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Molecular Interaction of Benzalkonium Ibuprofenate and its Discrete Ingredients with Human Serum Albumin

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Studying the interaction of pharmaceutical ionic liquids with human serum albumin (HSA) can help investigating whether or not ionic liquid formation can enhance pharmacological profile of the discrete ingredients. In this respect, in the present work, the interactions of Benzalkonium Ibuprofenate, as a well-known active pharmaceutical ionic liquid, Benzalkonium Chloride, and also Sodium Ibuprofenate with HSA were studied by molecular dynamics (MD) and docking simulations. First, molecular dynamics simulation using the GROMACS 4.5.0 package was employed to obtain the equilibrium HSA structure at pressure 1 bar and temperature of 310 K. Then, molecular docking approach by AutoDock Vina using a genetic algorithm was employed to find the binding sites of the three ligands on HSA. It was revealed that the three ligands can bind to the same residues at Sudlow site II. It was also found out that steric and electrostatic interactions play major roles in the interaction of aforementioned three ligands with HSA but the contribution of these interactions in HSA binding is altered by ionic liquid formulation.

Keywords: Ionic liquid, Human serum albumin, Benzalkonium Ibuprofenate, Molecular dynamics simulation, Docking

INTRODUCTION

Ionic liquids (ILs) are salts which melt below 100 ºC and their molten forms are solely constituted of ions. Some ionic liquids are unique in being task-specific and possessing a potential spectrum of utilities [1]. As an example, a modular, IL-based strategy allows compartmentalized molecular level design of a wide range of new materials with tunable biological, as well as the known physical and chemical properties of ILs. Such materials can be considered as 'tunable' active pharmaceutical ingredients (APIs) with novel performance enhancement and delivery options [2-3]. IL strategies can take advantage of the dual nature (discrete ions) of ILs to realize enhancements which may include controlled solubility, bioavailability or bioactivity, stability, elimination of polymorphism, new delivery options (*e.g.*, slow release or the IL-API as 'solvent'), or even customized pharmaceutical cocktails [4]. [Benzalkonium][Ibuprofenate] (Fig. 1) is one example of these APIs, consisting of both anti-bacterial Benzalkonium Chloride and anti-inflammatory Sodium Ibuprofenate as its cation and anion, respectively. The resulting yellow gel, with melting point of -41 ºC has exhibited dual therapeutic functions [5].

A potent protein carrier for a broad range of drugs is human serum albumin (HSA), the most prominent protein in plasma, which has also the capability to bind and transport a wide spectrum of compounds such as non-esterified fatty acids, heme, bilirubin, thyroxine, and bile acids [6-8]. As a drug carrier, HSA may aid in the selective delivery of drugs to the target, facilitating the process of drug access into the cell or causing a reduction in the amount of drug available for the receptor, by its rapid removal from the circulation [9]. Therefore, strong affinity/binding toward HSA can decrease the concentrations of free drugs in plasma and improve the pharmacodynamics and pharmacokinetic properties for the drugs, whereas weak binding can lead to a short lifetime or poor distribution [10].

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Fig. 1. Chemical structure of [Benzalkonium][Ibuprofenate] composed of Benzalkonium Chloride (n = 5) and Sodium Ibuprofenate.

In this study, the interaction of Benzalkonium Chloride, Sodium Ibuprofenate and their corresponding API, Benzalkonium Ibuprofenate, with HSA was studied, using molecular dynamics (MD) simulation and automated molecular docking approaches. In this regards, in the first step, in order to obtain the equilibrium structure of HSA the crystal structure of HSA in a water box was subjected to MD simulation. In the second step, molecular docking simulation was performed to find binding sites of ligands with HSA.

Methods

Molecular dynamic simulation. MD simulations on HSA structure, retrieved from chain A of 2BXD entry code of protein data bank, were performed using the GROMACS 4.5.0 package [11-13] with GROMOS96 43a1 force field [14]. The protein was immersed in a box of 65264 extended simple point charge (E/SPC) water molecules [15] and was neutralized by adding 14 Na^+ counter ions. The energy of this system was minimized using 60 ps of the steepest descent method [16]. Then, 20 ps position restraining simulations were performed to relieve close contacts before the actual simulations. Finally, 10 ns MD simulations were carried out at the NPT canonical ensemble [17] under the periodic boundary conditions, in all three dimensions, by applying the leap-frog algorithm [18] with a time step of 2 fs.

HSA, water molecules and the ions were served by Berendsen thermostat [19] at 310 K and pressure of 1 bar. The particle mesh Ewald (PME) method [20-21] for long-range electrostatics, a 7 Å cut off for van der Walls interactions and Coulomb interactions with updates every 10 steps, and the Lincs [22] algorithm for covalent bond constraints were used. Also, the initial atomic velocities were generated within a Maxwellian distribution [23-24] at the given absolute temperature. Then, the docking simulations were applied to the final structures of studied systems obtained from the molecular dynamic simulations.

Molecular docking simulation. The structures of Benzalkonium Chloride, Sodium Ibuprofenate and Benzalkonium Ibuprofenate were generated by HyperChem Professional 7.0 [25]. Then, their configurations were imposed to semi-empirical method AM1 with Polak-Ribiere algorithm to maintain their energy minimized structures. Prior to docking, the three ligands were optimized with Gasteiger-Hückel charges [26] by the package of MGL Tools [27]. The docking studies were performed by AutoDock Vina [28] using a genetic algorithm [29]. The receptor was taken from MD simulation and was in its most stable conformational state. AutoDock Vina has reported high accuracy in predicting binding free energies by setting the receptor rigid while appraising flexible ligands, with a comparatively low standard error [28]. Therefore, HSA conformational flexibility was neglected by rigid receptor docking.

RESULTS AND DISCUSSION

Molecular Dynamic Simulation

To examine the HSA conformational variations, the root mean square deviation (RMSD) was calculated, at 310 K, with respect to its initial structure. Figure 2 displays the RMSD evolution of HSA backbone in a water box as a function of time. The obtained mean RMSD value is about 5.3 Å for HSA and becomes relatively stable after 6 ns. In conclusion, the RMSD profile characterizes an equilibrium state for the protein. Consequently, the obtained final structure can be efficiently used in docking simulations to represent the corresponding physiological structure of HSA at the fixed temperature.

Molecular Docking Simulation

The outcomes of a standard docking simulation consist of: a) docking scores which are estimates on the strength of the protein-ligand interaction, and b) the binding sites of the ligand on the protein target. The binding sites and roles of each binding residue will be discussed.

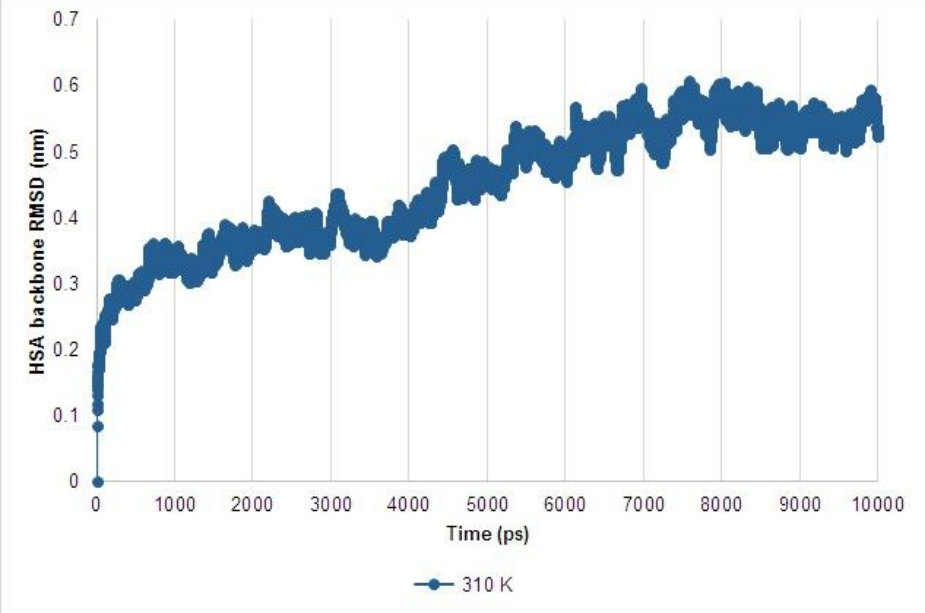

Fig. 2. RMSD evolution of HSA backbone at 310 K.

Binding sites. Although it is proved that HSA has several binding sites for each drug [30-32], the three studied ligands prefer to bind to just one site of HSA. Regardless of their net charge and overall structures, they tend to bind to similar binding site which is located at the space between domains I and III (see Figs. 3 and 4, the residue numbers should be added by one in all the figures since the starting pdb structure did not contain the first HSA residue). This site is known as Sudlow site II, which is the common binding site for many small aromatic drugs.

Benzalkonium chloride. Arg 145, Arg 186, Asp 108, 183 and 187, Glu 141, 153, 188, 425 and 520, Gly 189, His 146, Ile 142, Leu 154 and 185, Lys 190, Phe 149 and 157, Ser 193, and Tyr 161 stabilize the interaction of Benzalkonium Chloride with its binding site on HSA but Arg 144 and 160, and Lys 195, 199, 432, 456 and 519 destabilize this interaction. The obtained results suggest that there is electrostatic repulsion between positively charged Arg, Lys and Glu residues, with major contribution of Arg 186, Asp 108 and Glu 141, and positive nitrogen atom of Benzalkonium Chloride. The detailed binding analysis indicates the steric interaction with absolutely negligible

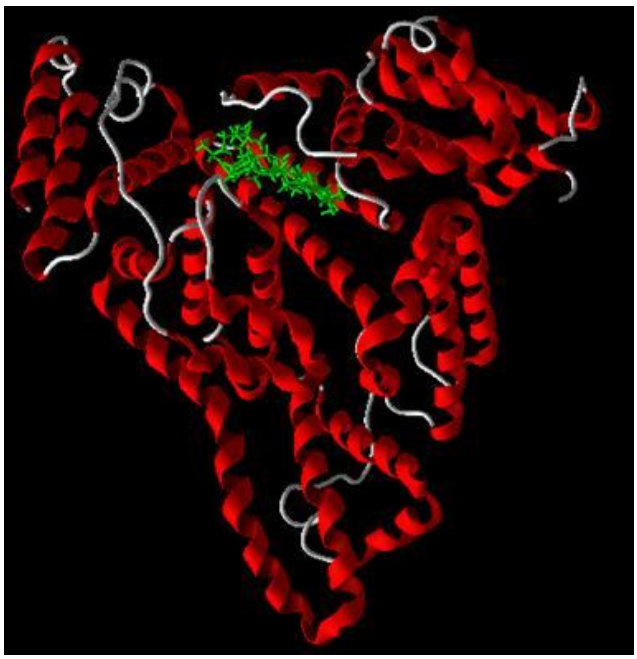

Fig. 3. Binding mode of Benzalkonium Chloride, Sodium Ibuprofenate and Benzalkonium Ibuprofenate on HSA.

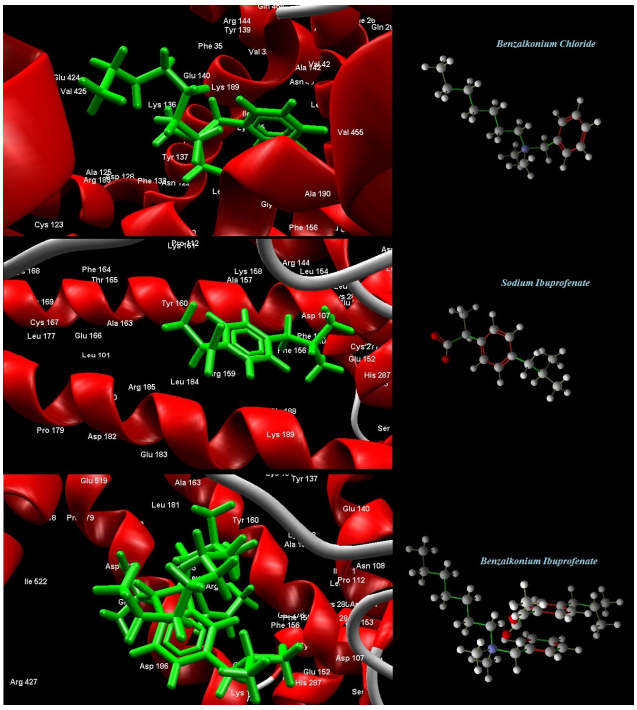

Fig. 4. Benzalkonium Chloride, Sodium Ibuprofenate and Benzalkonium Ibuprofenate in close contact with HSA residues, in addition to their structures prior to docking.

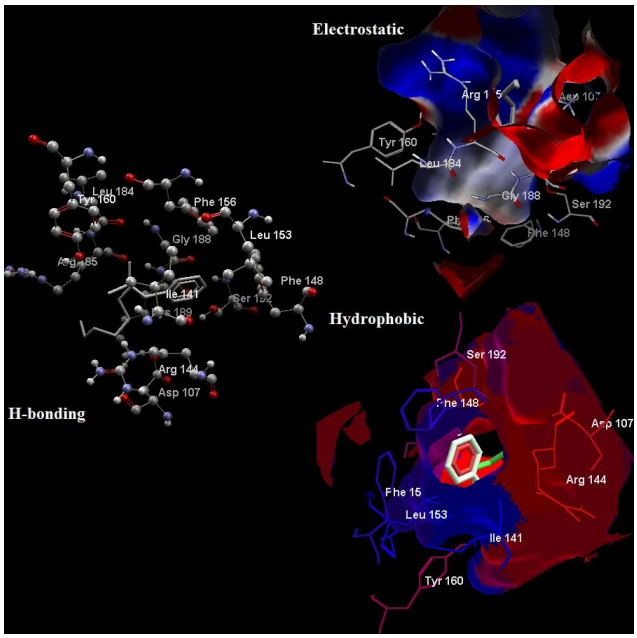

Fig. 5. H-bonding, Electrostatic and Hydrophobic views of Benzalkonium Chloride in HSA binding site. The hydrogen bonding contacts are shown by dashed blue lines. Green and orange colors in hydrophobic views correspond to hydrophilic and hydrophobic nature, respectively.

electrostatic repulsion, while no hydrogen bonding is observed. Figure 5 shows the H-bonding, Electrostatic and Hydrophobic views of Benzalkonium Chloride in HSA binding site.

Sodium ibuprofenate. Ala 143, Arg 144, 145, 186 and 197, Asp 108, Gly 189, His 146, Ile 142, Leu 154, Lys 106, 137, 190, 195, 432, 436, 519 and 524, Phe 149 and 157, Ser 192 and 193, and Tyr 161 let Sodium Ibuprofenate to relax in its binding site on HSA. Electrostatic repulsion between the negative charge on carboxylic acid oxygen atoms and negatively charged Asp residues 107, 183 and 187, and Glu residues 141, 425 and 520 are observed. Again, steric factor seems to be the principal stabilizing factor. Poor electrostatic interactions and non-significant hydrogen bonding (between carboxylic group of Ibuprofenate, Arg 145 and Lys 190 N-terminal moieties) were also detected. These non-steric interactions are a direct consequence of bearing the carboxylic group of Ibuprofenate structure. Figure 6 illustrates the H-bonding, Electrostatic and Hydrophobic views of Sodium Ibuprofenate in HSA binding site.

Benzalkonium ibuprofenate. Here, Arg 114, 145, 186 and 428, Asn 109, Asp 107 and 108, Glu 425 and 520, Ile 115, Lys 190, 432, 519 and 524, Pro 113 and 421, Thr 527, and Val 116 all stabilize its interaction with HSA by steric and electrostatic binding forces. The electrostatic forces are apparently arisen from the ionic nature of the ligand and noticeable presence of Arg 186, Asp 183 and Glu 520. As in Sodium Ibuprofenate, hydrogen bonding (between the carboxylic group and Arg 186 N-terminal moiety) plays a poor role in its interaction (see Fig. 7).

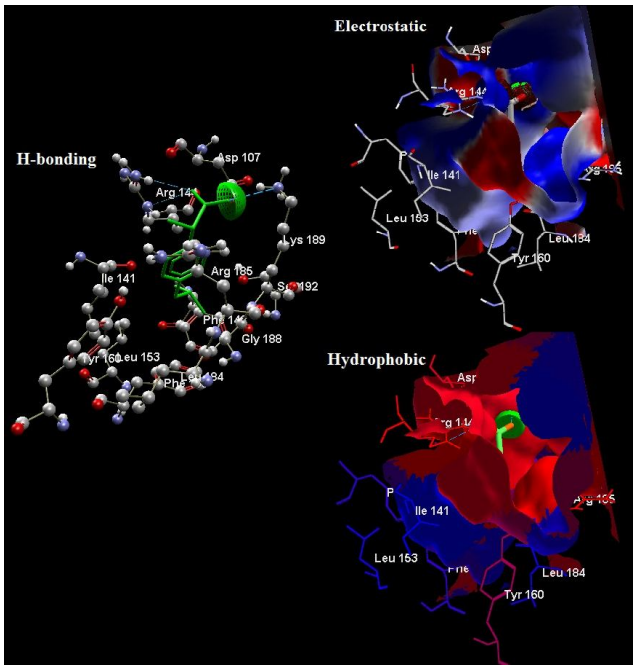

Fig. 6. H-bonding, Electrostatic and Hydrophobic views of Sodium Ibuprofenate in HSA binding site.

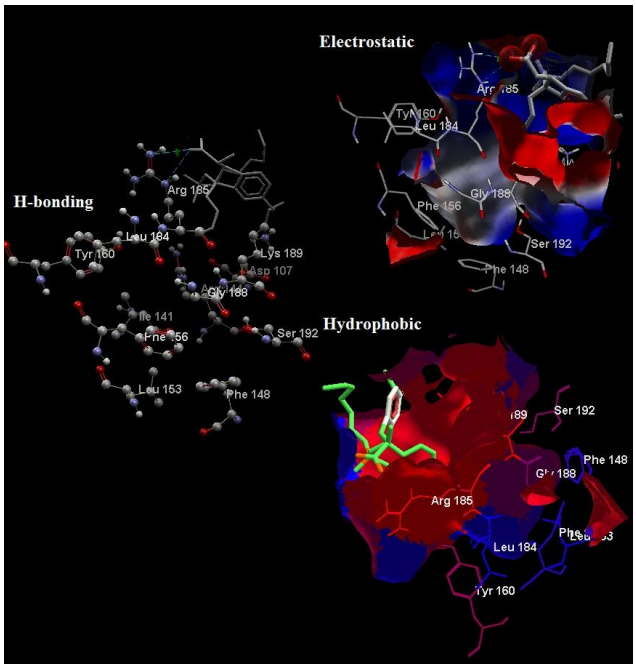

Fig. 7. H-bonding, Electrostatic and Hydrophobic views of Benzalkonium Ibuprofenate in HSA binding site.

CONCLUSIONS

In this study, the interaction of Benzalkonium Chloride, Sodium Ibuprofenate and their ionic liquid form, Benzalkonium Ibuprofenate, with human serum albumin was evaluated by molecular dynamics and docking simulations. Our detailed docking results showed that the three ligands can bind to the same residues, between domains I and III (*i.e.* Sudlow site II). However, the contributions of electrostatic, hydrogen bonding and steric interactions in binding of the ligands to HSA are altered by ionic liquid formulation.

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