Chemosphere 180 (2017) 253–258

Contents lists available at ScienceDirect

Chemosphere

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

Spectroscopic and molecular modeling approaches to investigate the interaction of bisphenol A, bisphenol F and their diglycidyl ethers with PPARα

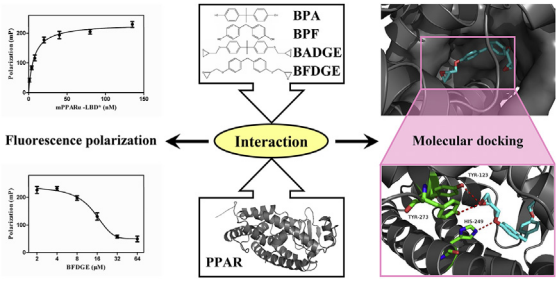
Jie Zhang [a], Tiehua Zhang [b], Tianzhu Guan [b], Ping Ruan [a], Dayong Ren [a], Weichang Dai [a], Hansong Yu [a, *], Tiezhu Li [a, **]

[a] College of Food Science and Engineering, Jilin Agricultural University, Changchun 130118, China
[b] College of Food Science and Engineering, Jilin University, Changchun 130062, China

HIGHLIGHTS

- Interaction of BPA, BPF, BADGE, and BFDGE with mPPARα-LBD* was investigated.
- The derivatives exhibited stronger binding potencies than their parent molecules.
- The mechanism of BPs-mPPARα-LBD* binding was illustrated by molecular docking.
- The proposed method can be used for preliminary screening of these compounds.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form
2 April 2017
Accepted 8 April 2017
Available online 9 April 2017

Handling Editor: Frederic Leusch

Keywords:
Peroxisome proliferator-activated receptor
Bisphenols
Fluorescence polarization
Molecular docking

ABSTRACT

A fluorescence polarization (FP) assay for the simultaneous determination of bisphenol A (BPA), bisphenol F (BPF), bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) was developed. The method was based on the competition between bisphenols (BPs) and fluorescein-labeled dexamethasone derivative (Dex-fl) for mouse peroxisome proliferator-activated receptor α ligand binding domain (mPPARα-LBD). A recombinant soluble protein derivative mPPARα-LBD* was prepared, then *in vitro* binding of 4 BPs to mPPARα-LBD* was investigated. Fluorescence polarization assay showed that these compounds exhibited different binding potencies with mPPARα-LBD*. Additionally, molecular dynamics simulations were performed to further understand the mechanism of BPs binding affinity for mPPARα-LBD*. Docking results elucidated that the driving forces for the binding of BPs to mPPARα-LBD* were predominantly dependent on hydrophobic and hydrogen-bonding interactions. Comparison of the calculated binding energies *vs.* experimental binding affinities yielded a good correlation ($R^2 = 0.7258$). The proposed method has potential for multi-residue detection of BPA, BPF, BADGE, and BFDGE.

1. Introduction

Bisphenol A (BPA) and bisphenol F (BPF) are industrially important chemicals used in the manufacture of polycarbonate plastics and epoxy resins. As the crosslinking agent, diglycidyl ethers based on BPA (BADGE) and BPF (BFDGE) are most commonly

* Corresponding author.
** Corresponding author.
E-mail addresses: yuhansong@jlau.edu.cn (H. Yu), tiezhu_li@163.com (T. Li).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.034
0045-6535/

used epoxy resin monomers (Nerín et al., 2002; Ruiz et al., 2007). It has been reported that all these bisphenols can migrate from interior coatings into canned foodstuffs (Hammarling et al., 2000; Theobald et al., 2000; Goodson et al., 2002; Cabado et al., 2008). Thus, many studies focused on the potential adverse health effects to the consumer when exposed to bisphenol-type contaminants from food packaging materials. The toxicities of BPA, BPF and their corresponding diglycidyl ethers have been proven to be mainly related to their estrogenic, anti-androgenic and cytotoxic effects (Satoh et al., 2004; Ramilo et al., 2006; O'Boyle et al., 2012; Švajger et al., 2016). Consequently, many countries have taken actions to limit the production and consumption of bisphenols (Lintschinger and Rauter, 2000). The specific migration limits (SMLs) with regards to foodstuffs for BPA, BADGE and BFDGE have been set in Europe by Commission Directive 2011/8/EU and 2002/16/EC at 0.6 mg/kg, 1 mg/kg and 1 mg/kg, respectively.

The most frequently used methods for the analysis of bisphenols are chromatographic techniques. Simultaneous determination of bisphenol A, bisphenol F and their diglycidyl ethers in food and environmental samples are performed by gas chromatography–tandem mass spectrometry (GC–MS/MS) (Vılchez et al., 2001; Jiao et al., 2012), liquid chromatography–fluorimetry (LC–FL) (Lintschinger and Rauter, 2000; Ballesteros-Gómez et al., 2007), and micellar electrokinetic capillary chromatography (MECC) (Gallart-Ayala et al., 2010). Although chromatography methods are reliable and accurate, they require precision equipments and skilled operators and the sample pretreatment procedure may be complicated.

Recently, increasing attention has been paid to the immunoassay methods due to the advantages of high specificity and low cost. Enzyme-linked immunosorbent assay (ELISA) (Kim et al., 2007), chemiluminescence enzyme immunoassay (CLEIA) (Yu et al., 2014), and time-resolved fluorescence immunoassay (TRFIA) (Du et al., 2015) have been reported for the quantitative analysis of BPA. In the heterogeneous assays mentioned above, multiple washing steps and a long reaction time are needed, both of which make them time-consuming. As a homogeneous competitive immunoassay, fluorescence polarization immunoassay (FPIA) requiring no separation or washing steps has been developed to detect BPA (Huang et al., 2015). In general, based on the antigen-antibody reactions, immunoassays are focused on detecting bisphenol A and unable to recognize multiple bisphenols simultaneously.

In comparison with antibodies, receptors exhibit the advantage of broad-spectrum binding to their ligands, making them suitable for simultaneous analysis of a group of compounds. As a multi-residue method, receptor assay has been applied to determine medicine residues, such as β-lactams, sulfonamides and so on (Liang et al., 2013; Zhang et al., 2013, 2016). However, few researches were carried out on the application of receptor assay for the detection of bisphenol analogues.

As a widely studied endocrine disrupting chemical (EDC), BPA has been confirmed to alter several metabolic functions through the expression of the nuclear receptors, such as estrogen receptors (ERs) (Matthews et al., 2001), glucocorticoid receptor (GR) (Zhang et al., 2017), and peroxisome proliferator-activated receptors (PPARs) (Grasselli et al., 2013). The transcriptional activity of PPARs is regulated by their distinct conformational states that are the results of ligand binding. It has been reported that BPA and its analogues, such as diglycidyl ethers and halogenated flame retardants, are ligands of PPARγ (Wright et al., 2000; Riu et al., 2011; Rogers et al., 2013). Hence, based on the binding between receptor and ligands, a soluble PPAR protein derivative was prepared for the determination of BPA, BPF, BADGE and BFDGE.

In the present work, a receptor-based competitive binding assay was established to monitor multiple bisphenols using fluorescence polarization. The binding potencies of 4 tested compounds with mPPARα-LBD* were evaluated. To understand the differences in mPPARα-LBD* binding affinity among these bisphenol structural analogues, molecular docking was employed to investigate the interactions of BPs with the receptor protein.

2. Materials and methods

2.1. Materials

4,4′-Isopropylidenediphenol (BPA), 4,4′-Dihydroxydiphenylmethane (BPF), bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) were purchased from Aladdin (Shanghai, China) and Sigma–Aldrich (St. Louis, MO, USA). The structures of the bisphenols above are shown in Table 1. Dexamethasone fluorescein (Dex-fl) was purchased from Invitrogen Molecular Probes (Eugene, OR, USA). Unstained protein molecular weight marker was purchased from Thermo Fisher Scientific (San Jose, CA, USA). All other reagents used were of analytical grade.

2.2. Preparation of mPPARα-LBD*

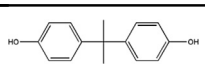
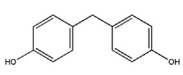
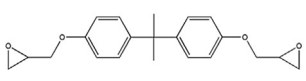
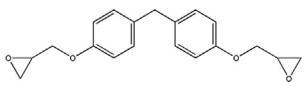
In order to improve the efficiency of soluble expression for target protein, the deletion of N-terminal amino acids from 202 to 266 was conducted to produce a new soluble protein named mPPARα-LBD*. The cDNA fragment was amplified from cDNA generated from mouse liver mRNA and then cloned into pET28a vector. The plasmids were transformed into *Escherichia coli* strain Rosetta (DE3). Expression of the target protein was induced with IPTG. The supernatant was applied to a Ni-NTA column to purify the recombinant protein. Homogeneity of the purified protein was confirmed by SDS-PAGE and Western blot analysis.

2.3. Binding of fluorescent tracer with mPPARα-LBD*

In the direct binding assay, the recombinant receptor was determined for the ability to bind Dex-fl using fluorescence polarization. FP experiments were carried out by a fluorescence spectrophotometer (F-7000, Hitachi, Tokyo, Japan) with excitation at 484 nm and emission at 520 nm through a pair of polarizers. Dex-fl (8 nM) was titrated with various concentrations of mPPARα-LBD*, and the increase of FP values upon the formation of Dex-fl-mPPARα-LBD* complexes was monitored. The dissociation constant ($K_{d,probe}$) of Dex-fl with mPPARα-LBD* was calculated according to the following equation:

$$Y = B_{max} * X/(K_d + X) \quad (1)$$

Table 1
IC_{50} values and dissociation constants ($K_{d,BP}$) of 4 bisphenols.

Chemical	Structure	IC50 (μM)	$K_{d,BP}$ (μM)
BPA		12.27	10.80
BPF		19.13	16.83
BADGE		8.58	7.55
BFDGE		13.69	12.05