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Binding interaction of atorvastatin with bovine serum albumin: Spectroscopic methods and molecular docking

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ABSTRACT

The interaction of atorvastatin with bovine serum albumin (BSA) was investigated using multi-spectroscopic methods and molecular docking technique for providing important insight into further elucidating the store and transport process of atorvastatin in the body and the mechanism of action and pharmacokinetics. The experimental results revealed that the fluorescence quenching mechanism of BSA induced atorvastatin was a combined dynamic and static quenching. The binding constant and number of binding site of atorvastatin with BSA under simulated physiological conditions (pH = 7.4) were $1.41 \times 10^5\ M^{-1}$ and about 1 at 310 K, respectively. The values of the enthalpic change (ΔH^0), entropic change (ΔS^0) and Gibbs free energy (ΔG^0) in the binding process of atorvastatin with BSA at 310 K were negative, suggesting that the binding process of atorvastatin and BSA was spontaneous and the main interaction forces were van der Waals force and hydrogen bonding interaction. Moreover, atorvastatin was bound into the subdomain IIA (site I) of BSA, resulting in a slight change of the conformation of BSA.

1. Introduction

Atorvastatin (Fig 1a), marketed by Pfizer as a calcium salt under the trade name Lipitor, belongs to the class of organic compounds known as diphenylpyrroles and is used for lowering blood cholesterol through inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase which catalyzes the conversion of HMG-CoA to mevalonate [1,2]. The primary use of atorvastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease. Meanwhile, research results also revealed that it is an adjuvant in cancer therapy [3–6].

As is well known, drugs, after entering the body, are absorbed resulting in entering the blood circulation, are distributed by the blood to the site of action, are metabolized to several different compounds by enzymes and are excreted from the body. The blood becomes the pivot of drug transport in vivo. Serum albumin is the most abundant protein in the plasma (approximately 60% of the total protein), which contributes to carrying several of endogenous ligands and drugs in vivo. The binding affinity of drugs with human serum albumin (HSA) significantly affects the distribution and the free, active concentration of them in vivo. Generally, the stronger the binding interaction between serum albumin and drugs is, the lower the concentration of free drugs in plasma, as the weak binding interaction of serum albumin with drugs can lead to a short lifetime or poor distribution of drugs in vivo. In other words, the binding interaction of drugs with serum albumin plays a key physiological role in transportation, distribution and metabolism of atorvastatin in vivo. Therefore, the research of the binding interaction of serum albumin with drugs is helpful for understanding the action mechanism and metabolic processes and is also a first step to clarify the detailed understanding of the pharmacology of drugs [7–11]. Currently, the research has been considered as one of the key topics in the field of life sciences, chemistry and medicine.

Bovine serum albumin (BSA, Fig 1b), containing a 583 amino acid residue protein, is monomer containing three homologous helical domains, namely, I(1–195), II(196–383) and III(384–583). Each domain is divided into two sub-domains (A and B). Because of the characteristics of the high homology with HSA and relatively inexpensive, BSA has the advantage on allowing the more thorough experimental measurements and profound investigations. At present, BSA has widely been used as evaluating binding interaction between drugs and serum albumin [12–18]. To the best of our knowledge, the study on the intermolecular interactions of atorvastatin with serum albumin using UV absorption spectroscopy, fluorescence spectroscopic titration, synchronous fluorescence spectroscopy, circular dichroism (CD), Fourier transform infrared spectroscopy (FT-IR) and molecular docking methods has not been reported.

The aim of this work was to acquire the detailed information about the binding interaction of atorvastatin with BSA such as the fluorescence quenching mechanism of BSA induced by atorvastatin, the binding constant (K_b) of atorvastatin–BSA complex, the specific binding site and binding mode of atorvastatin on BSA, and the effect of atorvastatin on the micro-environmental and conformational changes of

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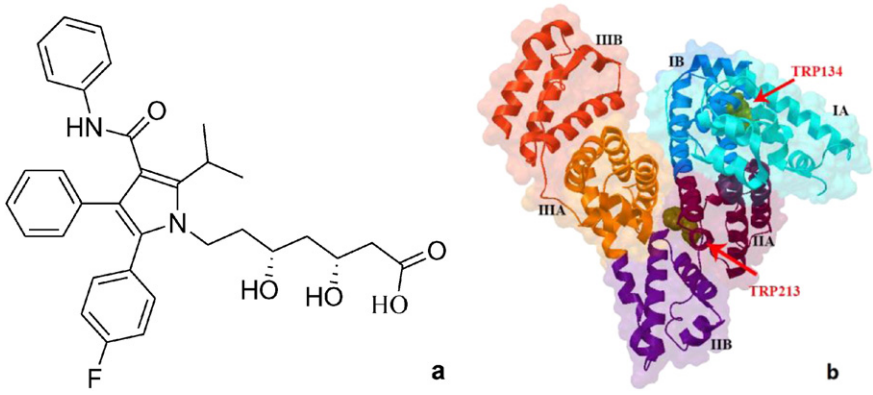

Fig. 1. Structures of atorvastatin (a) and BSA (b) from the Protein Data Bank (PDB ID: 3V03).

BSA, among others. To achieve our goal, the UV absorption spectroscopy, fluorescence spectroscopic titration, synchronous fluorescence spectroscopy, circular dichroism (CD), Fourier transform infrared spectroscopy (FT-IR) and molecular docking were carried out in this work. It can be expected that the study has great significance in helping to elucidate the store and transport process of atorvastatin in the body and the mechanism of action and pharmacokinetics.

2. Materials and methods

2.1. Reagents and solutions

BSA was purchased from Shanghai Shenhang Biotechnology Co., Ltd. (Shanghai, China), which was not purified before used. Atorvastatin calcium (≥99%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Disodium hydrogen phosphate (Na_2HPO_4 $12H_2O$) (≥99.0%) was purchased from Huzhou Hushi Chemical reagents Co., Ltd. (Zhejiang, China). Phosphoric acid (H_3PO_4) (≥85%) was purchased from Shanghai Lingfeng Chemical reagents Co., Ltd. (Shanghai, China). Sodium chloride (≥99.9%) was purchased from Zhejiang Zhongliang Chemical reagents Co., Ltd. Phenylbutazone (≥99.9%) was purchased from Hubei Hengshuo Chemical Co., Ltd. (Hubei, China). Ibuprofen (≥99.9%) was provided from Zhejiang University of Technology.

Na_2HPO_4–H_3PO_4 buffer solution (PBS) consisted of 0.06 mol L^{-1} Na_2HPO_4 in redistilled water and was adjusted to pH = 7.4 using H_3PO_4. The stock solution of atorvastatin (1.5×10^{-3} mol L^{-1}) was prepared in methanol. The stock solution of BSA (1.0×10^{-6} mol L^{-1}) was prepared in PBS containing 0.10 mol L^{-1} NaCl (pH = 7.4) (to maintain the ion strength). Phenylbutazone and ibuprofen were dissolved in methanol to form the corresponding stock solutions with the concentration of 1.5×10^{-3} mol L^{-1}. Above stock solutions were stored at 0–4 °C.

All other chemicals and solvents were of analytical reagent grade. Redistilled water was used throughout this work.

2.2. UV–vis spectra measurements

The UV–vis spectra of BSA and atorvastatin under simulated physiological condition (pH = 7.4) were recorded on UV-1601 Spectrophotometer with 10 mm quartz cell (Shimadzu corporation, Kyoto, Japan) from 200 to 320 nm. The corresponding atorvastatin solution was used as correct background.

2.3. Fluorescence spectra measurements

All fluorescence spectra of BSA solutions under simulated physiological condition (pH = 7.4) in the absence and presence of atorvastatin were recorded on a F96S Spectrofluorimeter with 10 mm quartz cell (Shanghai LengGuang Industrial Co., Ltd., Shanghai, China) from 300 to 450 nm at λ_{ex} = 289 nm at different temperatures. The corresponding buffer solution was used as correct background.

In this work, the inner-filter effect of fluorescence was ignored because the absorbance of atorvastatin at 337 nm was lower than 0.011 when the concentration of atorvastatin was 1.50×10^{-5} mol L^{-1}.

2.4. Synchronous fluorescence spectroscopy

The synchronous fluorescence spectra of BSA solutions in the absence and presence of atorvastatin was recorded on RF5301 spectrofluorimeter with 5/5 nm slit widths with a 10 mm quartz cell (Shimadzu corporation, Kyoto, Japan). The values of Δλ, which is the difference between the emission wavelength (λ_{em}) and the excitation wavelength (λ_{ex}), were set at 15 and 60 nm, respectively.

2.5. Circular dichroism

The measurement of circular dichroism (CD) spectra of BSA solutions in the absence and presence of atorvastatin was carried out on a Jasco J-815 Spectropolarimeter with a 5 mm quartz cell (Japan Spectroscopic Company, Tokyo, Japan) in the range of 200–260 nm at ambient temperature. The data was collected with an interval of 1 nm and with scan speed of 100 nm/min and the corresponding buffer solution was used as correct background.

2.6. FT-IR measurements

The FT-IR spectra of all BSA solutions in the absence and presence of atorvastatin were recorded on Nicolet 5700 FT-IR spectrophotometer (Thermo Nicolet, America) equipped with a Ge/KBr beamsplitter and a DTGS detector from 4000 to 1000 cm^{-1}. The sample solutions were placed between ZnS windows. For all spectra 100 scans recorded at 4 cm^{-1} resolution were averaged. The nicolet omnic software (Version 7.2) was used for all data manipulation. The data files were transferred to a computer for analysis using a digital curve-fitting program (Origin 8.0).

2.7. Molecular simulation

The crystal structure of BSA (PDB ID: 3V03) was extracted from the Protein Data Bank (http://www.pdb.org/pdb/home/home.do). The structure of BSA was optimized by energy minimization in a spherical water box with the radius of 49.1 nm with 0.15 M NaCl to maintain the charge neutrality. The BSA structure with the lowest energy was obtained using the CHARMM 27 force field, Langevin force field and the conjugate gradient method (30,000 cycles) implemented in NAMD 2.9 software from the Theoretical and Computational Biophysics Group (http://www.ks.uiuc.edu/Research/namd/). And, the temperature, cutoff and time step were set at 310 K, 12.0 and 2.0 fs, respectively.