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Magnetic nanoparticles-phenanthrene conjugates (MNPs-Phe) probe based competitive chemiluminescence enzyme immunoassay (MNPs-icCLEIA) for phenanthrene and other homologous polycyclic aromatic hydrocarbons

Yu Sun[a,b,1], Yansong Li[a,1], Xianmei Meng[b], Xingyu Meng[a,c], Bin Qiao[a], Zhaozhao Si[a], Pan Hu[a], Shiying Lu[a], Honglin Ren[a], Zengshan Liu[a], Ying Zhang[d], Lei Meng[d], Yu Zhou[a,d,e,*]

[a] Key Laboratory of Zoonoses Research, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun 130062, PR China
[b] Grain and Oil Food Processing Key Laboratory of Jilin Province, Jilin Business and Technology College, Changchun 130507, PR China
[c] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, PR China
[d] Beijing Hongwei Science & Technology Co., Ltd, 100000, PR China
[e] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou, 215000, PR China

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ABSTRACT

In this study, a magnetic nanoparticle-phenanthrene hybrid conjugates (MNPs-Phe) probe was synthesized and an indirect competitive chemiluminescence enzyme immunoassay (MNPs-icCLEIA) was developed for rapid and sensitive detection of phenanthrene (Phe) and its homolog in water samples. The developed MNPs-icCLEIA was based on competitive reaction between Phe in samples and the MNPs-Phe probe. In the presence of Phe, the monoclonal antibody (McAb) would be competitive captured by MNPs-Phe probe and Phe in samples. Then MNPs-Phe-McAb complexes would be separated by a magnetic field. After the reaction of horseradish peroxidase conjugated goat anti-mice IgG (HRP-IgG) with MNPs-Phe-McAb complexes, chemiluminescent substrate was added and chemiluminescence was produced. The linear range of the assay for Phe was from 1.85 to 71.51 ng mL^{-1} and the limit of detection (LOD) was 0.85 ng mL^{-1}. Compared with the traditional enzyme linked immunosorbent assay (ELISA), the sensitivity of the developed MNPs-icCLEIA was about 3 times higher and more than 90 min was saved to fulfill the procedure of developed method while the linear range was nearly the same. The recoveries of polycyclic aromatic hydrocarbons (PAHs) from three different water samples were 97.2%–103.4%, 95.8%–104.8% and 96.9%–103.5% respectively, which had a good correlation (R^2 = 0.9986) with those from ELISA. The results indicated that the developed MNPs-icCLEIA is sensitive, rapid and reliable for the detection of Phe and other homologous PAHs in environmental water.

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; Phe, phenanthrene; MNPs, magnetic nanoparticles; MNPs-Phe, magnetic nanoparticles-phenanthrene conjugates; CLEIA, chemiluminescence enzyme immunoassay; McAb, monoclonal antibody; HRP-IgG, horseradish peroxidase conjugated goat anti-mice IgG; LOD, limit of detection; ELISA, enzyme linked immunosorbent assay; EPA, Environmental Protection Agency; HPLC, high performance liquid chromatography; GC–MS, gas chromatography–mass spectrometry; DLLME-GC–MS, dispersive liquid–liquid microextraction coupled with gas chromatography–mass spectrometry; M-d-μSPE, magnetic-assisted dispersive micro-solid-phase extraction; UHPLC, ultra-high-performance liquid chromatography; FD, fluorescence detection; MSPE, magnetic solid phase extraction; HS-GC–MS, static headspace gas chromatography coupled to mass spectrometry; FLD, fluorescence detection; CLEIA, chemiluminescence enzyme immunoassay; TMB, 3,3',5,5'-tetramethylbenzidine; DMF, dimethyl formamide; CR, cross-reactivity; DCC, N, N'-dicyclohexylcarbodiimide; NHS, N-hydroxysuccinimide; PBS, phosphate-buffered saline; PBST, PBS containing 0.05% (v/v) Tween 20; CBS, sodium carbonate/bicarbonate buffer; BSA, bovine serum albumin; OVA, ovalbumin; RLU, relative luminescent unit; IC50, 50% inhibition; CV, coefficient of variation.

* Corresponding author at: Key Laboratory of Zoonoses Research, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun 130062, PR China.
E-mail address: zhouyurunye@sina.com (Y. Zhou).
[1] These authors contributed equally to this work.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) is a class of ubiquitous environmental pollutants [1]. Natural and anthropogenic processes such as incomplete combustion of organic materials and automobile exhausts can cause the production of PAHs [2–4]. Due to the diverse sources of PAHs, they are usually exist as complex mixture in the environment [5]. Many PAHs have been found to be mutagenic or carcinogenic [6–10]. Because of their extremely lipid soluble characteristic, PAHs are easily absorbed from the gastrointestinal tract of mammals [11]. Therefore, the US Environmental Protection Agency (EPA) has listed 16 PAHs as priority pollutants in which the minimum contaminant level was 200 ng L^{-1} in drinking water [12,13].

High performance liquid chromatography (HPLC) [14], UV/visible absorption spectrometry [15], gas chromatography-mass spectrometry (GC–MS) [16] and fluorescence spectrometry [17] were used as conventional analytical methods for analysis of PAH. A variety of multi-technique integrated methods have also been utilized to detect PAHs, such as DLLME-GC–MS [18], M-D-μSPE-UHPLC-FD [19], MSPE-HS-GC–MS [20] and magnetic solid-phase extraction-HPLC–FLD [21]. Despite sensitive, these techniques require expensive equipment, labor-intensive sample pre-treatments and skilled analysts. Enzyme immunoassays which are based on antibody or antigen conjugated to enzyme to identify and quantify the target analyte, are more convenient, reliable and simple [22,23]. In recent years, chemiluminescence enzyme immunoassay (CLEIA) has been applied in biomedical researches owing to their wide dynamic range, acceptable sensitivity and preclusion of radiation damage [23,24]. However, CLEIA requires relatively longer performance time, which limits its application. Magnetic nanoparticles (MNPs), as a special biomolecular carrier, has been recently proven to solve the problem due to their higher surface area for immobilization and easier separation from the reaction mixture with a magnet [25,26]. MNPs-CLEIA technique, combining the advantages of both MNPs and CLEIA, has been used in clinical diagnosis [27–30], pharmaceutical analysis [31], toxicological analysis [32,33], food safety and environmental analysis [34].

Herein, 2-Phenanthrene butanoic acid as a substitute to phenanthrene (Phe) was directly immobilized on the surface of amino-MNPs to synthesize a magnetic nanoparticle-phenanthrene hybrid conjugates (MNPs-Phe) probe (Fig. 1A). An indirect competitive chemiluminescence enzyme immunoassay (MNPs-icCLEIA) based on the probe was developed for rapid and sensitive detection of Phe and its homolog in water samples (Fig. 1B). In the proposed method, the monoclonal antibody (McAb) was firstly competitive reacted with MNPs-Phe probe and Phe in samples. Then horseradish peroxidase conjugated goat anti-mice IgG (HRP-IgG) was captured by MNPs-Phe-McAb complexes. After a magnetic separation washing step, chemiluminescent substrate was added. The relative luminescent unit (RLU) was inversely proportional to the amount of analyte present in the sample. The traditional enzyme linked immunosorbent assay (ELISA) was used as a comparison to the developed MNPs-icCLEIA, including recovery, detection time, linear range (IC_{15}-IC_{85}) and the limit of detection (LOD, IC_{10}). Up to now, there has been no report about the construction of MNPs probe based on PAHs.

2. Materials and methods

2.1. Materials and instrumentation

16 PAHs (Phe, acenaphthene, pyrene, naphthalene, fluorene, anthracene, fluoranthene, acenaphthylene, indeno [1,2,3-cd] pyrene, benzo [a] pyrene, benzo [a] anthracene, chrysene, benzo [k] fluoranthene, benzo [b] fluoranthene, dibenz [a,h] anthracene, benzo [g,h,i] perylene) and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl formamide (DMF), N, N'-Dicyclohexylcarbodiimide (DCC), N-Hydroxysuccinimide (NHS) and HRP-IgG were purchased from J&K Scientific (Beijing, China). 2-phenanthrene butanoic acid was synthesized by Department of Applied Chemistry, Faculty of Science, Nanjing Tech University. 96-well microplate was supplied by Costar Group, Inc. (NY, USA). Luminol substrate solution and amino-modified MNPs (amino-MNPs, 300 nm in diameter) were obtained from Thermo Fisher Scientific (San Jose, CA, USA) and BioCanal (Hangzhou, China), respectively. The coating antigen Phe-ovalbumin (Phe-OVA) and the McAb which has high cross-reactivity (CR) with Phe, acenaphthene, fluorene, fluoranthene, pyrene and indeno [1,2,3-cd] pyrene were produced in our laboratory [35]. All other chemical reagents were analytical-grade quality.

The absorbance values were recorded at 450 nm with a Bio-Tek ELx 800 Microplate Reader (Vermont, USA). Chemiluminescence intensities were measured by Tecan Infinite F200 microplate reader (Männedorf, Switzerland).

2.2. Buffers and solutions

Dilution buffer, phosphate-buffered saline (PBS, 10 mmol L^{-1}, pH 7.4); PBST solution, PBS containing 0.05% (v/v) Tween 20 (PBST); Coating buffer, sodium carbonate/bicarbonate buffer (CBS, 0.1 mol L^{-1}, pH 9.6); TMB solution, 50 mmol L^{-1} sodium citrate buffer (pH 5.0) containing 0.01% (w/v) TMB and 0.005% (v/v) H_2O_2; enzymatic stopping solution, 2.0 mmol L^{-1} H_2SO_4; PAHs standard solution, 1 mg mL^{-1} of each PAH was dissolved in DMF respectively; PAHs mixture solution (1 mg mL^{-1}): equal amount of Phe, acenaphthene, fluorene, fluoranthene, pyrene and indeno [1,2,3-cd] pyrene were dissolved in DMF. BSA solution: 136 mg bovine serum albumin (BSA) was dissolved in 8 mL PBS (pH 7.4). Distilled water was used throughout the study.

2.3. Preparation of MNPs-Phe probe

The procedure of preparing MNPs-Phe probe was depicted in Fig. 1A. First, 2 mg 2-phenanthrene butanoic acid was dissolved in 2 mL DMF. 1 mg DCC and 1 mg NHS were added into the solution. The reaction mixture was gently stirred at room temperature for 30 min to form NHS-activated Phe solution. Amino-MNPs was separated from the preservation solution with a magnet and re-dispersed immediately following removal of the magnet. After being washed three times with 1 mL DMF to remove preservation solution by magnetic separation process, 20 mg amino-MNPs were added into the NHS-activated Phe solution. The mixture was gently stirred overnight at 4 °C in the dark. The prepared MNPs-Phe probe was washed by magnetic separation washing step to remove the excess free 2-phenanthrene butanoic acid. The residual binding sites on MNPs surface were passivated by adding 1 mL 10% BSA into the as-prepared MNPs-Phe solution at room temperature for 4 h with gentle stirring. Finally, the probe was washed by another magnetic separation washing step, suspended in 5 mL PBS and stored at 4 °C for further use.

2.4. Immunoassay procedures of MNPs-icCLEIA

Microplate was blocked by adding 200 μL of 1% BSA into each well overnight at 4 °C and washed three times with PBST. Then the mixture solution of 100 μL MNPs-Phe probe, 50 μL McAb and 50 μL Phe or sample solution was added and incubated for 15 min at 37 °C. After a magnetic separation washing step, 100 μL of HRP-IgG was added and incubated with gentle shaking at 37 °C for 15 min.