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Studies on the interaction between scopoletin and two serum albumins by spectroscopic methods

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ABSTRACT

The interactions of scopoletin to bovine serum albumin (BSA) and human serum albumin (HSA) have been investigated by spectroscopic methods. The fluorescence tests indicated that the formation mechanism of scopoletin–BSA/HSA complexes belonged to the static quenching. The displacement experiments suggested that scopoletin primarily bound to tryptophan residues of BSA/HSA within site I (subdomain IIA). The binding distance of scopoletin to BSA/HSA was 2.38/2.34 nm. The thermodynamic parameters (ΔG, ΔH and ΔS) calculated on the basis of different temperatures revealed that the binding of BSA–scopoletin was mainly depended on van der Waals interaction and hydrogen bond, and yet the binding of HSA–scopoletin was strongly relied on the hydrophobic interaction and electrostatic interaction. The results of synchronous fluorescence, 3D fluorescence, UV–vis absorption, and FT-IR spectra showed that the conformations of BSA and HSA altered with the addition of scopoletin. In addition, the effects of some common ions on the binding constants of scopoletin to proteins were also investigated.

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1. Introduction

The interactions between drugs and bio-macromolecules have attracted great interest among researchers in several decades [1]. Among bio-macromolecules, serum albumins (SAs) are abundant proteins in the blood circulatory system. Moreover, they play important roles in the transport and deposition of endogenous and exogenous substances such as fatty acids, amino acids, drugs, etc. [2]. However, among albumins, bovine serum albumin (BSA) and human serum albumin (HSA) have been widely used as models in evaluating the interactions of ligands with proteins owing to their similar folding and well-known primary structure [3]. BSA shows 76% similarity with that of HSA [4], which has a single-chain with 582 amino acid residues. Moreover, it has two tryptophan residues (Trp134 and Trp212), that is to say, Trp-134 in the first domain is located on the surface of the molecule, and Trp-212 in the second domain is located within a hydrophobic binding pocket of BSA, whereas, HSA consists of a single polypeptide chain with 585 amino acid residues in which the single tryptophan residue (Trp214) measures the binding affinity of drugs to HSA [5]. In recent years, some research groups [6–13] have reported the interactions of different drugs with BSA/HSA by different techniques, such as, spectroscopic (FTIR, CD, fluorescence, synchronous fluorescence, UV–visible absorption and 3D fluorescence spectra), density functional theory (DFT) calculations, molecular docking and NMR spectroscopy.

Scopoletin (7-hydroxy-6-methoxycoumarin) (Scheme 1 [14]), as one of main coumarin constituents occurring in the stems of *Erycibe obtusifolia* Benth, is usually used for the treatment of various rheumatoid diseases in traditional Chinese medicines. It is well known that scopoletin possesses a wide range of biological activities [14–19], such as anti-depressant, anti-cholinesterasic, anti-nociceptive, anti-thyroid, anti-inflammatory, anti-oxidant, anti-hyperglycemic, anti-tumoral, anti-arthritic activity, and anti-proliferative agent. Recently, scopoletin has been reported to attenuate FGF-2-induced angiogenesis and act by directly preventing the stimulation action of FGF-2 and indirectly decreasing the production of VEGF, so it is possible that scopoletin and its analogs should become candidate drugs for angiogenesis-related diseases [20]. To the physical chemist, scopoletin is well known for its fluorescence very sensitive to the environment, which recommends it for exploring binding sites of bio-relevant targets [10]. Although the biological activities of scopoletin have been investigated intensively, the mechanism of action between scopoletin and proteins still remains unknown. However, knowledge of the interaction mechanism between scopoletin and proteins was of crucial importance for us to understand the pharmacology, pharmacodynamics, pharmacokinetic behavior of the drug and design its analogs with effective pharmacology properties. Therefore, we have thought it worthwhile to investigate the interactions of proteins with scopoletin.

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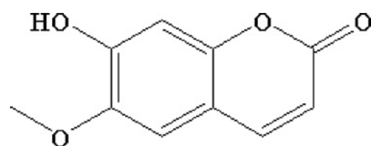

Scheme 1. Chemical structure of scopoletin.

In the present work, the interactions between scopoletin and BSA/HSA have been investigated in vitro using spectroscopic techniques under physiological conditions. The association constants, the thermodynamic parameters, the number of binding sites, the binding forces and the energy transfer distance of scopoletin–BSA/HSA complexes were estimated. At the same time, the effect of scopoletin on the microenvironment and conformation of BSA/HSA was also discussed. In addition, the binding mechanism of scopoletin with BSA/HSA was explored. We believe that these studies will be useful to design and synthesis of scopoletin derivatives and their pharmaceutical research.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA, Fraction V, 98% purity) purchased from Roche Company, and human serum albumin (HSA, 96% purity) purchased from Sigma-Aldrich, were used without further purification and their molecular weight were assumed to be 68,000 and 66,000, respectively. They were both dissolved in the Tris-HCl buffer solution (pH 7.4) to form a solution of 2.0×10^{-4} mol L^{-1} and then stored in the dark at 4 °C. Scopoletin (98% purity) was obtained from DELTA information center for natural organic compounds (Anhui, China). The stock solution of scopoletin was prepared by dissolving it in 5% absolute ethanol with the final concentration of 6.0×10^{-4} mol L^{-1}. Other materials were of analytical reagent grade and also used without further purification. Doubly distilled water was used throughout the experiment. Sample masses were accurately weighted on an electronic analytical balance ESJ180-4 (Shenyang Longteng Electronic Co., Ltd, China) with a resolution of 0.1 mg.

2.2. Fluorescence quenching measurements

All fluorescence measurements were carried out on a Cary Eclipse fluorescence spectrofluorometer (Varian, USA) equipped with a xenon lamp source and 1.0 cm quartz cell and a thermostat bath.

Fluorometric titration experiments: 3.0 mL of BSA/HSA solution (pH 7.4) was added accurately to the quartz cell and then titrated by successive additions of scopoletin solution with the concentration of 6.0×10^{-4} mol L^{-1} using a 50 μL microsyringe to attain a series of final concentrations. Titrations operated manually and mixed moderately. Fluorescence emission spectra were measured at 293, 298, 304 and 310 K with the width of the excitation and emission slit both adjusted at 5 nm. The excitation wavelength was 280 nm, and fluorescence emission spectra were recorded in the range of 250–590 nm. Synchronous fluorescence spectra were recorded from 240 to 320 nm at $\Delta\lambda=15$ and $\Delta\lambda=60$ nm, respectively. Also appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background.

Three-dimensional fluorescence spectra of free BSA/HSA and scopoletin–BSA/HSA complex were measured under the following conditions: the initial excitation wavelength was set at 200 nm with increment of 2 nm, and the emission wavelength was recorded between 200 and 500 nm at a scanning rate of 24,000 nm/min, and other scanning parameters were the same as those of fluorescence quenching spectra.

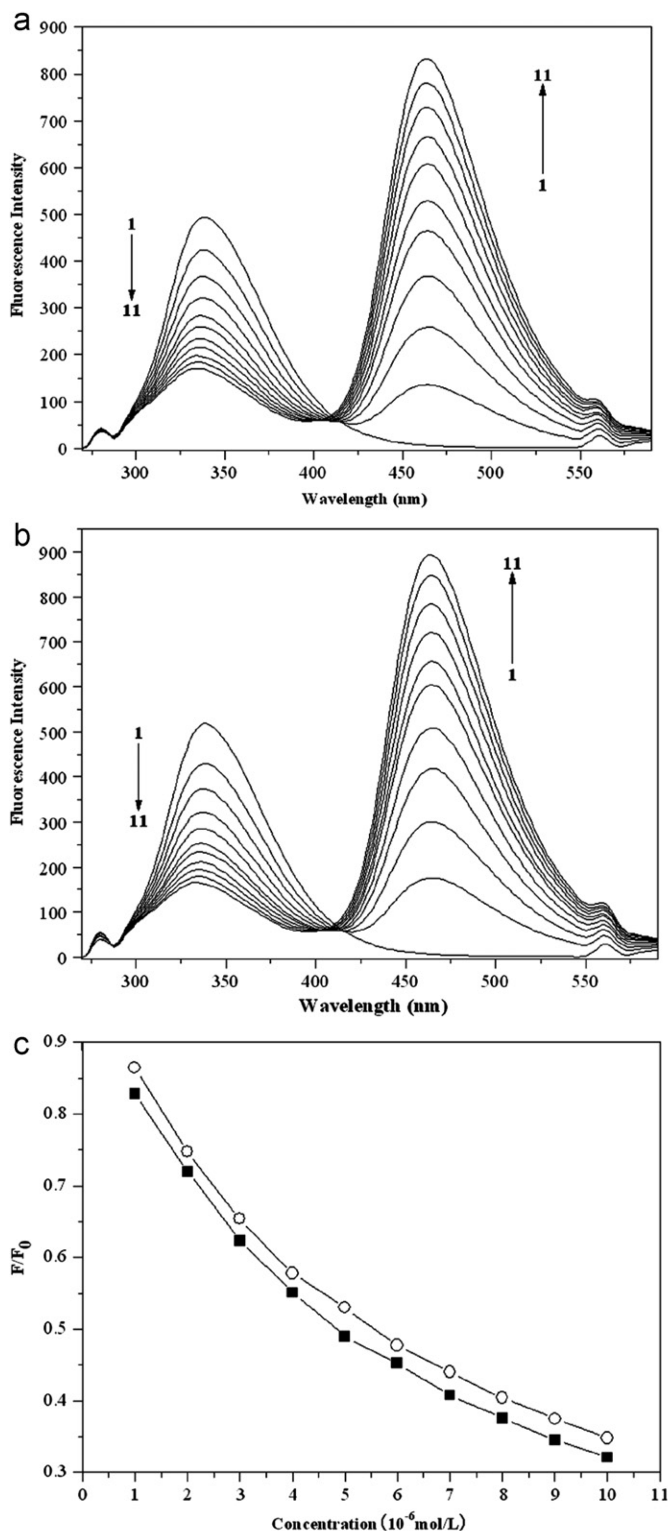

Fig. 1. The effects of scopoletin on the fluorescence intensities of BSA (a) and HSA (b), and (c) normalized fluorescence intensities of BSA (○) and HSA (■) with different scopoletin concentrations. Conditions: $T=298$ K, $\lambda_{ex}=280$ nm; buffer, Tris-HCl; pH 7.4; c(BSA)=c(HSA)=5.0 μM; c(scopoletin) 1–11: 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μM, respectively.

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