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Spectroscopic analysis and molecular modeling on the interaction of jatrorrhizine with human serum albumin (HSA) ☆

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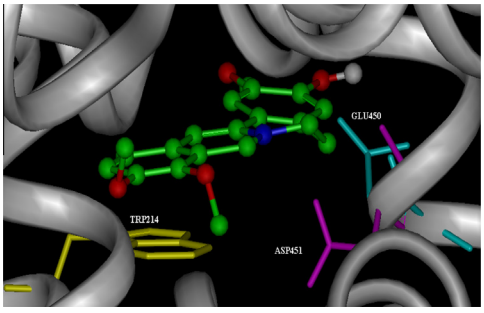
HIGHLIGHTS

- This paper attests that jatrorrhizine has high binding affinity with HSA.
- Jatrorrhizine effectively quenched the fluorescence of HSA molecules through static mechanism.
- The energy transfer from HSA molecules to jatrorrhizine occurs with high probability.
- Experimental data show that jatrorrhizine interacts with HSA mainly via electrostatic force.
- Molecular calculation shows that π–π staking and hydrogen bond also stabilize the complex on site I.

GRAPHICAL ABSTRACT

The binding behavior of jatrorrhizine with HSA was studied by UV–vis, fluorescence and molecular modeling examinations. Jatrorrhizine has high binding affinity with HSA by effectively quenched the fluorescence of HSA molecules via static mechanism. The binding sites, the type of interaction and the energy transfer process were revealed. The thermodynamic parameters indicate that electrostatic force plays an important role in the binding. The energy transfer from HSA molecules to jatrorrhizine occurs with high probability.Furthermore, the molecular modeling was performed to explore the possible binding site and assess the microenvironment around the bound jatrorrhizine.



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ABSTRACT

In this work, the interaction of jatrorrhizine with human serum albumin (HSA) was studied by means of UV–vis and fluorescence spectra. The intrinsic fluorescence of HSA was quenched by jatrorrhizine, which was rationalized in terms of the static quenching mechanism. The results show that jatrorrhizine can obviously bind to HSA molecules. According to fluorescence quenching calculations, the bimolecular quenching constant (k_q), apparent quenching constant (K_{SV}) at different temperatures were obtained. The binding constants K are 4059 L mol^{-1} and 1438 L mol^{-1} at 299 K and 304 K respectively, and the number of binding sites n is almost 1. The thermodynamic parameters determined by the Van't Hoff analysis of the binding constants (ΔH −12.25 kJ mol^{-1} and ΔS 28.17 J mol^{-1} K^{-1}) clearly indicate that the electrostatic force plays a major role in the process. The efficiency of energy transfer and the distance between the donor (HSA) and the acceptor (jatrorrhizine) were calculated as 22.2% and 3.19 nm according to Föster's non-radiative energy transfer theory. In addition, synchronous fluorescence spectroscopy reveals that jatrorrhizine can influence HSA's microstructure. That is, jatrorrhizine is more vicinal to tryptophane (Trp) residue than to tyrosine (Tyr) residue and the damage site is also mainly at Trp residue. Molecular modeling result shows that jatrorrhizine–HSA complex formed not only on the basis of electrostatic forces,

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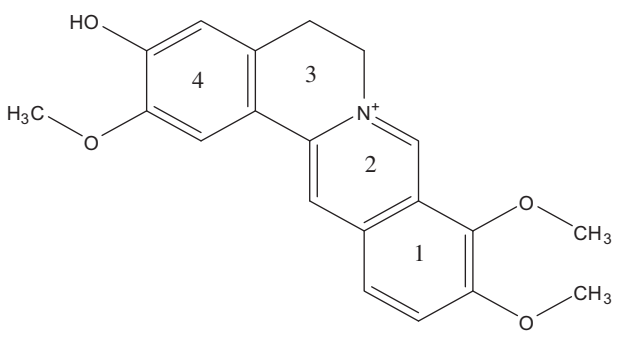
but also on the basis of π–π staking and hydrogen bond. The research results will offer a reference for the studies on the biological effects and action mechanism of small molecule with protein.

Introduction

Studies on supramolecular interactions of drugs or organic dyes with biological molecules have significantly contributed to the understanding of the structures and functions of bio-macromolecules and some biophysical processes [1]. By binding to HSA, most drugs circulate in plasma and reach the target tissues and their distribution is mainly controlled by HSA [2]. Therefore, drug binding to proteins has becomes an important determinant of pharmacokinetics, e.g. prolonging in vivo half-life, restricting the unbound concentration and affecting distribution and elimination of the drug [3]. Human serum albumin (HSA) is the most abundant protein in human blood plasma and has high affinity to many endogenous and exogenous compounds, serving as a solubilizer and transporter for drugs and other organic molecules to their targets [4]. For these reasons, HSA is commonly used as a model protein for biophysical and physicochemical studies [5]. The globular protein consists of a single polypeptide chain of 585 amino acid residues and composed of three structurally similar domains (I, II, and III). Many drugs could interact with protein by multiple binding sites, thus the protein serve as an important regulator of intercellular fluxes.

Fluorometric method has been widely used because of its high sensitivity, selectivity, convenience and providing more information. The spectral change observed upon binding of a fluorophore to protein is a powerful tool for investigating the topology of binding site and the conformational changes and characterization of bound substrate [6].

Jatrorrhizine (structure shown in Scheme 1) is one of the major bioactive components isolated from tinospora cordifolia's roots [7,8]. The structure and medication of jatrorrhizine are same with another alkaloid, berberine. Because of its excellent pharmacological activity, antibacterial, antifungal and parasite fighting abilities, it is necessary to study the interaction between jatrorrhizine and protein for understanding the mechanism of drug action at molecular level. The understanding of the interaction of jatrorrhizine with HSA is of utmost importance in formulating safe drugs and effective dosages. Researches on some cationic alkaloids with protein have received rapidly rising interesting [9,10]. For example, Zhang et al. [11] studied the interactions between three protoberberine alkaloids and Bcl-2 by fluorescence spectroscopy. They found that three protoberberine alkaloids could quench the intrinsic fluorescence of Bcl-2 by static mechanism. Li et al. [12] studied the interaction between jatrorrhizine and human gamma globulin (HGG) in AOT/isooctane/water microemulsions by using fluorescence quenching, UV absorption spectroscopy, circular dischroism (CD) spectroscopy and dynamic light scattering. Chen et al. [13] studied the interaction of jatrorrhizine with HSA using fluorescence, but the results are limited to binding constants. The detailed and systematic investigations on the interaction of jatrorrhizine with HAS is still rarely been reported and are very limited.



Scheme 1. The chemical structure of jatrorrhizine.

We have previously reported the spectrocopic studies to character the interaction of some alkaloids with ctDNA [14,15]. In this paper, the complexation behavior of jatrorrhizine with HSA was studied by UV–vis, fluorescence and molecular modeling examinations. The fluorescence quenching mechanism of HSA by jatrorrhizine, the binding sites, the type of interaction and the energy transfer process were revealed. Attempts were also made to investigate the thermodynamic parameters for the reaction and the effect of jatrorrhizine on the protein secondary structure. The results indicate that electrostatic force played an important role in the binding due to the opposite charge on the jatrorrhizine and the protein. Furthermore, the molecular modeling was performed to explore the possible binding site and assess the microenvironment around the bound jatrorrhizine. The binding properties of jatrorrhizine to HSA elucidated in this study may provide some useful clues for future application of this kind of drugs as a photosensitizer for anticancer treatment.

Materials and methods

Materials

HSA was purchased from Sigma Chemical Corporation. A 1×10^{-4} mol L^{-1} stock solution was prepared by dissolving the solid HSA in 0.5% NaCl solution and stored at 0–4 °C. Jatrorrhizine (AR grade) was obtained from the national institute for the control of pharmaceutical and biological products (Beijing, China), and a stock solution (1×10^{3} mol L^{-1}) was prepared by dissolving an appropriate amount of jatrorrhizine into doubly distilled water. Tris–HCl (pH = 7.40) buffer solution was used to control the solution pH.

Apparatus and methods

The absorption spectra were obtained on a UV-2450 dual-beam UV–vis spectrophotometer (Shimadzu, Japan). A F-4500 spectrofluorometer (Hitachi, Japan) equipped with a 150 W xenon lamp and a thermostatic bath was used for the measurement of fluorescence spectra. Instrument settings, i.e. the slit widths (excitation at 5.0 nm; emission at 5.0 nm). Fluorescence spectra of HSA were obtained with excitation at 296 nm. One centimeter quartz cells were used through out the experiments. Solution pH values were measured with a pHS-3C pH meter (Shanghai REX Instrument Factory, China) with a combined glass electrode.

Procedures of fluorescence measurement

HSA stock solution was transferred to a 10 mL volumetric flask, diluted to the final volume with a pH 7.40 Tris–HCl buffer solution. The interaction between jatrorrhizine and HSA was investigated by fluorometric titration. An exact 2.5 mL portion of 1×10^{-5} mol L^{-1} HSA solution was added to a 1.0 cm quartz cell, and then titrated by successive additions of 1.0×10^{-2} mol L^{-1} jatrorrhizine with a 5 mL microsyringe to attain a series of final concentrations. The