In Silico Prediction for the Binding Of AMPA-Receptor with Glutamate in Owl and Falcon

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Abstract

The AMPA-receptor is a subtype of the ionotropic glutamate receptor that is linked to ion channels and can control cell excitability by gating calcium and sodium ions into the cell.This receptor affinity to bind with glutamate may be different between owl and falcon and affect their ability to transfer glutamate neurotransmitters to cells. This study was conducted to predict the biological affinity of both owl and falcon AMPA-receptors to bind with glutamate and to assess the possible consequences of this binding on the vision strength in both birds.AMPA-receptor 4 isoform-X1 primary structures of both owl and falcon were retrieved from NCBI. Both amino acid sequences were compared to find out the ratio of homologous sequences. Subsequently, 3D structures were generated for both proteins and superimposed to assess the ratio of homologous domains. Molecular docking reactions were performed between both AMPA-receptors with glutamate to assess the strength and mode of interaction between this ligand and both proteins. Root mean square fluctuations (RMSF) for both docked complexes were conducted to compare the stability of each receptor upon being docked with glutamate.it was shown that both investigated receptors hared considerable similarity in their amino acid sequences as only a few differences were found. 3D impositions showed a relatively low level of differences between both proteins. Molecular docking reactions showed that such differences were sufficient to induce considerable differences in docking strength and patterns of interaction between glutamate and these receptors. Glutamate was bound with owl AMPA-receptor with a higher docking score (-5.0 kcal/mol) than that found in the docked AMPA-receptor/glutamate complex (-4.3 kcal/mol). These results were confirmed by RMSF values that showed more fluctuations of flacon AMPA-receptor complex than that found in owl AMPA-receptor complex.our study showed that owl AMPA-receptor can bind with glutamate with more efficiency compared with falcon AMPA-receptor whether in terms of the strength and mode of binding. This higher affinity induces better transmission to glutamate throughout the cell membranes. Accordingly, owls may exhibit a better vision than that found in falcons. Further *in vitro* studies are suggested to provide a more in-depth interpretation for this finding.

Keywords: Falcon, Owl, AMPA-receptor, glutamate, aminoacid.

1. Introduction

The conformational search was performed using the coarse-grain-based MD method at 300 K using the Langevin thermostat. The total number of steps and other settings were left at default to ensure a better simulation of the system. MD runs were used to anticipate root mean square variation (RMSF). The result files were retrieved from the UNRES website, and the simulations were shown and commented on using Microsoft Excel 2016.. Other specialized cells termed glial cells (or simply glia) give structural and metabolic support to neurons in the nervous system (Tortora and Derrickson, 2016 ; Ari Kristina and Hanifah, 2021). Electrically, through gap junctions, and chemically, through neurotransmitters, cells communicate with one another.

Chemical synaptic transmission permits nerve impulses to be transferred between cells that are not electrically connected. The chemical messenger, or neurotransmitter, is responsible for transmitting the signal from the presynaptic neuron to the postsynaptic cell across the extracellular space.In the CNS1, glutamic acid (Glu) is the primary excitatory neurotransmitter. (Glu) is an amino acid that does not penetrate the blood–brain barrier and is thus non-essential. It is made from glucose and numerous precursors in the mitochondria of neurons. (Glu) is produced and then released into the cytoplasm, where it accumulates in synaptic vesicles via a Mg2+/ATP-dependent mechanism. Exocytosis, a process that relies on intracellular Ca2+, stimulates the release of (Glu) from the synapse when the nerve impulse propagates towards the axon terminal. The particular receptors for (Glu) can then engage with it (Flores-Soto, et. al.,2012; Phuc, et. al.,2021)

The principal mediators of excitatory synaptic transmission in the brain are glutamate receptors (GluRs). AMPA receptors (AMPARs), other ionotropic glutamate receptor (iGluR) family members, N-methyl-D-aspartate receptors (NMDARs), and kainate receptors are all cation permeable receptor tetramers (NMDARs and KARs)(Yi, et. al.,2019; Kumar, et. al.,2021)

In computer-aided drug development, in silico identification for possible hits has become a common method. This approach can aid with bioactivity rationalization or optimization by narrowing the search for a prospective lead chemical from a vast the quantity of compound databases, selecting possible hits via high-throughput molecular docking, or understanding potential hits' mechanistic interactions. The use of a molecular docking technique in the creation of new inhibitors is highlighted in this paper, with possible antivirals for dengue illnesses as examples [8, 9] and use in component of plant [10].

In 2017 Mahendran,R., andhis team study in silico treatment Alzheimer's disease [11] while Tamilanban T and et. al. in 2020 used this software to detect protein its responsible for Alzheimer's disease [12] also have been used in study of the breast cancer [13].

2. In silico Methods

Protein structure designing

The primary structure of both AMPA-receptors for both owls and falcons were conducted by retrieving the referring amino acid sequences of both receptors. The whole amino acid sequences of both receptors were retrieved online from the national center for biotechnology (NCBI) server (http://www.ncbi.nlm.nih.gov). After the retrieval of both proteins, the entire amino acid sequences were compared with each other by their alignment using the Edit Seq tool ver. 7.1.0 (DNA STAR, Lasergene). Subsequently, the 3D structures of both AMPA-receptors were generated by Phyre2 (protein homology/analogY recognition engine), an online three-dimensional model prediction software [10]. The virtual visualizations of both receptors were performed by using PyMOL-v1,7.0.1 software (www.shrodinger.com).

Molecular docking

Before conducting docking reactions, the 3D conformer of glutamate was retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). After obtaining the required 3D confirmers in the form of SDF structure of glutamate (PubChem CID_4525487), both 3D structures of owl and falcon receptors were docked with this ligand using AutodockVina inbuilt in PyRx software [14, 15, 16]. The virtual screening conditions were the same for both investigated ligands, in which a grid box of was set in include the entire AMPA-receptor in both cases. After completing docking experiments, protein/ligand interactions were ranked according to binding strengthobserved in both cases, and results calculated in kcal/mol. Each protein/ligand was considered for downstream analyses to unmask the pattern of such interactions with further details.

Interactions of the ligand with critical amino acid residues

The atomic interactions between protein/ligand complexes were conducted in both owl and falcon AMPA-receptors. The binding energy of the best binding pose of each docked protein-ligand complex was visualized to discover the pattern of these ligand-amino acid residues' interactions. Using BIOVIA Discovery Studio software, several kinds of AMPA-receptor/glutamate interactions were assessed and described. Further 2D and 3D graphical representations of a variety of interactions of best binding poses were analyzed, and detailed interactions were shown.

MD simulation

The 3D files of the targeted AMPA-receptors were tailored to comprehend dynamic behavior after being conjugated with the glutamate ligand since they were static and devoid of hydrogen atoms. Molecular dynamic (MD) simulations of AMPA-receptors with glutamate were performed using UNRES server 12 to examine the dynamic stability of the AMPA-receptors when coupled with this ligand. The coarse-grain-based MD approach was done at the temperatures of 300 K using the Langevin thermostat that was used to execute the conformational search. To ensure a better simulation of the system, the total number of steps and other settings were kept at default.. To forecast root mean square fluctuation, MD runs were done (RMSF). The UNRES server was used to retrieve output files, and Microsoft Excel 2016 was used to display and comment simulations.

3. Results and Discusion

initial primary amino acid residues comparisons were performed between the investigated owl and falcon AMPA-receptors. Results found that both types of receptors shared a high ratio of homology in almost all aligned sequences. However, only a few exceptions were observed in both cases. Concerning owl receptors, it was found that this protein consisted of more amino acid residues than that found in its falcon counterparts. Owl AMPA-receptor has 902 amino acid residues, while falcon AMPA-receptor has 732 amino acid residues. Moreover, it was found that the amino acid Val residue that positioned in 353 of owl receptor was substituted with Ile in falcon counterpart (Figure 1).

10 20 30 40 50 60 70 80 90 100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
MRIICRQLVLLFSGFWGLAMGAFPSSVQIGGLFIRNTDQEYTAFRLAIFLHNTSPNASEAPFNLVPHVDNIETANSFAV
TNAFCSQYSRGVFAIFGLYDK
AMPA-receptor, falcon-
110 120 130 140 150 160 170 180 190 200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
RSVHTLTSFCSALHISLITPSFPTEGESQFVLQLRPSLRGALLSLLDHYEWNRFVFLYDTDRGYSILQAIMEKAGQNSW
QVSAICVENFNDASYRRLLED
AMPA-receptor, falcon-
210 220 230 240 250 260 270 280 290 300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
LDRRQEKKFVIDCEIERLQNLLEQIVSVGKHVKGYHYIVANLGFKDISLERFMHGGANVTGFQLVDFSTPMVTKLMQ
RWKKLDQREYPGSETPPKYTSAL
AMPA-receptor, falcon.
310 320 330 340 350 360 370 380 390 400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
TYDGVLVMAETFRNLRRQKIDISRRGNAGDCLANPAAPWGQGIDMERTLKQVRIQGLTGNVQFDHYGRRVNYTM
DVFELKNTGPRKVGYWNDMDKLVLIQ
AMPA-receptor, falcon. I
410 420 430 440 450 460 470 480 490 500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
HEPTLGNDTSAIENRTVVVTTILEAPYVMFKKNHDTFEGNDKFEGYCVDLASEIAKHIGIKYKIAIVPDGKYGARDPET
KIWNGMVGELVYGKAEIAVAP
AMPA-receptor, falcon
510 520 530 540 550 560 570 580 590 600
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
LTITLVREEVIDFSKPFMSLGISIMIKKPQKSKPGVFSFLDPLAYEIWMCIVFAYIGVSVVLFLVSRFSPYEWHTEEPEDGK
EGPSDQPPNEFGIFNSLW
AMPA-receptor, falcon
610 620 630 640 650 660 670 680 690 700
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
FSLGAFMQQGCDISPRSLSGRIVGGVWWFFTLIIISSYTANLAAFLTVERMVSPIESAEDLAKQTEIAYGTLDSGSTKEF
FRRSKIAVYEKMWTYMKSAE
AMPA-receptor, falcon
710 720 730 740 750 760 770 780 790 800
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
PSVFTRTTAEGVARVRKSKGKFAFLLESTMNEYIEQRKPCDTMKVGGNLDSKGYGVATPKGSPLRNAVNLAVLKLNE
QGLLDKLKNKWWYDKGECGSGGG
AMPA-receptor, falcon
810 820 830 840 850 860 870 880 890 900
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AMPA-receptor, owl
DSKDKTSALSLSNVAGVFYILVGGLGLAMLVALIEFCYKSRAEAKRMKLTFSEAIRNKARLSITGSVGENGRVLTPDCP
KAVHTGTAIRQSSGLAVIASD
AMPA-receptor, falcon
AMPA-receptor, owl LP
AMPA-receptor, falcon..

Figure 1: Amino acid alignment between AMPA-receptors of owl and falcon using Edit-seq tool of LASER GENE software. colored dots refer to the similarity between both aligned proteins, and dashes refer refers to the absence of any homologous sequences. Single code of amino acids used in the alignment tool.

Using the Phyre2 modeling tool, the 3D structures of both owl and falcon AMPA receptors were generated.Both 3D structures appeared to be similar in terms of their 3D arrangements with few differences (Figure 2A). However, to discover the pattern and the extent of these differences, the root mean square deviation (RMSD) values of both investigated proteins were estimated. Due to the importance of RMSD value in the determination of the extent of protein differences, both proteins were aligned using the PyMol tool. The observed RMSD value was found to be -2.304 (Figure 2B). When the RMSD value is zero, both proteins have an entire fitness between each other. Thus, the RMSD value of -2.304 has indicated the presence of no big 3D differences between both proteins as both of them shared a high ratio of similar domains [17, 18].

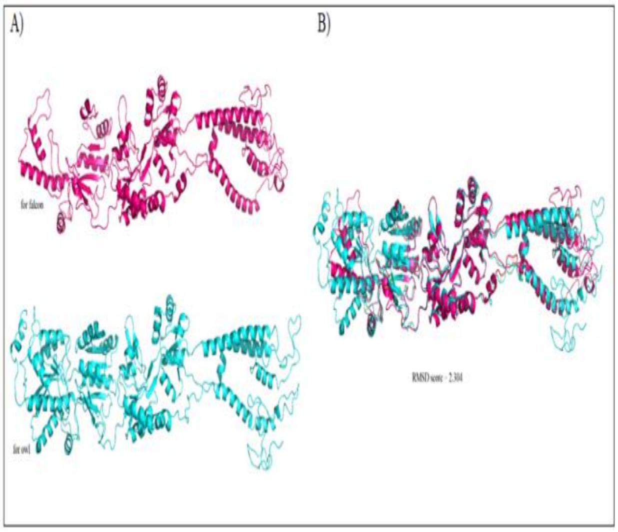

Figure 2: Three-dimensional structure and superimposition of glutamate receptor 4 isoform X1 of falcon and owl. A) 3D structure of glutamate receptor 4 isoform X1 for falcon and owl respectively. B) Superimposition of both receptors using the PyMol tool. falcon and owl receptors were shown in pink and cyan colors respectively.

After generating a 3D structure for the entire proteins of AMPA-receptors for owl and falcons, both involved receptors were docked against glutamate to determine the strength and mode of interactions with this neurotransmitter. Molecular docking reactions conducted between owl AMPA-receptor and glutamate indicated that glutamate can bind with AMP-receptor with a docking score of -5.0 [19]. This docking score of such ligand-receptor complex was generated by two types of bonding reactions; hydrogen bonds and slat interactions. With regard to hydrogen bonds, four hydrogen bonds were formed between glutamate and four different amino acid residues [20]. The involved amino acid residues with the direct hydrogen bond interactions are Asn36, Thr37, As70, and Tyr287 (Figure 3). In addition to the strong hydrogen bonds observed between glutamate and four amino acid residues, two salt bridges were formed between glutamate and Asp38 too. In the salt bridge case, both donor and acceptor atoms are fully charged [21, 22]. Thus, the bonding energy observed between ligand and receptor is significantly higher than that observed in hydrogen bonds in which only one involved atom is fully charged or both donor and acceptor are partially charged.

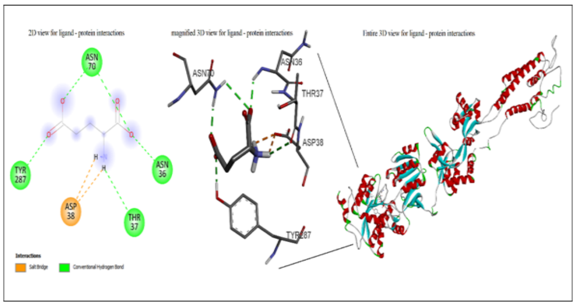

Figure 3: Docking between owl AMP-receptor and glutamate using AutodockVina. Both 2D and 3D ligand-receptor interactions were shown. The docking score between ligand (glutamate) and owl AMP-receptor is -5.0 kcal/mol.

Compared with owl AMPA-receptor, falcon AMPA-receptor had showna low docking score with glutamate (docking score -4.3). Glutamate formed six hydrogen bonds with six amino acid residues, namely Lys210, Asn211, Thr212, Ser240, Ala241, and Arg245 (Figure 4). Though falcon AMPA-receptor bound with glutamate ligand with six hydrogen bonds, the observed docking score was less than that found with owl AMPA-receptor/glutamate interactions. This is due to the absence of the two salt bridges observed in the case of the owl AMPA-receptor/glutamate complex. So, the reason behind such a lower docking score was attributed to the absence of any contribution of these bonds with these protein-ligand interactions, and all observed ligand-protein interactions were attributed to the hydrogen bonds.

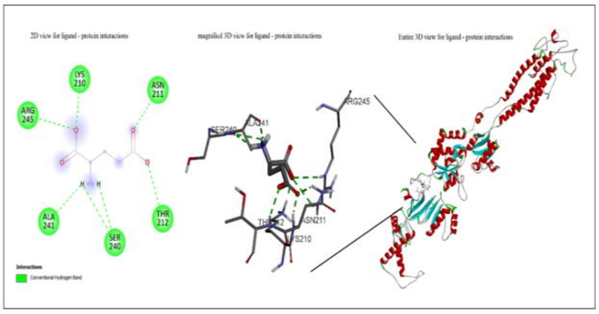

Figure 4: Docking between falcon AMP-receptor and glutamate using AutodockVina. Both 2D and 3D ligand-receptor interactions were shown. The docking score between ligand (glutamate) and owl AMP-receptor is -4.3 kcal/mol.

Another layer of confirmation of the stronger binding between owl AMPA-receptor with glutamate was came from the MD simulations. RMSF values showed that owl AMPA-receptor had shown less frequent simulations than that found in the falcon counterpart. As well, RMSF values indicated that all amino acid residues involved in the direct interactions with glutamate in the owl AMPA-receptor showed less frequent fluctuation compared with the falcon AMPA-receptor. This was obvious in the positions occupied by these amino acid residues, Asn36, Thr37, As70, and Tyr287. Since owl-AMPA-receptor has shown less frequent fluctuations in terms of its binding with glutamate, more frequent stability was observed in such complex.

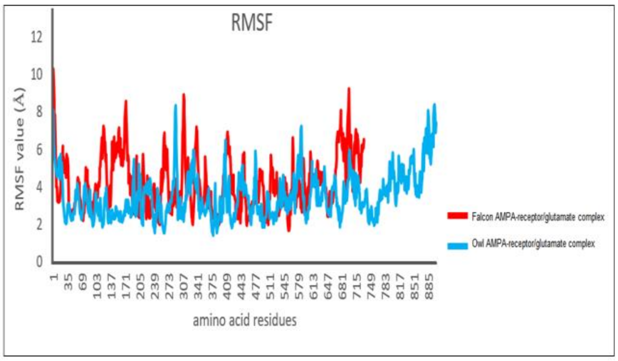

Figure 5: Root mean square fluctuation (RMSF) simulation for owl AMP-receptor/glutamate complex and owl AMP-receptor/glutamate complex using UNRES server

4. Conclusions

docking results showed that falcon AMPA-receptor has exhibited higher affinity in terms of the direct interaction with the neurotransmitter glutamate than that found in the falcon AMPA-receptor/glutamate interactions. Further support for this static observation was came from the dynamic simulation, in which RMSF values showed more stabilized interactions of owl AMPA-receptor than that found in the falcon counterpart. This observation can be manifested in more potentially efficient neurotransmission for glutamate and better vision ability in the owl. To confirm this *in silico*-based observations, intensive *in vitro*-based experiments should be done in the wet lab to prove this finding.

5. Acknowledgment

The authors would like to thank Al-Fadil Foundation for Study Services, Training and Development / Babylon Branch for their kind support us to complet this study.

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