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Atomic-scale investigation of the interactions between tetrabromobisphenol A, tetrabromobisphenol S and bovine trypsin by spectroscopies and molecular dynamics simulations

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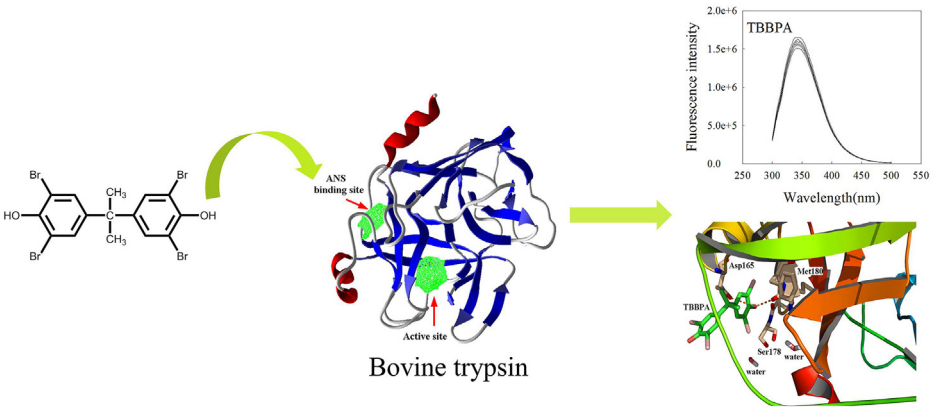
HIGHLIGHTS

- The interaction of TBBPA/TBBPS with bovine trypsin was deciphered for the first time.
- The fluorescence of bovine trypsin was quenched in a concentration-dependent mode.
- TBBPA and TBBPS bind at the ANS binding site with distinct binding modes.
- TBBPS has a higher binding affinity toward bovine trypsin than TBBPA.
- Our *in vitro* and *in silico* approach is helpful to assess risk of TBBPA-related BFRs.

GRAPHICAL ABSTRACT



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ABSTRACT

Tetrabromobisphenol A (TBBPA) and its replacement alternative tetrabromobisphenol S (TBBPS) are used widely as brominated flame retardants (BFRs). However, the potential risk of their effects on bovine trypsin remains largely unknown. We investigated the effects of TBBPA and TBBPS to bovine trypsin by the fluorescence spectroscopy, circular dichroism and molecular dynamics (MD) simulations. They statically quenched the intrinsic fluorescence of bovine trypsin in a concentration-dependent mode and caused slight red-shifted fluorescence. The short and long fluorescence lifetime decay components of bovine trypsin were both affected, partly due to the disturbed microenvironmental changes of Trp215. The β-sheet content of bovine trypsin was significantly reduced from 82.4% to 75.7% and 76.6% by TBBPA and TBBPS, respectively, possibly impairing the physiological function of bovine trypsin. TBBPA and TBBPS bind at the 8-anilinonaphthalene-1-sulfonate (ANS) binding site with an association constant of $1.09 \times 10^4\ M^{-1}$ and $2.41 \times 10^4\ M^{-1}$ at 298 K, respectively. MD simulations revealed that van der Waals interactions and hydrogen bond interactions are dominant for TBBPA, whereas electrostatic interactions are critical for TBBPS. Our *in vitro* and *in silico* studies are beneficial to the understanding of risk assessment and future design of environmental benign BFRs.

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1. Introduction

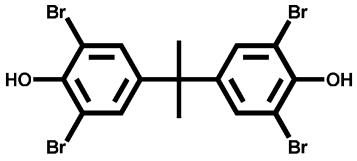
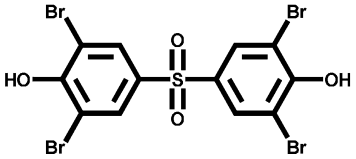
The environmental occurrence and potential toxicity of brominated flame retardants (BFRs) caused an increasing public concern and their fate and risk are becoming an emerging research hotspot in recent years [1–5]. Tetrabromobisphenol A (TBBPA) is the most commercially used BFR with a high production volume (HPV) and has worldwide demand of >200,000 tons/year [6], bearing ~60% of the total BFR market. TBBPA acts as a reactive flame retardant and an additive flame retardant. It has been widely used for the production of various consumer products, such as printed circuit boards, electric appliances and various plastic resins [4]. As a replacement alternative, tetrabromobisphenol S (TBBPS) can retard flame better than TBBPA and also has wide applications for many resins.

The environmental release of TBBPA and TBBPS from existing or discarded products caused their residues in air, water, sediment, soil, food [7–12], human serum and breast milk [13,14]. Exposure to TBBPA was reported to cause carcinogenic effects, hepatotoxicity, immune response neurotoxicity, and disruption of endocrine system [15]. TBBPA was recently reported to activate proinflammatory responses in human first trimester trophoblasts [16], and have association with *in vivo* endocrine and reproductive toxic effects [17,18]. It can mimic hormones and disrupt the binding, transport, and regulation of hormones [19,20], leading to endocrine disruption. It was suggested as obesogens in zebrafish larvae (*Danio rerio*) and zebrafish PPARγ activator [21]. TBBPA can specifically interact with and activate PPARγ [22–24], and the binding mode was elucidated at the atomic level [25]. TBBPA was also reported to interact with other proteins such as bovine serum albumin [26]. However, the potential risk of TBBPA and TBBPS on enzymes such as bovine pancreatic trypsin remains largely unknown to date.

Bovine trypsin belongs to one part of the digestive system for vertebrates and is an archetypical member of serine protease (EC 3.4.21.4) from the PA clan superfamily. It is a water-soluble globular protein produced in the pancreas with 223 amino acids. Bovine trypsin has diverse biological functions such as immune responses, digestion, hemostasis, and cancer metastasis. Its catalytic site consists of one catalytic triad composed by His57, Asp102 and Ser195, responsible for the hydrolysis of C-terminal side of lysine and arginine as well as amide linkages and ester of substrates. Due to its essential physiological function, it has often been chosen as target protein to study the structural effects of small molecules to trypsin and thereby the function [27–30]. However, there still lacks studies on the interactions of TBBPA and TBBPS with bovine trypsin. The increasing toxicological studies on TBBPA and TBBPS call for detailed information on the molecular-level events for their interactions with bovine trypsin. Such investigations can shed light on the effect of TBBPA and TBBPS on the conformational changes of trypsin and also facilitate the evaluation of their influence on the normal physiological function of bovine trypsin.

In this present study, we investigated the interactions of TBBPA and TBBPS (Table 1) with bovine trypsin using fluorescence lifetime assay, steady-state fluorescence spectroscopy and circular dichroism (CD). The effect of TBBPA and TBBPS on conformational changes and secondary structural changes was probed. Molecular dynamics (MD) simulations were also innovatively applied as a complement to these *in vitro* assays. The binding mode of TBBPA and TBBPS to bovine trypsin was elucidated by MD simulations at the atomic level. Their binding free energies were decomposed and the predominant force for their interactions was deciphered. Our *in vitro* and *in silico* studies facilitate the risk assessment of TBBPA-related materials and offer a framework to elucidate the interactions of various BFRs with target receptors.

Table 1
The structures of TBBPA and TBBPS.

Compound	Structure	CAS
TBBPA		79-94-7
TBBPS		39635-79-5

2. Materials and methods

2.1. Reagents and chemicals

TBBPA, TBBPS, 8-anilinonaphthalene-1-sulfonate (ANS) and rose bengal with a purity of 97% were purchased from Sigma–Aldrich (USA). Bovine trypsin (Catalogue T1426, Sigma–Aldrich) was used without further purification. All other chemicals were of analytical grade. The stock solution of trypsin was prepared in 0.02 M PBS buffer (pH = 7.4) and was stored at 4 °C. All aqueous solutions were prepared with distilled water (18.2 MΩ, Millipore, Bedford, MA).

2.2. Steady-state fluorescence spectroscopy

All measurements of fluorescence spectra were performed in a 1-cm quartz cell using FluoroMax®-4 spectrofluorometer (Horiba Jobin Yvon IBH). Fluorescence emission spectra were recorded from 300 nm to 500 nm with an excitation wavelength of 280 nm at 298 K, 304 K and 310 K, and slits of excitation and emission were set at 4 nm. The background fluorescence of the buffer was subtracted, and the inner-filter effect was eliminated according to the following equation:

$$F_e = F_m e^{(A_1+A_2)/2} \quad (1)$$

where F_e and F_m are the corrected and measured fluorescence, respectively; A_1 and A_2 are the sum of the absorbance of proteins and ligands at the excitation and max emission wavelengths, respectively.

2.3. Time-resolved fluorescence spectroscopy

The time-resolved fluorescence decay was measured on FluoroMax®-4 spectrofluorometer (Horiba Jobin Yvon IBH) equipped with a FluoroHub module using the time-correlated single-photon counting (TCSPC) method. The lifetimes of trypsin with or without ligands were measured using a picosecond 280 nm diode laser (NanoLed-280 nm) at an emission wavelength of 340 nm. The slit of 4 nm was used for both excitation and emission. Fluorescence decay profiles were analyzed by the DAS6 Decay Analysis software (Horiba Jobin Yvon IBH) using the two-exponential decay method. The quality of model fitting was assessed by chi-square values (0.9–1.2).

2.4. Ligand displacement assay

The ligand displacement assays were carried out to determine the binding location of TBBPA and TBBPS. Bengal was reported to bind at the active site and ANS binds at the binding site neighboring