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Fate and transport of free and conjugated estrogens during soil passage

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ABSTRACT

Endocrine disrupting chemicals, such as the free estrogens 17β-estradiol (E2), estrone (E1) and the conjugated estrogen estrone-sulfate (E1-3S) are found at low concentration levels in the environment. This is somehow contradictory to the strong sorption and high degradation potentials found in laboratory experiments. In particular, the fate and transport behavior of conjugated estrogens is poorly understood, and the importance of enzymes triggering the transformation pathways has received little attention. To address these deficiencies, the present research uses packed laboratory soil columns with pulse injections of free estrogens, either E2 or E1, or E1-3S, to provide sound evidence of the transformation pathways. It is further shown that (i) transport of free estrogens is subject to strong retardation and degradation, (ii) the transport of conjugated estrogens is less retarded and only to a minor degree affected by degradation, and (iii) arylsulfotransferase is the enzyme triggering the transformation reaction.

1. Introduction

The fate and transport of endocrine-disrupting chemicals (EDCs) in the soil–water environment is of major concern as they can interfere with the endocrine system in humans and aquatic life (Hanselman et al., 2003; Khanal et al., 2006). EDCs are released to the environment by humans and livestock, and appear to be almost omnipresent in spite of their low solubility and strong sorption affinity. They are frequently detected at trace concentrations in parts per trillion range in wastewater, surface waters, groundwater and, most recently, in drinking water (Kolpin et al., 2002; Campbell et al., 2006; Hernando et al., 2006; Benotti et al., 2009; Liu et al., 2009; Kostich et al., 2013; Esteban et al., 2014). In recent years, several studies have indicated that exposure to EDCs in the aquatic environment might lead to adverse effects on fish reproduction. More generally, there is evidence that exposure to EDCs has adverse effects on the metabolism, development, growth and reproduction of organisms (Colborn et al., 1993; Campbell et al., 2006; Brack et al., 2007; Diamanti-Kandarakis et al., 2009; Burkhardt-Holm, 2010; Leet et al., 2011; Caldwell et al., 2012).

The high estrogenicity of 17β-estradiol (E2), a biologically-active natural estrogen, has attracted the strongest attention. There is considerable research dealing with either E2 or its metabolites estrone (E1) and estriol (E3) focusing on their fate in the environment (Khanal et al., 2006). Laboratory studies mainly emphasized the strong sorption of E2 or E1 as demonstrated in batch experiments and their high removal capacity in either naturally or in engineered systems (Hanselman et al., 2003; Khanal et al., 2006; Liu et al., 2009). But these findings seem to contradict the fact that the various estrogens are frequently detected in the environment (Hernando et al., 2006; Liu et al., 2009; Esteban et al., 2014). Fewer studies deal with the fate and mobility of estrogens during transport through soil (for example, Das et al., 2004; Casey et al., 2005; Fan et al., 2008; Steiner et al., 2010). They give insight into the transport of unconjugated, free estrogens (e.g. E2, E1, E3); which in turn emphasizes the need for a better understanding of the degradation processes that operate during their movement in the subsurface.

Estrogens exhibit a large degree of chemical variability that strongly influences the fate of the molecules and their transport properties (Hanselman et al., 2003; Khanal et al., 2006; Liu et al., 2009). Unconjugated estrogens are less polar, sorb stronger, but are biologically more active as they allow binding and activation of nuclear estrogen receptors in tissues. Conjugated estrogens have

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added either sulfate or glucuronide groups, rendering them more polar and thus more mobile or easier to excrete.

In the human body, the level of biologically-active estrogens is of particular interest as they are responsible for the progression of estrogen-dependent cancers (Secky et al., 2013). In the literature, two potential pathways for the sulfate conjugation of E1 are described: a) the sulfatase pathway, which converts the inactive estrogen to the active estrogen, and b) the sulfotransferase pathway, which converts the active estrogen to the inactive form (Secky et al., 2013). Both pathways are triggered by enzymes, and the correct function of their interplay is of great importance for human health and well-known in medical sciences, albeit the sulfate conjugation or de-conjugation is only one of several physiological transformation pathways. Arylsulfatase is the enzyme responsible for the conversion of the inactive to the active estrogen (e.g. E1-3S to E1), whereas arylsulfotransferase converts the active to the inactive estrogen (e.g. E1 to E1-3S) (Secky et al., 2013). While the detection of arylsulfatase is described in soil (e.g. in Scherr et al., 2009b), arylsulfotransferase has not been determined, to date, in soil matrices.

It is common knowledge that estrogens are excreted by humans or animals either as free or as sulfate- or glucuronide-conjugated estrogens, among other transformation products (Hutchins et al., 2007; Shore and Pruden, 2010; Zheng et al., 2013). As they enter the environment, the conjugated estrogens themselves might serve as the precursors of the free estrogens, dependent on the conditions. Given the molecular structures of the free and conjugated estrogens, varying affinity and mobility properties in soil are expected. The free and conjugated estrogens themselves show different endocrine disrupting potentials. Therefore, the knowledge of all compounds present along with the soil and hydrochemical properties is important to understand their fate and transport behavior. Recent studies have examined the fate and transport of sulfate- or glucuronide-conjugated estrogens in soils (Scherr et al., 2008, 2009a, 2009b; Shrestha et al., 2012). For example, in the study of Scherr et al. (2008) which is based on batch experiments, a fast degradation of estrone-3-sulfate (E1-3S) to the metabolite E1 was observed; however, transport experiments were not performed. Hence, in none of the studies has the formation of sulfate-conjugated estrogens as metabolites of unconjugated estrogens been investigated.

We showed recently that a certain amount of free E2 can be transformed to E1 and subsequently to E1-3S in soils (Goeppert et al., 2014). The present study focuses on understanding the transport behavior reflected in breakthrough curves at the column outlet and the identification of the enzymes operating. From these analyses, the metabolic pathway from E2 to E1, and subsequently to E1-3S, which might be triggered by the enzyme arylsulfotransferase, was validated.

2. Material and methods

2.1. Chemicals

The following steroids were purchased from Sigma–Aldrich, Steinheim, Germany: estrone, E1, [53-16-7], >99% purity; estrone-3-sulfate, E1-3S, [1240-04-6], >98% purity; 17β-estradiol, E2, [50-28-2], ≥98% purity; and estriol, E3, [50-27-1], ≥97% purity. Isotopically-labeled estrone-^{13}C (estrone 3,4-$^{13}C_2$, [53-16-7]) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Details of other compounds used for this study are given in the Supplementary material (S1).

Sterile Whatman® Puradisc™ FP30 syringe filters with a cellulose acetate membrane and a pore size of 0.45 μm were purchased from Whatman, Dassel, Germany. Water was purified and deionized on a milli-Q purification system (Millipore Corp., USA).

2.2. Preparation of estrogen stock solutions and background solution

Stock solutions of each hormone were prepared in acetonitrile and diluted with nanopure water. Five mL aliquots of water samples and calibration solutions were spiked with 10 μL (300 ng L^{-1}) of internal standard, isotopically-labeled estrone-^{13}C (E1 3,4-$^{13}C_2$). The estrogen stock solutions were stored at 4 °C in the dark. The limits of detection and quantification were calculated according to ISO 11843. The standards were linear ($R^2 \geq 0.99$, $n = 10$) and the limits of detection (LOD) according to ISO 11843 were 1.55 ng L^{-1} (E1), 2.8 ng L^{-1} (E2) and 1.03 ng L^{-1} (E1-3S). The composition of local tap water (Chefetz et al., 2008) was chosen as background solution for the tests and prepared by diluting stock solutions. Further details are given in the Supplementary material. Sodium azide (0.02%) was added to the water samples to prevent microbial activity.

2.3. Soil

A sandy clay loam soil with a clay content of 25.3% (mainly montmorillonite), 14% silt, 48.6% sand, 12.1% coarse sand, 0.5% organic matter and specific surface area 68 m^2 g^{-1} was obtained from Bet Dagan, Israel. More detailed information on Bet Dagan soil is provided in the supplementary material (Table SM1). The non-autoclaved soil was sieved (1 mm mesh) and air-dried at room temperature (22 °C). Blank tests confirmed that Bet Dagan soil (BDS) was hormone free prior to the study.

2.4. LC-MS/MS analysis

Analysis of free and conjugated estrogens was performed using an online solid-phase extraction liquid-chromatography tandem mass spectrometry system (online SPE LC-MS/MS, Waters, Aqua Analysis system) that comprised three 515 HPLC pumps, a 1525 binary HPLC pump, a 1525μ binary HPLC pump, a 2777C sample manager with a 5 mL syringe, a hot sleeve column heater with temperature controller and a triple quadrupole mass spectrometer (Quattro micro API) with electrospray ionization (ESI). The two SPE extraction columns that operate in parallel were Waters Oasis® HLB columns (25 μm, 2.1 × 20 mm) and the analytical column was a Waters XBridge™ C^{18} column (3.5 μm, 2.1 × 50 mm). The detailed analysis procedure is provided in the Supplementary material (S2).

The retention times were 5.42 min for E1 and E2, and 5.23 min for E1-3S. The estrogens were analyzed using the parameters and gradient system given in Table SM2 and S2 (supplementary material). The total chromatographic run time for one sample including online SPE LC-MS/MS was 15 min. All analyses were run in MRM mode (Multiple Reaction Monitoring), which allowed a separation of each compound on the basis of the fragmentation and retention times.

2.5. Photometric analysis of enzyme activities

The enzyme activity of both arylsulfatase (ASTS) and arylsulfotransferase (ASULT) in Bet Dagan soil was analyzed with a Perkin Elmer Lambda 35 spectrophotometer. Analysis of ASTS was done by a method from the literature (Scherr et al., 2009b; Tabatabai and Bremmer, 1970). Briefly, the 0.5 g of soil was adjusted to 60% maximum water holding capacity in 2.0 mL acetate buffer (pH 4.65) and incubated for 4 h at 25 °C with 0.5 mL 50 mM 4-nitrophenylsulphate in acetate buffer (pH 4.65); 0.5 mL of 0.5 M