Chemosphere 181 (2017) 536–543

Contents lists available at ScienceDirect

Chemosphere

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

Binding of Bisphenol-F, a bisphenol analogue, to calf thymus DNA by multi-spectroscopic and molecular docking studies

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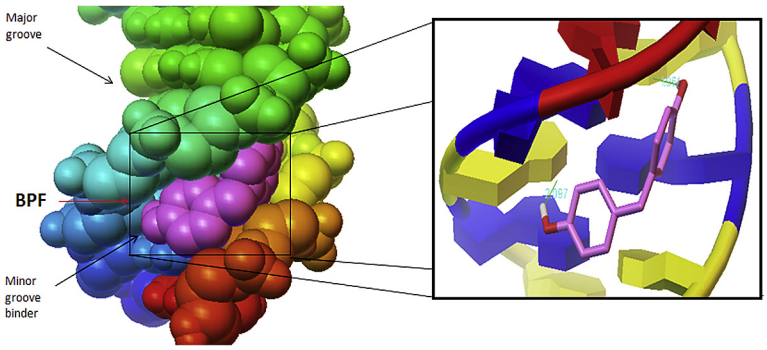
HIGHLIGHTS

- Interaction between Bisphenol-F and calf thymus DNA was studied.
- Multi-spectroscopic, voltammetric and molecular docking techniques were employed.
- BPF binds to ctDNA through groove binding mode.
- BPF was found to be genotoxic under *in vitro* conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 February 2017
Received in revised form
17 April 2017
Accepted 23 April 2017

Handling Editor: A. Gies

Keywords:
Bisphenol-F
Calf thymus DNA
Binding mode
Molecular docking
Interaction

ABSTRACT

BPF (Bisphenol-F), a member of the bisphenol family, having a wide range of industrial applications is gradually replacing Bisphenol-A. It is a recognized endocrine disrupting chemical (EDC). EDCs have been implicated in increased incidences of breast, prostate and testis cancers besides diabetes, obesity and decreased fertility. Due to the adverse effects of EDCs on human health, attempts have been directed towards their mechanism of toxicity especially at the molecular level. Hence, to understand the mechanism at the DNA level, interaction of BPF with calf thymus DNA was studied employing multi-spectroscopic, voltammetric and molecular docking techniques. Fluorescence spectra, cyclic voltammetry (CV), circular dichroism (CD) and molecular docking studies of BPF with DNA were suggestive of minor groove binding of BPF. UV–visible absorption and fluorescence spectra suggested static quenching due to complex formation between BPF and ctDNA. Hoechst 33258 (HO) and ethidium bromide (EB) displacement studies further confirmed such mode of BPF interaction. Thermodynamic and molecular docking parameters revealed the mechanism of binding of BPF with ctDNA to be favorable and spontaneous due to negative ΔG and occurring through hydrogen bonds and van der waals interactions. BPF induced DNA cleavage under *in vitro* conditions by plasmid nicking assay suggested it to be genotoxic.

1. Introduction

Bisphenol-F (bis(4-hydroxyphenyl)methane; BPF) (Fig. 1) is a member of the bisphenol family which contains two phenol rings linked by a methylene bridge. It has wide-ranging industrial applications including manufacture of epoxy resins and polycarbonates and has progressively replaced BPA (Liao and Kannan, 2014a; Molina-Molina et al., 2013). Due to its increasing applications, it has now emerged as an environmental contaminant and

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http://dx.doi.org/10.1016/j.chemosphere.2017.04.115
0045-6535/

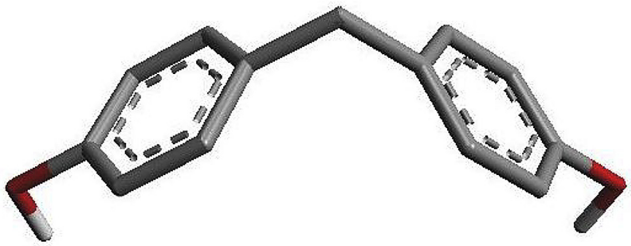

Fig. 1. The molecular structure of Bisphenol-F.

has been detected in several environmental samples (Usman and Ahmad, 2016) including indoor dust (Liao and Kannan, 2013). Moreover, it has become a contaminant in food stuffs (Liao and Kannan, 2014a; Gallart-Ayala et al., 2011) and personal care products (PCPs) (Liao and Kannan, 2014b). In fact, it is more toxic than its metabolites dihydroxybenzophenone (DHB) and BPF-OH in terms of genotoxicity and endocrine toxicity (Cabaton et al., 2009). It was also found to exhibit moderate cytotoxicity and liver toxicity (Audebert et al., 2011; Higashihara et al., 2007). The Environment Protection Agency of USA has confirmed it as an Endocrine Disrupting Chemical (EDC) (Zhang et al., 2013). A report concluded BPF to be as hormonally active as bisphenol-A (BPA) (Rochester and Bolden, 2015). EDCs have been associated with increased incidences of breast, prostate and testis cancers besides diabetes, obesity and decreased fertility (Ahmad et al., 2017; De Coster and van Larebeke, 2012). These adverse effects of EDCs on human health have aroused attention to study different aspects of their toxicity with particular reference to the toxicity mechanism of bisphenol-F.

DNA is repository of genetic information and plays an important role in vital biological processes such as gene expression, gene transcription, mutagenesis and carcinogenesis. It commonly serves as the main molecular target of drugs and toxic substances. Hence, in recent years, there has been a focus on the binding interactions of small molecules with DNA in order to understand their toxic or chemotherapeutic effects (Wang et al., 2014). DNA as a target of many drugs and environmental toxicants has had its binding mechanism studied extensively; the three major non-covalent modes of interaction being: (i) intercalative binding distorting the DNA structure, (ii) groove binding causing little distortion, and (iii) electrostatic interaction which is the weakest binding force of the three. The binding interactions of toxic substances with calf thymus DNA (ctDNA) are investigated to better understand their toxicological mechanism at molecular level and to design new chemicals with low toxicity (Tao et al., 2015).

To accomplish the above mentioned purpose the interaction of BPF with ctDNA was studied by multiple spectroscopic techniques comprising UV–Vis absorption, fluorescence and circular dichroism (CD). Moreover, methods such as cyclic voltammetry (CV), DNA cleavage assay and molecular docking analysis were also employed. The mechanism of interaction between BPF and ctDNA was obtained through analyses of the specific binding site, quenching mechanism, binding constants, binding energy and thermodynamic parameters.

2. Materials and methods

2.1. Materials

Calf thymus DNA (ctDNA), Hoechst 33258 (HO), ethidium bromide (EB) and agarose superior grade were purchased from SRL, India. BPF (>99% purity) was obtained from TCI chemicals, India. pBR322 plasmid was purchased from Thermo Fisher Scientific. Stock solution of ctDNA was prepared by dissolving in potassium phosphate buffer (50 mM, pH 7.4) and stored at 4 °C. The purity of DNA was checked by observing the ratio of absorbance at 260 nm to that of 280 nm. The ratio was in the range of 1.8–1.9, indicating that the DNA was free of protein. The concentration of ctDNA in stock solution was determined by taking the UV absorption at 260 nm and using a molar absorption coefficient ε_{260} = 6600 $M^{-1}cm^{-1}$ (Reichmann et al., 1954). The stock solution of BPF was prepared by dissolving in DMSO.

2.2. Methods

2.2.1. UV–vis absorption measurements

UV-1800 Shimadzu UV-spectrophotometer equipped with 1 cm quartz cuvettes was used to measure the UV–Vis absorption spectra of BPF and the mixtures of DNA-BPF solutions at room temperature in the range of 235–300 nm. The spectra were recorded with a fixed concentration of BPF (25 μM) titrated with varying concentrations of DNA (5–50 μM). Also, the spectra were recorded with a fixed concentration of DNA (25 μM) titrated with varying concentrations of BPF (2–12 μM). The corresponding solutions of ctDNA or BPF depending on the experiment were taken as the reference solution.

2.2.2. Fluorescence measurements

Shimadzu RF-5301pc spectrofluorophotometer was used for the fluorescence spectra measurements of BPF-ctDNA system. A 3 ml solution of 25 μM BPF was successively titrated with increasing concentrations of DNA. The fluorescence emission spectra were measured at 298 and 310 K in the range of 290–340 nm with the excitation wavelength fixed at 276 nm.

Iodine quenching experiments were conducted by taking the spectra of BPF (25 μM) in the presence and absence of ctDNA (50 μM) while being titrated with KI (60–160 mM). The emission and excitation range of wavelength selected at 280–400 nm and 276 nm respectively to calculate the binding constant.

For the displacement assays, the ctDNA-EB and ctDNA-Hoechst33258 complexes (DNA- 25 μM; EB and Hoechst 33258–2.5 μM) were titrated with varying concentrations of BPF. The emission spectrum of ctDNA-EB complex was recorded in the range 555–650 nm with the excitation wavelength 471 nm. However, the emission spectrum and excitation wavelength of ctDNA-Hoechst 33258 complex were selected at 350–700 nm and 343 nm respectively.

2.2.3. Viscosity measurement

The viscosity measurements were carried out using Oswald capillary viscometer at 25°C (Arjmand and Yousuf, 2013). The concentration of ctDNA was kept constant (100 μM) with varying concentrations of BPF. The flow time was measured using a stop watch. The relative viscosity $(\eta/\eta_0)^{1/3}$ was then plotted against the ligand/DNA concentration ratio, where η and η_0 are the viscosity of DNA+ BPF and DNA alone respectively.

2.2.4. Circular dichroism spectra measurements

All CD spectra were obtained using a JASCO-J-813 spectropolarimeter. The spectra of ctDNA incubated with BPF with the molar ratio of 0 and 1 were recorded in the range of 220–320 nm. These CD spectra were taken in Tris-HCl (pH 7.4) at room temperature.

2.2.5. Cyclic voltammetry

CV studies were performed on a Potentiostat/Galvanostat (Model 263A, USA). A three electrode configuration was used