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Molecular modeling and spectroscopic studies on the interaction of the chiral drug venlafaxine hydrochloride with bovine serum albumin

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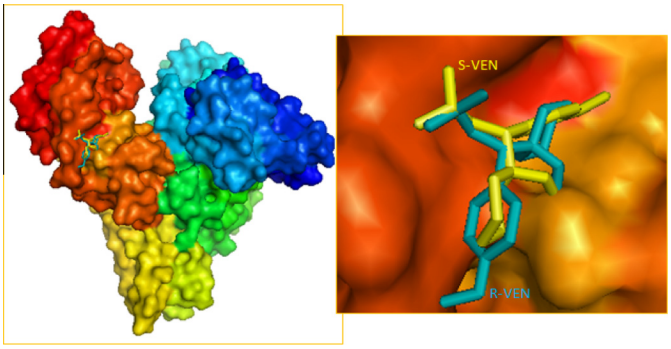
HIGHLIGHTS

- Spectroscopic techniques combination with molecular modeling were used.
- The hydrogen bonding and weak van der Waals force played a major role in the interaction.
- Through the site marker competitive experiment, VEN located in subdomain IIIA of BSA.
- Molecular docking studies showed that both S and R isomers have similar interactions with BSA.

GRAPHICAL ABSTRACT

In present work we have studied the interaction of racemic mixture of the antidepressant drug "S,R-Venlafaxine hydrochloride (VEN)" with BSA.



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ABSTRACT

This study was designed to examine the interaction of racemic antidepressant drug "S,R-venlafaxine hydrochloride (VEN)" with bovine serum albumin (BSA) under physiological conditions. The mechanism of interaction was studied by spectroscopic techniques combination with molecular modeling. Stern–Volmer analysis of fluorescence quenching data shows the presence of the static quenching mechanism. The thermodynamic parameters indicated that the hydrogen bonding and weak van der Waals interactions are the predominant intermolecular forces stabilizing the complex. The number of binding sites (n) was calculated. Through the site marker competitive experiment, VEN was confirmed to be located in subdomain IIIA of BSA. The binding distance (r = 4.93 nm) between the donor BSA and acceptor VEN was obtained according to Förster's non-radiative energy transfer theory. According to UV–vis spectra and CD data binding of VEN leaded to conformational changes of BSA. Molecular docking simulations of S and R-VEN revealed that both isomers have similar interaction and the same binding sites, from this point of view S and R isomers are equal.

Introduction

Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms. Bovine serum albumin (BSA) being the major macromolecule in blood plasma of animals accounting to about 60% of the total protein corresponding to a concentration of 42 g dm^{-3} [1]. It consists of a single chain of 582 amino acids, globular nonglycoprotein cross-linked with 17 cysteine residues (eight disulfide bonds and one free thiol). It is divided into three linearly arranged, structurally distinct and evolutionarily related domains (I–III); each domain is composed of two subdomains (A and B) [2]. BSA has two tryptophans, embedded in two different domains: Trp-134, located on the surface of domain I and Trp-213, located within the hydrophobic pocket of domain II. The binding cavities associated with subdomains IIA

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and IIIA are also referred to as site I and site II according to the terminology proposed by Sudlow et al. [3]. The most exceptional property of a serum albumin is that it serves as a depot protein and a transport protein for numerous endogenous and exogenous compounds [4]. The exogenous substances that bound to protein with a high affinity are drugs.

In this work, BSA is selected as our protein model because of its medically important, abundance, low cost, ease of purification, unusual ligand-binding properties, stability [5,6], and the results of all the studies are consistent with the fact that human and bovine serum albumins are homologous proteins [6–8].

Venlafaxine "1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl] cyclohexanol hydrochloride" commercially known as "Effexor" is a representative of a new class of antidepressants. For VEN there is one chiral center. VEN is administered as a racemate, composed of equal amounts the S-(+) and R-(−) enantiomeric forms (Fig. 1) and is usually categorized as a serotonin–nor epinephrine reuptake inhibitor (SNRI) but it has been referred to as a serotonin–norepinephrine–dopamine reuptake inhibitor. Its higher solubility in water results in burst effect with sudden peak levels of drug in blood. In humans, 90% of the total VEN decrease is accounted for by enantioselective O-desmethylation through cytochrome P450 (CYP) CYP2D6 leading to the active metabolite O-desmethylvenlafaxine (ODV) and 10% for no-enantioselective N-desmethylation by CYP3A4, leading to N-desmethylvenlafaxine (NDV) [9].

A key process in the development of new drugs is elucidation of the nature of the interaction between the drug molecule and the target protein. Since albumin serves as a transport carrier for drugs, it is important to study the interactions of these drugs with this protein. The effectiveness of these compounds as pharmaceutical agents depends on their binding ability.

Fluorescence and UV–vis absorption spectroscopy are effective techniques to study the small molecules–proteins interactions, because of their sensitivity, reproducibility and convenience. These approaches can reveal the binding affinity of small molecules with proteins and help to understand their binding mechanisms. Circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy are reliable methods for analyzing the contents of secondary conformation forms of proteins, which can explain the conformational changes of proteins induced by ligands [10].

In present work, we use several spectral methods including fluorescence, UV–vis absorption and CD spectroscopy combination with molecular docking to obtain information on the quenching mechanism, thermodynamic parameters, the special binding site and the effect of VEN on the secondary structure of BSA, which could reveal important information for the study of the structure–activity relationship of the drug and BSA.

Experimental details

Apparatus

Fluorescence measurements were performed with a JASCO spectrofluorimeter Model FP-6200 equipped with a thermostat bath, using a 1.0 cm quartz cell. UV–vis absorption spectra were measured on an Agilent UV–vis spectrophotometer Model 8453 using a 1.0 cm cell. CD measurements were recorded on a JASCO spectropolarimeter Model J-810, using a 0.1 cm quartz cell. pH measurements were carried out with a digital pH-meter with a combined glass-calomel electrode.

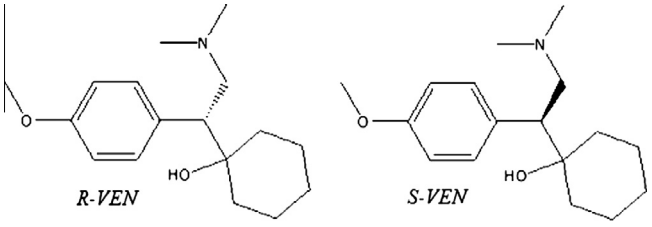

Fig. 1. Molecular structure of R and S-venlafaxine hydrochloride.

Reagents

Bovine serum albumin (BSA) was purchased from Sigma–Aldrich. All BSA solutions were prepared in the 0.1 M phosphate buffer (pH 7.4), BSA solutions stored in the dark at 4 °C. A stock solution of VEN was prepared by dissolving its crystals in 50 mM of the phosphate buffer at pH 7.4. Doubly distilled water was used to prepare the buffers.

Procedure

Fluorescence spectra

Fluorescence measurements were performed by keeping the concentration of BSA constant (5×10^{-6} M) while varying the rVEN concentration from 0.0 to 4.7×10^{-5} M at different temperatures (278, 288, 310).

Displacement experiments

The displacement experiments were performed using the site probes including ibuprofen and warfarin by keeping the concentration of protein and probe constant (each of 5×10^{-6} M). The fluorescence quenching titration was used to determine the binding constants of VEN–BSA systems in presence of above site probes for sites I and II.

UV–vis absorption studies

The absorbance measurements were performed by keeping the BSA concentration constant (1×10^{-5} M) while varying the rac-VEN concentration from 0 to 1.9×10^{-5} M (ri = [VEN]/[BSA] = 0.0, 0.7, 1.1 and 1.9). The samples were incubated at room temperature for 10 min and the spectra were recorded in the range of 200–400 nm.

Circular dichroism (CD) measurements

Circular dichroism (CD) measurements were conducted by keeping the concentration of BSA constant (3×10^{-6} M) while varying the VEN concentration from 0 to 1.8×10^{-6} M (ri = [VEN]/[BSA] = 0.0, 0.4, 0.5 and 0.6).

Molecular docking

MGL tools 1.5.4 with AutoGrid 4 and AutoDock 4 were used to set up and perform blind docking calculations between R and S-VEN with BSA. The structure of free BSA (PDB id: 3V03, chain A) obtained by X-ray crystallography was used as a template. Receptor (BSA) and ligands (R and S-VEN) files were prepared using AutoDock Tools. The BSA was enclosed in a box with number of grid points in $x \times y \times z$ directions, $126 \times 126 \times 126$ and a grid spacing of 0.703 Å. Lamarckian genetic algorithms, as implemented in AutoDock, were employed to perform docking calculations. All other parameters were default settings. For each of the docking cases, the lowest energy docked conformation, according to the Autodock scoring function, was selected as the binding mode. The output from AutoDock was rendered with PyMol.

In AutoDock, the overall docking energy of a given ligand molecule in its active site is expressed as follows:

$$\Delta G = \Delta G_{\mathrm{vdw}} \sum_{ij} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) + \Delta G_{\mathrm{hbond}} \sum_{ij} \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} + E_{\mathrm{hbond}} \right) + \Delta G_{\mathrm{elec}} \sum_{ij} \frac{q_i - q_j}{\varepsilon(r_{ij}) r_{ij}} + \Delta G_{\mathrm{tor}} N_{\mathrm{tor}} + \Delta G_{\mathrm{sol}} \sum_{ij} S_i V_j e^{(-r_{ij}^2/2\sigma^2)}$$