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E-assay concept: Detection of bisphenol A with a label-free electrochemical competitive immunoassay

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ABSTRACT

A label-free electrochemical immunosensor was developed by electropolymerization of *N*-(3-(4-(2-(4-hydroxyphenyl)propan-2-yl)phenoxy)propyl) 3-(5-hydroxy-1,4-dihydro-1,4-dioxonaphthalen-2(3)-yl)propionamide (JugBPA). By combination with an antibody directed to bisphenol A (αBPA), this conducting polymer-based biosensor can detect BPA directly with a limit of detection of 2 pg mL^{-1}. Square wave voltammetry shows that the polymer film presents a current decrease upon anti-BPA binding and an opposite current increase upon BPA addition in solution. This electrochemical immunosensor (E-assay) also shows high selectivity towards closely related compounds (bisphenol A dimethacrylate, and dibutyl phthalate). The E-assay concept described here could be a promising tool for simple, low-cost and reagentless on-site environmental monitoring.

1. Introduction

Bisphenol A (BPA) is found to be an endocrine disruptor due to its estrogen-like properties, which brings adverse effects to human in mimicking or interfering with hormone receptors (Hengstler et al., 2011). People are exposed to BPA through plastic food containers or some paper articles in everyday life (Liao and Kannan, 2011). In addition, BPA was also found to be an environmental pollutant in ecosystems by affecting reproduction and development of aquatic organisms (Kang et al., 2007; Vandenberg et al., 2007). In this context, detection and monitoring of industrial pollutants in food or in the environment is essential.

There have been lots of researches on BPA detection using traditional analytical methods or immunoenzymatic methods such as mass spectroscopy (Brock et al., 2001; Kim et al., 2003), high performance liquid chromatography (Noda et al., 1999), UV–vis spectrophotometry (Poorahong et al., 2012) and enzyme-linked immunosorbent assay (De Meulenaer et al., 2002). These methods present low detection limits and good selectivities, but require expensive instruments and/or prolix processes. Moreover, these methods cannot be easily used for application on a regular basis, so that there is a real challenge to develop alternative or complementary analytical tools. Biosensors appear to be reliable candidates to answer this question.

In the recent years, electrochemical biosensors have attracted more and more attention. A number of electrochemical methods have been used for BPA detection, for instance BPA electro-oxidation using differential pulse voltammetry (DPV), linear sweep voltammetry (LWV), cyclic voltammetry (CV) or chronocoulometry (CC). Enhancement of the electrochemical signal was achieved in recent years by using magnetic beads or nanoparticles (Huang et al., 2012; Niu et al., 2013; Yin et al., 2009; Zhang et al., 2013). Huang et al. developed an electrochemical biosensor based on molecularly imprinted polymers (MIPs) to detect BPA (Griffete et al., 2012; Huang et al., 2011a; Huang et al., 2011b). Impedimetric immunosensors for BPA detection were also reported (Piao et al., 2008; Rahman et al., 2007).

In this work, we developed an electrochemical immunosensor for BPA detection based on a functional conducting copolymer onto which BPA is covalently coupled (Fig. 1). First, a multifunctional monomer, *N*-(3-(4-(2-(4-hydroxyphenyl)propan-2-yl)phenoxy)propyl) 3-(5-hydroxy-1,4-dihydro-1,4-dioxonaphthalen-2(3)-yl) propionamide (JugBPA), was synthesized and characterized (see Supporting information). This monomer can be electropolymerized through the hydroxyl group of the juglone moiety while the quinone group provides a stable and well-defined electroactivity in neutral aqueous medium (Rubin et al., 2010) and the BPA moiety is used as the capture probe for anti-BPA (α-BPA) antibody. Co-electropolymerizaton of 5-hydroxy-1,4-naphthoquinone (juglone) and JugBPA leads to poly(Jug-co-JugBPA) while the

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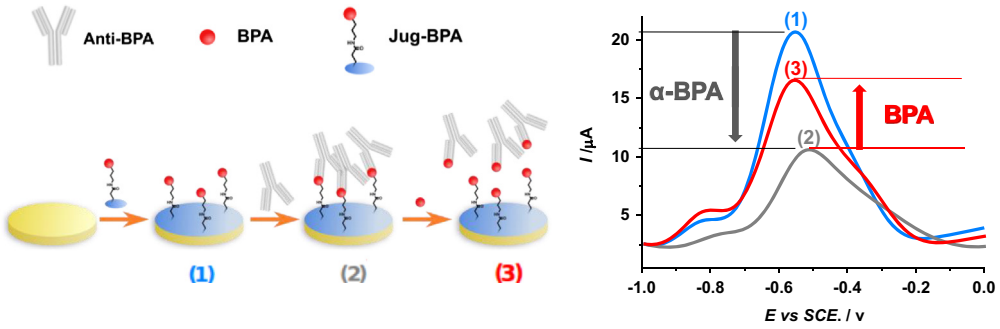

Fig. 1. Working principle of the BPA E-assay.

quinone and BPA groups are preserved within the polymer. The principles that underpin transduction are the following (Fig. 1). The copolymer contains quinone groups which exchange protons and sodium ions (Na^+) in neutral saline media, when oxidized or reduced (Rubin et al., 2010). Besides, BPA coupled to the electroactive polymer acts as a capture probe able to selectively interacts with the binding site of α-BPA, leading to poly(Jug-co-JugBPA/α-BPA). α-BPA being much bigger than the JugBPA probe (150,000 g mol^{-1} vs. 514 g mol^{-1}), it generates strong steric hindrance which decreases the apparent diffusion coefficient of counterions (Piro et al., 2007). Because the redox current of the polymer is limited by diffusion, α-BPA complexation leads to a current decrease (*signal-off*) (Fig. 1, step 2). Conversely, when free BPA is put into contact with poly(Jug-co-JugBPA/α-BPA), a competitive equilibrium occurs (Fig. 1, step 3) between BPA in solution and copolymer-bound JugBPA (α-BPA preferentially binds to free BPA than to JugBPA due to a higher association constant). As a consequence, α-BPA is dissociated from the electrode surface and the current increases back (*signal-on*). This original transduction process based on competitive complexation has been applied successfully to detect atrazine (Piro et al., 2013; Tran et al., 2012). This architecture provides an excellent selectivity because the useful signal can be easily distinguished from the background signal coming from non-specific interactions with the surface (e.g. adsorption).

In this study, we demonstrated a selective determination of BPA over a broad concentration range, with a limit of detection of ca. 2 pg mL^{-1} BPA (10 fM), which is lower than other label-free and reagentless electrochemical sensors for BPA (Piao et al., 2008; Rahman et al., 2007; Xu et al., 2012; Zhang et al., 2013).

2. Experimental

2.1. Chemicals and biological

N,*N*-diisopropylehtylamine (DIEA), 3-bromopropylaminehydrobromide, 5-hydroxy-1,4-naphthoquinone (juglone), 1-naphtol (1-NAP), lithium perchlorate and phosphate buffered saline (PBS, 0.137 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4) were purchased from Sigma Aldrich. Silver nitrate ($AgNO_3$) and succinic acid ($(CH_2–COOH)_2$) are from Fluka; bisphenol A (BPA), bisphenol A dimethacrylate, dibutyl phthalate (DBP), di-tert-butyl dicarbonate (Boc_2O), triethylamine (Et_3N) and trifluoroacetic acid (TFA) are from Alfa Aesar; *N*,*N*'-dicyclohexylcarbodiimide (DCC) is from Acros. All other reagents used (NaOH, HCl, H_2SO_4, K_2CO_3) and solvents (methanol (MeOH), dichloromethane (DCM), diethyl ether (Et_2O), chloroform ($CHCl_3$), dimethyl sulfoxide (DMSO), petroleum ether (PE) and ethyl acetate), were practical grade (PA). Acetonitrile (MeCN) is HPLC grade, distilled over calcium hydride before use. Thin-layer chromatography was performed on TLC plastic sheets of silica gel 60F254 (layer thickness 0.2 mm) from Merck. Column chromatography purification was carried out on silica gel 60 (70–230 mesh ASTM, Merck). Alumina slurry is from ESCIL, Chassieu, France. Aqueous solutions were made with bi-distilled or MilliQ water. α-BPA is a rabbit IgG antibody (MW = 150 kDa) from Interchim, France. Peroxidase-labeled purified secondary antibody to rabbit IgG is from P.A.R.I.S Biotech, France. Bisphenol A (BPA) ELISA kit was provided by Abnova, Taiwan.

2.2. Methods and apparatus

For electrochemical experiments, a conventional one-compartment, three-electrode cell was used with a VMP3 potentiostat from Biologic, the data being collected by EC-lab® software. Glassy carbon (GC) working electrodes (surface area, 0.07 cm^2) were from BASi Analytical Instruments. The counter electrode was a platinum grid and the reference electrode a commercial saturated calomel electrode (SCE, Metrohm). Square wave voltammetry (SWV) was used to characterize α-BPA complexation and BPA detection. The electrolytic solution was argon-saturated PBS (deaerated with argon for 20 min before and during all experiments). The SWV scans were repeated until complete stabilization of the electrochemical signal (i.e. no difference observed between two successive SWV scans). All electrochemical experiments were conducted at room temperature. NMR spectra were recorded in $CDCl_3$ or MeOD at 298 K on a Bruker Avance II 400 MHz spectrometer. Data were processed using TOP-SPIN software (Bruker). Hexamethyldisiloxane (HMDS) was used as an internal standard. Chemical shifts are given in ppm and peak multiplicities are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. MS data were collected from a LCT Premier XE (Waters).

2.3. Synthesis of JugBPA

Syntheses of the intermediate products are described in Supplementary information. First, juglone was carboxyethylated via a published substitution reaction (Piro et al., 2011; Salmon-Chemin et al., 2001) to give 3-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2(3)-yl)propanoic acid, Jug-COOH **1**. To synthesize *N*-(3-(4-(2-(4-hydroxyphenyl)propan-2-yl)phenoxy)propyl) 3-(5-hydroxy-1,4-dihydro-1,4-dioxonaphthalen-2(3)-yl)propionamide **5** (JugBPA), **1** (80 mg, 0.325 mmol) was dissolved in distilled HPLC grade DCM (15 mL) in the presence of DIEA (67.9 μL, 0.39 mmol), DCC (60 mg, 0.39 mmol) was then added. The solution was stirred vigorously for 30 min to allow dicyclohexylurea (DCU) to be formed and 3-(4-(2-(4-hydroxyphenyl)propan-2-yl)phenoxy)1-propylammonium trifluroacetate (80 mg, 0.325 mmol) in DCM (10 mL) was added dropwise. The mixture was refluxed for 2 h. After evaporating DCM, diethyl ether (60 mL) was added and stirred for 30 min at room temperature. After filtration, the

solution was washed by 60 mL 1 M $KHSO_4$, 60 mL 10% $KHCO_3$, and 60 mL H_2O, then dried over $MgSO_4$, filtered and diethyl ether was evaporated. The crude product was purified by column chromatography on silica gel (ethyl acetate/DCM, 1:4, v/v) to afford JugBPA as a brown oil product (63.5 mg, 38% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.92 (s, 1H, OH), 7.60–7.53 (m, 2H, ArH_{JUG}), 7.22 (dd, J=7.6, 2.0 Hz, 1H, ArH_{JUG}), 7.11 (d, J=8.8 Hz, 2H, ArH_{BPA}), 7.08–7.03 (m, 2H, ArH_{BPA}), 6.79 (s, 1H, ArH_{JUG}), 6.76–6.70 (m, 4H,ArH_{BPA}), 6.05 (s, 1H,NH), 3.98 (dd, J=7.7, 3.6 Hz, 2H, CH_2), 3.45 (dt, J=12.1, 6.1 Hz, 2H, CH_2), 2.88 (t, J=5.7 Hz, 2H, CH_2), 2.47 (dd, J=9.2, 5.6 Hz, 2H, CH_2, 1.96 (d, J=6.1 Hz, 2H, CH_2),1.61 (s, 6H, CH_3). HRMS calc. 514.2244, found 514.2230.

2.4. α-BPA affinity to JugBPA

The affinity of JugBPA, as well as BPA to α-BPA was determined using a commercial ELISA kit (Abnova, Taiwan). The data were collected using an Envision 2014 multilabel plate reader (Perkin Elmer). The detailed experimental protocol is given below.

Primary anti-BPA antibody, mixed with various concentrations of BPA or JugBPA in the supplied dilution buffer containing 10% methanol, was added into a 96-well immunoplate coated with BPA (100 μL/well). The incubation was maintained at room temperature for 1 h and the plate was then washed with the same buffer (three times). The diluted secondary antibody (peroxidase-labeled purified antibody to rabbit IgG, 100 μL/well) was added and the incubation was repeated at room temperature for 1 h. After aspirating the liquid from the wells and washing the wells with the buffer solution, TMB substrate (100 μL/well) was added and the plate was kept at room temperature in darkness for 10 min. Stop solution (100 μL/well) was finally added and the absorbance of each well at 450 nm was measured.

2.5. Electrochemical methods

To prepare the working electrodes, their surface were polished with 0.3 μm alumina slurry on microfiber pads for 1 min. The residual alumina particles were removed by sonication in distilled water then MeCN for 30 s, respectively. The electrodes were air-dried before use. The electropolymerization medium was 1×10^{-2} M juglone+1×10^{-3} M JugBPA+1×10^{-3} M 1-naphthol+0.1 M $LiClO_4$ in anhydrous MeCN. Potentials were scanned from 0.6 V to 1.15 V (vs. SCE) at a scan rate of 50 mV s^{-1} for 15 scans. The poly(Jug-co-JugBPA)-modified electrodes were sonicated in MeCN to remove adsorbed juglone and JugBPA monomers. Next, the modified electrodes were put into a cell containing argon-saturated PBS and scanned from −0.95 V to 0.1 V at 50 mV s^{-1} until complete stabilization. Then, square wave voltammograms (SWV) were recorded. All electrochemical studies were performed at room temperature and argon was continuously passed over the solutions during measurements.

2.6. Bioelectrode preparation

2.6.1. α-BPA complexation

For complexation between α-BPA and JugBPA on the electrode surface, 5 μL of 5×10^{-6} M α-BPA were dropped on poly(Jug-co-JugBPA)-modified electrodes at room temperature for 2 h, shielded by an eppendorf tube to avoid evaporation. Then, electrodes were rinsed three times with PBS and immersed in PBS for 1 h at room temperature to remove physisorbed α-BPA. Finally, SWV was performed to record the quinone electroactivity after this complexation step. In order to check the complexation specificity, we also used two control antibodies non-specific to BPA, α-OVA (anti-ovalbumin IgG antibodies) and α-CBZ (anti-carbamazepine IgG antibodies), under the same conditions as applied for α-BPA.

2.6.2. Sensitivity and selectivity of BPA detection

For BPA detection, the electrodes modified with poly(Jug-co-JugBPA)/α-BPA prepared as described in Section 2.6.1 were dipped into BPA solutions (concentrations varying from 0.01 nM to 50 nM in 10% MeOH in water) for 2 h at 37 °C, then rinsed three times with PBS and kept in PBS at 37 °C for 30 min. SWVs were subsequently recorded. Each concentration was triplicated. Selectivity was checked with a structural analog of BPA, bisphenol A dimethacrylate, and another common plastic additive, dibutyl phthalate (DBP), following the same procedure as for BPA detection.

3. Results and discussion

3.1. Affinity of α-BPA to JugBPA and BPA

The combining site of an antibody is able to react with more than one antigen by molecular mimicry; this is called cross-reactivity (Berzofsky and Schechter, 1981). Cross-reacting antigens generally share a structure similar to that of the immunizing antigen, but the antibody presents a lower affinity for them than for the true antigen. This cross-reactivity depends also on the relative concentrations of cross-reactants (Dankwardt, 2001). To compare the affinity of α-BPA to BPA and to JugBPA, ELISA experiences were carried out. As shown in Fig. SI.5, the α-BPA generated from BPA can also bind to JugBPA, but with lower affinity, as expected. From the binding constant of BPA to the α-BPA we used (10^5–10^6, data given by the provider) and from data of Fig. SI.5, we estimated that the binding constant of JugBPA to α-BPA is one order of magnitude lower, i.e 10^4–10^5. The lower affinity of α-BPA to JugBPA would make the competitive detection step easier and more effective; we can take advantage of this to improve the sensitivity/selectivity of the sensor (Khor et al., 2013).

3.2. Electrochemistry of poly(Jug-co-JugBPA)-modified electrodes

3.2.1. Poly(Jug-co-JugBPA) electrosynthesis

The electrochemical synthesis of the copolymer films was carried out by electrooxidation of 1×10^{-2} M juglone+1×10^{-3} M JugBPA+1×10^{-3} M 1-naphthol+0.1 M $LiClO_4$ in anhydrous MeCN on GC electrodes, by 15 potential scans at 50 mV s^{-1} between 0.4 and 1.20 V vs. SCE. The irreversible electrooxidation peak of the monomer occurs at ca. 0.86 V during the first scan, then gradually shifts to higher potentials. The oxidation current keeps increasing (Fig. SI.1), indicating the formation of the copolymer on the electrode surface. The structure of poly(Jug-co-JugBPA) is presented in Fig. SI.2. It consists of a conjugated backbone which includes the quinone groups along with the BPA group on the lateral chain. FT-IR characterization has shown that the JugBPA/Jug ratio is less than 5%. This structure is similar to the one obtained in a previous work with a glutathione-modified juglone (Reisberg et al., 2010).

3.2.2. Electrochemical characterizations

The electroactivity of poly(Jug-co-JugBPA) films was investigated in PBS in the negative potential range corresponding to the quinone electroactivity domain in this medium. As shown in Fig. SI.3, a main couple is detected at −0.54/−0.64 V with two shoulders centered at −0.26/−0.40 V and −0.77/−0.80 V vs. SCE. The main redox couple corresponds to that of quinone at the polymer/solution interface (Jug monomer in PBS presents only one couple at similar potentials) whereas the two others are attributed to the electroactivity of the internal layers of the film. The redox system is highly stable with respect to cycling, and the potential range is sufficiently low to avoid oxidation interferences from

other electroactive species that may be present (including BPA, the oxidation potential of which being around 0.45 V vs. SCE on glassy carbon). The effect of the scan rate on the CV peaks was investigated and shows a clear dependence of the faradic peak current as a function of $v^{1/2}$ (Fig. SI.6), which indicates a diffusion-controlled process.

3.2.3. α-BPA immobilization

SWV was used to characterize the binding of α-BPA to polymer-bound BPA on the electrode. SWVs of a poly(Jug-co-JugBPA)-modified electrode are shown in Fig. 2. The potential was swept from −1.0 V to 0 V vs. SCE (oxidation scan). Three oxidation peaks appear in this range; the main peak presents a maximum around −0.54 V vs. SCE, a smaller peak appears around −0.77 V vs. SCE, and a shoulder is situated at −0.26 V/SCE. These peaks correspond to those observed on CV in Fig. SI.3. Before binding, the current is high for the poly(Jug-co-JugBPA)-modified film (black spot, curve 1), then the formation of the α-BPA/JugBPA complex causes the current to diminish after α-BPA addition (open circles, curve 2), as expected. To exclude any artifact, two unrelated antibodies, α-OVA and α-CBZ, were used as control and the results were compared with that of α-BPA. In all these experiments, an antibody concentration of 5 μM was used. With α-BPA, the current decreased by ca. 55%, whereas it was only ca. 25% for α-OVA, and 28% for α-CBZ (see Fig. 3). One can deduce that barely half the current decrease is non-specific and due to adsorption, which is not surprising considering the heavy weight of antibodies, prone to adsorb on any surface. This is, in analytical terms, a huge drawback, which can be turned into an advantage if using a competitive assay, as described in Section 3.3.

3.3. BPA detection

To detect BPA, poly(Jug-co-JugBPA)/α-BPA-modified electrodes were incubated for 2 h in a BPA solution (10% MeOH). After rinsing the electrodes and keeping them in PBS for 30 min at 37 °C, SWV was performed in Ar saturated PBS. Results are shown in Fig. 2 (triangles, curve 3). The addition of BPA causes the current to increase (45% for [BPA]=10 nM), which indicated that α-BPA is removed from the electrode surface, due to the competitive binding between free BPA in solution and immobilized JugBPA (1).

$$\text{poly(Jug}-\text{co}-\text{JugBPA)/}\alpha\text{–BPA} + \text{BPA}_{\text{sol}} \rightleftarrows \text{poly(Jug} - \text{co} - \text{JugBPA)} + \text{BPA/}\alpha\text{–BPA}_{\text{sol}} \quad (1)$$

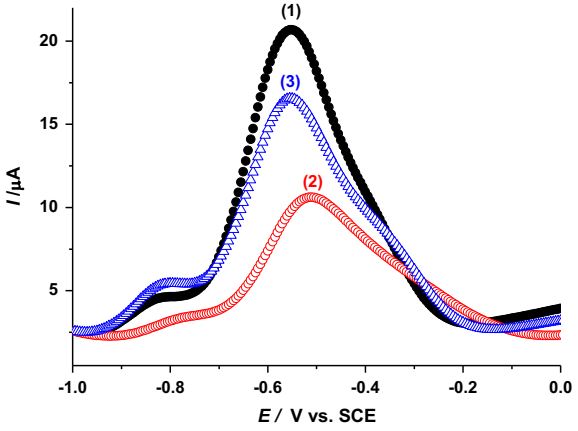

Fig. 2. SWV of poly(Jug-co-JugBPA)-modified GC electrode (black spot ●, curve 1); after complexation with α-BPA, [α-BPA]=5 μM (open circles ○, curve 2), and after addition of BPA, [BPA]=10 nM (triangles △, curve 3).

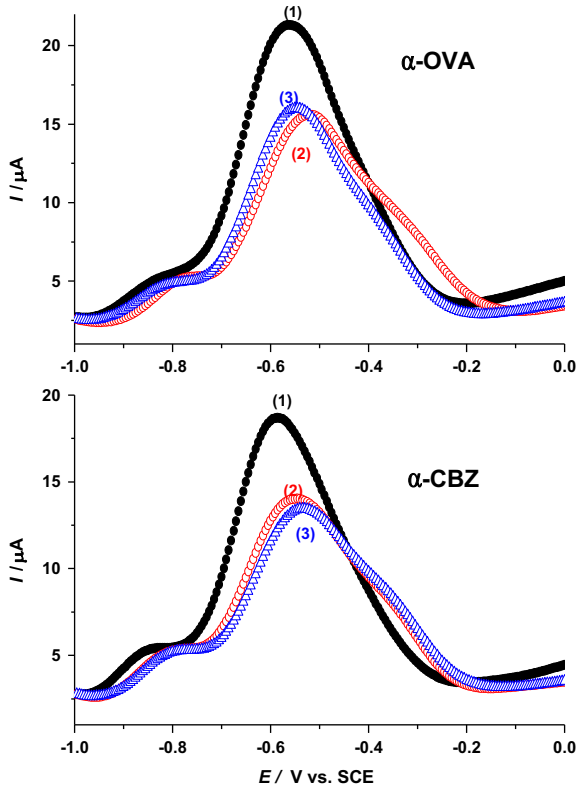

Fig. 3. SWV of poly(Jug-co-JugBPA)-modified GC electrode (black spot ●, curve 1); After addition of α-OVA and α-CBZ, [α-OVA]=[α-CBZ]=5 μM (open circles ○, curve 2); and after addition of BPA, [BPA]=10 nM (triangles △, curve 3). No α-BPA was added before addition of the non-specific antibodies.

It is proposed that the dissociation of the JugBPA/α-BPA complex strongly enhances the ionic flux through the polymer/electrolyte interface, therefore leads to current increase. The lower affinity of α-BPA for JugBPA than for BPA (natural substrate of α-BPA), combined with the higher availability of BPA in solution compared to JugBPA on the surface probably promotes the competitive decomplexation.

3.3.1. Selectivity

To demonstrate the selectivity of the BPA recognition, poly(Jug-co-JugBPA)/α-OVA and poly(Jug-co-JugBPA)/α-CBZ electrodes (as described in Section 3.2.3) were compared to poly(Jug-co-JugBPA)/α-BPA electrodes. Upon addition of 10 nM BPA, the current did not increase significantly for the α-OVA and α-CBZ-treated electrodes but increased of ca. 45% for the α-BPA-treated ones (triangles, curves 3 of Figs. 3 and 2.).

We also checked the selectivity of poly(Jug-co-JugBPA)/α-BPA electrodes to BPA compared with a structural analog, bisphenol A dimethacrylate, and another common plastic additive, dibutyl phthalate (DBP) (structures are given in Fig. 4.) with concentrations varying from 0.01 nM up to 50 nM. Results are shown in Fig. 5. For both BPA dimethacrylate (spots) and DBP (triangles), we observed a slight current decrease instead of the strong current increase observed for BPA (square). These results show that the immunosensor is selective.

3.3.2. Sensitivity

SWV responses were measured for poly(Jug-co-JugBPA)/α-BPA electrodes as a function of the BPA concentration to determine the detection limit of the sensor as well as its sensitivity and linearity domain. Results are shown in Fig. 5 for BPA concentrations

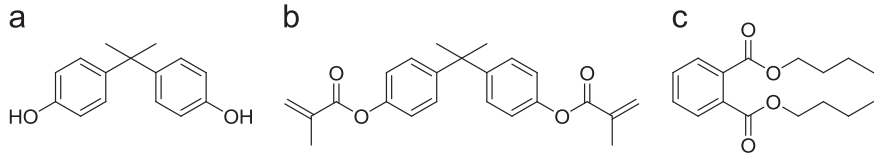

Fig. 4. Structures of BPA, BPA dimethacrylate and dibutyl phthalate. (a) BPA, (b) BPA dimethacrylate and (d) dibutyl phthalate.

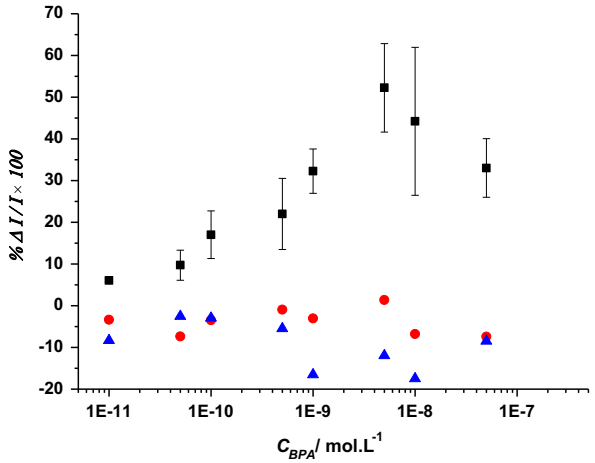

Fig. 5. Percentage of $\Delta I/I$ measured at −0.45 V vs. SCE, after addition of BPA (square ■), BPA dimethacrylate (spot ●) or DBP (triangle ▲) for concentrations from 0.01 nM up to 50 nM. $\Delta I = I_{\text{after decomplexation}} - I_{\text{before decomplexation}}$ and $I = I_{\text{before decomplexation}}$. Data points for BPA addition show mean ± SD ($n=3$).

between 0.01 nM and 50 nM and are expressed as relative current changes ($\Delta I/I$), where $\Delta I = I_{\text{after decomplexation}} - I_{\text{before décomplexation}}$ and $I = I_{\text{before decomplexation}}$. They display a linear relationship between $\Delta I/I$ and [BPA] from 0.05 nM to 5 nM. The current increases to reach a maximum for [BPA] ~5 nM, then slightly decreases for higher concentrations (which is attributed to BPA adsorption on the electrode surface). The detection limit (0.01 nM) corresponds to 2.28 pg mL^{-1} BPA, that is extremely competitive compared to other label-free immunosensors (Xu et al., 2012). This limit of detection is fully compatible with detection in food in contact with plastic containers, the limit being 0.6 mg kg^{-1} (European Food Safety Authority, 2006), and much lower than the actual limit of detection in tap water, i.e. 0.4 ng mL^{-1}.

4. Conclusion

We fabricated an electrochemical immunosensor for BPA with a "signal-on" detection using square wave voltammetry, based on a multifunctional conducting copolymer, poly(Jug-co-JugBPA). Poly (Jug-co-JugBPA) acts both as the complexation and the transduction element. JugBPA retains its properties when included in the polymer backbone and presents higher specificity for α-BPA than for other antibodies like α-OVA or α-CBZ. The presence of the α-BPA/JugBPA complex is characterized by a current decrease attributed to steric hindrance generated by antibodies, which modifies the cations transport rate at the polymer-solution interface and modifies the redox kinetics of the quinone group. The proposed transduction scheme takes advantage of the cross-reactivity of α-BPA, which means that BPA free in solution is able to displace the equilibrium and removes the formerly complexed antibody from the electrode surface, so that the poly(Jug-co-JugBPA) electroactivity is enhanced. The current decrease following α-BPA antibody complexation (or adsorption of any other proteins) is counterbalanced by the current increase upon addition of BPA. A very low detection limit (0.01 nM) is achieved. Two molecules related to BPA, BPA dimethacrylate and DBP, cause a slight signal decrease instead of an increase and are therefore easily discriminated. We are actually working on a generalization of this electrochemical immunosensor (E-assay) to other organic molecules such as drug residues.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.09.062.

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