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Modulating Immune System of Mosquitoes with A Novel Salicylate Derivative: A Bioinformatics-Based Study

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Abstract

The present study aims to compare the interactions of a novel derivative of salicylic acid, 5-(2-nitroethynl) salicylic acid (5-SANA, salicylic acid nitroalkene), and salicylic acid with the critical residues of mosquito's juvenile hormone binding protein (mJHBP). Given the better water solubility of 5-SANA and salicylic acid, it is believed that they can interact quicker than the natural substrate, juvenile hormone (JH), with the cavity of mJHBP, a circulating protein in mosquito's haemolymph. Prior research has shown that a knockout of mJHBP weakens the immune responses of mosquitoes against infections. The study aims to demonstrate the unique binding capability of 5-SANA with the mJHBP protein using computational methods, namely, the molecular docking followed by molecular dynamics simulations. The results revealed that 5-SANA molecule interacts with the key amino acid Tyr 129 of mJHBP, just like the JH molecule, and stabilizes the ligand-enzyme complex. The H-bond interaction of 5-SANA with Tyr 129 was maximally stable (99%) during the 100-nanosecond molecular dynamics simulation and thereby may prevent JH from binding. On the contrary, pure salicylic acid didn't exhibit H-bond interaction with Tyr 129 for the entire interval. It is also known that mJHBP protein plays a role in the gonotropic cycle of mosquitoes. This gives the hope that water-soluble 5-SANA may prove to be a birth control pill for preventing the proliferation of mosquitoes besides rendering the antibacterial defence defective during the developmental stages and affect the mechanisms underlying haematopoiesis and so lays the ground for further studies leading to the alleviation of suffering of mankind.

Keywords: 5-SANA; mJHBP; salicylic acid; MD simulation; Mosquito

INDTRODUCTION

Mosquitoes have developed powerful protection against a wide range of infections. However, they can also be the victims of many entomopathogenic infections caused by pathogens. As the responses to these infections are one-to-one adaptations, the expression and diversity of immunity and infection response genes are often different. Therefore, targeting the response mechanism found most commonly in all mosquitoes has become a prerequisite. Juvenile hormone (JH) is a key regulator of insect development and reproduction. The interaction of JH with mosquito juvenile hormone-binding protein (mJHBP) plays a critical role in insect reproduction [1]. mJHBP is present in the hemolymph of pupal and adult male and female mosquitoes, such as *Aedes aegypti,* and is a *member* of the odorant-binding protein (OBP) family. The immune system of insects gets activated in response to some stimuli through induced receptor activation to produce molecules such as antimicrobial peptides (AMPs) through various pathways.

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The physiological processes of mosquitoes are regulated by hormones. JH plays a vital role in antibacterial immunity [2]. JH is synthesized by the corpora allata near the brain and transported in the haemolymph by the circulating JH binding protein, mJHBP. *Research studies, through CRISPR-Cas9 gene editing technology, have shown that the lack of production of mJHBP weakens the immune system responses towards bacterial infections* [3].

EXPERIMENTAL

To understand the regulatory mechanism of the interaction between JH and mJHBP, a new water-soluble derivative of salicylic acid, 5-(2-nitroethynl) salicylic acid (5-SANA), along with salicylic acid, was studied computationally as an inhibitor for mJHBP. The novel molecule, 5-SANA, has been reported as a weight loss-promoting molecule and safe through phase 1 clinical trials [4].

Molecular docking studies of ligand and biomolecules depict the ligand's principal binding modes with the biomolecule in three-dimensional conformations. This helps to identify the critical residues participating in ligand binding and the efficiency of ligand binding [5]. In the present study, the selected ligand molecules, viz., salicylic acid and 5-SANA, were used to study their binding modes with the identified target, mJHBP protein. For the study, the structure of ligand molecules was collected from the PubChem server and was prepared for molecular docking studies under the OPLS4 force field at pH 7.2 ± 0.2 using the LigPrep module of the Schrödinger suite 2021-2 [6].

The crystal structure of mJHBP protein was collected from the PDB database (PDBID: 5V13) and submitted for the prep-wizard of the Schrödinger suite 2021-2 for structure optimization. The pre-processing was done at physiological pH (7.0 ± 2.0) using the Epik module. The structure was then energy minimized by protonating the ionizable protein groups using the PROPKA module at pH 7.0 with the OPLS4 force field [7]. The grid box centre was set by picking the JH molecule present in the crystal structure of 5V13 [1]. The best docked conformation was selected by observing the docking score and glide energy.

MD simulations were carried out to understand the structural stability of mJHBP upon interacting with the selected ligands. MD simulations were performed using the Desmond module implemented in the Schrödinger suite 2021-2. The MD simulation system was built using the SPC solvent model with the OPLS3e force field [8]. The periodic boundary conditions were set using a 10 Å water buffer around the protein in an orthorhombic simulation box. The unit cell was neutralized using Na^+ and Cl^- ions. The NVT ensemble was run at 300K, and the interactions were observed for a time scale of 100 nanoseconds, and the key binding residues that stabilized the binding were observed [9–11].

RESULTS AND DISCUSSION

The molecular docking studies identified the best binding conformation of ligand molecules with the mJHBP target. A negative Glide score of -7.415 by 5-SANA indicated the release of free energy, making the reaction spontaneous. The binding was compared with salicylic acid, with the Glide score of -6.571. Both the molecular interactions involved the participation of Trp53 and Tyr129 residues, though Tyr129, the only key residue chosen by JH in the crystal structure deposited. Further, the shape of the binding pocket in both interactions suggests the preferential selection of 5-SANA over salicylic acid (Figure 1 A & B).

The Root mean-square deviations observed in the molecular dynamics simulation studies indicated the stable binding of 5-SANA with the mJHBP target. There were no drastic conformational variations observed in terms of RMSD throughout the time scale (Figure 2A). The stability of the binding was mainly maintained by hydrogen bonds formed between the 5-SANA and the target protein. Such hydrogen bonding interactions were in line with the molecular docking studies. Amino acid Tyr 129 interacted with 99 % stable hydrogen bond, while Tyr33 interacted with 98% stability (Figure 2B).

Trp53 and Tyr133 displayed hydrophobic interactions with the target protein (Figure 2C). Further, Tyr129 had a constant H-bond contact with the 5-SANA throughout the time scale (2D).

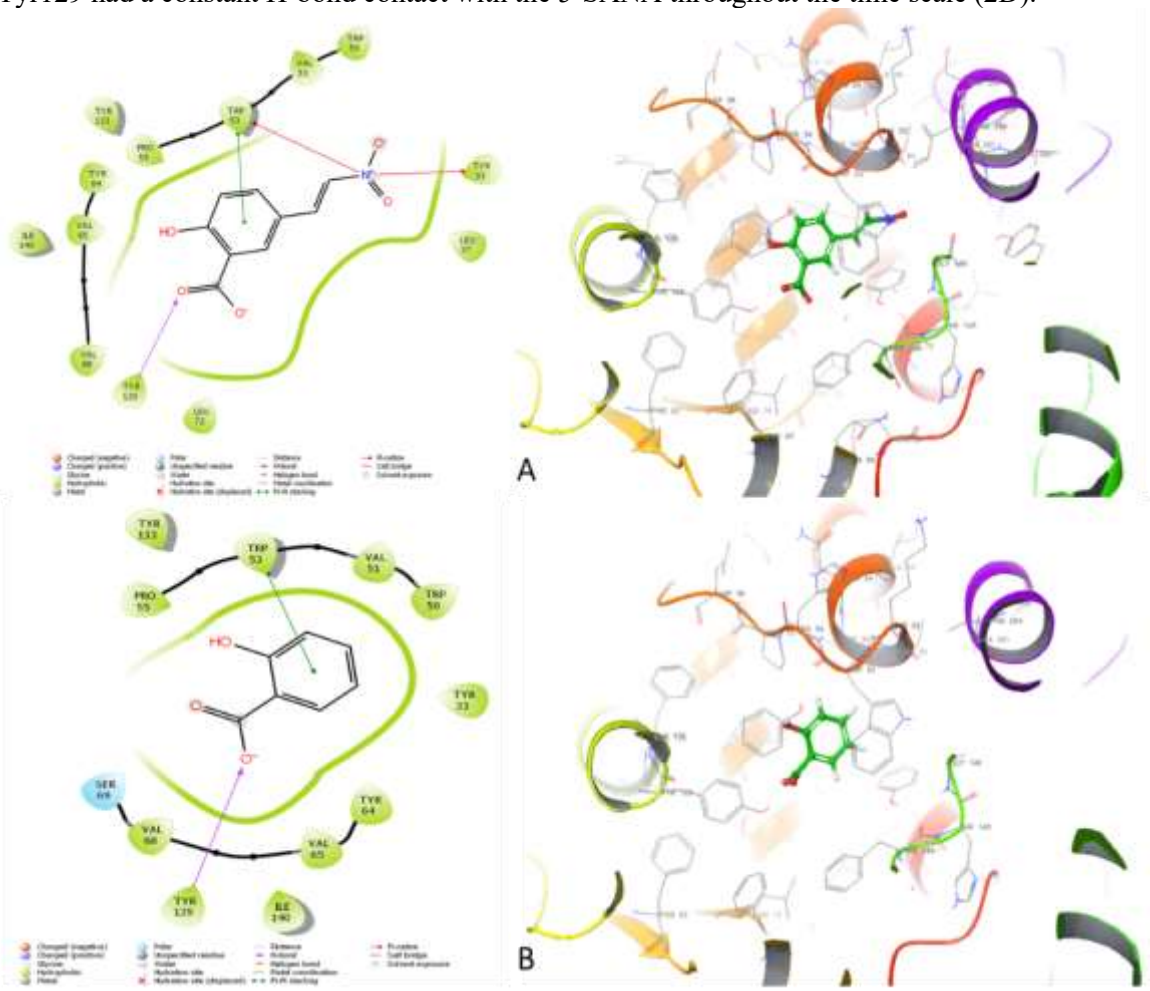

Figure 1.A Binding interactions of 5-SANA (A) and Salicylic acid (B) with mJHBP.

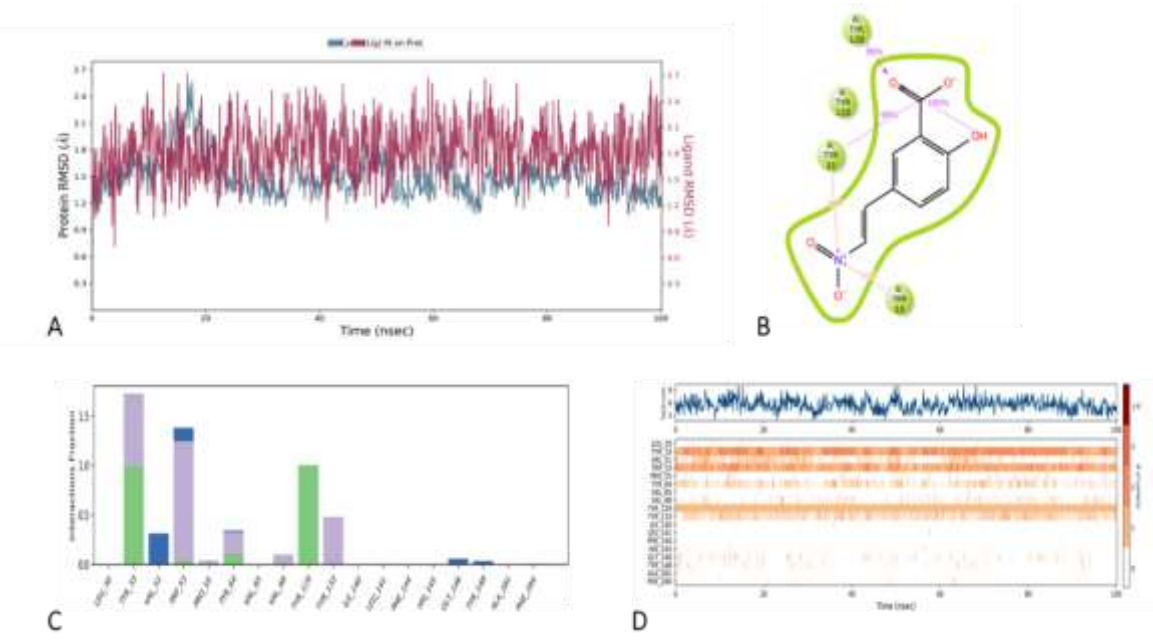

Figure 2. MD simulation studies of 5-SANA-mJHBP complex illustrating the RMSD (A), ligand binding (B), contacting residues (C) and number of contacts (D).

Similar parameters were studied by the MD simulations of the salicylic acid-mJHBP target complex. No major structural deviations were observed in the salicylic acid-mJHBP complex in terms of RMSD (3A). However, in contrast to the molecular docking interactions, amino acids Ser69 (99%) and Leu74 (94%) formed the stable hydrogen bond (3B) and in addition, Arg73 and Tyr75 were also involved in intermediate hydrogen bond formation with the salicylic acid. The H-bond with Tyr 129 was completely missing for the entire time scale but was substituted by hydrophobic interactions. (Figure 3C & 3D).

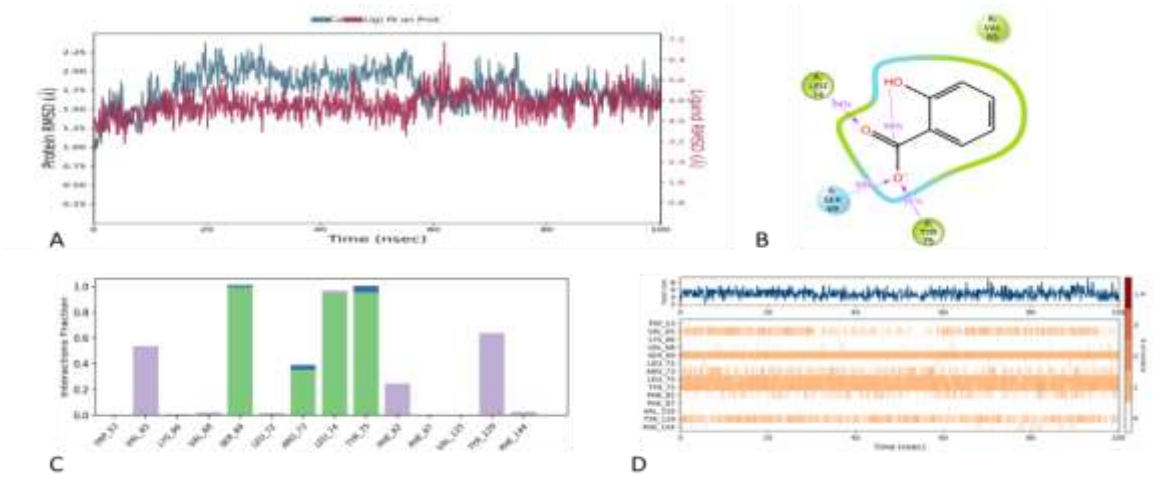

Figure 3. MD simulation studies of salicylic acid-mJHBP complex illustrating the RMSD (A), ligand binding (B), contacting residues (C) and number of contacts (D).

CONCLUSION

The findings from the current computational studies suggest a stronger preference of 5-SANA as a substrate for mJHBP enzyme, 5-SANA can bind sooner than JH due to its ` water solubility and possibly create a ground for weakening the immune system of mosquitoes. The real time experiments in this direction are expected to yield promising findings.

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