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TECHNICAL NOTE

Detection of bisphenol A using a novel surface plasmon resonance biosensor

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Abstract We present a compact surface plasmon resonance (SPR) biosensor for the detection of bisphenol A (BpA), an endocrine-disrupting chemical. The biosensor is based on an SPR sensor platform (SPRCD) and the binding inhibition detection format. The detection of BpA in PBS and wastewater was performed at concentrations ranging from 0.05 to 1,000 ng/ml. The limit of detection for BpA in PBS and wastewater was estimated to be 0.08 and 0.14 ng/ml, respectively. It was also demonstrated that the biosensor can be regenerated for repeated use. Results achieved with the SPR biosensor are compared with those obtained using ELISA and HPLC methods.

Keywords Endocrine disruptors · Bisphenol A · Biosensor · SPR sensor

Introduction

Endocrine-disrupting chemicals (EDCs), including bisphenol A (BpA), are a class of pollutants which have been examined for over a decade. EDCs are able to mimic or antagonize the effects of endogenous hormones in an organism [1]. BpA is an organic compound commonly used as an intermediate in the production of polycarbonate plastics, epoxy coatings, etc. Due to its widespread use, BpA has become a significant environmental contaminant present in wastewater [2], river water, and sediments [3, 4]. Conventional methods for the determination of BpA in environmental samples include high-pressure liquid chromatography (HPLC) [5], liquid chromatography [6], and gas chromatography [7] coupled with mass spectrometry. In recent years, immunochemical methods, such as enzyme-linked immunosorbent assay (ELISA), have been applied to the detection of BpA, with the limits of detection ranging from 0.05 to 500 ng/ml [8]. However, these methods are rather laborious and time consuming. Therefore, there is a need for rapid, low-cost screening methods for the detection of EDCs in environmental samples [9, 10]. Biosensors, such as quartz crystal microbalance [11] and surface plasmon resonance (SPR) biosensors [12] present attractive alternatives to laboratory methods. Typical limits of detection for SPR biosensors for BpA are around 1 ng/ml [13, 14].

We present a rapid and sensitive method for the determination of BpA in wastewater using an SPR biosensor. The SPR biosensor utilizes a special surface plasmon resonance coupler and disperser (SPRCD) structure and the binding inhibition detection format. The results of the SPR measurements are compared to those obtained using ELISA and HPLC methods.

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Experimental

Materials

BpA and buffers: sodium acetate (SA; 10 mM, pH 5.0), phosphate-buffered saline (PBS; 10 mM phosphate, 2.9 mM

KCl, 137 mM NaCl, pH 7.4), PBS containing 0.75 M NaCl (PBNa; 10 mM phosphate, 2.9 mM KCl, 0.75 M NaCl, pH 7.4), and sodium hydroxide (NaOH), methanol (MeOH) were purchased from Sigma-Aldrich, Czech Republic. HS-$(CH_2)_{11}$-$(O(CH_2)_2)_4$-OH (OH-OEG_4-AT), and HS-$(CH_2)_{11}$-$(O(CH_2)_2)_6$-OCH_2-COOH (COOH-OEG_6-AT) were purchased from ProChimia, Poland. Ethanolamine hydrochloride (EA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Biacore, Sweden. Conjugate of BpA with bovine serum albumin (BSA–BpA) was produced by Vidia, Czech Republic. Polyclonal chicken antibody for BpA (anti-BpA) was provided by HenAntibody, Czech Republic.

SPR biosensor

We employed a portable six-channel SPR biosensor based on a special diffractive structure referred to as the surface plasmon resonance coupler and disperser (SPRCD) [15]. For the detection of BpA using the binding inhibition format, the BSA–BpA conjugate was immobilized on the SPRCD sensor surface. Prior to the immobilization, the sensor chip was rinsed with absolute ethanol and deionized water, dried with a stream of pure nitrogen and placed in an O_2-plazma cleaner in order to remove organic contaminants. After completing the plasma cleaning process, the surface was blown with a stream of nitrogen. Then the surface was functionalized with a mixed self-assembled monolayer (SAM) by incubating the cleaned substrate in degassed 80% ethanol with a mixture (7:3) of OH-OEG_4-AT and COOH-OEG_6-AT alkanethiols. After immersing the chips in a mixed thiol solution (200 μM) and heating it up to 40 °C for 10 min, the chips were stored in a dark place at room temperature for up to 1 day. The COOH-OEG_6-AT alkanethiols terminated with a carboxylic head group were used to anchor BSA–BpA by amino coupling, while OH-OEG_4-AT alkanethiols terminated with a hydroxyl group were used to form a stable low-fouling background. Subsequently, the chip was removed from the solution, rinsed with absolute ethanol and deionized water, and dried with a stream of nitrogen. The rest of the immobilization procedure was performed with the chip loaded in the SPRCD instrument. The terminal carboxylic groups on the mixed SAM-coated surface were activated using a (1:1) mixture of NHS (11.51 mg/ml) and EDC (76.68 mg/ml) for 5 min. BSA–BpA (5 μg/ml) dissolved in SA buffer was flowed along the sensor surface for 35 min to attach BSA–BpA to the sensor surface. The high ionic strength PBNa buffer was used to remove non-covalently bound BSA–BpA conjugates. Finally, the sensor surface was treated with 1 M EA to deactivate residual carboxylic groups. The immobilization procedure was performed at a flow rate of 30 μl/min and a temperature of 25 °C.

Detection of BpA

The detection of BpA was performed using the binding inhibition detection format. In this format, a sample containing BpA is incubated with a fixed concentration of anti-BpA antibody and then the amount of unreacted anti-BpA is detected by the sensor with the BSA–BpA conjugate immobilized on the sensor surface. To determine the appropriate concentration of anti-BpA for the inhibition assay, different concentrations of anti-BpA in PBS were run across the sensing surface and the sensor response was measured.

Initially, the sensor response to known concentrations of BpA in PBS was measured to establish a calibration curve. Prior to each measurement, stock solutions of BpA in methanol were prepared. By diluting the stock solutions 100 times with PBS, a set of standard samples with a BpA concentration ranging from 0.05 to 1,000 ng/ml was prepared. The BpA samples were mixed with a fixed concentration of anti-BpA and incubated for 15 min under gentle stirring. The detection experiments consisted of the following steps: establishing a baseline, detection of the unreacted antibody, wash-out, regeneration, and establishing a new baseline. The baseline was established by flowing PBS across the sensor surface coated with BSA–BpA for 10–15 min. Then, either a pure anti-BpA at fixed concentration (reference channel) or its mixture with a BpA sample (sensing channel) was flowed through the flow-chamber for 15 min, followed with PBS to wash the loosely bound molecules away from the sensor surface. Regeneration of the sensor surface was performed by injecting 10 mM NaOH for 5–10 min, followed by 10–15 min washing with PBS to establish a new baseline (Fig. 1). The

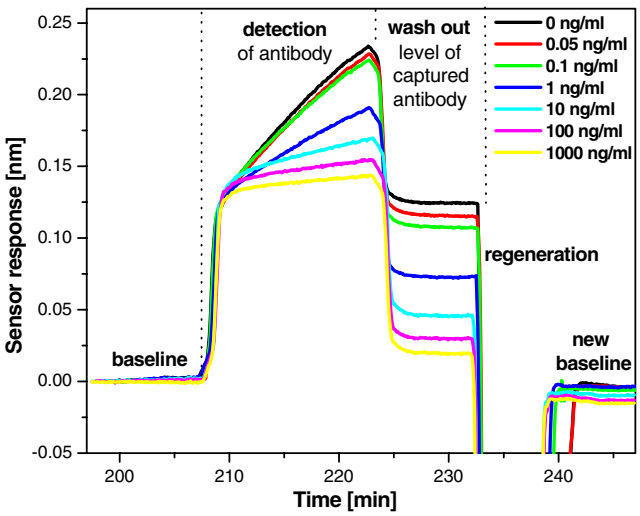

Fig. 1 Sensor response to BpA in PBS for different concentrations of BpA (0.05–1,000 ng/ml)

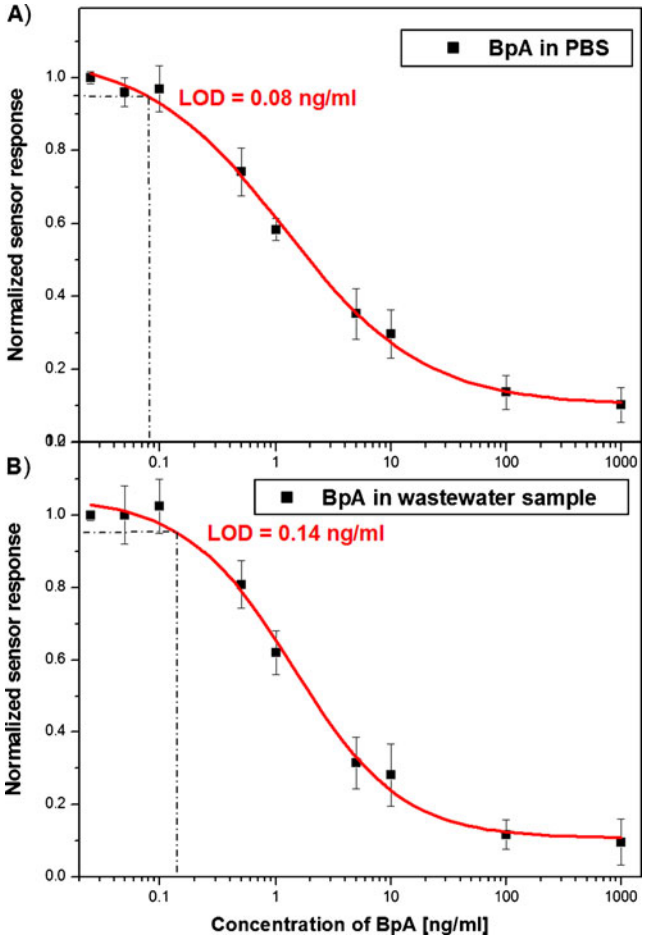

Fig. 2 Calibration curves for BpA in PBS and wastewater samples. The sensor response was normalized with the response obtained for blank samples in each cycle

sensor response was determined as the difference of the sensor response in PBS acquired before and after the injection of the sample. Prior to the detection of BpA samples, reference anti-BpA was injected into each channel to compensate for potential discrepancies among the sensing channels. In each detection cycle, five sensing channels were used to analyze the sample while the sixth channel was used as a reference channel through which pure anti-BpA solution diluted in PBS with 1% MeOH was flowed. The sensor response to the binding of the antibody in the absence of BpA was used to normalize the sensor response for each sample in any particular detection cycle.

Prior to analysis with an SPRCD biosensor, wastewater samples were homogenized and filtered through glass fiber filters (GFAPFF04700, GFAPFC047C0, GFAP2504700). An SPE column (SB-S1000-TAK-S) was washed with 1 ml of MeOH and 1 ml of distilled water; then the filtered water was added. Subsequently, the column was washed with 1 ml of 5% MeOH in H_2O and eluted 1 ml 100% MeOH. The eluate was evaporated under nitrogen and dissolved in 100 μl DMSO followed by 100% MeOH to final volume 1 ml. The solution was split into eight containers and each was spiked with a different concentration of BpA (100,000; 10,000; 1,000; 500; 100; 50; 10; and 5 ng/ml). Prior to the SPR experiments, the solutions were diluted a 100 times with PBS. The detection of BpA in wastewater was performed using the same procedure as that used for the detection of BpA in PBS.

Results and discussion

All the experimental data were processed as follows. Initially, each channel was normalized to the level of the fixed concentration of anti-BpA captured in each sensing channel prior to the detection experiments. Afterwards, the sensor responses to mixtures of BpA and anti-BpA in measuring channels were normalized to the response produced by the reference channel (in the appropriate measurement cycle) to account for potential aging of the sensing surface. Calibration curves for BpA in PBS and wastewater samples were determined from six independent chips. Each BpA sample was measured in duplicate on each chip. The obtained calibration curves for BpA in PBS and wastewater samples were fitted with four-parameter logistic function $\left(f(c) = \frac{(A_1 - A_2)}{1+(c/c0)^p} + A_2\right)$ and are presented in Fig. 2. We believe that standard deviations for individual BpA concentrations were in large part affected by inaccuracies in the dilution steps. The limit of detection (LOD) was determined as the concentration for which the sensor response was equal to three standard deviations of the sensor response in the absence of BpA. The LOD for BpA in PBS and wastewater samples was estimated to be 0.08 ng/ml and 0.14 ng/ml, respectively. The slightly higher value of LOD for BpA in wastewater compared to that in PBS is believed to

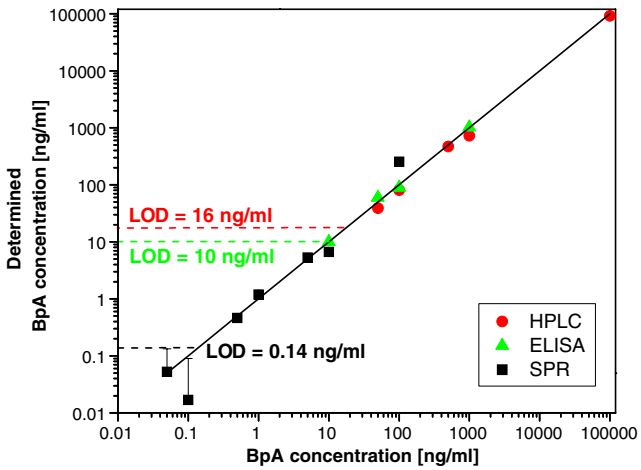

Fig. 3 Determination of BpA in wastewater samples using SPR, HPLC and ELISA methods

be associated with the non-specific adsorption of molecules present in wastewater samples to the sensor surface. The repeatability of the sensor was evaluated for a series of chips. The response of the SPRCD biosensor to the anti-BpA antibody was found to fall within 13% (the relative standard deviation) of the mean value. Our experiments also demonstrated that the biosensor can be used for at least ten detection cycles without a significant loss in the sensor sensitivity (data not shown).

ELISA and HPLC methods were used to analyze the same wastewater samples and the results were compared with those obtained with the SPR biosensor (Fig. 3). A commercial BpA competitive ELISA kit (VIDIA) was used for the detection of BpA in wastewater samples diluted 10× in a VIDIA Dilution buffer. The optical density was measured with an ELISA reader SLT Spectra. The concentrations of BpA were determined from the calibration curve interrelating the output signal (optical density) and the concentration of BpA in BpA standards. The LOD for BpA in wastewater samples for the ELISA method was determined to be 10 ng/ml. HPLC quantification of BpA in wastewater samples was performed using a Waters HPLC system. The analyte was separated in a Waters PAH C_{18} 5 μm column using aqueous acetonitrile gradient elution and measured at λ=280 nm. The calibration curve was determined using linear regression analysis. The correlation coefficient was determined to be R^2=0.999. The LOD for BpA was estimated from the limit of quantification (LOQ= 39 ng/ml) of the used HPLC method to be about 16 ng/ml.

As follows from Fig. 3, concentrations of BpA in wastewater samples determined by the SPR biosensor agree closely with those obtained using ELISA and HPLC. The SPR biosensor is considerably more sensitive than the ELISA and HPLC methods; in terms of the limit of detection, the SPR biosensor surpasses ELISA and HPLC by a factor of 70 and 110, respectively.

Conclusions

The detection of BpA in PBS and wastewater samples was performed using an SPR sensor and the binding inhibition detection format. The limits of detection were determined to be 0.08 ng/ml and 0.14 ng/ml for BpA in PBS and wastewater samples, respectively. The results obtained using the SPR sensor were compared with those obtained with the ELISA and HPLC methods. The limits of detection for competitive ELISA and HPLC were estimated to be 10 and 16 ng/ml, respectively. Moreover, it was demonstrated that the SPR sensor surface can be regenerated at least ten times using 10 mM NaOH without diminishing the activity of the immobilized BSA–BpA. These results suggest that the developed SPR biosensor may provide an efficient platform for the rapid and sensitive detection of BpA in environmental samples.

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