simplicity, high throughput and sensitivity. Actually, we have established an enzyme-linked immunosorbent assays (ELISA) for simultaneous determination of TBBPA DHEE and TBBPA MHEE with a LOD of 0.7 ng/mL using our produced antibody capable of recognizing both pollutants [8], but the sensitivity of this method can't meet the demands of the trace containments determination. Furthermore, the conventional ELISA has the shortcomings of low tolerance to matrix interferences [9,10], so suitable and more sensitive immunoassays are needed.

The luminol–H_2O_2 based chemiluminescence immunoassay (LCLIA) is an attractive immunoassay that promises simple, high sensitivity, low background and pollution-free, widely used in fields like biological toxins, pesticide residues and organic pollutants detection [11]. However, the matrix effect could significantly influence the sensitivity of LCLIA in complex samples [12]. To address these problems, some nanoparticles have been introduced into LCLIA system by virtue of their unique characters. Recently, chemiluminescence (CL)-functional gold nanoparticles (AuNPs) as substrate solution and signal reporters have been applied in CL system [13–15], which drew much attention at the field. For example, based on luminol functionalized AuNPs as labels, Cui's group developed a novel and ultrasensitive electrochemiluminescence strategy for DNA detection [16], in this work, the luminol functionalized AuNPs was synthesized by a very simple one-pot method, showing an ideal application in bioassay with the advantages of excellent labeling property, ECL activity and stability. Except for luminol, luminol analogues-functional AuNPs has also been a hot topic in CL measurement [17–19]. Han et al. established a label-free and sensitive eletrochemiluminescence immunosensor for the detection of copeptin using an excellent CL-functional AuNPs (Cu^{2+}-Cys-ABEI-GNPs-CS) that exhibited great ECL properties without any coreactant [17]. Although there are so many researches committed to the synthesis and application of CL-functional gold nanoparticles in CL detection, only few study focus on the application of functional luminol materials in LCLIA [16,20]. At the same time, Au nanoclusters (AuNCs), as one special size of gold nanoparticles, could be applied in LCLIA owing to their molecular-like properties, including luminescence [20,21] and unique catalytic performance [22,23].

Enlightened by this, we synthesized a kind of excellent luminol-functional Au nanoclusters (AuNCs@Pep@luminol) to improve LCLIA performance (shown in Scheme 1). In this work, alkaline phosphatase (ALP) was labeled on the secondary antibody (Ab_2) as a signal tag, catalyzing the disodium phenyl phosphate (PPNa) to rapidly produce large amounts of phenol which could effectively restrain the chemiluminescence intensity of AuNCs@Pep@luminol for signal amplification. After optimized and evaluated, the proposed method was used to investigate the occurrence of TBBPA-DHEE/TBBPA-MHEE in a typical area.

2. Experimental section

2.1. Materials and equipments

$HAuCl_4$, luminol, disodium phenyl phosphate ($C_6H_9Na_2O_6P$, PPNa), NaOH, $NaHCO_3$, Na_2CO_3, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, NaCl, KH_2PO_4, KCl, H_2O_2, Gelatin, and Tween 20 were bought from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). ALP, pepsin (Pep), horseradish peroxidase (HRP), goat anti-rabbit IgG/ALP were obtained from Sigma-Aldrich (USA). The polyclonal antibodies against TBBPA-DHEE and TBBPA-MHEE were synthesized in our lab (shown in Supplementary Information). All the chemicals were analytical grade and used without further purification. The protein solutions were stored at 4 °C before use.

Buffers and solutions: A carbonate buffer solution (CBS) composed of 1.59 g Na_2CO_3 and 2.94 g $NaHCO_3$ was dissolved in 1.0 L pure water. The blocking buffer was obtained by adding 1% gelatin to the CBS solution. The washing buffer was phosphate-buffered saline (PBS) containing 0.01 mol/L phosphate and 0.05% Tween 20 at pH 7.4.

An UV–vis spectrophotometer (Model: UV-2600, SHIMADZU, Japan), a transmission electron microscope (TEM) (Model: Tecnai 12, Philips, Holland), and a microplate reader (Model: Infinite M1000 Pro, TECAN, Switzerland) were used for the measurement.

2.2. Synthesis of AuNCs@Pep@luminol

The AuNCs@pepsin@luminol was synthesized according to the previous work with some modifications [20]. Firstly, 10 mL $HAuCl_4$ solution (5 mM) was mixed with a solution containing 200 mg Pep and stirred vigorously for 5 min. After that, 1 mL NaOH solution (1 M) was added, and the mixture was kept reacting at 37 °C for 12 h. Finally, the product was centrifuged at 3000 rpm, obtaining the AuNCs@pepsin.

To prepare the AuNCs@Pep@luminol, the luminol was dissolved in 10 mL NaOH solution (0.1 M) at the concentrations of 5, 2.5, 1.25, 0.625, 0.5 mM. The different luminol solutions were mixed with the prepared AuNCs@pepsin and kept 30 min, then, the solutions was centrifuged and dialyzed. In the process, the colour of the solution changed from light yellow to brown, and finally to purple.

2.3. LCLIA procedure

The novel LCLIA was implemented in 96-well microplate. Firstly, 100 μL/well coating solution was added in the microplate overnight at 4 °C. In order to prevent nonspecific binding, after washing, the microplate was blocked with blocking buffer and incubated at 37 °C for 45 min. Washing again, 50 μL/well primary antibody (Ab_1) and 50 μL/well TBBPA-DHEE standards or samples were mixed together in each well. After the steps of incubation and washing, 100 μL/well ALP-secondary antibody (ALP-Ab_2) was added. 30 min later, 25 μL/well PPNa solution and AuNCs@Pep@luminol were successively put in after washing. Another incubation, 50 μL/well HRP/H_2O_2 were added in each well. 10 min later, the relative light unit (RLU) was measured by a microplate reader.

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