Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 203 (2018) 301–307

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Spectroscopic and molecular modeling studies on the interactions of fluoranthene with bovine hemoglobin

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ARTICLE INFO

Article history:
Received 10 February 2018
Received in revised form 16 May 2018
Accepted 27 May 2018
Available online 30 May 2018

Keywords:
Fluoranthene
Bovine hemoglobin
Interaction
Fluorescence spectroscopy
Molecular modeling

ABSTRACT

This study aims to investigate the interaction between fluoranthene (FLA) and Bovine hemoglobin (BHb) by ultraviolet-visible (UV–vis) absorption, fluorescence, synchronous fluorescence, circular dichroism (CD) spectroscopy and molecular docking method. The results showed that the fluorescence intensity of BHb was declined with the increase of FLA concentration. The binding procedure was spontaneous mainly driven by hydrophobic force. The number of binding sites were 0.709 (298 K), and 1.41 (310 K). The binding constants were equal to 4.68×10^3 mol·L^{-1} at 298 K and 6.17×10^5 mol·L^{-1} at 310 K. The binding distance between FLA and the tryptophan residue of BHb was 4.50 nm. The results of UV–vis spectra, synchronous fluorescence and CD spectra revealed that FLA could change the conformation of BHb, which might affect the physiological functions of hemoglobin. Moreover, molecular modeling results showed that the fluorescence experimental results were in agreement with the results obtained by molecular docking.

1. Introduction

The environmental contamination problem of polycyclic aromatic hydrocarbons (PAHs) is a major concern due to their recalcitrance, toxicity, mutagenicity, and carcinogenicity [1]. Fluoranthene (FLA), a PAH with four fused aromatic rings, is found in barbecued foods [2], polluted air [3], automobile exhaust [4], and smoke from residential and commercial heating with coal or wood [1]. Moreover, FLA is a toxic material, which enters the body from food. Meanwhile, it was regarded as an important cancerogen [5]. The toxicological effects of FLA and the underlying mechanism are long-term focused problem in food and environmental science.

The main approach of PAHs enter the human body are respiration, skin contact and absorption in ingestion [6], they are also the main means by which PAHs get into the blood [7]. Red blood cells (RBCs) are the most numerous cells in human blood, which have important physiological functions for oxygen transportation. RBCs are primary targets attacked by environmental pollutant [8]. PAHs have good lipid solubility [9], therefore, PAHs can enter the biofilm lipids of RBCs easily and penetrate into the cell interior. Hemoglobin (Hb) is the major component and the only non-membrane protein of RBCs [10]. Therefore, the FLA can combine with Hb easily and affect its biological activity.

Some studies have been conducted about the effect of PAHs on Hb. The influence of FLA on the RBCs of F-344 rats were investigated and observed that the RBC counts, Hb concentration levels were decreased in the FLA-treated group [11]. By in vivo tests, Shugart L et al. demonstrated that administration of benzo[*a*]pyreneto pregnant mice led to adduct formation in the Hb of the mother and offspring [12]. The above researches are helpful for us to understand the toxicity of PAHs in RBCs. However, the underlying mechanism of FLA interaction with Hb is still undefined.

The amino acid sequences of bovine hemoglobins (BHb) are up to 85% similarity to human hemoglobins (Hb) [10]. In the present study, BHb was used to study the interaction of FLA with BHb in vitro instead of human hemoglobin. Common methods, including UV–visible, fluorescence, FTIR, CD spectroscopy, equilibrium dialysis and potentiometry can be applied to study the binding of different ligands to proteins [13]. Binding constant (K_b), number of binding sites (n) for the BHb-FLA complexes were analyzed and caculated with UV–vis and fluorescence quenching data. Synchronous fluorescence and CD measurement were carried out to study the change of BHb in microenvironment. At last, Molecular simulation technology was used to investigate the probable binding sites between FLA and BHb interaction.

2. Materials and Methods

2.1. Materials

FLA was obtained from J&K Chemicals (Beijing, China). BHb were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). PBS was obtained from Meilun Biotech Co., Ltd. (Dalian, China). All BHb solutions were prepared in phosphate buffer at pH 7.4.

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https://doi.org/10.1016/j.saa.2018.05.091
1386-1425/

2.2. Fluorescence Measurements

Information regarding binding mechanism and binding site of the ligand to protein can be measured by fluorescence methods [14,15]. The inner filter effect (IFE) was considered and corrected in fluorescence measurements [16]. The fluorescence measurements were taken with Hitachi F-7000 type Fluorescence Spectrophotometer (Hitachi High-Technologies Co., Ltd., Tokyo, Japan) with 1.0 cm quartz cell. The excitation wavelength was 280 nm, and the fluorescence emission spectra of BHb solutions were recorded from 200 to 500 nm under 10/10 nm slit widths at 298 and 310 K.

2.3. Synchronous Fluorescence Measurements

Synchronous fluorescence spectra of BHb with different FLA concentrations were studied at 298 K, and the wavelength interval (Δλ) between excitation wavelength (λex) and emission wavelength (λem) was respectively set at Δλ = 15 nm and Δλ = 60 nm (Δλ = λem − λex) over the wavelength range of 200–400 nm.

2.4. UV–Vis Spectroscopy Measurement

UV–Vis Spectroscopy was obtained by UV-2700 spectrophotometer (Shimadzu Co., Kyoto, Japan) with the equipment of 10 mm quartz cells. The native BHb and BHb-FLA solutions are under pH 7.4. The spectra measured from 200 nm to 370 nm.

2.5. Circular Dichroism (CD) Measurements

CD spectra were obtained by JASCO J-810 Spectrophotometer (Japan Spectroscopic Company, Tokyo, Japan) with a 1.0 mm quartz cuvette at 298 K. The scan rate was 100 nm·min^{-1} and the scan range was 200–350 nm with an interval of 1 nm.

2.6. Molecule Docking Investigation

The AutoDock 4.2 program was applied to explore the interaction between FLA and BHb [17]. The structure of FLA was downloaded from The PubChem Project (https://pubchem.ncbi.nlm.nih.gov) [18] and optimized with ChemDraw Software (CambridgeSoft, USA). The crystal structure of BHb was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) (PDB ID: 1G09). All the ligands and water molecules were removed, and then hydrogen atoms were added to the protein structure. The possible conformation of FLA banding to BHb was calculated by Lamarckian genetic algorithm (LGA) due to higher efficiency. The docking site by which the ligands bond to BHb was defined at the site with grid box at the size of 126 Å × 106 Å × 126 Å, spacing of 0.375 Å, and grid center of −0.235, 60, and 13.62, the running times of genetic algorithm, the evaluation times and the maximum number of generation were set at 100, 2,500,000 and 27,000, respectively. Based on the binding energy and the geometry matching, the most favorable docking model was selected for further analysis [15].

3. Results

3.1. Fluorescence Quenching Mechanisms

It has been reported that BHb could emit endogenous fluorescence due to the residues of tryptophan, phenylalanine and tyrosine [19]. In this study, the fluorescence spectra of BHb with or without FLA were shown in Fig. 1. There was a fluorescence emission peak around 340 nm after being excited at 280 nm. With the gradually increased concentration of FLA, the fluorescence intensity of BHb decreased, indicating that FLA interacted with BHb and quenched the fluorescence of BHb.

The diverse mechanisms of quenching are usually classified as either dynamic quenching or static quenching [20]. For the dynamic quenching, increased temperature will lead to faster diffusion and vast collisional quenching, therefore the quenching constant values will rise with the increasing temperature, while the reverse results would be detected for static quenching. To analyze the fluorescence quenching mechanism, the Stern-Volmer equation was utilized [21]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + K_q\tau_0[Q] \quad (1)$$

$$K_q = K_{SV}/\tau_0 \quad (2)$$

where F_0 and F represent the fluorescence intensities of FLA with or without the quencher respectively, [Q] means the concentration of the quencher, K_{sv}, K_q, and τ_0 represent the Stern-Volmer dynamic quenching constant, K_q is the quenching rate constant of the biomolecule, and the average lifetime of the fluorophore in the absence of quencher $\tau_0 = 1 \times 10^{-8}$ s [20].

In Fig. 2, a linear correlation was found in the plot. Meanwhile, as shown in Table 1, the values of K_q at two different temperatures were

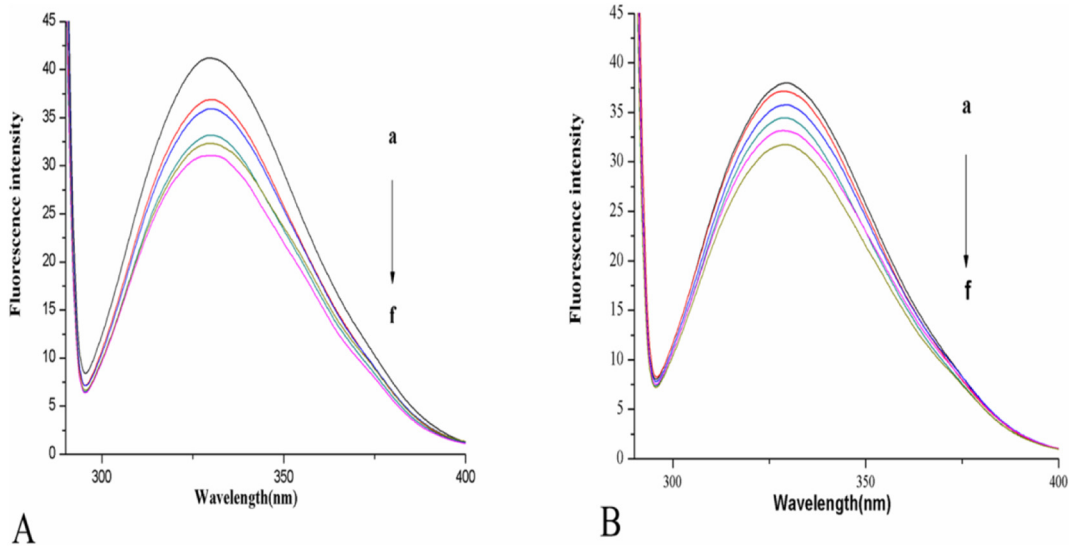

Fig. 1. Fluorescence quenching spectra of BHb in the absence (a) and presence (b-f) of fluoranthene (pH = 7.4). BHb = 1×10^{-5} mol·L^{-1} and fluoranthene (b–f) 0.5–2.5 × 10^{-5} mol·L^{-1} at 298 K (A) and 310 K (B). The spectra was taken at λex = 280 nm and λem = 290–400 nm.