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Effects of perfluorooctane sulfonate on the conformation and activity of bovine serum albumin

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ABSTRACT

Perfluorooctane sulfonate (PFOS) is among the most prominent contaminates in human serum and has been reported to possess potential toxicity to the human body. In this study, the effects of PFOS on the conformation and activity of bovine serum albumin (BSA) were investigated in vitro. The results indicated that the binding interaction of PFOS with BSA destroyed the tertiary and secondary structures of protein with the loss of α-helix structure and the increasing of hydrophobic microenvironment of the Trp or Tyr residues. During the thermal denaturation protein, PFOS increases the protein stability of BSA. The proportion of α-helix decreased on increasing the PFOS concentration and the microenvironment of the Trp or Tyr residues becomes more hydrophobic. The results from molecular modeling indicated that BSA had not only one possible binding site to bind with PFOS by the polar interaction, hydrogen bonds and hydrophobic forces. In addition, the BSA relative activities were decreased with the increase of PFOS concentration. Such loss of BSA activity in the presence of PFOS indicated that one of the binding sites in BSA is located in subdomain IIIA, which is in good agreement with the fluorescence spectroscopic experiments and molecular modeling results. This study offers a comprehensive picture of the interactions of PFOS with serum albumin and provides insights into the toxicological effect of perfluoroalkylated substances.

1. Introduction

Perfluorooctane sulfonate (PFOS) is one of the most widely used perfluoroalkylated substances (PFASs), which were listed among persistent organic pollutants [1]. Since the late 1990s, PFOS has attracted considerable attention and was listed in Annex B of the Stockholm Convention on May 2009 as persistent organic pollutant [2]. A series of tests on animals revealed that PFOS has toxic effects, such as reproductive and immunological toxicities, and causes disorders of the endocrine system, genotoxicity and cancerogenicity [3–5]. Because of its low degradation, high bioaccumulation, potential toxicity and long-range transport capacity, PFOS can reach high concentrations in blood plasma of wildlife and humans [6].

Recently, investigations on the concentration of PFOS in human whole blood serum were the research hotspot from the perspective of toxicology [1,2,7]. In addition, much attention has been paid to the binding interactions of PFOS with serum proteins. Beesoon and Martin used ultra-filtration devices to examine the dissociation constants of PFOS with human serum albumin [8]. Salvalaglio et al. determined the structure and energies of the binding sites of PFOS on human serum albumin by molecular modeling [9]. However, the effects of PFOS on the conformational structure, stability, and activity of serum albumin are still not well known.

Serum albumin plays an important role in the transportation and metabolism of a wide variety of endogenous and exogenous compounds including drugs and nutrients mostly through the formation of noncovalent complexes at specific binding sites in living organisms [10,11]. Therefore, the structure and functional changes of serum albumin can affect the absorption, distribution, metabolism, and excretion of small molecules binding with the carrier protein. Many studies have shown that the conformational changes of serum albumin are induced by its interaction with toxicity molecules [12–14].

In spite of the recent thrust in research in the field of the binding affinity of PFASs with serum albumin [15,16], the interactions between PFOS and transport proteins in aqueous medium remain far from being completely understood and hence demand meticulous exploration of the topic from varied viewpoints, such as the effects of PFOS on the conformation and activity of proteins. A systematic and thorough understanding of PFOS with serum albumin is still demanded. Herein, our efforts are delivered to study the effects of PFOS on conformation,

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stabilization, dynamics and functionality of serum albumin with the use of a combination of multi-spectroscopy methods and molecular modeling approach.

2. Materials and Methods

2.1. Materials

BSA (A1933, lyophilized powder, ≥98%) was purchased from Sigma-Aldrich Chemical. PFOS was purchased from Aladdin Industrial Corporation (Shanghai, China). All other chemicals used were of analytical purity or higher. Experiments were carried out in 0.05 mol/L phosphate-buffer (pH 7.40).

2.2. Circular Dichroism (CD) Experiments

CD spectra of BSA in the absence and presence of PFOS were obtained by using a Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, Surrey, UK) equipped with temperature control quantum for temperature control. For the CD experiments, a 0.05 mol/L phosphate buffer of pH 7.40 was exclusively prepared in ultrapure water. The 4.0×10^{-6} mol/L BSA solution in the presence and absence of PFOS was recorded from 200 to 260 nm with scanning speed set at 30 nm/min in 1 mm path length cells under constant nitrogen flush. For each CD spectrum, the CDNN software was used to analyze the secondary structure of BSA. The temperature of thermal denaturation of BSA in the absence and presence of PFOS was varied from 20 to 90 °C in 5 °C steps, with 350 s increments. The Global Analysis Software was used to analyze the melting temperature (T_m) and the enthalpy changes at the melting temperature (ΔH_m) of BSA.

2.3. UV–Vis Absorption

The UV–vis absorption measurements were carried out at room temperature using a SPECORD S600 spectrophotometer (Jena, Germany). The influence of PFOS on the esterase activity of BSA was examined with the *p*-nitrophenyl acetate by following the formation of *p*-nitrophenyl at 400 nm ($\lambda_{abs} = 405$ nm, $\varepsilon = 17{,}700$ L/mol/cm) using fixed wavelength absorption spectral measurements [17]. The reaction mixtures contained 4.0×10^{-6} mol/L BSA and 4.0×10^{-5} mol/L *p*-nitrophenyl acetate in the absence and presence of PFOS at pH = 7.4 and T = 310 K.

2.4. Fluorescence Measurements

Fluorescence measurements were performed in a LS-50B Spectrofluorometer (Perkin-Elmer USA). The excitation wavelength was 280 nm and the emission spectra were recorded from 300 to 500 nm for steady-state fluorescence spectra. Synchronous fluorescence spectra of BSA were recorded at 15 nm or 60 nm. In addition, for three-dimensional fluorescence spectra, the emission wavelength range was selected from 270 to 500 nm, and the initial excitation wavelength was set from 200 to 340 nm with increments of 10 nm. During the fluorescence experiments, the slit widths of all fluorescence experiments were 5.0 nm/5.0 nm and the protein concentration in each sample was 4.0×10^{-6} mol/L while PFOS concentrations were varied from 0.0 to 2.0×10^{-3} mol/L.

2.5. Molecular Docking Simulation

The native structure of BSA (PDB ID: 3V03) was taken from RCSB Protein Data Bank [18,19]. The molecular structure of PFOS was optimized by using Gaussian 09 density functional theory (DFT) method at the B3LYP/6-311++G level of theory [20]. Docking studies were performed with AutoDock 4.2.3 software which utilizes the Lamarckian Genetic Algorithm (LGA) implemented therein [21]. During the modeling docking study, a grid size was set to 126–126–126 with spacing of 0.415 Å in order to recognize all possible binding sites of PFOS in BSA. The GA population size, the maximum number of energy evaluation, and the number of GA runs were set at 150, 2,500,000, and 100, respectively. All other docking parameters were default parameters. Finally, the Molegro Molecular Viewer software (Molegro-a CLC bio company, Aarhus, Denmark) was used to analyze the docking results [22].

3. Results and Discussion

3.1. Effect of PFOS on BSA Conformation

3.1.1. CD Spectra

To understand the structural changes of BSA related to the interaction with PFOS, CD spectral method was used in this work. In the far-UV wavelength region (200–250 nm) CD spectrum of native BSA shows two negative minima at 208 nm and 222 nm, which are the characteristics for the α-helix structure. From Fig. 1(A) it is clear that the secondary conformation of BSA changes in the presence of PFOS as the ellipticity increases on increasing the concentration of PFOS. The secondary structural percentages of α-helix, β-sheet, β-turn and random coil in BSA with the increase of PFOS concentrations were plotted in Fig. 1(B). It can be seen that the proportion of α-helix decreased from 62.8% in native BSA to 55.8% on increasing the PFOS concentration, which indicates some loss of α-helix but as it remains more than 50% implying that BSA retains its structure in α-helix form with a partial unfolding in the presence of PFOS [23].

3.1.2. UV–Vis Spectra

The absorption spectral characteristics of BSA, PFOS and BSA–PFOS systems are shown in Fig. 2. In the protein molecular sequence, the aromatic amino acids (Trp, Tyr and Phe) can give an absorption peak at about 280 nm. The absorption spectrum of a protein is sensitive to the microenvironment surrounding these aromatic amino residues and to know the complex formation in solution [24]. It can be seen from Fig. 2 that there was a decrease at the absorption spectra peak 280 nm. A reasonable

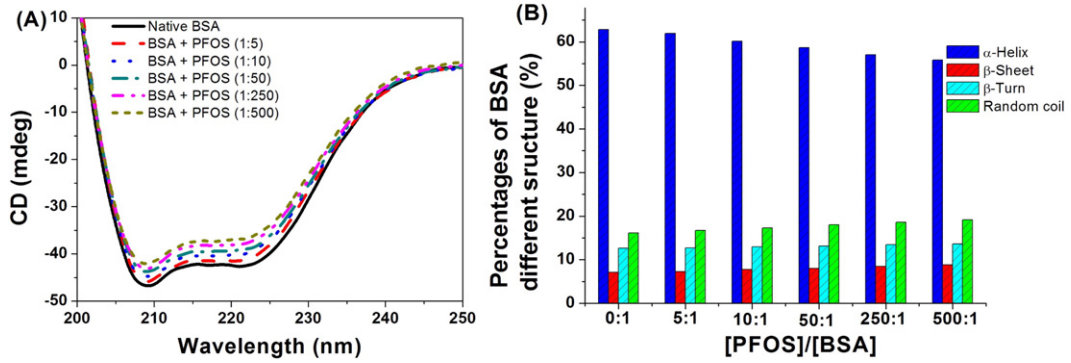

Fig. 1. (A) Effect of PFOS on the far-UV CD spectrum of BSA (4.0×10^{-6} mol/L). (B) Plots of the percentages of the different structures of BSA in the absence and presence of PFOS.