

Structural Analysis and Binding Modes of Benzodiazepines with Modeled GABA_A Receptor Subunit Gamma-2

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ABSTRACT

Activation of chloride gated GABA_A receptors regulates the excitatory transmission in the epileptic brain. Positive allosteric modulation of these receptors via distinct recognition sites is the therapeutic mechanism of antiepileptic agents which prevents the hyperexcitability associated with epilepsy. These distinct sites are based on subunit composition which determines binding of various drugs like benzodiazepines, barbiturates, steroids and anesthetics. The binding of antiepileptic agents to this recognition site increases the affinity of GABA_A receptor for modulating the inhibitory effects of GABA-induced chloride ion flux. In the pentameric complex structure of these receptors, the α/β interface locates the binding site of agonists and the α/γ interface forms the benzodiazepine (BZD) binding site on extracellular domain. Thus the γ subunit is shown as highly required for functional modulation of the receptor channels by benzodiazepines. The present study initiates the binding analysis of chosen benzodiazepines with the modeled GABA receptor subunit gamma-2. The extracellular domain of γ subunit of human GABA_A is modeled and docking studies are performed with diazepam, flunitrazepam, and chlordiazepoxide. The results revealed the binding modes and the interacting residues of the protein with the benzodiazepines.

Keywords - GABA, GABA_A receptors, epilepsy, benzodiazepine drugs

1. INTRODUCTION

Regulation of the synaptic localization of ligand-gated ion channels contributes to excitatory and inhibitory synaptic passing on. The imbalance between excitatory and inhibitory synaptic transmission in key brain areas is implicated in the pathophysiology of epileptic seizures, in which there is a decrease in the inhibition mediated by the neurotransmitter GABA. GABA mediates fast synaptic inhibition by interaction with GABA_A receptors. This has been the target of several clinically relevant anticonvulsant drugs [1, 2]. Many anti-epileptic and anti-convulsive drugs act by

enhancing the inhibitory action of these receptors by binding to modulatory compounds which result in the decrease of the excitatory effects leading to seizures. Therefore, by exerting its antiepileptic influence, GABA_A receptors play fundamental role in the epileptic brain [3, 4, 5]. GABA_A receptors are ligand gated chloride ion channels that can be opened by GABA with pentameric assemblies of subunits arranged around a membrane-spanning pore [2]. The pentameric structural design of this receptor channel, resulting from five of at least 19 subunits, grouped in the eight classes alpha (α 1-6), beta (β 1-3), gamma (γ 1-3), delta (δ), pi (π), epsilon (ϵ), theta (θ), and rho (ρ 1-3) permits an immense number of putative receptor isoforms [6,7]. Each subunit have a long extracellular amino terminus (around 200 amino acid), thought to be responsible for ligand channel interactions and forms agonist binding site, and four transmembrane (TM) domains and a large intracellular domain between TM3 and TM4 [8, 9]. The major receptor subtype of the GABA_A receptor consists of α ₁, β ₂, and γ ₂ subunits, and the most likely stoichiometry is two α subunits, two β subunits, and one γ subunit [10]. This differential composition of subunits exerts the distinct pharmacological and functional properties of GABA_A receptors and their sensitivity towards GABA [11].

The vital importance of subunit combination is the determination of the specific binding effects of allosteric modulators and clinically important drugs such as benzodiazepines (BZs), barbiturates, steroids, and general anaesthetics, some consultants, polyvalent cations, an ethanol. These drugs modulate GABA-induced chloride ion flux by interacting with separate and distinct allosteric binding sites [12]. Drugs and endogenous ligands bind either to the extracellular domain or channel domain of the GABA_A receptors and act as positive or negative allosteric modulators [8]. In the heterogeneity of these receptors, the presence of alpha and beta subunits are needed for functional channels while gamma subunits are required to mimic the full repertoire of native receptor for responses to drugs. The α and β subunits are implicated in forming the binding site for the GABA and agonistic site while the $\alpha\gamma$ interface is responsible for BZD recognition site

[13]. Thus the γ subunit has been shown as required one for functional modulation of the receptor channels by benzodiazepines [14, 15]. Benzodiazepines belong to the classic ligands of this site and exert their anxiolytic, sedative, muscle relaxant, and anticonvulsive action by positive allosteric modulation of different isoforms of the GABA_A receptor channel containing γ subunit.

By taking this diversity role of GABA_A receptors, in our present study, benzodiazepine drugs diazepam, chlordiazepoxide, and flunitrazepam are taken to perform docking studies with GABA receptor. This leads to know the binding analysis of the drug with the receptor. To stimulate the structure-based design of new drugs that target restricted altered implicated in GABA related epilepsy medication and minimize side effects, it is vitally important to find the 3D structures of GABA_A receptor subtypes. The extracellular γ subunit of GABA_A receptor (GABRG2) is modeled using the crystal structure of 3RHW as a template and further evaluation is done by PROSA and PROCHECK. Docking studies with benzodiazepine drugs revealed the binding modes and binding energies and the important residues involved in binding of receptor with the ligand.

2. METHODOLOGY

2.1 Sequence Alignment and Homology modeling

The protein sequence of human GABA_A receptor subunit gamma-2 (GABRG2) that has 456 amino acid residues is taken from SWISS PROT database [16]. The fasta sequence of extracellular domain (40-273) is retrieved followed by BLAST against PDB database to identify homologous protein structures. From the BLAST results, based on the highest identity and similarity to our target protein, the crystal structure of 3RHW from *C.elegans* is used as template for homology modeling [17]. Using crystal structural coordinates of template, the homology model of extracellular domain of GABRG2 (40-273) is modeled on the basis alignment of target and template. Further, the modeled protein energy minimization and loop refinement are carried out by applying CHARMM forcefields and smart minimization algorithm followed by conjugate gradient algorithm until the convergence gradient is satisfied. These procedures are performed by Discovery Studio [18].

2.2 3D structures Validation

The final refined model of GABRG2 is validated and evaluated by using Rampage by calculating the Ramachandran plot, PROSA and RMSD. RAMPAGE server considers dihedral angles ψ against ϕ of amino acid residues in protein structure. [19]. The PROSA is applied to analyze energy criteria comparing with Z scores of modeled protein and 3D template structure [20]. Root mean

square deviation (RMSD) is obtained by superimposition of 3RHW (template) and modeled GABRG2 using SPDBV.

2.3 Ligand generation and Optimization

The structures of chosen benzodiazepine drugs taken for binding analysis are downloaded from PubChem. Hydrogen bonds are added and by applying the CHARMM forcefield, energy minimization is carried out with the steepest descent method which follows by the conjugate gradient method till it satisfies the convergence gradient. The benzodiazepine drugs taken are diazepam, chlordiazepoxide, flunitrazepam etc.

2.4 Docking studies

This study uses LigandFit method for accurate docking of ligands into protein active sites. The final energy refinement of the ligand pose (or) pose optimization occurs with Broyden-Fletcher Gold Farbhanno (BFGS) method. Thus docking analysis of the taken compounds with GABRG2 is carried out using Discovery Studio (DS) to predict the binding affinities based on various scoring functions. Their relative stability is also evaluated using binding affinities.

3. RESULTS AND DISCUSSION

The extracellular domain(40-273) of human GABA_A receptor subunit gamma-2 is retrieved in fasta format from the SWISS PROT database(Accession No:P18507, Entry name: GBRG2_HUMAN, Protein name: Gamma-aminobutyric acid receptor subunit gamma-2).The BLAST results yield X-ray structure of 3RHW from *C.elegans* with a resolution of 2.60 Å and 31% identity to our target protein. The DS performs sequence alignment and homology modeling. The sequence alignment is done using sequence analysis protocols called "Align multiple sequences". Inputs of protocols are human GABRG2 sequence and 3RHW template, which align them and give the alignment output as shown in Fig. 1. The theoretical extracellular domain (40-273) structure of human GABRG2 is modeled using build homology models under the protein modeling protocol cluster of DS. Three molecular models of GBRG2 are generated and the build models are B99990001, B99990002 and B99990003. Out of three models, Model of B99990003 as shown in Fig. 2 has the lowest value in Probability Density Functions (PDF) - Total Energy (1174.27), PDF Physical Energy (599), and Discrete Optimized Protein Energy (DOPE) score (-21385.31), which indicates it as the best model. The energy refinement method gives the best conformation to the model. For optimization and loop refinement, the CHARMM forcefield and steepest descent are applied with 0.001 minimizing RMS gradient and 2000 minimizing steps.

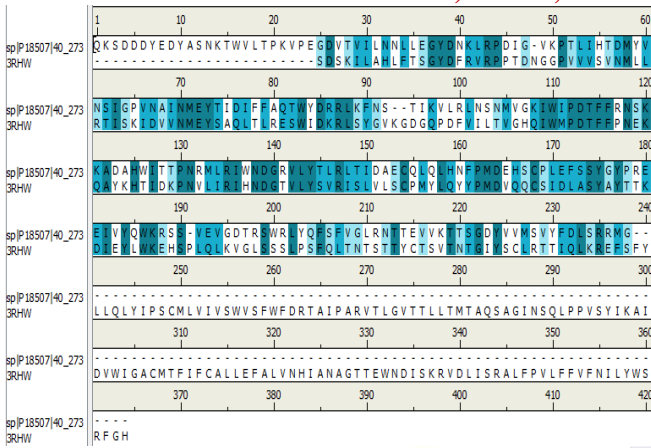


Figure 1: Sequence alignment of GABA_A receptor subunit gamma-2 from *Homo sapiens* with templates (PDB code: 3RHW) done using Discovery Studio.

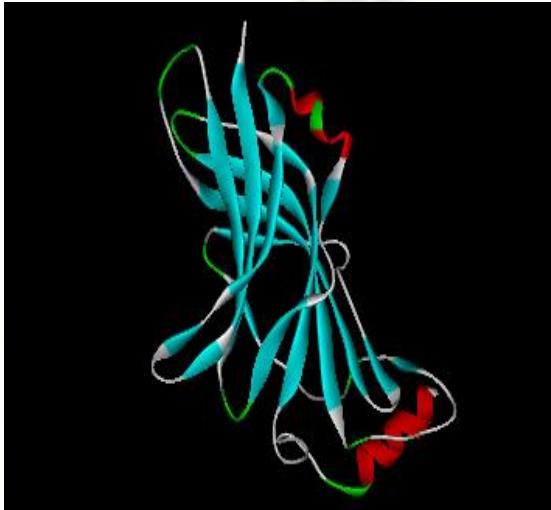


Figure 2: Modeled structure of GABRG2

Geometric evaluations of the modeled 3D structure are performed using Rampage. The Ramachandran plot of our model shows that 87.9% of residues are found in the favored and 9.7% allowed regions and 2.4% are in the outlier region as compared to 3RHW template (96.7%, 3.3%, and 0%) respectively. The Ramachandran plot for GABRG2 model and the template (3RHW) are shown in Fig. 3 (a) & Fig. 3(b) and the plot statistics are represented in Table 1.

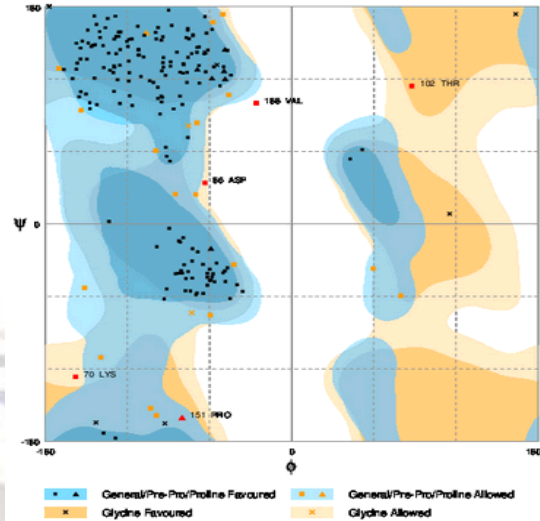


Fig 3(a): Ramachandran plot of GABRG2 from *Homo sapiens*

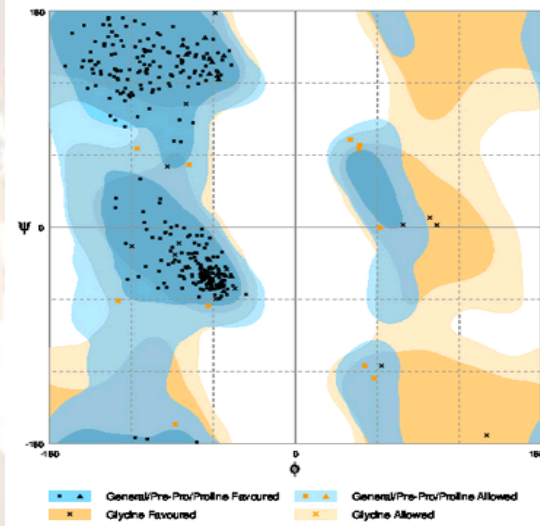


Fig 3(b): Ramachandran plot of 3RHW from *C.Elegans*

Validation is carried out by ProSA to obtain the Z-score value for the comparison of compatibility. The Z-score plot shows spots of Z score values of proteins determined by NMR (represented in dark blue color) and by X ray (represented in light blue color) using ProSA program. The two black dots represent Z-Scores of our model (-4.92) and template (-5.4). These scores indicate the overall quality of the modeled 3D structure of GABRG2.

RMSD (1.54 Å) is calculated between the main-chain atom of model and template. It indicates close homology. This ensures the reliability of the model. Superimposition between target and template structure is done using SPDBV.

Table 1: Ramachandran plot values showing number of residues in favored, allowed and outlier region through RAMPAGE evaluation server.

Structure	Number of residues in favored region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)
GABRG2	87.9	9.7	2.4
3RHW	96.7	3.3	0

energies with respective protein active site residues is done using different scoring functions. Two important parameters have been considered for selecting potential compounds among the given input: (i) Hydrogen bond details of the top-ranked pose and (ii) prediction of binding energy between the docked ligands and the protein using various scores.

Table 2: Summary of docking information of the top ranked poses in each protein

Docking Scores/information with GABRG2	Chlordiazepoxide	Flunitrazepam	Diazepam
Lig Score 1	2.1	2.1	1.26
Lig Score 2	3.7	3.91	3.48
-PLP1	52.78	54.38	56.41
-PLP2	46.97	56.95	53.0
JAIN	0.29	0.73	1.29
-PMF	88.6	104.12	105.66
DOCK SCORE	30.815	34.104	28.214

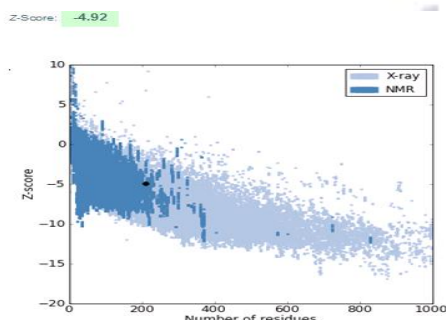


Fig 4(a): The plot of Z-Score of GABRG2

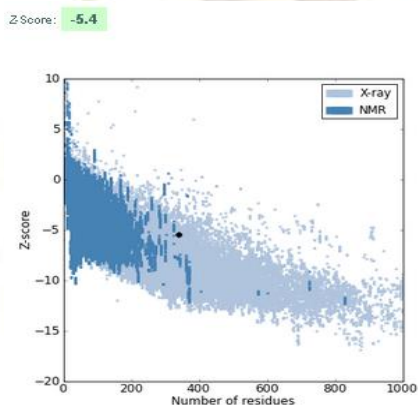


Fig 4(b): The plot of Z-Score of 3RHW (template)

To study the binding modes of the chosen benzodiazepine drug molecules in the active sites of modeled human GABRG2, molecular docking study is performed by LigandFit program of DS. Apart from these, other input parameters for docking are also considered for evaluating the GABRG2 docking efficacy with ligands.

In this work, GABRG2 is docked with benzodiazepine ligand. As a result, ten different conformations are generated for each protein, but only top ranked docked complex score is considered for binding affinity analysis. The different score values as shown in the Table 2 include Ligscore 1, Ligscore 2, PLP 1, PLP 2, JAIN, PMF and DockScore. The benzodiazepine drugs (diazepam, chlordiazepoxide, and flunitrazepam) are docked in to the active site of GABRG2 in order to find the receptor ligand binding orientation, binding affinity, binding free energies of the GABRG2 against the selected compounds. Therefore, rescoring of the best docked poses based on their interaction

By enlarging this interaction analysis, the hydrogen bond interaction is contributed as a major parameter. The Hydrogen bonding interaction of the protein with compounds (Fig 2) are analyzed. Each of the compounds having the highest dockscore with GABRG2 is taken for H-Bond analysis. Results are analyzed using Hbond Monitor of DS involved in formation of hydrogen bond with amino acids. The results revealed that chlordiazepoxide is having a dock score of 30.815 K.cal/mol with one hydrogen bond, Flunitrazepam having a dockscore of 34.104 with two hydrogen bonds and diazepam having a dockscore of 28.214 with one hydrogen bond.

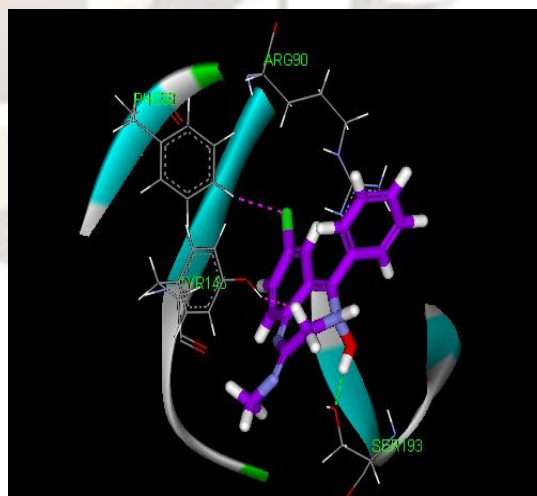


Figure 5(a):Hydrogen bond interaction with chlordiazepoxide

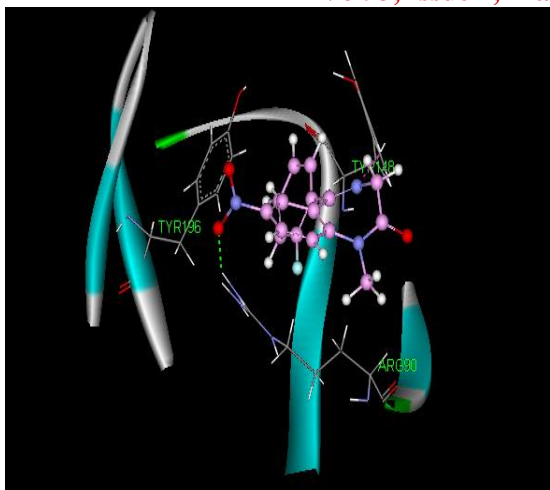


Figure 5(b):Hydrogen bond interaction with flunitrazepam

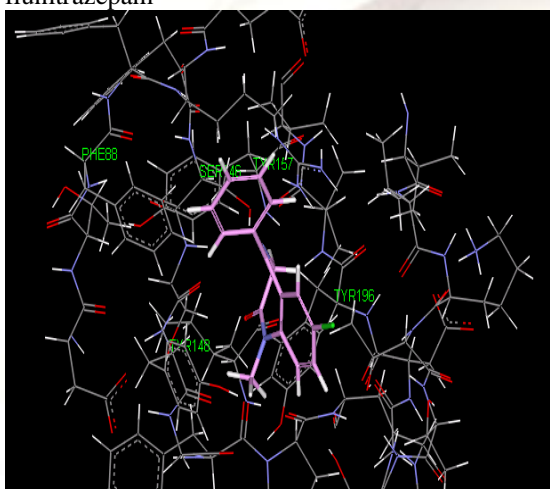


Figure 5(c): Hydrogen bond interaction with diazepam

Figures 5(a), 5(b), and 5(c) show hydrogen bond interactions of GABRG2 with chlordiazepoxide, flunitrazepam and diazepam. The green dotted lines represent the hydrogen bond formations, and green letters show the amino acids involved in the bonding and GABRG2.

Fig. 5(a) shows the amino acid residues involved in hydrogen bond interactions with GABRG2 with the ligand chlordiazepoxide. The interacting amino acids are PHE 88, SER193, PHE88, ARG90 and TYR146. Fig. 5(b) shows the amino acid residues involved in hydrogen bond interactions of GABRG2 with Flunitrazepam. The interacting amino acids are LYS 54, ASP 21, ARG 77, LEU22 and SER 119. Fig. 5(c) shows the amino acid residues involved in hydrogen bond interactions with GABRG2 and diazepam. The interacting amino acids are PHE 88, SER 46, TYR157, and TYR148.

4. CONCLUSION

In the present study, the homology model of the human GABRG2 receptor is modeled and molecular docking studies are performed to

illustrate the binding mechanism of GABRG2 to the selected ligands chlrodiazepoxide, flunitrazepam and diazepam. These studies revealed the binding modes and the interacting amino acid residues involved in recognition of the compound. Among three drug interactions with GABRG2, flunitrazepam shows the highest binding affinity (Dock score 34.104) and good hydrogen bond interactions with active site residues. The results obtained from this study would be useful in understanding the modulatory mode of GABRG2 with benodiazepine drugs.

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