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

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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.

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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

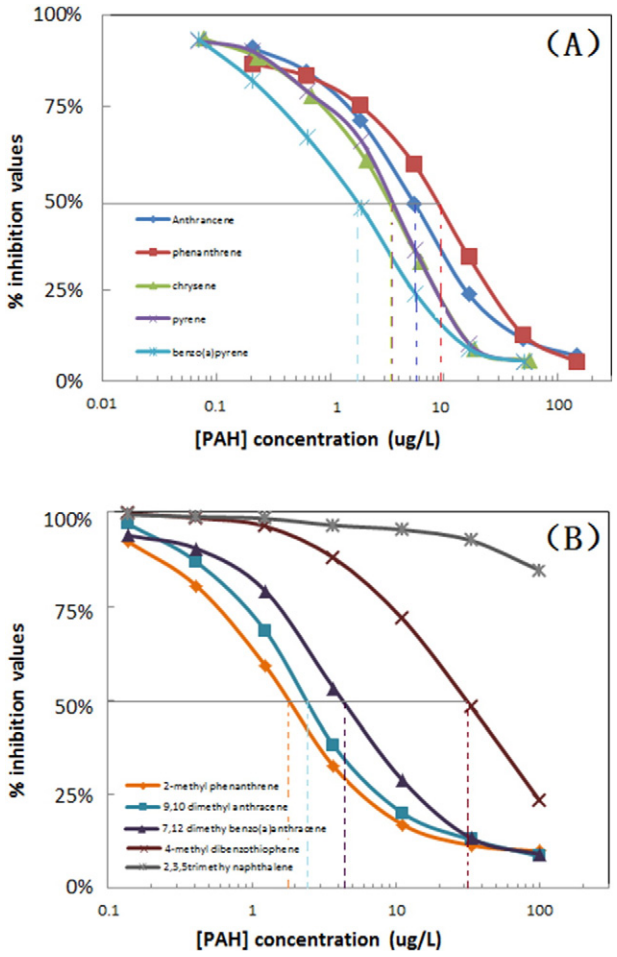

Fig. 1. ELISA inhibition curves for mAb 2G8 against different PAHs. (A) mAb 2G8 binding against class unsubstituted PAHs, with IC_{50} values between 1.68 and 9.44 μg/L.(B) mAb 2G8 binding against class alkylated PAHs, with IC_{50} values between 1.92 and 31 μg/L. Each data point is the average of 3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

three times with TTBS and then developed using a solution containing 7.7 μmol of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 10 μl of 30% H_2O_2 in 10 ml of citrate buffer (10 mM citric acid, pH 4.0). Absorption values were measured at 405 nm with a microtiter plate reader.

Plate wells containing hybridomas demonstrating significant reaction with PBA-BSA were selected for the second step screening procedure. Indirect competitive ELISA (icELISA) was employed by using the original PAH derivative (PBA) as inhibitor to ascertain the relative affinity for the free PAH apart from the KLH or BSA. The icELISA procedure was similar to that of indirect ELISA, except that high and zero concentrations of PBA working solutions (50 μl respectively mixed with the same volume of supernatants from one hybridoma well were added) were used. The inhibition curves were obtained through the calculation of the difference between the concentrations of PBA working solutions and inhibition values. The inhibition values were expressed as % B/B_0, where B was the absorbance at each concentration of PBA and B_0 was the absorbance in the absence of PBA [3].

Hybridomas presenting good inhibition with PBA were selected for clonal expansion. Clonality was ensured via limiting dilution [8]. Multiple icELISAs were employed for the final competitive screening assay after the chosen cell lines had been fully cloned. The icELISA procedure was in the same format as described above except that multiple free PAHs were used as inhibitors to characterize the antibody. Both unsubstituted and methylated PAHs were used, includinganthracene, phenanthrene, chrysene, pyrene, benzo(a)pyrene, 2-methylphenanthrene, 9,10-dimethylanthracene, 7,12 dimethybenz(a)anthracene, 4-methyl dibenzothiophene and 2,3,5-trimethyl naphthalene.

2.5. Selectivity determinations

The antibody was prepared on a large scale as ascetic fluid, by inoculating the hybridoma cells into pristine-primed BALB/c mice. The IgG fractions were separated and purified by the protein A column method [12]. The selectivity of the purified antibody was determined by calculation of the Krel values [22]. The Krel was calculated by following formula: Krel = (IC_{50} of reference analyte)/(IC_{50} of analyte X). Typically, the homologous analyte that corresponds to the immunization hapten was used as the reference analyte. For the selectivity comparison, pyrene was defined as the reference analyte in this study.

2.6. Biosensor development

The KinExA Inline biosensor was prepared according to our previous publications with small modifications [5,25]. Briefly, the immobilized antigen PBA-BSA was coated onto polymethylmethacrylate beads and the anti-PAH mAb 2G8 was tagged with the fluorescent dye Alexa Fluor 647 by following the standard protocol provided by Thermo Fisher Scientific [2]. The automated sample handling program of the biosensor was described previously [5].

Several calibration curves were obtained by using different PAH formulations as standards to evaluate mAb 2G8's performance on the biosensor. The formulations were phenanthrene, pyrene, a PAH mixture solution (containing equal concentrations of anthracene, benzo(a)pyrene, chrysene, phenanthrene and pyrene) and the commercial PAH mixture containing 24 PAHs, National Institute of Standards & Technology (NIST) Standard Reference Material 2260 (SRM 2260), Aromatic Hydrocarbons in Toluene. The biosensor response (dV) was obtained via a six-point calibration curve within the most linear range of the instrument. For each formulation standard, the calibration curves and biosensor linear range were determined by log-linear regression analysis. To assess the accuracy of the KinExA Inline Biosensor in determining total PAH concentrations in environmental samples a variety of aqueous samples were evaluated by GC–MS and biosensor, including sediment pore water and water-soluble fractions derived from Prudhoe Crude Oil and creosote.

2.7. GC–MS analyses of total PAHs of estuarine samples

The above environmental samples were analyzed by gas chromatography–mass spectrometry (GC–MS). The method was based on the techniques developed previously [27]. Briefly, the water samples were filtered using a 0.45 μm Millipore filter, transferred to a precleaned separatory funnel, surrogate standards (1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12},

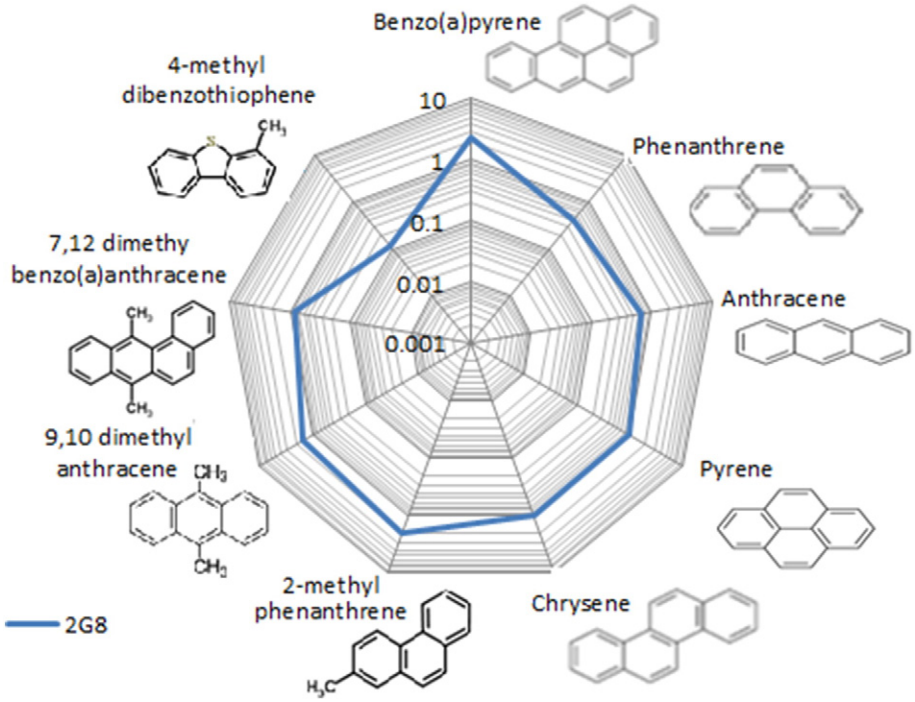

Fig. 2. Radar plot of Krel values of 2G8 with unsubstituted and methylated PAHs. ($Krel_{2G8}$ = IC_{50} of pyrene/IC_{50} of PAH X).

Table 1
Comparison of sensitivity of 2G8 with commercially available anti-PAH mAbs.

Compound	IC_{50}μg/L		
	2G8[a]	10C10[b]	4D5[b]
Benzo(a)pyrene	1.68 ± 0.97	2.77 ± 1.01	2.77 ± 1.01
Chrysene	3.84 ± 1.85	4.57 ± 0.91	5.48 ± 0.91
Pyrene	3.84 ± 1.69	6.88 ± 1.21	4.25 ± 1.01
Phenanthrene	9.44 ± 0.84	6.95 ± 0.89	11.41 ± 1.43
Anthracene	5.88 ± 1.29	8.20 ± 0.89	12.65 ± 1.60

[a] The IC_{50} values of 2G8 were means ± standard deviations for 5 times repetition.
[b] 10C10 and 4D5 both commercially available anti-PAH mAbs, the IC_{50} values for them came from analysis in [14].

perylene-d_{12}, and 1,1′-binaphthyl; Ultra Scientific, Kingston, RI, USA) added, and extracted three times with 20 ml of dichloromethane (Burdick & Jackson, Muskegon, MI, USA). The volume was reduced under a gentle stream of nitrogen using a TurboVap® evaporator (Zymark Corp., Hopkinton, MA, USA) and the internal standard p-terphenyl (ChemService, West Chester, PA, USA) was added. The extracts were analyzed on a Varian 3400 Gas Chromatograph using a Varian CP-8200 Autosampler coupled to a Saturn 4D GC/MS/MS ion trap MS (Varian Inc., Walnut Creek, CA, USA) operated in electron ionization (EI) mode (70 eV). It was equipped with a split/splitless injector maintained at 320 °C. The carrier gas was He and injections were made in splitless mode on a DB5, 60 m × 0.32 mm × 0.25 μm film thickness capillary column from J&W Scientific (Folsom, CA, USA). The GC temperature program was 75 °C to 350 °C at 4 °C/min with an initial hold of 1 min, final hold time was 1.25 min. The manifold and the transfer line temperatures were 270 °C and 315 °C, respectively. Scans were 100 to 500 m/z for 6 to 71 min; selected ions were used to quantify the targeted analytes. A seven to ten point calibration curve was used for the analyses of individual analytes with the Varian MS Workstation software package, version 5.2 (Varian). The limit of detection was approximately 0.01 μg/L per analyte. A laboratory blank (deionized water) was processed with each set of water samples.

3. Results and discussion

3.1. Immunization

The PBA-KLH conjugate induced four out of five Balb/c mice to produce hapten-selective antibodies 3 weeks after the initial immunization. The titers of antibodies were enhanced with the increase of inoculation times. After six weeks post-immunization, one of the five mice tested (mouse E) gave the highest sera titer (33,333 U/ml). The sera of mouse E also presented reaction capability to some free PAHs, including phenanthrene, pyrene and benzo(a)pyrene (data not shown). Since the main aim of this study was to develop an anti-PAHs antibody with generic selectivity, mouse E was chosen for B-lymphocyte donation for the subsequent fusion experiments.

3.2. Monoclonal antibody screening

Fifteen to twenty days after cell fusion, 590 colonies were visible to the naked eye in the 96-well tissue culture plates. A three-step screening procedure was applied to develop generic antibodies against PAHs, including indirect ELISA, icELISA and multiple icELISA. Antibodies produced by a hapten-carrier antigen include antibodies against the hapten, the carrier and also various mixtures of these molecules [10]. So the carrier protein used for screening was BSA, which is different from the immunization protein (KLH) thus preventing any positives due to the protein carrier (KLH) during the antigen screening process. Thirty-eight positive hybridomas were screened out by the first step. Finally 4 stable clones were obtained after the entire three-step screening procedure. All of these four clones demonstrated significant inhibition for free PAHs (Table S2). One of the clones produced (2G8) was selected for further detailed examination based on its general selectivity and high sensitivity to a variety of 3–5 ring PAH.

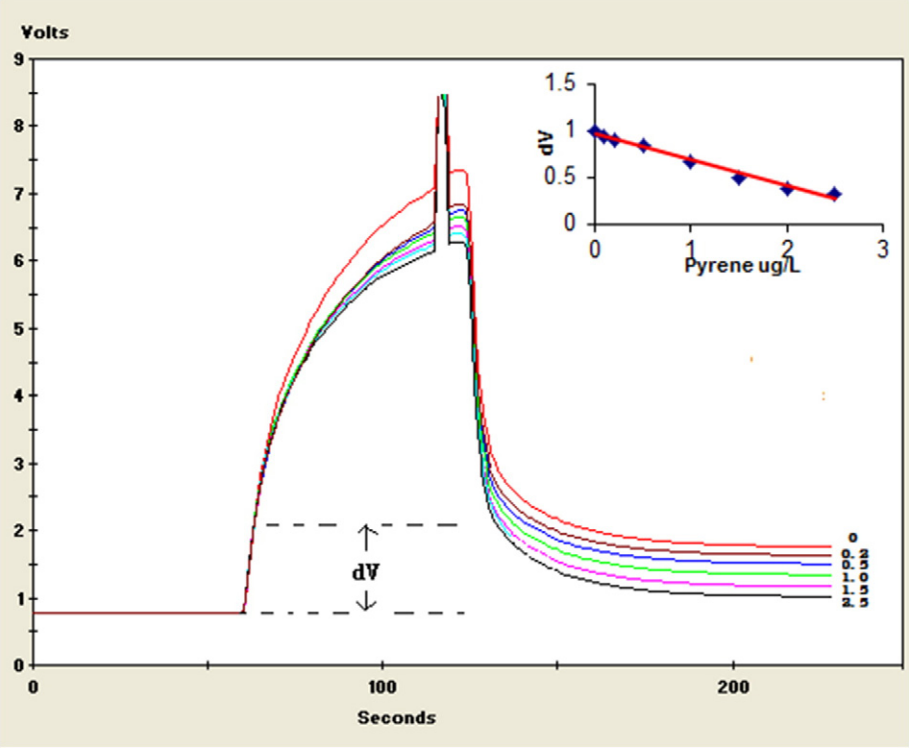

Fig. 3. Trace plots obtained for the Biosensor during the assessment of known concentrations of pyrene (concentration depicted on right, μg/L). *Inset*: The relationship between signal inhibition and concentration of pyrene (standard curve).

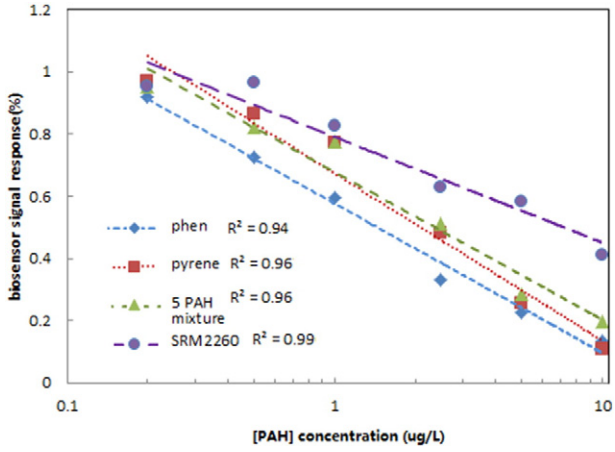

Fig. 4. Calibration curves for solutions containing either phenanthrene, pyrene, SRM 2260 Aromatic Hydrocarbons in Toluene (Table S1) and 5 PAH mixture (phenanthrene, anthracene, chrysene, pyrene, and benzo(a)pyrene). The log-linear lines of best fit are displayed for each solution tested. The coefficients of variation (n = 2) were between 0.9% and 4.8%.

3.3. Evaluation of mAb 2G8

To determine whether the mAb 2G8 was a suitable antibody to be used in the biosensor for total PAHs detection, we evaluated the sensitivity and selectivity of purified mAb 2G8 by icELISAs. Unsubstituted free PAHs as well as commonly detected environmental methylated PAHs were employed as inhibitors in icELISAs. The inhibition curves of 2G8 against 10 different PAHs are presented in Fig. 1. IC_{50} values (concentration of inhibitor that produces a 50% decrease of the maximum normalized response) were marked as an indicator of the antibody sensitivity. 2G8 demonstrated similar low IC_{50} values when unsubstituted PAHs were employed (Fig. 1(A)), with IC_{50} values ranging from 1.68 μg/L to 9.44 μg/L. In addition, 2G8 also demonstrated good inhibitions against methylated PAHs, with IC_{50} values against 2-methylphenanthrene, 9,10-dimethylanthracene, 7,12 dimethybenz(a)anthracene, 4-methyl dibenzothiophene ranging from 1.92 μg/L to 31 μg/L (Fig. 1(B)). The exception was 2,3,5-trimethyl naphthalene. 2G8 was not very sensitive to this compound, with an IC_{50} value of 350 μg/L (data not shown). The results demonstrated that 2G8 has low affinity for 2-ring PAHs.

The validation of the antibody-based immunoassay for total PAH quantification in environmental samples is difficult, as PAHs commonly occur as complex mixtures. It is well documented that methylated species may comprise a large fraction of the PAHs in petrogenic samples [20]. For this reason, the definition of total PAHs was broadened to include the sum of unsubstituted and methylated PAHs [25]. Therefore, 5 methylated PAHs which are frequently found in environmental samples were also tested. The inhibition curves of 2G8 against methylated PAHs indicated that 2G8 is very sensitive against not only unsubstituted PAHs but also for most of methylated PAHs.

The selectivity of 2G8 for the evaluated PAHs was also determined by Krel value, which permits an easy relative comparison of the selectivity between the various analytes. In regards to PBA, which was our original hapten in this study, we defined pyrene as the reference analyte to calculate Krel values (Krel for pyrene was set to 1.00). The Krels of 2G8 for different PAHs are shown in Fig. 2 by radar plots. It can clearly be seen that the length of each ray from the central point is almost the same, which demonstrated 2G8 performed nearly uniformly in regards to Krel values with a wide range of PAHs, including 5 unsubstituted PAHs and 4 methylated PAHs. As an example, the Krel value of 2G8 for anthracene is 0.65 and the Krel value for 9,10-dimethylanthracene is 1.54. Both values are within a factor of the reference value 1.00.

Previous studies have been published evaluating antibody selectivity for the 16 EPA priority pollutants [18,23]. However, binding with methylated PAHs should also be included to evaluate the utility of quantifying total PAH in complex environmental mixtures. Although some of the previous studies showed sensitive antibodies that had some cross-reactivity with selected PAHs, our current antibody, 2G8 has shown nearly uniform selectivity for most 3–5 ring, methylated, and unsubstituted PAHs tested; and is well suited for the analysis of total PAH concentrations in environmental samples.

Focused on the potential application to quantifying complex mixtures, the IC_{50} values of 2G8 were compared with two commercially available PAH mAbs 10C10 and 4D5 [6,14] (Table 1). The results showed that the sensitivity of 2G8 was comparable with commercially available 10C10 and 4D5 against the five PAHs evaluated in this study. The mAbs 10C10 and 4D5 were originally derived by Gomes and Santella [6] using 6-amino-BaP-BSA (BaP 6-isocyanate hapten) as the immunogen. It is probably the reason why 10C10 and 4D5 showed the highest affinity for BaP. Meanwhile, due to the 4 ring PBA-KLH immunogen used in this study, the selectivity of 2G8 for benzo(a)pyrene, pyrene, chrysene, phenanthrene and anthracene were closer, especially the IC_{50} values of 2G8 against anthracene at 5.88 ± 1.29 μg/L.

Antibody selection plays a central role in immunoassay development. Understanding the antibody affinity and selectivity properties determines the applicability of the assay. A good generic PAH antibody would allow accurate total PAH assessments of environmental samples from a wide range of sources as opposed to a selective one. As is discussed above, 2G8 possesses the following features: (1) very sensitive to both 3–5 ring unsubstituted and methylated PAHs (Fig. 1); (2) nearly uniformly recognition with different classes of PAHs (Fig. 2); (3) superior IC_{50} values and more consistent selectivity compared with commercially available anti-PAH antibodies (Table 1). These findings imply that the generic anti-PAH antibody 2G8 was an excellent candidate to employ in our biosensor system.

3.4. Calibration curves for the 2G8 biosensor

To evaluate the application of 2G8 in the biosensor, pyrene standard solutions were used to assess the signal response of 2G8 across a range of PAH concentrations (Fig. 3). A 2G8 concentration of 3.8 μg/ml resulted in a voltage change (dV) of approximately 1 V in blank water samples. The biosensor relies on a competitive inhibition of antibody binding to free PAH in solution vs. bound PAH within the detection cell which is similar to an icELISA, therefore, the biosensor signal (dV) decreases with the increase of the inhibitor (pyrene) concentration. In addition, there is a linear relationship between pyrene concentration and dV.

Phenanthrene, pyrene, the 5 PAHs mixture and the SRM2260 were employed to generate calibration curves. The log-linear curves for the four calibration standards are shown in Fig. 4. The concentrations of these solutions ranged from 0.2 to 10.0 μg/L and each standard was

Table 2
Comparison of PAH determinations between biosensor and GC–MS for environmental samples.

Sample ID[a]	Biosensor PAH μg/L	GC–MS PAH	
		3–5 ring μg/L	Total μg/L
MP5-1	13.36	20.28	66.93
MP5-3	1.90	4.91	6.37
MP5-4	7.53	9.38	19.91
MP5-5	5.22	7.70	12.53
MP5-7	5.81	8.48	12.38
MP5-8	98.72	85.69	1131.19
MP5-9	7.67	11.13	112.76
ATL Wood Sed H_2O	47.57	37.17	140.46
Prudhoe Crude Oil	36.03	16.03	314.38
Creosote	379.23	285.48	2301.19

[a] Sample IDs MP5 1–9 are sediment pore water samples.

analyzed in duplicate. Three of the calibration curves have comparable slopes with good correlation ($r^2 = 0.94–0.99$). Near overlapped calibration curves were obtained from phenanthrene, pyrene and 5 PAH mixture, which demonstrates that the biosensor signal response is similar when either pyrene or a 3–5 ring PAH mixture is employed for calibration. However, the calibration curve of SRM2260 deviates slightly from the other curves with less detector response due to the presence of 2 ring PAHs in the SRM2260 mixture (Table S1) that are not recognized by our 2G8 antibody.

3.5. Environmental sample analysis

To evaluate the accuracy of the 2G8 biosensor for the quantification of total PAHs in a variety of environmental samples, the results from the biosensor and conventional GC–MS were compared. A total of 10 environmental samples ranging from water-soluble fractions derived from oil and creosote to sediment pore waters were analyzed (Table 2). The PAH concentrations of samples ranged from less than 10 to over 1000 μg/L. Samples that exceeded the upper limit of calibration (2.5 μg/L) were diluted with deionized water. Comparable results were obtained for total 3–5 ring PAH concentrations with the biosensor and GC–MS (Table 2). However, if we include 2-ring PAH concentrations in the results from GC–MS, the limitations of the selectivity of 2G8 to 3–5 ring compounds are evident. We continue to work on development of mAbs against 2 ring PAHs to enhance the ability to detect these compounds that are present in high concentrations in some environmental samples. The biosensor results for the water soluble fraction derived from oil are higher than GC–MS and are likely explained by the complex nature of this sample that included many substituted PAHs that were below the quantitation limit of GC–MS but were detected by the antibody and contributed to the reported total.

4. Conclusion

A very sensitive generic mAb (2G8) against both 3–5 ring unsubstituted and methylated PAHs has been developed through a three-step hybridoma screening procedure. 2G8 has been successfully applied on the KinExA Inline Biosensor for quantifying total 3–5 ring PAH concentrations in a variety of aqueous environmental samples including sediment pore water. The high sensitivity and small sample volumes (1–5 mls) required by the biosensor allows convenient analysis of difficult to obtain environmental samples such as sediment pore waters. Total 3–5 ring PAH quantification compared favorably with GC–MS and the method can also be applied as a screening tool for sample prioritization for further GC–MS analysis when compound specific analysis is required. The method is low cost on a per sample basis, is rapid when compared with conventional chromatographic methods and shows great promise for rapid monitoring of PAH pollution.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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References

[1] Agency for Toxic Substances and Disease Registry, Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs), Department of Health and Human Services, Atlanta, GA, USA, 1995.
[2] G.P. Anderson, N.L. Nerurkar, Improved fluoroimmunoassays using the dye Alexa Fluor 647 with the RAPTOR, a fiber optic biosensor, J. Immunol. Methods 271 (2002) 17–24.
[3] L. Anfossi, M. Calderara, C. Baggiani, C. Giovannoli, E. Arletti, G. Gianfranco, Development and application of solvent-free extraction for the detection of aflatoxin M1 in dairy products by enzyme immunoassay, J. Agric. Food Chem. 56 (2008) 1852–1857.
[4] M.G. Barron, T. Podrabsky, S. Ogle, R.W. Ricker, Are aromatic hydrocarbons the primary determinant of petroleum toxicity to aquatic organisms? Aquat. Toxicol. *46* (1999) (253–268).
[5] E.S. Bromage, T. Lackie, M.A. Unger, J. Ye, S.L. Kaattari, The development of a real-time biosensor for the detection of trace levels of trinitrotoluene (TNT) in aquatic environments, Biosens. Bioelectron. 22 (2007) 2532–2538.
[6] M. Gomes, R.M. Santella, Immunologic methods for the detection of benzo[a]pyrene metabolites in urine, Chem. Res. Toxicol. 3 (1990) 307–310.
[7] Z. Grabarek, J. Gergelya, Zero-length crosslinking procedure with the use of active esters, *Anal. Biochem.* 185 (1) (1990) 131–135.
[8] E. Harlow, D. Lane, Monoclonal antibodies, *Antibodies*: *A Laboratory Manual*, New York, Cold Spring Harbor Laboratory Press 1988, pp. 139–243.
[9] S.S. Hecht, S.G. Carmella, P.W. Villalta, J.B. Hochalter, Analysis of phenanthrene and benzo[a]pyrene tetraol enantiomers in human urine: relevance to the bay region diol epoxide hypothesis of benzo[a]pyrene carcinogenesis and to biomarker studies, Chem. Res. Toxicol. 23 (2010) 900–908.
[10] A.H. Heussner, I. Moeller, B.W. Day, D.R. Dietrich, E. O'Brien, Production and characterization of monoclonal antibodies against ochratoxin B, Food Chem. Toxicol. 45 (5) (2007) 827–833.
[12] N. Kobayashi, H. Oiwa, K. Kubota, S. Sakoda, J. Goto, Monoclonal antibodies generated against an affinity-labeled immune complex of an antibile acid metabolite antibody: an approach to noncompetitive hapten immunoassays based on anti-idiotype or anti-metatype antibodies, J. Immunol. Methods 245 (2000) 95–108.
[13] K.G. Kropp, P.M. Fedorak, A review of the occurrence, toxicity, and biodegradation of condensed thiophenes found in petroleum, Can. J. Microbiol. 44 (1998) 605–622.
[14] K. Li, R. Chen, B. Zhao, M. Liu, A.E. Karu, V.A. Roberts, Q.X. Li, Monoclonal antibody-based ELISAs for part-per-billion determination of polycyclic aromatic hydrocarbons: effects of haptens and formats on sensitivity and specificity, Anal. Chem. 71 (1999) 302–309.
[15] M.H. Lin, Y.J. Liu, Z.H. Sun, S.L. Zhang, Z.H. Yang, C.L. Ni, Electrochemical immunoassay of benzo[a]pyrene based on dual amplification strategy of electron-accelerated Fe_3O_4/polyaniline platform and multi-enzyme-functionalized carbon sphere label, Anal. Chim. Acta 722 (2012) 100–106.
[16] Y.Y. Lin, G.D. Liu, C.M. Wai, Y.H. Lin, Magnetic beads-based bioelectrochemical immunoassay of polycyclic aromatic hydrocarbons, Electrochem. Commun. 9 (2007) 1547–1552.
[17] E. Manoli, C. Samara, Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis, Trends Anal. Chem. 18 (1999) 417–428.
[18] D. Matschulat, A. Deng, R. Niessner, D. Knopp, Development of a highly sensitive monoclonal antibody based ELISA for detection of benzo[a]pyrene in potable water, Analyst 130 (2005) 1078–1086.
[19] S. Pérez, M. la Farré, M.J. Garćia, D. Barceló, Occurrence of polycyclic aromatic hydrocarbons in sewage sludge and their contribution to its toxicity in the ToxAlert® 100 bioassay, Chemosphere 45 (2001) 705–712.
[20] C. Pies, T.A. Ternes, T. Hofmann, Identifying sources of polycyclic aromatic hydrocarbons (PAHs) in soils: distinguishing point and nonpoint sources using an extended PAH spectrum and n-alkanes, J. Soils Sediments 8 (2008) 312–322.
[21] D.L. Poster, M.M. Schantz, L.C. Sander, S.A. Wise, Analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples: a critical review of gas chromatographic (GC) methods, Anal. Bioanal. Chem. 386 (2006) 859–881.
[22] D. Pressman, A. Gorossberg, *The structural basis of antibody specificity*, in: E. Davis (Ed.), Microbial and Molecular Biology Series, W A Benjamin Inc., New York, 1968.
[23] T. Scharnweber, M. Fisher, M. Suchanek, D. Knopp, R. Niessner, Monoclonal antibody to polycyclic aromatic hydrocarbons based on a new benzo[a]pyrene immunogen, Fresenius J. Anal. Chem. 371 (2001) 578–585.
[24] C.R. Spier, E.S. Bromage, T.M. Harris, M.A. Unger, S.L. Kaattari, The development and evaluation of monoclonal antibodies for the detection of polycyclic aromatic hydrocarbons, Anal. Biochem. 387 (2009) 287–293.
[25] C.R. Spier, G.G. Vadas, S.L. Kaattari, M.A. Unger, Near real-time, on-site, quantitative analysis of PAHs in the aqueous environment using an antibody-based biosensor, Environ. Toxicol. Chem. 30 (2011) 1557–1563.
[26] G.M. Troisi, L. Borjesson, Development of an immunoassay for the determination of polyaromatic hydrocarbons (PAHs) in plasma samples from oiled sea birds, Environ. Sci. Technol. *38* (2005) 3748–3755.
[27] M.A. Unger, E. Harvey, G.G. Vadas, M. Vecchione, Persistent pollutants in nine species of deep-sea cephalopods, Mar. Pollut. Bull. 56 (2008) 1498–1500.
[28] H. Yu, R.M. Jones, D.A. Blake, An immunosensor for autonomous in-line detection of heavy metals: validation for hexavalent uranium, Int. J. Environ. Anal. Chem. (2005) 85.