Computational Analysis of Interactions between Human and Bovine Proteins and Organophosphorus Pesticides

Nur Ain Nabillah Muhamad Affandi[1], Asmaa Zainal Abidin[1], Mohd Junaedy Osman[1]*, Fadhlul Wafi Badrudin[2], Jahwarhar Izuan Abd Rashid[1], Ahmad Farid Mohd Azmi[1], Syed Mohd Shafiq Syed Ahmad[1], Wan Md Zin Wan Yunus[3], Ong Keat Khim[1] and Noor Aisyah Ahmad Shah[1]

[1]Jabatan Kimia dan Biologi, Pusat Asasi Pertahanan, Universiti Pertahanan Nasional Malaysia, Kem Sg. Besi 57000 Kuala Lumpur, Malaysia

[2]Jabatan Fizik, Pusat Asasi Pertahanan, Universiti Pertahanan Nasional Malaysia, Kem Sg. Besi 57000 Kuala Lumpur, Malaysia

[3]Jabatan Sains Pertahanan, Fakulti Sains Teknologi Pertahanan, Universiti Pertahanan Nasional Malaysia, Kem Sg. Besi 57000, Kuala Lumpur, Malaysia

*Corresponding author (e-mail: junaedy@upnm.edu.my)

Organophosphates are widely used as pest control agents in the agriculture industry, as they disrupt the nervous systems of pests. Although they have been used as effective pesticides for many years, their effects on Human Serum Albumin (HSA) has sparked debates over their potential negative impact on the environment and human health. The toxicity of a pesticide is a result of complex formation between a plasma protein, such as serum albumin, and the pesticide. Since these proteins are not encapsulated with carbohydrates, they can easily be bound and transported by various molecules. In this report, the results of a molecular docking experiment focusing on the chemical interactions and scoring function between organophosphate pesticides (OPP) and serum albumin protein via Discovery Studio are described. This study provides a fundamental understanding of the molecular mechanisms underlying their bindings from the interactions via computational analysis. The 2D interactions were able to provide a theoretical foundation for understanding the interaction mechanisms between OPP and Human Serum Albumin (HSA) / Bovine Serum Albumin (BSA). The LIBDOCK and CDOCKER analysis revealed favourable binding with strong intermolecular forces (H-bonds, Van der Waals forces, electrostatic bonds, and hydrophobic effects) between methyl-paraoxon (M-P) with both HSA and BSA. The hydrophobic interactions stabilised M-P inside the active sites of the proteins ALA 291 and ALA 191 in HSA and PHE 550, ALA 527, LEU 574, VAL 575, VAL 546 PHE 506 and LEU 528 in BSA. Structural binding modes showed that hydrophobic interactions play an essential role in stabilizing pesticides inside the active sites of the protein, and the scoring function demonstrated the binding affinities between proteins and pesticides.

Keywords: Biovia Discovery Studio; molecular docking; binding affinity; scoring function

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Organophosphate pesticides (OPPs), such as dichlorvos (Dic), glyphosate (Gly), methamidophos (Metha), methyl-paraoxon (M-P), and quinalphos (Qui), which are extensively used to control various insects in crop production, are toxic chemicals. The World Health Organisation (WHO) classifies Dic as a highly hazardous pesticide [1]. Severe poisoning incidents have been reported resulting from exposure to non-targeted organisms due to extensive utilization of pesticides [2]. In addition, these compounds are known to be used as Chemical Warfare Agents (CWA) [3]. Hospital admissions due to OPP poisonings caused by occupational exposure, are commonly observed especially in agricultural nations.

A study delved into the intricate molecular interactions between OPPs and proteins, seeking to unravel the underlying mechanisms that govern their binding, influence on protein structure, and subsequent physiological implications. This is crucial for assessing the environmental and health risks associated with OP exposure, as proteins serve as pivotal molecular targets in various biological processes. In connection with this, investigations on Human Serum Albumin (HSA) have recently gained attention due to the potential toxic effects of OPPs. The inclusion of carbon and phosphorous acid derivatives as structural components of OPPs allows for easy absorption through the skin, lungs, and gastrointestinal (GI) tract. Once within the body, these substances often attach themselves to red blood cells (RBC), and specifically acetylcholinesterase (AChE), preventing the enzyme from doing its job, which causes AChE to build up at synaptic connections [4]. In the long run, organisms that

have been exposed to OPPs may experience population decline due to detrimental effects on reproductive organs, related cells, and their by-products [5].

Since OPPs may linger in the soil, air, surface water, and groundwater for a long time in the form of the parent molecule or hydrolysis products, they pose a serious risk to human health. Upon entering the body through drinking, eating, inhaling, and skin absorption, OPPs can produce adverse effects on body proteins, including albumin. Albumin is essential for osmotic pressure maintenance, transportation of compounds (including medications and hormones), and pH regulation. Studying the relationship between pesticides and serum albumin is crucial because it regulates the free concentration of pesticides within the body and hence determines their effects, out of all the potential interactions between pesticides and proteins.

HSA and Bovine Serum Albumin (BSA) are the two serum proteins that have been studied the most. Both proteins are found in the blood serum of their respective species: HSA is derived from humans, and BSA is derived from cows (bovines).

Although there are some differences between BSA and HSA, BSA has an almost 88 % sequence similarity with HSA; however, their binding affinity for the same ligand is invariably different [6,7]. HSA and BSA consist of three homologous domains (I–III) (Figure 1). Each of these can be divided into two subdomains (A and B). BSA is a single-chain transporting protein consisting of 583 amino acids with 20 tyrosyl residues (Lys, Glu, Ala, Phe, Arg, Ile, Gn, His, Met, Asp, Ser, An, Tyr, Cys, Leu, Pro, Val, Thr, Ileu, Gly) and two tryptophanes located in positions 134 and 213. HSA consists of 585 amino acids with 17 tyrosyl residues (Lys, His, Arg, Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe) and Trp located in position 214. HSA's tryptophan (Trp214) is in subdomain IIA, whereas BSA has two tryptophan moieties (Trp134 and Trp213) located in subdomains IA and IIA respectively. A comparison of the amino acid sequences of both serum proteins reveals that 15 amino acid residues in subdomain IIA of HSA are replaced in the bovine variety [7].

HSA exhibits remarkable binding capabilities, interacting not only with fatty acids, peptides, and proteins, but also with various low molecular weight endogenous and exogenous compounds under physiological conditions. It can accommodate medium to long saturated fatty acids (C10–C18), as well as long chain unsaturated fatty acids such as arachidonic and oleic acid. Additionally, HSA possesses a high-affinity binding site for metals at the N-terminus of the chain, primarily binding Zn(II), Cd(II), Cu(II), and Ni(II). Domains II and III serve as the primary binding sites owing to their nonpolar nature. Similarly, BSA exhibits multiple binding sites, notably sites I and II within the hydrophobic cavities of subdomains IIA and IIIA. Beyond these interactions, BSA demonstrates a binding affinity towards nucleic acids, proteins, coordination compounds and ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Pt^{2+}) [7].

The binding of pesticides to HSA and BSA has seldom been investigated. Silva et al. studied the binding of methyl parathion OPP to HSA and BSA to establish whether methyl parathion exhibited a high affinity to HSA and BSA [9]. This fundamental knowledge of the interactions between pesticides and plasma proteins holds considerable toxicological relevance within the realm of computational chemistry, exerting substantial influence on the distribution dynamics and elimination pathways of these chemical agents.

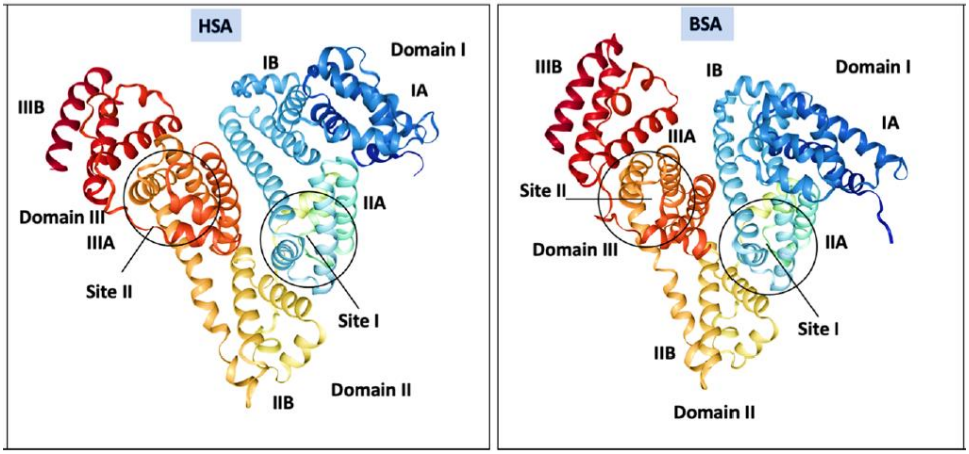

Figure 1. HSA and BSA with their domains and sites [8].

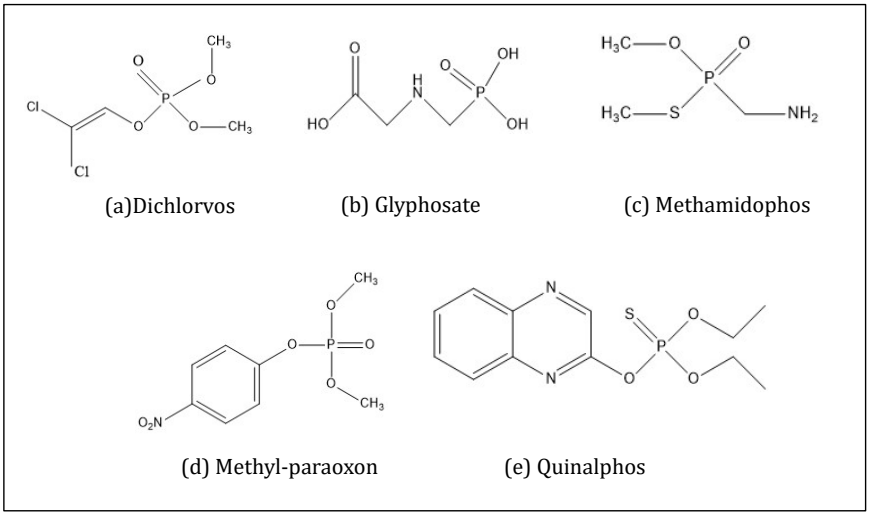

Figure 2. Chemical structures of the OPPs in this study.

In this paper, we describe the mechanisms of the interactions of five (5) OPPs (Dic, Gly, Metha, M-P, and Qui) with HSA and BSA, which were investigated using a molecular docking computational approach. The chemical structures of all OPPs used in this study are given in Figure 2. There are three objectives of performing docking: first, predicting the binding mode, second, virtual screening that separates the binders from nonbinders in a huge data set, and lastly, predicting the binding affinity of a smaller set of binders. Numerous studies have demonstrated that scoring functions are typically excellent at predicting the right binding modes for co-crystallized complicated structures. Furthermore, it has been shown that scoring functions can effectively enhance the identification of binders from a large dataset of binders and non-binders, making them valuable for virtual screening [10].

EXPERIMENTAL

Methodology

Sample Preparation: OPP Minimization and Protein Source Database-based Retrieval Method

The structures of OPP analogues as ligands were drawn in two dimensions (2D) using ChemDraw Professional 15.0 software and the drawing files were saved in pdb format. All 2D structures of OPP were generated in the software (BIOVIA Discovery Studio 2023). The energy minimizations were performed using a CHARMM-based force field on smart minimizer method with a maximum of 200 steps and a RMSD gradient of 0.1 kcal/mol [11].

For every complex position, the CHARMM energy is the interaction energy, a sum of interaction energy and ligand strain, which represents the ligand binding affinity [9]. After minimization was completed, the files then were saved as minimization ligand OPPs. The HSA and BSA structures were retrieved from the Protein Data Bank (PDB) with PDB ID: 2VDB and 4FS5 respectively.

Molecular Docking

Two molecular docking methods were performed in this study, LIBDOCK and CDOCKER, with Biovia Discovery Studio software. LIBDOCK is a docking program which uses protein site features referred to as Hot-Spots, while CDOCKER is a grid-based molecular docking method based on CHARMM. Prior to docking, ligands were removed and proteins were prepared according to the standard protocol of the Biovia Discovery Studio software tool to remove duplicates and eliminate compounds with undesirable properties, such as water molecules. Hydrogen atoms were added afterward.

In both cases, only the best docking pose for each OPP was saved for further analysis. The positions and sizes of active protein sites were determined according to their respective original ligands. With Biovia Discovery Studio software, we can define and edit binding sites under the Receptor-Ligand Interaction protocol. The LIBDOCK from a high throughput docking algorithm was used to dock compounds onto the active site of a receptor molecule with the selected ligand of each OPP. All docking and consequent scoring parameters used were kept at their default settings except for docking tolerance, which was set at 0.75 kcal/mol. Next, the CDOCKER from docking optimization was also performed and the default system was used for the scoring parameters. The input receptors were set for the prepared HSA and BSA and the input ligands were set for the prepared OPP.

Analysis of Molecular Docking Results

The interactions between ligands and receptor protein complexes, including hydrogen bonds, π-π bonds, π-sulfur bonds and charge attraction, were discovered using the Receptor-Ligand Interactions toolbar in BIOVIA Discovery Studio 2023. Further, all docked poses were scored and ranked, and the binding affinities of docker compounds were predicted. The binding modes of the best docked were analysed using the 2D diagrams of receptor-ligand complexes, and mainly included: the number of hydrogen bonds formed by the docked poses and the receptor protein complex, the number of hydrogen bonds formed with each amino acid residue and the distance between each interaction. The LIBDOCK score values and CDOCKER score energies were ranked. Finally, the lowest CDOCKER score energy was used to evaluate conformations during docking simulations.

RESULTS AND DISCUSSION

Table 1 lists interactions between the OPPs and serum albumin protein receptors observed with BIOVIA Discovery Studio 2023. The software allows researchers to see the two-dimensional interactions between ligands and protein receptors. Binding sites based on the docking score results are shown in Table 1. The 2D interactions, which included hydrogen bonding, π-π stacking effects, van der Waals forces, and hydrophilic and hydrophobic forces, represent the interactions of the OPPs with Human Serum Albumin (HSA) and Bovine Serum Albumin (BSA), providing a theoretical foundation for understanding the interaction mechanisms between these compounds.

From both LIBDOCK and CDOCKER virtual screenings, all ligands were ranked based on the ligands' scores. Important aspects of the binding process may be understood by predicting the binding mode and affinity of the tiny molecule within the target's binding sites. Predicting posture and affinity are common for empirical scoring functions. Docking procedures can utilise distinct scoring systems at every stage in this context. For example, binding modes can be predicted using a rapid scoring function, and affinities can be further predicted using a more complex scoring function designed specifically for affinity prediction [12]. Hence, these scoring functions were used as a guidance for docking and for scoring the resulting docking poses and binding affinities.

During the docking simulation, the LIBDOCK score values and CDOCKER score energies were examined to search for distinct conformers to obtain the optimal binding energy. Using the CDOCKER docking program, full ligand flexibility including types of bonds, angles, and distance could be obtained. In addition, the CDOCKER was useful for investigating the conformational spaces of macromolecules in a variety of docking studies [13]. The LIBDOCK scores and CDOCKER scores for bindings energies of HSA and BSA with 5 OPPs are listed in Table 2. The listed value is for the best pose, which yielded the highest LIBDOCK score and the lowest CDOCKER score energy. It is important to note that a higher LIBDOCK score indicates a greater likelihood of ligand-protein binding, while a lower CDOCKER score energy signifies stronger interactions between the ligand and the receptor protein [14, 15]. From the table, HSA and BSA bound and docked with Qui pesticide produced the highest LIBDOCK scores of 104.3300 and 102.2930 kcal/mol, respectively. These results showed that HSA and BSA had the highest chances of binding with Qui. However, higher CDOCKER score energies were obtained for HSA and BSA bound to Qui (-15.8045 and -17.8045 Kcal/mol respectively), showing that these were weak interactions. On the other hand, HSA and BSA bound and docked with M-P pesticide produced LIBDOCK score values of 98.2648 and 96.241 kcal/mol, respectively, and recorded the lowest CDOCKER score energies of -37.8242 kcal/mol and -33.8916 kcal/mol, respectively, predicting higher chances of strong binding interactions.

Table 1. Types and active protein sites of interactions between OPPs and serum albumin.

Category	Sub-Category	Type
Hydrogen Bonds	Classical	Conventional Hydrogen Bond
	Non-classical	Carbon Hydrogen Bond
Electrostatic	π-Charge	π-Cation
	π-Charge	π-Anion
Hydrophobic	π-Hydrophobic	π-π Stacked
	π-Hydrophobic	π-π T-Shaped
	π-Hydrophobic	Amide-π Stacked
	Alkyl Hydrophobic	Alkyl & π-Akyl
Miscellaneous	Sulfur	π-Sulfur

Table 2. LIBDOCK scores and CDOCKER energies for each OPP ligand with serum albumin proteins.

Receptors	Ligands	LIBDOCK score (kcal/mol)	CDOCKER energy (kcal/mol)
Human Serum Albumin (HSA)	Dic	67.5472	-14.8792
	Gly	84.0947	-39.5649
	Metha	54.8645	-26.2827
	M-P	**98.2648**	**-37.8242**
	Qui	104.3300	-15.8045
Bovine Serum Albumin (BSA)	Dic	66.2565	-18.4987
	Gly	68.1576	-33.3355
	Metha	52.4225	-23.6889
	M-P	**96.2410**	**-33.8916**
	Qui	102.2930	-17.8045

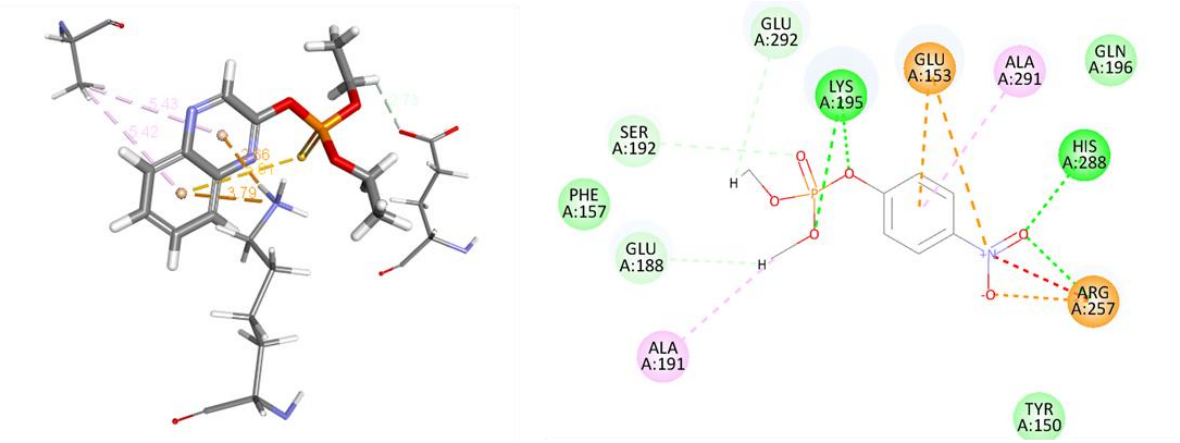

Figure 3. Interactions of M-P pesticide with HSA.

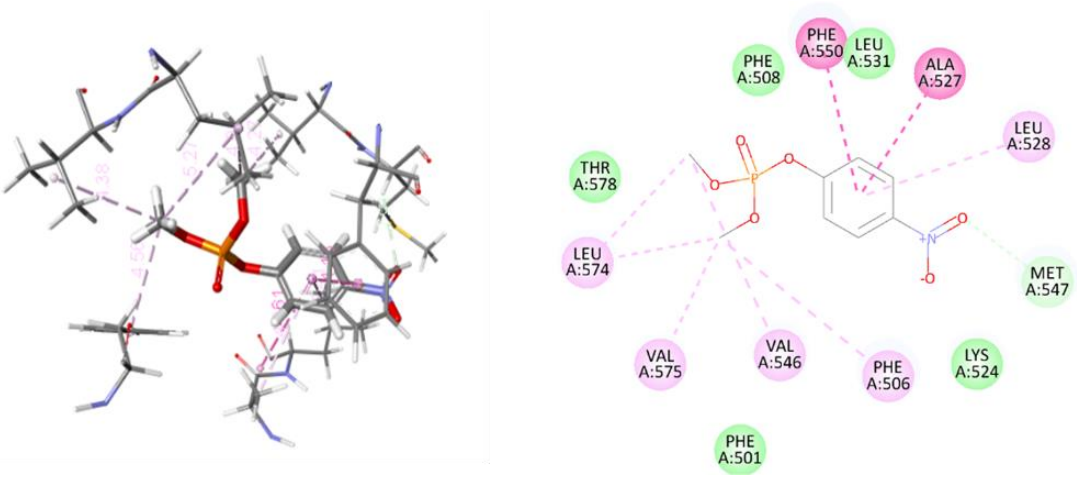

Figure 4. Interactions of M-P pesticide with BSA.

Predicted interactions between M-P with protein serum albumin (HSA and BSA) are shown in Figure 3 and Figure 4 respectively. It was expected that M-P formed two H-bonds at the active site of the HSA protein with three residues, LYS 195, ARG 257 and HIS 288. In addition, other interactions expected were the electrostatic bond π-anion interaction with two residues, GLU 153 and ARG 257, the hydrophobic bond alkyl interaction with two residues, ALA 291 and ALA 191, and Van der Waals forces with three residues, GLU 292, SER 192 and GLU 188.

Furthermore, M-P was expected to form hydrophobic bonds, amide π-stacked and alkyl interactions, and carbon hydrogen bonds at the active sites of BSA, as shown in Figure 4. The two residues for the amide π-stacked interactions were PHE 550 and ALA 527. For the alkyl interactions, five residues were connected between the pesticide and BSA: LEU 574, VAL 575, VAL 546 PHE 506 and LEU 528.

In both cases, strong interactions were expected to form by hydrogen bonds, of the electrostatic and hydrophobic category. In both cases, hydrophobic interactions were predicted and induced significant changes in biological activity [16, 17]. The predicted interactions and binding pockets of M-P pesticides with both proteins were similar, where the target ligand was at subdomain A despite different interactions predicted that favoured strong interactions in HSA-M-P. Previous studies described that HSA is made up of three homologous domains (I–III) that are joined by flexible loops and comprise subdomains A and B with helical folding patterns which easily trap the ligand [18-21]. The strong interactions between ligands and protein serum albumin play an important role in causing M-P to bind strongly with both protein serum albumins (HSA and BSA).

Based on these results, it can be concluded that the chemical structures of the OPPs affect their chemical interactions (results obtained from docking) with HSA and BSA. The chemical interactions between proteins (HSA and BSA) depended on steric compatibility, aromatic interactions, electrostatic interactions, the hydrogen bond network, hydrophobic effects and conformational changes. HSA/BSA-M-P was significantly influenced by the chemical structure of the pesticide. M-P contains a phosphoryl-oxygen double bond group (P=O), which is critical for its binding affinity to proteins. This particular group was more reactive due to the excellent electron withdrawing abilities of oxygen bound to phosphorus [22]. In addition, the existence of nitro groups (NO_2) which are also electron-withdrawing may enhance hydrogen bonding with polar residues of proteins [23], while alkyl chains may increase hydrophobic interactions with non-polar residues [24].

CONCLUSION

The binding mechanisms of five organophosphorus pesticides (Dic, Gly, Metha, M-P & Qui) with protein serum albumins (HSA and BSA) were studied using a molecular docking method to obtain LIBDOCK score values and CDOCKER score energies to predict the compounds' binding affinities with the target protein's active sites. The results showed high LIBDOCK score values and low CDOCKER energy values for M-P docked to proteins, which indicates a strong binding affinity is likely. The modes of interaction between HSA-M-P and BSA-M-P were mainly by H-bonds, Van der Waals forces, electrostatic bonds, and hydrophobic effects at the protein binding site (subdomain IIA) involving residues of ALA 291 and ALA 191 for HSA, and PHE 550, ALA 527, LEU 574, VAL 575, VAL 546 PHE 506 and LEU 528 for BSA. The data obtained for HSA and BSA is crucial to understand the binding characteristics of OPP with protein serum albumin, and can be used to explore applications such as sensors and decontamination materials.

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