Talanta 186 (2018) 497–505

Contents lists available at ScienceDirect

Talanta

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

Interactions of pyrene and/or 1-hydroxypyrene with bovine serum albumin based on EEM-PARAFAC combined with molecular docking

Jing Zhang[a], Linfeng Chen[b], Dan Liu[b], Yaxian Zhu[c], Yong Zhang[b,*]

[a] Key Laboratory of Estuarine Ecological Security and Environmental Health, Tan Kah Kee College, Xiamen University, Zhangzhou 363105, PR China
[b] State Key Laboratory of Marine Environmental Sciences of China (Xiamen University), College of Environment and Ecology, Xiamen University, Xiamen 361102, Fujian Province, PR China
[c] Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, PR China

ARTICLE INFO

Keywords:
Pyrene
1-Hydroxypyrene
Bovine serum albumin
Excitation-emission matrix spectra
Parallel factor analysis
Molecular docking

ABSTRACT

The interactions of pyrene (Pyr) and/or 1-hydroxypyrene (1-OHPyr) with bovine serum albumin (BSA) in binary and ternary systems were investigated using the excitation-emission matrix (EEM)-parallel factor analysis (PARAFAC) method combined with fluorescence quenching analysis and the molecular docking method. The results showed that the PARAFAC approach could be used to decompose the EEM spectra of Pyr, 1-OHPyr, and BSA in the binary and ternary systems. The binding constants of Pyr and 1-OHPyr with BSA increased from 1.01×10^6 and 1.62×10^6 L mol^{-1} to 2.09×10^6 and 1.86×10^7 L mol^{-1} in the ternary systems compared with the binary systems, respectively. Molecular docking revealed that in both binary and ternary systems, Pyr was bound between II A and III A regions of BSA, whereas 1-OHPyr was located in the I B region. Van der Waals forces dominated the formation of the BSA-Pyr complexes; however, for BSA-1-OHPyr complexes, in addition to Van der Waals forces, hydrogen bonds also played an important role in their binding as a hydrogen bond formed between 1-OHP and the amino residue of BSA. Moreover, the coexistence of Pyr and 1-OHPyr aggravated the conformation changes of BSA and led to a prominent decrease in the hydrophobicity of the micro-environment around tryptophan (TRP) residues. 1-OHPyr has a more severe influence on BSA conformation than Pyr in the ternary systems. This study will help to understand the combined effects of PAHs and their hydroxyl metabolites on proteins at the molecular level.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmentally persistent organic pollutants. Because of their highly hydrophobic and persistent characteristics, PAHs can accumulate in various organisms and pose a great potential hazard to human and animal health [1]. Serum albumin, serving as a major transport vehicle in plasma for numerous endogenous compounds (for example, fatty acids, hemin, bilirubin, and tryptophan) and exogenous compounds (drugs and pollutants), has important physiological functions [2–4]. Thus, the binding of PAHs to serum albumin not only determines the distribution and elimination of PAHs *in vivo* but is also associated with the toxicity of PAHs [5].

For decades, the interactions of PAHs with serum albumin and their effects on the structure and functions of the protein have attracted much interest [6–8]. Although some progress has been made in this field, few studies have focused on the complex interactions of PAHs and their metabolites with serum albumin. Inactive parent PAHs are primarily metabolized by cytochrome P450 enzymes in the human body, forming many more active oxy-derivatives, including epoxides and hydroxyl compounds. Thus, parent PAHs and their metabolites always coexist *in vivo*. Therefore, seeking proper methods to investigate the complex interactions of serum albumin with PAHs and their metabolites will help us to understand the actual protein toxicity of PAHs and assess their potential biological toxicity risk.

Excitation-emission matrix (EEM) fluorescence spectroscopy has been widely used to study the interaction between small molecules and proteins in recent years [9]. Compared with the conventional two-dimensional fluorescence spectra, EEM can determine the fluorescence intensity of fluorophores under varied excitation and emission wavelengths at the same time, which provides more detailed fluorescence information of the protein-ligand system [10]. However, serum albumin, PAHs, and their metabolites have fluorescence and their EEM spectra overlap. Thus, it is difficult to determine the fluorescence properties of each component in the ternary system without separation technology. The parallel factor analysis (PARAFAC) is based on the

* Corresponding author.
E-mail address: yzhang@xmu.edu.cn (Y. Zhang).

https://doi.org/10.1016/j.talanta.2018.04.066
Received 8 February 2018; Received in revised form 17 April 2018; Accepted 20 April 2018
Available online 22 April 2018

three linear decomposition theories [11] and uses the alternating least squares algorithm [12] to realize the decomposition of an EEM dataset. Combining PARAFAC with the EEM technology, and using "the mathematical separation" instead of "the physical or chemical separation", can well resolve the EEM data and distinguish the pure spectrum of each component in the mixture system, which could achieve a simple, rapid determination of multi-component fluorescent substances in solution [13,14]. Currently, the EEM-PARAFAC method has been successfully used in the study of the interactions between dissolved organic matter and environmental pollutants [15–17], and the interactions of proteins with a single drug molecule [18] or multiple drug molecules [19,20]. For instance, using combined EEM with PARAFAC methods, **Ni et al.** [21] studied the complex binding process of aspirin and ibuprofen with BSA. However, thus far, the role of the EEM-PARAFAC method in investigating the complex interactions of PAHs and their metabolites with serum albumin remains unclear. In recent years, molecular docking has been applied to simulate the binding process of a ligand into the active site of a protein, which can visually reveal their specific binding mode at the molecular level [22–24]. Therefore, a combinational use of experimental and molecular docking methods is promising and technically feasible to study the complex interactions of serum albumin with PAHs and their metabolites.

Pyrene (Pyr) is a typical high-molecular-weight PAH that is widely distributed in the environment [25]. The *in vivo* metabolite of Pyr, 1-hydroxypyrene (1-OHPyr), is commonly used as a biomarker for studying PAH exposure [26]. Thus, Pyr and 1-OHPyr are often selected as target compounds to investigate the interactions of PAHs or their metabolites with serum albumin. **Xu et al.** [27] investigated the interaction of Pyr with bovine serum albumin (BSA), which revealed that the number of binding sites and the binding constant of the Pyr–BSA complex were 1.20 and 2.63×10^6 L mol^{-1} at 298 K. **Ling et al.** [28] studied the interaction between Pyr and human serum albumin (HSA) using spectroscopy techniques and molecular modelling, which showed that the highest affinity sites for Pyr in HSA were subdomain I B and the site between subdomain II A and III A, and Pyr was largely stabilized by hydrophobic interactions with amino acid residues in HSA. **Carmona et al.** [29] studied the excited-state intermolecular-proton transfer reaction and energy transfer of 1-OHPyr with HSA, which demonstrated that 1-OHPyr formed a 1:1 complex with HSA with a binding constant of $(1.84 \pm 0.1) \times 10^6$ L mol^{-1} at (293 ± 1) K, and 1-OHPyr predominantly interacted with the hydrophobic pocket of subdomain IIA of HSA. Our previous study [30,31] showed that 1-OHPyr could bind to BSA with a binding constant of 2.40×10^6 L mol^{-1} at 291 K, and high concentrations of 1-OHPyr increased the content of the α-helix of BSA and exposed its tryptophan residue to a more hydrophilic microenvironment. The above work revealed meaningful information, and notably, the work only focused on the interaction of serum albumin with individual Pyr or 1-OHPyr. Due to variations in experimental conditions, and the materials and methods in these works, it is difficult to analyze the difference between the binding of Pyr and 1-OHPyr to serum albumin. Moreover, the effect of the binding of mixed Pyr and 1-OHPyr with serum albumin on Pyr and 1-OHPyr remains unclear. Therefore, under the same experimental conditions, a systematic study on the interaction of Pyr and 1-OHPyr with serum albumin and the corresponding effects on the structure of serum albumin in the binary and ternary systems is necessary.

In the present study, Pyr and 1-OHPyr were selected as a typical PAH and a PAH metabolite. BSA was selected as a model transport protein because of its well-characterized physical properties, good stability and high similarity to HSA [32]. This study aimed to study the *in vitro* interaction of Pyr and 1-OHPyr with BSA in binary and ternary systems, using the EEM - PARAFAC method combined with molecular docking. First, the fluorescence spectra of Pyr, 1-OHPyr, and BSA were decomposed by the EEM - PARAFAC method in different mixing systems. Then, the combined EEM - PARAFAC method with fluorescence quenching analysis, the quenching mechanism of BSA induced by Pyr or 1-OHPyr, and the binding constants of Pyr and 1-OHPyr with BSA were studied in both binary and ternary interaction systems. Moreover, the molecular docking method was employed to predict the specific binding modes of Pyr and 1-OHPyr with BSA in binary and ternary interaction systems. Finally, EEM spectroscopy was used to investigate the conformational changes of BSA induced by Pyr or 1-OHPyr in binary and ternary interaction systems. This is the first study on the complex interactions of PAH and its metabolites with protein under simulated physiological conditions. The results will provide important supporting data for revealing the combined toxic effects of PAHs and their hydroxyl metabolites on organisms.

2. Materials and methods

2.1. Materials

BSA (purity > 99.5%), Pyr (purity > 98%) and 1-OHP (purity > 99%) were purchased from Sigma Chemical Company (St. Louis, MO, USA) and were used without further purification. The stock solution of 4.0×10^{-5} mol L^{-1} BSA was prepared in 0.05 mol L^{-1} Tris-HCl buffer (pH = 7.40, containing 0.10 mol L^{-1} NaCl). Stock solutions of 1-OHP and Pyr were prepared individually in ethanol at concentrations of 2.0×10^{-3} mol L^{-1}. The stock solutions were stored at 277 K in the dark. All of the other chemicals that were used were of analytical reagent grade. Milli-Q water (18.2 MΩ cm) was used throughout the study.

2.2. EEM fluorescence spectra measurements of the Pyr/1-OHPyr – BSA systems

For all of the EEM spectroscopy measurements, 32 samples of different concentrations of Pyr, 1-OHPyr, and BSA were prepared with Tris–HCl buffer (Table 1), with each containing ethanol of no more than 0.5‰. Moreover, pure solutions of Pyr (6.0×10^{-5} mol L^{-1}), 1-OHPyr (7.5×10^{-5} mol L^{-1}), and BSA (1.0×10^{-6} mol L^{-1}) were also prepared in Tris–HCl buffer as reference samples. After equilibration at 25 ± 1 °C for 20 min, EEM measurements were carried out on an FLS 920 steady/transient fluorescence spectrometer (Edinburgh, UK), which was equipped with a 150-W xenon lamp. Samples were measured in the excitation range of 250–410 nm (every 5 nm) and emission range of 280–500 nm (every 2 nm) using a 10-mm quartz cuvette. The excitation and emission slits were both set at 1 nm. Thus, the EEM dataset had the dimensions of 33 (Ex.) × 111 (Em.) × 35 (Samples).

2.3. PARAFAC analysis procedures

Using MATLAB R2016b software (the MathWorks, Inc., Natick, MA, USA), the EEM dataset (size of 33 × 111 × 35) was decomposed by the PARAFAC method, according to the procedures described by [33]. First, the EEM spectra of the samples were corrected by subtracting the EEM spectra of the blank samples (Tris-HCl buffer) and removing the Rayleigh scattering and the two order Raman scattering peaks to reduce the scattering light and other background interference. Second, the 2–8 component model is used to decompose the EEM dataset by trilinear decomposition. To reduce the time of Matlab analysis, the relative fluorescence intensity values of the 35 EEM spectra were reduced by 100 times; thus, the relative fluorescence intensity values of the three components reported in this section were 0.01 times their practically measured values.

2.4. Molecular docking process

AutoDock 4.2.6 software and Autodock Tool (ADT) (the Scripps Research Institute, La Jolla, CA, USA) [34] were employed to perform the molecular docking processes of the binary and ternary interaction systems of Pyr, 1-OHPyr, and BSA. The 3-D structures of Pyr and 1-