Indian Journal of Biochemistry & Biophysics
Vol. 62, April 2025, pp. 426-430
DOI: 10.56042/ijbb.v62i4.14428

Bisphenol B: An inhibitor of mitochondrial electron transport chain protein

Shivani Gupta, Dastagiri Pinjari, Deepak Yadav, Deepak Kumar, Rajasri Bhattacharyya & Dibyajyoti Banerjee*

Department of Experimental Medicine and Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh

Received 16 October 2024; revised 05 November 2024

The toxicity of bisphenols is well-reported. Studies report mitochondrial dysregulation due to BPA. However, limited studies exist on other analogs of bisphenols. Moreover, the mechanism involved in such dysregulation is unknown. We have performed a study of Bisphenol B (BPB) with various proteins of mitochondria using *in silico* and *in vitro* studies. It was observed that BPB interacts with and inhibits the ETC complex III protein. This hints that the mitochondrial toxicity of BPB is due to the ETC complex III protein. However, more research in the field is required to unravel the toxicological effect of bisphenol on mitochondria fully.

Keywords: Mitochondria, Complex III protein, Toxicology, Molecular docking, Enzyme activity

Mitochondrial stress is a ubiquitous component of several diseases. Diabetes mellitus, hypertension, nonalcoholic fatty liver disease, obesity, and other common diseases of recent times are linked with abnormal mitochondrial function in one way or another[1]. Modern civilization has exposed us to numerous chemicals and many of them are mitochondrial toxins. Perhaps for such reasons, the above-mentioned chronic illness is tremendously increasing and not sparing the developed nations. So, screening of mitochondrial toxicity of environmentally related pollutants is an area that has generated considerable current interest[2].

Bisphenol compounds are among the most common phenols present in the environment. They are plastic leach out. Bisphenol A is one of the Bisphenols, and it is widely studied. It is concluded that it is bad for health. Nevertheless, other bisphenol analogs like Bisphenol B (BPB), Bisphenol C (BPC), etc., are also not safe, and many of them are endocrine disruptors. They also initiate the occurrence of chronic disorders[3].

Different bisphenols adversely affect mitochondrial function[4].BPB inhibits cellular respiration in yeast cell suspension[5]. Further, it was reported that BPB causes mitochondrial dysfunction in neuroblastoma cells[6]. However, not much is known about the mechanism of BPP-induced mitochondrial dysfunction. In this context, we have studied the interaction of BPB with mitochondrial proteins to understand BPP-induced mitochondrial dysfunction.

*Correspondence:
Phone: +91-9780134620
E-mail: dibyajyoti5200@yahoo.co.in
Suppl. Data available on respective page of NOPR

Materials

Chemicals

D-Mannitol, sucrose, Bovine serum albumin (BSA), Dithiothreitol (DTT) and BCA estimation kit were from Sigma. HEPES, disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium hydroxide were from Himedia. EDTA from Fisher Scientific. Bisphenol B from TCI. Protease cocktail inhibitor from Abcam. oxidized cytochrome C from SRL. Decylubiquinol was from Chemsworth. All other reagents were of analytical grade.

Equipment

Cold centrifuge, centrifuge tubes and Eppendorf, Dounce homogenizers, pestles, scissors,

Sample

Wistar rat's liver tissues

Methods

In silico

Molecular docking

The 3D structure of the mitochondrial proteins *i.e.*, NADH Dehydrogenase (PDB ID:5XTB, Mitochondrial ETC Complex I); Succinate Dehydrogenase (PDB ID: 6VAX, Mitochondrial ETC Complex II); Cytochrome B1 Complex (PDB ID: 5XTE, Mitochondrial ETC Complex III); Cytochrome C Oxidase (PDB ID: 5Z62, Mitochondrial ETC

Complex IV); Citrate Synthase (PDB ID: 5UZR); Monoamine Oxidase (PDB ID: 1GOS); Coproporphyrinogen Oxidase (PDB ID: 2AEX) was downloaded from the RCSB PDB database. Further, the 3D structure of BPB (Compound CID: 66166) was downloaded from the PubChem database. To understand the interaction of BPB with mitochondrial proteins/enzymes, molecular docking using the Autodock tool (version 4.2.6) was carried out. For docking, the known active site residue to proteins/enzymes was considered as the grid centre. Each docking run produced 10 confirmations. Pymol software (version 1.7.4.5) was used for visualizing the docked structure and calculating the non-bonded distances between ligands & the residues of proteins. Confirmation which has the highest negative binding energy is considered for further analysis[7].

Molecular Dynamics (MD) Simulations

The study uses MD simulation to understand the time-dependent behavior of biological systems. The GROMACS 2018.4 package is used, and the docked structure with the highest negative binding energy is considered. The ligand topology files are generated using the Swiss Param tool and CHARMM force field. The docked complexes are solvated in water using the TIP3P water model and neutralized using 11 sodium ions. Energy minimization is performed for 1,00,000 steps, and the system is equilibrated using NVT and NPT protocol. The simulation is run at 300K temperature and 1 bar pressure. Results are visualized using Pymol and Xmgrace plotting tools[7].

In vitro study

Isolation of mitochondria and determination of the effect of bisphenol B on enzyme activity of complex III of ETC

Before starting the experimental work, an IAEC clearance (Proposal number 864 (122nd) dated 29.08.2022) was obtained from the host institute. The control Wistar rats (starved overnight) were sacrificed by cervical dislocation, and the liver tissue was extracted. The mitochondria of liver tissue were then extracted using freshly prepared ice-cold extraction buffer (pH 7.4) by differential centrifugation method[8]. To obtain high quality and quantity of mitochondria the isolation was performed immediately after collecting the liver tissue. The samples were maintained on ice throughout the procedure. After the isolation of mitochondria, protein estimation was done using the Bicinchoninic acid reagent (BCA reagent). The Complex III enzymatic assay is based on the principal cytochrome oxidoreductase, reduces oxidized cytochrome C, and simultaneously oxidizes decyl ubiquinone to decyl ubiquinol[9].To understand the effect of BPB on the enzymatic activity of complex III, the mitochondrial fraction was incubated for two hours with different concentrations of BPB (0 to 12μM).

Statistical analysis

All the values obtained were represented as mean ± SD (n=6, if not mentioned otherwise). An unpaired t-test was used to analyze the data and a p-value <0.05 will be considered as significant.

Results

In silico study

Through molecular docking, we predicted the interaction of BPB with several mitochondrial proteins (located at the outer mitochondrial membrane, inner mitochondrial membrane, matrix, and intermembrane space).Out of the tested proteins, BPB interacted best with Coproporphyrinogen oxidase and Cytochrome B1 complex (ETC complex III protein). Table 1, Figure 1, and Suppl. Figures 1 to 29 shows the binding energy and the structures of the docked complex. Further, to understand the stability of the docked complex over time, an MD simulation run of BPB with cytochrome B1 complex protein for 100ns was performed. It was predicted that BPB interacted with the mitochondrial ETC complex III protein throughout the run time (Fig. 2). This hinted that BPB can potentially alter the enzyme activity of the Cytochrome B1 protein.

In vitro study

To confirm the results obtained in the *In silico* study further, we incubated the Wistar rat liver mitochondrial fraction; it was observed that BPB inhibited the enzyme activity in a dose-dependent manner. Except for100 nM, all the values showeda significant reduction in enzyme activity compared to control (Fig. 3).

Discussion

Mitochondria functioning is important for maintaining cellular homeostasis. However, dysregulation of mitochondrial function is linked with various diseases/disorders[10]. Toxic compounds like Bisphenolsare known to inhibit mitochondrial biogenesis and trigger cytochrome C release[11,12]. The ill effects of BPA are known, and regulations

Table 1 — Shows the Mean ± SD (n=6) of the binding energy (kcal/mol) of bisphenol B with mitochondrial proteins *i.e.*, NADH Dehydrogenase (Complex I), Succinate Dehydrogenase (Complex II), Cytochrome B1 Complex (Complex III), Cytochrome C Oxidase (Complex IV), Citrate Synthase, Monoamine Oxidase and Coproporphyrinogen Oxidase. All the values are represented as mean±SD (n=6)

Protein	Ligand	Active sites	Binding Energy (kcal/mol) Mean ± SD (n=6)
Mitochondrial ETC Complex I (NADH Dehydrogenase) (PDB ID:5XTB)	Bisphenol B	Arg 88	-2.93 ± 0.049
		Arg 199	-3.90 ± 0 .057
		Arg 224	-4.07 ± 0.114
		Phe 93	-3.62 ± 0.090
		Phe 229	-4.09 ± 0.038
		Phe 236	-3.83 ± 0.055
		Thr 95	-3.56 ± 0.063
Mitochondrial ETC Complex II (Succinate Dehydrogenase) (PDB ID: 6VAX)	Bisphenol B	Arg 340	-3.54 ± 0.12
		Arg 451	-3.99 ± 0 .024
		His 296	-4.45 ± 0.015
		His 407	-4.44 ± 0.018
		Thr 308	-4.3 ± 0.1
Mitochondrial ETC Complex III (Cytochrome B1 Complex) (PDB ID: 5XTE)	Bisphenol B	Cys 121	-5.84 ± 0.03
		Cys 124	-6.09 ± 0.29
		His 125	-6.03 ± 0.31
		Met 244	-5.81 ± 0.03
Mitochondrial ETC Complex IV (Cytochrome C Oxidase) (PDB ID: 5Z62)	Bisphenol B	His 378	-4.32 ± 0.05
		Ser 382	-3.87 ± 0.01
		Tyr 371	-4.39 ± 0.02
Citrate Synthase (PDB ID: 5UZR)	Bisphenol B	Arg 356	-2.68 ± 0.1
		Arg 428	-2.15 ± 0.04
		Arg 448	-2.84 ± 0.02
		His 265	-3.40 ± 0.09
		His 347	-3.94 ± 0.05
Monoamine Oxidase (PDB ID: 1GOS)	Bisphenol B	Cys 156	-3.35 ± 0.05
		Cys 365	-3.97 ± 0.02
		His 382	-4.57 ± 0.04
Coproporphyrinogen Oxidase (PDB ID: 2AEX)	Bisphenol B	His 258	-8.56 ± 0.1
		His 327	-5.68 ± 0.1
		Ser 244	-8.62 ± 0.02

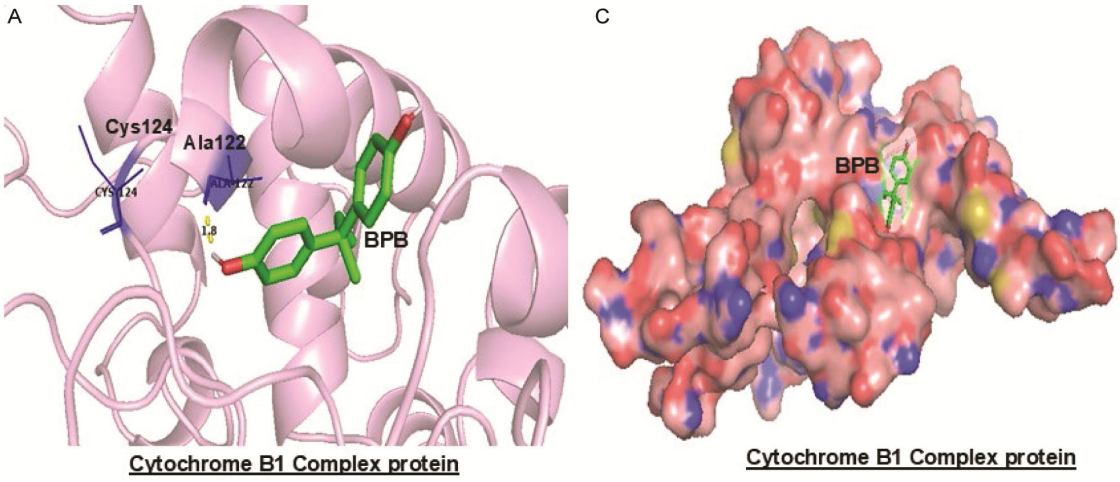

Fig. 1 — Shows the docked structure of Mitochondrial ETC complex III (Cytochrome B1 Complex protein) with bisphenol B (BPB, in stick mode), in cartoon (A) and surface (B) view. The active site residue of complex III Cys124 is highlighted in blue. A hydrogen bond was present between Ala122 of protein and BPB. The bond distances (Å) are labeled along with the residue involved.

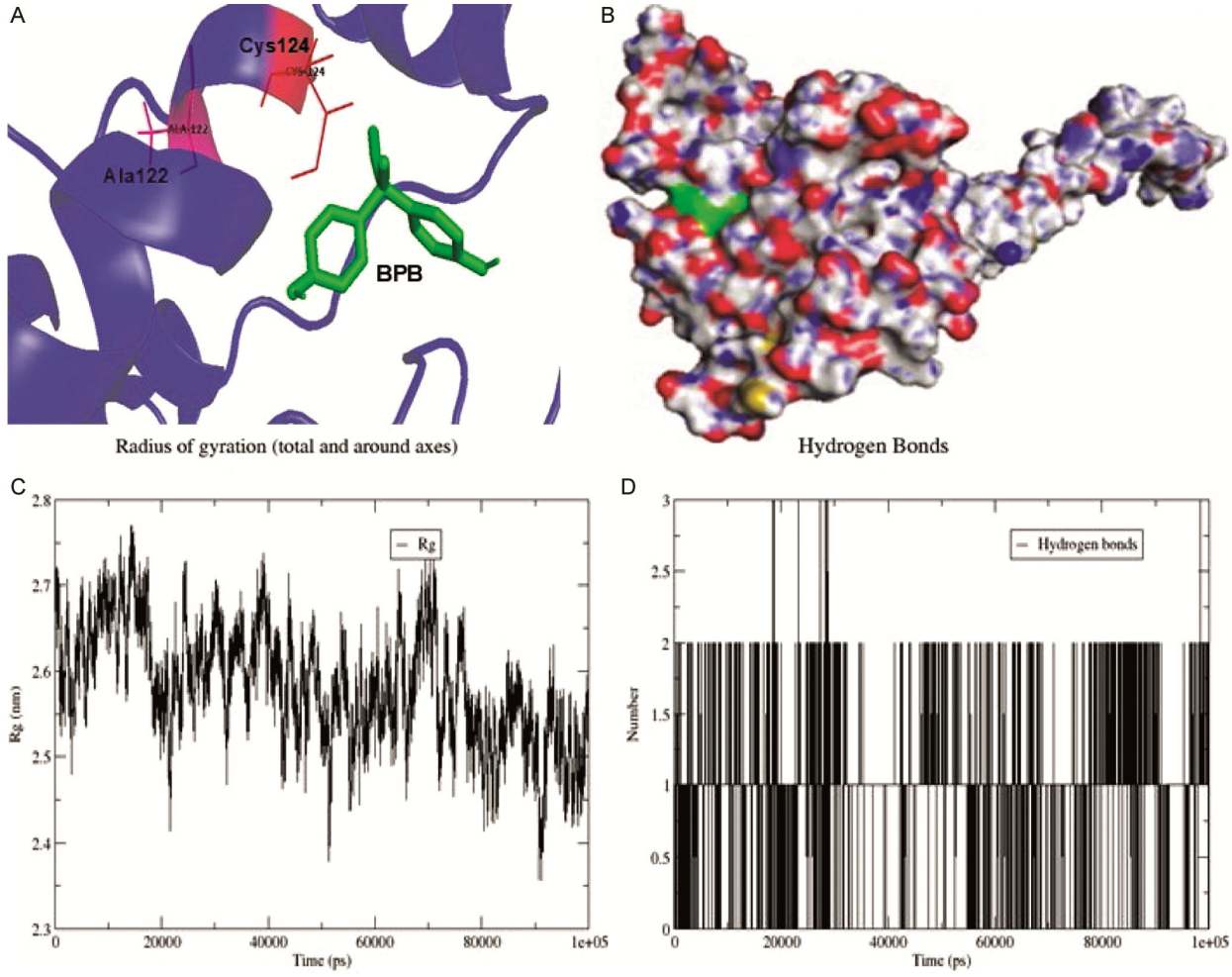

Fig. 2 — Shows the complex of Cytochrome B1 Complex protein, bonded with Bisphenol B (BPB, in stick mode highlighted in green) obtained after MD simulations run (100 ns) in cartoon (A) and surface (B) view. The BPB was found to be close to Ala 122. The other parameters, *i.e.*, the Radius of Gyration and hydrogen bonds of the complex for 100 ns, are shown in (C) and (D), respectively.

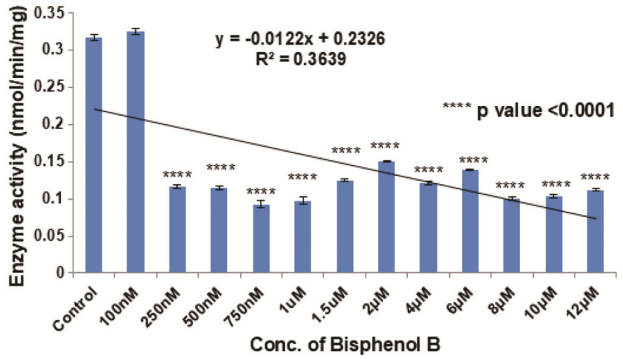

Fig. 3 — Shows the enzyme activity of Mitochondrial ETC complex III (Cytochrome B1 Complex) from mitochondrial lysate (obtained from Wistar rats) performed *in vitro* with various concentrations of BPB.

regarding its use are also in force. However, fewer studies on mitochondrial toxicity are there with other analogs of bisphenols[6,12]. In this context, this study proves beyond any doubt that BPB is a mitochondrial poison and inhibits ETC complex III activity (Figs 1-3). Therefore, in *in vivo* systems following BPB exposure, if the BPB reaches mitochondria, it will inhibit the ETC at complex III and thus can initiate chronic illness like other bisphenols in the long run if the exposure is continued[13].

Now plastic-related problems are well known. Like BPA, BPB can also leach out from plastics. BPB is a known endocrine disruptor, so it is a toxic substance. This study shows that apart from being an endocrine disruptor, it can inhibit mitochondrial ETC complex III enzymes. We believe that it is a new basis for exploring the toxicity profile of BPB from a mechanistic viewpoint (Fig. 4).

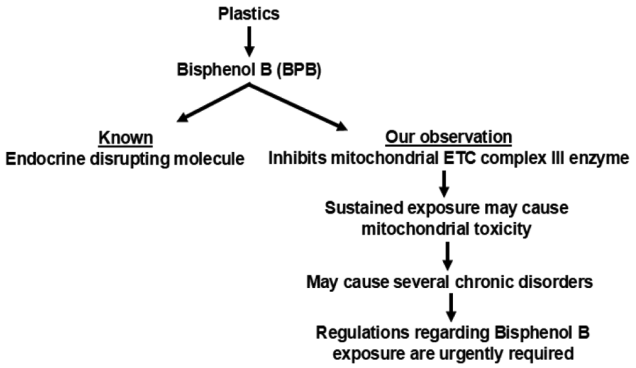

Fig. 4 — A flow diagram showing the possible new mechanism of toxicity of BPB.

Acknowledgment

RB acknowledges PGIMER Chandigarh for financial assistance (Sanction Order No: IM/213/28-08-23-1123).

Conflict of interest

All authors declare no conflict of interest.

References

1 Geto Z, Molla MD, Challa F, Belay Y & Getahun T, Mitochondrial dynamic dysfunction as a main triggering factor for inflammation associated chronic non-communicable diseases. *J Inflamm Res*, 13 (2020) 97.

2 Tang W, Chen J & Hong H, Discriminant models on mitochondrial toxicity improved by consensus modeling and resolving imbalance in training. *Chemosphere*, 253 (2020) 126768.

3 Catenza CJ, Farooq A, Shubear NS & Donkor KK, A targeted review on fate, occurrence, risk and health implications of bisphenol analogues. *Chemosphere*, 268 (2021) 129273.

4 Singh RD, Easson S & Thompson JA, Mitochondrial function after bisphenol S exposure: a safe substitute for BPA? *FASEB J*, 34 (2020) 1.

5 Dragone R, Grasso G & Frazzoli C, Amperometric cytosensor for studying mitochondrial interferences induced by plasticizers Bisphenol B and Bisphenol A. *Molecules*, 25 (2020) 5185.

6 Wang C, He J, Xu T, Han H, Zhu Z, Meng L, Pang Q & Fan R, Bisphenol A(BPA), BPS and BPB-induced oxidative stress and apoptosis mediated by mitochondria in human neuroblastoma cell lines. *Ecotoxicol Environ Saf*, 207 (2021) 111299.

7 Kumar D, Bhattacharyya R & Banerjee D, Pseudosterase activity-based specific detection of human serum albumin on gel. *Talanta*, 224 (2021) 121906.

8 Amigo I, Traba J & Rueda CB, Isolating Liver Mitochondria by Differential Centrifugation. *Bio Protoc*, 6 (2016) 1.

9 Spinazzi M, Casarin A, Pertegato V, Salviati L & Angelini C, Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. *Nat Protoc*, 7 (2012)1235.

10 San-Millán I, The Key Role of Mitochondrial Function in Health and Disease. *Antioxidants*, 12 (2023) 782.

11 Goyal S, Tiwari S, Seth B, Tandon A, Shankar J, Sinha M, Singh SJ, Priya S & Chaturvedi RK, Bisphenol-A inhibits mitochondrial biogenesis via impairment of GFER mediated mitochondrial protein import in the rat brain hippocampus. *Neuro Toxicol*, 85 (2021) 18.

12 Štefunková N, Greifová H, Jambor T, Tokárová K, Zuščíková L, Bažány D, Massányi P, Capcarová M & Lukáč N, Comparison of the Effect of BPA and Related Bisphenols on Membrane Integrity, Mitochondrial Activity, and Steroidogenesis of H295R Cells *in vitro* . *Life (Basel)*, 14 (2023) 3.

13 Chen, W., Zhao, H. & Li, Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. *Sig Transduct Target Ther*, 8 (2023) 333.