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Fluorescence-linked immunosorbent assay for detection of phenanthrene and its homolog

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ABSTRACT

A competitive fluorescence-linked immunosorbent assay (FLISA) was developed using rhodamine B isothiocyanate (RBITC) as the model fluorescent dye conjugate monoclonal antibody (McAb) for detection of Phe and its homolog (acenaphthene, fluorene, fluoranthene, pyrene and indeno [1,2,3-cd] pyrene) in water samples. The detection range of the assay for Phe was from 2.10 to 91.95 ng/mL. The limit of detection was 1.05 ng/mL, which was approximately 2-fold lower than that of traditional ic-ELISA. Compared with traditional ic-ELISA, more than 70 min was saved because of only one immunoreaction step was needed to accomplish the assay. The average recoveries of Phe and its homolog from domestic water, contaminated water and natural water were 100.7%, 100.8% and 101.2% respectively. The accuracy and precision of the developed FLISA were validated with GC-MS/MS. There were good correlation between the two methods from tap water, contaminated water and river water samples were 0.9994, 0.9935 and 0.9967, respectively. The results suggested that the proposed FLISA could be a potential alternative format for rapid, sensitive, and quantitative detection of Phe and its homolog in environmental water.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene (Phe) as ubiquitous contaminants, are released to the environment during incomplete combustion of fossil fuel and automobile exhausts. Generally, PAHs exist in the environment as complex mixture, 16 of which are listed by Environmental Protection Agency (EPA) of USA as priority pollutants [1]. Exposure to PAHs may cause serious threat to the health of humans such as carcinogenicity, teratogenicity and genotoxicity [2,3]. Due to their toxicity, the presence of PAHs in the environment have been received continuous attention [4–6].

Various hyphenated techniques have been proposed for the detection of PAHs, such as gas chromatography-mass spectrometry (GC-MS) [7], HPLC coupled to UV diode array detection (HPLC-UV-DAD) [8] and dispersive liquid–liquid microextraction based on solidification of floating organic drop and fluorescence (DLLME−SFO−MFD) [9]. Despite very sensitive and accurate of these methods, they require sophisticated instrumentation, time-consuming sample preparation procedures and skilled analysts [10,11]. Therefore it is necessary to explore a convenient and sensitive method for the detection of PAHs.

Immunoassays rely on antigen-antibody interaction, are efficient high-throughput screening techniques for medical diagnostics [12,13], environmental monitoring [14] and food safety analysis [15]. Compared to the instrumental analytical methods, the advantages of immunoassay are convenient, efficient and simple [16]. Among the current immunoassays, enzyme-linked immunosorbent assay (ELISA), owing to high selectivity and sensitivity, has become the main method for monitoring PAHs in environment and food [17–20]. Meanwhile, new immunoassay techniques are being developed for the detection of PAHs, such as real-time immuno-PCR assay (RT-IPCR) for pyrene [21], magnetic beads-based bioelectrochemical immunoassay for PAHs [22], fluorescence polarization immunoassay for benzo[a]pyrene, naphthalene, and anthracene [23] and color encoded microbeads-based flow cytometric immunoassay for benzo[a]pyrene [24]. Among these

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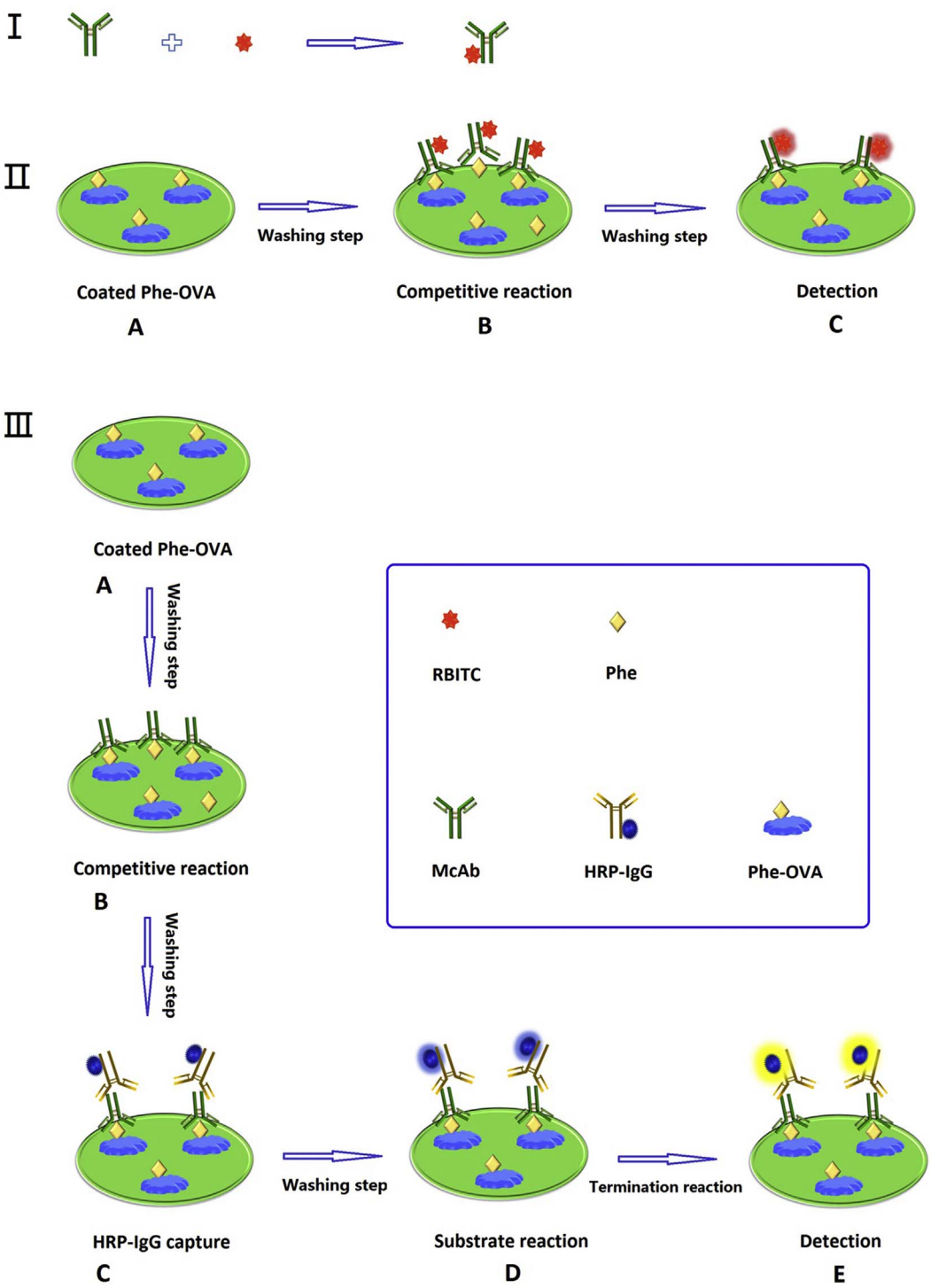

Fig. 1. Schematic diagram of preparation of RBITC-McAb probe and RBITC-McAb probe based FLISA procedure. (I) Preparation of RBITC-McAb probe. (II) The protocol of the FLISA. (A) The 96-well black microplate was coated with Phe-OVA (0.5 μg/mL, 100 μL/well). (B) The mixture solution of 50 μL RBITC-McAb probe (0.25 μg/mL) and 50 μL Phe or sample solution was added into the microplate. (C) After removing the Phe-RBITC-McAb complex by washing, the fluorescence intensity was measured by a multifunction microplate reader. (III) The protocol of the traditional ic-ELISA. (A) The 96-well microplate was coated with Phe-OVA (0.25 μg/mL, 100 μL/well). (B) The mixture solution of 50 μL McAb (14 ng/mL) and 50 μL Phe or sample solution was added into the microplate. (C) After removing the Phe-McAb complex by washing, 100 μL of HRP-IgG (200 ng/mL) was added. (D) After removing the unbound HRP-IgG by washing, 100 μL of TMB substrate solution was added. (E) After 10 min, 2 mol/L H_2SO_4 was added (50 μL/well) and the absorbance was read by a microplate reader.

methods, fluorescence immunoassays have recently attracted widespread interest because of their stronger optical signals and lower detection limit [25].

Herein, rhodamine B isothiocyanate (RBITC) was employed as model fluorescent dye to conjugate with monoclonal antibody (McAb). And a fluorescence-linked immunosorbent assay (FLISA) was developed based on RBITC-McAb probe for detection of Phe and homologous PAHs in water (Fig. 1). The results were also validated by GC-MS/MS with respect to its accuracy, precision and real sample assay. To the best of our knowledge, this is the first report that RBITC was used as fluorescence signal in immunoassay for sensitive detection of Phe and its homolog in water samples.