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Exploring the site-selective binding of jatrorrhizine to human serum albumin: Spectroscopic and molecular modeling approaches

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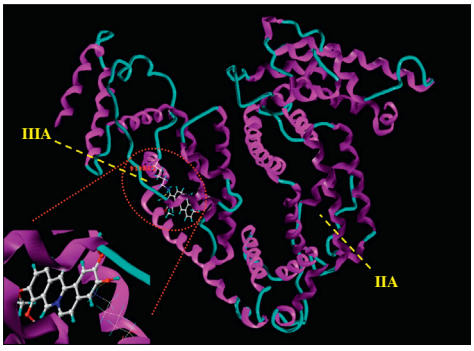
HIGHLIGHTS

- This work is focusing on deciphering the site-selective binding of jatrorrhizine to HSA.
- The spectroscopic methods and molecular modeling have been chosen as the study tools.
- The binding mechanisms, and a series of bind parameters have been explored.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper exploring the site-selective binding of jatrorrhizine to human serum albumin (HSA) under physiological conditions (pH = 7.4). The investigation was carried out using fluorescence spectroscopy, UV–vis spectroscopy, and molecular modeling. The results of fluorescence quenching and UV–vis absorption spectra experiments indicated the formation of the complex of HSA–jatrorrhizine. Binding parameters calculating from Stern–Volmer method and Scatchard method were calculated at 298, 304 and 310 K, with the corresponding thermodynamic parameters ΔG, ΔH and ΔS as well. Binding parameters calculating from Stern–Volmer method and Scatchard method showed that jatrorrhizine bind to HSA with the binding affinities of the order 10^4 L mol^{-1}. The thermodynamic parameters studies revealed that the binding was characterized by negative enthalpy and positive entropy changes and the electrostatic interactions play a major role for jatrorrhizine–HSA association. Site marker competitive displacement experiments and molecular modeling calculation demonstrating that jatrorrhizine is mainly located within the hydrophobic pocket of the subdomain IIIA of HSA. Furthermore, the synchronous fluorescence spectra suggested that the association between jatrorrhizine and HSA changed molecular conformation of HSA.

Introduction

Alkaloids are a large class of naturally occurring polyphenols widely distributed in plants. They have attracted considerable attention, specifically because alkaloids are of biological and physiological importance. Jatrorrhizine (2,9,10-Trimethoxy-5,6-dihydroisoquinolino [2,1-b]isoquinolin-7-ium-3-ol), a protoberberine alkaloid (Fig. S1, Supplementary material), is well known for its various biological activities [1]. Jatrorrhizine has been reported to have antimutagenic potency, antioxidant, antifungal, antibacterial antiplasmodial, antiamoebic activities [2–6]. In other studies, jatrorrhizine has been used for treating prostatitis, prostatomegaly, and infection of the urinary system diseases, might treat

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or prevent neurodegenerative diseases, exert therapeutic effects on the functional disorders of gastrointestinal tracts by regulating the contractions of gastrointestinal smooth muscles, and uses in the development of therapeutic and preventive agents for diabetic complications and diabetes mellitus [7–12].

Plasma protein binding is an important factor to understand the pharmacokinetics and pharmacodynamic properties of drug candidates, as it strongly influences drug distribution and determines the free fraction, which is available to the target [13]. In spite of these broad uses of jatrorrhizine mentioned above, its effects on plasma protein remain unclear. It is widely accepted in the pharmaceutical industry that the overall distribution, metabolism, and efficacy of many drugs can be altered based on their affinity to serum albumin [13–17]. In addition, many promising new drugs are rendered ineffective because of their unusually high affinity for this abundant protein [18]. Obviously, an understanding of the chemistry of the various classes of pharmaceutical interactions with albumin can suggest new approaches to drug therapy and design. Human serum albumin (HSA) is the most prominent protein in plasma, present in 6.0×10^{-4} M, contributes to about 80% of the blood osmotic pressure [19,20], and is composed of a single chain of 585 amino acid residues. It has three homologous α-helical domains (I, II, and III), and each domain is further divided into two subdomains (A and B). HSA contains two principal binding sites (i.e., Sudlow's site I and site II), which are located in the specialized cavities of subdomains IIA and IIIA, respectively [20–22]. HSA has long been the center of attention of the pharmaceutical industry due to its ability to bind various drug molecules and alter their pharmacokinetic properties [23–25], the finding may lead to a new perspective for scientific research of drug, and open new interdisciplinary fields [26–29]. Accordingly, it is important to understand and predict ligand/drug displacement interactions for a variety of endogenous and exogenous ligands/drugs. However, detailed investigations of the interaction of HSA with jatrorrhizine have yet to be conducted, this study will be the first one to look into the site-selective binding of jatrorrhizine to HSA and what could be the possible reasons.

In the present work, spectroscopic and molecular modeling approaches were performed in order to elucidate the site-selective binding of jatrorrhizine to HSA. The interaction information regarding quenching mechanisms, binding parameters, thermodynamic parameters, binding modes, site-selective binding site, and conformation investigation is reported here.

Materials and methods

Materials

HSA and warfarin were obtained from Sigma–Aldrich (St. Louis, MO, USA); Jatrorrhizine was obtained from national institute for control of pharmaceutical and biological products (Beijing, China); ibuprofen was obtained from Hubei biocause pharmaceutical Co., Ltd. (Hubei, China; the purity no less than 99.7%); the buffer Tris had a purity of no less than 99.5% and NaCl, HCl, etc. were all of analytical purity. All samples were dissolved in Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4), Appropriate blanks, run under the same conditions, were subtracted from the sample spectra.

Equipments and spectral measurements

Fluorescence spectra were recorded on F-2500 Spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. The widths of both the excitation slit and the emission slit were set to 2.5 nm. An excitation wavelength of 295 nm was chosen since it provides no excitation of tyrosine residues and therefore neither emission nor energy transfer to the lone indole side chain would be nonnegligible. Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background.

Three-dimensional fluorescence spectroscopy were recorded on F-4500 spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. The emission wavelength was recorded at 220–500 nm, the excitation wavelength was recorded at 200–350 nm.

The UV spectrum was recorded at room temperature on a TU-1901 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells.

Molecular modeling

Docking study was conducted by a Surflex-Dock program in Sybyl 8.1 package [30]. The crystal structure of HSA was obtained from RCSB Protein Database Bank (PDB code: 1H9Z, 2BXG chain a), the structure of the biopolymer was analyzed and prepared for the docking experiment. H atoms with H-bond orientation were added. All the ligands and water molecules were removed before the analysis. H atoms were added and the biopolymer was charged using AMBER7 FF99 method. The structure of jatrorrhizine was generated with sybyl8.1 package and the molecule was charged with Gasteiger–Marsili method, after that jatrorrhizine was energy minimized using Tripos Force Field.

Results and discussions

Fluorescence characteristics of HSA

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in quenching, including excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching.

In the experiment, the concentrations of HSA solution were stabilized at 1.0×10^{-5} mol L^{-1}, and the concentrations of jatrorrhizine varied from 0 to 2.4×10^{-5} mol L^{-1} at increments of 0.2×10^{-5} mol L^{-1}. The effect of jatrorrhizine on HSA fluorescence

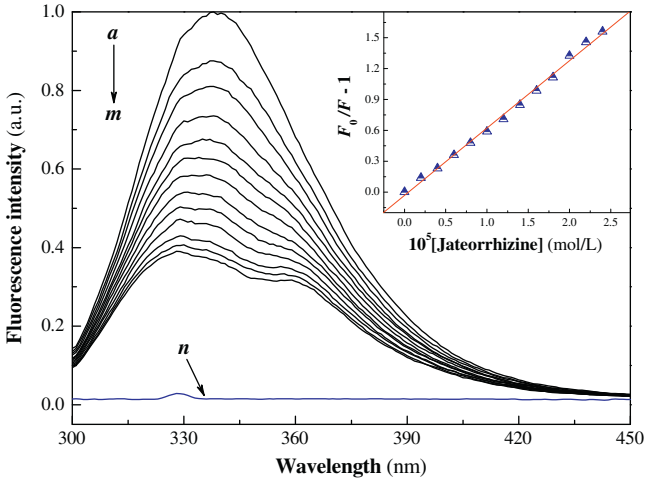

Fig. 1. Emission spectra of HSA in the presence of various concentrations of jatrorrhizine. c (HSA) = 1.0×10^{-5} mol L^{-1}; c (jatrorrhizine)/(10^{-5} mol L^{-1}), a – m: from 0.0 to 2.4 at increments of 0.20; curve n (blue line) shows the emission spectrum of jatrorrhizine only (T = 298 K, λ_{ex} = 295 nm). The inset corresponds to the Stern–Volmer plot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)