Binding of 2,2\',4,4\',6-Pentabromodiphenyl ether (BDE-100) and/or its metabolites to mammalian biliary carrier proteins.

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in the textile and electronics industries and are globally produced in the range of 150,000 tons annually.[1] Because they are very lipophilic, structurally similar to polychlorinated dibenzo-*p*-dioxins and biphenyls, environmentally persistent, and display an increasing number of toxicological effects, there is growing concern that this class of compounds may be emerging as a new environmental contaminant. Recent reports have documented their presence in human plasma,[2] milk,[3] and adipose tissue[4] and in aquatic species such as sperm whales, harbor seals, and whitebeaked dolphins.[5]

Only a few PBDE congeners are consistently found and reported in the environment, e.g. BDE-47, 99, 100, 153 and 154, and 209.[6] Of this group, only BDE-47[7] and 99[8] have been studied in mammals. Halogenated aromatic hydrocarbons can associate with endogenous carrier proteins in the urine and bile of rats, either as the parent or as metabolites.[9-11] Toxic and non-toxic dioxins, PCB's, and PBDE's all have this capacity. Based on its lipophilicity, BDE-100 would be expected to require carrier proteins for mammalian *in vivo* transport. The purpose of the association has not been established but may be part of the process involved in mammalian elimination of these xenobiotics. However, the association may affect the normal function of these carrier proteins. One of the purposes of the present research was to administer a single oral dose of BDE-100 to male rats and measure the amount eliminated in the urine and bile, as well as characterize the nature and extent of binding to any proteins in these excreta.

Experimental

2,2',4,4',6-Pentabromo-[^{14}C]diphenyl ether (BDE-100) was synthesized in-house. [^{14}C]Phenol (54.5 mCi/mmol; Sigma, St. Louis, MO) was diluted with cold phenol to a specific activity of 6,560 dpm/ug, then treated with bromine to form [^{14}C] 2,4,6-tribromophenol. A prolonged coupling reaction of 1-chloro-2,4-dinitrobenzene to 2,4,6-tribromophenol (27 h) in dry DMF and sodium hydride at 95^{B}C yielded [^{14}C] 2,4,6-tribromo-2',4'-dinitrodiphenyl ether (83% yield).[12] Reduction to [^{14}C] 2,4,6-tribromo-2',4'-diaminodiphenyl ether was accomplished with 5% platinum on charcoal with triethyl amine and formic acid.[13] [^{14}C]BDE-100 (>98% radiochemical purity by TLC/HPLC; >95% chemical purity by ^{1}H-NMR) was produced through a double Sandmeyer reaction with $NaNO_2$/Cu(I)Br in 16% HBr.[14]

[^{14}C]BDE-100 was administered orally (4.1 mg/rat in peanut oil; 0.9 μCi) to nine conventional male Sprague-Dawley rats (276-311g), and nine bile-duct cannulated rats (230-266g). The conventional rats were housed in stainless steel metabolism cages and the bile-duct cannulated rats maintained in restraining cages.[15] Urine, feces, and bile were collected every 24h for three days. Quantities of [^{14}C] in urine and bile were assayed in a liquid scintillation counter (LSC). Bile, because of limited amounts of radioactivity, was combined (0-72h) and chromatographed by size exclusion on Sephadex G-75 (4.5 x 90cm) and Sephacryl S-200 (2.2 x 85cm), as described previously.[16] The columns were eluted with 0.05 M phosphate buffer (pH 7.2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 13% acrylamide) and immunoblot analysis (Western blot) were performed, as described previously.[17] TLC analysis was performed on aliquots of bile after concentration by rotary evaporation. TLC plates (silica gel) were developed in 50:50 hexane:methylene chloride with a standard lane containing [^{14}C]BDE-100.

Results

Daily excretion of [^{14}C]BDE-100 into the urine or bile of male rats was minimal. Cumulative elimination into conventional or cannulated urine was less than 0.1% of the administered dose (Table 1). Cumulative biliary elimination was slightly higher (1.8%). Due to low levels of radioactivity in the daily collections from conventional and cannulated rats, analysis of urinary binding proteins was not conducted. Bile (0-72h) was pooled prior to G-75 chromatography (Figure 1). TLC analysis of bile showed that metabolism was necessary for BDE-100 elimination in the bile. These data suggest that metabolism of BDE-100 into water soluble metabolites by the xenobiotic metabolizing cytochromes was an unfavorable process.

A major portion (69.4 %) of the 0-72h biliary ^{14}C was bound to a protein because the ^{14}C and protein co-eluted from the G-75 column [major protein/^{14}C peak (major peak), Figure 1, Table 2]. After further chromatography on S-200, SDS-PAGE, and linear regression MW analysis with standards, it was determined that the ^{14}C was bound to a protein of 79-kDa (Figure 2). While minor protein peaks are observed at 30 and 56kDa in the SDS-PAGE (Figure 2), they are thought to be contaminates because they vary in intensity throughout the major peak in Figure 1. Also, the 79kDa protein was the most prominent protein band of the major peak in Figure 1. In addition, these peaks have rarely been observed in SDS-PAGE gels from other studies of xenobiotics binding to the 79kDa protein.[9,10,11,16,18] The amount of the biliary ^{14}C bound to the 79-kDa protein in the 0-72h bile was 53.9% (Table 2). Previous work[11] had also shown that this novel 79-kDa protein in rat bile binds polyhalogenated aromatics such as dioxins or their metabolites. The identity or role of this protein is not known, but our additional studies indicate it is an N-terminally blocked, monomeric protein with an isoelectric point of 5.7. A significant amount (15.5%, Table 2) of the biliary ^{14}C was also bound to albumin. The native function of the 79kDa protein and albumin may be affected by binding to BDE-100 metabolites.

In comparison to other PBDE's that have been studied, the extent of BDE-100 bound to the 79kDa bile protein, i.e. 53.9%, represented an intermediate level of binding. The daily average of BDE-99 and/or its metabolites bound to the 79kDa bile protein was only 32%, while this value was 90% in the case of BDE-209.[18] The basis of the association of each congener with the biliary 79kDa protein may be physiochemical in nature because BDE-209 has a higher octanol:water partition coefficient, i.e. k_{ow} 9.97 than does BDE-99, (k_{ow} 6.64)[19] or BDE-100 (k_{ow} 7.24)[20] and the degree to which these BDEs bind to the 79kDa protein may extend to the metabolites. Like $\forall_{2u}$ -globulin and albumin in urine, the 79kDa protein may be necessary to facilitate elimination of the lipophilic BDE-99 and 209 and their metabolites via the bile. Another possibility for the differences in the extent of biliary protein binding observed between these three congeners is that BDE-209 and/or its metabolites, and to a lesser extent BDE-100 , may serve as more specific ligands than BDE-99 for a binding site on the 79kDa protein.

Table 1: Recovery of ^{14}C in the urine and bile of male rats dosed orally with 2,2',4,4',6-pentabromo-[^{14}C]diphenyl ether (BDE-100).

Excreta	Percent of Dose Conventional (n=6)	Bile-duct Cannulated (n=10)
Urine		
0-24 h	0	0.05 ± 0.1
24-48 h	0.08 ± 0.22	0.001 ± 0.003
48-72 h	0	0.001 ± 0.003
Bile		
0-24 h	----	0.73 ± 1.5
24-48 h	----	0.43 ± 0.2
48-72 h	----	0.61 ± 0.3

Table 2: Protein binding of 2,2',4,4',6-pentabromo-[^{14}C]diphenyl ether (BDE-100) metabolites in rat bile.

Bile	% Unbound	% Bound	Albumin	79kDa
0-72 h		30.5	69.4	15.5 53.9

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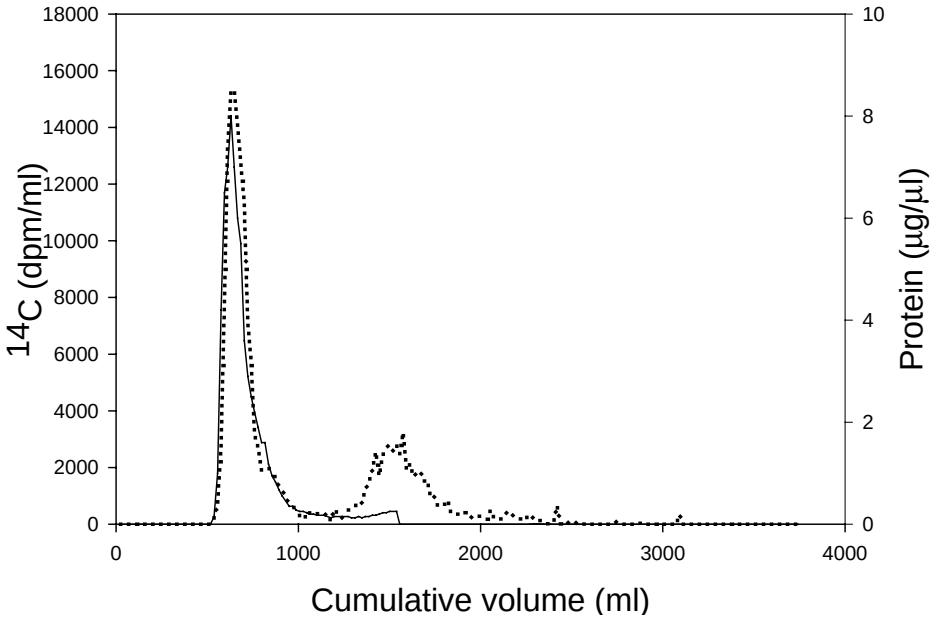

Figure 1. Distribution of radiolabel (…..) and protein (___) of 0-72h bile on a Sephadex G-75 gel filtration column.

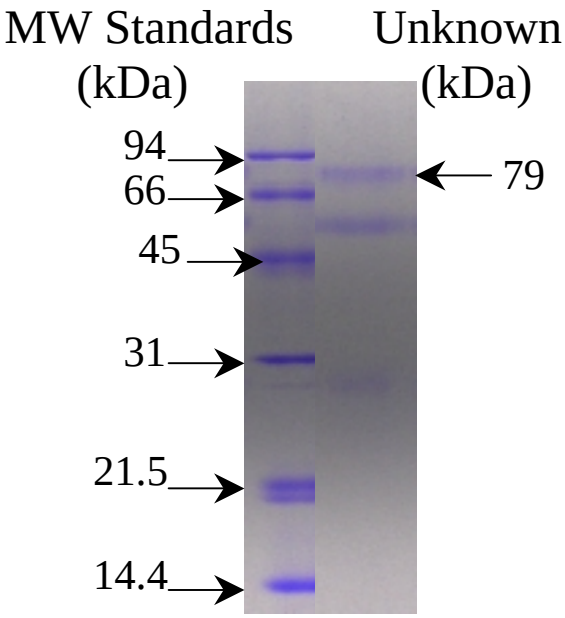

Figure 2. SDS-PAGE of the major radioactive/protein peak from G-75 gel filtration of 0-72h bile indicating the presence of unknown 79-kDa protein which binds to BDE-100 and/or its metabolites