Model and cell membrane partitioning of perfluorooctanesulfonate is independent of the lipid chain length

Wei Xie[a], Gabriele Ludewig[a], Kai Wang[b], Hans-Joachim Lehmler[a,*]

[a] Department of Occupational and Environmental Health, University of Iowa, College of Public Health, Iowa City, IA 52242, USA
[b] Department of Biostatistics, University of Iowa, College of Public Health, Iowa City, IA 52242, USA

ABSTRACT

Perfluorooctanesulfonic acid (PFOS) is a persistent environmental pollutant that may cause adverse health effects in humans and animals by interacting with and disturbing of the normal properties of biological lipid assemblies. To gain further insights into these interactions, we investigated the effect of PFOS potassium salt on dimyristoyl- (DMPC), dipalmitoyl- (DPPC) and distearoylphosphatidylcholine (DSPC) model membranes using fluorescence anisotropy measurements and differential scanning calorimetry (DSC) and on the cell membrane of HL-60 human leukemia cells and freshly isolated rat alveolar macrophages using fluorescence anisotropy measurements. PFOS produced a concentration-dependent decrease of the main phase transition temperature (T_m) and an increased peak width (ΔT_w) in both the fluorescence anisotropy and the DSC experiments, with a rank order DMPC > DPPC > DSPC. PFOS caused a fluidization of the gel phase of all phosphatidylcholines investigated, but had the opposite effect on the liquid-crystalline phase. The apparent partition coefficients of PFOS between the phosphatidylcholine bilayer and the bulk aqueous phase were largely independent of the phosphatidylcholine chain length and ranged from 4.4×10^4 to 8.8×10^4. PFOS also significantly increased the fluidity of membranes of cells. These findings suggest that PFOS readily partitions into lipid assemblies, independent of their composition, and may cause adverse biological effects by altering their fluidity in a manner that depends on the membrane cooperativity and state (e.g., gel versus liquid-crystalline phase) of the lipid assembly.

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1. Introduction

Perfluorooctanesulfonate and related chemicals belong to a class of organic fluorochemials in which all carbon–hydrogen bonds are replaced by carbon–fluorine bonds. PFOS-based chemicals, in particular perfluorooctanesulfonamides, have been used extensively in a range of industrial and consumer application, including water repellants on assorted fabrics, surfactants, waxes and gloss finish enhancers, anticorrosion agents and lubricants [1,2]. Like other perfluorinated compounds, the perfluorinated tail of PFOS-derived compounds is stable towards biological, chemical and thermal degradation because of the extreme stability of the carbon–fluorine bond. At the same time, PFOS-derived compounds are highly lipophobic and hydrophobic. These properties make PFOS-derived compounds uniquely useful for the above mentioned commercial applications. PFOS itself is the ultimate degradation product of PFOS-based chemicals [3–7] and has been detected

worldwide in the environment, wildlife and humans [2,8]. It is readily absorbed but poorly eliminated in wildlife and humans, with an estimated half-life of 4–5 years in humans [9]. Its prevalence and persistence in the environment raises environmental and public health concerns. As a result, the 3M Company as the worldwide major manufacturer of PFOS-based chemicals has phased out their production from 2000 to 2002 [8]. However, PFOS and related chemicals will remain an environmental and public health problem for many years to come due to the large scale commercial use and persistence of these chemicals.

A particular concern associated with exposure to PFOS-derived compounds is their developmental toxicity [10,11]. In humans, low level *in utero* exposure to PFOS correlates with low birth weight [12]. PFOS has also been reported to induce developmental toxicity and other adverse effects in rats, mice and Cynomolgus monkeys, which included decreased neonatal survival following *in utero* and postnatal exposure, reduction in mean post natal body weight, and a significant delay in sexual maturation [11,13]. *In vitro* studies have shown that PFOS interferes with mitochondrial bioenergetics [14], gap junctional intercellular communication [15], and fatty acid protein binding in the liver [16]. In addition, PFOS causes peroxisome proliferator activated receptor alpha (PPARα)-mediated hepatic peroxisome proliferation in rats and mice [17,18]. Studies

* Corresponding author at: The University of Iowa, Department of Occupational and Environmental Health, 100 Oakdale Campus #124 IREH, Iowa City, IA 52242-5000, USA. Tel.: +1 319 335 4211; fax: +1 319 335 4290.
E-mail address: hans-joachim-lehmler@uiowa.edu (H.-J. Lehmler).

with a structurally related chemical, perfluorooctanoic acid (PFOA), in PPARα null versus wild type mice demonstrate that knockout of PPARα abolishes the neonatal toxicity of PFOA [19]. However, a similar study with PFOS showed no differences in the neonatal mortality between PPARα null and wild type mice, thus suggesting that the developmental toxicity of PFOS is PPARα-independent [13].

Based on in vitro studies, we and others have hypothesized that interactions of PFOS with the lipid components of pulmonary surfactant may be a mechanism by which PFOS causes perinatal mortality in animal studies [20,21]. Despite the lipophobic character of PFOS-derived compounds, PFOS itself can partition into model mono- and bilayers [20,22], with an apparent partition coefficient of PFOS between DPPC model membranes and the bulk aqueous phase of approximately 6×10^4 [20,22]. At the air–water interface, PFOS more readily partitions into DPPC monolayers in the liquid-expanded compared to the liquid-condensed state [23]. PFOS causes changes in the fluidity of model monolayers [23], bilayers [20,22] and cell membranes [24], and increases the area per molecule of DMPC and DPPC at the air–water interface [23]. At the same time, molecular dynamic calculations indicate that PFOS improves the packing of phosphatidylcholine bilayers in the liquid-crystalline phase [23]. In addition, there are preliminary reports that PFOS alters the dynamic behavior of pulmonary surfactant [25] and probably is present in neonatal airways immediately after birth, as suggested by its presence in the amniotic fluid after gestational PFOS exposure [26].

Phosphatidylcholines with different hydrocarbon chain length (e.g., C_{14}, C_{16} and C_{18}) have different surface properties and display an increase in the cooperativity of their phase transition as a function of chain length. They are major components of biological membranes and other biological lipid-containing structures and fluids. For example, a saturated diacyl phosphatidylcholine, DPPC, is the major constituent of pulmonary surfactant, where it exists as a monolayer and undergoes rapid changes from liquid-expanded to liquid-condensed states with each respiratory cycle. The aim of this study is to contribute to our understanding of the effect of PFOS on the major component of pulmonary surfactant and other biological lipid assemblies by systematically investigating the interaction of PFOS with model lipid assemblies composed of phosphatidylcholine with different chain lengths and cell membranes of living cells using both steady-state fluorescent anisotropy and DSC.

2. Experimental

2.1. Chemicals and reagents

Potassium perfluorooctanesulfonate (PFOS) was obtained from Fluka Chemie GmbH (Buchs, Switzerland, batch #312421000). According to ^{19}F nuclear magnetic resonance analysis, this batch of technical grade PFOS contained approximately 82% linear, 10% isopropyl, 10% internally (including 1.2% α-methyl) and 0.4% t-butyl branched isomers of perfluorooctanesulfonate [27]. The PFOA potassium was synthesized by neutralization of the corresponding acid with an equimolar amount of potassium hydroxide. 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) were purchased from Avanti Polar Lipid (Alabaster, AL, USA). The fluorescence probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4 trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), were obtained from Molecular Probes (Eugene, OR, USA). All cell culture supplies were purchased from GIBCO BRL (Grand Island, NY, USA). Sterile 0.9% sodium chloride solution, tetrahydrofuran (THF), ethanol, methanol and chloroform were obtained from Fisher Scientific (Pittsburgh, PA, USA) and were HPLC or ACS grade.

All solvents and chemicals were used without further purification. Deionized water for the DSC studies was purified by distillation as described earlier [28,29], whereas deionized ultra-filtered water (>18 mΩ) for the fluorescence studies was obtained by reverse osmosis and ion exchange using a Millipore water system (Milli-Q, Millipore, Billerica, MA, USA).

2.2. Fluidity measurements using fluorescence anisotropy with model membranes

DPH and TMA-DPH-labeled multilamellar vesicle (MLV) suspensions of all three phosphatidylcholines were prepared as described by Xie et al. [22]. Subsequently, large unilamellar vesicle (LUV) suspensions were obtained by extruding the MLV suspensions ~15 times through a double-stacked polycarbonate membrane filter (pore size: 200 nm) using a LiposoFast extruder (Avestin Inc., British Columbia, Canada) above the melting temperature of the respective phospholipid [30]. Suspensions with PFOS concentrations ranging from 0 to 372 μmol/L (0–200 mg/L) were prepared by diluting the LUV suspensions 100-fold with an aqueous solution of PFOS. Fluorescence anisotropy in the lipid bilayer was measured using a LS55 Luminescence Spectrometer from PerkinElmer (Shelton, CT, USA). The temperature of the samples was controlled using a PerkinElmer PTP-1 Peltier System (Shelton). Steady-state DPH and TMA-DPH anisotropy were determined at $\lambda_{ex} = 350$ nm and $\lambda_{em} = 452$ nm [31] and $\lambda_{ex} = 360$ nm and $\lambda_{em} = 430$ nm [32], respectively. An excitation slit width of 10 nm, an emission slit width of 10 nm and a one second average sampling time were employed. Phosphatidylcholine suspensions were equilibrated at 40 °C (DMPC), 52.5 °C (DPPC) or 67.5 °C (DSPC) for 15 min and the DPH and TMA-DPH anisotropy values were recorded while cooling the sample to 10 °C (DMPC), 22.5 °C (DPPC) or 37.5 °C (DSPC) at a rate of 0.2°/min. The samples were stirred continuously at low speed. The temperature of the main fluid–gel phase transition (T_m), the transition width (ΔT_w) and the onset and offset of the transition were determined upon cooling from plots of the fluorescence anisotropy value as a function of temperature. All fluorescence anisotropy experiments were carried out at least in triplicates.

The apparent partition coefficient K of PFOS between the lipids bilayers and the bulk aqueous phase was estimated using the following equation [33]:

$$-\Delta T_m = \frac{R T_{m,o}^2}{\Delta H_m} \left(\frac{C_{PFOS,o} K}{55.5 + C_{Lipids} K} \right) \quad (1)$$

where ΔT_m is the change in melting temperature, R is the gas constant, $T_{m,o}$ is the melting temperature of hydrated lipids, ΔH_m is the phase transition enthalpy (31.4 kJ/mol [34]), $C_{PFOS,0}$ is the initial aqueous concentration of PFOS, and C_{lipids} is the lipid concentration.

2.3. Differential scanning calorimetry

Calculated amounts of PFOS were dissolved in chloroform–methanol (3:1, v/v) at the appropriate mole fractions, the solvent was removed and the PFOS–lipid mixture was hydrated using an excess of water (3 times by weight) [28,29,35,36]. Samples were heated above the lipid transition temperature for 5 min and vortexed for 2 min. This process was repeated approximately 8 times. Samples were stored at 4 °C overnight before collecting the DSC scans. Thermograms were recorded using a Thermal Analysis 2920 differential scanning instrument. The DSC cell was purged with 60 mL/min and the refrigerated cooling system (RSC) with 120 mL/min dry nitrogen, respectively. The hydrated samples were weighed into DSC aluminum pans, cooled to 4 °C at a rate of 10°/min and then heated from 4 °C to 80 °C with a rate of 5°/min [28,36,37]. All samples were subjected to two subsequent heating