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Binding interactions of perfluoroalkyl substances with thyroid hormone transport proteins and potential toxicological implications

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ABSTRACT

Perfluoroalkyl substances (PFASs) have been shown to cause abnormal levels of thyroid hormones (THs) in experimental animals, but the molecular mechanism is poorly understood. Here, a fluorescence displacement assay was used to determine the binding affinities of 16 PFASs with two major TH transport proteins, transthyretin (TTR) and thyroxine-binding globulin (TBG). Most of the tested PFASs bound TTR with relative potency (RP) values of 3×10^{-4} to 0.24 when compared with that of the natural ligand thyroxine, whereas fluorotelomer alcohols did not bind. Only perfluorotridecanoic acid and perfluorotetradecanoic acid bound TBG, with RP values of 2×10^{-4} when compared with that of thyroxine. Based on these results, it was estimated that displacement of T4 from TTR by perfluorooctane sulfonate and perfluorooctanoic acids would be significant for the occupationally exposed workers but not the general population. Structure-binding analysis revealed that PFASs with a medium chain length and a sulfonate acid group are optimal for TTR binding, and PFASs with lengths longer than 12 carbons are optimal for TBG binding. Three mutant proteins were prepared to examine crucial residues involved in the binding of PFASs to TH transport proteins. TTR with a K15G mutation and TBG with either a R378G or R381G mutation showed decreased binding affinity to PFASs, indicating that these residues play key roles in the interaction with the compounds. Molecular docking showed that the PFASs bind to TTR with their acid group forming a hydrogen bond with K15 and the hydrophobic chain towards the interior. PFASs were modeled to bind TBG with their acid group forming a hydrogen bond with R381 and the hydrophobic chain extending towards R378. The findings aid our understanding of the behavior and toxicity of PFASs on the thyroid hormone system.

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1. Introduction

Perfluoroalkyl substances (PFASs) are a class of organic chemicals that have all hydrogen atoms of the hydrocarbon backbone substituted by fluorine atoms (Lau, 2012a). Over the past few decades, PFASs have been widely used in a variety of industrial and commercial applications, such as cooking utensils, food packaging, and water- and stain-resistant materials (Lindstrom et al., 2011). Consequently, PFASs have been widely and frequently detected in wildlife and humans, with perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) having the highest concentrations (Calafat et al., 2007; Lau 2012b; Olsen et al., 2007).

In 2000, 3M, the major manufacturer of PFOS in the USA, announced the phase out of its production of PFOS. Additionally, the US Environmental Protection Agency initiated the PFOA Stewardship Program with industry as a scheme to eliminate emissions and product content of these chemicals by 2015 (EPA, 2011, 2013). Thus, a downward trend for PFOS and PFOA in the general population of USA has been noted (Kato et al., 2011). However, there are still other producers of PFOS-related compounds around the world, which likely maintain the unfavorable global emission levels. Moreover, increasing use of alternative shorter carbon-chain PFASs, such as perfluorobutane sulfonate (PFBS), perfluorohexanoic acid (PFHxA) and perfluorohexane sulfonate (PFHxS), has resulted in increasing concentrations of these PFASs in the environment and humans (Kato et al., 2011). In

Abbreviations: K, lysine; G, glycine; R, arginine.

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addition, perfluortelomer alcohols such as 8:2 fluorotelomer alcohol (FTOH-8:2) degrade to PFOA and possibly perfluorononanoic acid (PFNA) in biological systems (Martin et al., 2005), which may also contribute to exposure of PFASs to humans. Because of this persistent and widespread exposure to PFASs, there is significant concern about the potential harmful environment and health effects associated with PFASs use.

Extensive laboratory studies have demonstrated that PFASs exposure has been associated with many negative effects, including endocrine disruption, neurotoxicity, disruption to fetal development, and possible carcinogenic effects (Lau et al., 2007). In recent years, there is a growing concern about the disruption effect of PFASs to the thyroid hormone (TH) system (Berg et al., 2015; Webster et al., 2014). Numerous animal studies have suggested that exposure to PFASs may impair thyroid function (Liu et al., 2011; Yu et al., 2009). Langley and Pilcher (1985) and Gutshall et al. (1988) initially reported that a single dose of perfluorondecanoic acid (PFDA) significantly reduced THs levels, lowered body temperature, and depressed heart rate in rats. Several studies have also shown a depression of serum THs levels in PFOS-exposed rats (Lau et al., 2003; Seacat et al., 2003; Thibodeaux et al., 2003). Furthermore, some human epidemiologic studies have indicated the potential disruptive effects of PFASs on human thyroid function (Dallaire et al., 2009). For example, the results of a large-scale survey of the general adult population of the USA have indicated that higher concentrations of serum PFOA and PFOS are associated with current thyroid disease (Melzer et al., 2010). Lopez-Espinosa et al. (2012) suggested associations of serum PFOS with increase of total T4 level and of serum PFOA with hypothyroidism in the children living near a chemical plant located in the USA.

THs play important roles in many biological processes, including modulation of growth, differentiation of many organs, energy homeostasis, and numerous key metabolic pathways (Brent, 2012). The THs, including triiodothyronine (T3) and thyroxine (T4), are produced by the thyroid gland and transported in human plasma by primarily binding to two proteins, transthyretin (TTR) and thyroxine-binding globulin (TBG), responsible for 20% and 75% binding activity, respectively. After getting into the target cells, the THs exert their function via binding to the TH receptors (Yen, 2001). Thus, there are at least four different levels at which chemicals may interfere with thyroid homeostasis: at the receptor level, in THs biosynthesis, in THs metabolism, and in THs transport in the bloodstream (Gutshall et al., 1989; Ren et al., 2015). Competitive binding of chemicals with transport proteins might displace THs from the proteins and disturb the normal levels of THs in the blood stream. Some drugs such as diethylstilbestrol and some pollutants such as hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and polychlorinated biphenyl have been reported to possibly disrupt the thyroid functions by competitive binding to TTR or TBG (Cao et al., 2010; Liu et al., 2012; Ren and Guo 2012; Ucan-Marin et al., 2010). Gutshall et al. (1989) examined the displacement of radio-labeled T4 from rat albumin in vitro by PFDA, suggested that PFDA may disrupt the TH system by displacing circulating hormone from their plasma protein binding sites. Chang et al. (2008) observed a decrease in total T4, a transient increase in free T4, a transient decrease in TSH in circulation, and an increase in urinary excretion of labeled tracer from radio-labeled T4 following a single dose of PFOS, suggesting that PFOS may also act TH system disruption effect by displacing THs from their binding proteins in circulation. Based on the information obtained from previous studies, it is therefore worthwhile to investigate the binding of PFASs with TH transport proteins as a potential disruption mechanism of thyroid function.

Currently, information about the interaction of PFASs with TH transport proteins is limited, with only one report presenting the binding affinities of PFASs to TTR. Weiss et al. (2009) investigated the binding potencies of 24 PFASs towards human TTR by a radioligand-binding assay. They found the binding potencies of PFASs with TTR were 12.5–50 times lower than the natural ligand thyroxine (T4). Simple descriptors based on the two-dimensional molecular structures of the compounds suggested a dependence on molecular size and functional groups (Kovarich et al., 2012; Weiss et al., 2009). However, the binding mode and mechanism of the structure-dependent binding potency between PFASs and TTR was not revealed in these studies. In addition, the binding potencies of PFASs with TBG, another major TH transport protein, are unknown.

In the present study, the binding of PFASs to human TH transport proteins (both TTR and TBG) were examined, and critical structural features that define the interactions were characterized. We quantitatively assessed the binding affinities of 16 PFASs to TTR and TBG by a fluorescent competitive binding assay. To identify critical residues involved in the interaction between TH transport proteins and PFASs, we prepared three mutants and compared the binding potencies of PFASs with these mutants. Furthermore, molecular docking was used to simulate the interactions of these compounds with TH transport proteins in an effort to understand the structural basis of the determined PFASs-binding activities.

2. Materials and methods

2.1. Chemicals

Human wild-type TTR and TBG were purchased from Calbiochem (San Diego, CA, USA). Human TTR mutant [TTR with substitution of K15 with glycine, TTRmutK15G] and the two human TBG mutants [TBG with substitution of R378 with glycine, TBGmutR378G and TBG with substitution of R381 with glycine, TBGmutR381G] were prepared by Zhongding Biotechnology Co. Ltd. (Nanjing, China). T4 was purchased from Sigma-Aldrich (St. Louis, MO, USA). The fluorescence probe, fluorescein-thyroxine (F-T4), was prepared in our laboratory according to a method reported previously (Smith, 1977). The set of PFASs selected for testing was based on environmental relevance and on a broad variation in chemical functionalities, i.e., carbon (C) chain length (from C4 to C14), and different charged end groups (carboxylate, sulfonate, hydroxyl). Sixteen PFASs, including ten perfluoroalkyl acids: perfluorobutyric acid (PFBA), PFHxA, perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, perfluoroundecanoic acid, (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTA), and perfluorotetradecanoic acid (PFTdA); three perfluoroalkyl sulfonates: PFBS, PFHxS, and PFOS; and three perfluorotelomer alcohols: 6:2-, 8:2-, and 10:2- perfluorotelomer alcohol (FTOH-6:2, FTOH-8:2, FTOH-10:2) were used in the experiments (Fig. 1). PFOS was purchased from Alfa Aesar (Ward Hill, MA, USA). The remaining 15 compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). All 16 PFASs were dissolved in DMSO with concentrations of 20 or 50 mM. All other reagents were of the highest quality available.

2.2. Spectroscopic measurements

Steady-state fluorescence was measured on a Horiba Fluoromax-4 spectrofluorometer (Edison, NJ, USA). For polarization measurements, the fluorescent molecule was excited with vertically polarized 485 nm light, and its vertical ($I_{\parallel}$) and horizontal ($I_{\perp}$) fluorescence emission intensities at 520 nm were measured. The polarization value, indicated in millipolarization

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