Spectroscopic and Computational Molecular Docking studies on the protein-drug interactions

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***Abstract*—Investigation of the protein-drug active substance interactions has great importance in the fields of medicine, chemistry, pharmaceutical, biomedical and toxicology. In this study, binding properties of a potential anti-cancer drug agent ifosfamide with bovine serum albumin (BSA), one of the main ligand transporters in blood plasma, was analyzed by using ultraviolet and visible light (UV-Vis) spectroscopy along with molecular docking studies. The UV-Vis spectra of the constant BSA solution ($20x10^{-6}$ M) in complexes with various concentrations of ifosfamide ($20x10^{-6}$ M to $140x10^{-6}$ M) were obtained at physiological pH. Besides, the BSA protein was docked with ifosfamide drug active substance via computational molecular docking method. Amino acids in the binding sites of the BSA protein and the binding distances of these amino acids to the ligand (ifosfamide), their scores and RMSD values were determined, revealing that the interaction is a spontaneous process. Both molecular docking and the spectral results demonstrated that the anti-cancer drug agent binds to BSA via non-covalent interactions, resulting in minute conformational changes in BSA.**

Keywords—anti-cancer drug; protein; UV-Vis spectroscopy; binding; molecular docking.

I. INTRODUCTION

Analysis of the protein-drug interactions is important in the fields of chemistry, medicine, biomedical, pharmacology, toxicology and biology.[1],[2] The aim of this work is to investigate the interaction of selected protein-drug active substance with bovine serum albumin (BSA) by using both spectroscopic (UV-Vis spectroscopy) and molecular docking methods.

Recently, molecular docking methods have become an important tool for drug discovery. It has been widely used for modelling the interaction between small molecules and protein. The interactions between protein and molecule or structure of protein and molecule can be explored with the molecular docking processes. This method has also a crucial role in computer aided drug design.[3]–[5]

In this current study, it was aimed to reach the best possible conformation between protein and ligand by using the molecular docking simulation program. Therefore, in our study, the binding properties of the drug active substance, which serve as the basic information for pharmacological and toxicological studies, and the interaction of ifosfamide (an anti-cancer drug agent) with BSA was investigated in order to reveal the conformational changes in the structure of BSA upon binding.

Investigation of the binding properties of anti-cancer drugs on blood proteins is important because such drug active substances are transported by plasma proteins in the circulatory system in the treatment of cancer. Determination of reversible/irreversible binding of the drug agent to the plasma proteins is also crucial for maintaining cytotoxic activity of the drug in the course of blood circulation.[6]

Serum albumin is the most abundant protein in mammals therefore it is the most commonly studied protein in the literature. Due to their remarkable binding capacity, serum albumin is responsible for the distribution of various drugs, nutrients and other molecules.[1],[3] BSA is a globular protein with a molecular weight of ~66.5 kDa and consists of 585 amino acids. BSA comprise mainly of α-helical secondary structure.[7]

Therefore, the BSA protein was used as a model to investigate possible chemical and binding properties to drug active substance ifosfamide by using UV-Vis spectroscopy. Also, molecular docking simulation programs were applied to understand the dynamics of protein-drug active substances interaction.

II. METHODOLOGY

A. Samples

Albumin from bovine serum (Sigma-Aldrich, A7906) and ifosfamide (Sigma-Aldrich, I4909) of analytical grade were purchased and used without further treatment. Stock solutions of the BSA protein and ifosfamide were prepared by dissolving the lyophilized powder in 0.05 M potassium phosphate buffer pH 7.4.

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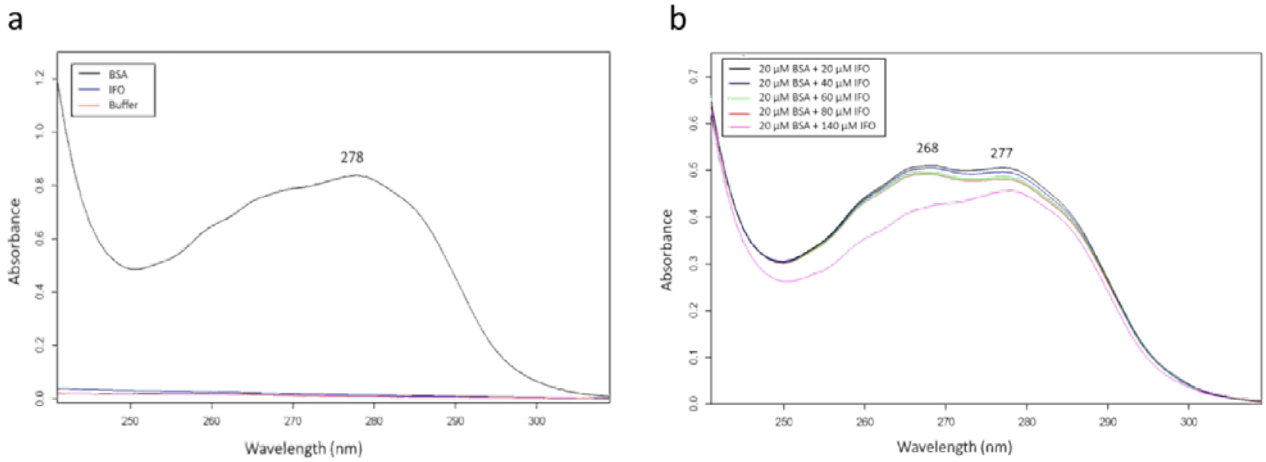

Fig.1. UV-Vis absorption spectra of BSA and BSA-ifosfamide complexes. **a)** The spectrum showing the presence of aromatic amino acids in BSA. The samples were measured after being heated at 37°C for 30 min; blank BSA in KPi buffer ($10x10^{-6}$ M), ifosfamide (IFO) (0.014 M) and KPi buffer (0.05 M). **b)** The spectra of the BSA and ifosfamide complexes. From top to bottom: BSA ($20x10^{-6}$) and ifosfamide complexes with various concentrations of $20x10^{-6}$, $40x10^{-6}$, $60x10^{-6}$, $80x10^{-6}$, and $140x10^{-6}$ M.

B. UV-Vis Spectroscopy

The UV-Vis spectroscopy measurements were performed by using a Perkin Elmer LAMBDA™ 750 UV/Vis/NIR Spectrophotometer. "ES quartz" cuvettes having a pathlength of 10 mm and a capacity of 0.7 mL were used.

Firstly, 0.05 M potassium phosphate buffer (KPi) and $30x10^{-6}$ M BSA stock solution were prepared at physiological pH 7.4. Then, the stock BSA solution was diluted to $20x10^{-6}$ M. Afterwards, 0.014 M stock ifosfamide solution in KPi was prepared.

The $20x10^{-6}$ M BSA solution was chosen as the constant value for the measurements while the ifosfamide concentration was varied ($20x10^{-6}$, $40x10^{-6}$, $60x10^{-6}$, $80x10^{-6}$, $140x10^{-6}$ M). Then, to start the binding reaction, the BSA protein and drug agent (ifosfamide) solutions were mixed inside a vial at equal amounts of volumes. Prior to UV-Vis spectroscopy measurements, each complex solution was incubated in a thermo shaker for 30 min at 37°C with 300 rpm. After 30 min, each protein-drug complex was measured immediately at room temperature.

C. Computational Molecular Docking

The three-dimensional coordinates of BSA were fetched by its ID number from the Protein Data Bank (PDB ID:4F5S) in UCSF Chimera 1.14. Before docking analysis, the crystal structure of BSA was prepared with the Dock Prep tool. Water molecules were removed; polar hydrogens and Gasteiger charges were added. Then, ifosfamide was fetched by its ID number from the PubChem (PubChem CID:3690). To recognize the binding sites in BSA, the grid size was set to 75, 100, and 75 along the X-, Y-, and Z-axes with a 0.375 Å grid spacing. The grid centre along the X-, Y-, Z-axes was set to 25, 10 and 20 Å. The AutoDock Vina tool was finally used to calculate possible bindings and energies. The output from AutoDock Vina was further analysed with PyMOL and Chimera.

III. RESULTS

D. UV-Vis Spectroscopy Results

Absorption spectrum was obtained by conducting absorbance scanning in a certain wavelength range (200-400 nm) in order to obtain information about the (bio)chemical structure of the protein molecule. As observed in Fig. 1a, the absorption maximum around 278 nm arise from the existence of aromatic amino acids such as tyrosine, tryptophan, and phenylalanine in the BSA protein.[8] It is clear that blank buffer and ifosfamide solutions do not give rise to any absorbance peak in this spectral region.

The absorption spectra of the BSA and ifosfamide complex were also shown in Fig. 1b. The BSA concentration was kept constant ($20x10^{-6}$ M) while the ifosfamide concentration was varied from $20x10^{-6}$ M to $140x10^{-6}$ M. Upon addition of drug agent, the change in the absorption spectrum is clearly observed that the absorption intensity decreases with increasing amount of the ifosfamide. This pattern of change, termed as hypochromism,[9] indicates binding of ifosfamide to the hydrophobic parts of the BSA protein with a slight change in the conformation. The maximum absorption peak at 278 nm shifts slightly.

E. Computational Molecular Docking Results

When the ligand docked to the protein, the score reflects the potential energy change. This change of potential energy reveals free binding energy. Reactants will respond spontaneously or any outside vitality source will be required to allow them to respond. Basically, in this study, a negative sign signifies that reaction will happen spontaneously. The more negative values of the scoring function show how strong the bond between protein and ligand is. Basics of thermodynamics state that response with negative free energy is unconstrained. Negative binding free energy values during molecular docking mean that the visitor is bound to the host.[10],[11] In our study, when the docking of ifosfamide and BSA protein has

been done, totally 9 poses were found as shown in the Fig. 2 below.

S	Score	RMSD lb.	RMSD u.b.	HBonds (all)	HBond Ligand Atoms	HBond Receptor Atoms
V	-5.0	0.0	0.0	1	1	1
V	-4.9	13.499	14.743	0	0	0
V	-4.9	3.575	5.374	1	1	1
V	-4.7	13.647	15.142	0	0	0
V	-4.7	20.136	21.398	0	0	0
V	-4.7	1.919	1.984	1	1	1
V	-4.6	19.81	21.118	0	0	0
V	-4.6	21.868	23.292	2	2	2
V	-4.6	20.95	21.999	1	1	1

Fig. 2. Optimum binding values for BSA. The score values mimic the potential energy changes, a higher negative score values correspond to strong binding between protein and ligand, so that, -5 was found as the lowest free energy for the best possible conformation of BSA-ifosfamide complex. First row: values that define the best possible binding site of BSA.

Since considering the RMSD (the root of mean square deviation) values the protein stability and conformational change of the protein can be analyzed, it is important to take pose which has the lowest RMSD values as RMSD values correspond to the deviation of the ligand from X-Ray coordinates.[12] In this study, pose 1 was decided to have the lowest RMSD values, which interacts with the target protein active site residues.

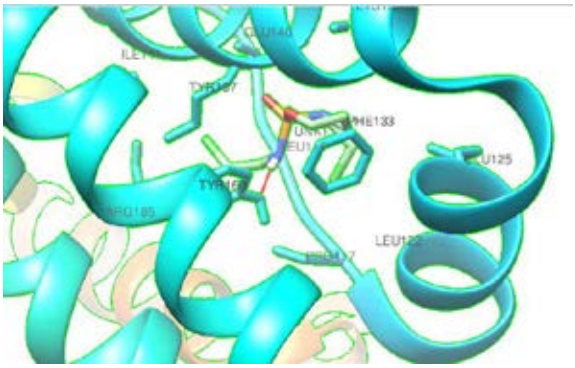

Fig. 3. Pose with lowest RMSD values. The red line between Tyr 160 and ifosfamide indicates the presence of only one hydrogen bond occurs.

Based on the molecular docking studies, it was found that the ifosfamide binds to Tyr 160 via H-bonding as depicted in Fig. 3 above. We know from the X-ray crystallographic structure that Tyr 160 is located on the helix9 in the subdomain IB.

Apart from the H-bond, the van der Waals interactions are also observed between ifosfamide and amino acids, including TYR 160, LEU 115, LEU 122, TYR 137, PHE 133, GLU 125 and GLU 140. The hydrophobic interactions are also noticed between amino acids of TYR 137, PHE 133, GLU 125, GLU 140 and ifosfamide.

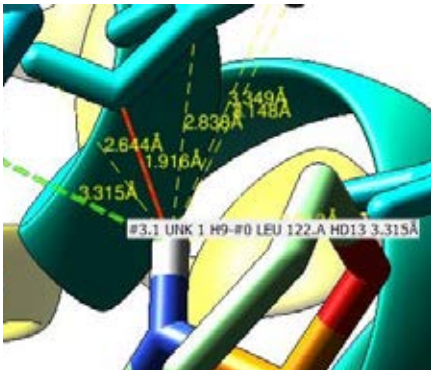

Fig. 4. The binding distance of the pose with the lowest RMSD values.

It is also feasible to reveal the distance between the atoms of protein and drug agent. As shown in Fig. 4, the distance between Tyr 160 and ifosfamide was found as 1.916 Å. Yellow dashed lines represent the distance of hydrogen bond interactions between BSA and ifosfamide for the other possible poses.

IV. CONCLUSIONS

In conclusion, our aim was to understand and analyze the dynamics and the interactions between protein and drug by combining the UV-Vis spectroscopy and molecular docking methods.

According to literature review, researchers studied with BSA protein to analyze the interactions between different types of drug active substances. Since ifosfamide is an active drug substance used for treatment of various cancer types, UV-Vis spectroscopy was used to determine chemical, structural and binding properties of different samples. The obtained results show the chemical and structural properties of all samples. When the UV-Vis spectrum is examined, information about the structure of BSA protein is obtained and thus the spectrum is interpreted, accordingly.

Based on the UV-Vis spectrum, when the drug agent is mixed with the BSA, the absorption intensity at 278 nm diminishes with increasing amount of ifosfamide. This hypochromism effect indicates binding of ifosfamide to the hydrophobic sites of the BSA protein with a slight change in the conformation. The absorption peak at 278 nm shifts slightly, as well.

For molecular docking, UCSF Chimera 1.14 which is a software package of AutoDock Vina 4.2 was used to determine the binding properties (binding energy, binding mode, conformational change, etc.) of BSA. Thus, protein-drug interactions which have the three-dimensional structure were obtained by molecular docking. Herein, ifosfamide was docked with BSA. In this way, binding energies, scores, and RMSD values, amino acids in binding sites and poses were obtained by using UCSF Chimera and AutoDock Vina 4.2. Based on the score and RMSD values, the best conformation was obtained computationally for the BSA-ifosfamide complex. The binding site of the drug

molecule is located on site I of BSA, which is energetically favorable.

Molecular docking showed that the interaction of BSA with ifosfamide is spontaneous. Both docking and UV-vis spectroscopy results are consistent that the anti-cancer drug agent ifosfamide binds to the BSA protein with non-covalent interactions (H-bond, van der Waals interactions and hydrophobic interactions), resulting in minute conformational changes in the BSA structure.

Further work is in progress to analyze the protein-drug interactions and to calculate the binding constants by using different spectroscopic techniques and by combining with molecular docking methods.

Acknowledgment

We are grateful to the Izmir University of Economics for all the physical and technical support during the project. This work was supported by a grant from the Scientific and Technological Research Council of Turkey (TUBITAK 2209-A programme) (Project no: 1919B011903382).

References

[1] Shen, H., Gu, Z., Jian, K., & Qi, J. (2013). In vitro study on the binding of gemcitabine to bovine serum albumin. Journal of Pharmaceutical and Biomedical Analysis, 75, 86-93. doi:10.1016/j.jpba.2012.11.021

[2] Sood, D., Kumar, N., Rathee, G., Singh, A., Tomar, V., & Chandra, R. (2018). Mechanistic Interaction Study of Bromo-Noscapine with Bovine Serum Albumin employing Spectroscopic and Chemoinformatics Approaches. Scientific Reports, 8(1). doi:10.1038/s41598-018-35384-6

[3] Jahanban-Esfahlan, A., Panahi-Azar, V., & Sajedi, S. (2015). Spectroscopic and molecular docking studies on the interaction betweenN-acetyl cysteine and bovine serum albumin. Biopolymers, 103(11), 638-645. doi:10.1002/bip.22697

[4] Chi, Z., Liu, R., Teng, Y., Fang, X., & Gao, C. (2010). Binding of Oxytetracycline to Bovine Serum Albumin: Spectroscopic and Molecular Modeling Investigations. Journal of Agricultural and Food Chemistry, 58(18), 10262-10269. doi:10.1021/jf101417w

[5] Suryawanshi, V. D., Anbhule, P. V., Gore, A. H., Patil, S. R., & Kolekar, G. B. (2011). Spectroscopic Investigation on the Interaction of Pyrimidine Derivative, 2-Amino-6-hydroxy-4-(3,4-dimethoxyphenyl)-pyrimidine-5-carbonitrile with Human Serum Albumin: Mechanistic and Conformational Study. Industrial & Engineering Chemistry Research, 51(1), 95-102. doi:10.1021/ie202005c

[6] Gullo, J. J., Litterst, C. L., Maguire, P. J., Sikic, B. I., Hoth, D. F., & Woolley, P. V. (1980). Pharmacokinetics and protein binding of cis-dichlorodiammine platinum (II) administered as a one hour or as a twenty hour infusion. Cancer Chemotherapy and Pharmacology, 5(1), 21-26. doi:10.1007/bf00578558

[7] Bujacz, A., & Bujacz, G. (2012). Crystal Structure of Bovine Serum Albumin. doi:10.2210/pdb4f5s/pdb

[8] Schmid, F. (2001). Biological Macromolecules: UV-visible Spectrophotometry. Encyclopedia of Life Sciences. doi:10.1038/npg.els.0003142

[9] Karami, K., Jamshidian, N., & Zakariazadeh, M. (2019). Synthesis, characterization and molecular docking of new C,N-palladacycles containing pyridinium-derived ligands: DNA and BSA interaction studies and evaluation as anti-tumor agents. Applied Organometallic Chemistry, 33(3). doi:10.1002/aoc.4728

[10] Saha, S., & Chowdhury, J. (2019). Binding Interaction of Juglone with Lysozyme: Spectroscopic Studies Aided by In Silico Calculations. Journal of Photochemistry and Photobiology B: Biology, 193, 89-99. doi:10.1016/j.jphotobiol.2019.02.006

[11] Usha, T., Middha, S. K., & Goyal, A. K. (2014). Molecular docking studies of anti-cancerous candidates in Hippophae rhamnoides and Hippophae salicifolia. Journal of Biomedical Research. doi:10.7555/jbr.28.20130110

[12] Yusuf, D., Davis, A. M., Kleywegt, G. J., & Schmitt, S. (2008). An Alternative Method for the Evaluation of Docking Performance: RSR vs RMSD. Journal of Chemical Information and Modeling, 48(7), 1411-1422. doi:10.1021/ci800084x