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Different binding mechanisms of neutral and anionic poly-/perfluorinated chemicals to human transthyretin revealed by *In silico* models

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HIGHLIGHTS

- Binding interactions between poly-/perfluorinated chemicals and human transthyretin are dependent on the chemical forms.
- Poly-/perfluorinated chemicals can have electrostatic interactions, hydrogen bonds, electron donor – acceptor interactions with human transthyretin.
- Fluorine atoms in poly/perfluorinated chemicals affect the binding interactions via inductive effects.
- Two mechanism-based QSAR models were developed.

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ABSTRACT

Chemical forms-dependent binding interactions between phenolic compounds and human transthyretin (hTTR) have been elaborated previously. However, it is not known whether the binding interactions between ionizable halogenated alphatic compounds and hTTR also have the same manner. In this study, poly-/perfluorinated chemicals (PFCs) were selected as model compounds and molecular dynamic simulation was performed to investigate the binding mechanisms between PFCs and hTTR. Results show the binding interactions between the halogenated aliphatic compounds and hTTR are related to the chemical forms. The ionized groups of PFCs can form electrostatic interactions with the $-NH^{+}_{3}$ groups of Lys 15 residues in hTTR and form hydrogen bonds with the residues of hTTR. By analyzing the molecular orbital energies of PFCs, we also found that the anionic groups (nucleophile) in PFCs could form electron donor – acceptor interactions with the $-NH^{+}_{3}$ groups (electrophile) in Lys 15. The aforementioned orientational interactions make the ionized groups of the PFCs point toward the entry port of the binding site. The roles of fluorine atoms in the binding interactions were also explored. The fluorine atoms can influence the binding interactions via inductive effects. Appropriate molecular descriptors were selected to characterize these interactions, and two quantitative structure-activity relationship models were developed.

1. Introduction

Due to the high stability of C–F bonds in poly- and perfluorinated chemicals (PFCs), PFCs are persistent in the environment and biological systems, which may lead to a low PFCs elimination rate in human bodies. It has been estimated that the geometric mean half-lives of serum elimination is 3.5 years for perfluorooctanoic acid (PFOA), 4.8 years for perfluorooctane sulfonic acid (PFOS), and 7.3 years for perfluorohexane sulfonic acid (PFHxS) (Olsen et al., 2007; Bartell et al., 2010; Steenland et al., 2010). The long-term retention of PFCs in serum means that the human body may suffer from continuing exposures to PFCs, which may cause a variety of adverse effects on human health (Apelberg et al., 2007; Fei et al., 2007; Hoffman et al., 2010; Nelson et al.,

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2010; Lopez-Espinosa et al., 2011; Toft et al., 2016). Thus, insight into the retention mechanism of PFCs in human serum is important in assessing their health risks.

Some previous studies showed that PFCs mainly occur in protein-rich tissues, e.g., blood and liver (Han et al., 2003; Kelly et al., 2009; Bischel et al., 2010), which is contrary to the performance of typical persistent organic pollutants that usually accumulate in the adipose tissues. Many proteins are important carriers of endocrine signaling molecules (e.g. hormones). Thus, PFCs binding to the proteins may result in the decrease of available protein binding sites for the signaling molecules, altering their homeostasis (Zhang et al., 2013a). Likewise, epidemiological investigation indicates that human exposure to PFCs may alter thyroid hormone (THs) homeostasis (Wang et al., 2014; Webster et al., 2014; Berg et al., 2015), and cause thyroid disease (Melzer et al., 2010). Thus, PFCs binding to the active sites of THs transport proteins may be an important mechanism for their long-term retention in human serum.

In human serum, THs are delivered to target tissues through three transport proteins: transthyretin (TTR), thyroxine-binding globulin (TBG) and albumin (Hulbert, 2000; Miller et al., 2009). Previous studies indicated that some PFCs could bind to TTR, TBG and albumin (Chen and Guo, 2009; Weiss et al., 2009; Ren et al., 2016). PFCs bound to TTR, TBG and albumin could be transported to normally inaccessible tissues, such as fetal compartment and brain, which may decrease the level of THs in these sites (Boas et al., 2006, 2012). It has been reported that PFCs can cross the placental barrier in both human and rodents (Luebker et al., 2005; Zhang et al., 2013b; Wang et al., 2014). It is the TTR that is responsible for the transfer of THs over that barrier to the fetal compartment (Landers et al., 2009; Mortimer et al., 2012). Thus, PFCs may influence the fetal THs homeostasis through competing the binding sites of TTR with THs. In addition, TTR is also a primary carrier of T_4 in the cerebrospinal fluid (Boas et al., 2006, 2012). Hence, identifying the binding mechanism of PFCs with TTR is vitally important.

To date, more than twenty PFCs have been tested for their binding potency with human TTR (hTTR) (Weiss et al., 2009; Ren et al., 2016). The experimental results indicated that the hTTR binding potency of all the tested PFCs is 12.5–50 times lower than that of 3,3′,5,5'-tetraiodo-L-thyronine (T_4), which implies that the PFCs exhibit moderate affinity to hTTR. In our previous study, it was found that ionized forms of phenolic compounds binds stronger than the corresponding neutral forms with hTTR (Yang et al., 2013). Crisan et al. (2014) also indicated that the ionization effects should not be neglected when modeling the binding modes of ligands with proteins. However, it is not clear whether PFCs follow the similar binding mechanisms with hTTR. The pK_a values for some PFCs e.g. perfluorinated alkyl carboxylic acids and sulfoacids are less than 4 (Goss, 2008; Ahrens et al., 2012; Vierke et al., 2013), which means that these compounds ionize completely under the experimental condition of pH = 8.0. Why the anionic forms of perfluorinated alkyl carboxylic acids and sulfoacids exhibit moderate hTTR binding potency? Besides, experimental results also showed that non-fluorinated fatty acids did not bind with hTTR (Weiss et al., 2009). However, the same fatty acid with fluorine substitution in its molecular structure has detectable binding potency with hTTR (Weiss et al., 2009; Ren et al., 2016). What is the role of fluorine atoms in the binding interactions between PFCs and hTTR? To clear those issues, it is necessary to employ an efficient method to identify the underlying mechanisms of PFCs binding to hTTR.

In silico approaches have been considered to be efficient methods for identifying the binding mechanisms between ligands and biomacromolecules (Rabinowitz et al., 2008; Li et al., 2010; Yang et al., 2016). Two classification models and a quantitative structure-activity relationship (QSAR) model have been developed to predict the binding potency of PFCs with hTTR (Kovarich et al., 2012; Papa et al., 2013). However, the binding mechanisms of PFCs with hTTR are still obscure and need further research. Thus, it is the purpose of this study to reveal the binding mechanism between different chemical forms of PFCs and hTTR, and to construct mechanism-based QSAR models for screening other fluorinated compounds with similar structures.

2. Materials and methods

2.1. Data set

Experimental data on the competing potency of twenty-four PFCs and six fatty acids measured by Weiss et al. (2009) were selected (Table 1). The competitive interactions of the thirty compounds with T_4 binding to hTTR were determined by radiolabeled ligand displacement assay under the condition of pH = 8.0. Fifteen PFCs have detectable binding affinity with hTTR. The biological endpoint is half-maximal inhibitory concentration (IC_{50}), which represents the concentration of a chemical when it replaces 50% of T_4 that is bound to hTTR. The molecular structures of all the studied compounds were listed in Table S1 of the Supplementary material.

The present study focuses on investigating the binding mechanisms between acidic compounds and hTTR. Among the thirty compounds, 26 compounds contained -COOH, -SO_3H, -SO_2H or -OH groups in their molecular structures, which were considered to be acids. According to Rayne and Forest (2009), the primary (perfluorooctane sulfonamide) and secondary (*N*-methyl perfluorooctane sulfonamide and *N*-ethyl perfluorooctane sulfonamide) substituted perfluoroalkyl sulfonamides were also treated as acids. Therefore, the final data set with twenty-nine acidic compounds after the removal of one alkaline compound (i.e., N, *N*-dimethyl perfluorooctane sulfonamide) was used to perform the modeling. The data set was divided into training set (20 compounds) and validation set (9 compounds) with the ratio of 2:1 for QSAR modeling. The distribution between training and validation sets was made somewhat arbitrarily in such a way that the compounds in each of the sets would contain at least one example of each basic function group (i.e. -COOH, -SO_3H, -SO_2H, -OH, -NH_2).

The logarithm of relative competing potency of a chemical with T_4 binding to hTTR (log*RP*) was employed to scale its binding potency. The *RP* was defined as:

$$RP = \frac{IC_{50,T_4}}{IC_{50,\text{Competitor}}}. \quad (1)$$

where $IC_{50,T4}$ and $IC_{50,\text{Competotor}}$ are the half-maximal inhibitory concentration of T_4 and model compounds, respectively.

In order to model both the active and inactive compounds, the inactive compounds were included in the development of the QSAR models. For the inactive compounds, the IC_{50} was given a value of 6.25×10^{-4} M (Yang et al., 2011). According to Weiss et al. (2009), the IC_{50} of T_4 is 6.1×10^{-8} M. Thus, the log*RP* of inactive compounds is −4.011.

2.2. Molecular modeling

Three successive simulation steps were performed, including molecular docking, molecular dynamic simulation, and binding pattern analysis.

2.2.1. Molecular docking

The potential bioactive conformations of the PFCs binding to hTTR were determined by adopting the CDOCKER protocol in docking simulation. The initial structure of hTTR for the docking