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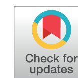
Research paper

Unravelling the binding mechanism of benproperine with human serum albumin: A docking, fluorometric, and thermodynamic approach



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ABSTRACT

The interaction between benproperine (BEN) and human serum albumin (HSA) has been simulatively and experimentally investigated based on docking, fluorometric, thermodynamic, and spectroscopic approach. The blind Autodock docking study first recognized the hydrophobic cavity of HSA at Domain IB as the probable binding site for BEN. BEN bound to HSA via a static quenching mechanism, resulting in the formation of BEN-HSA complex confirmed by fluorescence quenching and time-resolved fluorescence. Fluorescence titration and isothermal titration calorimetry (ITC) revealed that the binding mode between BEN and HSA owning moderate affinity (binding constant at 10^4 magnitude) was mainly driven by electrostatic attraction and hydrophobic interaction. Circular dichroism (CD) spectra suggested upon Addition of BEN induced the conformational changes on HSA with α-helix decreasing. Due to the conformational rearrangements, BEN-HSA complex was stabilized by several non-covalent bonds. This work first clarified the progress in binding mechanism of benproperine with human serum albumin and then provided fresh insights into this drug transportation and metabolism.

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1. Introduction

Benproperine (BEN, 1-[1-methyl-2-[2-(phenylmethyl)phenoxy] ethyl]-piperidine) has been widely used as a central and peripheral nonnarcotic antitussive agent. Originally, BEN was first synthesized by the Pharmacia Research Laboratory and its antitussive activity has been found approximately superior to that of codeine while devoid of undesirable side-effects [1–3]. As a cough suppressant, BEN is allowed to be taken orally in usual doses of 25–50 mg two to four times daily in forms of embonate or phosphate [4]. After oral administration and gastrointestinal absorption, BEN enters the bloodstream. The therapeutic efficacy of BEN is directly related to its free concentration in blood plasma; thus, the binding ability towards plasma proteins is one of the critical pharmacokinetic parameters during its transport and metabolism process [5]. However, to the best of our knowledge, the mechanism by benproperine binds to plasma proteins appears to be primarily through the formation of complexes and that has not been studied up to now.

Among these proteins, human serum albumin (HSA) is a principal extracellular protein (50%–60% of total) in human blood plasma and helps maintain the osmotic pressure [6]. HSA exhibits dualism because of the hydrophobic and hydrophilic properties of its amino acids which causes HSA to interact with amphiphilic molecules [7]. HSA can distinguish to a variety of endogenous and exogenous compounds and binds to them with moderate to high association constant (10^4–10^6 L mol^{-1}), so that it regulates the transportation of various drugs [5,8]. Furthermore, HSA is capable of binding reversibly to various drugs to increase the solubility, decrease toxicity [9], and protect the bound ligands against oxidation in plasma [10]. Given its exceptional abilities, HSA is commonly chosen as target in drug-protein interaction for understanding the pharmacokinetics and pharmacological effects of drugs.

The present work is a comprehensive in vitro study on the interaction of benproperine with human serum albumin by establishing a docking, fluorometric, and thermodynamic approach based on Autodock strategy, fluorescence titration, time-resolved fluorescence, isothermal titration calorimetry, and circular dichroism. The binding properties such as quenching mechanism, binding parameters, acting forces, binding mode, and conformational

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changes were discussed. These techniques are complementary, and their findings were consistent with one another. Study of the binding nature benproperine with human serum albumin is of interest to provide support for the continued clinical investigation of the drug.

2. Results and discussion

2.1. Molecular docking of BEN binding to HSA

Molecular docking studies based on AutoDock program package provide insight into the potential interactions between small molecules and biomacromolecule, which may be goal-oriented the experimental results. To visualize the binding of BEN on HSA theoretically, the AutoDock strategy was used to search entire HSA and simultaneously optimize the conformations of the peptides for locating all possible binding sites to hold BEN [11,12]. In the present study, a total of 15 multimember conformational clusters were gathered from 300 docking runs. As seen in Fig. 1a, the highest populated cluster containing the maximum conformations (36%) of docking procedure was located at the lowest energy scale. 91% binding modes (expect cluster No. 9 and 11 from energy low to high) of BEN were settled down on Domain IB [13] of HSA. The optimum pose in front of the first class with lowest c (-7.69 kcal mol^{-1}) was chosen as the most authentic binding orientation.

As shown in Fig. 1a–b, BEN inserted into a hydrophobic cavity of HSA at Domain IB and mainly surrounded by the residues Tyr 138, Tyr 161, Met 123, Leu 115, Pro 118, Arg 117, Val 116, Leu 182, Ile 142, Leu 139, Leu 154, Phe 157, Ala 158, Leu 135, Phe 134, and Phe 165 that contributed to hydrophobicity of BEN to HSA in different degree. The docking results of the BEN-HSA system provided the electrostatic energy was -0.04 kcal mol^{-1} and had potential π-π stacking interactions between aromatic ring closing to oxygen atom of BEN and Tyr 161 and Tyr 138. Furthermore, the residues of HSA active amino acids with rose pink involved in alkylation and the rest of residues were in favor of van der Waals forces for the interaction process. The location of BEN on HSA was characterized by varying forces which played significant roles in stabilizing BEN-HSA complex. These observations of docking simulations promote visual understanding and may provide structural basis for developing bonding mechanism (such as fluorescence quenching and thermodynamic parameter changes) of BEN to HSA.

2.2. Fluorescence quenching mechanism

Fluorescence spectroscopy was chosen to study the affinity of BEN to HSA owning to its exceptional sensitivity, convenience, and abundance of theoretical foundation. When HSA interacts with BEN, its intrinsic fluorescence intensity, which is mainly contributed by tryptophan, tyrosine, and phenylalanine [14], may be decreased by various molecular interactions. As displayed in Fig. 2a, HSA fluorescence emission spectra with and without BEN generated an obvious band absorption peak at 337 nm, which was gradually quenched with increasing BEN concentrations, whereas, BEN alone did not emit fluorescence at the highest concentration from 300 to 450 nm. This fluorescence quenching could be attributed to excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collision frequently categorized into dynamic or static quenching [15–17]. To explore the quenching mechanism of BEN to HSA qualitatively, the fluorescence data at different temperatures were analyzed using the Stern-Volmer equation [18,19]:

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of a quencher, respectively; $[Q]$ is the quencher concentration; K_{SV} is the Stern-Volmer quenching constant can be determined using the linear regression of the plot of F_0/F against $[Q]$. Fig. 2a(inset) shows good linearity of the F_0/F plots for HSA versus BEN concentrations. The obtained quenching constant K_{SV} values were $(2.74 \pm 0.028) \times 10^4$ L mol^{-1} (25 °C, $R = 0.9974$) and $(1.99 \pm 0.024) \times 10^4$ L mol^{-1} (37 °C, $R = 0.9987$). In present system, K_{SV} values decreased with increasing temperature, which indicated that the quenching of HSA by BEN was initiated by complex formation via the static quenching process rather than dynamic collision based on principles of fluorescence spectroscopy [20].

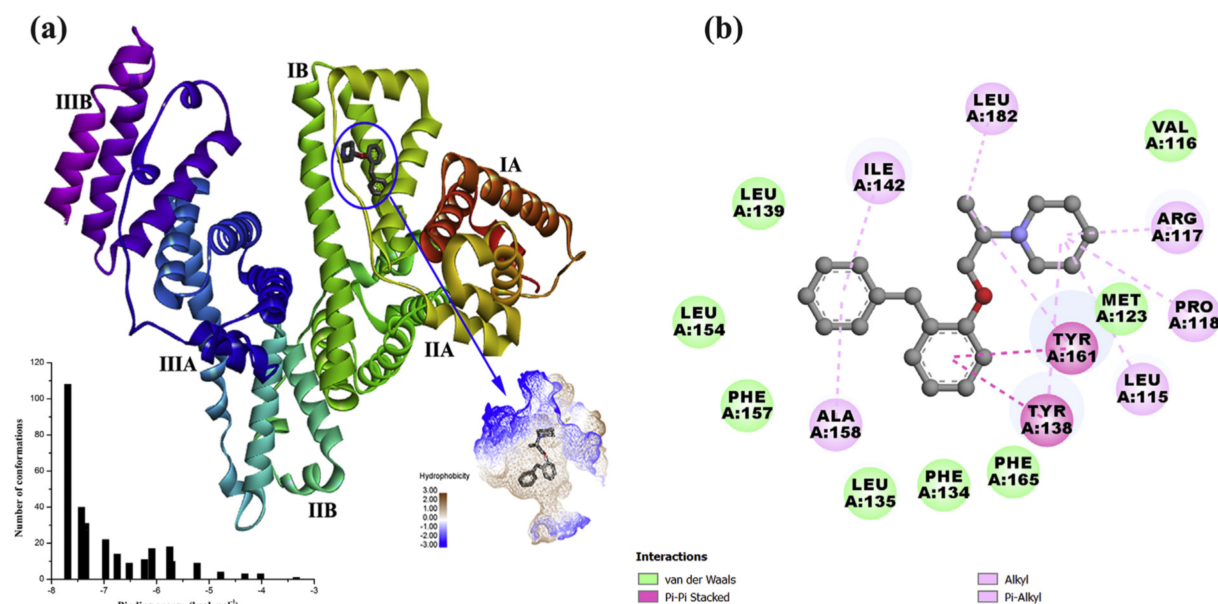

Fig. 1. 3D portrait (a) and schematic diagram (b) of optimal BEN-HSA conformation generated by AutoDock Version 4.2.5.1. The pictures in left bottom and right bottom in (a) shows the cluster analyses and the hydrophobic cavity of BEN-HSA system.

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