Synthesis of *p*-Hydroxybromodiphenyl Ethers and binding to the Thyroid Receptor

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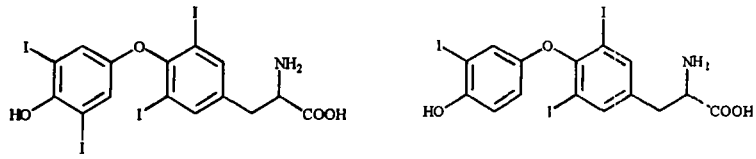
Introduction

Metoxylated polybrominated diphenyl ethers (MeO-PBDEs) have been found in various biotic samples from the Baltic Sea (1,2) and also in human blood (3). Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been detected in salmon from the Baltic Sea (2) and in mice and rats dozed with 2,2',4,4'-tetrabromodiphenyl ether (4). The sources to the MeO-PBDEs and OH-PBDEs in the environment are not known. Potential sources are (*i*) industial activities (*ii*) metabolites of PBDEs, and (*iii*) natural products, since OH-PBDEs have been found in tropical marine sponges (5).

There is no complete toxicological evaluation currently available on PBDEs in general. Recent studies indicate that some of the PBDE congeners exert neurotoxic effects similar to some of the polychlorinated (6). The close structural resemblance with the thyroid hormones may be of importance for the decreased levels of thyroxine (3,3',5,5'-tetraiodo-L-thyronine (T_4)) and thyroid hyperplasia observed in animals dosed with PBDEs (7-9). The aim of the present study was to synthesize the OH-PBDEs with potentially high affinity for the receptors according to molecular modelling (10) and to determine their actual affinity for the thyroid hormone receptor.

The principal physiological effects of the thyroid hormones, 3,3',5,5'-tetraiodo-L-thyronine (T_4) and its 5'-deiodinated congener T_3 (Figure 1), are associated with energy metabolism, the cardiovascular system, and lipid metabolism (11). The actions of thyroid hormones are mediated by soluble thyroid hormone receptors (THRs). There are two sub-types of THR, designated as α and β. THRs belong to the super family of nuclear receptors that have a zinc-finger DNA-binding motif (12). The motif enables the ligand activated THR to bind to response elements (TREs) situated on DNA upstream of THR-regulated genes. The transcription of these genes is then up or down regulated in response to the hormone. The IC_{50} for binding of T_3 to THR is approximately 0.2 nM and T_4 binds with ~ 10% of T_3's affinity. *In vivo*, T_4 is converted to T_3 by deiodinases.



(T_4) (T_3)

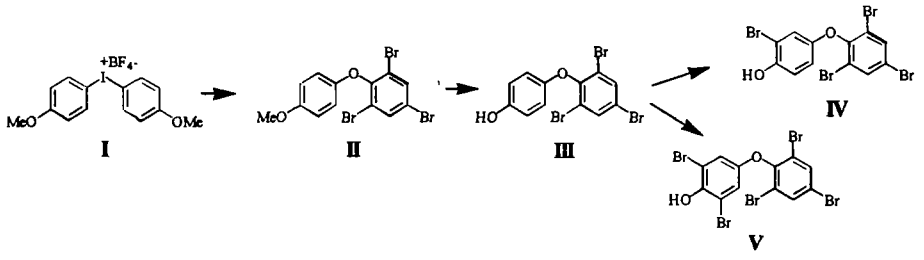
Figure 1. Chemical structures of 3,3',5,5'-tetraiodo-L-thyronine (T_4) and its 5'-deiodinated congener T_3.

A combination of crystallographic studies of rat THR-α (13), experimental, QSAR, and computational chemistry studies (11) has resulted in a profound knowledge of the prerequisites of binding to the thyroid hormone receptor. The 4'-hydroxyl group of the ligand, acting as a hydrogen bond acceptor or donor, is a crusial prerequisite of binding (11). A hydrophobic substituent in the 3'-position, such as an iodine, phenyl or cyclohexyl ring, increases the affinity whereas hydrophilic substituents markedly decreases the affinity (11). Substituents smaller than an iodine atom have lower affinity than analogues with iodine (11). The affinity of a ligand is decreased by the presence of a 5'-substituent larger than a hydrogen atom, possibly due to steric limitations (11). The size of substituents in the 3-, 5- and 3'-positions are less restricted (13). The diphenyl ether oxygen atom is buried within a hydrophobic core of the receptor and is not involved in hydrogen bonding. This oxygen is thus not important for the bindning and also compounds with a methylene group or a sulfur would bind to the receptor (11). The carboxylate anion of the 1-position is important for the affinity, most probably due to electrostatic interactions with a charged amino acid in the receptor (11). Removal of the amino group on the side chain increases however the affinity slightly (11).

Material and Methods

Receptor affinity measurements. The affinity of a compound was determined by its ability to compete with ^{125}I-triiodothyronine (^{125}I-T_3) for binding to the human THR-α_1 (hTHR-α_1). The receptor protein was obtained from nuclear extracts of sf-9 cells infected with recombinant baculovirus encoding for the hTHR as described previously (11). The compounds to be tested were dissolved in dimethyl sulfoxide and serial dilutions of them were prepared in the same solvent. An aliquot (4 µL) of the compound solution was mixed with ^{125}I-T_3 (final concentration = 200 pM) and hTHR (final concentration = 20 pM) diluting to a total volume of 204 µL with an aqueous buffer containing potassium chloride (400 mM), potassium hydrogenphosphate (17 mM), potassium dihydrogenphosphate (3 mM), magnesium chloride (1 mM), EDTA (0.5 mM), glycerol (8.7 %), monothioglycerol (6 mM), and 0.5% unprogrammed rabbit reticulocyte lysate and incubated for 18-20 hr at +4° C. The incubation was terminated by separation of THR-bound ^{125}I-T_3 from free ^{125}I-T_3 on Sephadex G-25 columns as described previously (11) and the THR-bound ^{125}I-T_3 was measured in a gamma-counter and plotted versus the concentration of the compound in competition curve. IC_{50} values were determined for each compound as the concentration of compound required to inhibit 50% of the binding of ^{125}I-T_3 to hTHR.

Synthesis. The synthesis of 4,4'-dimethoxydiphenyliodonium tetrafluoroborate (I) and the following coupling with 2,4,6-tribromophenolate resulting in 4-(2,4,6-tribromophenoxy)anisole (II), were performed according to Yokoyama *et al.* (14), cf. Scheme 1. Compound (II) was then demetylated with boron tribromide to yield 4-(2,4,6-tribromophenoxy)phenol (III). This compound was brominated resulting in the formation of 2-bromo-4-(2,4,6-tribromophenoxy)-phenol (IV) and 2,6-dibromo-4-(2,4,6-tribromophenoxy)phenol (V), depending on the amount of the bromine added.



Scheme 1.

Results and Discussion

4-Hydroxydiphenyl ether had the lowest affinity for THR-α and THR-β, cf. Table 1. The affinity of the analogue without 3',5'-bromines[1] (III) was also very low, whereas the triiodothyronine (T3) analogue (IV), in which the bromine substituents correspond to the iodines in T3 had the highest affinity of the here presented compounds. The thyroxine (T4) analogue (V) had about one third of the affinity of the T3 analogoue (VI). These results are all in agreement with the previously known Structure-Affinity Relationships for thyroid hormone receptor binding. The binding mode of the brominated diphenylethers is most probably the same as for the thyroid hormones. The lower affinities compared to thyroid hormones is most probably mainly due to the lack of a 4-carboxyl group. The bromines in 3-,3'- and 5-positions increase the binding by hydrophobic interactions compared to hydrogen substituents, although bromine atoms are not as effective as iodine substituents in this context.

Table 1.

Receptor bindning (IC_{50})	**THR-α (nM)**	**THR-β (nM)**
T_3	0.39	0.29
T_4	1.3	3.6
Triac	0.13	0.037
4-hydroxydiphenyl ether	B_{min}>Ref B_{min}	8800
4'-hydroxy-1,3,5-tribromodiphenyl ether (III)	>1000	>1000
4'-hydroxy-1,3,3',5-tetrabromodiphenyl ether (IV)	198.9	41.1
4'-hydroxy-1,3,3',5,5'-pentabromodiphenyl ether (V)	543.7	181.3

[1] To facilitate the comparison between the structures of the hydroxybromodiphenyl ethers synthesized in the present study and the thyroid hormones, the numbering system used for the thyroid hormones are used.

PBDEs are currently being used as flame retardants in large amounts.The global production was estimated to 40.000 tonnes/year in 1992 by WHO (7). The levels are much lower than those of the PCBs, but while the concentrations in e.g. human mother's milk are decreasing, those of the PBDEs are increasing (15). The findings that 4-hydroxybromodiphenyl ethers has significant affinity for the thyroid hormone receptor may have far-reaching implications, since these compounds may be formed as metabolites from PBDEs. There is also the possibility that PBDEs with sub-optimal bromine substitution patterns for receptor binding may be metabolized by dehalogenation or abiotic degradation in the environment to analogues that are better ligands. The PBDEs may thus constitute potential endocrine disruptors. It is therefore important to assess the relative occurrence of brominated diphenylether isomers in the environment, to investigate their metabolism, and to assess the thyromimetic potency of these compounds, in order to clarify their role as endocrine disruptors.

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