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Biotransformation and responses of antioxidant enzymes in hydroponically cultured soybean and pumpkin exposed to perfluorooctane sulfonamide (FOSA)

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ABSTRACT

Perfluorooctane sulfonamide (FOSA) is an important perfluorooctane sulfonate (PFOS) precursor used for commercial applications. In order to investigate the transformation and responses of selected antioxidant and degradation enzymes of FOSA in the plants, *in vivo* exposure of soybean (*Glycine max* L. Merrill) and pumpkin (*Cucurbita maxima* L.) were conducted in the solution-plant microcosms. FOSA was readily taken up by soybean and pumpkin roots and translocated to shoots, and metabolized to PFOS, perfluorohexane sulfonate (PFHxS) and perfluorobutane sulfonate (PFBS). Although morphological and biomass effects were not visible, significant changes in oxidative stress response were observed except for thiobarbituric acid reactive substances (TBARS). Superoxide dismutase (SOD) and peroxidase (POD) activities were significantly increased by 19.2–30.8% and 19.2–20.7% in soybean (8–12 d) respectively, and increased by 39.2–92.8% and 21.1–37.6% in pumpkin (3–12 d) respectively, suggesting an activation of the antioxidant defense system in the plants exposed to FOSA. Glutathione-S-transferase (GST) activities were decreased in soybean (2–12 d) with 9.0–36.1% inhibition and increased in pumpkin (3–12 d) with 22.5–47.3% activation respectively; cytochrome P450 (CYP450) activities were increased markedly in soybean and pumpkin with 13.2–53.6% and 26.7–50.2% activation respectively, giving indirect evidences on the involvement of CYP450 and GST in degradation of FOSA in plants.

1. Introduction

Per- and polyfluoralkyl substances (PFASs), including perfluoroalkyl acids (PFAAs) and their precursors, have been widely used in a variety of products due to their lipophobic and hydrophilic characteristics (Paul et al., 2009). Perfluorooctane sulfonate (PFOS) is the dominant PFASs which is extremely persistent in the environment and bioaccumulative in food webs, and has toxicological effects in biota (Houde et al., 2011). After PFOS and perfluorooctanesulfonyl fluoride (PFOSF) were added to the list of Stockholm Convention on Persistent Organic Pollutants (POPs), their production and use significantly decreased in many countries (UNEP, 2009). However, the production and application of the products containing PFOS and its precursors (PreFOS) are increasing in some places of the world (Löfstedt Gilljam et al., 2016). Perfluorooctane sulfonamide (FOSA) is a PreFOS, which is used as surfactants, as well as intermediates in the synthesis of other PFASs (Fromme et al., 2009). The release of FOSA into the environment may occur directly from FOSA-based products during application, disposal or FOSA residuals or indirectly through the degradation products of other PreFOSs, including N-ethyl perfluorooctane sulfonamido ethanol (N-EtFOSE) (Mejia Avendano and Liu, 2015; Xu et al., 2004), N-ethyl perfluorooctane sulfonamido acetate (N-EtFOSAA) (Higgins et al., 2007), N-methyl perfluorooctane sulfonamidoacetic acid (N-MeFOSAA), N-ethyl perfluorooctane sulfonamide (N-EtFOSA) (Fu et al., 2015), and (N-ethyl perfluorooctanesulfonamido) ethanol-based phosphate diester (diPAP) (Peng et al., 2014). Thus, FOSA can be found in air (Eriksson and Kärrman, 2015; Haug et al., 2011), water (Boulanger et al., 2004), soil (Houtz et al., 2013), wildlife (Houde et al., 2011), humans (Bonefeld-Jørgensen et al., 2014) and consumer products (Fromme et al., 2007). Soil is an important reservoir of FOSA in the environment due to its strong adsorption capacities to soil/sediment (Ahrens et al., 2011). While toxic to biota and humans (Bonefeld-Jørgensen et al., 2014; Olufsen and Arukwe, 2015; Slotkin et al., 2008), FOSA could be transformed to PFOS through biotic and abiotic pathways (Bizkarguenaga et al., 2016; Chen et al., 2015; Ross et al., 2012).

Bioaccumulation and metabolic transformation of PFAA precursors in plants are important behaviors in the ecosystems due to their adverse effects on the environment and human health. Many previous studies

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indicated that ionic PFAAs (Bizkarguenaga et al., 2016; Felizeter et al., 2012) and their precursors (Zhang et al., 2016; Zhao et al., 2018b) could be bioaccumulated by plants. Fluorotelomer alcohols (FTOHs), which are widely recognized as precursors of perfluoroalkyl carboxylates (PFCAs), could be taken up by soybean from solutions and biodegraded to PFCAs (Zhang et al., 2016). Our previous study has shown that N-EtFOSA could be taken up by crops and biotransformed to different carbon chain length PFASs (Zhao et al., 2018b). Bizkarguenaga et al. (2016) reported that FOSA could be accumulated by carrot and lettuce from soil. In the soil-plant microcosm, the biodegradation products of FOSA in plants are the integrated effects of plant biotransformation and plant uptake from contaminated soils. In previous studies, biodegradation of FOSA in soils have been reported (Mejia Avendano and Liu, 2015; Zhao et al., 2018a). However, little work has been done to clarify the responses of biodegradation and antioxidant enzymes in plants exposed to FOSA. Plants possess a number of antioxidant and oxidative biotransformation systems that protect them from oxidative damage and metabolize xenobiotics. Thiobarbituric acid-reactive substances (TBARS) assay is an indicator of cytotoxic product of lipid peroxidation (Trevisan et al., 2001). Superoxide dismutase (SOD) and peroxidase (POD) are involved in protect cells from oxidative stress (Tewari et al., 2008). Cytochrome P-450 (CYP450), glutathione-transferase (GST), and POD are known to be involved in plant metabolism of many organic pollutants (Huang et al., 2013; Monferran et al., 2007; Zhai et al., 2013; Zhang et al., 2016).

This study was conducted with crops cultivated in solution, spiked or unspiked with FOSA, to evaluate the biodegradation and physiological responses of FOSA in plants. Soybean (*Glycine max* L. Merrill) and pumpkin (*Cucurbita maxima* L.) were chosen as the model plants because they are important economic and vegetable crops, respectively. In order to minimize the interference of soil microbes on the biodegradation of FOSA, plants hydroponic greenhouse experiments were performed. The concentrations of FOSA and its degradation products were determined in exposure solutions and different parts of soybean and pumpkin plants. The TBARS values and activities of enzymes, including SOD, POD, GST and CYP450 in soybean and pumpkin roots over different exposure times were analyzed to identify the biodegradation and physiological responses in plant exposed to FOSA.

2. Materials and methods

2.1. Chemicals

Standards of perfluorooctane sulfonamide (FOSA, 90%), perfluorobutane sulfonate (PFBS, 98%), perfluorohexane sulfonate (PFHxS, 98%), perfluoroheptanoic acid (PFHpA, 98%) and perfluorooctanoic acid (PFOA, 98%) were obtained from J&K Chemical Ltd (Beijing, China). Perfluorooctane sulfonate (PFOS, 98%) was from Shanghai Aladdin Reagent Co., Ltd. (China). Native FOSA standard was from Wellington Laboratory (Guelph, ON, Canada). Perfluorohexanoic acid (PFHxA, 98%) was bought from Matrix Scientific (Shanghai, China). All solvents, including methanol (Dikma Technology Inc., Beijing, China), ammonium acetate (ANPEL Scientific Instrument Co., Ltd., Shanghai, China), dichloromethane (DCM) and methanol for extraction (Dalian Bono Biochemical Reagent Ltd., Dalian, China) were of high-performance liquid chromatography (HPLC) grade. Other chemicals and reagents used were of reagent grade. Milli-Q water (18.2 MΩ) was used throughout the experiment.

2.2. Hydroponic exposure of plant

Soybean (*Glycine max* L. Merrill) and pumpkin (*Cucurbita maxima* L.) seeds were purchased from Hebei Shenhe seeds Co., Ltd (Cangzhou, China). Seeds were surface-sterilized with 10% H_2O_2 solution for 15 min, and rinsed and soaked with autoclaved deionized water overnight at room temperature. Then seeds were germinated on sterilized quartz sand beds for 7 days at 27 °C in the dark. After germination, uniform seedlings with the height of 5–6 cm were transferred to 250 mL glass bottles (5 plants pot^{-1}) containing 200 mL of sterile 1/4-strength Hoagland's nutrient solution (Zhang et al., 2016) for cultivation. The containers were wrapped with aluminum foil paper to prevent the photolysis of FOSA.

Standard solutions of FOSA dissolved in methanol were gradually diluting with sterile nutrient solution and mixed thoroughly. The initial concentration of FOSA determined in the spiked exposure solutions were 1.856 nmol mL^{-1} (0 d). The volume of methanol in the exposure solution was below 1‰ (v/v) to minimize cosolvent effects. The soybean and pumpkin seedlings were transferred to the glass containers immediately after FOSA spiking (named S and P group, respectively). Blank controls with soybean and pumpkin seedlings cultured in nutrient solution with the same concentration of methanol as the test groups but without FOSA (named CS and CP group, respectively), and unplanted controls with FOSA but without seedlings as incubation solution (named Solution-12 d group) were set up simultaneously. The experiments were conducted in a growth chamber (14 h, 25 °C, day; 10 h, 22 °C, night) and were set in triplicate (n = 3). All glass containers were positioned randomly and re-randomized on each other day. During hydroponic exposure, approximately 10 mL d^{-1} of sterile nutrient solution was added into each container to compensate for the transpiration losses. Plant seedlings were harvested from each container at days of 1, 2, 3, 5, 8 and 12, and at each sampling time, three containers were sacrificed (n = 3) in each group. Root samples were thoroughly rinsed with sterilized distilled water, and rinses were collected and then combined with the exposure solutions for the analysis of FOSA and its metabolites in solutions. Plant seedlings were wiped with paper towel and weighed immediately as fresh weight (FW), and sampled as root and shoot parts. The fresh root samples (1, 2, 3, 5, 8 and 12 d) were used to evaluate biological responses. At the end of the experiments (12 d), part of the shoot and root samples were freeze-dried for 48 h in a lyophilizer, and then homogenized and stored at − 20 °C in polypropylene (PP) tubes before PFASs content analysis.

2.3. Chemical extraction and instrumental analysis

The extraction and cleanup methods of FOSA and its metabolites in solution (12 d) and plant samples (roots and shoots/12 d) were based on our previous study (Zhao et al., 2018b)

2.3.1. Plant samples

Approximately 0.1 g (0.5 g) of homogenized root (shoot) sample was extracted with 5 mL of dichloromethane (DCM) in a 50 mL polypropylene (PP) centrifuge tube. The mixture was vortexed and extracted in an ultrasonic bath for 30 min. Then 5 mL of methanol was added and shaken for 1 h at 250 rpm, and centrifuged for 30 min (10,000 rpm). This extraction process was repeated once and the extracts were evaporated under gentle nitrogen (N_2) to almost dryness, and then reconstituted in 5.0 mL of methanol. The extracts were cleanup using Clearnert Pesticarb-SPE cartridge (500 mg/6 mL, Bonna-Agela Technologies) according to our previous study (Zhao et al., 2018b).

2.3.2. Solution samples

In brief, 20 mL of filtered solution was purified using Cleanert PEP cartridges (500 mg/6 mL, Bonna-Agela Technologies). The cartridges were preconditioned by passage of 10 mL of methanol, followed by 10 mL of water (1 drop s^{-1}). Solution samples were then loaded on the cartridge, and then pumped until dry. The target analytes were eluted with 10 mL of methanol and evaporated under a gentle stream of N_2 to approximate 1 mL. The extract was centrifuged (12,000 rpm, 30 min) and the supernatant was transferred into an auto sampler and diluted with methanol to 1 mL.