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COMMUNICATION

Surface plasmon resonance biosensor for the ultrasensitive detection of bisphenol A

Chadtová Song Xue[1] · Gedeonová Erika[1] · Homola Jiří[1]

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Abstract
Bisphenol A (BpA) is a chemical that is extensively used in common plastic products, such as food and drink containers. It can leach from the plastics and penetrate into the human body, where it acts as an endocrine disruptor with significant risks to human health. In order to minimize the exposure of human populations to BpA, methods for the detection of BpA are needed. In this work, we present a novel surface plasmon resonance (SPR) biosensor for ultrasensitive detection of BpA. Our approach combines a binding inhibition assay with functionalized gold nanoparticles for the enhancement of sensor response. We demonstrate that the developed biosensor enables the detection of BpA in a wide range of concentrations (0.01 to 100,000 ng/mL) with an extremely low limit of detection—5.2 pg/mL.

Keywords Bisphenol A · Surface plasmon resonance biosensor · Binding inhibition assay · Functionalized gold nanoparticles

Introduction

Bisphenol A (BpA, 2,2-bis (4-hydroxyphenyl) propane) is an industrial chemical that is used in the synthesis of polycarbonate plastics, epoxy resins, and other polymeric materials, which are major components of commonly used plastic items, including food and beverage containers, microwave ovenware, or baby feeding bottles. It has been established that residues of unreacted BpA can leach from these containers into their content (foods, drinks) [1, 2] and thus penetrate into the human body, where it may interfere with the human endocrine system. It has been demonstrated that exposure of the human organism to even low concentrations of BpA may result in developmental, neurological, reproductive, and other health problems [3, 4]. Based on these findings, the European Food Safety Authority (EFSA) has set the tolerable daily intake of BpA as 4 μg per 1 kg of body weight per day (European Food Safety Authority CEF, 2015). Therefore, the development of rapid, robust, and cost-effective methods for the detection of low levels of BpA is being pursued worldwide. Nowadays, the detection of BpA is performed by several well-established analytical methods, such as high-pressure liquid chromatography (HPLC), mass spectrometry coupled with liquid chromatography (LC-MS), or gas chromatography (GC) [5]. However, these methods are rather time-consuming and require cumbersome laboratory equipment with high operating costs and complex sample preparation [5]. Hence, various approaches based on sensors and biosensors [6] have been developed as an attractive alternative to conventional methods. While optical biosensors based on fluorescence provide high sensitivity and enable detection of BpA down to tens of picogram per milliliter [7, 8], they require the addition of labels to generate the sensor response. Therefore, the development of optical label-free methods, among which the surface plasmon resonance (SPR) biosensor is one of the most advanced, has also been pursued. As the sensor response generated by small molecules such as BpA is usually not sufficient to allow for the detection of small analytes at low levels using standard detection schemes, alternative approaches based on BpA-induced conformational changes in the functional molecular coating [9] or indirect assay formats have been used. Hegnerova et al. used the binding inhibition format and a SPR label-free biosensor to detect BpA in drinking water with a limit of detection as low as 40 pg/mL [10] or in wastewater with a limit of detection of 140 pg/mL [11]. In recent years, there have been numerous attempts to improve the limit of detection (LOD) by using functionalized gold nanoparticles, especially in the sandwich

✉ Homola Jiří
homola@ufe.cz

[1] Institute of Photonics and Electronics, Czech Academy of Sciences, Chaberská 57, 182 51 Prague, Czech Republic

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detection formats [12, 13]. However, the use of functionalized nanoparticles in the binding inhibition detection formats has so far been rather seldom [14, 15].

In this work, we present a SPR biosensor that combines the binding inhibition detection format with functionalized gold nanoparticles to enable rapid detection of trace amounts of BpA.

Materials and methods

BpA, bovine serum albumin (BSA), streptavidin from *Streptomyces avidinii*, methanol (MeOH), and all buffers (sodium acetate (SA10; 10 mM, pH 5.0), phosphate-buffered saline (PBS; 10 mM phosphate, 2.9 mM KCl, 137 mM NaCl, pH 7.4), high ionic strength PBS containing 0.75 M NaCl (PBS_{high}; 10 mM phosphate, 2.9 mM KCl, 0.75 M NaCl, pH 7.4)) were purchased from Sigma-Aldrich, Czech Republic. Conjugate of BpA with BSA (BpA-BSA; 3.5 mg BpA/10 mg BSA), and polyclonal antibody against BpA (Ab(BpA); egg fraction with total protein concentration of 40 mg/mL) were produced by Vidia, Czech Republic. Oligo-ethylene glycol thiols 11-mercapto-hexa (ethyleneglycol) undecyloxy acetic acid ($HS\text{-}C_{11}\text{-}(EG)_6\text{-}OCH_2\text{-}COOH$) and 11-Mercapto-tetra (ethyleneglycol) undecanol ($HS\text{-}C_{11}\text{-}(EG)_4\text{-}OH$) 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. EZ-Link™ $NHS\text{-}PEG_4$-Biotin was purchased from Thermo Fisher Scientific, Czech Republic. Biotin-modified Ab (BpA) (b-Ab(BpA)) was prepared according to EZ-Link™'s instructions. Streptavidin-modified gold nanoparticles (S-AuNPs) were prepared and functionalized following the procedure described by Springer et al. [12]. All buffers were prepared using deionized Milli-Q water (Merck, Czech Republic).

In this study, we used a six-channel laboratory SPR platform based on the wavelength spectroscopy of surface plasmons (Plasmon VI) with dispersionless microfluidics developed at the Institute of Photonics and Electronics, Czech Academy of Sciences [16]. In this SPR biosensor, the sensor response is expressed as a shift in the wavelength for which the resonance occurs and is directly proportional to the mass of biomolecules attached to the surface of the sensor. The SPR chips used in this study were prepared by coating microscope glass slides (Marienfeld, Germany) with thin layers of titanium (1–2 nm) and gold (48 nm) via e-beam evaporation in vacuum. All experiments were performed at a temperature of 25 °C and a flow rate of 20 μL/min.

The surface of the SPR chip was modified by a self-assembled monolayer (SAM) of mixed thiols on which BpA-BSA conjugate was immobilized using the amino-coupling as described previously [12]. Briefly, a clean SPR chip was immersed in a 3:7 M mixture of $HS\text{-}C_{11}\text{-}(EG)_6\text{-}OCH_2\text{-}COOH$ and $HS\text{-}C_{11}\text{-}(EG)_4\text{-}OH$ (ethanol solution, total concentration of 0.2 mM) and was incubated for 10 min at 40 °C and for at least 12 h at room temperature in the dark. Prior to use, the chip was rinsed with ethanol and Milli-Q, dried with a stream of nitrogen, and immediately mounted into the SPR biosensor. First, a mixture of 12.5 mM NHS and 62.5 mM EDC (in Milli-Q water) was injected (for 10 min) to activate the carboxylic groups. Then, BpA-BSA conjugate (or BSA) at concentration of 30 μg/mL in SA10 buffer was pumped (for 20–25 min) until the surface reached saturation. PBS_{high} buffer was applied (for 5 min) to remove non–covalently attached BpA-BSA from the surface, and finally, 1 M EA was injected (for 10 min) to deactivate unreacted carboxylic groups.

The assay for the detection of BpA was based on the binding inhibition format and consisted of three consecutive steps: (1) incubation of the b-Ab(BpA) with the analyzed sample, (2) binding of the unreacted b-Ab(BpA) to the BpA-BSA immobilized on the SPR chip, and (3) binding of S-AuNPs to the previously captured b-Ab(BpA) (Fig. 1).

In order to establish a calibration curve, sensor response was measured for BpA in PBS with an addition of 250 μg/mL of BSA (PBS_{BSA}) with concentrations of BpA ranging from 0 to 10,000 ng/mL. Prior to each measurement, stock samples with particular concentrations of BpA in 100% methanol were prepared. The stock samples were then diluted by PBS_{BSA} to 1:100, and prior to the detection experiments, they were further diluted by PBS_{BSA} containing a fixed concentration (1:200 dilution) of b-Ab(BpA) to 1:10. The mixture was gently stirred for 10 min and then injected to the flow-cell interfaced with the SPR chip (for 15 min) and the binding of b-Ab(BpA) was recorded. Then, the following solutions were injected: PBS_{BSA} (for 10 min), S-AuNPs (optical density—

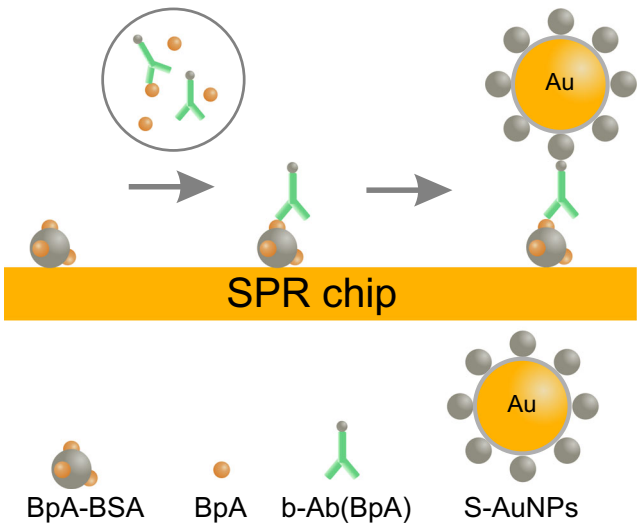

Fig. 1 Assay for detection of BpA

0.1; for 10 min), and PBS_{BSA} (for 5 min). To minimize the interfering effects, all measurements were performed with the reference compensation. The reference sensor response was obtained from a channel in which BSA was immobilized (instead of BpA-BSA) and the channel was exposed to a zero concentration of BpA in the detection step; otherwise, the reference channel was treated in the same manner as the sensing channels. The sensor response was calculated as the difference between the sensor responses in PBS_{BSA} acquired 5 min before and after the S-AuNPs binding. This value was normalized to the BpA-BSA surface coverage in the sensing channel and sensor response obtained in the reference channel was subtracted from that of the sensing channel.

Results and discussion

The reference-compensated sensor responses and the calibration curve obtained for BpA concentrations ranging from 0.001 to 10,000 ng/mL are shown in Fig. 2. Each data point was obtained from at least three independent SPR chips. The curve was fitted using the sigmoidal Boltzmann function. The limit of detection (LOD; BpA concentration corresponding to the sensor response of a blank sample from which a triplicate of its standard deviation was subtracted) was determined to be 5.2 pg/mL. The blank sample was represented by a sample with zero concentration of BpA; standard deviation of the sensor response to the blank sample was determined from eight measurements. The demonstrated LOD is compatible with the monitoring of BpA at the levels set by EFSA—assuming a 70-kg person, who consumes 2.8 L of water per day, the tolerable daily intake for BpA corresponds to a BpA concentration of 100 ng/mL. This makes the developed biosensor a promising candidate for the detection of BpA in liquid samples (e.g., river water or drinking water) and extracts from other foods. The demonstrated LOD also compares favorably with those obtained with SPR biosensors employing binding inhibition format and functional gold nanoparticles for other small molecules (e.g., 82 pg/mL for 17β-estradiol [14] and 18 pg/mL for ochratoxin [15]).

Conclusion

In this work, we report on a novel SPR biosensor that combines a binding inhibition assay with functionalized gold nanoparticles to allow for the detection of trace concentrations of BpA. We demonstrate that the biosensor can detect BpA rapidly (< 60 min) over a wide concentration range (> 6 orders of magnitude) and achieve an extremely low limit of detection (< 6 pg/mL). This limit of detection is the lowest limit of detection for BpA achieved with label-free optical affinity biosensors. In conjunction with emerging portable SPR biosensor platforms, the reported assay presents a promising avenue for rapid and sensitive detection of BpA in the field.

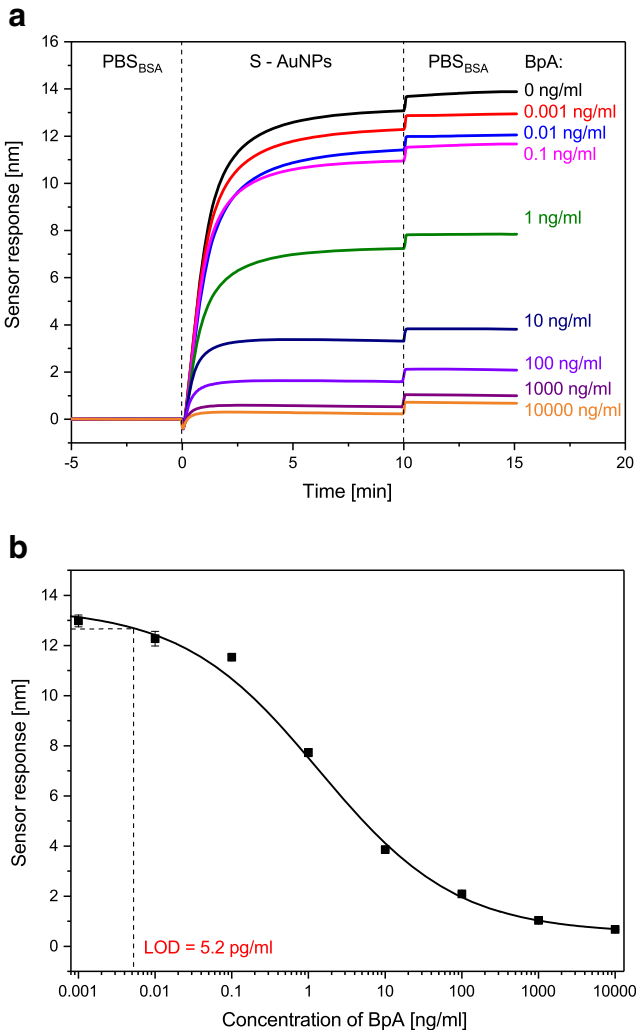

Fig. 2 **a** Reference-compensated sensor responses to the final step of the assay (binding of S-AuNPs) for different concentrations of BpA (0.001–100,000 ng/mL). **b** Calibration curve for the detection of BpA established by binding inhibition assay with S-AuNP enhancement

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Compliance with ethical standards

This study did not involve any human participants or animals.

Conflict of interest The authors declare that they have no conflict of interest.

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