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Debromination of polybrominated diphenyl ether-99 (BDE-99) in carp (*Cyprinus carpio*) microflora and microsomes

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Abstract

Based on previous findings in dietary studies with carp (*Cyprinus carpio*), we investigated the mechanism of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) debromination to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) using liver and intestinal components. *In vitro* aerobic and anaerobic experiments tested the ability of carp intestinal microflora to debrominate BDE-99. No debromination of BDE-99 to BDE-47 was observed in microfloral samples; therefore, carp enzymatic pathways were assessed for debromination ability. After sixty-min incubation, intestine and liver microsomes exhibited $83 \pm 34\%$ and $106 \pm 18\%$ conversions, respectively, of BDE-99 to BDE-47; with no significant ($p > 0.05$) difference between organ debromination capabilities. Microsomal incubations with BDE-99, enzyme cofactors and competing substrates assessed the potential mechanisms of debromination. The presence of NADPH in the microsomal assay did not significantly ($p > 0.05$) affect BDE-99 debromination, which suggest that cytochrome P450 enzymes are not the main debrominating pathway for BDE-99. Co-incubation of BDE-99 spiked microsomes with reverse thyronine (rT3) significantly ($p < 0.05$) decreased the debromination capacity of intestinal microsomes indicating the potential of catalytic mediation via thyroid hormone deiodinases. The significant findings of this study are that intestinal microflora are not responsible for BDE-99 debromination, however, it is an endogenous process which occurs with approximately equal activity in intestine and liver microsomes and it can be inhibited by rT3.

Keywords: Carp; Polybrominated diphenyl ether; Debromination; Metabolism; Deiodinase; Thyroid

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been manufactured, since the 1970s and are primarily used as flame retardants for plastics, textiles and electronics. PBDEs are ubiquitous contaminants, since they are not covalently bound to materials and migrate into air, water and soil. As recalcitrant and hydrophobic compounds that primarily bind to sediment and soils, PBDEs may contaminate an environment for years. The mono- through hexabrominated (lower) BDE congeners are generally more bioaccumulative than the more brominated hepta- through deca-compounds (Darnerud et al., 2001; De Wit, 2002; McDonald, 2002).

A pattern of lower congener accumulation including substantial increases in BDE-47, -99 and -100 have been noted in wildlife and human tissues (Hites, 2004). In exposed organisms, higher brominated BDE congeners are minimally absorbed but can be debrominated to lower congeners where they are accumulated in tissues (Kierkegaard et al., 1999; Hakk and Letcher, 2003; Stapleton et al., 2004a). With PBDE bioaccumulation comes the possibility of adverse health effects to exposed individuals and higher trophic level organisms that consume them. Indeed, studies have shown that tetrabromo- and pentabromo-BDE congeners can have neurodevelopmental, endocrine and

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hepatic effects (De Wit, 2002; Darnerud, 2003; Gill et al., 2004). Since PBDE congeners can biomagnify with increasing trophic level (Booij et al., 2002; Boon, 2002; Ohta et al., 2002), and lower brominated congeners are more bioavailable for absorption, the transformation of PBDEs is important to determining environmental exposure and risk.

Stapleton et al. (2004b) established that common carp (*Cyprinus carpio*) debrominate BDE-99 (2,2′,4,4′,5-pentabromodiphenyl ether) within 1.5–3.5 h after dietary *in vivo* exposures of 400 ng g^{-1} day^{-1}. The debrominated product, BDE-47 (2,2′,4,4′-tetrabromodiphenyl ether) bioaccumulated in carp whole body tissues, liver and intestines during the 62 day exposure. Multiple pathways may be responsible for the debromination of BDE-99 including transformation by intestinal microflora (anaerobic and aerobic bacteria) or catalysis by endogenous enzymes.

Under anaerobic conditions, bacteria have been shown to debrominate di- through deca- brominated BDE congeners (Rayne et al., 2003; Gerecke et al., 2005; He et al., 2006). Similarly, the identified carp intestinal bacteria are predominately classified as anaerobes (*Bacteroidaceae)* or facultative anaerobes (*Vibrionaceae* and *Enterobacteriaceae*), although the quantity and composition can vary by season and handling stress (Sugita et al., 1997; Mahmoud et al., 2004).

Fish-endogenous systems capable of transforming xenobiotics include phase I (CYPs) and II enzymes. Dietary studies indicate that at least 15% of administered BDE congeners undergo phase I and II reactions to produce metabolites, hydroxylated (OH), methoxylated, glucoronidated and glutathione substituted, (Hakk and Letcher, 2003) other than the debrominated congener. To date, fish and mammalian studies implicate CYP -1A, -2B, and -4A3 enzymes in hydroxylating PBDEs (Meerts et al., 2000; Chen et al., 2001; Zhou et al., 2001; Kuiper et al., 2004). The phase II uridine diphosphate glucoronosyl-transferases (UDPGTs) and glutathione *S*-transferases (GSTs) have also been associated with PBDE metabolism via conjugative processes (Tjarnlund et al., 1998; Hakk et al., 2002; Jenssen et al., 2004; Fernie et al., 2005; Skarman et al., 2005). Dehalogenation reactions may also be catalyzed by bacterial dehalogenases, iodotyrosine dehalogenase (IYD) and/or iodothyronine deiodinases (DIs).

PBDEs have been shown to affect thyroid hormone concentrations in turbot and rats (Jenssen et al., 2004; Branchi et al., 2005). It is known that several brominated flame retardants can bind to the plasma transthyretin (TTR) protein (Meerts et al., 2000) which may result in an increased excretion of unbound thyroid hormones. Another hypothesis for modification of thyroid hormone levels may be that PBDEs are competitively or non-competitively interacting with the deiodinase enzymatic process. The structural similarities between PBDEs and thyroid hormones add substance to this hypothesis as these compounds possess two phenyl rings attached by an ether linkage and a meta-substituted halogen. Likewise, the loss of the meta-substituted halogen is analogous in BDE-99 debromination and the deiodination of thyroid hormones by DIs. Given these similarities, it is hypothetically possible that PBDEs may also be debrominated by DIs.

There are three isoforms of DIs, which are abbreviated D1, D2, D3, and function to metabolize thyroxine (T4), thyronine (T3) and reverse thyronine (rT3). For example, an enzymatic (outer ring-ORD) deiodination occurs when D1 removes the iodine from the meta-carbon of the hydroxylated phenyl ring of rT3. Fish species have been shown to have DI activity with species and organ-specific differential expression of the enzymes observed (MacLatchy and Eales, 1992; Mol et al., 1993; Mol et al., 1998). Additionally, Friedman et al. (2006) describes IYD, a mammalian enzyme located in thyroid, liver, kidney and intestines that deiodinates mono- and diiodotyrosines that may carry out other dehalogenation reactions.

To our knowledge, mechanistic research has not been undertaken to examine the debromination reaction nor the ability of carp intestinal microflora to metabolize PBDEs. Further, there are not any reported investigations into the ability of deiodinases to potentially debrominate PBDEs. The present research investigated and compared BDE-99 debromination pathways and potential competitive interactions by employing carp liver and intestinal microsomes and intestinal microflora.

2. Materials and methods

2.1. Animal care

Juvenile carp (*C. carpio*) were obtained from Hunting Creek Fisheries (Thurmont, MD) and allowed to acclimate to Chesapeake Biological Laboratory (Solomons, MD) holding conditions for a period not less than three weeks. In agreement with approved animal care guidelines, carp were held in tanks with flow-through ambient water (18–22 °C) and a 12 h photoperiod. Carp were fed once daily at an amount that was 3–5% of total body weight.

2.2. Chemicals and reagents

BDE congeners were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). $^{13}C_{12}$-labeled 2,3′,4,4′,5-pentabromo-BDE (BDE-118L) was used as an internal standard. The recovery standard was 4′-fluoro-2,3,3′,4,5,6-hexabromoDE (FBDE-160) from Chiron (Trondheim, Norway). All solvents used were HPLC-grade. All other reagents were ACS grade or higher and purchased from either Fisher Scientific (Pittsburgh, PA USA), Sigma Aldrich (St Louis, MO USA) or EMD (Gibbstown, NJ, USA).

2.3. Anaerobic and aerobic microfloral incubations

Food material and fish sacrifice time were in accordance with Stapleton et al. (2004b). Briefly, carp were acclimated to food containing 80% blood worm (San Francisco Bay