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Application of molecular modelling and spectroscopic approaches for investigating the binding of tanshinone IIA to human serum albumin

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ABSTRACT

The interaction of tanshinone IIA and human serum albumin (HSA) has been characterised by molecular modelling, fluorescence, Fourier transform infrared (FT-IR) and circular dichroism (CD) spectroscopic methods. The results of molecular modelling suggested that tanshinone IIA located within the binding pocket of subdomain IIA of HSA is held mainly by hydrophobic forces. Fluorescence titration revealed that tanshinone IIA could quench the intrinsic fluorescence of HSA. The binding constants at three temperatures (296, 303, and 310) K are ($6.42 \cdot 10^4$, $1.54 \cdot 10^5$, and $4.35 \cdot 10^5$) $dm^3 \cdot mol^{-1}$, respectively. In addition, the studies of CD spectroscopy and FT-IR spectroscopy showed that the binding of tanshinone IIA to HSA changed molecular conformation of HSA.

1. Introduction

Tanshinone IIA (structure shown in figure 1), a derivative of phenanthrenequinone, is the major component isolated from *Salvia miltiorrhiza* (Danshen, in Chinese) which is a well-known traditional Chinese herbal medicine. It has been reported to display a great variety of pharmacological activities including prevention of angina pectoris and myocardial infarction [1], anticancer [2,3], and antioxidant [4] properties. Moreover, recent research indicates that tanshinone IIA may possess complex inhibiting and inducting action on CYP1A [5].

Human serum albumin (HSA), the most abundant carrier protein in blood circulation, plays a major role in the transport and deposition of many endogenous and exogenous drugs ligands in blood [6,7]. A number of the relatively insoluble endogenous compounds and a wide variety of drugs can bind to albumin and other serum components, which implicates HSA's role as a carrier [8,9]. Due to the availability of hydrophobic pockets inside the protein network and the flexibility of the albumins to adapt its shape [10], serum albumin can increase the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells *in vivo* and *in vitro*. Therefore, investigating the binding of the drug to HSA can provide useful information of structural features that determine the therapeutic effectiveness of drugs. Binding studies have become an important research field in life sciences, chemistry and clinical medicine. There are some works [11–13] underway to study the interaction of the drug with protein by the fluorescence technique, Fourier transform infrared spectroscopy (FT-IR), circular dichroism (CD) spectroscopy, and molecular modelling. However, none of the investigations determines in detail the tanshinone IIA–HSA binding constants and the effect of tanshinone IIA complexation on the protein structure.

In this paper, molecular modelling and multi-spectroscopic methods were employed to demonstrate the interaction of tanshinone IIA–HSA. First, the molecular docking was performed to reveal binding tanshinone IIA to HSA through SGI FUEL workstations (theoretical model). Then, thermodynamic data of binding (including binding mechanism, binding constant) of tanshinone IIA to HSA were studied under simulative physiological conditions utilising the fluorescence method. The effect of tanshinone IIA on the structure of HSA was also examined using Fourier transform infrared spectroscopy and circular dichroism spectroscopy.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA) was purchased from Sigma Chemical Company. All HSA solutions were prepared in pH 7.40 buffer solution, and HSA stock solution was kept in the dark at $T = 277$ K. Tanshinone IIA (analytical grade) was obtained from the National Institute for Control of Pharmaceutical and

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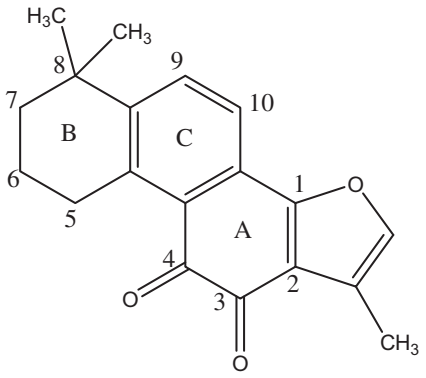

FIGURE 1. The chemical structure of tanshinone IIA.

Bioproducts, China. The stock solution ($1.0 \cdot 10^{-4}$ mol · dm^{-3}) was prepared in ethanol. NaCl (analytical grade, 1.0 mol · dm^{-3}) solution was used to maintain the ion strength at 0.1. The buffer (pH 7.40) consists of tris (0.2 mol · dm^{-3}) and HCl (0.1 mol · dm^{-3}). The pH was checked with a suitably standardized pH meter. All reagents were of analytical reagent grade and distilled water was used throughout the experiment.

2.2. Apparatus and methods

Molecular modelling was investigated through SGI FUEL WORKSTATION. The crystal structure of HSA in complex with R-Warfarin was taken from the Brookhaven Protein Data Bank (entry codes 1 h9z) [14]. The potential of the 3-D structure of HSA was assigned according to the Amber 4.0 force field with Kollman-all-atom charges. The initial structures of all the molecules were generated by molecular modelling soft-ware SYBYL 6.9 [15]. The geometries of these compounds were subsequently optimised using the Tripos force field with Gasteiger-Marsili charges. The FlexX program was applied to calculate the possible conformation of the ligands that bind to the protein.

All the fluorescence spectra were performed on a RF-5301PC Spectrofluorophotometer (Shimadzu, Japan), using 5 nm/5 nm slit widths. The excitation wavelength was 280 nm, and the emission wavelengths were red at (300 to 480) nm.

Fluorescence titration experiments: 3.0 cm^3 solution containing appropriate concentration of HSA was titrated manually by successive addition of a $1.0 \cdot 10^{-4}$ mol · dm^{-3} ethanol stock solution of tanshinone IIA (to give a final concentration of ($3.3 \cdot 10^{-7}$ to $3.0 \cdot 10^{-6}$) mol · dm^{-3}) with trace syringes, and the fluorescence intensity was measured (excitation at 280 nm and emission at 335 nm). All experiments were measured at different temperature (296, 303, and 310) K. The temperature of sample was kept by recycled water throughout the experiment.

The UV absorbance spectra were recorded using a CARY-100 UV–vis spectrometer (Varian, USA) equipped with 1.0 cm quartz cells.

FT-IR measurements were carried out at room temperature on a Nicolet Nexus 670 FT-IR spectrometer (USA) equipped with a Germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. All spectra were taken via the attenuated total reflection (ATR) method with a resolution of 4 cm^{-1} and 60 scans. The infrared spectra of HSA and tanshinone IIA–HSA complex (the molar ratio of tanshinone IIA to HSA was 2:1) were obtained in the featured region of (2200 to 1300) cm^{-1}. Corresponding absorbance contributions of buffer and free tanshinone IIA solutions were recorded and subtracted with the same instrumental parameters. The subtraction criterion was that the original spectrum of the protein solution between (2200 and 1800) cm^{-1} was featureless [16,17].

Circular dichroism was made on a Jasco-20 automatic recording spectropolarimeter (Japan), using a 2 mm cell at T = 296 K. The spectra were recorded over the range of 200 to 300 nm. The results are expressed as molar ellipticity ($[\theta]$) in deg · cm^2 · dmol^{-1}. The α-helical content of HSA was calculated from the $[\theta]$ value at 208 nm using the equation: α%helix = $\{(-[\theta]_{208} - 4000)/(33{,}000 - 4000)\} \cdot 100$ as described by Lu *et al.* [18].

3. Results and discussion

3.1. Molecular modelling study of the interaction between tanshinone IIA and HSA

The application of molecular modelling by the computer method has been employed to predict the interaction of tanshinone IIA and HSA. The crystallographic analyses of HSA have revealed that the protein, a 585 amino acid residues monomer, contains three homologous a-helical domains (I–III): I (residues 1–195), II (residues 196–383), III (residues 384–585), and each containing two subdomains (A and B) [19]. It is reported that HSA has binding sites in sub-domains IIA and IIIA, which are corresponding to site I and site II, respectively, and a single tryptophan residue (Trp-214) is in subdomain IIA [20,21]. There is a large hydrophobic cavity present in sub-domain IIA that many drugs can band at. The best energy ranked result is shown in figure 2. From figure 2, it can be seen that tanshinone IIA binds within the subdomain IIA of the protein (The Warfarin Binding Pocket), and it is important to note that the tryptophan residue of HSA (Trp-214) is in close proximity to B ring of tanshinone IIA, suggesting the existence of hydrophobic interaction between them. Further, the finding provides a good structure basis to explain the efficient fluorescence quenching of HSA emission in the presence of tanshinone IIA. On the other hand, figure 2 shows that the residues Arg-218 and Arg-222 of HSA are suitable to form intermolecular H-bond with 1-O. In addition, Lys-195 is also able to form H-bond with 3-O. The result indicated that the formation of hydrogen bonds decreased the hydrophilicity and increased the hydrophobicity to stability the (tanshinone IIA + HSA) system. Therefore the results obtained from modelling indicate

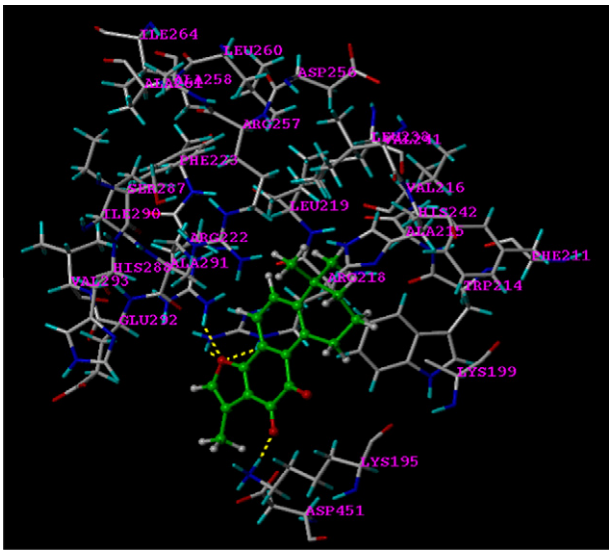

FIGURE 2. The interaction mode between tanshinone IIA and HSA. The residues of HSA are represented using line and tanshinone IIA structure is represented using ball and stick model. The hydrogen bond between tanshinone IIA and HSA is represented using dashed line.