Chemosphere 198 (2018) 442–449

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Interactions of perfluorooctanoic acid and perfluorooctanesulfonic acid with serum albumins by native mass spectrometry, fluorescence and molecular docking

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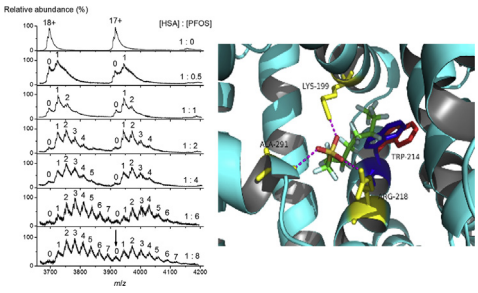
HIGHLIGHTS

- The detailed interactions of PFOA/PFOS with albumin were investigated.
- Binding constants of protein complexes were calculated according to ESI-MS spectra.
- The binding ability of PFOS with albumins was stronger than that of PFOA.
- The pocket around Trp 214 in HSA was demonstrated as one of the primary binding site.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 November 2017
Received in revised form
26 January 2018
Accepted 28 January 2018
Available online 3 February 2018

Handling Editor: I. Cousins

Keywords:
Perfluorooctanoic acid (PFOA)
Perfluorooctanesulfonic acid (PFOS)
Protein complexes
Serum albumins
Native mass spectrometry

ABSTRACT

The binding information of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) with bovine and human serum albumins was investigated and characterized in details by using a combination method of electrospray ionization mass spectrometry (ESI-MS), fluorescence, circular dichroism (CD) and molecular docking (MD). The ESI-MS analysis revealed that maximally eight PFOA or PFOS molecules could bind to serum albumins at high mole ratios of PFOA/PFOS. Association constants were measured by ESI-MS and suggested that PFOS had a better binding affinity than PFOA. PFOA and PFOS were likely to bind with serum albumins in more than one pocket. The CD data demonstrated that binding of PFOA and PFOS could change the conformation of serum albumins with decreasing α-helix content, which may affect the protein physiological function. The phenomenon of protein fluorescence quenching by the binding of PFOA and PFOS indicated that the hydrophobic pocket proximate to Trp 214 in human serum albumin might be one of the dominated binding sites. This assumption was further confirmed by MD simulation. Consistent to ESI-MS observation, MD results also displayed a stronger binding affinity of PFOS than PFOA according to the calculated binding free energy, which is probably ascribed to one more hydrogen bond formed in the PFOS-bound protein complexes.

1. Introduction

Perfluorinated compounds (PFCs) produced since the 1950s have been widely employed in industry as surfactants in the emulsion polymerization of perfluoropolymers such as poly

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https://doi.org/10.1016/j.chemosphere.2018.01.152
0045-6535/

(tetrafluoroethylene) or fluorinated elastomers. They are used as protective coatings for paper and textile fabrics, for the production of semi-conductors or as a component of fire-fighting foam (Lindstrom et al., 2011). The manufacturing of PFCs and the use of products containing PFCs have led to a release of PFCs into the environment. Nowadays PFCs are found in diverse environmental compartments at concentrations ranging from pg kg^{-1} levels to μg kg^{-1} levels all around the world (Houde et al., 2006, 2011; Delinsky et al., 2010; Zhang et al., 2010; Zareitalabad et al., 2013), and their persistence, bioaccumulation potential, and associated toxicity make them of high concern (Lau et al., 2007; Lindstrom et al., 2011). Among PFCs perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) have attracted most attentions due to their high proportion of environmental contaminations (Houde et al., 2006; Lau et al., 2007; Delinsky et al., 2010; Zhang et al., 2010; Houde et al., 2011; Lindstrom et al., 2011; Zareitalabad et al., 2013). Previous research suggested that the half-lives of PFOS and PFOA are approximately 5.4 and 3.8 years in humans (Olsen et al., 2007), respectively, and therefore the relatively long half-life arose concerns about potential health effects.

Serum albumin, as the most abundant protein in blood, is vital important to maintain osmotic pressure and pH, and it can also bind various exogenous and endogenous molecules and transport them between tissues and organs (Peters, 1985). It has been reported that serum albumin is the primary target protein and can form strong complex with PFCs (Han et al., 2003; Chen and Guo, 2009; Wu et al., 2009; Bischel et al., 2010; D'Eon et al., 2010; Hebert and MacManus-Spencer, 2010; MacManus-Spencer et al., 2010; Qin et al., 2010; Salvalaglio et al., 2010; Bischel et al., 2011; Luo et al., 2012; D'Alessandro et al., 2013; Chen et al., 2015), which explains why PFCs can enrich in blood with a quite high concentration. Hence, the investigation of interactions of PFCs with albumin is crucial for a better understanding of the biological process and toxic mechanism of PFCs *in vivo*.

Various spectroscopic and computational methods have been applied to study the interactions between PFCs and serum albumin or other proteins, especially fluorescence spectroscopy (FS) and molecular docking (MD) (Bischel et al., 2011; Qin et al., 2011; Chen et al., 2015; Ng and Hungerbuehler, 2015; Yue et al., 2016). In the last two decades, electrospray ionization mass spectrometry (ESI-MS) has become a popular analytical tool for studying noncovalent interactions of biomolecule complexes (Han et al., 2003; Bischel et al., 2010, 2011; D'Eon et al., 2010; D'Alessandro et al., 2013), because ESI is a "soft" ionization technique by which the weak noncovalent interaction in the protein-ligand complex can be preserved during the transfer of ions from the liquid to gas phase (Camilleri and Haskins, 1993). ESI-MS has the merits of direct determination of the stoichiometry of ligands bound to protein, and the association constants with high sensitivity and low consumption of sample (Loo, 1997; Zhang et al., 2003; De Vriendt et al., 2004; Sundqvist et al., 2005).

Regarding the binding affinity of PFCs to bovine serum albumin (BSA) or human serum albumin (HSA), binding constants (K_a) from different studies showed a wide range from 10^2 to 10^6 L mol^{-1} for PFOA and PFOS (Han et al., 2003; Chen and Guo, 2009; Bischel et al., 2010; Hebert and MacManus-Spencer, 2010; Qin et al., 2010; Chen et al., 2015), with obvious differences for different methods (MacManus-Spencer et al., 2010). Chen and Guo (2009) reported that the K_a values of PFOA and PFOS to HSA were 2.7×10^5 L mol^{-1} and 2.2×10^4 L mol^{-1} for Trp site, and PFOS of 7.6×10^6 L mol^{-1} for Sudlow site II by FS method. Chen et al. (2015) calculated the K_a values of PFOA and PFOS to BSA at the level of 10^5 L mol^{-1} with a higher value for PFOS, and the binding of PFOA to BSA took place in Sudlow site I, whereas PFOS was mainly located in Sudlow site II and partially bound into site I. Salvalaglio et al. (2010) estimated the binding free energies (ΔG) of PFOA and PFOS to HSA by MD method, and the ΔG of PFOA to the Trp 214 site was −8.0 kcal mol^{-1}, the highest among the PFOA complexes, while the PFOS binding site with the highest energy, −8.8 kcal mol^{-1}, was located near the Trp 214 site. Luo et al. (2012) proposed a crystal structure of HSA-PFOS with two binding sites at fatty acid site 3/4 (FA 3/4), which overlapped with Sudlow site II and fatty acid site 6 (FA 6), which was adjacent to Trp. Whether or not PFOS possess a better binding affinity than PFOA with serum albumin proteins is still obscure. Moreover, it has not reached an agreement on the predominant binding sites and the numbers of the binding sites of PFCs to serum albumins. In addition, the binding properties of PFCs with serum albumin and the relationship of potential toxicity and structures of PFCs are still unrevealed (Ding and Peijnenburg, 2013). The purpose of this work is to investigate the detailed binding properties of PFOA and PFOS to serum albumins by a combination method of ESI-MS, FS, circular dichroism (CD) spectroscopy and MD. The binding abilities of PFOA and PFOS to BSA and HSA were obtained and compared according to the association constants calculated by ESI-MS, competitive MS experiments and MD data. The binding pockets and dominant binding sites of PFOA and PFOS to HSA were characterized by FS and MD. CD and FS probed the conformational change of serum albumins when binding with PFOA or PFOS. This detailed study of the interactions of PFOA and PFOS with serum albumins would provide help for understanding the toxic mechanism of perfluoroalkyl carboxylates and perfluoroalkyl sulfonates *in vivo*.

2. Materials and methods

2.1. Materials and solutions

Essentially fatty acid free and IgG free bovine serum albumin (BSA, ≥ 99%), essentially fatty acid free human serum albumin (HSA, ≥ 96%), and PFOS (dissolved in methanol, 100 μg mL^{-1}, AR) were purchased from Sigma-Aldrich (St. Louis, MO, USA). PFOA (AR), ammonium acetate (chromatographic grade, ≥ 99%); warfarin (≥98%) and ibuprofen (≥98%) were obtained from Aladdin Industrial Corporation (Shanghai, China). Ultrapure water was produced by Mole Scientific Instrument Co. Ltd. (Shanghai, China). Serum albumins were used without further purification.

A 10 mmol L^{-1} ammonium acetate buffer (pH 7.4) was selected to dissolve BSA, HSA and PFOA because the volatility of ammonium acetate could minimize the pollution of ESI-MS. PFOA stock solution of 100 μmol L^{-1} were prepared with the buffer in polypropylene bottle. The commercial PFOS of 100 μg mL^{-1} (about 200 μmol L^{-1}) was used as stock solution. BSA and HSA stock solutions of 100 μmol L^{-1} were prepared fresh daily in the buffer at room temperature and stored at 4 °C.

2.2. ESI-MS

All mass spectrum analysis was conducted on an Agilent 6520 electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF) equipped with a Agilent 1200 HPLC in the positive-ion mode. The operating parameters were optimized as follows: gas temperature, 250 °C; drying gas, 9 L min^{-1}; nebulizer, 25 psig; capillary voltage, 3500 V; fragmentor, 125 V; skimmer, 30 V; sample injection volume, 10 μL. Mass signals were collected over the scan range m/z 600–4800.

Albumin-PFOA/PFOS complex solutions at varying protein-ligand mole ratios were prepared and diluted with ammonium acetate buffer to a final albumin concentration of 5 μmol L^{-1}. After incubated for 45 min at 37 °C, the complex solutions were injected into LC-ESI-Q-TOF with an automatic sampler.