International Research Journal of Biochemistry and Bioinformatics (ISSN-2250-9941) Vol. 3(7) pp. 130-139, October, 2013
DOI: http:/dx.doi.org/10.14303/irjbb.2013.016
Available online http://www.interesjournals.org/IRJBB

Full Length Research Paper

Putative ligand-target docking studies of human AMPA selective Ionotropic glutamate receptors reveal that β-ODAP has high binding affinity compared to tyrosine and glutamate

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Abstract

Neurolathyrism is a neurological disorder engendered by excessive consumption of *Lathyrus sativus* (Grass pea) comprising large amounts of the neurotoxin, β-N-Oxalyl-L, α, β-diaminopropionic acid (*β*-ODAP), a structural analogue of glutamate. *β*-ODAP acts by binding at AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) selective Ionotropic glutamate receptors (iGluRs), and blocking glutamate transporters in the neural milieu. This might direct a sustained increase in the concentration of both ODAP and glutamate in neuronal synapses, triggering excitotoxic degeneration of neurons. We propose that incidence of Neurolathyrism is preferably due to neuronal damage caused by high affinity binding of *β*-ODAP rather than glutamate excitotoxicity, which is usually purported in various neurodegenerative disorders. Our present *in silico* study using Accelrys Discovery Studio (ADS), CHARMm force field, SMART (Hybrid protocol of Steepest Descent and Conjugate Gradient Method) protocol and experimental results we obtained justify selective high affinity interaction of *β*-ODAP (dock score of 104.079) with iGluR protein [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] when compared to ligands of Glutamate (dock score- 29.000), Tyrosine (dock score- 39.654). This data supports our proposition that *β*-ODAP but not glutamate binds and acts at synapse by causing intensive calcium influx and mitochondrial energy deprivation of motor neurons, ultimately leading to spastic paralysis.

Keywords: AMPA selective Ionotropic glutamate receptors, Glutamate, Tyrosine, *β*-ODAP, *Lathyrus sativus*, Accelrys Discovery Studio, CHARMm force field, SMART (Hybrid protocol of Steepest Descent and Conjugate Gradient Method), Neurolathyrism.

INTRODUCTION

Neurolathyrism, a form of spastic paraparesis (S.L.N. Rao, 2011) is an ancient (Yu-Haey Kuo et al., 2007) and non-progressive (Kuniko Kusama-Eguchi, et al., 1996) neurodegenerative disorder. It is associated with the ingestion of seeds and foliage of *Lathyrus sativus* (grass pea) as staple diet for 2-3 months, which contains a neurotoxic amino acid β-ODAP (β-N-Oxalyl-L, α, β-diaminopropionic acid) (B. Peter Nunn et al., 2011). It is a

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motor cortical neuron disorder characterized by irreversible paralysis of the lower extremities. This disorder predominantly targets the upper motor neurons (betz cells and the corticospinal tracts) of the cortex of brain (S.A. Lipton, 2007), anterior horn cells and axons in the pyramidal tracts of lumbar region and pyramidal tract neurons of the spinal cord (V. Ravindranath, 2002, A. Hirano et al., 1976). Neurolathyrism is endemic in several regions of Asia and Africa (J. Hugon, et al., 2000, R. Tekle Haimanot et al., 2005). During drought and famine when other crops failed, it was the only survival food for the poor. This crippling disease with sudden onset affects preferentially the most active young men in destitute remote rural areas and living in a hand-to-mouth economy (Fernand Lambein, 2007).

The major toxic component of the pulse is β-ODAP. It is a non-protein, neuroexcitatory amino acid which was identified by (Rao et al., 1964) and (Murti et al., 1964). This neurotoxic amino acid has the potential to act as an agonist at certain glutamate receptors in the neural milieu (S.M. Ross et al., 1989, S. Pearson et al., 1981). The amino acid L- Glutamate acts as the neurotransmitter at the majority of excitatory synapses in the brain and spinal cord of vertebrates (Raymond Dingledine et al., 1999). It mediates nearly 50% of all the synaptic transmissions in the CNS and its involvement is implicated in all aspects of normal brain function, movement, cognition and development (I.J. Reynolds et al., 1995). Although glutamate is the native ligand of iGluRs, β-ODAP acts as a complementary agonist at the iGluRs and elicits mitochondrial dysfunction (V. Ravindranath, 2002), due to the accumulation of oxidative stress and injurious reactive oxygen species (ROS) by increasing the influx of many cation permeable channels primarily Ca^{2+} (M.L. Mayer et al., 1987). Ionotropic glutamate receptors (iGluR) mediate excitatory synaptic transmission in vertebrates and invertebrates through ligand-induced opening of transmembrane ion channels. These receptors activate a cation-selective ion channel permeable to Na^{+} and K^{+}, with differing degrees of permeability to, and block by, the divalent cations Ca^{2+} and Mg^{2+} (M. Hollmann et al., 1994). iGluRs are important in the development and function of the nervous system and are essential in memory and learning. They are implicated in various dysfunctions ranging from Alzheimer's, Parkinson's and Huntington's diseases, schizophrenia, epilepsy and Rasmussen's encephalitis to stroke (S.W. Rogers, 1994, S. G. Carriedo et al., 1996). Ca^{2+} permeable AMPA-receptors have been characterized in motor neurons (A.Victor Derkach, et al., 2007). β- L- ODAP acts as an agonist majorly at AMPA selective Ionotropic glutamate receptors (AMPAR). These receptors are predominant transducers of rapid excitatory transmission in the mammalian CNS (S.L.N. Rao, 2001).

Though β-ODAP has been implicated in the pathogenesis of Neurolathyrism so far there is no conclusive evidence to support the binding interaction of β-ODAP to the glutamate receptors. According to the molecular modelling studies carried out by Dr. S.L.N. Rao (L.L. Dugan et al., 1995), β-ODAP is conformationally cognate more with tyrosine and less with glutamate. In order to establish a clear link between β-ODAP, tyrosine, glutamate interaction and binding affinity with AMPA selective Ionotropic glutamate receptors present study has been undertaken.

MATERIALS AND METHODS

Crystal structure of the Ligand binding domain of *Homo sapien* AMPA ionotropic Glutamate Receptor (iGluR2) was downloaded from Protein Data Bank (PDB). The DOI of the receptor is 10.2210/pdb3rn8/pdb [Crystal structure of iGluR2 ligand binding domain from *Homo sapiens* (PDB ID: 3RN8)]. This three dimensional structure of iGluR (3RN8) is vital, to obtain a comprehensive understanding of interactions of various ligands at the molecular level. Glutamate, Tyrosine and β- ODAP were the three different ligands used in the study to analyze their respective binding interactions with AMPA glutamate receptor.

Crystallographically analysed Glutamate, Tyrosine and *β*-ODAP structures were retrieved from ChemSpider (http://www.chemspider.com/) which is a free accessing web server structure-centric community for chemists and is integrated with a multitude of other online services.

Accelrys Discovery Studio (ADS) was used to find the Active Binding Site (ABS), Energy Minimization and Protein-Ligand docking of the protein. CHARMm force field was applied on protein to perform energy minimization using the SMART (Hybrid protocol of Steepest Descent and Conjugate Gradient Method) protocol. Ligandfit module was implemented for AMPA Ionotropic Glutamate Receptor protein with Glutamate, Tyrosine and *β*-ODAP by Ligand docking.

Docking studies

Polar hydrogen atoms were added to Glutamate Receptor protein, and non-polar hydrogen atoms were merged with electrostatic forces assigned for the ligands. No restraint bonds were maintained and created the allowable binding receptor surface. From the ranked binding site, we selected the bigger site for docking. Out of all the docked conformations one best ranked conformation was selected from the highest docking energy, with not more than 2.5 Å rmsd (root-mean-square deviation). The H-bond, Van Der Waals (VDW), and other binding interactions were then analyzed by ADS. Structural quality statistical validations (performed by PROCHECK program) and physiochemical interactions/contacts in protein-ligand docked complex were analysed by PDBSum online tool

Name	Forcefield	Initial Potential Energy (kcal/mol)	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Angstrom)	Final RMS Gradient (kcal/(mol x Angstrom)
3RN8	CHARMm	-11161.90752	-15353.77477	-2239.19380	-10465.42773	194.09886	0.09919

Figure 1. Energy Minimization of [Crystal structure of iGluR2 ligand binding domain from Homo sapiens (PDB ID: 3RN8)] 3RN8.pdb protein structure using Accelrys Discovery Studio by applying CHARMm Forcefield.

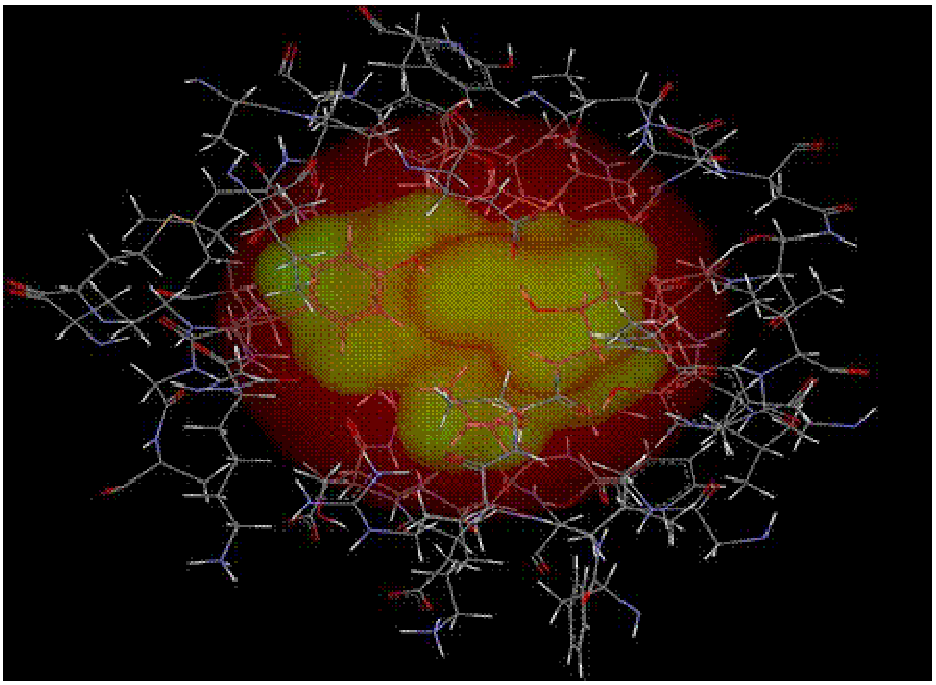


Figure 2. Biggest Active Binding Site in [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] 3RN8.pdb

(http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html).

Evaluation of the protein-ligand interactions

The refined quality of the final protein-ligand complex was assessed by subjecting it to a series of tests for its internal consistency and reliability. To determine the quality of glutamate receptor protein structure [Crystal structure of iGluR2 ligand binding domain from Homo sapiens (PDB ID: 3RN8)] PROCHECK program was used and the stereochemical quality of the protein structure was additionally evaluated by Ramachandran Plot.

RESULTS

Glutamate receptor protein crystal structure (10.2210/pdb3rn8/pdb) was retrieved from protein data bank (PDB) and its energy was minimized to -15353.77477 (kcal/mol) (Figure 1: Energy Minimization) by SMART minimiser of ADS software**.** The three dimensional (3D) structure of iGluRs [Crystal structure of iGluR2 ligand binding domain from Homo sapiens (PDB ID: 3RN8)], when compared to its putative ligands (Glutamate, Tyrosine and *β*-ODAP) is vital to obtain a comprehensive understanding of interactions of various ligands at the molecular level.

Glutamate, Tyrosine and *β*-ODAP were used as ligands to study their binding interaction with the active binding sites of Glutamate Receptor. Carbon rich regions of this receptor are considered for the prediction of binding sites from amino acid sequence information. Based on carbon content Flood-Filling algorithm of ADS predicted the biggest active site binding residues as Glu110, Ile198, Tyr210, Asn345, Tyr346, Arg335, Leu215, Arg108, Arg180, Glu112, Leu111, Glu187, Glu189, Ser188, and Phe95. The summaries for the active binding-site residues and H-bond interactions for Glutamate, Tyrosine and *β*-ODAP are presented in Figure 2: Biggest Active Binding Site of 3RN8.pdb. These

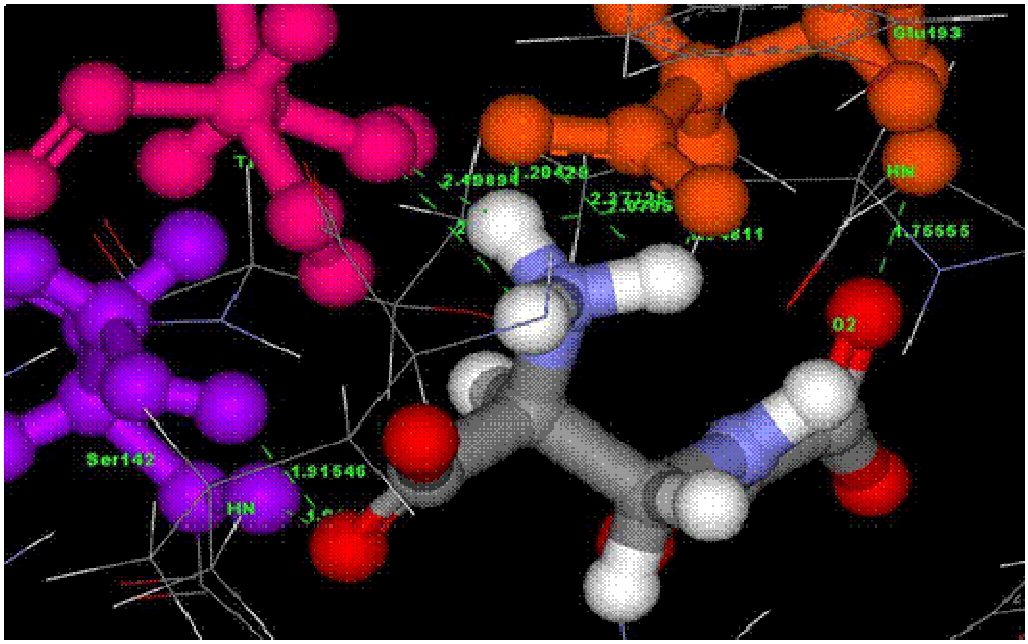
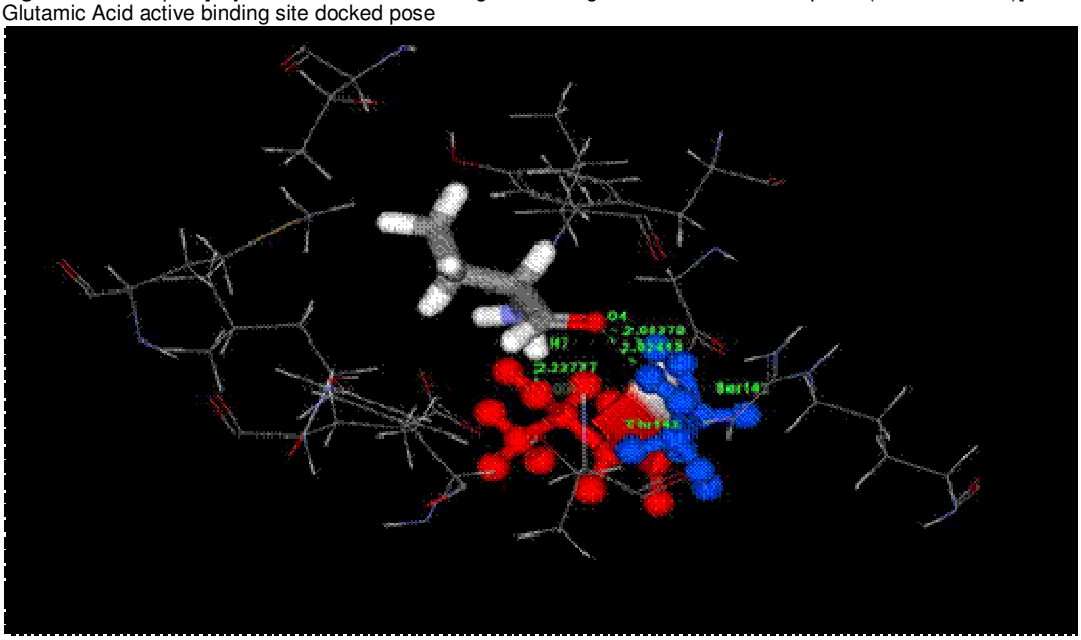


Figure 3. 3RN8.pdb [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] - ODAP active binding site docked pose

Figure 4 . 3RN8.pdb [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] – Glutamic Acid active binding site docked pose



docked ligands showed lowest internal energy indicative of their most favourable conformation. The spatial arrangement of the Glutamate, Tyrosine and β-ODAP bound to the active binding site of Glutamate Receptor protein (Figure 3: 3RN8.pdb-ODAP, Figure 4: 3RN8.pdb-GlutamicAcid, Figure5: 3RN8.pdb-TYROSINE) and 3RN8.pdb protein PROCHECK Statistics-Ramachandran Plot analysis validated 100.00% geometric structural quality model (Figure 6: PROCHECK Statistics-Ramachandran Plot). Ramachandran Plot analysis confirmed high quality results for both the Glutamate-3RN8, Tyrosine-3RN8 & *β*-ODAP-3RN8 docked complex as indicative from the maximum amino acids in geometric structural conformations of most favoured regions (89.8%) and additional allowed regions (10.2%). The topology of the 3RN8.pdb is provided in

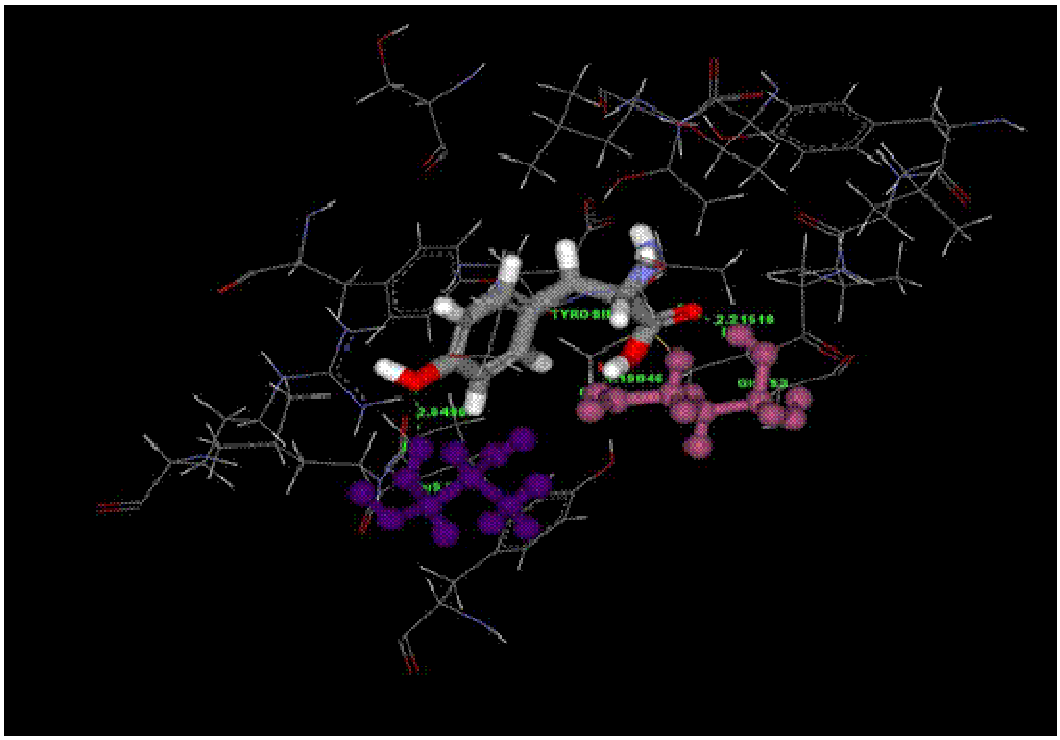


Figure 5. 3RN8.pdb [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] - TYROSINE active binding site docked pose

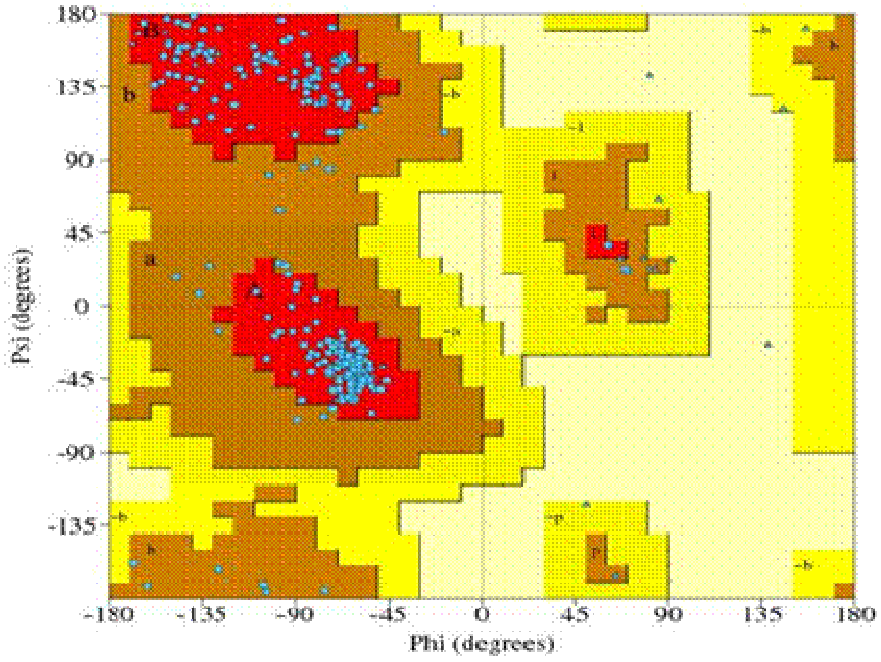


Figure 6. PROCHECK Statistics (Ramachandran Plot Analysis for 3RN8.pdb) 203 residues are in most favoured region (89.8%) and 23 residues are in additionally allowed region (10.2%). Glycine and Proline residues were 23 and 7 respectively. Overall the 3RN8.pdb is having 100.00% structural quality. The G-Factor of the structure was measured with excellent overall average score of -0.01 (values <-0.5 are unusual structure).

(Figure 7: Secondary Structure Topology of 3RN8.pdb)

According to the estimated free energy it has been observed that *β*-ODAP strongly interacted with Glutamate Receptor when compared to Tyrosine and its native ligand glutamate. The hydrophobic interactions were assessed by PROCHECK analysis and the results for

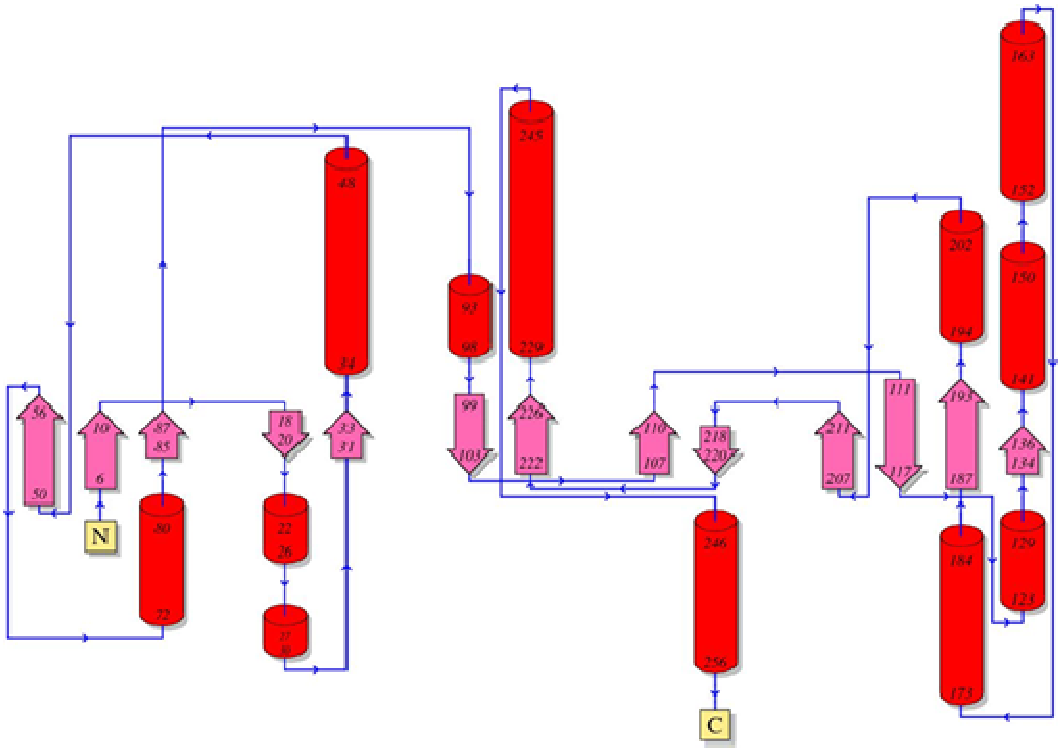


Figure 7. Secondary Structure Topology of 3RN8.pdb. 2D Red color barrel shape indicates Alpha-Helix and pink color 2D arrow mark shape indicates Beta-Sheets secondary structure. The secondary structure topologywere numbered on 2D shapes according to their connectivity shown in blue color arrowed line.

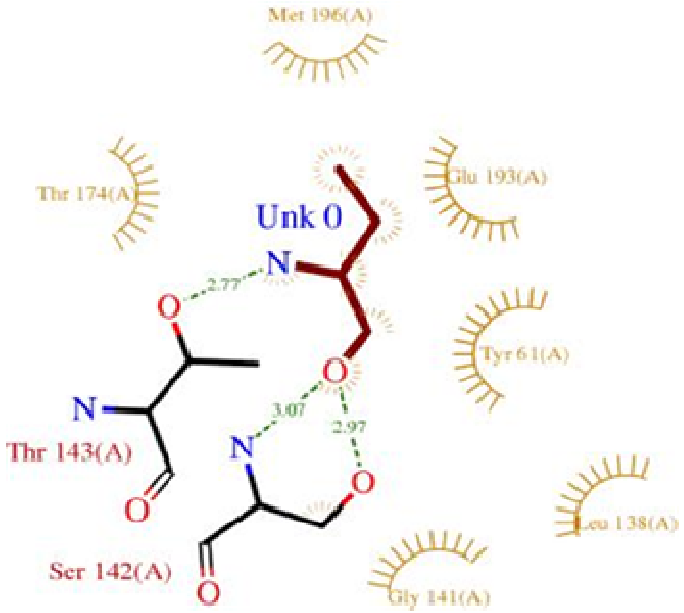


Figure 8. 3RN8.pdb [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] - Glutamate hydrophobic interactions using Ligplot. Thr143 and Ser142 were forming hydrogen bonds. The residues forming hydrophobic interactions were Thr174, Met196, Glu193, Tyr611, Leu138, and Gly141.

each ligand-target i.e., Glutamate, ODAP, and Tyrosine with 3RN8.pdb protein structure were shown in Figure 8, Figure 9 and Figure 10 respectively.

The docking scores of 3RN8 [Crystal structure of

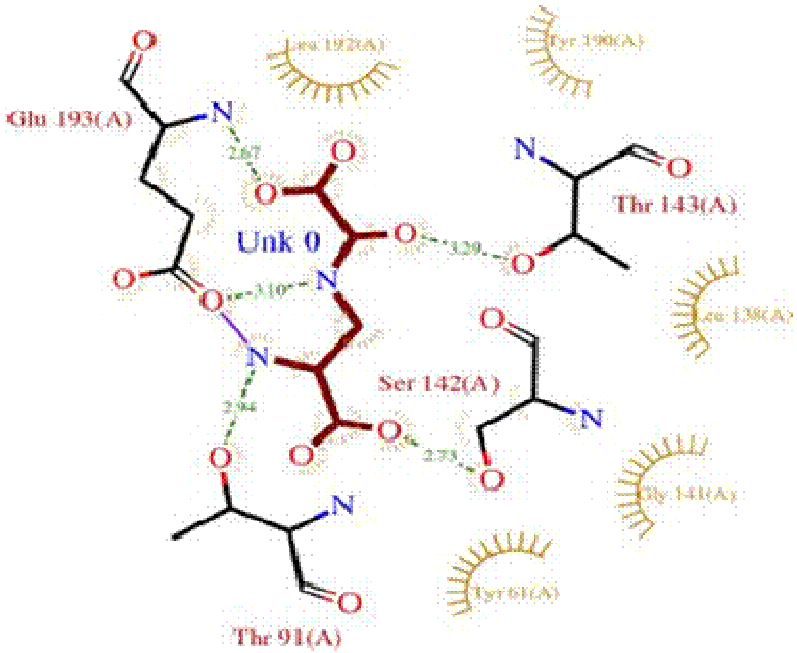


Figure 9. 3RN8.pdb [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] – ODAP hydrophobic interactions using LigPlot. Glu193, Thr91, Thr143, and Ser142 were forming five hydrogen bonds depicted in dotted green line with distance. The residues forming hydrophobic interactions for stabilizing the structure were Leu192, Tyr190, Leu138, Gly141, and Tyr61.

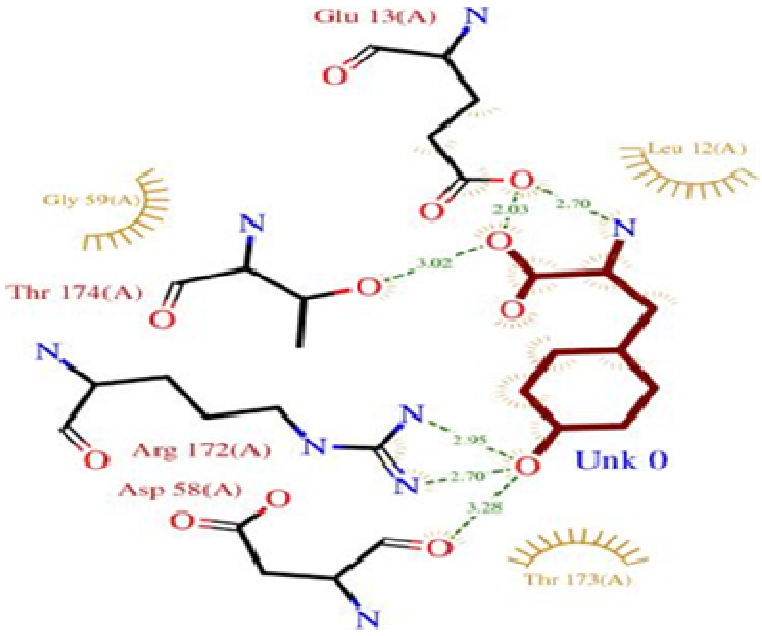


Figure 10. 3RN8.pdb [Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] – Tyrosine hydrogen bond and hydrophobic interactions generated by Ligplot. Asp58, Arg172, Thr174, and Glu13 were forming six hydrogen bonds depicted with dotted green line measured in distance. The residues forming hydrophobic interactions with Tyrosine for stabilizing the 3RN8.pdb were Gly59, Leu12, and Thr173.

iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)] the estimated free energy of binding for Glutamate, Tyrosine and *β*-ODAP are 29.000, 39.654 and 104.079 respectively (shown in Table1). Based on the dock score, *β*-ODAP is proved to be a staunch analogue of glutamate and might thus compete with

Table 1. Dock scores of 3 different ligands with glutamate receptor of Crystal structure of iGluR2 ligand binding domain from homo sapiens (PDB ID: 3RN8)

Protein (3RN8) - Ligand	Dock Score
Glutamate	29
Tyrosine	39.654
β-ODAP	104.079

it for docking at the target (glutamate receptor) in neural milieu. The binding affinities of the three ligands to iGluRs is in the following order - ODAP > Tyrosine > Glutamate. The obtained results demonstrate that Glutamate Receptor protein interaction is more potent with *β*-ODAP due to its multiple and strong hydrogen bond interactions between Glutamate receptor protein residues.

DISCUSSION

Even though Neurolathyrism is known since times immemorial, its exact mechanism of action still remains elusive. In earlier studies it was hypothesized that ODAP is involved in motor neuron degeneration, but till to date conclusive evidence which supports its mechanism is lacking. So, we have primarily attempted to unveil the basic interaction of three different ligands (via Glutamate, Tyrosine and *β*-ODAP) with AMPA selective glutamate receptors *in silico*. We have chosen Glutamate as, it is the native ligand at glutamate receptors; *β*-ODAP because of its analogous nature to glutamate and Tyrosine because of its conformational cognateness towards *β*-ODAP (L.L. Dugan et al., 1995). The substantial and high affinity binding of *β*-ODAP than glutamate to the receptors on the post synaptic neuron as shown in our study leads to toxicity in a variety of ways as reported in earlier studies: I) Dysfunction of mitochondria has been reported in Neurolathyrism in several studies (V. Ravindranath, 2002). Minor alterations in mitochondrial function can lead to oxidative damage and deleterious pathological changes in neurons (R.S. Kenchappa et al., 2003). II) The inhibition of mitochondrial complex I presumably through glutathionylation of critical thiol groups in subunits of complex I (B.A. Warren et al., 2004). III) Considerable evidence supports a link between Ca^{2+} influx and glutamate receptor mediated neurodegeneration. Mitochondria triggers production of ROS (reactive oxygen species), when stimulated by *β*-ODAP (B.A. Warren et al., 2004). IV) Suppression of cystine transporter [X_c^- antiporter] activity (L. Diwakar et al., 2007). V) Inhibition of glutathione synthesis at the cysteine supply step by inhibiting cystathionine-c-lyase .

CONCLUSION

The present findings significantly felicitate in understanding the role of *β*-ODAP in binding to glutamate receptors and triggering motor neuron death in Neurolathyrism. Further research can be done based on results to explore the pathways that are inhibited in motor neuron

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How to cite this article: Ankulu M, Aparna N, Shirfule A, Vankudavath RN, Khandare AL (2013). Putative ligand-target docking studies of human AMPA selective Ionotropic glutamate receptors reveal that β-ODAP has high binding affinity compared to tyrosine and glutamate. Int. Res. J. Biochem. Bioinform. 3(7):130-139

1. Ramachandran Plot statistics

		No. of residues	%-tage
		------	------
Most favoured regions	[A,B,L]	203	89.8%*
Additional allowed regions	[a,b,l,p]	23	10.2%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
		----	------
Non-glycine and non-proline residues		226	100.0%
End-residues (excl. Gly and Pro)		3	
Glycine residues		23	
Proline residues		7	

Total number of residues		259	

Based on an analysis of **118** structures of resolution of at least **2.0** Angstroms and *R*-factor no greater than **20.0** a good quality model would be expected to have over **90%** in the most favoured regions [A,B,L].

2. G-Factors

Parameter	**Score**	**Average Score**
---------	-----	-----
Dihedral angles:-		
Phi-psi distribution	-0.25	
Chi1-chi2 distribution	0.11	
Chi1 only	-0.29	
Chi3 & chi4	0.48	
Omega	-0.57*	
		-0.19
		=====
Main-chain covalent forces:-		
Main-chain bond lengths	0.49	
Main-chain bond angles	0.08	
		0.25
		=====
OVERALL AVERAGE		-0.01
		=====

G-factors provide a measure of how **unusual**, or out-of-the-ordinary, a property is.
Values below -0.5* - unusual

Values below **-1.0**** - highly unusual

Important note: The main-chain bond-lengths and bond angles are compared with the Engh & Huber (1991) ideal values derived from small-molecule data. Therefore, structures refined using different restraints may show apparently large deviations from normality.