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Molecular docking of bisphenol A and its nitrated and chlorinated metabolites onto human estrogen-related receptor-gamma

Sainath Babu [a], Nadeem A. Vellore [b], Agasthya V. Kasibotla [a], Harlan J. Dwayne [c], Michael A. Stubblefield [c], Rao M. Uppu [a,*]

[a] *Department of Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA, United States*
[b] *Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, United States*
[c] *Department of Mechanical Engineering, Southern University and A&M College, Baton Rouge, LA, United States*

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ABSTRACT

A xenoestrogen and known endocrine disruptor, bisphenol A (BPA) binds the human estrogen-related receptor-gamma (ERRγ) with high affinity (Kd ≈ 5.5 nM). It is likely that BPA undergoes oxidative biotransformation by hypochlorite/hypochlorous acid ($^{-}$OCl/HOCl) and peroxynitrite (PN) and the products formed in these reactions may serve as secondary estrogens and contribute to the toxicodynamics of BPA. Therefore, in the present study we have examined the formation of chlorinated and nitrated BPA in reactions of BPA with $^{-}$OCl/HOCl and PN(+CO_2) performed around the neutral pH. We have identified four major products in these reactions and they include 3-chloro-BPA (CBPA), 3,3′-dichloro-BPA (DCBPA), 3-nitro-BPA (NBPA) and 3,3′-dinitro-BPA (DNBPA). Towards understanding the toxicodynamics and estrogenic activity of BPA in biological systems, we have performed molecular docking of BPA, CBPA, DCBPA, DNBPA and NBPA onto the ERRγ using AutoDock 4.2 software and compared the binding energies with those of estradiol, the natural ligand. Based on the genetic algorithm, the three best conformations were selected and averaged for each ligand and a detailed analysis of molecular interactions based on free energies of binding (kcal/mol) was computed. The results indicate the following rank order of binding to ERRγ: BPA (−8.78 ± 0.06) > CBPA (−8.53 ± 0.41) > NBPA (−7.36 ± 0.74) > DCBPA (−5.24 ± 0.17) > DNBPA (−4.95 ± 0.78) > estradiol (−4.94 ± 1.04). The docking studies revealed that the OH group of one of the phenyl rings forms a hydrogen bond with Glu275/Arg316, while the OH group of other phenyl ring was bound to Asp346. These results suggest that both BPA and its putative chlorinated and nitrated metabolites have strong binding affinity compared to estradiol.

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1. Introduction

Bisphenol A (BPA; Fig. 1), a xenoestrogen and semi-persistent organic pollutant in urban environments, adversely affects the endocrine system even at low doses in experimental animals and humans [1–3]. Recent studies show that BPA binds strongly to the human estrogen-related receptor-gamma (ERRγ; Kd ≈ 5.5 nM) as compared to the estrogen receptor (ER) itself [4,5]. ERRγ belongs to the group orphan nuclear receptors, and these are closely related to ER. As on date, three different ERR proteins (α, β, γ) have been identified [5]. The function of ERRγ is not well known, however, high levels of this receptor are expressed in the fetal brain and in other tissues of adult rodents and humans [6,7].

Approximately 20–25% of BPA is known to undergo metabolic transformation by enzymes of cytochrome P450 system [8,9] and, possibly, neutrophil and macrophage derived oxidants, viz., hypochlorite/hypochlorous acid ($^{-}$OCl/HOCl) and peroxynitrite (PN). The direct reaction of BPA with $^{-}$OCl/HOCl has been studied in the context of bioremediation and treatment of waste water and industrial effluents [10,11]. These reactions mainly produce chlorinated BPA. Interestingly, the reaction of BPA with PN has never been studied; however, an analogy of reactions of PN (±CO_2) with most phenolic compounds [12–14] suggests nitrated BPA could be the major products. Studies have shown that chlorinated and nitrated BPA exhibit greater toxicity than native BPA itself and elaborates mutagenic and/or genotoxic effects [15–17]. Therefore, it is likely that these chlorinated and nitrated BPA if formed in biological systems can serve as secondary estrogens and relay, at least, in part, the toxic effects of BPA.

In order to understand the molecular targets for BPA and its putative chlorinated and nitrated products and the likely disruption of endocrine function, in the current study, we carried out the reactions of $^{-}$OCl/HOCl and PN (+CO_2) with BPA around the neutral pH. We identified the major products formed in these

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* Corresponding author. Fax: +1 225 771 5350.
E-mail address: rao_uppu@subr.edu (R.M. Uppu).

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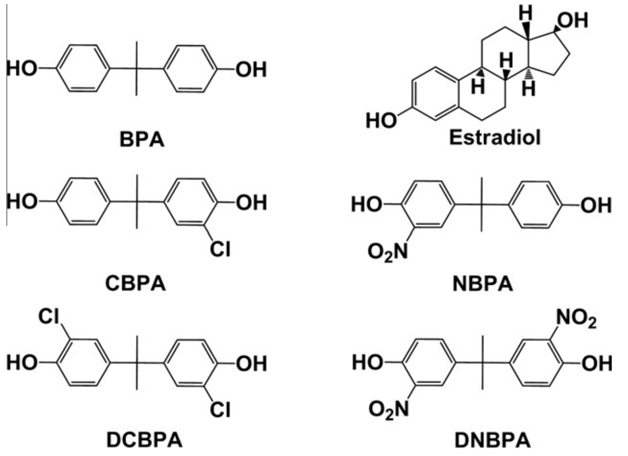

Fig. 1. Structures of estradiol, BPA, nitrated BPA (NBPA and DNBPA), and chlorinated BPA (CBPA and DCBPA).

reactions, and investigated their estrogenic activities along with BPA based on molecular docking onto ERRγ.

2. Materials and methods

2.1. Materials

Chemicals and reagents were obtained as follows: bisphenol A, H_2O_2 (30%), isoamyl nitrite (96%), dichloromethane (DCM), DMSO-*d*$_6$, MnO_2 (granular), 1-methylimidazolium hydrogen sulfate ($[MIM]^+$ HSO_4^-), $NaHCO_3$, $NaNO_3$, potassium phosphate monobasic, sodium phosphate dibasic, and sodium hypochlorite (chlorine content: *ca*. 5%) from Sigma (St. Louis, MO); diethylenetriaminepentaacetic acid (DTPA) from ACROS Organics (New Jersey, NJ); HNO_3 (70%) and trifluroacetic acid (TFA) from Fisher Scientific (Fair Lawn, NJ); and acetonitrile and acetone from Mallinckrodt (Philipsburg, NJ). Peroxynitrite (PN) and 3,3′-dinitro-BPA (DNBPA) were synthesized as described in our previous publications [18,19]. Water used in all experiments was deionized to a final resistance of 18.0 MΩ/cm or higher.

The concentration of $^{-}$OCl and H_2O_2 in stock solutions was determined using $\varepsilon = 350\ M^{-1}\ cm^{-1}$ at 292 nm [20] and $\varepsilon = 41\ M^{-1}$-cm^{-1} at 240 nm [21], respectively. Working standards of PN and $^{--}$OCl/HOCl (2 or 5 mM) were prepared by dilution of the respective stock solutions with water and used immediately (usually within 5 min). Stock solutions of BPA (10 mM) were prepared in 0.01 M NaOH and stored in aliquots of 1 mL each at −20 °C until use.

2.2. Reactions of BPA with PN and $^{-}$OCl/HOCl

BPA (200 μM) was allowed to react with 0 to 500 μM of PN (or $^{-}$OCl/HOCl) in 2 mL of 0.08 M phosphate buffer, pH 7.0 that also contained 0.1 mM DTPA. In reactions performed with PN, $NaHCO_3$ was included in the assay at a final concentration of 10 mM [22]. The reaction in all cases was initiated by the addition of PN (or $^{-}$OCl/HOCl). Throughout the course of addition of PN (or $^{-}$OCl/HOCl), which typically took 5 s, the contents were constantly stirred and stirring continued for an addition 10 s. Before further analysis by reversed phase HPLC (see below), the reaction mixtures were left at room temperature (25 ± 1 °C) for 5–15 min.

2.3. RP-HPLC analysis of BPA and its oxidation products

The reaction mixtures of PN-BPA and $^{-}$OCl/HOCl-BPA were analyzed using a Hypersil Gold LC18 column (150 × 3.6 mm) and a mobile phase consisting of 0.1% TFA and 50% acetonitrile in water. The flow of the mobile phase was set at 1 mL/min and the eluent was monitored at 292 nm. A Thermo Scientific Spectra System SCM 1000 equipped with a Spectra System P4000 (quaternary pump) and a Spectra System UV2000 (dual wavelength detector) was used for the purpose of sample analysis.

2.4. Synthesis and characterization of NBPA

Nitration of BPA was performed using a combination of sodium nitrate and an acidic ionic liquid, $[MIM]^+$ HSO_4^- [23]. BPA (3.32 g; 20 mmol) and $[MIM]^+$ HSO_4^- (5.72 g; 20 mmol) were dissolved in 80 mL of acetonitrile. To this mixture, 1.7 g (20 mmol) of $NaNO_3$ was added and stirred overnight at 25 ± 1 °C. At the end, the mixture was filtered and the filtrate was evaporated under reduced pressure at *ca*. 50 °C. The thick brown liquid-like residue was extracted with hot hexane, which was then evaporated under reduced pressure.

The yellow liquid residue, dissolved in dichloromethane, was analyzed by GC/MS/EI using an Agilent Technologies 7890A GC equipped with the Agilent Technologies 5975 C V L triple-axis MSD and a HP-5MS capillary column (length: 30 m; internal diameter: 0.25 mm; and film thickness: 0.25 μm). Helium was used as the carrier gas (total flow: 3 mL/min; split ratio: 1:50) with temperature programming as follows: 40 °C for 2 min (isothermal); 20 °C/min up to 150 °C (ramp 1), 150 °C for 3 min (isothermal); 20 °C/min up to 300 °C (ramp 2), and 300 °C for 2 min (isothermal) (total run time: 20 min; temperature of the inlet port: 270 °C).

When analyzed by GC/MS/EI, the nitro product resolved as a single peak at 17.34 min. The ion chromatogram of this product showed a molecular ion ($M^{\cdot+}$) at *m/z* 273 and a daughter ion at *m/z* 258 (100%; base peak; $[M-15]^+$), confirming that the chemical identity of the product is 3-nitro-BPA.

2.5. Synthesis and characterization of CBPA, DCBPA and TCBPA

BPA (1.37 g; 6 mmol) was allowed to react with excess $^{-}$OCl/HOCl (*ca*. 25 mmol) in 20 mL of 50% methanol in water, as described by Fukazawa et al. [10]. Following an overnight stirring, aliquots (200 μL each) were subjected to RP-HPLC analysis using an Econosphere C8-column (250 × 10 mm; particle size: 10 μ) and a mobile consisting of 50% acetonitrile in water that also contained 0.1% TFA (flow: 3 mL/min). The eluent was monitored at 292 nm. A Lab Alliance series II/III liquid chromatograph equipped with a Lab Alliance model 500 UV–Vis detector was used. Peaks with significant absorbance were collected and pooled individually from several HPLC runs and concentrated by flash evaporation.

GC/MS/EI analysis of chlorinated BPA was performed using a Hewlett Packard-6890 GC equipped with HP-5973 MS and a RTX-5MS capillary column (length, 30 m; internal diameter, 0.25 mm; and film thickness, 0.25 μm). Helium was used as the carrier gas (total flow: 1 mL/min; splitless) with temperature programming as follows, 150 °C for 2 min (isothermal); 30 °C/min up to 270 °C (ramp), and held at 270 °C for 6 min (isothermal) (total run time: 12 min; temperature of the inlet port was 260 °C). All three products resolved as single peaks. Each of them showed $M^{+\cdot}$ corresponding to the predicted molecular weight of 262 for NBPA, 296 for DCBPA, and 330 for TCBPA. In each case, the daughter fragment corresponded to $[M-15]^+$.

2.6. Preparation of receptor structure

The structure of ligand binding domain of ERRγ was obtained from Research Collaboratory for Structural Bioinformatics (RCSB)-Protein Data Bank (PDB). The PDB entry 2E2R was selected for

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