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Regular paper

Binding of the bioactive component Jatrorrhizine to human serum albumin

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Abstract

The interaction between Jatrorrhizine with human serum albumin (HSA) were studied by fluorescence quenching technique, circular dichroism (CD) spectroscopy, and Fourier transform infrared (FT-IR) spectroscopy. Fluorescence data revealed the presence of a single class of binding site on HSA and its binding constants (K) are 7.278×10^4, 6.526×10^4, and 5.965×10^4 $L\cdot mol^{-1}$ at 296, 303, and 310 K, respectively. The CD spectra and FT-IR spectra have proved that the protein secondary structure changed in the presence of Jatrorrhizine in aqueous solution. The effect of common ions on the binding constants was also investigated. In addition, the thermodynamic functions standard enthalpy (ΔH^0) and standard entropy (ΔS^0) for the reaction were calculated to be -10.891 $kJ\cdot mol^{-1}$ and 56.267 $J\cdot mol^{-1}$ K^{-1}, according to the van't Hoff equation. These data indicated that hydrophobic and electrostatic interactions played a major role in the binding of Jatrorrhizine to HSA. Furthermore, the displacement experiments indicated that Jatrorrhizine could bind to the site I of HSA, which was also in agreement with the result of the molecular modeling study.

Keywords: Jatrorrhizine; Human serum albumin; Binding constant; Fluorescence quenching; FT-IR spectroscopy

1. Introduction

Serum albumins have been one of the most studied proteins for many years. They are the most abundant of the protein in blood plasma, accounting for about 60% of the total protein corresponding to a concentration of 42 $g\cdot L^{-1}$ [1,2] and provide about 80% of the osmotic pressure of blood [1]. Albumins have been used as a model protein for many and diverse biophysical and physicochemical studies. They play an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood [3]. Recently, the three-dimensional structure of human serum albumin (HSA) has been determined through X-ray crystallographic measurements [4]. The globular protein consists of a single polypeptide chain of 585 amino acid residues and has many important physiological functions [5]. The globular protein is composed of three structurally similar domains (I, II and III), each containing two subdomains (A and B), and is stabilized by 17 disulfide bridges. Aromatic and heterocyclic ligands were found to bind within two hydrophobic pockets in subdomains II A and III A, site I and site II [2,4]. There are typical sites of coordination for several substances such as amino acids, fatty acids, hormones and drugs [2]. The multiple binding sites underlie the exceptional ability of HSA to interact with many substances and make this protein an important regulator of intercellular fluxes and pharmacokinetic behavior of many drugs. The molecular interactions are often monitored using optical techniques because these methods are sensitive and relatively easy to use. Among these, fluorescence spectroscopy has been widely used to investigate the interaction of drug and protein.

Jatrorrhizine (structure shown in Fig. 1) is one of the bioactive components isolated from a number of medicinal plants, such as *Tinospora cordifolia* (roots), *Berberis julianae* Schneid, *Jatrorrhiza palmate* Miers and so on. Also, Jatrorrhizine has antibacterial, antifungal and parasite-

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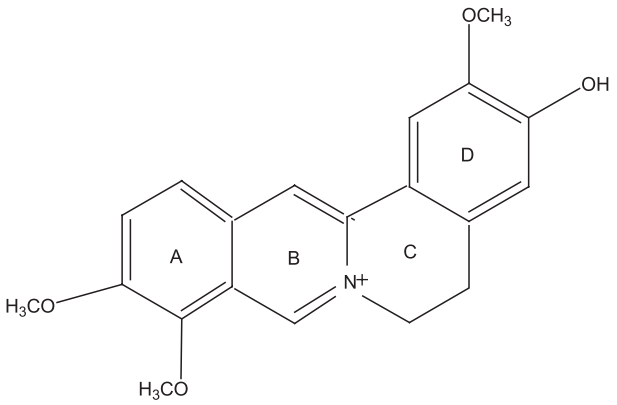

Fig. 1. The chemical structure of Jatrorrhizine.

fighting properties [6]. Given all these, Jatrorrhizine is therefore very worthy of study. However, its interaction mechanism with protein is poorly understood. So, we have been interested in examining the effect of Jatrorrhizine on HSA that can be used as a model for Jatrorrhizine–protein interaction. Investigating the influence of the drug on protein structure not only can provide usable information for realizing the pharmacological action of active components in Chinese herbs, but can also illuminate its binding mechanisms.

In this paper, we have studied the binding of Jatrorrhizine to HSA at three temperatures under physiological conditions, utilizing the fluorescence method together with the circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy. Partial binding parameters of Jatrorrhizine to HSA have been calculated. In addition, a comparative study of HSA interaction with Jatrorrhizine and phenybutazon (PB), fluofenamic acid (FA) or digitoxin (Dig) should help understand preferential binding at the molecular level.

2. Materials and methods

2.1. Materials

HSA (fatty acid-free), purchased from Sino-American Biotechnology Company (China), was used without further purification and its molecular weight was assumed to be 66500. All HSA solutions were prepared in the pH 7.40 buffer solution and the HSA stock solution was kept in the dark at 4 °C. Jatrorrhizine chloride, phenybutazon (PB), fluofenamic acid (FA) and digitoxin (Dig) were of analytical grade, and purchased from the National Institute for Control of Pharmaceutical and Bioproducts (China), and the stock solutions were prepared in absolute ethanol. NaCl (analytical grade, 1.0 $mol \cdot L^{-1}$) solution was used to maintain the ionic strength at 0.1. Buffer (pH 7.40) consists of Tris (0.2 $mol \cdot L^{-1}$) and HCl (0.1 $mol \cdot L^{-1}$), and the pH was adjusted to 7.40 by adding 0.5 $mol \cdot L^{-1}$ NaOH. Solutions of common ions (2.0×10^{-5} $g \cdot mL^{-1}$) were prepared. The pH was checked with a suitably standardized pH meter.

2.2. Apparatus and methods

Fluorescence spectra were measured with a RF-5301PC spectrofluorophotometer (Shimadzu), using 5/5-nm slit widths. The excitation wavelength was 295 nm, and the emission was read at 300–500 nm.

Fluorometric titration experiments: 3.0 mL solution containing appropriate concentration of HSA was titrated by successive additions of a 1.0×10^{-3} $mol \cdot L^{-1}$ ethanol stock solution of Jatrorrhizine (to give a final concentration of 3.3×10^{-6}–2.0×10^{-5} $mol \cdot L^{-1}$). Titrations were done manually by using trace syringes, and the fluorescence intensity was measured (excitation at 295 nm and emission at 343 nm). All experiments were measured at three temperatures (296, 303, and 310 K). The temperature of sample was kept by recycled water throughout the experiment.

Circular dichroism (CD) measurements were made on a Jasco-20c automatic recording spectropolarimeter (Japan) in cell of 2 mm pathlength at room temperature. CD spectra (200–250 nm) were taken at an HSA concentration of 3.0×10^{-6} $mol \cdot L^{-1}$, and the results are taken as molar ellipticity ($[\theta]$) in deg cm^2 $dmol^{-1}$; the α-helical content of HSA was calculated from the $[\theta]$ value at 208 nm using the equation [7]:

$$\%\text{helix} = \left\{\left(-[\theta]_{208} - 4000\right)/(33000 - 4000)\right\} \times 100$$

FT-IR measurements were carried out at room temperature on a Nicolet Nexus 670 FT-IR spectrometer (America) equipped with a Germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. All spectra were taken via the Attenuated Total Reflection (ATR) method with resolution of 4 cm^{-1} and 60 scans. Spectra processing procedures: spectra of buffer solution were collected at the same condition. Then, we subtracted the absorbance of buffer solution from the spectra of sample solution to get the FT-IR spectra of proteins. The subtraction criterion was that the original spectrum of protein solution between 2200 and 1800 cm^{-1} was featureless [8]. Fourier self-deconvolution and secondary derivative were applied to this range respectively to estimate the number, position and width of component bands. Based on these parameters, curve-fitting process was carried out by Galactic Peak solve to get the best Gaussian-shaped curves that fit the original protein spectrum. After the identification of the individual bands, the representative structure of HSA was calculated using the area of their respective component bands.

The crystal structure of HSA in complex with R-warfarin was taken from the Brookhaven Protein Data Bank (entry codes 1 h9z) [9]. The potential of the 3-D structures of HSA was assigned according to the Amber 4.0 force field with Kollman-all-atom charges. The initial structures of all the molecules were generated by molecular modeling software Sybyl 6.9 [10]. The geometries of these compounds were