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Modeling of αk/γ2 (k = 1, 2, 3 and 5) interface of $GABA_A$ receptor and docking studies with zolpidem: Implications for selectivity

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Abstract

The three-dimensional models of the αk/γ2 (k = 1, 2, 3 and 5) interface of $GABA_A$ receptors, which included the agonist-binding site, were constructed and validated by molecular modeling technology. To investigate the mechanism of α subunit selectivity of zolpidem, docking calculations were used to illustrate the potential binding modes of zolpidem with different α subtypes. The results revealed that there were three reasons resulting in the distinct binding affinity of zolpidem to different α subtype. Firstly, the number of hydrogen bonds of agonist–receptor complex would determine the magnitude of binding affinity. Secondly, the His residue in loop A of α subunit was indicated as a key role of benzodiazepine binding. Thirdly, the side chain of Glu in loop C reduced the affinity of zolpidem to those receptors containing α2, α3 or α5 subunits.

Keywords: αk/γ2 interface; Homology modeling; Zolpidem; Docking study; Selectivity

1. Introduction

$GABA_A$ receptors are the main inhibitory neurotransmitter in the mammalian central nervous system [1]. They are members of superfamily of ligand-gated ion channel (LGIC) that include the nicotinic acetylcholine receptors, glycine receptors and serotonin receptors too [2]. Molecular cloning has revealed that $GABA_A$ receptors are heterogeneous since they were formed by different isoforms of several subunit classes. To date, 7 subunit families and 16 subunit subtypes [α (1–6), β (1–3), γ (1–3), δ (1), ε (1), η (1), and θ (1)] have been found in mammalian species [3,4]. Most $GABA_A$ receptor subtypes in vivo are believed to be composed of α, β and γ subunits and the physiological and pharmacological properties of individual subtypes depend on their precise subunit composition. It is reported that the anxiolytic drugs of the benzodiazepine family exert their soothing effects by potentiating the responses of $GABA_A$ receptors to GABA [5]. However, not all $GABA_A$ receptor in the brain can recognize the benzodiazepins. It is known that the particular α subunit isoform is the primary determinant of benzodiazepine recognition. For the six α isoforms, only α1, α2, α3, and α5 determine benzodiazepine pharmacology and α4 or α6 subunit does not recognize benzodiazepine. If the α1 subunit in the most common $GABA_A$ receptor is replaced by α4 or α6, the receptor would fail to recognize the benzodiazepine. Biochemical and mutational analysis show that this insensitivity is due, at least in part, to a single amino acid substitution: an arginine residue in α4 and α6 subunits replaces histidine that is present in the extracellular N-terminal domain of α1, α2, α3, and α5 subunits [6,7]. Also, replacing this arginine in the α6 subunits with the corresponding histidine in the α1 subunits confers sensitivity to diazepam, CL218872, and zolpidem [8]. Additionally, mutational studies show that γ2 subunit is a necessary subunit for benzodiazepine binding [9].

Heterologous expression studies further confirm that $GABA_A$ receptors containing α1 subunit display a type I BZ-binding profile, while the receptors containing α2, α3 or α5 subunits display BZII-type pharmacology [10]. Diazepam, CL218872 and zolpidem can distinguish types I and II benzodiazepine pharmacology. They all display a high affinity to the α1 subunit-containing receptors; however, CL218872

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and zolpidem are different from diazepam in having reduced affinity to α2-, α3- or α5-containing receptors. Zolpidem, the most widely prescribed hypnotic in the market, is able to distinguish $GABA_A$ receptors on the basis of their α subunit isoform composition: it has a high affinity for those receptors which contain α1, a relative low affinity for those receptors which contain α2 or α3 and a very low affinity for those receptors which contain α5 [11–13]. The affinity of zolpidem for subtypes 1, 2, 3 and 5 are 17 nM, 291 nM, 357 nM and >15 μM, respectively [14].

LGIC structures at atomic level have not been resolved up to now due to the difficulty in crystallizing integral membrane proteins. Recently, the crystal structure of acetylcholine-binding protein (AChBP) from *Lymnaea stagnalis* has been determined [15,16], which is a water-soluble protein with high homologous to the extracellular domains of ligand-gated ion channels and has been proved to be a good model for modeling the N-terminal domain of the members of the LGIC receptor superfamily [17–21]. Based on the crystal structure of AChBP, models of GABA receptor have been constructed. However, most previous studies were focused on the construction of α1β2γ2 subtype, which was the most common subunit combination in the native brain. To our best knowledge, little attention was paid on the modeling for other subtypes of $GABA_A$ receptors. Moreover, little information of interaction mode of agonists in the benzodiazepine-binding site of GABA receptors was available.

To guide the structure-based design of subtype-specific $GABA_A$ receptor agonists, it is valuable to understand the differences of molecular details of the binding site in α1, α2, α3 and α5 subtypes. The interaction mode analysis of agonist–receptor complex will also gain insights into the causes that zolpidem has different affinity with α1, α2, α3 or α5 subtypes, respectively. In this work, using molecular modeling technology, the three-dimensional models of the α*k*/γ2 (*k* = 1, 2, 3 and 5) interface that include the agonist-binding site are constructed and validated. The docking studies of zolpidem with our models would be helpful to explore the architecture of the binding pocket of different receptor isotypes that might be the leading determinant for the subunit selectivity of zolpidem.

2. Methodology

2.1. Sequence alignment

The multiple alignments between AChBP and the extracelluar domains of human GABA receptor α1, α2, α3, α5 and γ2 subunits were generated using FUGUE, which is a program of recognizing distant homologues by sequence-structure comparison [22].

2.2. Structures model

Based on the structure coordinates for A and B chains of AChBP and the alignment file produced by FUGUE, α*k* and γ2 models were separately developed by automodel class of MODELLER (version 8v2) [23]. In the model building, we employed an optimization method involving conjugate gradients and molecular dynamics to minimize violations of the spatial restraints. For each subunit, 20 models were obtained from the MODELLER calculation and each model consisted of five structures with different loop conformations. The best model for each subunit was determined by the lowest value of the MODELLER objective function. To evaluate the fold of the selected model, MODELLER was used for calculating the discrete optimized potential energy (DOPE) score of each residue [24]. In comparison with the DOPE scores of the template structure, the area with the highest DOPE scores in the energy profile of the model structure was refined by using the loopmodel module of MODELLER [25]. The final model for each subunit was evaluated with PROCHECK [26,27].

The interface of two subunits was generated by superimposing the independently produced α*k* and γ2 models onto A and B chains of AChBP. The generated dimmer was then energy minimized with the GROMOS96 implementation of SPDBV [28]. Ten Ångström cut-off was adopted for non-bonded interactions. The minimization protocol included 2000 steps of steepest descent, followed by 2000 steps of conjugate gradients.

2.3. Docking study

The structure of zolpidem was built with standard bond length and angle using ChemDraw and then optimized with the MM2 force field of Chem3D program (default settings: step interval, 2.0 fs; frame interval, 10 fs; terminate after 1000 steps; heating/cooling rate, 1.0 kcal/atom ps and target temperature, 300 K) [29]. Docking studies were carried out by using the program AUTODOCK3.05 [30]. This program starts with a ligand molecule in an arbitrary conformation, orientation, and position and finds favorable dockings in a protein-binding site using both simulating annealing and genetic algorithms.

The program AutoDockTools (ADT), which has been released as an extension suite to the Python Molecular Viewer, was used to prepare the protein and the ligand [31]. For the macromolecule, polar hydrogens were added, and then Kollman United Atom charges and atomic solvation parameters were assigned [32]. For the ligand, hydrogens were added before computing Gasteiger charges, and then the non-polar hydrogens were merged [33].

The grid maps of docking studies were computed using the AutoGrid3 included in the Autodock3 distribution. Grid center was centered on the active site and 45 × 45 × 45 points with grid spacing of 0.375 were calculated. The GA-LS method was adopted to perform the molecular docking. The parameters for GA were defined as follows: a maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; mutation and crossover rates of 0.02 and 0.8, respectively. Pseudo-Solis & Wets parameters were used for local search and 300 iterations of Solis & Wets local search were imposed. The number of docking runs was set to 50. Both Autogrid and Autodock computations were performed on SGI Octane 2 graphics workstation.

After docking, all structures generated were assigned to clusters based on a tolerance of 1 Å all-atom RMSD from the

lowest-energy structure. The final docked representation of the potential binding mode of zolpidem was chosen based on the selection of the compound possessing the lowest docked energy within the most populated cluster. The docking result in all cases showed that the lowest docked energy conformation was included in the largest cluster found. The further detailed inspection of independent zolpidem conformers in the cluster with the highest population revealed that most had nearly identical orientation. SPDBV was used to extract all amino acid residues within 5.0 Å of the ligand surface. Hydrogen bonding and hydrophobic interactions between docked zolpidem and amino acid residues within the interface of dimer were analyzed using Ligplot (Version 4.22) [34].

3. Results and discussion

3.1. Sequence alignment

The amino acid sequences of α1, α2, α3, α5 and γ2 subunits of human $GABA_A$ receptor were retrieved from Swiss-Prot/TrEMBL database (the accession numbers were P14867, P47869, P34903, P31644 and P18507, respectively). The amino acid residues in the intracellular domains were removed since they were outside benzodiazepine (BZ) binding site. Sequence and structure of the acetylcholine-binding protein (AChBP) were obtained from the RSCB protein data bank at 2.7 Å resolutions (PDB code: 1i9b).

It is well known that the sequence identity between the extracellular domain of $GABA_A$ receptor subunits and the AChBP is less than 20%, but the secondary structure similarity of the both is estimated as about 80% [17]. To improve the reliability of the alignment, ClustalW [35] was previously used to align AChBP with Cys-loop ligand-gated ion channels and particularly with those that are similar to AChBP (such as nicotinic ACh receptor) [36]. However, ClustalW uses the global alignment algorithm, which is not suitable when the sequences to be aligned differ greatly. For this reason, FUGUE, which quantifies sequence alignment in the context of 3D structure, was used to create the alignment and thus greatly improved the alignment accuracy. Fig. 1 shows the final multiple alignments of amino acid sequences of AChBP with the amino terminal domains of α1, α2, α3, α5 and γ2 subunits. The alignments were annotated using the program JOY [37]. Regions containing insertions relative to template are the greatest sources of uncertainty in homology modeling. In our model, only small infrequent gaps can be observed at loop domain, so it can be concluded that the alignment of six sequences was reliable.

3.2. Model building

The α models and γ2 model were obtained from independent MODELLER calculation based on A and B chains of AChBP, respectively. Each subunit was constructed with 20 models and each model consisted of five structures with different loop conformations. The objective function values for the original 20 models were tightly clustered. After refinement of the initial model with the different loop conformations, a big gap was found between the objective function value of the refined model and that of the initial model, suggesting that the accurate conformation of the loop might lead to the decreasing objective function value. We selected the best model with the lowest value of the MODELLER objective function, which is reported in the second line of the model PDB file. Although the values of the objective function in MODELLER is not an absolute measure, in the sense it could be used to rank models calculated from the same alignment.

For the chosen models, MODELLER was used to calculate the discrete optimized potential energy (DOPE) score for each residue. Compared with the template, the evaluation for the chosen model showed 13 residues with relative positive energy in each model (Fig. 2). This region of each subunit was then refined by using the loopmodel module of MODELLER. The final model was the one with the lowest MODELLER objective function number. DOPE score profiles of α1 subunit before and after loop refinement were shown in Fig. 2. The refined DOPE score profile showed an evident improvement compared to the profile of the initial model. The score at almost each residue from E138 to A150 of α1 subunit was less than the score of the corresponding residues of the unrefined model.

Based on the previous experimental results, the subunits in the dimer were arranged with αkγ2 (k = 1, 2, 3 and 5) order (clockwise from intracellular view) which was supposed to create a BZ-binding site in the interface of α and γ subunits [38,39]. In our modeling process, to maintain the complementarity between subunits at their interface, α and γ models were constructed based on A and B chains of AChBP, respectively, which could guarantee that the model structure did not show major differences with the AChBP structure. The dimer interface was created by superimposing αk (k = 1, 2, 3 and 5) and γ2 subunits onto A and B chains of AChBP, followed by subsequent energy minimization which could remove steric clashes at the subunits interfaces. As expected, the backbone atoms of the predicted models and AChBP were well overlapped (RMSD values computed using SPDBV were 0.64 Å for α1γ2, 0.64 Å for α2γ2, 0.59 Å for α3γ2 and 0.63 Å for α5γ2, respectively) due to the algorithm used by MODELLER and the low number of gaps in the alignment. Additionally, the backbone conformations of the constructed models were evaluated by the inspection of the Phi/Psi Ramachandran plot obtained from PROCHECK analysis. The Ramachandran plots showed that the four dimer models had good quality with 85–88% of the residues in the most favored regions but no residues in disallowed conformations (the template had 86.6% residues in most favored regions).

3.3. Docking study

3.3.1. Location of the putative binding site

The binding site for benzodiazepine ligand was known to be located at the interface of two adjacent α/γ subunits. According to experimental data, the benzodiazepine binding site was lined by various residues including H102, Y160, T207 and Y210 in the α1 subunit, and F77 and S142 in the γ2 subunit

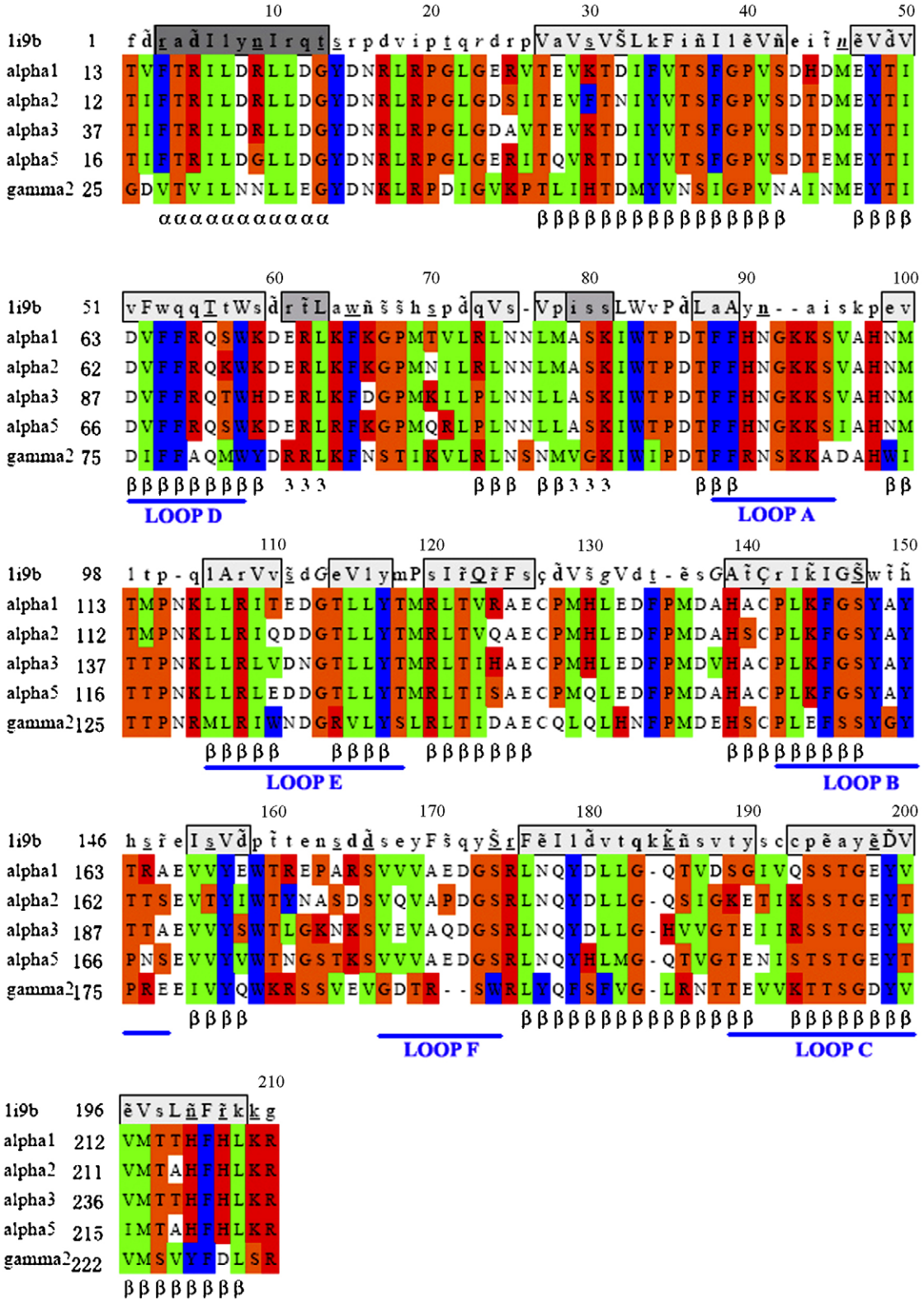

Fig. 1. The amino acid sequence of the extracellular domain of the $GABA_A$ receptor subunits were aligned with that of AChBP. The alignments were annotated using the program JOY. Key to alignment: a-helix, α; b-strand, β; 3_{10} helix, 3; solvent accessible, lower case 'x'; solvent inaccessible, upper case 'x'; hydrogen bond to main-chain amide, boldface 'x'; hydrogen bond to main-chain carbonyl, underlined 'x'; disulfide bond, cedilla 'c'; positive f-torsion angle, italic 'x'.

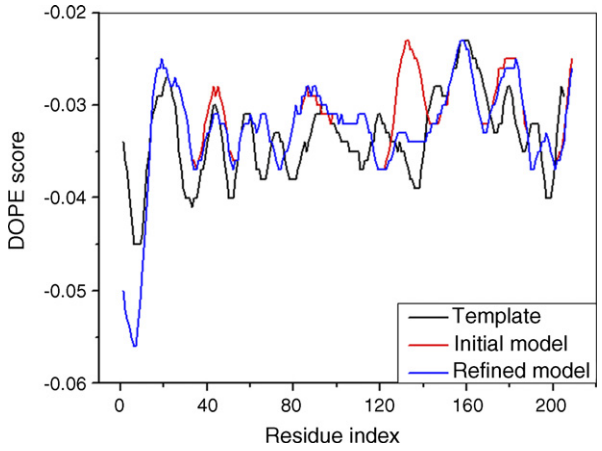

Fig. 2. DOPE score profiles of the template and α subunit before and after loop refinement.

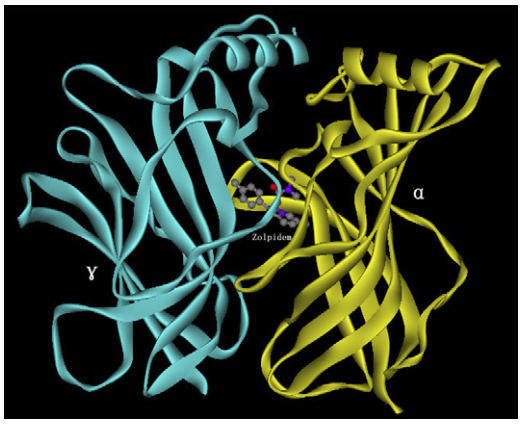

Fig. 3. The ribbon representation of α/γ dimer defined the putative agonist-binding pocket at the subunit interface. zolpidem was docked into subunit interface and the bottom of the structure was in contact with the cell membrane. Zolpidem was shown as scaled ball-and-stick colored by atom charge. Yellow: α subunit, cyan: γ subunit.

[10,13,7,6,40–42]. It was suggested that these residues were part of, or close to the binding pocket of the benzodiazepine ligand. The residues discussed above were all homologous to residues that were suggested to form the binding site of acetylcholine (ACh) in the nicotinic acetylcholine receptor and the recently crystallized acetylcholine-binding protein (AChBP) [15,43]. Moreover, the alignment of $GABA_A$ receptor subunits with nicotinic acetylcholine (ACh) receptor implicated that the binding sites for benzodiazepine and ACh had the similar architecture. Although the structure of AChBP had not yet been crystallized in complex with ACh, there was a HEPES buffer molecule presented in the putative binding pocket at each intersubunit interface [15]. Mutational experiments further proved that the residues, which surrounded HEPES, were conserved with the residues in the *n*ACh receptor that had been implicated experimentally in agonist binding [16,44]. Therefore, in our docking study, the binding position was defined by the position of HEPES in the AChBP structure. The docking of zolpidem into α1γ2, α2γ2, α3γ2 and α5γ2 was performed with the program AUTODOCK3.05. The docking representation of α1/γ2 interface with zolpidem was shown in Fig. 3.

Table 1
Residues within 5 Å of zolpidem in loops A, B and C of α*k* (*k* = 1, 2, 3 and 5) subunit and loops D and E of γ2 subunit

Subtype	αk subunit					γ2 subunit			
	Loop A	Loop B		Loop C		Loop D		Loop E	
α1γ2	**Phe100**	Lys156	**Phe157**	Val203	**Gln204**				
	Phe101	**Gly158**	**Ser159**	Thr207	**Gly208**				
						Phe77		**Ser142**	
	His102	**Tyr160**	Ala161	Glu209	**Tyr210**				
	Asn103	Tyr162	Thr163	Val211					
α2γ2	**Phe99**								
	Phe100	Lys155	**Phe156**	Glu200	**Ile202**				
	His101	**Gly157**	**Ser158**	**Lys203**	Glu208	**Phe77**		Ser142	
	Asn102	**Tyr159**	Ala160	Tyr209					
	Gly103								
α3γ2								Met130	Leu131
	His126	Ser183	**Tyr184**	Glu225	**Ile227**	Phe77	Ala79		
								Arg132	**Leu140**
		Ala185		Arg228		**Gln80**	**Met81**		
								Tyr141	**Ser142**
α5γ2					Gly202	Thr203			
								Met130	Leu131
			Tyr163	Ala164	Glu204	Ile206			
								Arg132	Leu140
			Tyr165x	Ile206	Ser207	Ser209			
								Tyr141	Thr142
					Thr210	Gly211			

The boldface represented the residues that had direct contact with zolpidem, while the italic and the box represent the conserved residues which played the key role in determining the zolpidem binding.

3.3.2. Predicted interactions of zolpidem with the interface of α1/γ2 dimer

Using an RMSD-tolerance of 1.0 Å, docking of zolpidem to α1/γ2 interface resulted in four multi-member conformational clusters from 50 runs and the major cluster included 22 similar conformations. The lowest docked energy structure in this cluster showed that 21 amino acid residues were within 5.0 Å of the zolpidem surface (shown in Table 1). Among them, 19 residues were from α1 subunit. In the receptor–agonist complex, three hydrogen bonds were formed between zolpidem and the residues that were in loops B and C of α1 subunit (Fig. 4a). The detailed analysis of hydrogen bond showed that

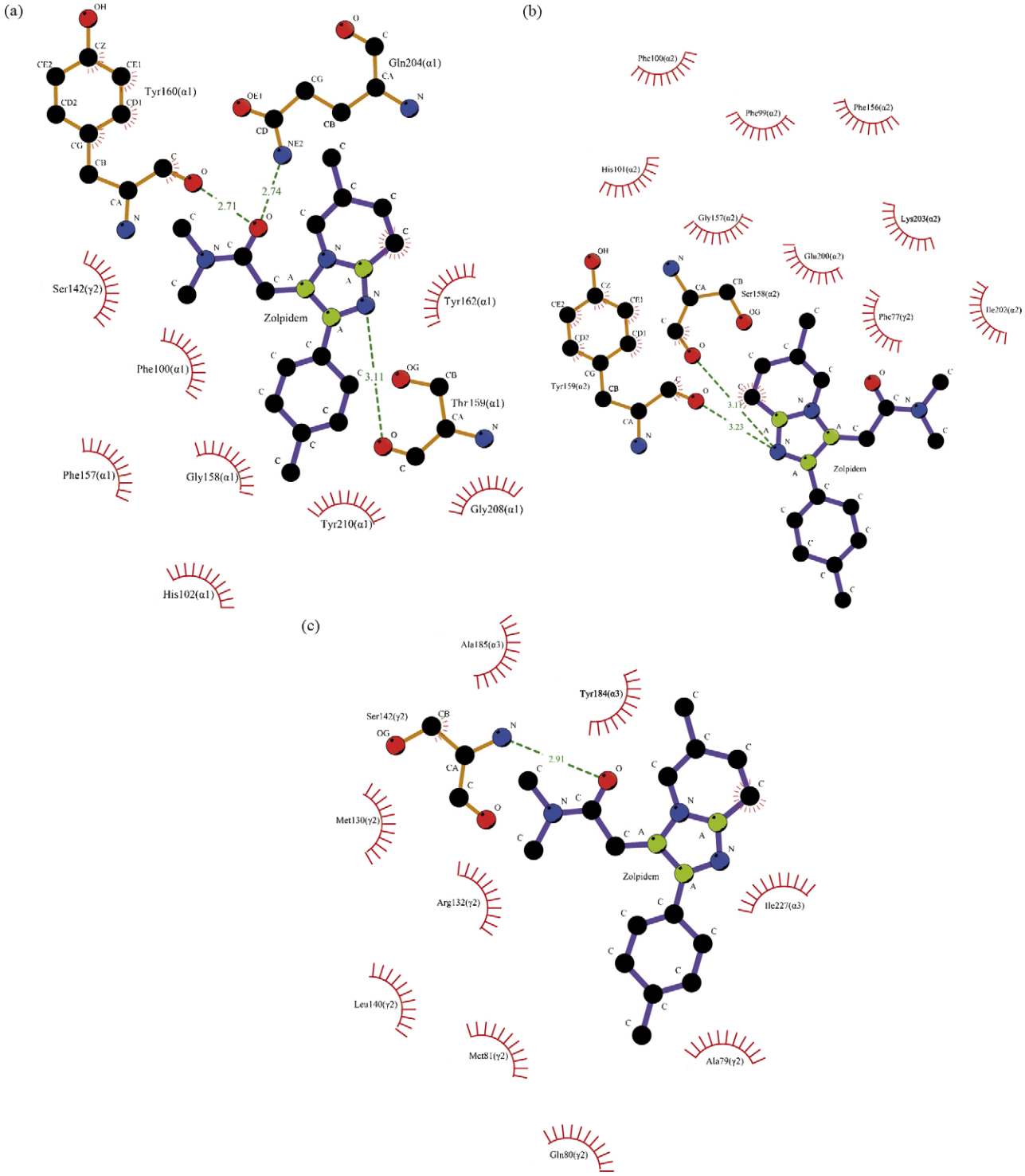

Fig. 4. Two-dimensional representation for the predicted hydrogen bonding and the hydrophobic interaction of zolpidem with the residues from α1/γ2 (a), α2/γ2 (b) and α3/γ2 (c) interface of $GABA_A$ receptor. Hydrogen bonds were drawn as green dotted lines and hydrophobic interactions were illustrated with spokes by the Ligplot 4.22.

the oxygen of acetamide had the trend to form two hydrogen bonds: one to NE2 from Gln204 and the other to backbone carbonyl group of Tyr160. Additionally, the third hydrogen bond was found between nitrogen of the imidazole and carbonyl group of Ser159. On the other hand, the pyridine ring of zolpidem was sandwiched between Tyr160 and Tyr162 and surrounded by Phe100 and His102 in loop A, Phe157 and Gly158 in loop B, Gly208 and Tyr210 in loop C and Ser142 in loop E through hydrophobic interactions.

Mutational study had proved that His102 in the α1 subunit was a major determinant of benzodiazepine binding. The substitute of His102 by the equivalent Arg in α6 subunit led to insensitivity to the classical BZ antagonists such as diazepam, zolpidem and CL218872 [6,40]. Site-directed technology showed that H102C labeled with a reactive group could attach to the C atom in diazepam by covalent interaction, and the C atom in diazepam was usually connected with a Cl atom [45]. However, His102 had the hydrophobic contact with pyridine moiety of zolpidem in our model.

Aromatic residues network, which was formed by Phe100, Phe157, Tyr160, Tyr162 and Tyr210 of α1 subunit, lined the putative binding site and had direct contact with zolpidem that showed similar to those residues surrounding the quaternary ammonium of HEPES in the AChBP [15]. Site-directed mutagenesis further demonstrated that the modulation of benzodiazepine was dramatically impaired when two tyrosine residues on the a1 subunit (Tyr160 and Tyr210) were mutated to serine. The Y210S substitution resulted in a seven-fold increase in the EC_{50} for BZ diazepam, and the Y160S substitution nearly completely abolished BZ-mediated potentiation [42].

The Phe77 and Thr142 in the γ2 subunit have been suggested to be part of the binding pocket for the ligand of the benzodiazepine binding site [13,40]. Previous study implied that Thr142 was involved in mediated conformational change via a hydrogen bond with Tyr160 of a1 subunit, which was in agreement with our docking study [46]. The proximity of the hydroxyl group of Tyr160 to loop E allowed H-bonds to be formed with the backbone carbonyl of Thr142 (the predicted H-bond distance was 2.6 Å). Phe77 in our model had not direct contact direct with zolpidem, which might improve the stability of binding pocket through the aromatic side chain.

3.3.3. Predicted interactions of zolpidem with α2/γ2 and α3/γ2 dimer

Docking result of α2/γ2 dimer with zolpidem showed that nitrogen of imidazole ring was predicted to be involved in the formation of hydrogen bonding, interacting with main-chain carbonyl group from Ser158 and Tyr159. Moreover, favorable hydrophobic contacts were observed between pyridine moiety of zolpidem and 11 amino acid residues (Fig. 4b).

Table 1 shows that 15 residues of α3/γ2 interface were located within 5.0 Å of the agonist surface. Only one hydrogen bond was formed between Ser142 of γ2 subunit and acetamide of zolpidem. Ten amino acid residues took part in the formation of hydrophobic pocket (Fig. 4c). Photoaffinity labeling studies had suggested that the $GABA_A$ α subunit was a major determinant of BZ binding and specificity [47,48]. However, in this docking result, most residues contributing to the formation of agonist-binding pocket came from γ2 subunit, which might influence the affinity of zolpidem to $GABA_A$ receptors containing α3 subunit. No particular interaction was implicated between His126 and zolpidem although this residue was located in the vicinity of zolpidem. This result may lead to high K_d values of zolpidem to α3 subtype because the presence of His at position 102 of α1 subunit (the equivalent position in other α subunits) could determine the high affinity of the benzodiazepine agonist.

The interaction of the ligand with the receptor was mainly mediated by hydrogen bond and hydrophobic interactions. According to three docking study discussed above, the number of residues taking part in the direct hydrophobic interaction with zolpidem had little change among the different subtypes; however, the lower number of hydrogen bonds between zolpidem and α(k = 1, 2 and 3)/γ2 dimer might be one of the reasons for the decreased affinity presented in the different subtypes.

Another important amino acid residue, which was located in the 5.0 Å binding site and determined the low selectivity of α2/α3 subunit to zolpidem, was identified as Glu200/Glu225 in loop C [43]. Mutational study showed that a single amino acid change, from a glutamate to a glycine at position 225 in the α3 amino acid sequence, led to at least 10-fold increasing in binding affinity to CL218872 and zolpidem. Reversely, glycine residue at position of the 201 of the α1 subunit, if mutated to glutamate which occupied the homologous position in the α3 subunit, produced a receptor that displayed BZII-type pharmacology [9,10]. Docking result showed that the large side chain of Glu likely hindered zolpidem to access the binding site from loop Wingrove et al. also proposed the carboxylic acid side chain of the glutamate residue, which was presented in α2/α3 subunit, had more charge and larger volume than glycine in α1 subunit [49]. Both reasons suggested that the lower affinity of zolpidem to α3-containing receptors was possibly assigned to inhibition of binding by either steric hindrance or charge repulsion.

3.3.4. Predicted interactions of zolpidem with α5/γ2 interface

Eighteen residues were located the binding site though no particular interactions were implicated between these residues and zolpidem. In this model, no residues from loops A and E were within 5 Å of zolpidem. The absence of His113 in the binding pocket and steric effect of Glu204, which were homologous to His102 in α1 subunit and Glu225 in the α3 subunit, respectively, might lead to a very low affinity of zolpidem to those receptors containing α5 subunit.

4. Conclusion

The three-dimensional model of the αk/γ2 (k = 1, 2, 3 and 5) dimer had been generated using the crystal structure of acetylcholine-binding protein (AChBP) from *L. stagnalis* as structural template. To investigate the mechanism of α subunit selectivity to benzodiazepine pharmacology, the potential binding modes of zolpidem with different subtypes were illustrated for the first time by means of docking studies.

Docking results revealed different binding affinity of zolpidem to four subtypes can be attributed to the following reasons: at first, the affinity was affected by the number of hydrogen bonds of the complex. Zolpidem has three hydrogen bonds with those receptors which contain α1 subunit, two and one hydrogen bonds for those receptors which contain α2 and α3, respectively. In addition, no hydrogen bond was found between agonist and α5 subtype. Thus, the number of hydrogen bonds between zolpidem and four subtypes maybe counted for the binding affinities of zolpidem to GABA receptors containing different α subunits. Additionally, the histidine of loop A was indicated as a major determinant of benzodiazepine binding site. In our docking model, a histidine at position 102 of α1 subunit (the equivalent position at 101 in α2 subunit) had direct interaction with pyridine ring of zolpidem through hydrophilic contact. However, no direct interaction was found between His126 and zolpidem although this residue positioned at binding pocket in α3 subtype. No residue in loop A of α5 subunit contributed to the formation of the binding site. Lastly, Glu of loop C led to the low affinity of zolpidem to those receptors containing α2, α3 or α5 subunits because of large side chain itself, which was homologous to Gly201 of α1 subunit. A number of mutational analyses had suggested Gly201 was involved in forming the benzodiazepine recognition site; however, others had suggested that this residue was possibly involved in the coupling of ligand binding to channel gating and was not directly involved in the initial binding [50–52]. Our model supported the latter.

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