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Binding of the bioactive compound 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone to human serum albumin

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Abstract

5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone is one of the bioactive components isolated from *Artemisia* plants possessing antitumor therapeutic activities. In this paper, its binding properties and binding sites located on human serum albumin (HSA) have been studied using UV absorption spectroscopy, fluorescence spectroscopy and Fourier transform infrared (FT-IR) spectra. The results of fluorescence titration revealed that 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone could strongly quench the intrinsic fluorescence of HSA by static quenching and there was only one class of binding sites on HSA for this drug. The binding constants at four different temperatures (289, 298, 310, and 318 K) were 1.93, 1.56, 1.22, and 0.93×10^5 L mol^{-1}, respectively. The FT-IR spectra evidence showed that the protein secondary structure changed with reduction of α-helices about 27.6% at the drug to protein molar ratio of 3. The thermodynamic functions standard enthalpy change (ΔH^0) and standard entropy change (ΔS^0) for the reaction were calculated to be -18.70 kJ mol^{-1} and 36.62 J mol^{-1} K^{-1} according to the van't Hoff equation. These results and the molecular modeling study suggested that hydrophobic interaction was the predominant intermolecular force stabilizing the complex, and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone could bind to the site I of HSA (the Warfarin Binding site).

Keywords: 5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone; Human serum albumin; Binding; FT-IR spectroscopy; Molecular modeling

1. Introduction

Human serum albumin (HSA) is the most abundant plasma protein synthesized exclusively in the liver. HSA constitutes over half of the total plasma proteins, a concentration of 35–50 g/L, in a healthy individual [1]. It is a globular protein consisting of a single peptide chain of 585 amino acids. As the major soluble protein constituent of the circulatory system, it has many physiological and pharmacological functions. One of its main functions is to regulate plasma osmotic pressure between the blood and tissues and it is chiefly responsible for the maintenance of blood pH. Another very important role of albumin is that it also functions as a transport molecule. This role is based on albumin's unique ability to bind a variety of exogenous and endogenous compounds, such as metal cations, fatty acids, amino acids and diverse drugs [2–4]. It has been shown that the distribution, free concentration and the metabolism of various drugs can be significantly altered as a result of their binding to HSA [1]. The effectiveness of drugs depends on their binding ability. Investigating the interaction of drugs to HSA can be used as a model for elucidating the properties of drug–protein complex, as it may provide useful information of the structural features that determine the therapeutic effectiveness of drugs, and it has become an important research field in life sciences, chemistry, and clinical medicine. So far, there have been some reports that investigate the interaction of proteins with drugs by fluorescence technique [5,6], FT-IR [7–9], CD spectroscopy [10], but the binding of Chinese herbal medicine active component to HSA has seldom been investigated.

Flavonoids are a group of phytochemicals distributed ubiquitously throughout the plant kingdom that exhibit a wide variety of biological effects in mammalian organisms [11]. They are important effective constituents of some medicines especially of Chinese herbal medicines. Currently there is growing evidence for flavonoids with a wide range of therapeutic activities (anticancer, antitumor, anti-inflammatory, anticoagulant, etc.) of high potency and low toxicity [12,13]. The antioxidative

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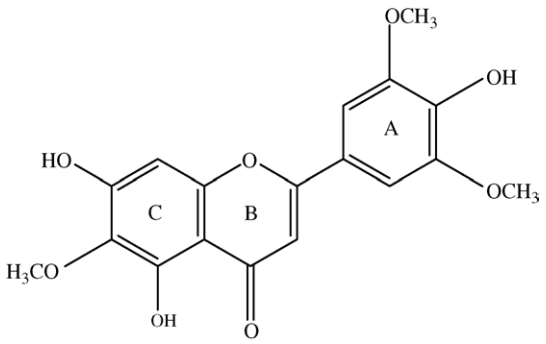

Fig. 1. Chemical structure of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone.

effects of flavonoids have been widely studied [14]. A number of biochemical and molecular biological investigations have revealed that proteins (including enzymes) are frequently the 'targets' for therapeutically active flavonoids of both natural and synthetic origin [15]. However, until now, very little has been known about the mode of interaction of such flavonoid compounds with their respective target proteins at molecule level. 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (its structure was shown in Fig. 1) is one of the bioactive components isolated from *Artemisia* plants. Like other flavonoid bioactive component, this drug possesses anticancer activities. In laboratory, it was found that it has the effect of inhibition on cellular growth of lung carcinoma A549 cells and the expression of PCNA. Nowadays, some works on the binding of flavonoid to protein have been carried out [16–23]. They were genistein [17], quercetin [18,20,21], 3-hydroxyflavone [19,23], fisetin [22], yet the binding of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone to HSA at molecule level has not been studied until now.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA, 70024-90-7, 98% purity) was purchased from Sigma Chemical Company, was used without further purification and its molecular weight was assumed to be 66,500. 5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone was provided by the Lanzhou Institute of Chemical Physics, Chinese Academy of Science. Tris (hydroxymethylaminomethane) was of biochemical grade and was purchased from The Shanghai Chemical Reagent Head Factory (Shanghai, China). 1.0 mol L^{-1} NaCl solution was used to keep the ion strength at 0.1. 0.05 mol L^{-1} Tris–HCl buffer solution containing 0.1 mol L^{-1} sodium chloride was selected to keep the pH of the solution at 7.40. 3.0×10^{-5} mol L^{-1} HSA stock solution was prepared in the pH 7.40 Tris–HCl buffer solution and kept in the dark at 4 °C. 1.0×10^{-3} mol L^{-1} stock solution of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone was prepared in anhydrous methanol. All other reagents were of analytical grade and doubly distilled water was used throughout all the experiments.

2.2. Apparatus and methods

Fluorescence emission spectra were measured with a RF-5301PC Spectrofluorimeter (Shimadzu, Japan) equipped with a xenon lamp source and 1.0 cm quartz cells, using 5 nm/5 nm slit widths. The excitation wavelength was 280 nm, and the emission wavelengths were read in the range of wavelength from 290 to 500 nm. A CARY-100 UV-visible spectrophotometer (Varian, USA) equipped with 1.0 cm quartz cells was used for scanning the UV–vis absorption spectra in a given wavelength range at room temperature. All pH values measurements were made with a PHS-10A digital pH meter (Xiaoshan, China).

Fluorometric titration experiments: 3.0 mL solution containing appropriate concentration of HSA was titrated in the pH 7.4 Tris buffer solution by successive additions of stock solution of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (to give a final concentration of 6.0×10^{-6}–1.80×10^{-5} mol L^{-1}). The drug was added from concentrated stock solution so that volume increment was negligible. Titrations were done manually by using trace syringes, and the fluorescence intensities were recorded at excitation and emission wavelengths of 280 and 336 nm. All experiments were performed at four temperatures (289, 298, 310, and 318 K). An electronic thermo regulating water-bath (NTT-2100, EYELA, Japan) was used for controlling the temperature. The data herein obtained were analyzed by the modified Stern–Volmer equation and Scatchard equation to calculate the binding parameters.

FT-IR measurements were performed at room temperature on a Nicolet Nexus 670 FT-IR Spectrometer (America) equipped with a germanium attenuated total reflection (ATR) accessory, a deuterated triglycine sulphate (DTGS) 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. The infrared spectra of HSA and the 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone–HSA complex (the molar ratio of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone to HSA was 3:1) were obtained in the featured region of 1800–1300 cm^{-1}. Corresponding absorbance contributions of buffer and free 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone solutions were recorded and digitally subtracted with the same instrumental parameters.

3. Results and discussion

3.1. Interaction of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone with HSA

This study was designed to investigate the binding properties (including binding mechanism, the binding constants, the binding sites, and binding mode) of the interaction of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone with HSA and the effect of this drug on the HSA conformation changes. First, the fluorescence emission spectra of HSA solutions with different concentrations of 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone were collected. The effect of drug on HSA fluorescence intensity is shown in Fig. 2. From Fig. 2, it can be seen that the HSA had a strong fluorescence emission band at 336 nm while 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone had no intrinsic