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Exploring the interaction between rotenone and human serum albumin

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ABSTRACT

Investigation of interaction between rotenone (Rot) and human serum albumin (HSA) is provided. Fluorescence, UV–visible absorption, circular dichroism (CD), and molecular docking methods have been utilized to study the interaction. The static fluorescence quenching was observed and the binding site of Rot–HSA was shown near the warfarin site. The association for Rot and HSA was spontaneous process driven by enthalpy and the dominant driving force was hydrogen bonding interaction. The result was accordant with the molecular docking result. Furthermore, the microenvironment of tyrosine residue was influenced by the binding of rotenone *via* the synchronous fluorescence spectra. However, Rot had little effect on the conformation of HSA, which supported by the CD method and 3D-fluorescence spectra. Taking all of results into consideration, we conclude Rot changes the conformation of HSA slightly.

1. Introduction

As the most abundant protein in blood circulation, human serum albumin (HSA) plays an important role in many processes of life, for instance, absorption, transportation, distribution, and metabolism of drugs [1]. It has been reported that a quite number of the relatively insoluble endogenous compounds and a wide variety of exogenous compounds can bind to albumin and other serum components, which implicates serum albumin is a vital carrier and storage for transportation of drugs [2]. Meanwhile, serum albumin helps to strengthen pharmacological effect, increase blood volume, maintain pH balance, and equilibrium osmotic pressure. The interactions between drugs and serum albumin are connected with the efficiency evaluation of drugs. Furthermore, clarifying pharmacological mechanism *in vivo* will contribute to clinical medication. Based on this, we choose human serum albumin as the model protein due to its universal existence, wide sources and specific structure.

Rotenone (Rot) is a kind of powerful pesticide. As is known to all that human beings cannot live without energy supply and the generation of energy is closely related to respiratory chain in mitochondria. Since Rot can inhibit cellular respiration by interfering with Complex I on respiratory chain, it is widely used in study about mitochondria [3]. Recent studies have demonstrated that Rot shows potential anticancer activity against several cancer cell lines, where it interacts with microtubules and these findings are particularly interesting as they highlight the possibility of Rot being a candidate for anticancer therapy [4,5]. It has been indicated that Rot can increase reactive oxygen species (ROS), reduce mitochondrial membrane potential ($\Delta\Psi_m$), induce mitochondrial permeability transition (MPT), active apoptotic mitochondrial pathway, and induce cell death in a variety of cells *via* inhibiting mitochondrial Complex I [6]. However, it also has been confirmed that Rot is a more potent mitochondrial membrane permeability transition pore (mPTP) inhibitor than cyclosporin A (CsA) in various cell types [7]. It has been widely accepted that mitochondrial respiratory chain including Complexes I and III mainly is one of the most important sites of ROS production under physiological conditions [8,9] and several groups have shown that molecules that stimulate formation of ROS can result in apoptosis [10]. Furthermore, Rot can induce ROS production in mitochondria, which is closely related to apoptosis induced by Rot [3,11,12]. In addition, the viewpoint, which Complex I deficiency is involved in dopaminergic neuron death and Parkinson disease pathogenesis, is supported by the finding that Parkinsonism disorder will be induced upon treatment of laboratory animals with the Complex I inhibitor Rot [13]. Apart from this, Rot and rotenoids also have been found to have anti-adipogenic activity [14]. The action of uncouplers is mediated by some proteins and it is important to study the interaction between mitochondrial uncouplers and human serum albumin [15]. Therefore, we are interested in studying the binding of Rot with HSA in order to identify the mechanism Rot interacts with HSA.

In this paper, the interaction between Rot and HSA was studied systematically by fluorescence, UV–visible absorption, molecular docking, and circular dichroism (CD). We utilized fluorescence

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spectra and UV–visible absorption spectra to verify static quenching mechanism for Rot binding to HSA. Furthermore, the binding site was recognized as near warfarin site by site-competitive experiment. This conclusion was supported by the molecule docking method. Three-dimensional fluorescence spectra and the CD method further showed that Rot slightly affected the conformation of HSA.

2. Materials and methods

2.1. Materials

As listed in table S1, Rot and human serum albumin (HSA) were purchased from Sigma Chemical Company (St. Louis, Missouri, United states). HSA was dissolved in PBS (pH 7.4) at the concentration of $1.0 \cdot 10^{-5}$ mol · L^{-1}. Warfarin was purchased from Jiangsu Hengrui Medicine Co. Ltd. (Jiangsu, China), and Ibuprofen was purchased from Hubei Biocause Heilen Pharmaceutical Co. Ltd. (Hubei, China). Both of them were prepared by N,N-dimethylformamide. All other reagents were of analytical reagent grade and all solutions were prepared with aseptic double-distilled water.

2.2. Apparatus

LS-55 spectrofluorimeter (Perkin-Elmer) was used to measure fluorescence spectra. The absorption spectra of HSA and Rot were measured with a UNICO 4802 UV–visible double-beam spectrophotometer. Circular dichroism photomultiplier (Applied Photophysics Limited, UK) was used to measure CD spectra.

2.3. Fluorescence spectra

The fluorescence spectra were recorded at (298, 304, 310, and 316) K respectively with HSA ($1.0 \cdot 10^{-5}$ mol · L^{-1}) and different concentration of Rot. The excitation was 285 nm and the slit widths were 15/6 nm with a 1 cm quartz cell. The emission spectra wavelengths range from (300 to 450) nm. The appropriate blank fluorescence for buffer was subtracted to correct the background fluorescence. The site-competitive replacement experiments were performed with the addition of Rot into the HSA-site marker system. Synchronous fluorescence spectra were obtained at a fixed excitation wavelength ($\Delta\lambda$) (15 or 60) nm. The 3D fluorescence spectra were measured in the condition which the excitation wavelength was 200 nm and the emission spectra were obtained from (200 to 500) nm. The number of scanning was 31 at the increment of 5 nm per scanning.

2.4. UV–visible absorption spectra

The UV–visible absorption spectra were measured with a 1 cm quartz cell at the wavelength ranging from (200 to 800) nm. The concentrations of HSA and Rot were both $1.0 \cdot 10^{-5}$ mol · L^{-1}. The absorbance of HSA, Rot and HSA–Rot complex whose mole ratio is 1:1 were recorded respectively.

2.5. Circular dichroism (CD) spectra

CD spectra were detected with a 0.1 cm quartz cell at room temperature. The concentration of HSA was still $2.0 \cdot 10^{-6}$ - mol · L^{-1}, while the molar ratio value of Rot/HSA was varied as 0, 0.5, 1, 5, 10, 20, 30. The scanning wavelength was from (200 to 260) nm. The contents of different secondary structure were shown when CD spectra were analyzed by SELCON3 software.

2.6. Molecular docking investigation

Molecular docking is a good simulation mean to explore the interaction between small molecules and macromolecules. Sybyl 8.1 software was used to study the interaction. Sketch molecule module was used to generate the structure of Rot. Then the molecule was optimized in Tripos Force Field. The 3D structure of HSA was obtained from Protein Data Bank. The original structure of HSA was optimized prior to investigations. In Biopolymer module, random polar H was added to the protein and the charge was also added by the AMBER7 FF99 method. The optimized structure of Rot and HSA were prepared for docking. Docking study was carried out by Surflex-Dock program in Sybyl 8.1 software. We chose ligand model to generate the protomol. The protomol is a computational representation of the intended binding site to which putative ligands are aligned. In the generation of protomol, the threshold was kept at 0.50 and the bloat was set at 0. The other parameters during the docking program were determined through a number of attempts. Finally, they were set as follow: additional starting conformation per molecule: 7; angstroms to expand search grid: 6; max conformation per fragment: 20; max number of rotatable bonds per molecule: 100. Based on the operation above, we finished the docking program of Rot and HSA.

3. Results and discussion

3.1. Quenching mechanism of HSA by Rot

We first used fluorescence spectroscopy to study the interaction between HSA and Rot. As shown in figure 1, when excitation wavelength was set at 285 nm, the maximum fluorescence intensity of HSA decreased with the addition of Rot. The emission wavelength of maximum fluorescence intensity occurred at 355 nm, and its position remained unchanged as the concentration of Rot increased. This phenomenon showed that Rot quenched the intrinsic fluorescence of HSA. Generally speaking, fluorescence quenching mechanism includes dynamic quenching and static quenching. They are distinguished by different temperature dependent properties [16]. In practical terms, this means that the quenching rate constant is directly proportional to temperature in the case of dynamic quenching, and by contrast, the quenching rate constant is inversely proportional to temperature in the case of static quenching. In order to confirm the quenching mechanism, we explored the

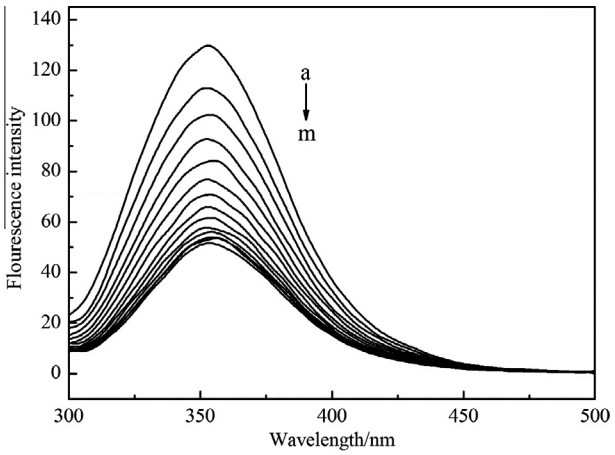

FIGURE 1. Effect of rotenone on fluorescence spectrum of HSA (T = 298 K, λ_{ex} = 285 nm). c (HSA) = $1.0 \cdot 10^{-5}$ mol · L^{-1}; c (rotenone) = $1.0 \cdot 10^{-6}$ mol · L^{-1}, molar ratio of Rot and HSA (a–m): 0, 1.67, 3.33, 5.00, 6.67, 8.33, 10.00, 11.67, 13.33, 15.00, 16.67, 18.33, 20.00, respectively.