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Spectroscopic studies on the interaction between tetrandrine and two serum albumins by chemometrics methods ☆

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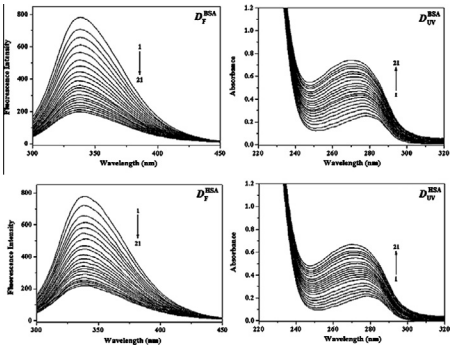
HIGHLIGHTS

- The binding modes of TETD and BSA/HSA have been established.
- The binding sites on BSA/HSA by TETD were discussed.
- MCR-ALS was applied to interpret the interaction between TETD and BSA/HSA.
- Investigating the structural changes of proteins.
- Further information was extracted by chemometrics methods.

GRAPHICAL ABSTRACT

The interactions of tetrandrine (TETD) with two serum albumins (BSA and HSA) have been investigated using multispectroscopic and chemometrics methods at different temperatures under imitated physiological conditions. These spectra data measured were further analyzed by multivariate curve resolution-alternating least squares (MCR-ALS) and the concentration profiles for three species (BSA/HSA, TETD and TETD-BSA/HSA) existed in the interaction procedure.



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ABSTRACT

The binding interactions of tetrandrine (TETD) with bovine serum albumin (BSA) and human serum albumin (HSA) have been investigated by spectroscopic methods. These experimental data were further analyzed using multivariate curve resolution-alternating least squares (MCR-ALS) method, and the concentration profiles and pure spectra for three species (BSA/HSA, TETD and TETD-BSA/HSA) existed in the interaction procedure, as well as, the apparent equilibrium constants K_{app} were evaluated. The binding sites number n and the binding constants K were obtained at various temperatures. The binding distance between TETD and BSA/HSA was 1.455/1.451 nm. The site markers competitive experiments indicated that TETD primarily bound to the tryptophan residue of BSA/HSA within site I. The thermodynamic parameters (ΔG, ΔH and ΔS) calculated on the basis of different temperatures revealed that the binding of TETD-BSA was mainly depended on the hydrophobic interaction strongly and electrostatic interaction, and yet the binding of TETD-HSA was strongly relied on the hydrophobic interaction. The results of synchronous fluorescence, 3D fluorescence and FT-IR spectra show that the conformation of proteins has altered in the presence of TETD. In addition, the effect of some common ions on the binding constants between TETD and proteins were also discussed.

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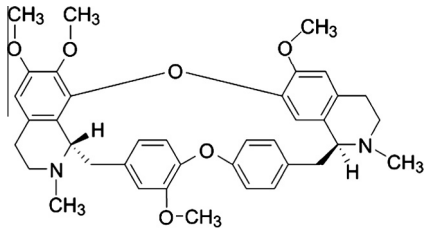

Scheme 1. Chemical structure of TETD.

Introduction

Tetrandrine (TETD, a bis-benzylisoquinoline alkaloid, Scheme 1), an extract from the root of Hang-Fang-Chi (*Stephania tetrandra S. Moore*), has been reported to have many pharmacological effects including immunosuppressive, non-selective Ca^{2+} channel Blocking, anti-proliferative, anti-oxidant, anti-fibrotic and anti-tumor (such as, breast cancer, lung cancer, neuroblastoma, Burkitt's lymphoma, hepatocellular carcinoma, colon cancer, nasopharyngeal carcinoma, glioma and leukemia) activity [1–5]. For several decades, TETD has been used in China to treat patients with arthritis, arrhythmia, hypertension, inflammation, and silicosis [2]. Moreover, TETD exhibits synergistic effects with chemotherapeutics in restricting tumor cell proliferation [6], but the molecular mechanisms underlying its anti-tumor efficacy remain poorly understood. Later, Cheng et al. [7] investigated the mechanisms by utilizing two-dimensional gel electrophoresis (2DE), and their studies suggested that TETD could inhibit HepG2 cells proliferation and might exert its effect on liver cancer at least partially through regulating these proteins' expression directly or indirectly. However, further study of these proteins will be helpful to further elucidate anti-tumor mechanism of TETD and identify new targets of drugs for chemotherapy. In addition, TETD can reduce portal venous pressure, prevent diabetes, ameliorate the reperfusion injury in porcine intestinal transplantation, inhibit calcium channels, lipid peroxidation and proliferation of vascular smooth muscle cells [8]. Gong et al. [9] reported that TETD might alleviate the fulminant hepatic failure (FHF) induced by LPS/D-GalN and reduce TNF-α production through inhibiting NF-κB activation. Recently studies [10,11] have demonstrated that TETD may provide a therapeutic approach for AD (Alzheimer's disease), and inhibit HSFs (hypertrophic scar fibroblasts) at least partially through induction of Smad7 and decrement of Smad2 resulting in inhibition of TGF-β1 (transforming growth factor β1) transcription and its intracellular signaling. In 2007, Wang et al. [12] reported that the binding of effective component (such as, TETD) of Chinese herbs with HSA were investigated by spectroscopic methods. Latterly, they [13] studied the interaction between TETD and BSA by fluorescence spectroscopy. But, identifying binding sites between TETD and BSA/HSA, further exploring the structural change of BSA/HSA (such as, FTIR, 3D fluorescence spectra methods) and investigating the influences of common ions on the interaction of proteins with TETD need still be discussed. However, in order to understand detailedly its pharmacological importance, the interactions of proteins with TETD have been investigated thoroughly because the binding phenomena can not only provide some useful information for understanding pharmacological actions, bio-information, bio-distribution, etc., but also illustrate its binding mechanism at a molecular level and design its analogues with effective pharmacological properties.

Serum albumins (SAs) are the most abundant protein in plasma, and participate in the binding and transportation of various ligands, such as fatty acids, hormones and drugs [14]. So, serum albumin is often employed as a model for studying the interaction of drug with protein in vitro. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Among albumins, bovine serum albumin (BSA) and human serum albumin (HSA) have been widely used as models in evaluating the interactions between ligands and proteins owing to their similar folding and well-known primary structure [15].

In recent years, some research groups [16–26] have reported the interactions of different drugs with proteins by different techniques, such as, spectroscopic (FTIR, CD, fluorescence, synchronous fluorescence, UV–vis absorption and 3D fluorescence spectra), HPLC, electrochemistry, capillary electrophoresis, density functional theory (DFT) calculations, molecular docking, NMR spectroscopy, isothermal titration calorimetry (ITC), scanning electron microscope (SEM) and transmission electron microscopy (TEM) analyses. However, in the multicomponent reaction systems reported above, if researchers are hoping to estimate simultaneously, the amounts of the small molecule, the biopolymer and their complex product, the analytical tasks should become quite difficult and challenging by the common methods of data interpretation. Therefore, in order to solve the difficult questions, chemometrics methods such as multivariate curve resolution-alternating least squares (MCR-ALS) method [27] are applied. Up to now, a few research groups [28–32] reported the interactions between various small molecules (such as drugs) and biopolymer (such as, polynucleotide, BSA and DNA) by fluorescence, UV–vis spectra, electrochemistry and other methods with the aid of MCR-ALS method. Moreover, in these binding reaction systems, further information can be obtained, for example, the formation of the complex, the concentration profiles and pure spectra for each species.

In the present work, the interaction between TETD and BSA/HSA has been investigated in vitro using fluorescence, UV–vis and FTIR spectra techniques under physiological conditions. The association constants, the thermodynamic parameters, the number of binding sites, the binding forces and the energy transfer distance of TETD-BSA/HSA complexes were estimated. At the same time, the effect of TETD on the microenvironment and conformation of proteins was also discussed. Moreover, the MCR-ALS method was applied to resolve the two-way UV–vis and fluorescence spectra data so that it can help us to understand further the complex kinetic processes and extract the equilibrium profiles of the reacting species. In addition, the binding mechanism of TETD with BSA/HSA was explored. We believe that these studies will be useful to design and synthesis of TETD derivatives with high activity and their pharmaceutical research.

Experimental

Materials

Bovine serum albumin (BSA, FractionV, 98% purity, M_r = 68000) purchased from Roche Company, and human serum albumin (HSA, 96% purity, M_r = 66000) purchased from Sigma–Aldrich, were used without further purification. 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. Tetrandrine (TETD, 98% purity) was obtained from Shanghai Source Leaf Biological Technology Co., Ltd. (Shanghai, China). The stock solution of TETD was prepared by dissolving the crystals into 10% acetone 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 weighed on an electronic analytical balance ESJ200-4 (Shenyang Longteng Electronic Co., Ltd., China) with a resolution of 0.1 mg.