COMPETITIVE BINDING OF PERFLUORINATED COMPOUNDS TO THE THYROID HORMONE TRANSPORT PROTEIN TRANSTHYRETIN

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Introduction

Perfluorinated organic compounds (PFCs) are a class of substances that is characterized by a partially or fully fluorinated alkyl chain and a terminal functional group, i.e. alcohol, sulfonate, carboxylate or amide group with different substitutions next to the sulfone. The many C-F bonds result in great stability under extreme heat and chemical stress, and the fluorinated chain is also oleophobic (oil repelling). These unique properties contribute to the widespread use of PFCs in a variety of commercial products, such as household surface finishes, food packaging, water- and stain-resistant materials, fire-fighting foams, etc.

Environmental research initially focused on the compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the predominant perfluorinated contaminants found in the environment. However, a variety of other PFCs are now being found worldwide in the environment, animals and humans, from urban to remote areas in all trophic levels [1-5]. The structure of many PFCs and their behaviour within the body of organisms is comparable to free fatty acids (FA), and as such they bind to liver-fatty acid binding protein and protein albumin, which is mainly present in blood, liver and eggs [6, 7]. It is suggested that the combined polar and hydrophobic nature of fluorine-containing compounds can lead to increased affinity for natural proteins, despite the relatively weak dipolar interactions that characterize the hard C-F dipole [8]. Highest levels of PFCs in rodents, humans and marine animals are accordingly found in the protein rich blood and liver, followed by lower levels in kidney, heart, fat, testis and muscle [4, 7, 9-12].

The toxicology of PFCs has recently been extensively reviewed [3, 9, 13]. Among other observations, altered thyroid hormone levels after PFC exposure have been found in monkeys and rodents [7, 10, 11, 14, 15]. By altering thyroid hormone levels, PFCs may affect foetal and neonatal development, especially since PFOS can cross the placental barrier in both humans [16] and rodents [10, 11].

The aim of this study was to screen the binding capacity of the perfluorinated compounds and structurally similar fatty acids to the thyroid hormone transport protein transthyretin (TTR). The set of PFCs selected for testing was based on environmental relevance and on a broad variation in chemical functionalities, i.e. carbon chain lengths (from 4 to 14 carbons), fluorination grade (from fully to non-fluorinated chains) and different functional groups at the heads of the molecules (carboxylates, sulfones, sulfonamides, alcohols etc.). All 24 compound structures are represented in Table 1. TTR binding potency was compared to the natural hormone thyroxine (T_4) and the inhibition at the highest competitor concentration is expressed as a percentage.

Materials and Methods

The TTR binding assay was performed according to Lans and co-workers [17] with modifications [18]. All standards were obtained in highest purity available (95-99.5%). The incubation mixture consisted of human TTR (Sigma-Aldrich; 30 nM), Tris-HCl buffer (pH 8.0; 0.1 M NaCl, 0.1 mM EDTA) with a mixture of ^{125}I-labeled T_4 (T_4*, L-3'-5'-^{125}I-Thyroxine, PerkinElmer Life and Analytical Sciences; 4400 Ci mmol^{-1}; 100,000 cpm), unlabeled T_4 (Sigma-Aldrich; 55 nM), and competitor (10-10000 nM), which was incubated overnight at 4ºC in a final volume of 200 µl. Further details of the study are given in a submitted manuscript [19].

Results and Discussion

The binding to TTR at the maximum concentration of the competitors is given in Table 1.

Without performing an adequate structure-activity relationship evaluation, three structural parameters can be identified to influence the TTR binding potency, i.e. functional end-groups, carbon chain length and the degree of fluorination.

The highest potency of TTR binding was attributed the perfluorinated sulfone, i.e. PFHxS, followed by carboxylates of different carbon chain length. The sulfonamide was also shown to bind to TTR, whereas if the functional amide group was "protected" by an alkyl chain no binding was detected, i.e. FOSA. An alcohol as

functional end-group was not available to bind to TTR (i.e. FTOH and FOSE). Earlier studies have reported that a hydroxyl group at the *para* or *meta* positions in the phenolic compounds are essential for TTR binding [17, 20, 21]. An amyloid fibril inhibition assay, where almost 80 compounds were tested, reported that the functional group changed the affinity for TTR, where the nitro substituents were less effective than carboxylates, but most potent where the hydroxyl group on an aromatic skeleton [22], and the latter is stated in several other reports as well [17, 20, 21, 23]. The effects of functional groups on TTR-binding potency are reflected by their bioaccumulation in rainbow trout, where sulfonates bio accumulates to a greater extent than carboxylates of equivalent carbon chain length [24].
The optimum PFC chain length for binding to TTR is 6-10 carbons (Table 1), implicating that a shorter chain is not long enough to reach both the inner and outer binding site, whereas a longer chain length is hindering an optimum binding. In a similar way, PFC carbon chain length affects the bioaccumulation factor (BAF) and depuration rate constant in rainbow trout [24], the gap junction intercellular communication disturbances in rats and dolphins [25]. The effects peaked around a chain length of eight carbon atoms, except regarding the bioaccumulation of the perfluorinated carboxylates, where the BAF increased with chain length (C8 to C14). Perfluorinated sulfonates and carboxylates with chain lengths shorter than seven and six carbon atoms, respectively, could not be detected in most tissues and were considered to have insignificant BAFs in rainbow trout [24].
TTR binding was highest for fully fluorinated PFCs. Compounds with equal functional groups and carbon chain length but with less fluorine substituents, i.e. FHUEA (C8F12) - PFOA (C8F15), and DoFHpA (C7F12) - PFHpA (C7F13), clearly show a decrease in binding potency with fluorination degree. The structurally similar but non-fluorinated fatty acids did not bind to TTR at all. Earlier studies with chlorinated and brominated compounds also revealed that higher halogenated compounds had higher TTR-binding affinity than lower halogenated compounds [20, 21]. A suggestion of the binding orientation of the PFCs to the TTR can be done based on structural studies using other compounds. The fluorinated anti-inflammatory drug flufenamic acid was determined by X-ray crystallography to occupy the innermost halogen-binding pocket with the CF_3 substituent on the inner phenyl ring, interacting with Ser-117, Thr-119, Leu-110 and Ala-108, providing Van der Waals contacts [26]. The carboxylate group on the flufenamic acid outer phenyl ring is located near the entrance of the binding site, forming electrostatic interactions with the side chains of the Lys-15 residues from opposing TTR subunits. This 'reversed' binding mode, i.e. with the hydroxyl group pointing towards the mouth of the binding pocket, was also the exclusive binding site for the penta- and tri-bromophenols [21]. Whether this 'reversed' binding mode is also the most important orientation for TTR-binding by PFCs remains to be verified by structural methods.
The levels of the fluorinated compounds detected in blood of animals and humans are due to a strong association of the PFCs to proteins. Given its high affinity and concentration in serum, albumin is generally believed to be the main carrier for PFCs in blood [6], but results from the present study suggest that TTR may contribute to the retention of PFCs in blood.
The TTR-binding potency of the tested PFCs was only one tenth of the natural ligand T4 and was also less than for many other environmental pollutants [17, 20, 21, 23]. Despite their relatively weak TTR-binding affinity, PFCs may contribute to adverse effects on thyroid hormone homeostasis, and the chemical structure suggests that many other non-aromatic and non-phenolic halogenated compounds can have similar TTR-binding potentials. The results also contribute to the understanding of the presence of PFCs in serum, where TTR levels in serum contribute to the bioaccumulation of PFCs in humans and wildlife.

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Table 1. A summary of all analyzed non- and fluorinated compounds are given and the maximum binding competition of analytes is expressed as binding of T_4 (%) at max concentration (10 uM) of the competitor.

Abbreviation	End group	Full name	MW (g mol^{-1})	% T_4 bound at 10 µM
T_4	-OH	Thyroxine	776.9	
C6 FA	-COOH	Hexanoic acid	116.2	99
C8 FA	-COOH	Octanoic acid (Caprylic acid)	144.2	100
C10 FA	-COOH	Decanoic acid (Capric acid)	172.3	89
C12 FA	-COOH	Lauric acid	200.3	97
C14 FA	-COOH	Myristic acid	228.4	97
C18 FA	-COOH	Stearic acid	284.5	128
PFBA	-COOH	Perfluorobutyric acid	214.0	106
PFHxA	-COOH	Perfluorhexanoic acid	314.0	43
PFHpA	-COOH	Perfluoroheptanoic acid	364.1	7
PFOA	-COOH	Pentadecafluorooctanic acid	414.0	4
PFNA	-COOH	Heptadecafluorononanoic acid	464.0	18
PFDcA	-COOH	Perfluordecanoic acid	514.0	46
PFUnA	-COOH	Perfluoroundecanoic acid	563.9	74
PFDoA	-COOH	Perfluorododecanoic acid	614.0	91
PFTdA	-COOH	Perfluortetradecanoic acid	713.9	71
DoFHpA	-COOH	7H-Dodecafluoroheptanoic acid	346.1	45
FHUEA	-COOH	2H-Perfluoro-2-octenoic acid (6:2)	358.1	47
PFBS	$-SO_3$	Perfluorobutanesulfonate	300.0	69
PFHxS	$-SO_3$	Perfluorohexanesulfonate	400.0	3
PFOS	$-SO_3$	Perfluorooctanesulfonate	500.0	1
L-PFDS	$-SO_3$	Perfluorodecanesulfonate	622.1	122
L-PFOSI	$-SO_2$	Perfluorooctanesulfinate	506.1	6
FTOH (6:2)	-OH	2-Perfluorohexyl ethanol	364.1	117
FTOH (8:2)	-OH	2-Perfluorooctyl ethanol	464.1	117
N-MeFOSE	$-NR(CH_2)_2OH$	2-(N-methylperfluoro-1-octanesulfonamido) ethanol	557.2	119
N-EtFOSE	$-NR(CH_2)_2OH$	2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	571.3	122
FOSA	$-NH_2$	Perfluorooctanesulfonamide	499.2	32
N,N-Me2FOSA	$-NR_2$	N,N-dimethyl perfluorooctanesulfonamide	527.2	115
N-MeFOSA	-NRH	N-methyl perfluorooctanesulfonamide	513.2	114
N-EtFOSA	-NRH	N-ethyl perfluorooctanesulfonamide	527.2	112