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MOLECULAR INTERACTIONS OF GABA ANALOGUES AGAINST THE α+β-INTERFACE OF $GABA_A$ RECEPTOR: DOCKING AND MOLECULAR DYNAMICS STUDIES

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$GABA_A$ receptor (GABAaR) is a primary mediator of inhibitory neurotransmission, and considered as the target protein for drug designs. In this work, homology modeling, docking (cDocker) and molecular dynamics simulations (GROMACS) were combined to study the bindings of GABAaR with GABA analogues. It was found that the binding pocket of GABA analogues was located at the α+β-interface of GABAaR. In contrast to the series of 5-aminopentanoic acid, GABA, 2-methyl-GABA, 3-methyl-GABA and 4-methyl-GABA more fit the binding pocket, with the binding energies (E_{int}) of -41.20, -38.57, -40.17 and -40.22 kcal mol^{-1}, respectively. Further structural analysis revealed that short main chain is favorable to the binding and might confer the high activity. The additional methyl group may be benefit for the selectivity of drugs. Furthermore, the α1 subunit residue Glu127 and β2 subunit residues Val394 and Leu399 played the additional essentials for the bindings. This work also pointed out how to effectively reinforce the bindings of agents. We hope that the results will be helpful for designing of novel neuromodulatory drugs.

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*Keywords:*GABA analogues; $GABA_A$ receptor; Interaction mechanisms; Molecular modeling; Molecular dynamics

1. Introduction

γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter of mammalian central nervous system [1], which primarily acts as an endogenic ligand of the ionotropic $GABA_A$ receptor (GABAaR)[2].Once GABA integrates with the GABAaR, the conformation of receptor within the cell membrane would be changed with the quick activation of channel pore, and then the chloride anions (Cl^-) can selectively be conducted through the channel[3].Because the resting potential for chloride in most neurons can well be compared with the resting membrane potential, the activation of GABAaR tends to stabilize or hyperpolarise the resting potential, contributing to the early part of the inhibitory postsynaptic potential (IPSP)[4-5].This motion blocks the depolarization effect of excited neurotransmitter and the generation of action potential, reducing the activity of neuron[6].It is now clear that the mild inhibition of neuronal firing by psychoactive drugs, which hinder the activity of GABAaR, can cause a reduction of anxiety in the patient[5-6].

GABAaR is a member of the large "Cys-loop" super-family, consisting of numerous subunit isoforms embedded in the nerve cell membranes [7]. And the most common type in the brain is a pentamer composed of two α subunits, two β subunits and a γ subunit ($\alpha_2\beta_2\gamma$) [8]. Among these subunits, α and β subunits play the essential role in producing a GABA-gated ion channel [9].

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GABAaR has been known as key target protein of numerous pharmacologically and clinically important drugs, such as benzodiazepine, picrotoxinin, barbiturate and steroid [3, 10-12].Binding sites for several drugs have been preliminarily identified throughout the modeling studies, as a number of multiple solvent-accessible pockets within the GABAaR protein[13]. For example, the benzodiazepine binding site is located at the α+γ-interface of GABAaR[14], whereas the binding site for GABA might be mapped to the interface between an α and a β subunit[15]. So far, the extracellular α+β-interface of GABAaR has not been systematically investigated as the possible binding pocket for selective agents, although drugs interacting with this interface should be able to modulate the αβ, αβγ and αβδ receptors and should thus exhibit a much broader action than the available psychoactive drugs[12, 16].

The recent studies indicated that GABA analogues might inhibit the excitability of ion channels and reduce the activity of neurons, as a class of anticonvulsant agents[17-18]. For example, 3-methyl-GABA represents the rather anticonvulsant activity *in vitro*[17-18].Characterizing the bindings at the α+β-interface of GABAaR will contribute to the in-depth understanding on the interactions, and ultimately enable the design of improved agents[12]. Here, the bindings of GABA analogues (Figure 1) with the α+β-interface of GABAaR will be further systematical studied via the molecular modeling methods, in order to explain the interaction mechanism and disclose the importance of functional groups on the binding affinities. This approach has been employed to study the interactions between substrates and the influenza virus neuraminidase[19-20]and identify the novel druggable loop domain[21]. We hope that the results can aid to understand and characterize the detailed interaction profiles, and guide the synthetic and medicinal chemists to discover the novel agents for the treatment of epilepsy and convulsion.

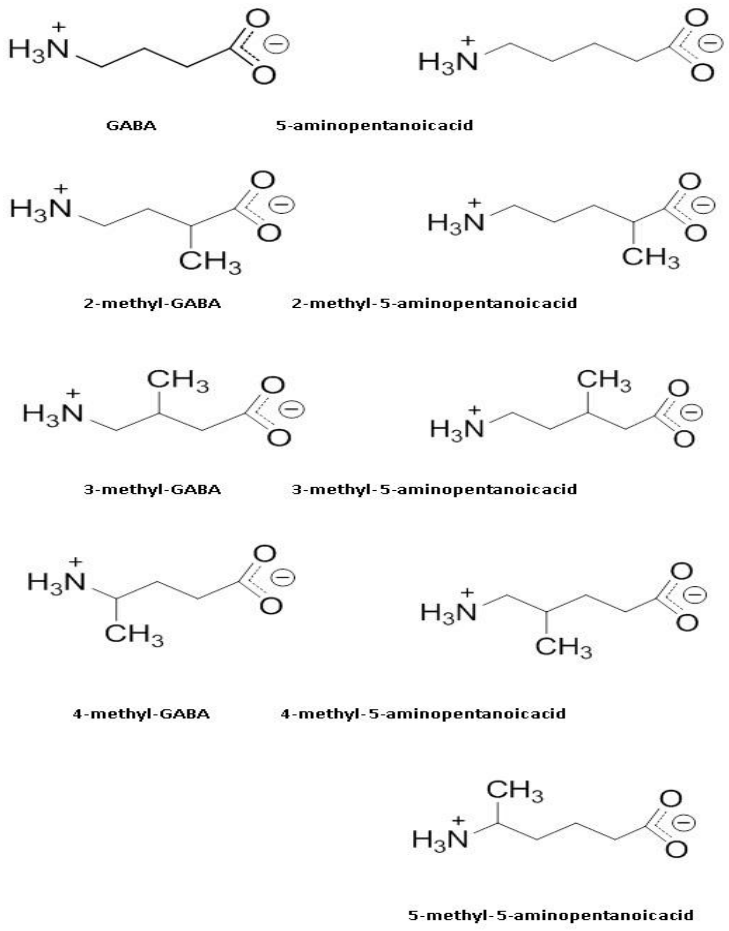

Fig. 1. Chemical structures of GABA analogues used in this study.

2. Methodology

Structure model and docking studies were performed with the different modules implemented in Discovery Studio software package[22] and the molecular dynamic (MD) simulations were performed using GROMACS4.5.5 program[23-24].

2.1.Homology modeling

In accord to previous reports[25-27], primary sequences of α1 (accession number: P62813) and β2 (accession number: P63138) subunits of $GABA_A$ receptor (GABAaR) were taken from the Swiss-Prot database (http://www.uniprot.org), and the initial model of GABAaR was conducted through the MODELER module[22, 27].Briefly, web-FASTA program was performed in the RCSB Protein Data Bank (http://www.rcsb.org) to determine the best templates. The crystal structures of glutamate-gated chloride channel (entry code 3RHW)[28] and β3 subunit of GABAaR (entry code 4COF)[29] were used as templates. The generated dimer was energy-minimized by a 1000-step steepest descent minimization, followed by conjugate gradient minimization, until converged to 0.01 $kcal \cdot mol^{-1} \cdot Å^{-1}$.

The energy-minimized dimer was adequately equilibrated by 100.0-ns MD simulations using GROMACS4.5.5 program[23-24] and Charmm27 force field [30-31].The dimer was immersed into a SPC (simple-point-charge) water box, with a distance of 10.0 Å extended from any solute atom[32]. The system was neutralized with NA^+ anions[33]. The NPT ensemble was applied (300 K, 1 Bar)[34] and the particle-mesh Ewald (PME) algorithm was used to handle the long-range electrostatics [35]. A cutoff equal to 10.0 Å was used for short-range electrostatics and van der Waals interactions. The covalent bonds involving hydrogen was constrained with the LINCS algorithm[36].Coordinates were saved every 10.0 ps, with the time step of 2.0 fs. The reliability of structure model was checked by Profile-3D module [22] and Procheck program[37].

2.2. Docking and MD simulations

Docking simulations were performed using the cDocker protocol[27, 38] in the Discovery Studio environment, features for its grid-based method that the residues are held rigid and ligands are free to move[27]. Geometry and partial atomic charges of GABA analogues(Figure 1) were conducted throughout the "Minimize Ligands" tools[22]using the Charmm force field[39], with a convergence criterion of 0.001 $kcal \cdot mol^{-1} \cdot Å^{-1}$.The binding site sphere was assigned with a sphere of 10.0 Å. Combining random rotations and simulated annealing method, the optimal orientations of ligands within the GABAaR protein were probed, on the basis of interaction energies and geometrical matching qualities[26-27]. The optimal docked complexes were then minimized by conjugate gradient method, until converged to 0.01 $kcal\ mol^{-1}\ Å^{-1}$. The energy-minimized docked complexes were then sufficiently equilibrated by 100.0-ns MD simulations, using GROMACS4.5.5 program[23-24] and Charmm27 force field [30-31].

3. Results and Discussion

3.1. Model building

As shown in Figure 2, the amino acid sequences of α1 and β2 subunits of GABAaR(Accession codes P62813 and P63138) were aligned with anion-selective Cys-loop receptor(PDB ID: 3RHW) and β3 subunit of GABAaR (PDB ID: 4COF) via the MODELER module[22, 40]. The sequence identity and similarity between the α1/β2 dimer and template 3RHW (4COF) were calculated to be 39.0 % (62.0%) and 60.0 % (77.0 %), respectively. The putative binding pocket of GABA was located at the interface of α1 and β2subunits (Figure 3), consistent with previous reports[12, 26].

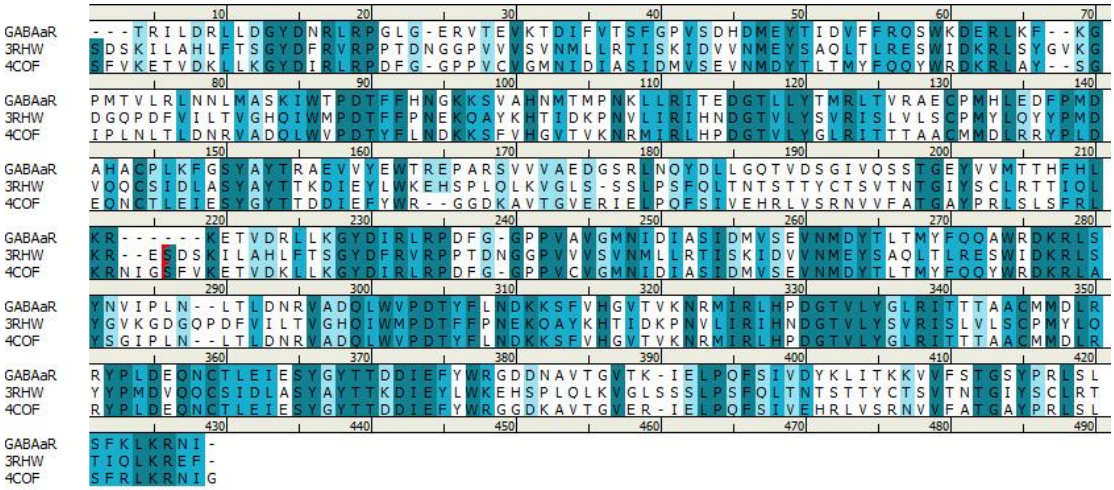

Fig. 2.Sequence alignment between GABAaR and templates (3RHW and 4COF). The identity of sequence was represented by the depths of blue colour.

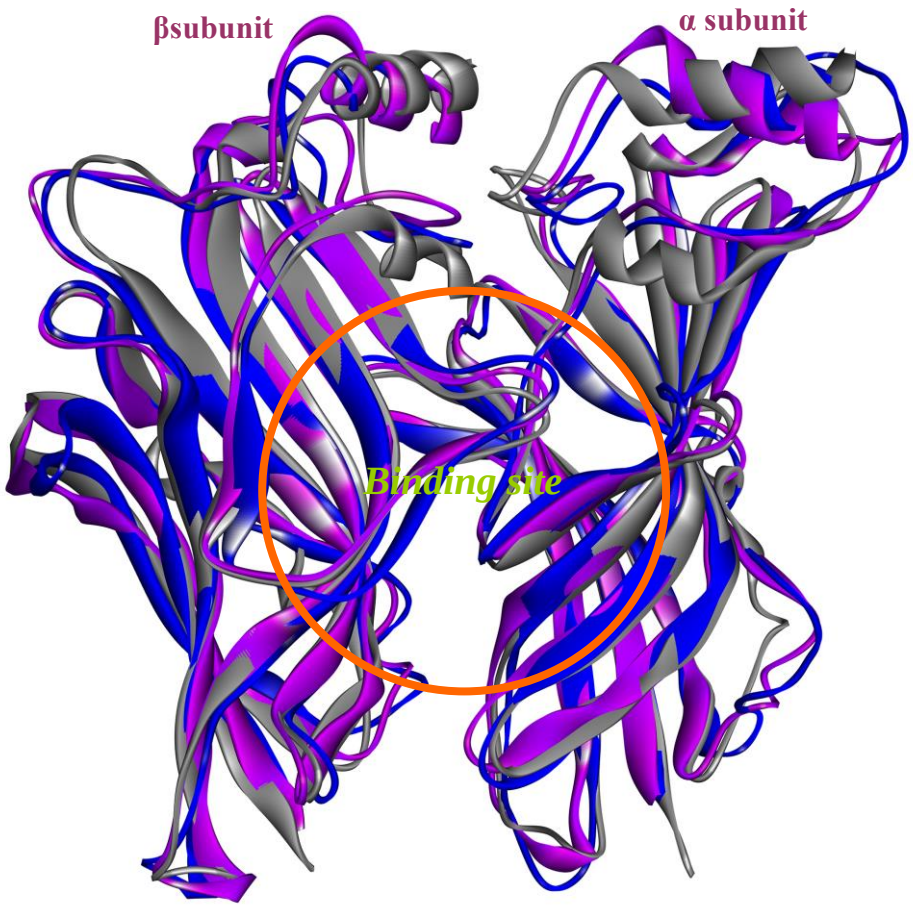

Fig. 3.The propeller structure of GABAaR. GABAaR is in blue ribbon. Regarding as the templates, the structures of glutamate-gated chloride channel (3RHW) and β3 subunit of GABAaR (4COF) are also shown in purple and gray ribbon.

The total energies and backbone atom root–mean–square deviations (RMSD) in Figure 4 revealed that the generated model reaches the equilibrium since about 60.0 ns and remain rather stable afterwards. The time-evolution backbone gyration radiuses (Rg) also confirmed the state of equilibrium(Figure 4).The refined α1/β2 dimer model had good quality with the verify score of 134.06, much higher than the critical score of 84.58 (Figure 5). And only 1.8 % of the residues were found to be located in the disallowed regions in the Ramachandran plot (Figure 6). Furthermore, the backbone RMSD between refined α1/β2 dimer and β3 subunit of GABAaR(crystallographic structure) was calculated to be 0.52 Å. Therefore, the stable structure is reasonable, and then could be used to study the interactions involving with the GABA analogues. With the conformation diversity, the binding properties of these GABA analogues somewhat differ from each other, which will be discussed in the following section.

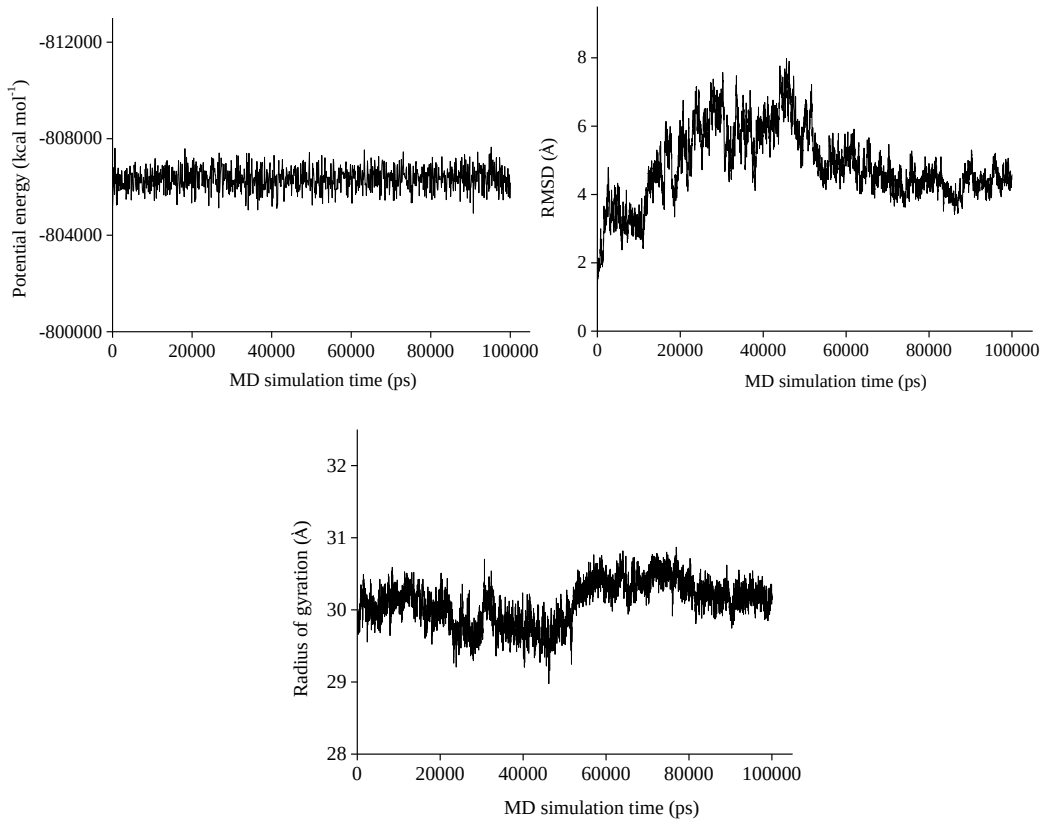

Fig. 4.Variation of the potential energy, backbone-atom RMSD and radius of gyration (Rg) during the 100.0 ns MD simulation on GABAaR.

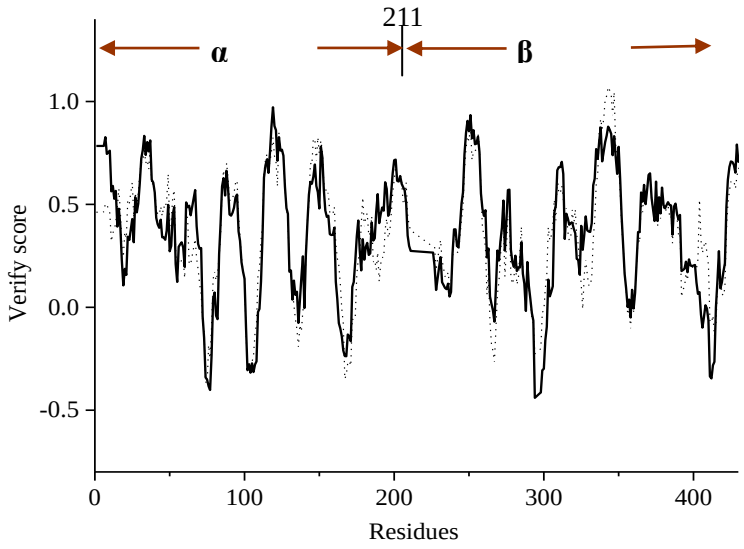

Fig.5.Profile-3D score of the GABAaR model.
Unoptimized and optimized models are in dashed and solid lines, respectively. Profile-3D score of GABAaR is shown in line with higher verifying scores indicating that the primary sequence is compatible with the 3Dstructure. If the overall quality score is positive, some or all of the structure is reasonable.

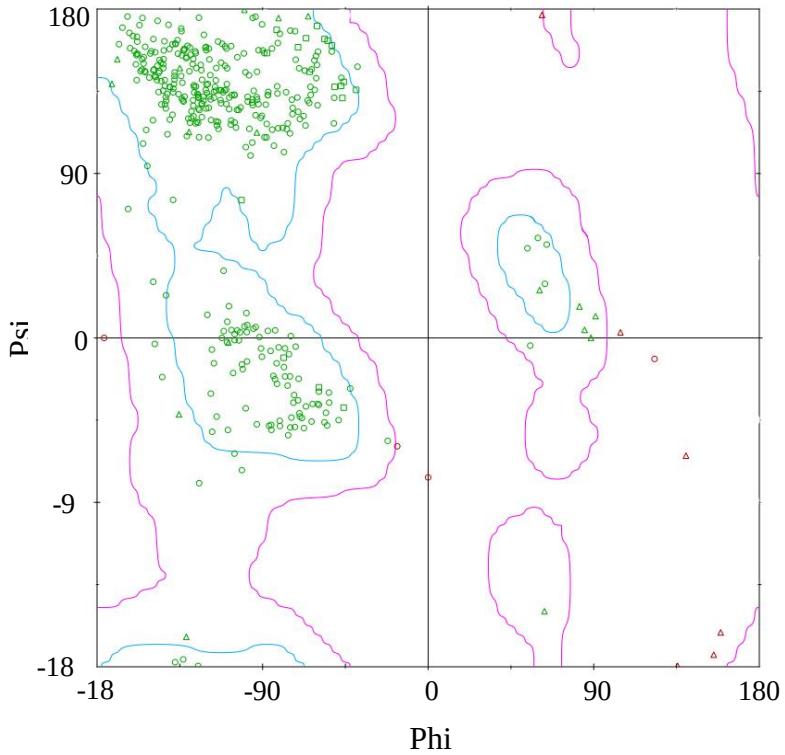

Fig. 6.Ramachandran plots of the GABAaR model.
Ramachandran plot shows phi-psi torsion angles of all residues in the structure. The coloring/shading on the plot represents the different regions: the darkest areas (in red) correspond to the "core" regions representing the most favorable combinations of phi-psi values. The percentage of residues in the "core" regions is one of the better indications of stereochemical quality.

3.2.Characteristics of the GABA analogues bindings

In accord with previous reports[12, 26], the interface of α1 and β2 subunits, characterized by the twisted and parallel β-sheet, was determined to be the binding location of GABA analogues(Fig.7). As the total energies and backbone root-mean-square deviations (RMSD) in Figure8 show, the docked complexes reached equilibrium since about 60.0 ns and remain rather stable afterwards. Therefore, the trajectories from 60 ns to 100 ns were selected for analysis. GABA, 2-methyl-GABA, 3-methyl-GABA and 4-methyl-GABAcould bind to the GABAaR, with the similar binding locations (Table 1 and Figure 7).The cDocker interaction energies (E_{int}) were calculated to be -41.20, -38.57, -40.17 and -40.22 kcal mol^{-1}, respectively. Simultaneously other five compounds in the Figure 1 move far from the binding pocket, agrees with the experiments[3, 6, 10, 13-14].Associated as the distinctions of E_{int} values, the binding poses of GABA, 2-methyl-GABA, 3-methyl-GABA and 4-methyl-GABA were somewhat different from each other, such as the H-bonding interactions (Table 1 and Figure 9).Detail information will be discussed latter.

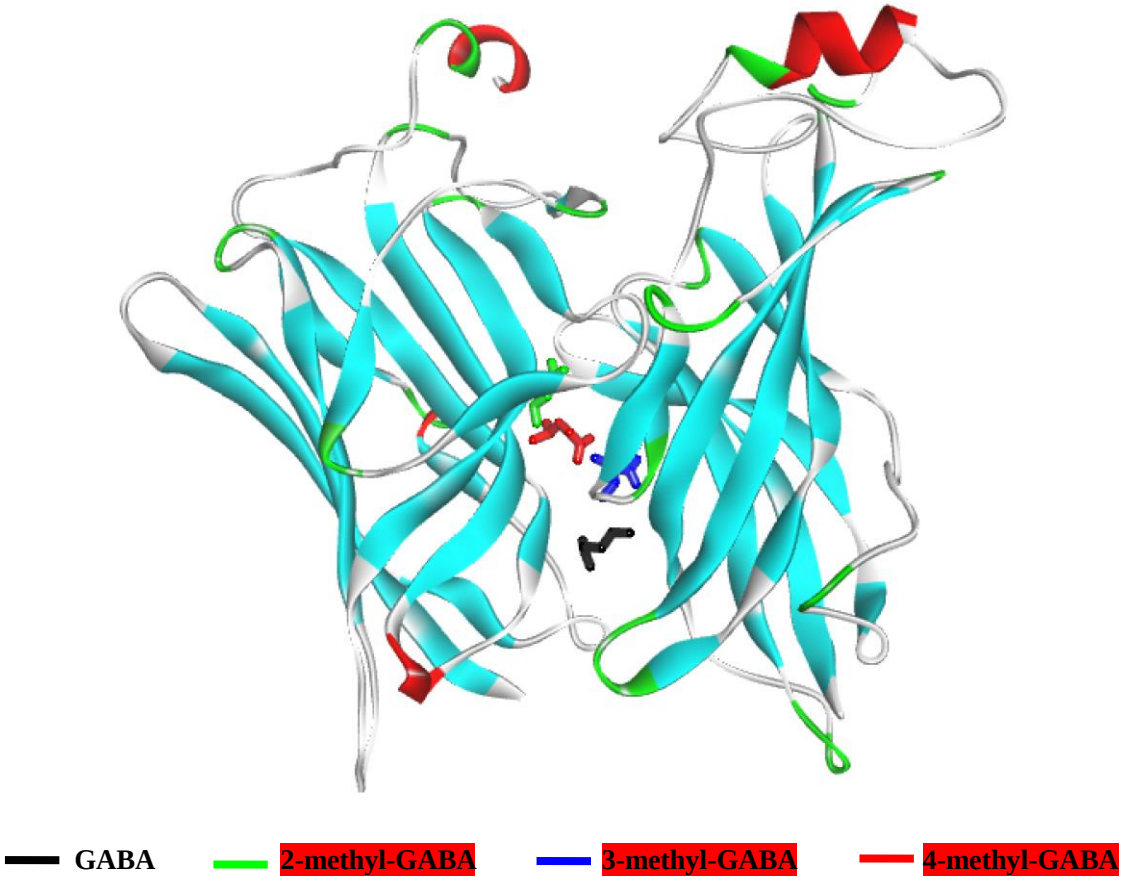

Fig. 7. The superposed binding positions of compounds within GABAaR after 100.0 ns MD simulations. The compounds are represented by stick models.

Table 1. The cDocker energy (E_{total}), cDocker interaction energy (E_{int}) and H-bonding information between $GABA_A$ receptor and various compounds [a]

Compound [b]	E_{total}	E_{int}	H-bond NO.	Connecting residues of H-bonding interactions [c]
GABA	-44.14	-41.20	3	Glu127 (1), Thr395 (2)
2-methyl-GABA	-43.51	-38.57	4	Ser194 (1), Thr196 (1), Asp257 (2)
3-methyl-GABA	-41.85	-40.17	2	His91 (1), Lys145(1)
4-methyl-GABA	-43.86	-40.22	1	Val394 (1)

[a] Energy units in $kcalmol^{-1}$;
[b] Other five compounds move far from the receptor;
[c] The numbers of related H-bonds are given in parentheses.

The pocket that binds to GABA was mapped to the α+β-interface, consistent with the results of reverse genetics and electrophysiological experiments[41-42].The negatively charged carboxylate group of GABA was docked towards the residuesVal394 and Thr395(β2subunit), with the formation of one and one H-bond (Figure9A). The lengths of the two H-bonds equal to be 2.0 and 1.8 Å, respectively. An additional H-bond was observed between the amine group of GABA and the side chain of residue Glu127 (α1 subunit), with the length of 2.4 Å. The simulated results are consistent with the previous experiments that the carboxylate and amine groups of GABA agonists are critical for the activity, associated with the contributions of electrostatic, H-bonding and cation–π interactions [43]. Furthermore, favorable hydrophobic contacts were observed between GABA and the hydrophobic portions of 6 residues (Figure9A). It disclosed that GABA holds the residue Glu127 of α1 subunit, as well as the residues Ser260, Val394, Thr395, Leu399 and Pro401 of β2 subunit, leading to the steric hindrance approach to the α+β-interface[7, 11], and then modulate the conformation of GABAaR[12, 16], very well compared to the homology modeling and docking results of Wen et. al. [26].

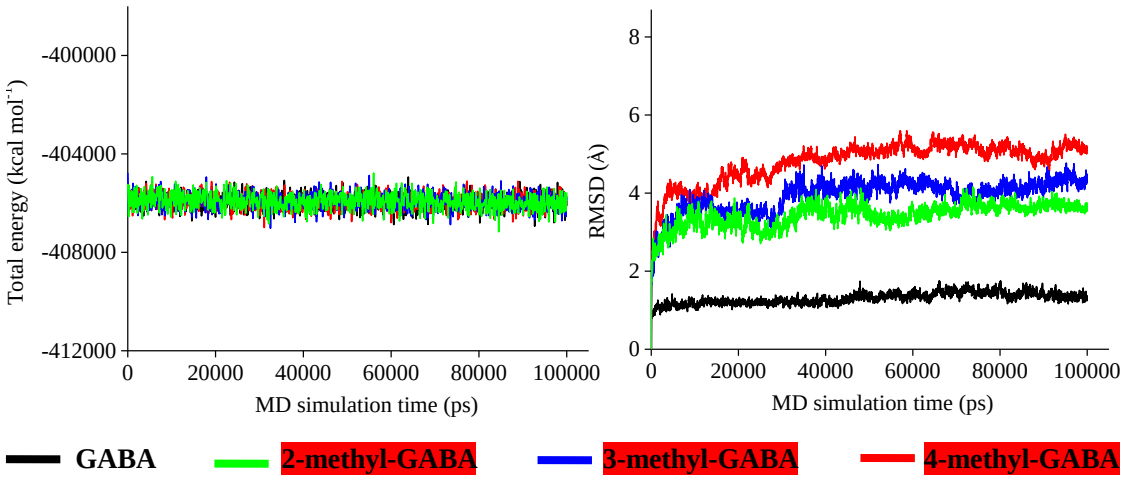

Fig. 8.Variation of the potential energy and backbone-atom RMSD during the MD simulations on GABAaR in complex with various compounds.

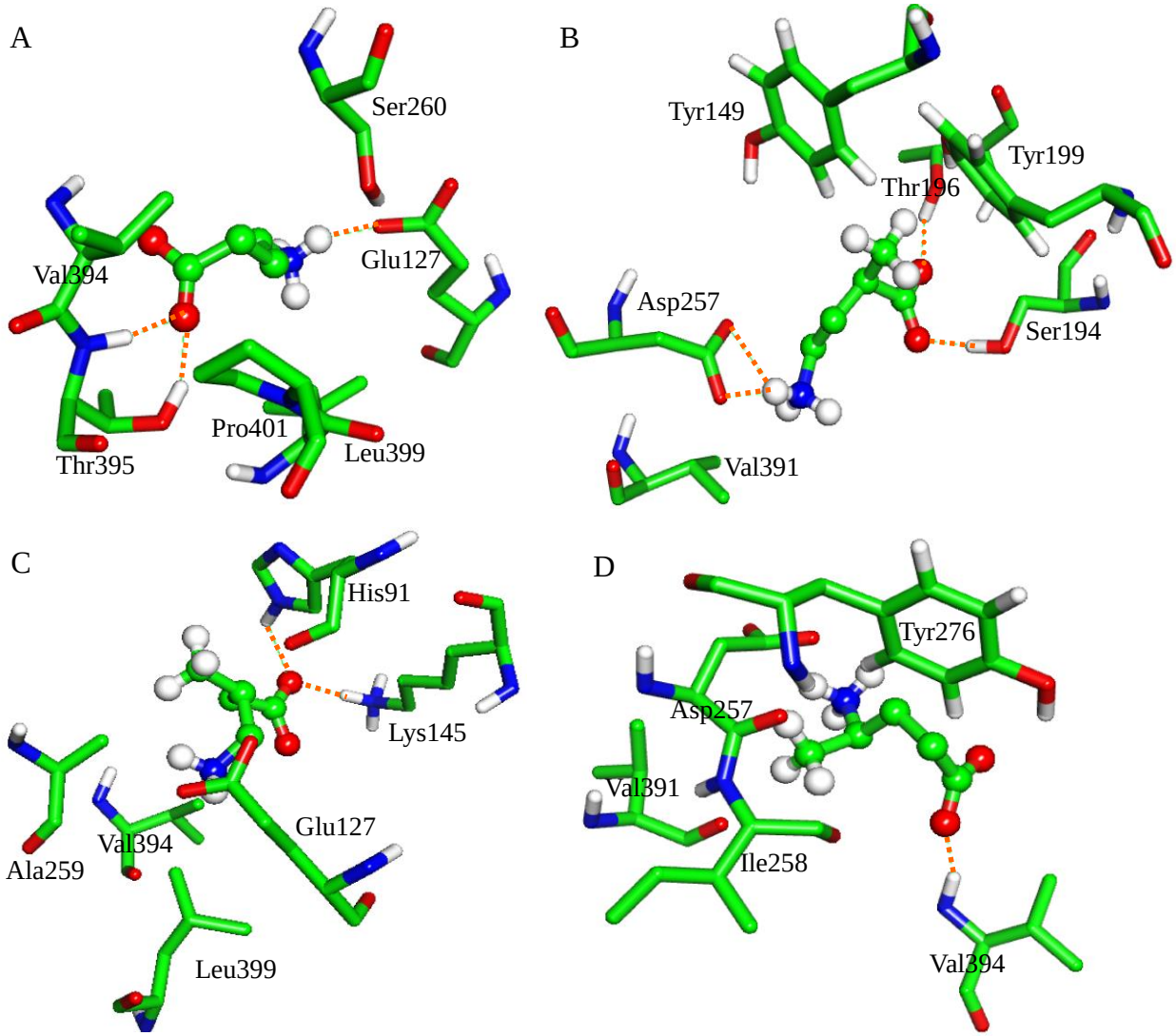

Fig.9.A close view of the binding modes of GABA (A), 2-methyl-GABA (B), 3-methyl-GABA (C) and 4-methyl-GABA (D) with GABAaR. Key residues are represented by stick models. Compounds are represented by ball and stick models. The oxygen, nitrogen, carbon and hydrogen are colored in red, blue, green and white, respectively. The important H-bonds are labeled in the dashed golden lines.

Regarding as 2-methyl-GABA, its binding location is close to that of GABA(Figures9A and 9B). With the addition of methyl group (C_2 position), the carboxylate group of 2-methyl-GABA turned towards the side chains of residues Ser194 and Thr196 (α1 subunit) and had the trend to form two H-bonds, with the lengths of 1.7 and 1.7Å, respectively(Figure 9B). Besides, it was found that the amine group of 2-methyl-GABA interacts with the side chain of residue Asp257 of β2 by the bidentate H-bond interaction, with the lengths of 2.0 and 2.4Å. The essential residues for the binding are Tyr149, Ser194, Thr196 and Tyr199 of α1 subunit, as well as Asp257 and Val391 of β2 subunit (Figure 9B). The carboxylate group of 3-methyl-GABA was sandwiched between the residues His91 and Lys145(α1 subunit)and exerted two H-bonds, with the lengths of 2.3 and 2.0Å, respectively(Figure 9C).The amine group of 3-methyl-GABA was observed to have interactions with the residues Glu127 (α1 subunit) and Val394 (β2 subunit),surrounded by the residues Ala259 and Leu399 of β2 subunit (Figure 9C). In addition, there was rather strong cation–π interaction between the amine group of 3-methyl-GABA and the imidazole functional group of residue His91, with the distance and minimum angle of 6.4Å and

45°, respectively. 3-methyl-GABA had the interactions with the α1 subunit residues His91, Glu127, Lys145 and the β2 subunit residues Ala259, Val394 and Leu399. In 4-methyl-GABA-GABAaR complex, merely one H-bond was formed between the carboxylate group of ligand and the side chain of residue Val394 (2.2 Å). With the addition of methyl group (C_4 position), the amine group of 4-methyl-GABA moved far from the α+β-interface, with no H-bonds (Figure 9D). In addition, no residues of α1 subunit were within 4.0 Å of the ligand surface. In our simulations, residues Asp257, Ile258, Tyr276, Val391 and Val394 of β2 subunit were lined surrounding 4-methyl-GABA.

3.3. Implications for rational drug design

As described above, GABA instead of other compounds had rather well interaction energy (E_{int}) and selectivity against the GABAaR. To further investigate binding features, some structure-activity relationships of the nine compounds (Figure 1) were explored for the drug designs. The maximal structural differences of GABA, 2-methyl-GABA, 3-methyl-GABA and 4-methyl-GABA were focused on the methyl group(Figure 1);in contrast to the series of 5-aminopentanoic acid, it was the length of main chain. The simulated results revealed that the four carbons of main chain may fit the binding pocket of GABAaR and probably confer a higher bioactivity in the ulterior drug design, whereas the longer main chain does not. Compared with GABA, the additional methyl groups of 2-methyl-GABA, 3-methyl-GABA and 4-methyl-GABA introduced the decreasing of interaction energy (E_{int}) (Table 1) and the reorientation of carboxylate and amine groups of ligands (Figure 9).When the methyl group was at the C2 site, the generated compound(2-methyl-GABA) had the lowest interaction energy (E_{int}) and the slight instability of docked complex (E_{total}), with the values of -38.57 and -43.51 kcalmol^{-1}, respectively (Table 1). When the methyl group was at the C3 or C4 site, the interactions (E_{int}) between the compounds and the GABAaR were improved (Table 1), associated with more hydrophobic interactions involving the methyl groups with the nearby residues (Figure 9).At the same time, the additional methyl groups introduced the 3-methyl-GABA and 4-methyl-GABA bound close to the edge of α+β-interface, the former preferred to the α1 subunit and the latter preferred to the β2 subunit (Figure 9). According to the identified charge and dipole requirement, the methyl group should be benefit for the selectivity of GABA agonists, and given enough attention in the drug designs[43].

As Table 1 and Figure 9 show, residue Glu127 (α1 subunit) played the important roles for the bindings, consistent with previous reports that the residue might form the H-bonds with the GABA agonists[40, 43]. In our simulations, the residues Val394 and Leu399 of β2 subunit could stabilize the interactions with GABA analogues via the hydrophobic interactions (Figure 9).It indicated that the three residues may be the additional elements for the bindings[40, 43]. With the low toxicity and side effects, GABA analogues throw new light on the rational design of novel psychoactive drugs. Furthermore, the residues surrounding the GABA analogues were hydrophobic or aromatic, such as His91 (α1 subunit) or Tyr276 (β2 subunit) (Figures 9C and D). Hence, the additional aromatic groups or analogy donors, such as the C=C structure, might increase the binding affinity of ligands.

4. Conclusions

In order to the in-depth understanding on the interactions of GABA analogues with the GABA receptor (GABAaR), and ultimately enable the design of novel psychoactive drugs, homology modeling, docking and molecular dynamics methods were combined to analyze their bindings. Meanwhile, this work also provides useful insights for the structure-activity relationships and how to effectively modify the functional groups.

The putative binding pocket of GABA was located at the α+β-interface of GABA receptor. It was found that the series of 5-aminopentanoic acid(Figure 1) have no strong interactions with the residues involved in receptor binding. At the same time, GABA, 2-methyl-GABA, 3-methyl-GABA and 4-methyl-GABA could fit the binding pocket of GABAaR, with the distinct binding poses. For example, the H-bonding numbers were 3, 4, 2 and 1, respectively (Table 1).The interaction energies (E_{int}) were calculated to be -41.20, -38.57, -40.17 and -40.22 kcal mol^{-1},

respectively.

GABA instead of other compounds had the best selectivity to the GABAaR. It was found that short main chain(four carbons) may fit the binding site of GABAaR and probably confer the high bioactivity. The methyl group addition introduced the reorientation of carboxylate and amine groups (GABA analogues), associated with more hydrophobic contributions (Table 1 and Figure 9). Besides, the methyl group may be beneficial for the drug selectivity. The α1 subunit residue Glu127 and β2 subunit residues Val394 and Leu399 played the additional essentials in stabilizing the interactions with GABA analogues. In addition, the modifications of GABA were explored compared with the properties of the surrounding residues. We hope that the results will be helpful for designing novel neuromodulatory drugs.

Acknowledgements

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