Sensing and Bio-Sensing Research 7 (2016) 115–120

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

A highly sensitive monoclonal antibody based biosensor for quantifying 3–5 ring polycyclic aromatic hydrocarbons (PAHs) in aqueous environmental samples

Xin Li, Stephen L. Kaattari, Mary A. Vogelbein, George G. Vadas, Michael A. Unger *

Department of Aquatic Health Sciences, Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA 23062, United States

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 3 February 2016
Accepted 4 February 2016

Keywords:
Monoclonal antibody
PAH
Pore water
Biosensor
Pyrene

ABSTRACT

Immunoassays based on monoclonal antibodies (mAbs) are highly sensitive for the detection of polycyclic aromatic hydrocarbons (PAHs) and can be employed to determine concentrations in near real-time. A sensitive generic mAb against PAHs, named as 2G8, was developed by a three-step screening procedure. It exhibited nearly uniformly high sensitivity against 3-ring to 5-ring unsubstituted PAHs and their common environmental methylated PAHs, with IC_{50} values between 1.68 and 31 μg/L (ppb). 2G8 has been successfully applied on the KinExA Inline Biosensor system for quantifying 3–5 ring PAHs in aqueous environmental samples. PAHs were detected at a concentration as low as 0.2 μg/L. Furthermore, the analyses only required 10 min for each sample. To evaluate the accuracy of the 2G8-based biosensor, the total PAH concentrations in a series of environmental samples analyzed by biosensor and GC–MS were compared. In most cases, the results yielded a good correlation between methods. This indicates that generic antibody 2G8 based biosensor possesses significant promise for a low cost, rapid method for PAH determination in aqueous samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds composed of two or more blended aromatic rings, are an important class of environmental pollutants [4,13]. PAHs are a concern because several of its members have been known to cause cancer in humans [9]. The U.S. Environmental Protection Agency (EPA) has identified 16 PAHs as priority pollutants because of their suspected carcinogenicity and high toxicity [1]. Environmental PAHs are typically present in complex mixtures and are mainly formed during the incomplete combustion of organic matter [17]. Because PAHs are found naturally in the environment but are also man-made, one can be exposed to PAHs in a number of ways, including air, water and food [19]. Traditionally, PAHs are analyzed by high performance liquid chromatography (HPLC) combined with ultraviolet (UV) absorption or fluorescence detection; or by gas chromatography (GC) combined with mass spectrometry or flame ionization detection [17,21]. These methods are sensitive, but are dependent on sophisticated equipment and often require complicated sample preparation steps, which can increase the time and effort for analysis.

Antibody-based immunoassays are widely employed in environmental PAH analysis because of their low cost, rapidity and sensitivity [15,16,26]. Therefore, a number of monoclonal antibodies (mAbs) against PAHs have been described in the recent past [14,23,24]. Although those mAbs differ in terms of their sensitivity and selectivity, they generally target one or a few select PAHs, and many have been developed to be selective to benzo(a)pyrene (BaP). Environmental samples typically contain complex mixtures of PAHs so there is a need for the rapid simultaneous determination of total PAHs in one analytical step for environmental fate studies where mapping of PAH gradients is needed. A near real-time assay for total PAH concentrations will allow the analyst to evaluate spatial or temporal changes in total PAH concentrations not economically feasible by traditional methods. To develop immunoassays for the quantitative detection of total PAHs in environmental samples, it is necessary to have antibodies with generic high affinity against PAHs.

Our laboratory has employed a fast, highly sensitive, automated system, KinExA Inline BioSensor [28], (Sapidyne Instruments) to serve as a biosensor for PAH detection, which allows near real-time assessment of PAHs in aquatic samples [25]. The technology is based on fluid phase interaction of the target with a selective antibody and detection of fluorescence inhibition [5]. This method requires only 3 min for a quantitative response and an additional 7 min for sensor regeneration. The next phase of development was to seek new PAH mAbs with broad selectivity that could be employed in the biosensor to assess PAH concentrations in a wide range of environmental samples. Therefore, the focus of this project was the development and evaluation of a generic anti-PAH mAb as well as evaluating it in our inline biosensor system for the accurate detection of total PAH concentrations in environmental samples.

* Corresponding author.
E-mail address: munger@vims.edu (M.A. Unger).

http://dx.doi.org/10.1016/j.sbsr.2016.02.003

2. Methods

2.1. Chemicals and reagents

PAH standards (phenanthrene, anthracene, pyrene, chrysene, benzo[a]pyrene) were obtained from Sigma Aldrich (St. Louis, MO, USA). Keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) and 1-pyrene butyric acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). Anhydrous N,N-dimethylformamide (DMF) was from Acros Organics (Morris Plains, NJ, USA). Bicinchoninic acid (BCA) protein assay Kit, N-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC) were obtained from Pierce (Rockford, IL, USA). HAT medium supplement, Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI-1640 medium with L-glutamine and fetal bovine serum (FBS) were obtained from Hyclone (Logan, UT, USA). Goat anti-mouse immunoglobulin G secondary antibody conjugated with horseradish peroxidase (GAM IgG-HRPO) was purchased from Jackson Immunoresearch (West Grove, PA, USA). Tissue culture plates and microtiter plates were supplied by Costar (Corning, NY, USA). The polymethylmethacrylate beads were obtained from Sapidyne Instruments (Boise, ID, USA). The fluorescent dye AlexaFluor 647 came from Invitrogen (Carlsbad, CA, USA). All other reagents were of analytical grade and purchased from Fisher Scientific (Pittsburgh, PA, USA).

Balb/c mice were bred at our own facility from stock originally purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the College of William and Mary.

2.2. Preparation of PAH-protein conjugates

Since PAHs are low molecular weight and devoid of antigenicity, they must be coupled to a protein carrier in order to elicit an immune response. For this study a derivative of pyrene, 1-pyrene butyric acid (PBA) was chosen as the hapten for immunization. The conjugations of the immunogen 1-pyrene butyric acid-KLH (PBA-KLH) and the screening antigen PBA-BSA were both carried out by the method described in the Pierce N-hydroxysulfosuccinimide (S-NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) protocol adapted from Grabarek and Gergely [7] with some modifications. Briefly, PBA hapten was dissolved in MeOH at 1 mg/ml. S-NHS and EDC were added to each of two aliquots of the hapten solution at a final concentration of ~500 mM and ~200 mM respectively, and rotated for 15 min at room temperature. To quench the EDC, 2-mercaptoethanol (20 mM) was added to each aliquot and rotated for 10 min. Any precipitate was removed by centrifugation at 12,000 rpm for 5 min at room temperature; the supernatant contained the activated hapten. A 1 mg/ml solution of both BSA and KLH was made in 0.1 M sodium phosphate, pH = 7.5. The PBA was then conjugated to the carrier by mixing 1 ml of the activated hapten to either 10 ml of BSA or 20 ml of KLH to yield a molar ratio of ~20:1 (PBA:BSA) and ~50:1 (PBA:KLH). The mixtures were covered in foil and allowed to react for ~2–3 h on a rotator at room temperature. To remove excess reactants, the two conjugates were dialyzed separately in 1× PBS with a minimum of three 1 h 1 L changes, including one overnight, at 4 °C. The conjugates were filter-sterilized through 0.22 μm filters, and a final protein concentration was determined by bicinchoninic acid (BCA) protein assay (Pierce) and read on a microtiter plate reader (MTX Lab Systems, Vienna, VA, USA) using a 540 nm filter.

2.3. Immunization and generation of hybridoma cells

Five 10-week-old female Balb/c mice were immunized with 100 μl of a 1:1 FCA emulsion containing 50 μg of PBA-KLH by intraperitoneal injection. Antisera were collected from the tail vein of each mouse at 3 weeks and 6 weeks post-immunization and assayed for antibody titer determination by using an enzyme-linked immunosorbent assay (ELISA) procedure [8]. The ELISA for titer was carried out as described by Spier et al. [24]. The mouse whose antiserum exhibited the highest titer was given an intravenous booster 3 days before the spleen was removed. The booster injection used a 10 μg dose of PBA-KLH antigen in Phosphate-buffered saline (PBS).

The boosted mouse was sacrificed; the spleen was removed aseptically and fused with SP2/0 myeloma cells using the 50% polyethylene glycol method as described elsewhere [8]. The resulting cells were distributed into 10 96-well tissue culture plates at 150 μl/well. After visible colonies had formed, about 10–15 days post-fusion, culture supernatants were screened for antibodies against PAH using a three-step screening procedure as described in the following section.

2.4. Three-step screening procedure

In the first step, an indirect ELISA was employed to screen positive wells and eliminate antibodies that react with KLH. Briefly, microtiter plates were coated with PBA-BSA conjugate with 5 μg/ml and incubated at 4 °C overnight and blocked with Tween Tris buffered saline (TTBS, 0.1% Tween 20, 50 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 8.0) for 1 h at room temperature with slight horizontal shaking at 300 rpm. The culture supernatants were added to the wells for another 1 h at room temperature on a rotator. After washing 3 times with TTBS, the plate was incubated with GAM IgG-HRPO (dilution 1/2000 in PBS, 100 μl/well) for 1 h at room temperature. The plates were washed

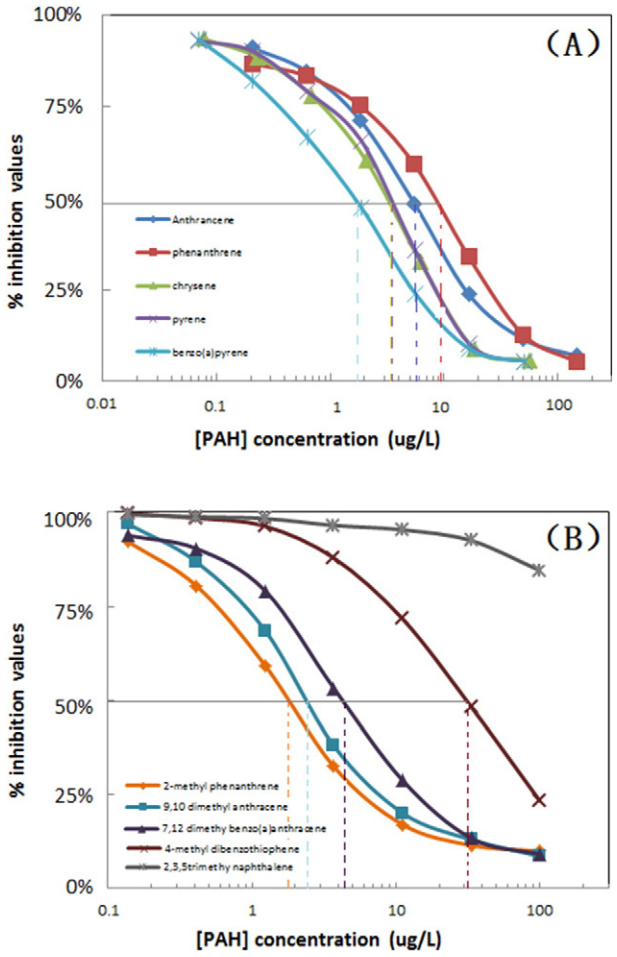

Fig. 1. ELISA inhibition curves for mAb 2G8 against different PAHs. (A) mAb 2G8 binding against class unsubstituted PAHs, with IC_{50} values between 1.68 and 9.44 μg/L.(B) mAb 2G8 binding against class alkylated PAHs, with IC_{50} values between 1.92 and 31 μg/L. Each data point is the average of 3 times duplicate samples. The coefficients of variation (n = 3) were between 0.5% and 7.9%.