Chemosphere 74 (2009) 556–562

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Factors controlling the biodegradation of 17β-estradiol, estrone and 17α-ethinylestradiol in different natural soils

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ARTICLE INFO

Article history:
Received 8 July 2008
Received in revised form 15 September 2008
Accepted 16 September 2008
Available online 5 November 2008

Keywords:
Estrogens
Mineralization
Sorption
Degrading microbial community

ABSTRACT

We conducted a series of laboratory microcosm incubations with [^{14}C]-labeled 17β-estradiol (E2), estrone (E1) and 17α-ethinylestradiol (EE2) in 17 different natural soils to characterize hormone mineralization. A significantly higher mineralization was observed for E1 (2.0–37.6%) and E2 (4.2–50.2%) than for EE2 (0.5–2.6%) in all test soils after 21 days. Soil physical or chemical parameters were not related to estrogen mineralization. Although sorption parameters varied greatly for E2 (K_F = 21.9–317.5 mL g^{-1}), for E1 (K_F = 46.0–517.5 mL g^{-1}) and for EE2 (K_F = 29.9–326.1 mL g^{-1}) this apparently did not control estrogen bioavailability since it showed no effects on hormone mineralization.

In order to elucidate the controlling factors, experiments with combined additions of radiolabeled estrogens and different substrates were conducted. Additions of ammonium nitrate or alanine to soil samples generally increased EE2 mineralization, thus indicating N-limitation. Additions of glucose induced higher E2 and EE2 degradation in comparison to control samples which is attributed to co-metabolism. Additions of saw dust, catechol or streptomycin influenced the microbial population in the test soils and affected the mineralization of E2 and EE2. Thus, we clearly demonstrate that different microbial communities are responsible for E2 and EE2 degradation in soils. We suggest that EE2 is mineralized by white-rot fungi and E2 by bacteria.

1. Introduction

Studies have shown that the disposal of animal manures, waste water and sewage sludge to agricultural land can lead to the transfer of steroid hormones like the natural 17β-estradiol (E2) and the synthetic contraceptive 17α-ethinylestradiol (EE2) into surface and ground waters (Shore and Shemesh, 2003). In soils Finlay-Moore et al. (2000) detected background E2 concentrations of 55 ng kg^{-1} which increased to 675 ng kg^{-1} through broiler litter applications. Concentrations of estrogens in surface and ground waters are generally low. There, estrogen concentrations vary between 0.5 and 70 ng L^{-1} depending on their pollution source (Belfroid et al., 1999; Ternes et al., 1999). In effluents of different sewage treatment plants EE2 has been detected in concentrations ranging up to 42 ng L^{-1} and E2 concentration has been found ranging up to 64 ng L^{-1} (Ying et al., 2002). However, these compounds affect reproduction and development of fishes at concentrations as low as 1 ng L^{-1} (Rodgers-Gray et al., 2000; Vajda et al., 2008). Particularly EE2 has an estrogenic potency about ten times that of natural hormones (Ranney, 1977).

Colucci et al. (2001a,b) investigated the dissipation of E2 and EE2 in agricultural soils and clearly demonstrated different removal efficiencies of both compounds with E2 being degraded 7-times faster. Other studies confirmed a higher stability of EE2 in soils and sediments as well as in activated sludge (Ying et al., 2004; Braga et al., 2005; Ying and Kookana, 2005). Under anaerobic conditions, EE2 was found to be resistant to biodegradation while E2 degraded slowly (Cynthia and Londry, 2006).

Estrogen mineralization seems to be indirectly regulated by sorption processes but hypotheses about this are conflicting. Some studies (Casey et al., 2003; Das et al., 2004) report that hormones degrade while present in the soil sorbed phase or rapidly desorb to the liquid phase. On the other hand, Weber et al. (2005) investigated the E2 and EE2 degradation in activated sludge and attributed a decrease in hormone mineralization to sorption to solid sludge particles.

Furthermore, conflicting results about the transformation of E2 to E1 are reported. Colucci et al. (2001a) observed an abiotic oxidation of E2 to E1, while Ying and Kookana (2005) found E2 to only be biotransformed to E1. Moreover, Cynthia and Londry (2006) observed an aerobic and anaerobic oxidation of E2 to E1 and a reversible inter-conversion.

The elimination of estrogens in soils is relevant for their environmental fate but very little is known about the estrogen degrading microbial communities. Tamagawa et al. (2006) attributed

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doi:10.1016/j.chemosphere.2008.09.072

the E1 removal to ligninolytic enzymes from white rote fungi whereas Weber et al. (2005) found E2 and E1 to be degraded by a mixed culture of *Achromobacter xylosoxidans* and *Ralstonia sp*. But they found no EE2 mineralization through bacterial activity in the activated sludge. Vader et al. (2000) observed EE2 degradation by nitrifying bacteria in activated sludge. Chun et al. (2006) spiked soils with E2 and detected an increasing number of proteobacteria which seem to be E2 degraders. Biotransformation of E1 and E2 was attributed to the activity of *Chlorella vulgaris* by Lai et al. (2002).

In general, degradation of organic compounds is dependent on their bioavailability and on the ability of microorganisms to transform and degrade them. Co-metabolism is one possible mechanism of hormone degradation, but has not been investigated so far. Some parameters like pH, temperature and moisture seem to control the persistence of estrogens in the environment, especially in soils (Colucci et al., 2001a). A study by Vader et al. (2000) indicates that EE2 degradation may be N-limited because the estrogen was completely degraded by nitrifying sludge within 6 d.

Overall, the factors controlling estrogen degradation are unclear. A common approach to elucidate this is by varying certain soil parameters. Hamer and Marschner (2005) showed that additions of easily available substrates affected the mineralization of soil organic carbon. Puglisi et al. (2007) reported that substrate additions could increase mineralization of phenanthrene in soils. Rhizodeposition and root exudates can provide such substrates and enhance the degradation of PAHs in soils (Kamath et al., 2005; Rentz et al., 2005). Rentz et al. (2005) suggested that the increase was caused by co-metabolism through the stimulated microbial community.

One objective of this study was to investigate the dissipation, transformation and sorption of E2, E1 and EE2 in 17 different soil samples with different properties and land use. The other objective was to delineate some factors that control the microbial degradation of the estrogens. Therefore, different substrates for the stimulation and inhibition of microorganisms were added to selected soils spiked with the estrogens E2 and EE2.

2. Materials and methods

2.1. Chemicals

The unlabeled chemicals 17β-estradiol, estrone and 17α-ethinylestradiol were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). The labeled compounds [4^{14}C]-17β-estradiol, [4^{14}C]-estrone and [17^{14}C]-17α-ethinylestradiol were purchased from Hartmann Analytic (Braunschweig, Germany) and had a radiochemical purity between 98% and 99%. 17β-Estradiol and estrone were ^{14}C-labeled on the C-4 carbon of the steroid backbone and 17α-ethinylestradiol had the ^{14}C-label on the ethinylgroup in the 17-position. In all cases, the ^{14}C-label was in the most stable position of the molecule so that evolving $^{14}CO_2$ indicates estrogen mineralization.

Glucose, L-alanine, catechol (Sigma–Aldrich, Germany), ammonium nitrate, the antibiotic streptomycin (VWR, Germany) and untreated, ground saw dust (JRS, Rosenberg Germany) were used as unlabeled substrates.

2.2. Soils

Topsoil samples (Table 1) were taken from two grassland sites, two forest sites and 14 agricultural fields, air-dried and sieved (<2 mm).

Two soils were collected from a long-term experimental field in Metelen (Germany) of a cattle-grazed grassland (Mg) and of a control plot without grazing (Mco).

Table 1
Physical and chemical characteristics of the soil samples.

	pH	SOC	C/N	Sand	Silt	Clay
	[0.01 M $CaCl_2$]	[%]		[%]	[%]	[%]
Metelen(grasland)						
Mg	4.7	3.5	10.8	86	5	5
Mco	4.4	3.3	11.8	84	6	4
Köln (field)						
Kss	7.2	1.1	7.7	12	56	18
Km	7.2	1.0	7.8	13	58	18
Kco	7.4	0.7	7.2	13	53	18
Halle (field)						
HALm	6.1	1.6	10.8	69	20	7
HALco	5.5	1.3	12.1	68	18	10
Gumpenstein (field)						
Gmc	5.9	3.1	9.1	43	37	14
Glc	5.4	2.3	8.5	44	36	14
Gcoc	4.2	2.0	8.3	42	36	15
Gmf	6.1	3.5	9.3	45	35	14
Glmf	5.6	2.5	8.7	46	35	13
Gcof	5.2	2.2	8.7	42	35	15
Hamapil (orchard)						
HAMww	7.3	1.5	10.3	82	6	10
HAMfw	7.3	1.5	10.1	77	4	14
Bochum (forest)						
BOb	3.9	3.4	14.6	6	67	15
BOi	4.0	22.3	23.0	12	59	9

Samples from grassland and forest sites were collected from 0 to 10 cm. Samples from field and orchard soils were taken from depths of 0 to 30 cm. All values represent means (n = 3). SOC indicates soil organic carbon.

Three of the agricultural soils are from an experimental field operated from 1969 to 2004 near Cologne where different organic soil amendments were tested for their potential to enhance organic matter accumulation (Delschen, 1999). Samples were collected from all four replicates of the control (NPK fertilization) (Kco), the sewage sludge (Kss) and manure treatments (Km).

Two agricultural soils were taken from another long-term experimental field in Halle (Germany) from plots with manure treatments (Hm) and the control plot (Hco) with NPK fertilizer.

Six agricultural soil samples are from a long-term field experiment in Gumpenstein (Austria) which was established in 1962. Three of the treatments manure (Gm), liquid manure (Glm) and NPK fertilizer as control (Gco) from cropped (c) as well as from fallow (f) plots were selected.

Two agricultural soils are from Hamapil (Israel) an avocado orchard which has plots with a 15-year history of waste water and freshwater irrigation. No data is available on past waste water parameters but a high load of organic constituents can be assumed. Soils were collected from all four replicates of the freshwater control (HAMfw) as well as for the waste water irrigated plot (HAMww).

The forest soils were sampled in two different forest sites in Bochum (Germany). The sites are under forest, with spruce forest growing at Bochum Langendreer (BOl) and beech and oak at Bochum Bergen (BOb).

2.3. Batch sorption experiments

Sorption of [4^{14}C]-17β-estradiol, [4^{14}C]-estrone and [17^{14}C]-17α-ethinylestradiol on the γ-irradiated test soils (Table 1) was determined in batch experiments in 0.01 M $CaCl_2$ with hormone concentrations of 120, 60, 12, 6 and 1.2 μg g^{-1}.