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Binding of triclosan and triclocarban to pepsin: DFT, spectroscopic and dynamic simulation studies

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HIGHLIGHTS

- Density Functional Theory was used for determination feasible energy transitions.
- The binding properties of triclosan or triclocarban to pepsin was studied.
- Secondary structure of pepsin was perturbed on addition of triclosan or triclocarban.
- Triclosan had better binding capacity with pepsin than triclocarban.

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ABSTRACT

The use of antibacterial agents, triclosan (TCS) and triclocarban (TCC), in personal care products can result in direct human exposure. Density Functional Theory (DFT) was utilized to evaluate the electronic properties of TCS and TCC, and the determined energetically accessible transitions across the HOMO-LUMO gap. Choosing pepsin as a model protein, we explored the binding effects of TCS or TCC on pepsin by molecular docking and dynamic simulations. Titration of pepsin with TCS or TCC at pH 2.2 led to quenching of the pepsin intrinsic fluorescence via formation of a ground-state complex. The binding constants of the TCS/TCC-pepsin complexes, determined at 296 K, were $(7.053 \pm 0.030) \times 10^4\ M^{-1}$ and $(6.233 \pm 0.060) \times 10^4\ M^{-1}$, respectively. Analysis of the thermodynamic properties of each system at various temperatures demonstrated that the binding reaction is a spontaneous process driven by hydrophobic interactions. The spectroscopic results revealed that changes in the secondary structure of pepsin are induced by TCS or TCC. The thermal stability of pepsin was evaluated, and no change in thermal stability was observed upon substrate binding. However, the binding of either TCS or TCC to pepsin effectively reduced the activity.

1. Introduction

Triclosan (TCS) and triclocarban (TCC) (Fig. 1) are bacteriostat, which were historically used in household and personal care items such as detergent, mouthwash, shampoo, and soap (Kolpin et al., 2002; Liao and Kannan, 2014). The widespread use of these chemicals over the course of 30 years has resulted in their contamination of in aquatic environments (Chalew and Halden, 2009). The absorption of TCS or TCC by human matrices via casual contact with consumer products containing these chemicals is evident by analysis of urine, blood, serum, plasma, human milk and amniotic fluid samples (Fair et al., 2009; Sherburne et al., 2016; Wang et al., 2015; Xue et al., 2015; Iyer et al., 2018). According to the U.S. national population survey conducted in 2003, TCS was detected in 74% of urine samples (Calafat et al., 2008). Further studies demonstrated that the concentrations of TCS in breast milk samples ranged from un-detectable to 63 ng mL^{-1} (Azzouz et al., 2016; Toms et al., 2011). Moreover, exposure to the TCS has been correlated with adverse health effects, such as the occurrence of asthma in children, an increased risk of cancer (Iyer et al., 2018), and obesity problems (Lankester et al., 2013). TCS has been shown to weaken the contractile properties of cardiac and skeletal muscles *in vitro* and *in vivo*, decreasing muscle function (Cherednichenko et al., 2012). Similarly, adverse health effects are observed following exposure to TCC including methemoglobinemia, and

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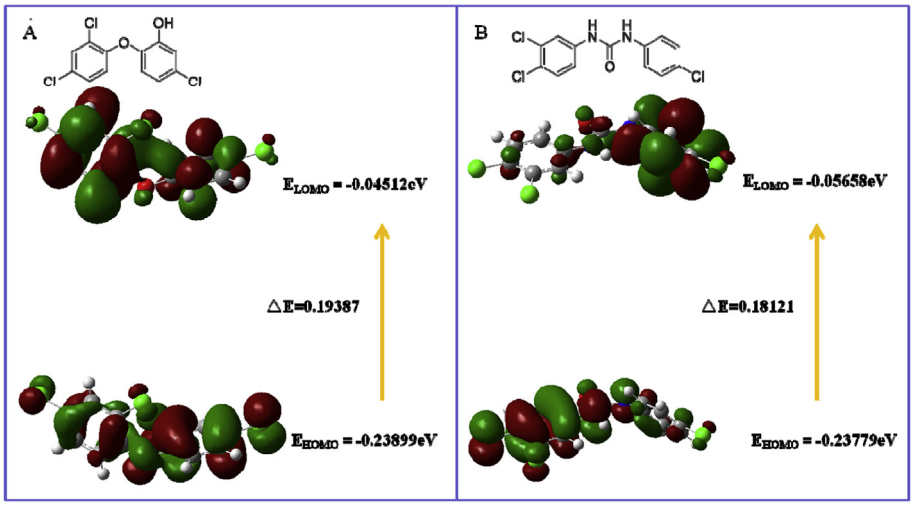

Fig. 1. Patterns of the HOMO, LUMO of TCS (A) and TCC (B) calculated at the B3LYP/6-311G(d,p) level.

inhibition of mammalian reproduction (Johnson et al., 1963; Chen et al., 2013). The disruption of endocrine activity by TCS and TCC has considerable scientific interest in these chemicals. In fact, in 2016 the U.S. Food and Drug Administration (FDA) moved to ban the use of TCS and TCC in over-the-counter consumer wash products (FDA, 2016; Albanese et al., 2017).

Use of TCS- or TCC-containing personal care products is a major source of human exposure to these chemicals (Liao and Kannan, 2014). While the exposure to these chemicals can lead to the adverse health effects described above, the mode of action of these chemicals has not been widely explored. The work described in this report seeks to probe the interaction of TCS or TCC with pepsin, an important protease in the human digestive system. Pepsin ia an aspartic protease secreted by the stomach in mammals. During digestion, pepsin specializes in severing the links between particular types of amino acids, breaking down dietary proteins into amino acids or peptides (Zeng et al., 2014). Crystallographic studies have shown that pepsin contains two structurally homologous domains: one, an N-terminal domain (residues 1–172) and the other, a C-terminal domain (residues 173–326) (Spelzini et al., 2008; Jin et al., 2008). The active binding sites are located in the cavity between these two domains. Two aspartic acid residues are present at the catalytic site and are protonated in the pH range required for good protein-degrading activity (pH 1.5–2.5) (Mohseni-Shahri et al., 2018). Previous studies have demonstrated that the binding of small molecules to pepsin can result in enzyme inhibition. For example, Nan et al. demonstrated an energy transfer from pepsin to the small molecules myricetin and dihydromyricetin upon introduction of these substrates into the hydrophobic cavity of pepsin (Nan et al., 2016). Considering the greater role in human's digestive system, pepsin and small molecules were selected as a model system to investigate the effects on their structural properties or activities.

In present work, a detailed description of the binding of TCS/TCC to pepsin and the resulting structural alterations, is reported. We analyzed the binding of TCS/TCC to pepsin via several spectroscopic techniques and further probed the system by molecular modeling calculations. The frontier molecular orbitals of TCS and TCC were used to investigate their chemical reactivity, and extensive computer simulations of the pepsin and TCS/TCC interactions were reported. The thermal stability of pepsin was studied by differential scanning calorimetry (DSC), and enzyme activity assays for pepsin were performed in the presence of TCS/TCC. The results of this study provide insight into the mechanism of toxicity of TCS and TCC, which has larger implications for the development of government regulations and risk prevention measures.

2. Materials and methods

2.1. Reagents and materials

The following reagents were purchased from J&K Chemicals (Beijing): pepsin from porcine gastric mucosa (lyophilized powder, ≥2500 units/mg protein) and analytical grade TCS and TCC. Pepsin was dissolved in a phosphate buffer (pH 2.2) to prepare 3.0×10^{-6} M stock solutions. All other chemicals, including citric acid and sodium hydrogen phosphate were analytical grade and purchased from Aladdin.

2.2. Calculations

DFT calculations were performed using the Gaussian 09 package (Frisch et al., 2013). The structural and electronic properties of TCS and TCC were optimized using Becke's three-parameter hybrid method with the Lee-Yang-Parr (LYP) correlation functional (B3LYP)/6-31G(d). Geometric optimizations were performed without constraints on bond lengths, bond angles, or dihedral angles. Electrostatic transition energies including the HOMO and LUMO were generated at the B3LYP/6-31G level (Stratmann et al., 1998).

TCC/TCS–pepsin binding modes and energies were predicted using AutoDock 4.2 (Morris et al., 2009). The crystal structure of pepsin was obtained from the Protein Data Bank (ID: 5PEP). Water molecules and ions were removed from the structure of pepsin and the polar hydrogen atoms and Kollman charges were added. ADT was used to determine the initial binding position of the ligands by assigning the Gasteiger charges to atoms and merging nonpolar hydrogens. Default parameters of the Lamarckian genetic algorithm (LGA) were used for initiation of the docking simulation. An appropriate grid box size of $80 \times 80 \times 80$ Å^3 was selected and the pepsin was positioned at the center of the grid. The lowest energy docked-structure was further analyzed using Discovery Studio.

Molecular dynamics (MD) simulations were performed using the sander module implemented in the Amber 16 package (Fani et al., 2013). The ff14SB force field was used for the receptor (protein), and the GAFF force field for the ligand. The ANTECHAMBER module was used to calculate the AM1-BCC partial charges of the liand. Sodium ions were added to the receptor to neutralize the