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Apoplastic and symplastic uptake of phenanthrene in wheat roots☆

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ABSTRACT

The contamination of agricultural crops by polycyclic aromatic hydrocarbons (PAHs) has drawn considerable attention due to their carcinogenicity, mutagenicity, and toxicity. However, the uptake process of PAHs in plant roots has not been clearly understood. In this work, we first study the radial uptake of phenanthrene in hydroculture wheat roots by vacuum-infiltration-centrifugation method. The concentration-dependent kinetics of apoplastic and symplastic uptake at phenanthrene concentrations of 0–6.72 μM for 4 h can be described with the Langmuir and Michaelis-Menten equations, respectively; whereas, their time-dependent kinetics at 5.60 μM phenanthrene for 36 h follow the Elovich equation. The apoplastic and symplastic uptake increases with temperature of 15–35 °C. The apparent Arrhenius activation energies for apoplastic and symplastic uptake are 77.5 and 9.39 KJ mol^{-1}, respectively. The symplastic uptake accounts for over 55% of total phenanthrene uptake, suggesting that symplast is the dominant pathway for wheat root phenanthrene uptake. Larger volume of symplast in roots and lower activation energy lead to the greater contribution of symplast to total uptake of phenanthrene. Our results provide not only novel insights into the mechanisms on the uptake of PAHs by plant roots, but also the help to optimize strategies for crop safety and phytoremediation of PAH-contaminated soil/water.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread, hydrophobic, persistent organic pollutants deriving primarily from natural and anthropogenic incomplete combustion or pyrolysis of organic material (Lafontaine et al., 2015). PAHs have been attracting worldwide concern due to their carcinogenicity and toxicity to both humans and animals (Liu et al., 2014; Zhang et al., 2015; Ruby et al., 2016). PAH pollution in China is heavier than in other countries because of the rapid industrial development (Gao et al., 2007; Guo et al., 2011). The annual emission of PAHs in China was about 114,000 tons, comprising up to 21–29% of the global total (Zhang et al., 2009; Lin et al., 2016a). Over 90% of PAHs reside in surface soil (Gao et al., 2007). Crops/plants grown in the contaminated soil can be polluted with PAHs (Li and Ma, 2016). It has been observed that 16 priority PAHs varied from 8600 to 111,000 ng g^{-1} dry weight in Chinese vegetables from Minjiang River Estuary, China (Zhang et al., 2004). Over 20% of staple crops contain PAHs above the relative control limit (5 μg kg^{-1} benzo[*a*]pyrene) in China (Zhou and Sun, 2004). This poses a threat to human and animal health. Dietary intake has been identified as the major route of PAH exposure for the non-smoking population with plant-based foodstuffs (Menzie et al., 1992; Phillips, 1999; Kulhánek et al., 2005). Therefore, it is essential to shed light on the mechanisms underlying PAH uptake by plant roots for staple crop safety, risk assessment, and phytoremediation of PAH-contaminated soil or water.

Our previous results have confirmed that PAHs can move into plant roots via both passive and active transport, and the active uptake is mediated by the proton/PAH cotransport system (Zhan et al., 2010, 2012, 2015). However, the exact transport process of PAHs into plant roots is not yet fully understood (Dupuy et al., 2016). In general, solute and water movement across the root cortex to the xylem occurs via two different pathways: the apoplast (intercellular spaces and cell walls) and the symplast (passing from cell to cell through the plasmodemata) (Ma et al., 2015; Miller et al., 2016). In most roots, apoplastic movement is blocked by the Casparian strip of the endodermis. To enter the xylem, water and solute must bypass the impermeable endodermal barrier, briefly entering the endodermal symplast before rejoining the apoplast

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within the central cylinder. Nonetheless, the relative importance of the two pathways for PAH transport across the root cortex is unknown.

Although the significance of apoplastic and symplastic transport of nutrients and contaminants in plant roots has been well recognized (Sakurai, 1998; Sattelmacher, 2001; Sattelmacher and Horst, 2007; Nouchi et al., 2012), the information pertaining to the exact process and characterization of apoplastic and symplastic transport in plant roots is scarce. This is due to the difficulties inherent to the collection methods for apoplastic and symplastic fluid. Recently, the building of the vacuum-infiltration-centrifugation method in plant roots makes it easier to separate the apoplastic and symplastic fluid (Zhu et al., 2015).

In this study, we hypothesize that PAHs radially travel from soil solution to central cylinder, via the root apoplast as far as the root endodermis and then via the symplast to the tracheary elements, or via entry to the root symplast at the root epidermis and ongoing transport via the root cortical symplast or some combinations of these two pathways. The objectives of this study were (1) to characterize the apoplastic and symplastic uptake of PAHs by plant roots; (2) to evaluate the relative contribution of root apoplast and symplast to short-distance transport of PAHs; (3) to understand the radial transport process of PAHs in plant roots. To our knowledge, this is the first report of PAH radial transport in plant roots by the vacuum-infiltration-centrifugation method.

2. Materials and methods

2.1. Chemical

Phenanthrene, a model PAH (Huang et al., 1998, 2016; Xiao et al., 2004), was purchased from Fluka Chemical Corporation (phananthrene purity >97%), with molecular weight of 178.2 g mol^{-1}, and water solubility of 7.3 μmol L^{-1} at 25 °C (Cerniglia, 1992). All organic solvents used were of HPLC grade.

2.2. Plant preparation

Wheat (*Triticum aestivum* L.) seeds were surface-sterilized in 10% H_2O_2 for 10 min, then completely washed with Millipore water (MIlli-Q, Billerica, MA, USA) and germinated on moist filter paper for 4 days at 25 °C in the dark. Thereafter, the seedlings were removed into black plastic pots with 2500 mL half-strength aerated Hoagland solution for 5 days, and then shifted to full-strength Hoagland solution for another 5 days. The Hoagland solution was prepared with Millipore water of pH 5.5. Seedlings were cultured in a growth chamber under 16 h light (25 °C)/8 h dark (20 °C) and a relative humidity of 75% with a light intensity of 400 μmol m^{-2} s^{-1}. After growth in Hoagland solution, the seedlings were starved in Millipore water for a day and then used in the subsequent experiments of phenanthrene uptake.

2.3. Phenanthrene uptake experiments

Intact wheat seedlings were removed to 600 mL beakers containing 500 mL full-strength Hoagland solution (pH 5.5). To enhance the dissolution of phenanthrene in nutrient solution, phenanthrene stock solution prepared with methanol was added, and the methanol concentration in Hoagland solution was 0.05%. Each treatment had triplicates.

In the time course of root uptake of phenanthrene, wheat seedlings were exposed to 5.60 μmol L^{-1} phenanthrene at 15, 25 and 35 °C for 0, 2, 4, 8, 16, 24 and 36 h, respectively. The concentration-dependent uptake of phenanthrene was detected after 4 h in the solutions with phenanthrene concentrations of 0, 1.12, 2.24, 3.36, 4.48, 5.60 and 6.72 μmol L^{-1} at 15, 25 and 35 °C.

After harvest, plant roots were immersed in methanol for 3 min, rinsed with Millipore water to remove phenanthrene sorbed on root surface, and wiped with tissue paper (Schwab et al., 1998; Jiao et al., 2007).

2.4. Fractionation of phenanthrene in roots

The apoplastic fluid from intact wheat roots was obtained by the vacuum-infiltration-centrifugation method described previously by Zhu et al. (2015). 1 g of the above rinsed and wiped wheat roots was placed in a 50-mL glass beaker containing 40 mL of infiltration medium (50 mM trishydroxymethyl aminomethane (Tris)-HCl (pH 8.0), 0.6 M NaCl, 0.1% (v/v) β-mercaptoethanol). The glass beaker was then vacuum-infiltrated at 70 KPa for 10 min. After being blotted dry, the infiltrated roots were placed in a 5-mL glass syringe and centrifuged in a 50-mL centrifuge tube at 1000 g for 15 min at 4 °C. The process of vacuum-infiltration and centrifugation was repeated three times. Thereafter, the roots and syringe were rinsed with 5 mL dichloromethane/acetone (1:1, v/v) solvents. Both centrifuged and rinsed solution was stored at −80 °C until analysis of apoplastic phenanthrene. After removal of apoplastic phenanthrene, the remainder in the roots was symplastic phenanthrene.

2.5. Analysis of apoplastic and symplastic phenanthrene

Symplastic phenanthrene in wheat roots was extracted according to Zhan et al. (2010). Purification and analysis of apoplastic and symplastic phenanthrene were performed as described by Zhan et al. (2012). The details of extraction, purification and analysis are described in supplementary information (Text S1). The HPLC system (Thermo Scientific Dionex UltiMate 3000 Series) was composed of an automatic injector (WPS-3000SL), a quaternary analytical pump (LPG-3400SDN), a rapid separation thermostatted column compartment (TCC-3000RS) and a variable wavelength detector (VWD-3100). Separations were conducted with a reverse phase Symmetry C_{18} (ø 4.6 × 250 mm, 5 μm particle) column. The average recovery obtained by spiking plant samples with phenanthrene was 95.2% (relative standard deviation < 2.68%, n = 5) for the entire procedure from fractionation to determination. The detection limit was 48.5 pg phenanthrene. Analytical standards were measured before each series of analyses. Internal standard calibration was employed. None of the data in the study is corrected by recovery.

2.6. Statistical analyses

The comparison among different temperatures or between apoplast and symplast was performed with paired *t*-test at 95% confidence level. Apoplastic and symplastic uptake at each temperature was subjected to one-way analysis of variance (ANOVA) and compared using *Duncan*'s test at $P < 0.05$. Pearson's correlation analysis was employed to evaluate the fitting results. Statistical analyses were conducted with SAS software version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Time course of apoplastic and symplastic uptake of phenanthrene

The time course of apoplastic and symplastic uptake of phenanthrene is exhibited in Fig. 1. The apoplastic and symplastic uptake of phenanthrene was followed as a function of time with nonlinearity over 36 h. The uptake rate was initially fast and slowed down