Single-walled carbon nanotube release affects the microbial enzyme-catalyzed oxidation processes of organic pollutants and lignin model compounds in nature

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HIGHLIGHTS

- SWCNT release affects the peroxidase-catalyzed oxidation processes of lignin model compounds.
- Biodegradation processes of PAHs can be disturbed by the SWCNT.
- We assessed the impact of SWCNTs on the biodegradation of β-hexachlorocyclohexane mediated by haloalkane dehalogenase using molecular dynamics simulations.

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ABSTRACT

The question how microbial enzyme-catalyzed oxidation processes of organic pollutants and lignin model compounds (LMCs) are affected by the release of single-walled carbon nanotube (SWCNT) into the environment remains to be addressed at the molecular level. We have, therefore concentrated the effects of SWCNT on some important properties associated with enzyme activity and function during microbial oxidation of polycyclic aromatic hydrocarbons (benzo(a)pyrene, acenaphthene and anthracene), LMCs (2,6-dimethoxyphenol, guaiacol and veratryl alcohol) and β-hexachlorocyclohexane, including the behaviour of water molecules, hydrogen bonds (HBs) and hydrophobic interactions (HYs) between ligand and the enzyme, and conformational dynamics in N- and C-terminus. Our study revealed that SWCNT significantly affected the behaviour of water molecules within 5 Å of both these substrates and their respective enzymes during oxidation ($p < 0.01$), by increasing or decreasing the water number near them. SWCNT tended to significantly enhance or reduce the stability of atom pairs that formed the HBs and HYs ($p < 0.01$). N- and C-terminus conformations underwent transitions between positive and negative states or between positive state or between negative state in all analyzed complexes. Significant conformational transitions were found for all C-terminus, but only for a part of N-terminus after the inclusion of the SWCNT. These results showed that SWCNT release would significantly affect the microbial enzyme-catalyzed processes of organic pollutants and LMCs in nature.

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1. Introduction

The past decades have seen a significant progress in nanotechnology with wide applications (Yamada et al., 2011; Park et al., 2013; Shulaker et al., 2013; Zhang et al., 2014; Chen et al., 2016). However, such wide applications have been resulting in that nanomaterials (e.g., carbon nanotubes, CNTs) are released into the natural environment by accident and direct acting (Shvedova et al., 2012; Bhattacharya et al., 2014). Unfortunately, CNTs are expected to be persistent in nature due to their steady features (Berry et al., 2014; Flores-Cervantes et al., 2014). Also, there is evidence that CNTs are potentially toxic to living organisms and the environment (Chandrasekaran et al., 2014; Clar et al., 2015). However, up to now, the processes and mechanism for how CNTs disturb the natural environmental processes (e.g., enzyme-catalyzed oxidation) are still largely unclear.

Organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and β-hexachlorocyclohexane (β-HCH) are persistent, being difficult to completely remove in nature (Okai et al., 2013; Chen et al., 2015a). The contamination derived from them is not limited to water body, as they also can enter the soil system by direct release and leaching. Biodegradation has been identified as an effective strategy to remove PAHs and β-HCH from the environment. For example, *Trametes versicolor* laccase was found a good candidate for the oxidation of benzo(*a*)pyrene, acenaphthene and other PAHs (Torres et al., 2003). Naphthalene 1,2-dioxygenase (NDO) is capable of performing the oxidation of anthracene (Ferraro et al., 2006). Besides, haloalkane dehalogenase (Hde) is able to be utilized for the degradation of β-HCH (Okai et al., 2013). Evidently, due to wide distribution and inadequate disposal, lignin becomes waste and even pollutant in many cases (Chen et al., 2011, 2015b). The pollution problems derived from lignin accumulation and energy shortage have aroused intensive studies over the past decades in the subject of lignin biotransformation (Munk et al., 2015; Mycroft et al., 2015). Lignin structure is so complex that researchers often employ lignin model compounds (LMCs) for lignin degradation/synthesis studies, the latter being simple compounds that contain the structural units of lignin (Bu et al., 2012; Chen et al., 2015b; Deuss and Barta, 2016). Laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) have been demonstrated to be the main ligninolytic enzymes (Sánchez, 2009). The crystal structure of *Melanocarpus albomyces* laccase has been determined, consisting of three domains (A, 1—157; B, 158—341; C, 342—559) (Hakulinen et al., 2002). *M. albomyces* laccase catalyzes the oxidation of several LMCs, such as 2,6-dimethoxyphenol (DMP) (Andberg et al., 2009) and guaiacol (Kiiskinen et al., 2002). These LMCs were also reported to be the substrates of *Phanerochaete chrysosporium* MnP (Hu et al., 2009). Veratryl alcohol (belonging to LMC) was often used as the substrate for studying the properties of LiP (Ferapontova et al., 2006). The 3D structures of both MnP and LiP from *P. chrysosporium* are available in PDB database (Rose et al., 2013), such as 1YYD (Sundaramoorthy et al., 2005) for MnP and 1LLP (Choinowski et al., 1999) for LiP. Despite wide studies on enzyme-catalyzed oxidation of organic pollutants and lignin/LMCs, little research has been conducted on the effect of CNTs on their enzyme-catalyzed oxidation processes.

The oxidation of organic pollutants and lignin mediated by microbial enzymes often occurs in the environment (Sánchez, 2009; Chen et al., 2015a). CNTs have been detected in soil and water systems. If this process occurs in the CNT-polluted places, it would lead to the fact that this enzyme-catalyzed oxidation process may be disturbed by CNTs. This is because many studies show that the presence of CNTs affects the enzymatic activity and other properties (Mubarak et al., 2014; Tavares et al., 2015). In addition, CNTs themselves can be decomposed by various enzymes. It was found that the degrading efficiency of CNTs by horseradish peroxidase was very low, and that the degrading processes were slow with long half-lives (about 80 years) (Flores-Cervantes et al., 2014). Besides horseradish peroxidase, typical enzymes already known for their ability to degrade CNTs include myeloperoxidase (Kagan et al., 2010), lactoperoxidase (Bhattacharya et al., 2015), eosinophil peroxidase (Andón et al., 2013), lignin peroxidase (Chandrasekaran et al., 2014) and manganese peroxidase (Zhang et al., 2014).

Addressing the environmental problems derived from CNTs remains a great challenge. Previous studies provide little insight into whether CNT release would affect the enzyme-catalyzed oxidation processes in nature. Thus, it is still unclear how the presence of CNTs changes the contacts between various substrates and their corresponding enzymes that are the foundation of enzymatic reaction. To tackle this problem, we aim to use molecular dynamics (MD) simulations to investigate the effects of single-walled carbon nanotube (SWCNT) on the behaviour of water molecules, hydrogen bonds (HBs) and hydrophobic interactions (HYs), and conformational dynamics in N- and C-terminus during the enzyme-catalyzed oxidation of PAHs (Benzo(*a*)pyrene, acenaphthene and anthracene), lignin (lignin model compounds were used as representative, including DMP, guaiacol and veratryl alcohol), and β-HCH by comparing the conformations in the presence and absence of SWCNTs.

2. Materials and methods

2.1. Data on enzyme-catalyzed oxidation

We found enzyme-catalyzed oxidation information of organic contaminants and LMCs by searching the University of Minnesota Biocatalysis/Biodegradation Database (Gao et al., 2010), BRENDA database (Schomburg et al., 2002) and Google Scholar. Finally, we selected eight pairs of substrate-enzyme systems based on the following criteria: (1) the experimental 3D structures of enzymes must be available; (2) the substrate molecules must be small in size. These substrates and their corresponding enzymes were: laccase vs. guaiacol; LiP vs. veratryl alcohol; MnP vs. DMP; MnP vs. guaiacol; laccase vs. benzo(*a*)pyrene; laccase vs. acenaphthene; NDO vs. anthracene; Hde vs. β-HCH. For simplicity, we defined their abbreviations in Table 1.

2.2. Structure models of organic pollutants, LMCs and enzymes

Some experiment-determined 3D structures of the above selected enzymes available in RSC PDB database (Rose et al., 2013) (http://www.rcsb.org/pdb/home/home.do) enable the present study. The structures of substrate molecules were taken directly from ChemSpider (http://www.chemspider.com/) (Pence and Williams, 2010), while those of selected enzymes were downloaded from PDB with some simple treatment as described in our previous studies (Chen et al., 2011, 2015b).

2.3. Preparation of initial conformations

SWCNT-bound conformation models of the enzymes were generated using PatchDock (Schneidman-Duhovny et al., 2005), and then refined by FireDock (Mashiach et al., 2008). The model system adopted in each PatchDock run was composed of an enzyme and a SWCNT (6,6) with a length of 2 nm. Substrate molecules were docking into their corresponding enzymes with and without SWCNTs by Molegro Virtual Docker (MVD) (Thomsen and Christensen, 2006). MolDock score [Grid] was employed as the scoring function with 0.3 Å of grid resolution, whereas the selected algorithm was Moldock SE. The obtained candidates were ranked based on MolDock score and Re-Rank score to get the best pose which was selected as the final model for further studies.

2.4. MD simulations

Gromacs 4.6 packages (Pronk et al., 2013) were adopted to perform the simulations where OPLS-AA force field was employed (Kaminski et al., 2001). RESP charges of small molecules were obtained at the HF/6-31G* level. Other field parameters of small molecules were determined by MKTOP 2.0 (Ribeiro et al., 2008), a Perl script. For each pair of substrate and its corresponding enzyme, we constructed two systems, one with SWCNT and another one without SWCNT which was used as a control group with the same simulation conditions (Table 1, Figs. 1—4). Thus, a total of 16 complexes were presented. Each complex was solvated by SPC water model in two cubic boxes with the same size. 36,284—215,956