Journal of
Inflammatory Diseases
(Previous Title: The Journal of Qazvin University of Medical Sciences)

Summer 2021, Volume 25, Number 2

Research Paper

A Comprehensive Study of Human Serum Albumin Interaction With Trimethoprim Using Molecular Docking and Molecular Dynamics Methods: An Appropriate Tool for Drug Delivery Systems

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Citation Salehi M, Shariatifar H, Ghanbari Johkool MG, Farasat A. A Comprehensive Study of Human Serum Albumin Interaction With Trimethoprim Using Molecular Docking and Molecular Dynamics Methods: An Appropriate Tool for Drug Delivery Systems. Journal of Inflammatory Diseases. 2021; 25(2):99-104. http://dx.doi.org/10.32598/JQUMS.25.2.2125.1

http://dx.doi.org/10.32598/JQUMS.25.2.2125.1

Article info:
Received: 25 Apr 2020
Accepted: 16 Jul 2020
Publish: 01 Jul 2021

Keywords:
Human serum albumin, Trimethoprim, Molecular docking, Molecular dynamics, Protein data bank

ABSTRACT

Background: Human Serum Albumin (HSA) is one of the most prominent proteins in human blood. Trimethoprim (TMP) is an efficient antibiotic drug for treating pneumocystis pneumonia. Patients with HIV/AIDS and cancer are highly affected by this disease due to immune system deficiency.

Objective: This study aims to evaluate the Molecular Dynamics (MD) simulation of HSA with TMP for drug delivery systems.

Methods: In the first step, the 3D structure of HSA and TMP were determined by PDB (Protein Data Bank) and PubChem, respectively. Then, the molecular docking was done via AutoDock Vina software, and the best complex was selected based on the lowest binding energy. Finally, the structural characteristics of the above complex were evaluated.

Results: The results showed that TMP binds to the HSA molecule with a binding energy of -7.3 kcal/mol, and this binding causes changes in the third and second structures of the HSA. Thus, Root-Mean-Square Deviation (RMSD) and radius of gyration results proved the third structural change, and the results obtained from DSSP (Database of Secondary Structure assignment for all Protein entries) confirmed the second structural modification. The TMP-HSA complex formation is accompanied by hydrophobic interaction between residues of Tyr150, Ala291, His288, Leu238, Leu219, Lys199, Lys195, Glu153, and TMP. The TMP molecule had two hydrogen bonds with Arg222 residue and three with Ser192. Furthermore, the final PDB file of the MD simulation process showed that the TMP molecule reacted with HSA (IIA chain).

Conclusion: Because of the extensive application of TMP in infectious diseases and appropriate interaction with HSA, the complex could be used for the purposeful transport of nanoparticles in the future.

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1. Introduction

Albumin is the main blood protein with a half-life of 19 days with a blood concentration of 35-50 g/L. Besides the known functions of the protein, it can transfer various compounds produced in the body, creates osmotic pressure, and regulate blood acidity [1-4]. Bovine Serum Albumin (BSA) and Human Serum Albumin (HAS) are protein models which have received particular attention in molecular docking studies. The structure of albumin in human serum consists of 585 amino acids and a protein chain, of which 17 Tyr amino acids and one Trp are in residue 214 [5, 6]. Human serum albumin is made in the liver from a prealbumin precursor in the rough endoplasmic reticulum and released into the bloodstream; it plays an important role in transporting various drugs and hydrophobic elements, including hormones and hemopexin [7, 8]. The Cys34 in the albumin structure has the antioxidant ability and traps free radical groups through the thiol group. In most liver-biliary and kidney disorders, the inflammatory factors, including Nitric Oxide (NO) increase in the bloodstream.

Albumin plays a major role in detoxifying some factors from the liver and kidneys and protects the gastrointestinal tract and blood vessel walls against various toxic metabolites produced in the body. It has been shown that this blood carrier binds to these toxic metabolites through its second domain, thus reducing their toxic effects and removing them from the body [4, 9]. In recent years, HSA-based nanoparticles achieved more attention due to their high biocompatibility and biodegradability in physiologic conditions, surface modification, enhanced permeation and retention in tumors, specific release in tumor tissue, and lower immune response. Thus, these nanoparticles were applied to deliver various cancer drugs such as noscapine, docetaxel, and paclitaxel [4, 10, 11].

Among various properties of this protein, the main role of HSA is the transfer of various biological and drug molecules in the body to the target organs. Therefore, the interaction of the protein and different types of drugs has been explored in several studies because the interaction of drugs and HSA affects the structure of the drugs and their activity [12, 13].

Trimethoprim (TMP) is an effective antibiotic in treating pneumocystis pneumonia. This pneumonia severely affects patients with HIV/AIDS and cancer with immune system deficiency [14]. The chemical formula for TMP is 2,4-diamine and 1,2,3-trimethoxybenzene [15]. Trimethoprim, with the trade name of Bactrim, inhibits the dihydrofolate reductase enzyme in bacteria and less efficiently in mammalian cells and thus reduces the synthesis of thymine and DNA damage, thereby exerting its antibacterial effects [5]. This antibiotic could treat respiratory, blood, and urinary tract infections but is affected by blood factors such as albumin. It is eventually eliminated from the body by the renal system [15-17].

Molecular Dynamics (MD) simulations provide potent tools for exploring the conformational energy landscape accessible to biological molecules. Also, the rapid increase in computational power coupled with improvements in methodology makes it an exciting time for applying simulation to structural biology. The Molecular Dynamics (MD) simulation process has a great role in protein-ligand recognition and the protein conformational changes at the atomic level [18]. Thus, in the current study, the MD simulation method was applied to assess the physicochemical characteristics of TMP with HSA and analyze the affinity of this drug with HSA *in silico*.

2. Materials and Methods

Protein-ligand docking

The characteristics of TMP were obtained from PubChem, and the HSA crystal structure (entry code: 4K2C) was provided from Protein Data Bank (http://www.rcsb.org). The TMP molecular docking process to HSA was evaluated through a convertible docking process. The receptor and ligand side chain possess enough flexibility and could interact together using AutoDock Vina [19]. To determine the permissible torsions for the ligand, search space coordinates characterization, and add polar hydrogen atoms to the protein, we used the graphical AutoDock tool [20]. The HSA (IIA domain) can form a bond with the ligand on X=34, Y=40, and Z=42 in 1 Å spacing. Autodock software can specify the lowest binding energy of the TMP-HSA (domain II) complex for docking conformation, which was assumed as the primary conformations for the MD simulation process.

Molecular dynamics simulation of HSA-TMP

The conformational changes of the receptor-ligand complex were estimated using the MD method (GROMOS96 43a1 force field and the GROMACS 4.5.4 package software) [21]. The complexes provided by the method mentioned above were located in a simulation box full of water molecules. Afterward, the GROMACS software evaluated the HSA topologic parameters. Furthermore, the topologic characteristics of the TMP were

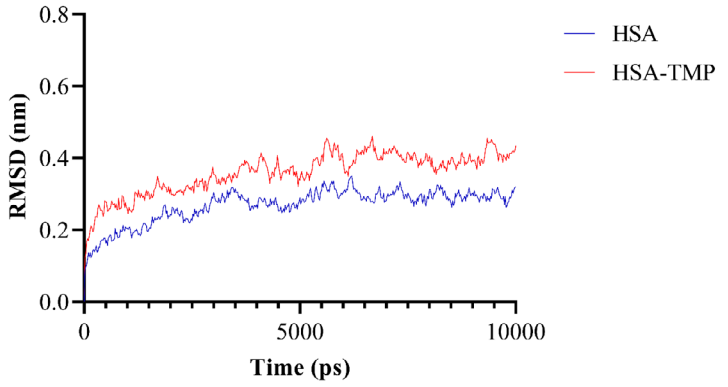

Figure 1. The Root-Mean-Square Deviation (RMSD) value of Cα of the free Human Serum Albumin (HSA) and Trimethoprim (TMP)-HSA complex

provided by the Dundee PRODRG server [22]. In the next step, a box of simple point charge water molecules was provided for the immersion of the complex mentioned above. For energy minimization, the steepest descent method (10000 steps), followed by the conjugate gradient method (10000 steps), was used for incompatible contact releases. The equilibration phase of the system (Position-restrained dynamics simulation, NVT/NPT) was performed at 310 K for 100 ps, followed by an MD production run for 10 ns [23, 24]. The atomic coordinates were recorded every 2.0 ps within the MD simulation process. In the next step, to recognize the MD process better, the RMSD (Root-Mean-Square Deviation) [25] and Rg (Radius of gyration) were measured [26]. The secondary structure analysis was done by the DSSP (Database of Secondary Structure assignment for all Protein entries) program [27]. Ultimately, the final PDB (Protein Data Bank) file of MD simulation was designed via PyMOL software [28]. Then, to analyze the H-bond and hydrophobic reactions of the complex, the LIGPLOT software was applied [29].

3. Results and Discussion

Molecular docking and molecular dynamics simulation

In the current study, to evaluate the interaction of TMP with HSA, we carried out the molecular docking process. Binding TMP to HSA and evaluating the interaction is important because the protein-ligand binding interaction can provide helpful knowledge about their structure as potential therapeutic agents [13]. In this study, selective side-chain residue flexibility is a valuable option provided by AutoDock Vina software [14]. The primary advantage of this option is providing a practical approach to ligand-protein interaction without a remarkable increase in computer processing time. First, to evaluate the structural modifications caused by ligand binding (TMP), the MD simulation process of free HSA (subdomain II) and the TMP-HSA complex was done, and the results were compared carefully. Then, three structural parameters of RMSD, Rg, and the secondary structure were measured. To predict

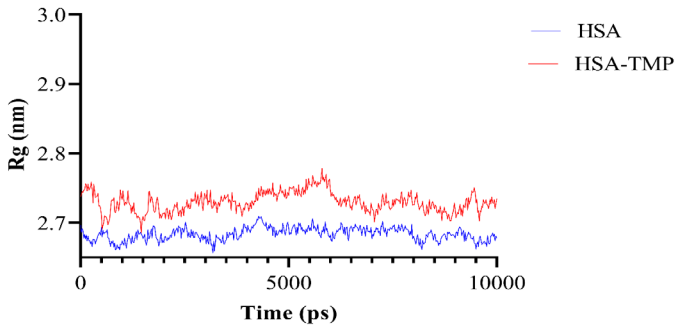

Figure 2. Radius of gyration values of the free Human Serum Albumin (HSA) and Trimethoprim (TMP)-HSA

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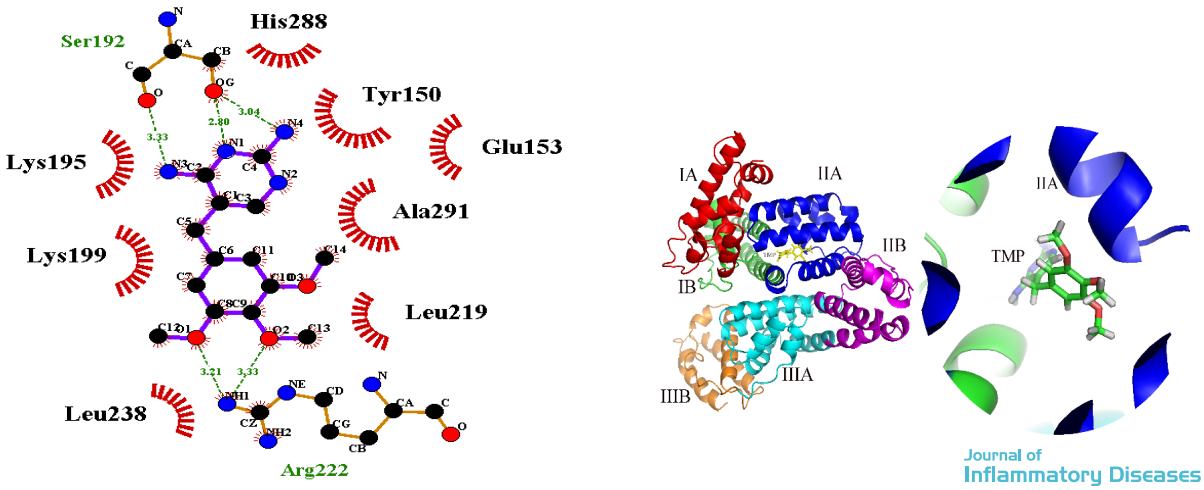

Figure 3. The interactions of Trimethoprim (TMP)-Human Serum Albumin (HSA) created by LIGPLOT and PyMOL software

Left) 2D images of TMP-HSA complex, Right) 3D images of TMP-HSA complex

the system equilibration during the simulation process, the RMSD was a helpful parameter that was applied [16].

Next, to prove the system equilibration, the RMSD profiles of free HSA and the TMP-HSA complex were evaluated during the 10 ns simulation process. The RMSD profiles of the free HSA and the TMP-HSA complex are shown in Figure 1, in which the system equilibration was obvious after 5 ns. The RMSD value of free HSA and the TMP-HSA complex were respectively 0.32 and 0.38 nm in the last 5 ns of the simulation. It should be mentioned that the stabilization happened for all the systems after 5 ns. The RMSD value of the complex is a little higher than free HSA. This phenomenon is due to more activity and interaction of HSA atoms in the presence of TMP, which leads to some conformational and structural modifications, confirmed by previous studies [30].

The Rg parameter shows the protein contraction during the simulation process [18]. To assess the TMP effects on HSA contraction during the simulation process, the gyration of each system was estimated (Figure 2). The average Rg values of free HSA and TMP-HSA complex were 2.67 and 2.72, respectively, for the final 2 ns of the simulation. Our results demonstrated no significant differences between the Rg values of free HSA and the TMP-HSA complex. Accordingly, the Rg value of HSA improves when it binds to its ligand (TMP), implying a less compact structure and partial unfolding of the protein after the simulation process. These results show that binding HSA to its ligand changes the HSA microenvironment, resulting in HSA conformational modifications during the simulation process, confirmed by previous studies [31].

The docking results demonstrated that TMP binds to HSA with the lowest binding energy of -7.3 kcal/mol. The 2D and 3D images of the TMP-HSA complex after 10 ns of the MD simulation process are illustrated in Figure 3. As depicted in the aforementioned 2D image, in the TMP-HSA complex, the TMP has hydrophobic interactions with Ala291, His288, Leu238, Leu219, Lys199, Lys195, Glu153, and Tyr150. It should be noted that the TMP possesses two hydrogen bonds with Arg222 residue and three hydrogen bonds with Ser192

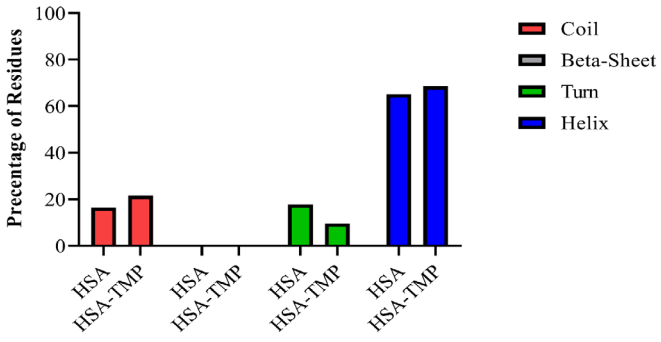

Figure 4. Secondary structure analysis of the free HSA and Trimethoprim (TMP)-HSA showing using the DSSP algorithm

(Figure 3A). Furthermore, the TMP structure in relation to the residues mentioned above in HSA is illustrated in a 3D image (Figure 3B).

The docking and MD results showed that TMP binds to HSA (IIA subdomain in site I) effectively [5]. Furthermore, the secondary structure analysis was carried out using the DSSP program [27]. It should be noted that the secondary structures of the free protein and the complex are illustrated in Figure 4. Usoltsev et al. reported that free HSA in DSSP program consists of β-turn (8.9%), α-helix (68.4%), irregular structure (12.9%), and β-sheet (0%). When TMP binds to HSA, the alpha helix content increases slightly, which confirms the results mentioned above [32].

4. Conclusion

Several studies confirmed that IIA/IIIA subdomains of HSA could be target sites for the docking process. The results demonstrated that TMP binding to HSA leads to conformational changes in the HAS, resulting in complex stability. The above changes were assessed via several parameters, including RMSD, Rg, and DSSP. The molecular docking and MD simulation approaches show that the TMP molecule binds to domain II of HSA protein. Because the MD simulation allows the evaluation of structural parameters with high accuracy, the results of this study can provide sound knowledge about structural changes in the third and second structure of HAS protein and conformational changes at the molecular level.

Ethical Considerations

Compliance with ethical guidelines

This article does not contain any studies with human participants or animals performed by any authors.

Conflict of interest

The authors declared no conflict of interest.

Funding

The authors received support from Qazvin University of Medical Sciences (Grant No.: IR.QUMS.REC.1398.229).

Acknowledgments

Qazvin University of Medical Science financially supported this work which is gratefully acknowledged.

Authors' contributions

All authors equally contributed to preparing this article.

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