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Binding Interactions of Psilocin and Serotonin in the 5-HT_{2A} Receptor

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Abstract

Psilocin is a molecule found in psilocybin mushrooms, which are typically consumed recreationally for their hallucinogenic effects. Recently, studies have shown that psilocin can have almost immediate antidepressant effects in patients who are treatment-resistant to medications that increase serotonin levels in the synapse. Researchers believe that the molecule works by suppressing activity in the medial prefrontal cortex and amygdala, which are both brain structures involved in the emotional aspect of depression. However, psilocin's exact mechanism of action and binding characteristics in the body remain unknown. Using Chimera for visualization and AutoDock Tools and AutoDock Vina for docking, psilocin and serotonin were separately docked in a crystallized 5-HT_{2A} receptor. Key residues were identified using existing information in the RCSB database. Once the ligands were docked, the lengths of the potential bonds between atoms of the ligands and the key residues within the receptor were measured to determine if they were close enough to each other to interact. Serotonin had multiple possible hydrogen bonds and hydrophobic interactions; however, psilocin only had one potential hydrophobic interaction. The main structural difference between psilocin and serotonin is the presence of the phosphate group in psilocin; therefore, studies of phosphate's binding properties within the 5-HT_{2A} receptor could potentially provide insight on the efficacy of psilocin.

Introduction

Psilocin, the active form of psilocybin, is a compound found in psilocybin mushrooms (Carhart-Harris et al.). Popularly known as a psychedelic, psilocin has shown therapeutic potential for individuals with treatment-resistant depression (Carhart-Harris et al.). There are limited studies on psilocin because of the hazards associated with hallucinations; however, some researchers have determined effective doses that are safe for patients (Mahapatra et al.). Psilocin is not meant for long-term use; therefore, the safe dose was a one-time 10 mg dose the first week and a one-time 25 mg dose the second week (Mahapatra). Another team of researchers gave their test subjects psilocin using a 0.6 milligrams per kilogram dose, which would be approximately 33 mg for the average-sized adults, and there were no serious adverse side effects (Brown et al.).

Currently, there are multiple classes of antidepressants including monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and serotonin and dopamine reuptake inhibitors (SDRIs). While some of the drugs block the action of monoamine oxidase, an enzyme responsible for the decomposition of monoamine neurotransmitters, others inhibit the reuptake mechanism. In general, these medications work by increasing the synaptic levels of the neurotransmitters implicated in depression. SSRIs target serotonin, SNRIs affect serotonin and norepinephrine, and SDRIs raise dopamine and serotonin levels. Since the exact mechanism of depression is unknown, physicians typically try the different types of antidepressants on patients until one leads to improvement. In some cases, individuals are prescribed multiple antidepressants that belong to different classes. Unfortunately, these conventional antidepressants take almost a month to be effective (Mahmoudi et al.). For some patients in the

midst of a major depressive episode, one month is too long. Absence of immediate care can result in suicide, which insinuates the need for a fast-acting antidepressant.

Preliminary research has suggested that psilocin works as an agonist for the 5-HT_{2A} receptor, which is a member of the serotonin receptor family of proteins (Spain et al.). It is proposed that this binding interaction leads to inhibition of the medial prefrontal cortex and amygdala, which are both brain structures implicated in depression. Specifically, fMRIs of individuals diagnosed with depression show heightened activity in these regions of the brain; therefore, the inhibitory effects of psilocin provide therapeutic potential (Mahapatra et al.).

Moreover, selective serotonin inhibitors, along with the other standard antidepressants, are only indirect agonists of the 5-HT_{2A} receptor, meaning they do not directly bind to the receptor (Carhart-Harris et al.). They potentiate the receptor by increasing levels of serotonin, dopamine, and/or serotonin in the synaptic cleft. The lack of direct binding to the receptor could account for the one month period before SSRIs, SNRIs, and SDRIs become effective. In contrast, psilocin binds directly to 5-HT_{2A} (Carhart-Harris et al.), which could explain its fast-acting properties (Dunn et al.).

While the direct binding can potentially account for the efficiency of psilocin, the reason behind the success of the compound in treatment-resistant patients is unknown. Even after one month of taking various standard antidepressants, some patients' depressive symptoms never improve, suggesting that the monoamine hypothesis is not applicable to everyone. If psilocin associates with 5-HT_{2A} in a similar manner as norepinephrine, serotonin, and dopamine, the antidepressant effects of psilocin would mimic those of the classic monoamine neurotransmitters after the one month period. Perhaps, the explanation behind the efficacy of psilocin lies within the binding interaction itself. Using docking and molecular visualization software, I will

separately dock serotonin and psilocin into the 5-HT_{2A} receptor to determine if more stabilizing interactions occur between psilocin and the key resides within the 5-HT_{2A} receptor in comparison to serotonin and the key residues within the 5-HT_{2A} receptor. The key residues are Tyrosine 370, Aspartate 155, and Tryptophan 336. Dopamine and norepinephrine will not be docked in this study because they are the less common neurotransmitters implicated in depression. Serotonin is almost always one of the targets in antidepressant medications. In addition to providing insight into the mechanism of action for psilocin, this research can contribute to the scientific community's understanding of the basis of depression.

Methods

Structure Model Selection

The PDB structure used for the 5-HT_{2A} receptor was 6A93, and its co-crystallized ligand was risperidone, also known as 8NU (Kimura et al.). Tyrosine 370, Aspartate 155, and Tryptophan 336 were identified as the key residues within the 5-HT_{2A} receptor using the "Ligand Interaction" tool on the RCSB website. In order to be considered a key residue, the amino acid had to show a dotted line connection to risperidone in the tool viewing window.

Crystal Structure Assessment

To determine if AudoDock Tools could predict the correct position of the ligand within the binding cavity of the 5-HT_{2A} receptor (Morris), the re-docking process was employed (Lewis). Using the Chimera software (Pettersen), Chain B of the 5-HT_{2A} receptor structure was removed. In addition, all of the ligands except risperidone were removed. The ligand and receptor were saved as separate PDBs.

The PDB of the ligand was opened in AutoDock Tools, and the root for the ligand tree was established. Next, the PDBQT version of the ligand, which includes the charges on the ligand, was saved. Then, the PDB file of the receptor was opened and saved as a PDBQT.

To establish the grid parameters, the box was centered on the ligand. The spacing was set to 1.0 angstrom. The center coordinates for x, y, and z were 14.321, -1.194, and 60.438 respectively. There were 20 points in the x-dimension, 22 points in the y-dimension, and 30 points in the z-dimension. Using the coordinate values, docking was done using AutoDock Vina (Trott).

Once docking was complete, the output file was opened in Chimera. With the exception of the key amino acid residues, the visible amino amino acids were removed for visualization purposes. The co-crystallized version of the ligand as well as the docked poses of the ligand were superimposed within the binding cavity of the 5-HT_{2A} receptor to determine if the software was able to predict the conformation of the ligand in the cavity. The pose that was most similar to the co-crystallized ligand was selected and saved.

Docking Serotonin and Psilocin

The 2D structures for serotonin (“Serotonin”) and (“Psilocin”) were downloaded from the PubChem database. The ligands were docked separately, following the same process. The ligands were saved as separate PDB files in Chimera. The root for each ligand tree was established, and each ligand was saved as a PDBQT file. Next, each ligand was docked using AutoDock Vina. The same grid parameters used in the re-docking step were used to dock serotonin and psilocin. The different outputs were opened in Chimera, and the most energetically favorable pose for each ligand was saved.

Identifying Ligand Interactions with Key Residues

Tyrosine 370, Aspartate 155, and Tryptophan 336 were displayed in the binding cavity. Separately, the docked poses of serotonin and psilocin were displayed within the receptor. In order for a hydrogen bond to occur, the distance between the hydrogen bond donor and acceptor must be 3.3 angstroms or less. For a hydrophobic interaction to be possible, the bond cannot exceed 3.8 angstroms in length. To determine if interactions were possible between the ligands and the receptor, the distance between atoms of the ligand and the key residues were measured using the "Structural Analysis" tool in Chimera.

Results

Re-docking of Risperidone

The 5-HT_{2A} structure that has co-crystallized risperidone (PDB ID 6A93) (Kimura) was used for re-docking because risperidone is a known drug that binds to the serotonin receptor. The most energetically favorable pose did not match the original ligand; therefore, the 8^{th} pose was used for comparison to the co-crystallized ligand. Sometimes the most energetically favorable pose is not similar to the co-crystallized ligand because the human body is unpredictable and often favors other aspects of a molecule other than its most energetically favorable position. The docked pose and co-crystallized ligand aligned with only slight discrepancies; therefore, this structure was used for further studies.

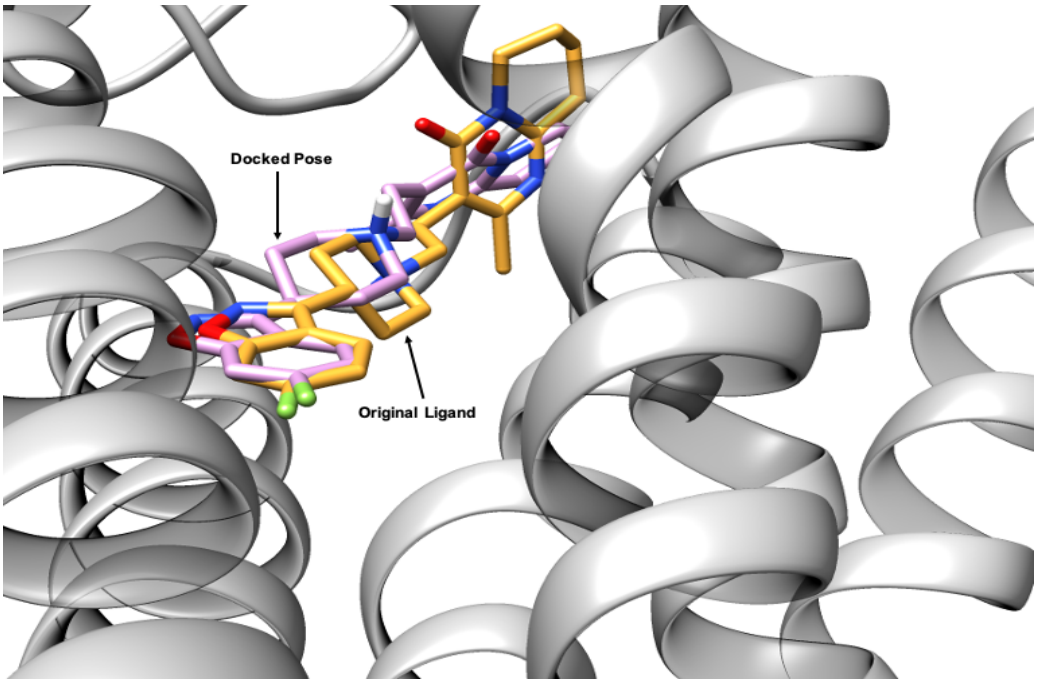

Figure 1: Docked pose of risperidone (pink) with original co-crystallized version of the ligand (orange). Atom-specific coloring key: blue = nitrogen; red = oxygen; white = hydrogen; green = fluorine.

Serotonin Docked in 5-HT_{2A} Receptor

The docked pose of serotonin suggested that there were three possible hydrogen bonds that can form between the ligand and two of the key residues. In addition, a hydrophobic interaction potentially occurred. Specifically, there is one potential hydrogen bond between Tyrosine 370 and serotonin, and Aspartate 155 is capable of forming two hydrogen bonds with serotonin because the bond lengths are less than 3.3 angstroms (Figure 2). There is a possible interaction between Tryptophan 336 and serotonin because the threshold for hydrophobic

interactions is 3.8 angstroms (Figure 2). Helices 2 and 7 are important in this binding cavity because the key residues are attached to them.

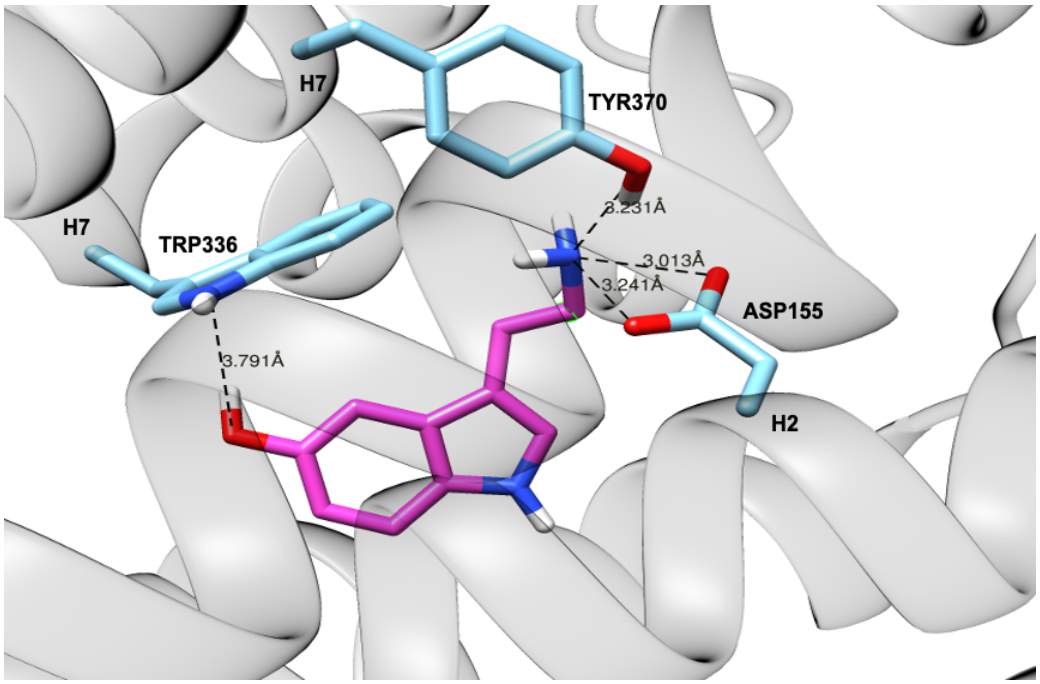

Figure 2: Distance measurements suggesting potential interactions between serotonin (pink) and the key residues of the 5-HT$_{2A}$ receptor (aqua). Atom-specific coloring key: nitrogen = blue; red = oxygen; white = hydrogen. Potential hydrogen bonds are represented by the black dotted lines.

Psilocin Docked in 5-HT$_{2A}$ Receptor

While psilocin has proven to be an effective antidepressant through activation of the serotonin receptor, the mechanism of action is not understood. Docking of the psilocin compound into the 5-HT$_{2A}$ receptor did not match the docked position of serotonin. When the distances between the atoms of the psilocin and the receptor were calculated, there was no evidence for possible hydrogen bonds. There is only one potential hydrophobic interaction between Aspartate 155 and psilocin because the distance between the selected atoms was 3.737 angstroms. Perhaps, there are more key residues that were not able to be identified with the

"Ligand Interaction" tool. The major difference between serotonin and psilocin is the presence of the phosphate group on the psilocin. The phosphate group is too far away from the key residues to form a bond. There were no ligands with phosphate in a 5-HT_{2A} receptor on the RCSB website; therefore, those key residues are unknown.

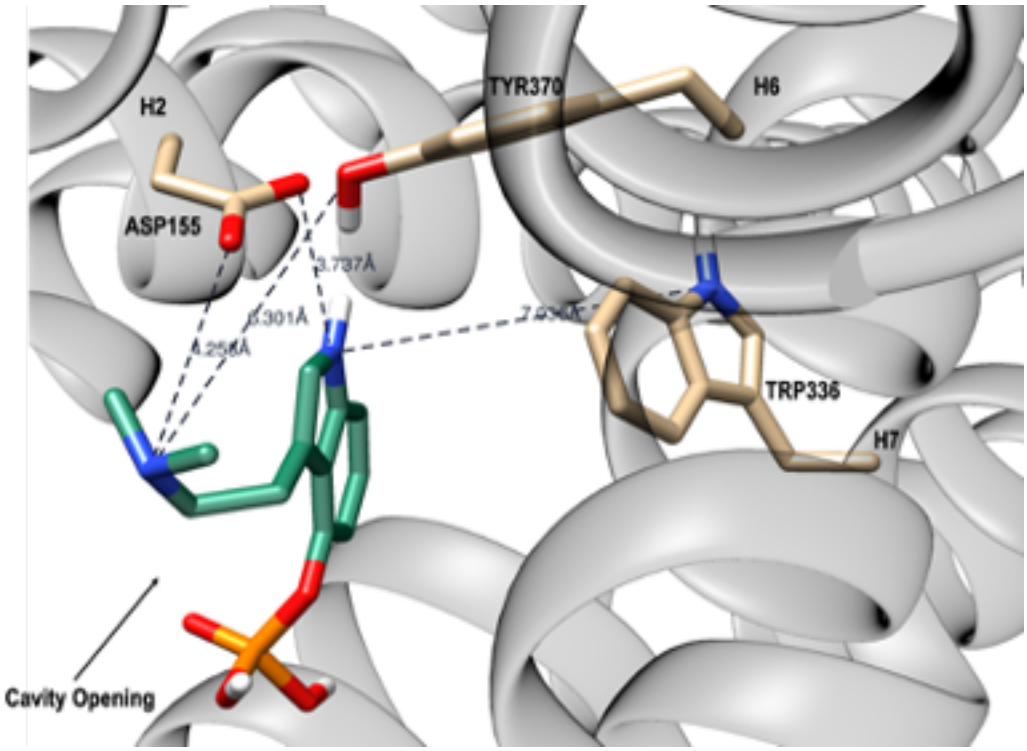

Figure 3: Potential interactions between psilocin (green) and key residues (tan) of the 5-HT_{2A} receptor. Atom-specific coloring key: nitrogen = blue; red = oxygen; white = hydrogen. The bond lengths are greater than 3.3 angstroms, indicating that hydrogen bonds between the ligand and receptor are not possible.

Conclusions

In contrast to the hypothesis, serotonin had more potential hydrogen bonds and hydrophobic interactions than psilocin. This could be attributed to the fact that 5-HT_{2A} is considered to be a receptor within the serotonin family. Evidently, this study showed that the efficacy of psilocin is likely not related to its interactions with Tyrosine 370, Aspartate 155, and Tryptophan 336. It is possible that the phosphate group component of psilocin is responsible for the interactions; however, there are not ligands with phosphate groups that bind to the 5-HT_{2A} receptor available in the RCSB database. Furthermore, the antipsychotic drugs, like Seroquel, that are known to bind to the 5-HT_{2A} receptor, do not contain phosphate groups either ("Seroquel").

In addition, the receptor is a G coupled-protein receptor; therefore, a signaling cascade begins after binding occurs (Narendra). Perhaps, psilocin has a unique effect on the signaling cascade that is responsible for its antidepressant effects.

For future studies, all amino acids on the 5-HT_{2A} receptor in proximity to the phosphate group of psilocin should be analyzed using the "Structural Analysis" tool in Chimera. Perhaps, there are key residues that only interact with the phosphate group instead of the other atoms in psilocin. In addition, several antipsychotic drugs are known to bind to the receptor (Kimura). Docking each of the drug molecules could provide insight into more key binding interactions that are occurring.

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