Chemico-Biological Interactions 232 (2015) 77–84

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Interaction of oridonin with human serum albumin by isothermal titration calorimetry and spectroscopic techniques

Xiangrong Li *, Zhenhua Yang

Department of Chemistry, School of Basic Medicine, Xinxiang Medical University, Xinxiang, Henan 453003, PR China

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 9 March 2015
Accepted 17 March 2015
Available online 24 March 2015

Keywords:
Human serum albumin
Oridonin
Isothermal titration calorimetry
Fluorescence spectroscopy
UV–vis absorption spectroscopy

ABSTRACT

Oridonin has been traditionally and widely used for treatment of various human diseases due to its uniquely biological, pharmacological and physiological functions. In this study, the interaction between oridonin and human serum albumin (HSA) was investigated using isothermal titration calorimetry (ITC), in combination with fluorescence spectroscopy and UV–vis absorption spectroscopy. We found that the hydrogen bond and van der Waals force are the major binding forces in the binding of oridonin to HSA. The binding of oridonin to HSA is driven by favorable enthalpy and unfavorable entropy. Oridonin can quench the fluorescence of HSA through a static quenching mechanism. The binding constant between oridonin and HSA is moderate and the equilibrium fraction of unbound oridonin $f_u > 60\%$. Binding site I is found to be the primary binding site for oridonin. Additionally, oridonin may induce conformational changes of HSA and affect its biological function as the carrier protein. The results of the current study suggest that oridonin can be stored and transported from the circulatory system to reach its target organ to provide its therapeutic effects. But its side-effect in the clinics cannot be overlook. The study provides an accurate and full basic data for clarifying the binding mechanism of oridonin with HSA and is helpful for understanding its effect on protein function during the blood transportation process and its biological activity in vivo.

1. Introduction

Serum albumin is the principal extracellular protein of the circulatory system, and accounts for about 60% of the total plasma proteins corresponding to a concentration of 42 mg mL^{-1} and providing about 80% of the colloid osmotic pressure of blood [1]. Human serum albumin (HSA) is the most studied serum albumin because its primary structure is well known and its tertiary structure has been determined by X-ray crystallography. It is a single-chain, non-glycosylated globular protein consisting of 585 amino acid residues, and 17 disulfide bridges assist in maintaining its familiar heart-like shape [2]. Crystallographic data show that HSA contains three homologous α-helical domains (I, II, and III): I (residues 1–195), II (196–383), and III (384–585), each of which includes 10 helices that are divided into six-helix and four-helix subdomains (A and B) [2]. A multitude of ligand binding sites are scattered over the entire protein. The principal regions of ligand binding sites in HSA are located in hydrophobic cavities in subdomains IIA and IIIA, called site I and site II, respectively [3]. There are nine distinct fatty acid binding sites, four thyroxine binding sites, several metal binding sites including albumin's N-terminus, and a site centered around residue Cys34 [4]. These multiple binding sites underline the exceptional ability of HSA to act as a major depot and transport protein, capable of binding, transporting and delivering an extraordinarily diverse range of endogenous and exogenous compounds in the bloodstream to their target organs [1]. Up to now, many literatures have reported the interaction between drugs and HSA [5–9].

Knowledge of interaction mechanisms between drugs and serum albumin are very important to understand the pharmacokinetics and pharmacodynamics of drugs. First, the drugs–HSA interaction plays a dominant role in the bioavailability of drugs because the bound fraction of drugs is a storeroom, whereas the free fraction of drugs shows pharmacological effects [10]. In addition, if drugs are metabolized and excreted from the body too fast because of low protein binding, drugs won't be able to provide their therapeutic effects. Alternatively, if drugs have high protein binding and are metabolized and excreted too slowly, it may increase the half-life of drugs in vivo and lead to undesired side effects [11]. Furthermore, very high affinity binding of drugs to serum albumin may prevent drugs from reaching the target at all, resulting in

* Corresponding author at: Department of Chemistry, School of Basic Medicine, Xinxiang Medical University, 601 Jin-sui Road, Hong Qi District, Xinxiang 453003, PR China. Tel.: +86 373 3029128.
E-mail address: 1842457577@qq.com (X. Li).

http://dx.doi.org/10.1016/j.cbi.2015.03.012
0009-2797/

insufficient tissue distribution and efficacy. In a word, the absorption, distribution, metabolism, and excretion properties of drugs can be significantly affected as a result of their binding to serum albumin. Besides, there is evidence of conformational changes of serum albumin induced by its interaction with drugs, which may affect serum albumin's biological function as the carrier protein [12]. Consequently, investigation of the binding of drugs to serum albumin is of great importance.

Oridonin, an ent-kaurane diterpenoid compound (molecular structure shown in Fig. 2), isolated from Rabdosia Rubesens, has various pharmacological and physiological effects such as antitumor, anti-inflammation, anti-bacteria, scavenging active oxygen free radicals, and has been widely used clinically [13]. Some spectroscopic studies on the interaction between oridonin and bovine serum albumin (BSA) have been published [14]. However, to our knowledge, an accurate and full basic data for clarifying the binding mechanisms of oridonin to HSA remain unclear. In the present work, a comprehensive investigation was performed for the binding properties of oridonin to HSA under the physiological conditions. Using isothermal titration calorimetry (ITC), in combination with different spectroscopic methods, the binding information, including thermodynamic parameters, quenching mechanism, binding parameters, the equilibrium fraction of unbound oridonin, high-affinity binding site, and conformation changes of HSA was investigated.

2. Experimental

2.1. Materials

HSA, oridonin, warfarin, and ibuprofen were purchased from Sigma–Aldrich Chemicals Company (USA). Oridonin was dissolved in 99.5% ethanol and then diluted with phosphate buffer solution of pH 7.40 (0.01 mol L^{-1} PBS). The stock solution of oridonin was prepared and used immediately because of oxidation under light and air. Double distilled water was used to prepare solutions. The HSA was dissolved in 0.01 mol L^{-1} PBS at pH 7.40. The HSA stock solution was prepared by extensive overnight dialysis at 4 °C against the buffer. The concentration of the HSA was determined on a TU-1810 spectrophotometer (Puxi Analytic Instrument Ltd., Beijing, China) using the extinction coefficient ε_{280} = 36,600 mol^{-1} L cm^{-1} [15]. The pH was determined using a pHS-2C pH-meter (Shanghai DaPu Instruments Co., Ltd., Shanghai, China) at ambient temperature. Sample masses were weighed accurately on a microbalance (Sartorius, BP211D) with a resolution of 0.01 mg. All other reagents were all of analytical reagent grade and were used as purchased without further purification.

2.2. Isothermal titration calorimetry (ITC)

Titration of HSA with oridonin was performed using a Model Nano-ITC 2G biocalorimetry instrument (TA, USA) at 298 K. All these solutions were thoroughly degassed prior to the titrations to avoid the formation of bubbles in the calorimeter cell. The sample cell was loaded with the phosphate buffer (PBS, 0.01 mol L^{-1}) or protein solution and the reference cell contained double distilled water. In a typical experiment, buffered HSA solution was placed in the 950 μL sample cell of the calorimeter and oridonin solution was loaded into the injection syringe. Injections were started after baseline stability had been achieved. Oridonin was titrated into the sample cell by means of syringes via 25 individual injections, the amount of each injection was 10 μL. The first injection of 10 μL was ignored in the final data analysis. The contents of the sample cell were stirred throughout the experiment at 200 rpm to ensure thorough mixing. Raw data were obtained as a plot of heat (μJ) against injection number and featured a series of peaks for each injection. These raw data peaks were transformed using the instrument's software to obtain a plot of enthalpy change per mole of injectant (ΔH^0, kJ mol^{-1}) against molar ratio. Control experiments included the titration of oridonin solution into buffer, buffer into HSA, and buffer into buffer, controls were repeated for the same HSA concentration used. The last two controls resulted in small and equal enthalpy changes for each successive injection of buffer and, therefore, were not further considered in the data analysis [16]. Corrected data refer to experimental data after subtraction of the oridonin into buffer control data. Estimated binding parameters were obtained from ITC data using NanoAnalyze software provided by the manufacturer. Fitting the data according to the independent binding model resulted in the stoichiometry of binding (n), the equilibrium binding constant (K), and enthalpy of complex formation (ΔH^0). The standard changes in free energy (ΔG^0) and entropy (ΔS^0) are calculated using the following equations [17]:

$$\Delta G^0 = -RT \ln K \quad (1)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (2)$$

2.3. Fluorescence measurements

The fluorescence measurements were performed on Cary Eclipse fluorescence spectrophotometer (VARIAN, USA) equipped with a 1.0 cm quartz cell and a thermostat bath. The HSA concentration was kept at 2×10^{-6} mol L^{-1}. The excitation and emission slit widths were fixed at 5 nm and a scan rate of 100 nm min^{-1}. Experiments were carried out at four temperatures (293, 298, 303 and 310 K) with recycle water keeping the temperature constant. The synchronous fluorescence spectra were scanned from 280 to 330 nm ($\Delta\lambda$ = 15 nm) and from 310 to 380 nm ($\Delta\lambda$ = 60 nm), respectively. In the site marker experiment, the oridonin was gradually added to the solution of HSA and site markers held in equimolar concentrations (2×10^{-6} mol L^{-1}). The markers used were warfarin for site I and ibuprofen for site II.

The fluorescence measurements are hindered by the inner-filter effect, which is that small ligands absorb the light at the excitation and emission wavelengths of proteins and leads to unreliable results [18]. Thus it is very important to subtract such an effect from the raw quenching data. The extent of this effect can be roughly estimated with the following equation [19]:

$$F_{cor} = F_{obsd} 10^{(A_{ex}+A_{em})/2} \quad (3)$$

where F_{cor} and F_{obsd} are the corrected and observed fluorescence intensities, respectively, whereas A_{ex} and A_{em} are the sum of the absorbance of protein and ligand at the excitation and emission wavelengths, respectively. The fluorescence intensity utilized in this study is the corrected intensity.

2.4. Absorbance measurements

UV–vis absorption spectra were recorded with a TU-1810 spectrophotometer (Puxi Analytic Instrument Ltd., Beijing, China) equipped with 1.0 cm quartz cells at 298 K. Buffer (control) and samples were placed in the reference and sample cuvettes, respectively.