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The interaction of a new Schiff base ligand with human serum albumin: molecular docking and molecular dynamics simulation studies

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Abstract

The interaction of a new heterocyclic Schiff base bearing pyridine and pyrimidine cycles, with human serum albumin (HSA) using molecular docking and molecular dynamics simulation methods was examined. Molecular docking studies showed that the ligand was bonded to the IB domain of the protein. It was found that there was one hydrogen bond interaction between HSA and the ligand. The standard Gibbs free energy for binding of the ligand to HSA was calculated as $-9.63 \text{ kcal.mol}^{-1}$. The results of the molecular dynamics simulation showed that the root mean square deviation (RMSD) of the non-liganded HSA and the HSA–ligand complex reached equilibration after 1000 ps. The study of the radius of gyration revealed that there was a

conformational change when the HSA–ligand complex was formed. Finally, analyzing the RMS fluctuations (RMSF) suggested that the structure of the ligand binding site remained approximately rigid during the simulation.

Keywords

Human serum albumin, molecular docking, molecular dynamics simulation, Schiff base ligand.

1. Introduction

Schiff bases fascinate a large number of chemists and biochemists due to their easy formation, high stability, their pharmacological properties and, notably, their anticancer activity [1-4]. Most Schiff base ligands show biological activities, such as antibacterial [5], anticancer [6, 7], anti-inflammatory [8], antifungal [9], antitumor [10], antiparasitic [11], antiviral [12] and anti-HIV [13].

The knowledge about interaction mechanisms of a drug with plasma proteins as a biomacromolecule is required to understand the drug's pharmacodynamics and pharmacokinetics [14]. Due to the function of plasma proteins as drug carriers, there are a large number of literatures about their binding to drugs [15, 16]. The interaction of plasma proteins with a drug has effects on the drug absorption, distribution and elimination in the circulatory system [17], and can prevent their rapid elimination from the blood stream [18]. Human serum albumin (HSA) is the most abundant plasma protein; it also is known as the dominant transporter plasma protein for endogenous and exogenous ligands (fatty acids, hormones and some drugs) [19]. The accommodation of a wide range of lipophilic molecules in HSA is possible as a result of its flexible structure [20]. The HSA-binding of a drug can increase its solubility in plasma, decrease its toxicity, protect it from oxidation, prolong its *in vivo* half-life as well as increase the pharmaceutical effect of the drug [21].

Recently, Ozedmir et. al. synthesized a new, heterocyclic Schiff base bearing pyridine and pyrimidine cycles (Figure 1) [22]. Aware of the therapeutic activity of Schiff

bases and in an attempt to obtain more insight into their biological activity, the HSA-binding of the mentioned compound has been investigated. The computational molecular docking method was used to evaluate the binding behaviour of the Schiff base ligand to HSA, quantitatively. In addition, to find out the behaviour of the Schiff base-HSA complex, a powerful computational method, molecular dynamics (MD) simulation has been used.

2. Methods

2.1. Molecular docking procedure

In this work, a docking study was carried out to indicate the HSA-binding site for the Schiff base compound. The 3D structures of the compound were generated using GaussView 5.0 software [23] and its geometry was optimized using Gaussian 03 [24] software by the B3LYP (Becke, three-parameter, Lee-Yang-Parr) method [25] at the level of 6-31g** [26]. The crystal structure of HSA (PDB ID: 1AO6) was taken from the Brookhaven Protein Data Bank (<http://www.rcsb.org/pdb>). The R-value and resolution of this file were 0.218 and 0.25 Å, respectively. It was prepared in pH=7.5. Water molecules of the protein pdb file were removed and missing hydrogen atoms and Gasteiger charges were added. Flexible-ligand docking was performed by AutoDock 4.2 molecular-docking program (<http://autodock.scripps.edu>) using the implemented empirical free energy function and the Lamarckian Genetic Algorithm [34]. The Gasteiger charges were added to prepare the macromolecule input file for docking and the Auto Grid was used to calculate the grids. A blind docking with 126 lattice points along the X, Y, and Z axes was performed to find the active site of the

ligands with the HSA. After determination of the active site, the dimensions of the grid map were selected as 60 points on a side with a grid point spacing of 0.375 Å, to allow the ligand to rotate freely. 200 docking runs with 25,000,000 energy evaluations for each run were performed.

2.2. *Molecular Dynamics Simulation*

The lowest binding free energy conformation of the complex was considered as the initial conformation for the MD studies. All MD studies were carried out using the GROMACS 4.5.6 (University of Groningen, Netherlands) package [27,28] and the GROMOS96 43a1 force field [29,30]. The Dundee PRODRG2.5 server was used to generate the topology parameters of the ligands [31]. The partial atomic charges of the ligand were calculated using Gaussian 03 [24] at the level of B3LYP/6-31G** [25,26]. The complex was located in a cubic box with periodic boundary conditions. The box volume was $11.36381 \times 11.36381 \times 11.36381 \text{ nm}^3$ and the minimum distance between the protein surface and the box was 1.0 nm. The box contents, filled with extended simple point charges (SPC), water molecules [32], and the solvated systems, were neutralized by adding 15 sodium ions (Na^+). Energy minimization was done through using the steepest descent method. Then, the system was equilibrated for 100 ps at the temperature of 300 K. Finally, a 20 ns MD simulation was carried out at 1 bar and 300 K. A Berendsen thermostat [33] at 300 K, the particle mesh Ewald (PME) method [34,35] for long range electrostatics, and a 9 Å cut off for van der Waals interactions and Coulomb interactions were used. The equation of motions was integrated by the leap-frog algorithm with 2 fs time steps. The atomic coordinates

were recorded to the trajectory file every 0.5 ps for later analysis. Finally, an all-bond constraint was used to keep the ligand from drifting in the MD.

3. Results and Discussion

3.1. Molecular docking study

HSA is the most abundant protein in human blood plasma and consists of 585 amino acids; it has three α -helical domains (I, II and III), each containing two subdomains (A and B) [36]. HSA transports drugs, hormones, fatty acids, and other compounds, and maintains osmotic pressure, among other functions [37,38]. Previous crystal structure studies have shown that most ligands are bound to binding site in subdomains IIA or IIIA [39–43]. Also, the D-shaped cavity in subdomain IB is the binding site of some compounds [44–46]. In this work, the selected Schiff base ligand was docked to the crystal structure of HSA. The obtained results from molecular docking showed that the ligand binds to the IB subdomain of HSA. The standard Gibbs free energy for binding of the ligand to HSA was $-9.63 \text{ kcal.mol}^{-1}$. The results indicated that the Arg (117) amino acid residue is involved in the hydrogen bond interaction in the HSA-ligand system. The results of the docking are presented in Fig. 2. The other amino acid residues shown in Fig. 2 (lower right) are involved in hydrophobic interactions with the Schiff base ligand. Thus it can be concluded that the interaction of ligand with HSA is not mainly hydrophobic and that the carbonyl groups in the structure of the ligand have a significant role in the binding process.

3.2. Analysis of MD trajectories

The beginning structure for the MD analyses was selected from the conformation with lowest docking energies. The trajectories were analyzed in terms of root mean square deviation (RMSD), radius of gyration (Rg), and root mean square fluctuation (RMSF) using the GROMACS routines.

3.2.1. Root mean square deviation of the trajectory of the HSA backbone

The stabilities of the trajectories for HSA and the HSA-ligand were examined using the RMSD of the backbone of HSA (Figure 3). A glance at the analysis in Fig. 3 shows that the RMSD of the two systems reached equilibrium and fluctuated around 0.027 and 0.017 nm for HSA and HSA-ligand, respectively at about 300 and 1000 ps, respectively. This means that both systems were equilibrated well and the RMSDs had only low fluctuations around their mean values.

3.2.2. Radius of gyration

The Rg for both systems were also determined and plotted against simulation time to study the protein compactness (Figure 4) showed that both systems stabilized after about 500 and 4000 ps for HSA and HSA-ligand, respectively. It can be clearly seen that the Rg was larger upon binding of the ligand, suggesting a more compact structure before the binding of Schiff base ligand to HSA. This indicates that the environment of the HSA changed during its interaction with the Schiff base ligand.

3.2.3. Root mean square fluctuation

The mobility of the HSA residues was evaluated by analysis of the RMSF of the C atoms of HSA in the presence of the Schiff base ligand (figure is not shown). The profiles of the atomic fluctuations were found to be very similar for HSA and the

HSA–ligand complex. The obtained results clearly indicate that the residues at the binding site had only small fluctuations in their interactions with the ligand. Also, the structure of the ligand binding site remained approximately rigid during simulation. In addition, the RMSFs of the atomic positions of the Schiff base ligand were calculated to examine its conformational variations (Figure 5). The results indicated that the ligand atoms showed limited fluctuations (<0.30 nm). Hence, it can be concluded that the interactions of HSA and the Schiff base ligand were stable during the simulation time.

4. Conclusions

Interaction of a new heterocyclic Schiff base bearing pyridine and pyrimidine cycles with HSA was examined since HSA plays a major role in transporting ligands to target places. The molecular docking studies indicated that the Schiff base ligand binds to the IB domain of HSA with one hydrogen bonding interaction. Therefore, it can be concluded that the interaction of this Schiff base ligand with HSA is not mainly hydrophobic. The standard Gibbs free energy for binding of ligand to the HSA was calculated to be -9.63 kcal.mol⁻¹. The MD study, in particular, made an important contribution toward understanding the effect of the binding of this ligand on the conformational changes of HSA and the stability of HSA–ligand complex system in aqueous solution. The MD simulation studies revealed that the RMSD of the HSA and HSA-ligand systems reached equilibrium and oscillated around the average value after 300 and 1000 ps simulation time, respectively. Analysis of R_g indicated that the ligand changed the conformation of the HSA during MD simulation. The similarity of

profiles of the atomic fluctuations of HSA and the HSA–ligand complex suggested that the structure of the ligand-binding site remained approximately rigid during the simulation.

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Figure 1. Optimized chemical structure of the Schiff base ligand containing (1) pyridine, (2) pyrimidine and (3) phenyl rings.

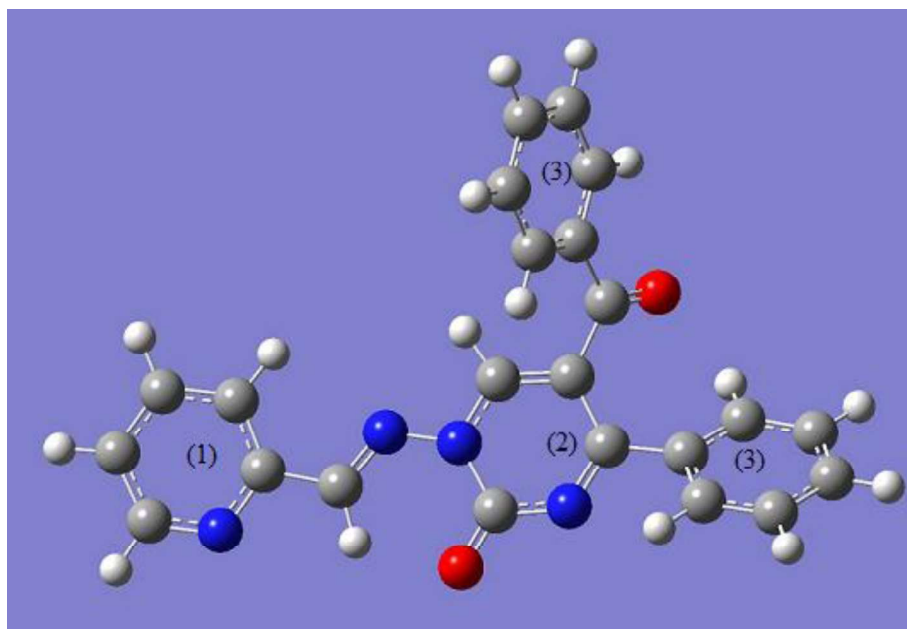


Figure 1.

Figure 2. The docking pose of the HSA-ligand complex.

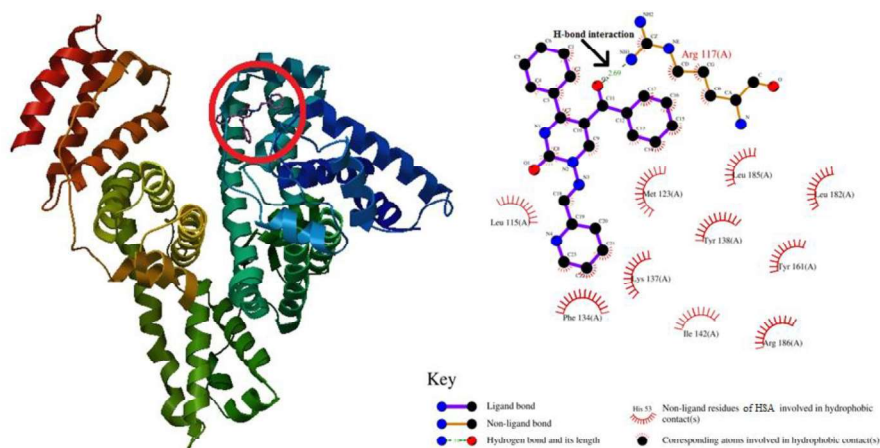


Figure 2.

Figure 3. Time dependence of RMSD. RMSD values of the backbones for non-liganded HSA and HSA–ligand complex during 20,000-ps MD simulation using the GROMACS package and the GROMOS96 43a1 force field at 300 K and 1 bar.

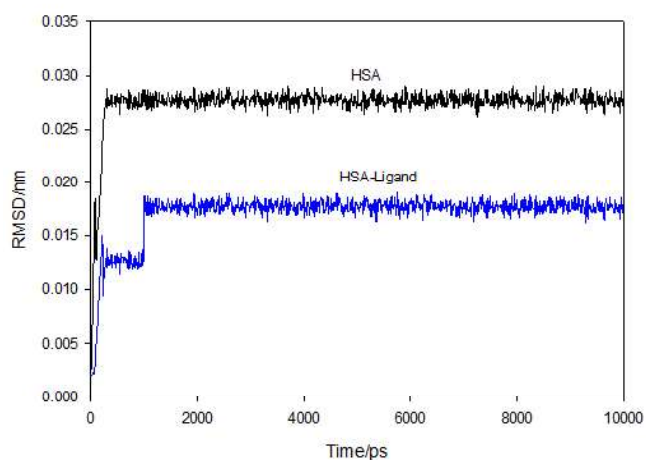


Figure 3.

Figure 4. Time dependence of Rg. Rg values for non-liganded HSA and HSA–ligand complex during 20,000-ps MD simulation using GROMACS package and the GROMOS96 43a1 force field at 300 K and 1 bar.

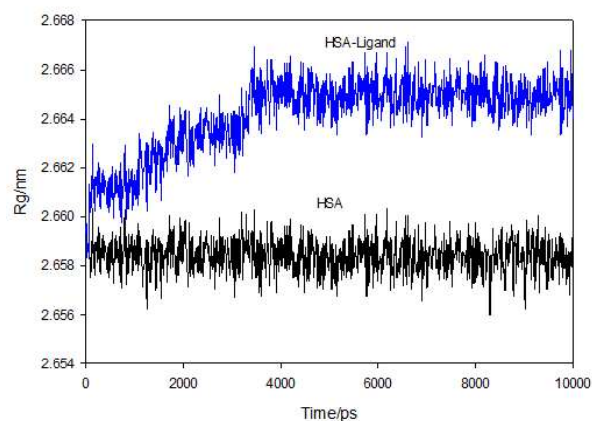


Figure 4.

Figure 5. Atomic fluctuations for the Schiff base ligand during 20,000-ps MD simulation using GROMACS package and the GROMOS96 43a1 force field at 300 K and 1 bar.

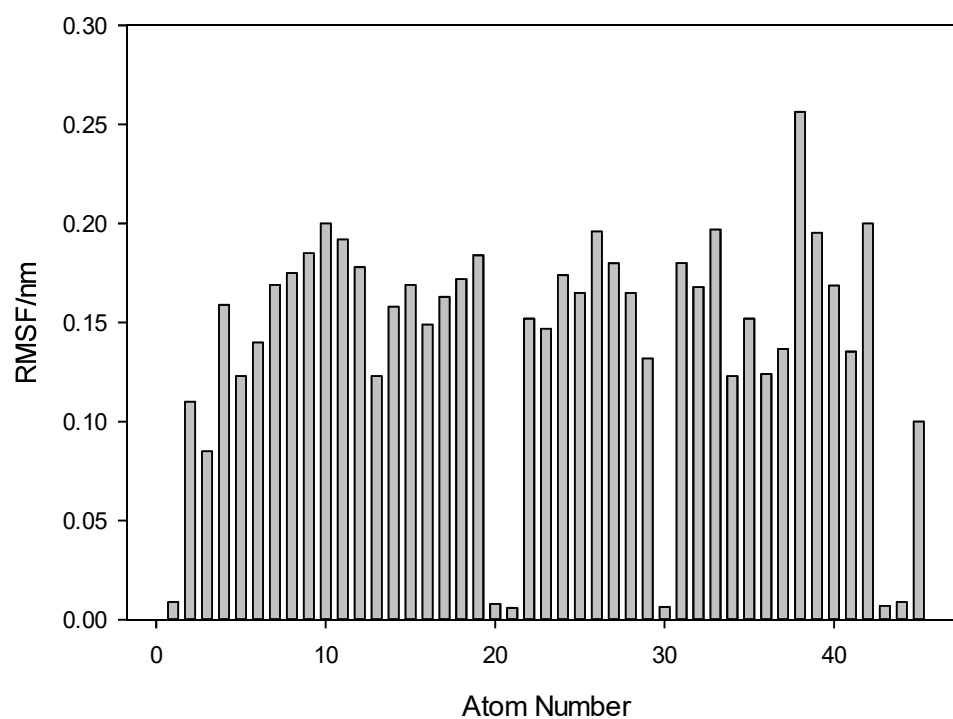


Figure 5.