Detail Spectroscopic Study of Perfluorooctanoic Acid (PFOA) Binding with Human Hemoglobin

N.L.D. Perera*, J. Betancourt, J. Miksovska, K.E. O'Shea

Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th street, Miami, Florida 33199, United States, npere281@fiu.edu

1 Introduction

Per and poly-fluoroalkyl substances (PFAS) including perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) are notorious pollutants with powerful negative health impacts [1]. In general, PFAS including PFOA are extremely stable in the environment and accumulate inside the human body [2]. PFOA is known as a developmental and immune system toxicant [3] which can also exert adverse alteration to hormone levels inside the human body [4] and induces suppression of a broad range of genes, thus leading to the classification of PFOA as a genotoxic compound [5,6]. Due to the elevated presence in the environment and biological matters, we chose to investigate the interactions between PFOA and hemoglobin (Hb).

Hb is an iron-containing metalloprotein in red blood cells of vertebrates that exists as a tetramer of globin chains with two α and two β subunits, with each subunit, carrying a heme prosthetic group [7]. Recent studies have suggested that it has the ability to bind a diverse set of molecules including physiological allosteric effectors, synthetic effectors, and small hydrophobic molecules such as acridines [8,9]. Hb is reported to strongly bind detergents such as sodium dodecyl sulfate (SDS) [10]. Given the presence of a negative charge with a long hydrophobic chain, and the surfactant-like properties of both SDS and PFOA we hypothesize it may also bind with Hb and alter the structural properties and critical function of this protein. With this in mind, steady-state spectroscopic techniques were employed to investigate the PFOA interactions with Hb and to characterize the structural properties of the Hb: PFOA complex.

2 Materials and Methods

Chemicals: PFOA, sodium dithionate, and Hb were purchased from Sigma-Aldrich. Dibasic sodium phosphate and monobasic sodium phosphate were purchased from Fisher Scientific. Argon and carbon monoxide gas was purchased from AirGas. All chemicals were used without further purification.

Sample preparation: Stock solutions of PFOA (1.0 mM), and Hb (1.0 mM) were prepared in a 50 mM sodium phosphate buffer (pH 7.4) and stored in polypropylene tubes. All the stock solutions were mixed well and filtered using microfilters of 0.45 μM pore size. Hb in CO bound form (CO-Hb) was prepared by a multiple-step process, first purging ferric hemoglobin protein (metHb) with argon gas for 20 minutes. Then protein was first reduced to deoxy-Hb by adding a small amount of freshly prepared sodium dithionite dissolved in 50 mM sodium phosphate buffer. The transition to deoxy-Hb was confirmed by observing the shift of the Soret band from 405 to 430 nm. The sample was then purged with CO gas to obtain the CO-Hb form. For the preparation of oxygen-bound Hb (oxy-Hb), Hb protein in the deoxy form was purged with air.

UV-Vis absorbance spectroscopy: The UV-Vis absorbance study was performed using a double beam spectrophotometer (Varian Cary 100 Bio,UV Visible Spectrophotometer). The absorbance spectra for metHb and CO-Hb samples were obtained in the presence of increasing concentrations of PFOA.

Fluorescence spectroscopy: The fluorescence emission of metHb was studied as a function of the concentration of PFOA employing 1,8-Cary Eclipse fluorescence spectrophotometer (Agilent technologies). The excitation wavelength was set at 280 nm and emission was measured from 300 to 400 nm with a 10 nm slit width for both excitation and emission.

CD spectroscopy: CD measurements were performed by dissolving metHb in 50 mM sodium phosphate buffer (pH = 7.4) to reach the final concertation of 10 μM in the presence of PFOA (0-395 μM). The alpha-helical content in the protein was calculated combiningequation 3 and 4.[11]

$$\text{MRE } (\theta) = [\text{observed CD (mdeg)}] / [\text{Cnl} \times 10] \qquad (3)$$

where MRE is the mean residue ellipticity (deg cm^2 $dmol^{-1}$), C is the concentration of Hb (M), n is the number of amino acid residues and l is the path length of the cuvette (cm). The alpha-helical content of Hb was then calculated using the equation 4:

$$\alpha - helix\ (\%) = \frac{-[\theta]-4000}{33000-4000} \times 100 \qquad (4)$$

3 Results

UV-Vis absorption: UV-Vis absorption spectroscopy was used to probe the binding of PFOA by metHb and CO-Hb. The changes in UV-spectra of metHB and CO-Hb as a function of PFOA concentration are shown in Figures 1 (a) and (b), respectively. The absorption spectrum of the metHb has the Soret band located at 405 nm (Figure 1(a)).

Upon PFOA addition the intensity of the Soret band decreased. The addition of PFOA to CO-Hb also resulted in a decrease in the Soret band absorbance at 420 nm (Figure 1 (b)). In addition, two isobestic points were present at 405 and 435 nm whereas no isobestic points were observed upon PFOA binding to metHb.

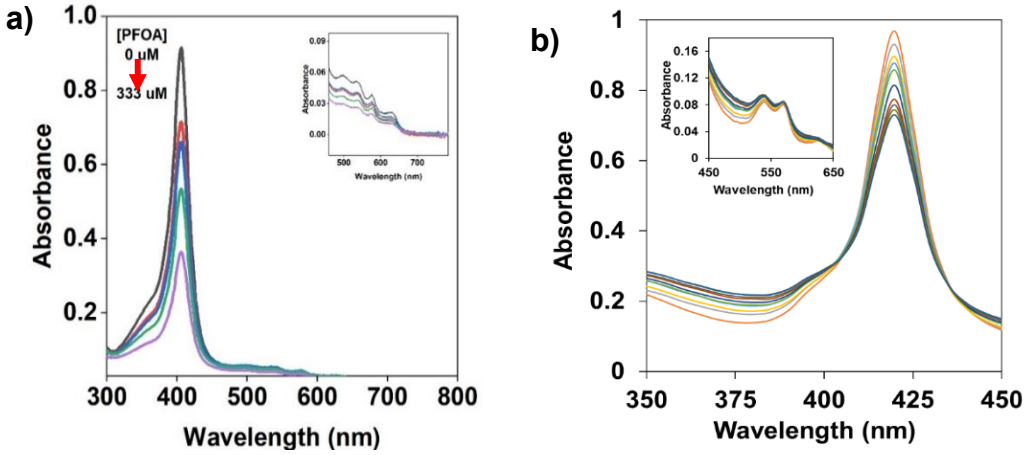

Figure 1: Absorption spectrum of metHb (panel a) and CO-Hb (panel b) as a function of increasing concentration of PFOA (0- 160) µM.

Steady-State fluorescence emission: Studies of the fluorescence emission spectra were investigated to probe the PFOA-induced conformational and structural change in metHb. Figure 2 shows the fluorescence emission spectra metHb in the presence of an increasing concentration of PFOA. The emission spectrum of metHb in the absence of PFOA has a maximum wavelength of 330 nm. As shown in Figure 2(a), the addition of PFOA to the metHb leads to the a hypsochromic shift of the fluorescence emission maximum from 334 to 317 nm (Figure 2(b)). Further addition of PFOA leads to an increase in fluorescence emission intensity.

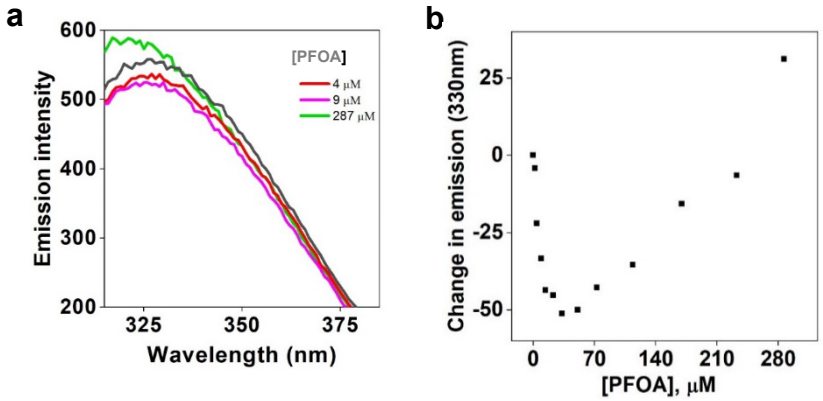

Figure 2: (a) Emission spectrum of metHb in the presence of PFOA (b) met Hb emission intensity at 330 nm as a function of PFOA concentration.

CD titration: The CD spectroscopy method is an excellent tool to determine the secondary structure content and the folding properties of proteins. Figure 3 shows the CD spectrum of metHb upon PFOA addition. The presence of α-helical secondary structures in the protein is evident by ellipticity minima at 208 and 220 nm. At low PFOA concentrations, less than 178 µM, PFOA addition to metHb has a negligible impact on protein secondary structure whereas at increased PFOA concentration (395 µM) the binding of PFOA leads to a decrease in alpha-helical content and the destabilization of the secondary structure of Hb.

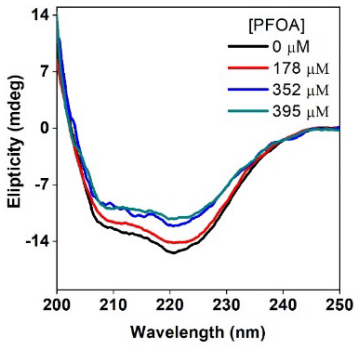

Figure 3: CD ellipticity of metHb as a function of increasing concentration of PFOA.

4 Discussion

UV-Vis absorption: It is known that the Soret band is an important feature for the spectral mapping of the conformational homogeneitysurrounding the heme group in heme proteins. [12,13]. The changes in the absorption spectrum of the metHb and CO-Hb are indicative of the PFOA binding to the vicinity of the heme group in these proteins. Interestingly, the overlay of the absorption spectra measured for PFOA binding to CO-Hb indicates the presence of two isosbestic points that are absent in the overlay of the absorption spectra for PFOA association to metHb. These results are consistent with two or more PFOA molecules binding to Hb in the absence of the gaseous ligand, whereas the presence of CO in the position of the axial ligand limits the number of PFOA molecules bound to the protein.

Steady-state fluorescence emission: Fluorescence spectroscopy is an important tool that can be used with the proteins containing tryptophan (Trp) and tyrosine (Tyr) residues to understand the conformational characteristics of proteins and their interactions with other ligands. The polarity of the Trp sites on the protein can be determined based on their emission maximum [14]. There are six Trp amino acid residues in Hb, which are responsible for the fluorescence emission with maximum emission at 334 nm [15]. A hypsochromic shift in the maximum emission wavelength in the presence of an increasing concentration of PFOA indicates either a change in the protein conformation that causes a less polar Trp environment or possibly a direct interaction between hydrophobic PFOA chain and Trp residues.

CD titration: CD results are consistent with the fluorescence spectroscopic studies that show a distinct impact on Hb structure at low and high PFOA concentrations. The bands at 208 and 220 nm are due to the π-π^* transition of alpha helixes and n-π^* transition of both alpha helices and the random coils respectively [11]. Specifically, at low PFOA concentrations, the PFOA molecule(s) binds to a specific binding site(s) either in the heme distal cavity or its vicinity without a significant impact on the overall protein structure which was suggested by the fluorescence and CD spectroscopic data. At PFOA concentrations above 100 μM, additional binding of the PFOA molecule leads to the partial unfolding and destabilization of the protein secondary structure.

5 Conclusions

In summary, the PFOA-induced changes to the structure and stability of Hb protein have been reported in this study, and the results were characterized by spectroscopic methods. The fluorescence emission spectroscopy data revealed PFOA could greatly enhance the fluorescence emission of Hb, while UV-Vis absorption spectrum results showed an attenuation of the absorbance intensities at 420 nm in the presence of increased PFOA concentration. Furthermore, the secondary structures of the proteins were altered by the addition of PFOA as confirmed by the CD data. These structural and conformational changes to the globular protein could affect their normal function and thereby pose a potential threat of toxicity.

6 References

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