Journal of Molecular Graphics and Modelling 27 (2009) 813–821

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Molecular modeling of ligand–receptor interactions in $GABA_C$ receptor

Dmitry I. Osolodkin, Vladimir I. Chupakhin, Vladimir A. Palyulin, Nikolay S. Zefirov *

Moscow State University, Department of Chemistry, Leninskie Gory 1/3, 119991 Moscow, Russia

ARTICLE INFO

Article history:
Received 8 August 2008
Received in revised form 24 November 2008
Accepted 12 December 2008
Available online 24 December 2008

Keywords:
$GABA_C$ receptor
GABA
TPMPA
Molecular dynamics
Ligand–receptor interactions

ABSTRACT

A new homology model of the GABA binding site of the $GABA_C$ receptor was built. Natural agonist GABA and antagonist TPMPA were docked into the receptor and molecular dynamics simulation of the complexes was performed to clarify binding poses of the ligands. It was shown that orientation of the ligand is defined by salt bridges between the ligand and the arginine (Arg104) and glutamate residues (Glu194 and Glu196) of the binding site. Different behavior and binding poses for agonist and antagonist was demonstrated by molecular dynamics simulation along with differential movement of the loop C during agonist and antagonist binding. Binding orientations of the ligands revealed that main binding forces in the GABA binding site should be electrostatic ones.

1. Introduction

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the vertebrate CNS. GABA activates three types of receptors, known as $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ and $GABA_C$ receptors belong to nicotinicoid (Cys-loop) family of ligand-gated ion channels, which also includes the nicotinic acetylcholine receptor (nAChR), serotonin receptor 5-HT_3 and glycine receptor. These receptors are pentameric ion channels; each subunit of the pentamer contains a big extracellular N-terminal ligand-binding domain (LBD), four transmembrane (TM) helices and a variable intracellular loop between TM3 and TM4. $GABA_C$ receptors appear as chloride channel-containing homopentamers consisting of ρ subunits, the most studied of them being ρ_1; the channel pore is formed by TM2 [1]. There is evidence that ρ subunits can form functional heteromeric receptors similar to those observed *in vivo* in complexes with certain $GABA_A$ receptor subunits [2], however such heteromeric receptors are not studied well.

$GABA_C$ receptor is an interesting therapeutic target. Subunits ρ_1 of $GABA_C$ receptor are expressed in retina and cerebellum. In the other brain regions such as thalamus, mesencephalon, and temporal and frontal cortex ρ_2 receptors are expressed more widely [3]. Selective competitive antagonists of $GABA_C$ receptors may be used as cognitive enhancers, anxiolytic agents [1] and sleep regulators [4]. Little is known about pharmacology of the receptor because of the lack of highly selective ligands possessing high activity. Such ligands may be found based on the homology model of the receptor 3D structure or QSAR studies.

Recently a number of structures of the acetylcholine-binding protein (AChBP) complexed with different ligands were solved [5–7]. The folding of this protein resembles that of nicotinicoid receptors LBD, so AChBP can be used as a template for homology modeling of them [8]. There are three homology models of the $GABA_C$ ρ_1 receptor structure built to date [9–11] based on the structures of AChBP from *Lymnaea stagnalis* in the form representing the structure of the LBD in the ligand-bound (activated) state. Conservative tyrosine residues Tyr102, Tyr198, Tyr200, Tyr241, and Tyr247 are shown to play the main role in the binding site formation [9]. The receptor–GABA interactions were studied with the help of docking methods [10,11] and QM/MM method [11]. It is supposed that the GABA carboxylate group forms a salt bridge with Arg104, Ser168, and Ser243 [10,11] and the ammonium group of GABA is engaged into the cation–π interaction [10,12] or forms a hydrogen bond with Tyr198 [11].

The Cys-loop ligand-gated ion channel superfamily is divided into two families (anionic and cationic channels) based on ion selectivity of the channel. Cationic channels (nAChR, 5HT_3) are most studied experimentally [13]. The activation of the Cys-loop LGICs requires ligand binding accompanied by the so-called "loop C" [14] closure and further channel opening. The movement of loop C may be independent from ligand binding

* Corresponding author. Tel.: +7 495 939 1620; fax: +7 495 939 0290.
E-mail addresses: dmitry_o@org.chem.msu.su (D.I. Osolodkin), chupvl@org.chem.msu.su (V.I. Chupakhin), vap@org.chem.msu.su (V.A. Palyulin), zefirov@org.chem.msu.ru (N.S. Zefirov).

Abbreviations: GABA, γ-aminobutyric acid; TPMPA, methyl-(1,2,3,6-tetrahydropyridin-4-yl)phosphinic acid; LGIC, ligand-gated ion channel; MD, molecular dynamics; nAChR, nicotinic acetylcholine receptor; 5HT_3R, 5-HT_3 receptor; AChBP, acetylcholine-binding protein; LBD, ligand-binding domain; TACA, trans-aminocrotonic acid.

1093-3263/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmgm.2008.12.004

and seems to be slow natural fluctuation [15]. The experimental confirmation of the above statements was obtained by Unwin et al. for nACh receptor studied by electron microscopy. The loop C and two neighboring beta sheets turn by 15° trapping the ligand inside the binding pocket [16]. The difference between cationic and anionic Cys-loop LGICs is due to different ion selectivity of the channel. The nature of the agonists of these groups also differs greatly, the simple ligand pharmacophore of the $GABA_A$ and Gly receptor is a zwitterionic ligand with three main pharmacophoric features: H-bond donor and H-bond acceptor and a steric link between them. The ligands of nAChR and $5HT_3R$ have distinct pharmacophoric features, H-bond acceptors in the acetylcholine and serotonin are weaker than those in GABA and glycine. It is known from AChBP and nicotinic acetylcholine receptor (nAChR) studies [15] that aromatic residues in the ligand-binding pocket form the so-called "aromatic box" during receptor activation. Cation–π interaction is believed to be the main driving force of the aromatic box formation for cationic and aromatic ligands such as nicotine, acetylcholine or serotonin, but there is a discussion about dominant forces for ligand recognition in GABA and Gly receptors [17].

We have built a model of the ρ_1 $GABA_C$ receptor LBD in apo-form by homology with AChBP from *Aplysia californica* (PDB id 2BYN [7]) and docked the well-known ligands GABA (native agonist) and TPMPA (selective competitive antagonist [18]) into the binding site. Then a molecular dynamics simulation of these models has been performed for a more thorough investigation of the binding site and clarification of ligand–receptor interactions. Amino acid residues Glu194 and Glu196 located in the loop B [14] were found to be important for ligand binding.

This article is an extension of a previously published short communication [19].

2. Methodology

2.1. Amino acid sequence alignment

Amino acid sequence of the template protein was extracted from the structure file from PDB (access code 2BYN [7]). The sequence for the human ρ_1 $GABA_C$ receptor subunit [20] was obtained from the SWISS-PROT server (access code P24046) [21]. The amino acid sequences of the target and template proteins were aligned on the FUGUE web server [22]. FUGUE algorithm takes into account the secondary structure information of the template protein and predicts the secondary structure of the target protein from its sequence. The alignment obtained was compared with the multiple sequence alignment of different AChBPs and Cys-loop LGICs, and manual corrections were made where necessary. The resulting alignment is shown in Fig. 1.

2.2. Homology modeling

Homology modeling was performed by means of a MODELLER 8v1 program [23]. Thirty different models of pentameric LBD were built and the simulated annealing procedure was applied to every model. The best one according to the MODELLER objective function and PROCHECK [24] Ramachandran plot statistics was chosen for subsequent optimization.

Further optimization was performed for LBD dimer in SYBYL 7.1 (Tripos Inc., 1699 South Hanley Rd., St. Louis, Missouri 63144, USA). All hydrogens were added and Kollman–All charges were assigned to the atoms of the receptor. Then 150 steps of the Powell minimization in the Tripos force field were performed. The final structure was used for docking studies.

2.3. Docking studies

Structures of GABA and TPMPA used for docking were prepared in SYBYL 7.1. Ionic state of ligands was assigned according to physiological pH level; both ligands were used in the zwitterionic form. Atomic charges were assigned by the Gasteiger–Hückel method. Structures of the ligand molecules were minimized within the Tripos force field by the Powell method.

Docking study was performed by means of DOCK 6.1 [25] software. Positions and dimensions of the grid box were chosen to fully include the ligand-binding cavity between two adjacent subunits which is characteristic for pentameric ligand-gated ion channels. Parameters of conformational search were tuned to perform an exhaustive search of the conformational space of ligands. The ligand poses were clustered based on the RMSD threshold of 2.0 Å. The simple energetic scoring function implemented in DOCK 6.1 was used for binding energy evaluation and the choice of the best docking solution to use in molecular dynamics simulation.

2.4. Molecular dynamics

Molecular dynamics simulations were run by means of the AMBER 8 software package [26]. To reduce computational time, the SHAKE algorithm was applied to all bond lengths and the dimeric complex was partially solvated with explicit TIP3P water molecules placed inside the spherical "cap" centered on the mass center of the ligand; the cap radius was set to 20 Å (Fig. 2). The movement of receptor atoms outside the cap was restrained with the force constant of 75 kcal/(mol $Å^2$). The AMBER FF99 force field was used for the receptor and water atoms, and General AMBER Force Field (GAFF [27]) and AM1-BCC charges [28] were used for the ligands.

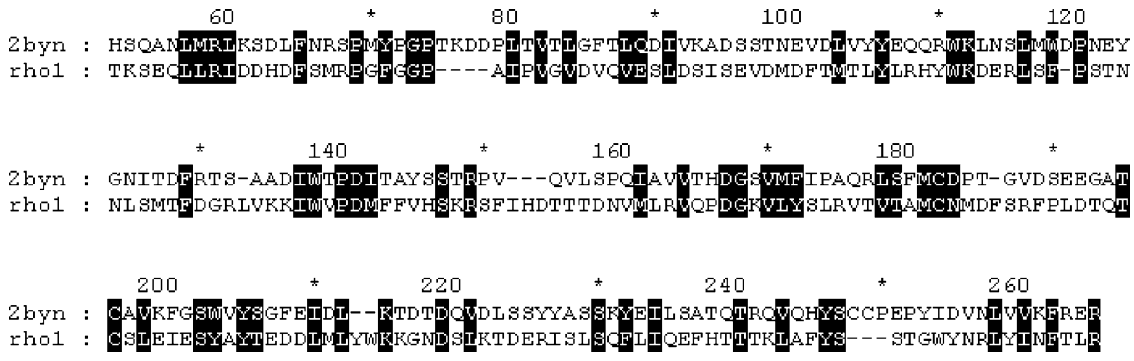

Fig. 1. Alignment of AChBP (2byn) and $GABA_C$ receptor LBD (rho1) sequences. Residue numbers are given according to rho1 sequence numeration with gaps counted; the first residue of the sequence is Thr52, the last residue of the sequence is Arg257. The 2byn sequence corresponds to the pdb file. Identical residues are highlighted.

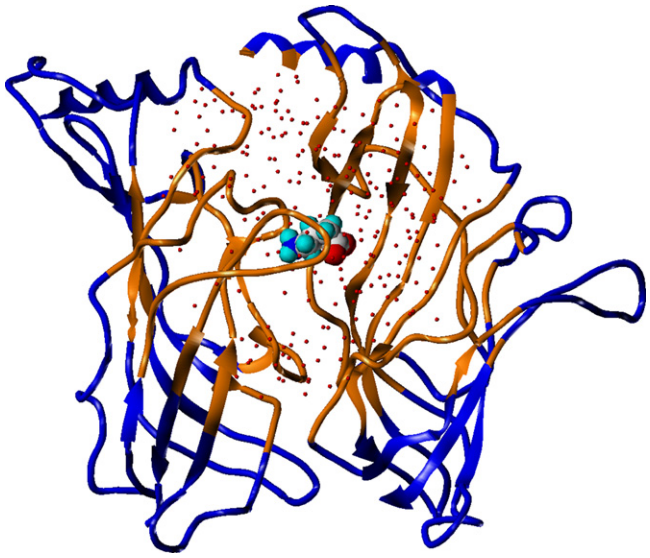

Fig. 2. Architecture of the system used for the MD simulation. Frozen residues are shown in blue; movable residues are shown in orange. GABA molecule is shown spacefilled, water molecules are given as small balls.

The system was minimized in 1000 steps by the steepest descent algorithm, heated from 0 to 300 K during 100 ps, and then the 10 ns production dynamics simulation was run.

2.5. Data analysis

Molecular dynamics trajectories were analyzed visually using VMD 1.8.4 [29], and mathematically using the ptraj module of AMBER 8 package. Default settings were used for the ptraj analysis.

3. Results

3.1. Molecular modeling

We have built a model of the homopentameric ligand-binding domain of the $GABA_C$ ρ_1 receptor in the apo-form. This domain includes 206 residues in each subunit: from Thr52 to Arg257. The first 50 residues could not be modeled based on AChBP template and probably represent a distinct domain. This hypothesis is confirmed by Domain Linker Prediction web service [30]; deletion of the first 17 amino acid residues does not influence receptor channel properties [31]. Residues from 258 to 458 represent transmembrane and intracellular domains.

Stereochemical quality of the selected model assessed by PROCHECK was found to be good (79.9% in core regions, 18.2% in allowed regions, 1.2% in generously allowed regions, 0.7% in disallowed regions). After energy minimization only 5 of 1030 residues (one residue per subunit) in the pentameric model were placed in disallowed regions of the Ramachandran plot; these residues are located far from the binding site. Two adjacent subunits rated best by PROCHECK were used for further docking and molecular dynamics study (Fig. 3).

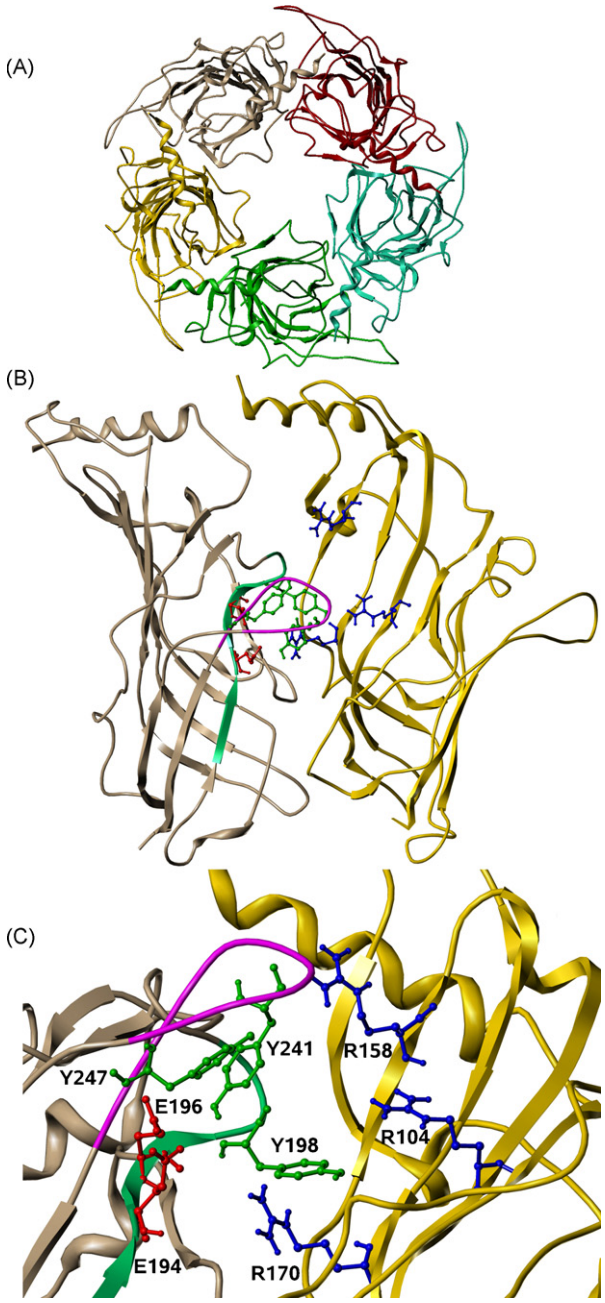

Fig. 3. (A) Top view of the model of the pentameric ligand-binding domain. (B) Overview of the dimer model used for docking and MD simulation. Important residues are shown in the binding site. Chain A is colored tan, chain B—yellow. Binding loop B of chain A is shown in green–blue color, loop C is in magenta. Arginine residues (Arg104, Arg158, Arg170 from chain B) are shown in blue, glutamate residues (Glu194, Glu196 from chain A) in red, tyrosine residues Tyr198, Tyr241, Tyr247 (chain A)) in green. (C) Closer bottom view of the binding site.

3.2. Ligand docking

The most studied ligands of $GABA_C$ receptor, GABA and TPMPA were docked into the model. The ligand-binding cavity between subunits (Fig. 3B) was investigated thoroughly to understand the ligand-binding mode. The main interactions orienting the ligand in the binding site should be salt bridges between the ligand molecule and arginine residues (Arg104, Arg158, Arg170) of chain B forming the complementary side of the binding pocket. Cationic center of the ligand may form cation–π interactions or hydrogen bonds with tyrosine residues (Tyr198, Tyr241, Tyr247 in chain A which forms the principal side of the binding site) or form a salt bridge with glutamate residues of the loop B placed deep in the binding cavity (Glu194, Glu196 in chain A). Serine residues Ser243 (chain A) and Ser168 (chain B) should not directly participate in the ligand binding: Ser168 forms a hydrogen bond with Arg104 and holds its initial orientation, whereas Ser243 is located at the edge of the loop C and can only take part in the initial seizure of the ligand molecule.

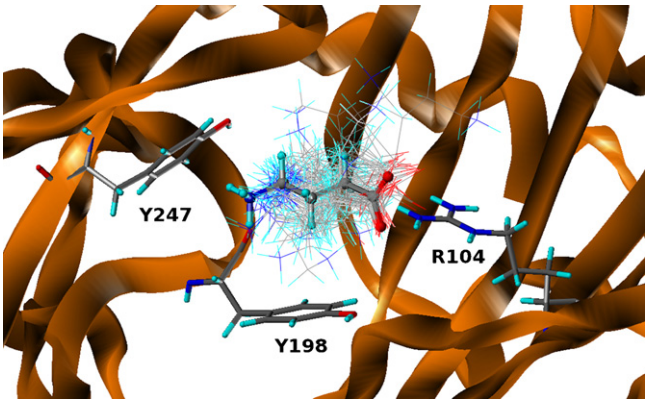

Fig. 4. A cluster of GABA docking results. Carboxy group of GABA forms a salt bridge with Arg104 (chain B), whereas amino group is preferentially positioned between Tyr247 and Tyr198 (both chain A). The most extended orientation of the ligand selected for further optimization is shown as ball-and-stick.

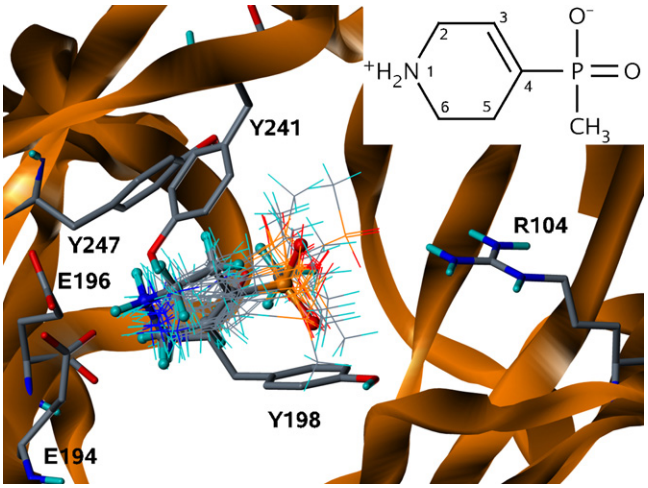

Fig. 5. TPMPA docking results. Amino group of the ligand forms salt bridges with Glu194 and Glu196 (chain A), methyl group forms a hydrophobic contact with the aromatic ring of Tyr198 (chain A), and Tyr241 and Tyr247 (chain A) residues form hydrophobic contacts with the carbon skeleton of the ligand. Arg104 does not form a salt bridge with the TPMPA phosphinic group. Orientation of the ligand selected for further optimization is shown as ball-and-stick. Inset: Structural formula of TPMPA. Atoms important for the building of the conformational descriptor (Fig. 6) are marked according to the IUPAC rules.

GABA docking results (Fig. 4) were rather ambiguous because of the great conformational flexibility of the ligand and rather big binding site volume compared to the volume of the ligand. About 100 different conformations were obtained, and no more than 60 of them were reasonable and placed the ligand molecule inside the binding cavity. The main interaction predicted by DOCK is a salt bridge between the carboxy group of GABA and Arg104 in chain B. The GABA aminogroup interacts with tyrosine residues (Tyr198 or Tyr247); the interactions may occur as H-bonds or as cation–π. An H-bond may also occur between carboxy group of GABA and phenolic hydroxyl of Tyr198, but such interaction requires a slight change of the hydroxyl orientation. Interactions with other charged or aromatic residues are not found by DOCK. The most extended (all-trans) conformation of GABA (distance between the nitrogen atom and the carboxylic carbon is 5.05 Å) in the most populated cluster (shown as ball-and-stick in Fig. 4) was chosen for further MD investigation. This choice was additionally dictated by the fact that TACA, which is fixed in the extended conformation, is one of the most potent $GABA_C$ receptor agonists known to date [1].

In contrast to GABA, TPMPA docking results are less ambiguous. This ligand is also oriented by salt bridge, but because of less conformational flexibility compared to GABA and bigger ligand volume only a few potential orientations of the ligand in the binding site are allowed. These orientations are determined by salt bridges of the ammonium group of TPMPA with Glu194 and Glu196 as well as by hydrophobic interactions between the ligand and Tyr247, Tyr241 and Tyr198 (Fig. 5). Potential salt bridge between the phosphinic group of TPMPA and Arg104 cannot be found simultaneously with TPMPA-Glu salt bridges due to the rigidity of the receptor molecule during docking. Steric hindrance between TPMPA and tyrosine residues may prevent the aromatic box from forming, being possible reason for antagonistic properties of TPMPA. The salt bridge formed by the phosphinic group of TPMPA should be stronger than that formed by the GABA carboxylic group, so the conformation of the antagonist in the binding site should be more stable and the full formation of the aromatic box is disfavored. Orientation of TPMPA in which main salt bridge is observed between ammonium group of the ligand and glutamate residues was chosen for further molecular dynamics investigation (Fig. 5).

3.3. Molecular dynamics

3.3.1. GABA

The results of GABA dynamics show that the orientation of the ligand predicted by docking is unstable. A good descriptor of GABA conformation in this case is the distance between the nitrogen atom and the carboxylic carbon which is plotted against time in Fig. 6A. In the initial conformation this distance equals 5.05 Å. The ligand molecule moves rather far from its starting position (RMSD plot is presented in Fig. 6B) and does not form interactions which are present during all the simulation (all conformations of GABA during MD simulation are shown on Fig. 7A). Distance between the nitrogen atom and the carboxylic carbon for the average pose equals 3.58 Å and corresponds to the folded conformation of GABA. The average pose is defined by an H-bond between the amino group of GABA and the backbone carbonyl oxygen of Tyr198 (chain A) and hydrophobic interactions with Arg158 and Leu166 of the chain B; carboxylate group of GABA is held by an H-bond with backbone amide or hydroxyl group of Ser168 (Chain B) (Fig. 7B). It should be noted that the salt bridge with Arg104 (chain B) changes into hydrogen bond (the distance between carboxylic oxygen of the ligand and guanidinium hydrogen of Arg104 is 2.84 Å). Nevertheless, these hydrogen bonds are not present during all the simulation: bond with Tyr198 is occupied only 23.8% of the simulation time, bond with Ser168 – 14.0%, and bond with Arg104 – 13.2%. Interestingly, the aromatic residues (Tyr 241, Tyr247, Tyr198, Tyr102 and His141) with the help of Val140 and Arg104 form a cluster in the binding site which may be related to the aromatic box.

The most interesting changes in the complex structure occur during heating of the system for the first 40 ps of dynamics. The amino end of the molecule goes out from the box between Tyr residues and forms a hydrogen bond with Ser248. The conformation of the GABA molecule changes from folded to extended and back. Tyr247 and Tyr198 participate in stacking interactions and the C-loop moves into the closed state (Fig. 8A and B). Further movement of the ligand molecule leads to the movement of the carboxylate group in the direction to Ser168; then sometimes the ligand forms an intramolecular salt bridge, but conformation of the ligand described earlier is more stable and appears more often.

3.3.2. TPMPA

The simulation of the receptor–TPMPA complex molecular dynamics showed that position of the ligand molecule in the

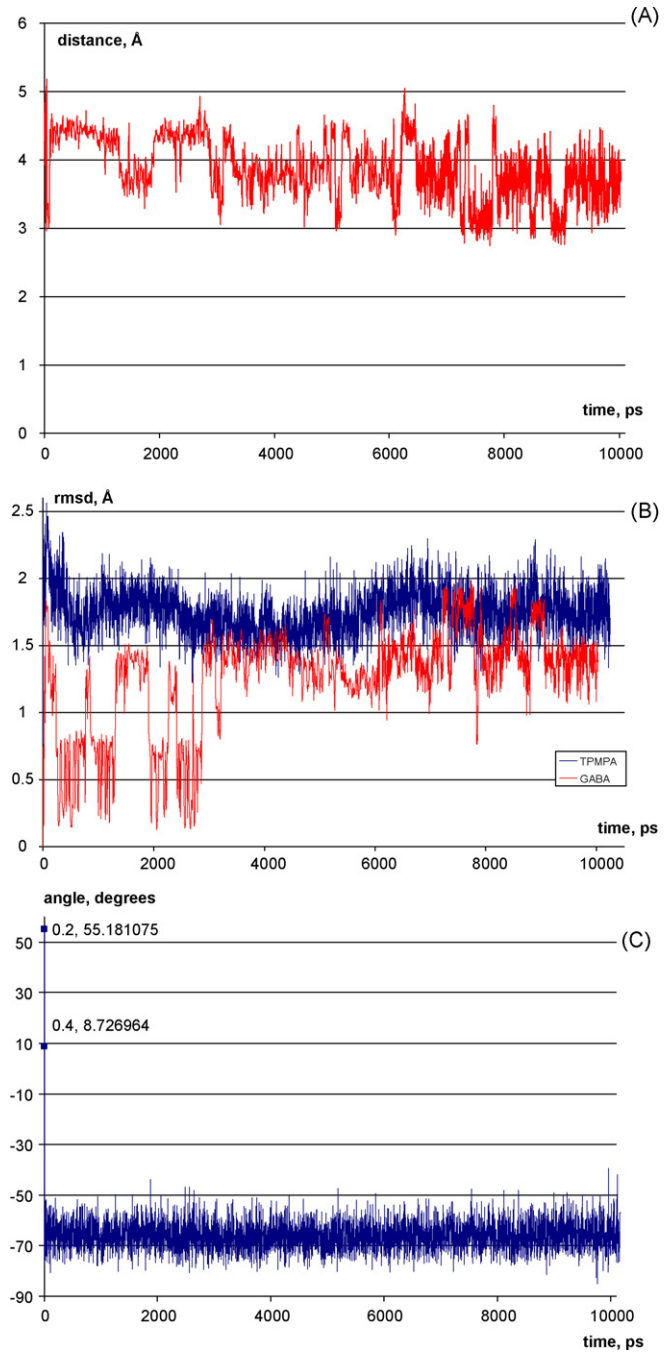

Fig. 6. Results of MD simulations for docked poses. (A) Distance (Å) between carboxylic carbon and nitrogen atoms of GABA plotted vs. time (ps); (B) RMSD vs. time plot for GABA (red line) and TPMPA (blue line) molecules; (C) TPMPA torsion angle C6–C5–N1–C2 (degrees) vs. time; note that initial value of this angle is about 55°.

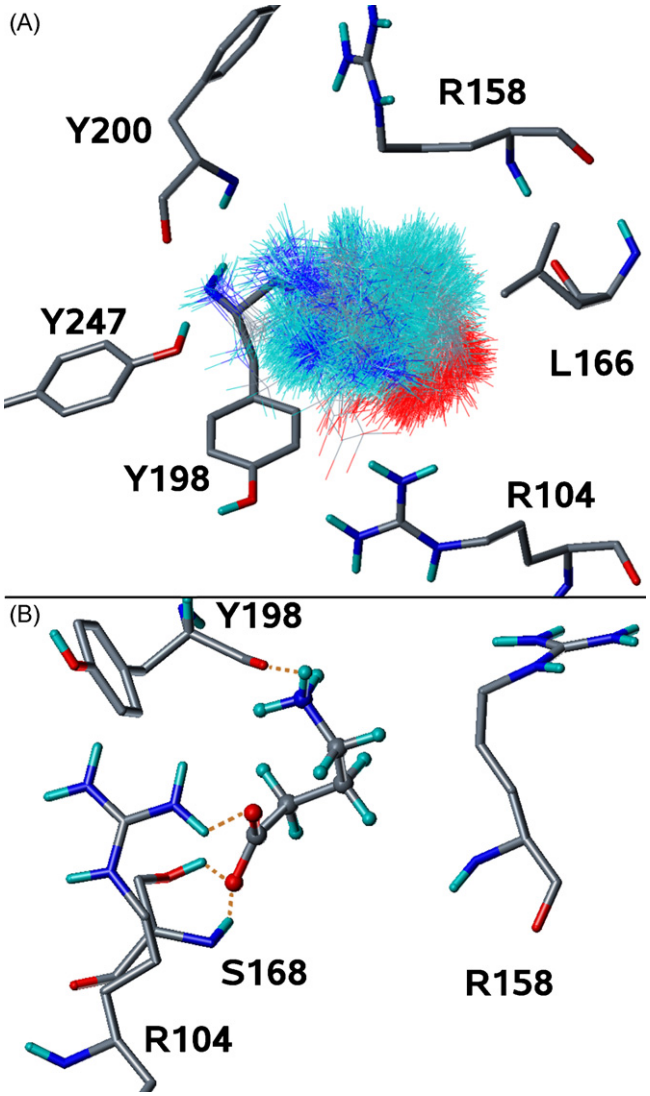

Fig. 7. (A) All conformations of GABA sampled during MD simulation in the average structure of the equilibrated binding site. (B) Average position of GABA in the equilibrated binding site according to MD simulation results. Nonpolar hydrogens of the receptor residues are omitted for clarity. A salt bridge appears between Arg104 and carboxy group of the ligand, so as hydrogen bonds with Ser168. Amino group of GABA is bound to the backbone carbonyl oxygen of Tyr198. The Arg158 sidechain and GABA carbon atoms form hydrophobic contacts.

binding site was predicted reasonably. There is no significant movement of the ligand molecule after heating the system (Fig. 6B) except the inversion of the cyclic system during the first 800 steps of simulation (Fig. 6C). The position of TPMPA is additionally stabilized by the formation of the salt bridge between the phosphinic group of the ligand and Arg104 (occupancy 91.2%) along with hydrogen bond between TPMPA and the phenolic hydroxyl of Tyr198 (occupancy 98.5%) and not very stable salt bridge with Arg170 (occupancy 57.6%) (Fig. 9). The salt bridge between Glu196 and TPMPA occurs during all the simulation (occupancy 99.7%). Glu194 forms a hydrogen bond with the phenolic hydroxyl of Tyr241 (occupancy 91.2%) and a salt bridge (occupancy 88.8%) not with the ammonium group of the ligand but with Arg170 because of the steric hindrance.

Interestingly, during the dynamics of the receptor–TPMPA complex some deformation of the C-loop can be observed, but rotational movement of the loop is completely absent (Fig. 8C and D) compared to the receptor–GABA complex simulation. Consequently, the aromatic box is not formed. Despite of the force restraints, the agonist and the antagonist cause different movements of the C-loop and aromatic residues in the binding site.

3.4. Secondary docking and molecular dynamics

Docking of GABA to the equilibrium structure of the receptor obtained after the receptor-TPMPA complex dynamics simulation was performed using the same parameters as those used during the first docking run. The obtained set of the ligand orientations was less diverse than that obtained after docking into the initial model. The main new feature of the most favorable GABA orientation according to the DOCK scoring function is the salt

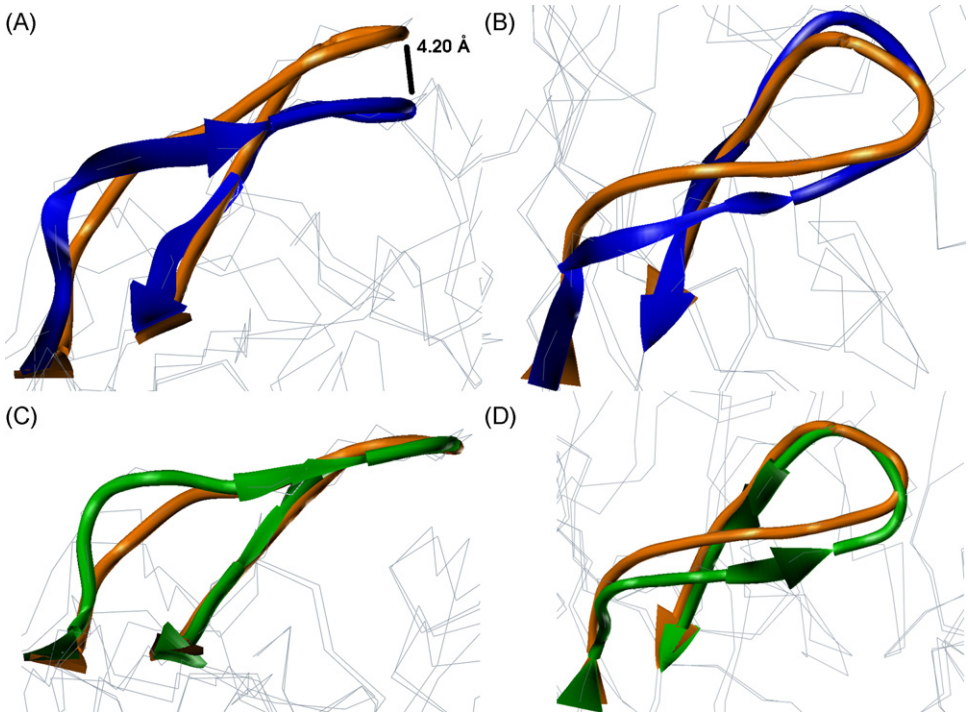

Fig. 8. Spatial alignments of the loop C for the initial (orange) and averaged over the dynamics simulation (other colors) structures. (A) Blue, average loop C conformation for the GABA simulation. Average distance between the turn backbone atom positions is about 4.2 Å. The direction of view is parallel to the receptor axis from the membrane side. (B) The same as (A), side view. (C) Green, average loop C conformation for the TPMPA simulation. No loop C rotation is observed. The direction of view is parallel to the receptor axis from the membrane side. (D) The same as (C), side view.

bridge between the amino end of GABA and Glu196. Hydrophobic interactions of GABA with Tyr247 and a salt bridge with Arg104 are more trivial. An additional H-bond is formed between the carboxy end of GABA and the hydroxyl group of Tyr198.

Molecular dynamics simulation of this complex was performed similarly to the previous MD runs. GABA orientation is stable during the dynamics (Fig. 10) despite the smaller occupancy of the key hydrogen bonds compared to TPMPA simulation due to greater conformational flexibility of the GABA molecule. The salt bridge between the protonated amino group of GABA and Glu196 remains present over the whole simulation (occupancy 84.5%) along with hydrogen bond between GABA aminogroup and the backbone oxygen of Glu196 (occupancy 61.3%). A number of salt bridges can be detected by ptraj between carboxy terminus of GABA and Arg104 due to atom movement and nonequivalence of their numbering; the most frequently appearing one is present for 75.9% of the simulation time. Tyrosine residues Tyr198, Tyr241 and Tyr247 form a small hydrophobic cavity for GABA backbone, and the OH-group of Tyr198 forms an H-bond (occupancy 89.6%) with the GABA carboxyl (Fig. 11). The salt bridge between Glu194 and Arg170 is present 29.6% of the simulation time, but in this case Glu194 also participates in binding the ammonium group of GABA (occupancy 80.7%).

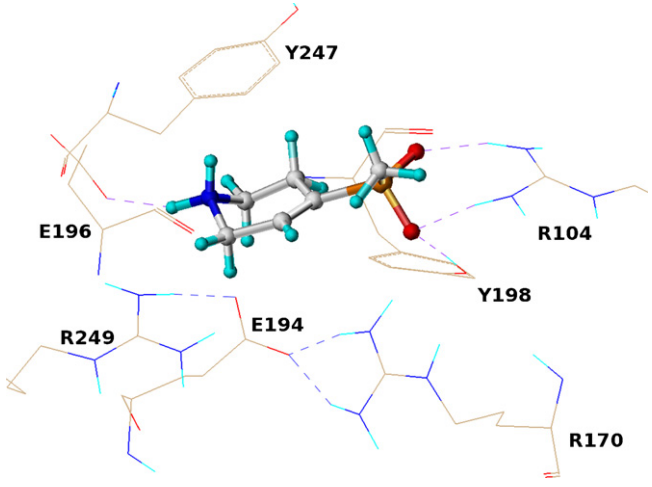

Fig. 9. Average position of TPMPA in the binding site according to MD simulation results. Nonpolar hydrogens of the receptor residues and Tyr241 (chain A) are omitted for clarity. The salt bridges appear between the ligand molecule and Glu196 (chain A) and Arg104 (chain B), as far as a hydrogen bond between the phosphinic group of TPMPA and Tyr198 (chain A) hydroxyl group. Glu194 (chain A) forms salt bridges with Arg249 (chain A) and Arg170 (chain B). Tyr247 (chain A) participates in the hydrophobic contact with TPMPA molecule.

4. Discussion

Mutation studies of the $GABA_C$ receptor [9,32] show that there are about 20 amino acid residues directly or indirectly participating in ligand binding and channel activation. Some of them may play a structural role in the binding site formation, whereas others directly participate in ligand binding.

Theoretical and experimental studies of $GABA_A$ receptor and the Cys-loop family include docking, molecular dynamics, QM/MM and QSAR studies [9,11,33]. A common description of ligand-binding mechanism in the Cys-loop ligand-gated ion channels includes formation of the aromatic box around the cationic center of the ligand due to cation–π interactions; this process causes a movement of the loop C closing the binding site, conjugated with the channel opening. Such binding mechanism is characteristic primarily for nAChRs and AChBPs, whose ligands are usually conformationally restricted cations [7,15]. Binding of zwitterionic ligands of glycine and GABA receptors may involve some different structural features. Namely, arginine residues on the complementary side of the binding site should form a salt bridge with the

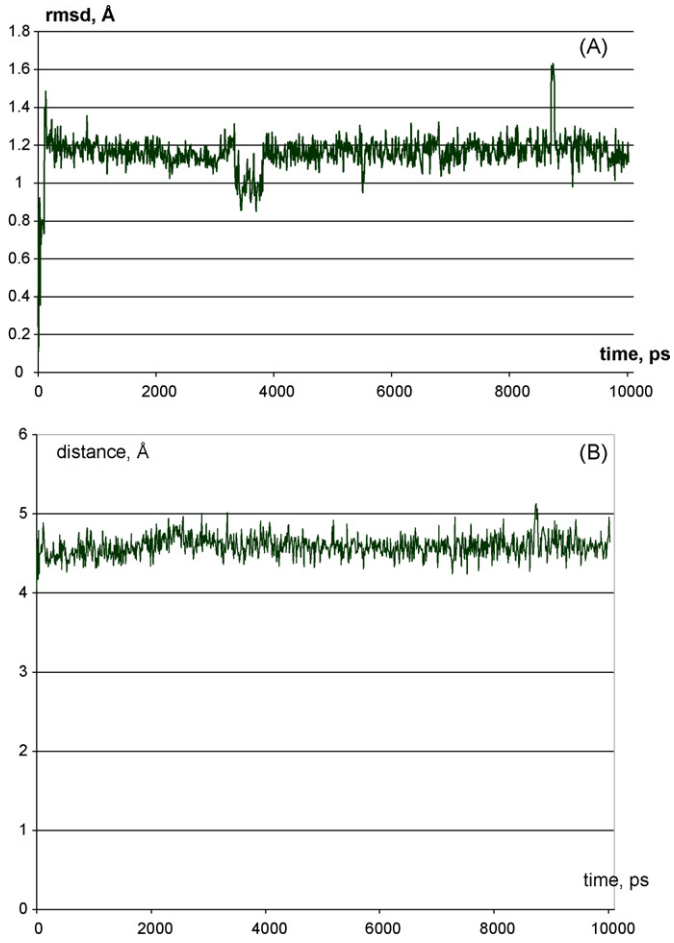

Fig. 10. (A) RMSD (Å) vs. time (ps) plot for GABA in the secondary MD simulation. (B) Distance (Å) between carboxylic carbon and nitrogen atoms of GABA plotted vs. time (ps).

carboxylic group of the ligand. This function was demonstrated by different researchers for Arg104 [10,11,34].

There is a point of view that main trapping force for the positively charged amino group of the zwitterionic ligands of the family is the cation–π interaction [10,35]. On the other hand, there

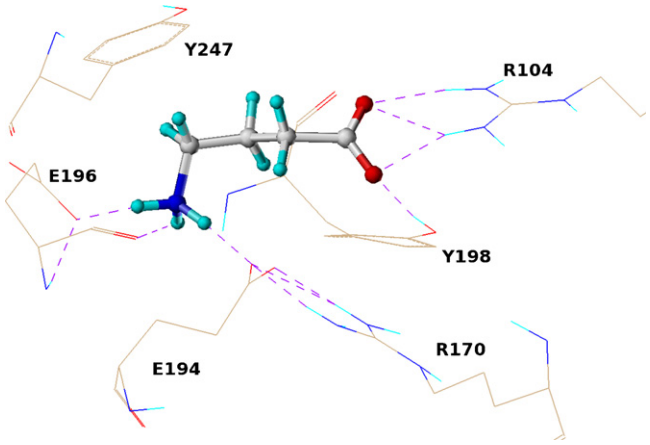

Fig. 11. Average position of GABA in the binding site according to the secondary MD simulation. Tyr241 (chain A) and nonpolar hydrogens of the receptor residues are omitted for clarity. Aminogroup of GABA forms salt bridges and hydrogen bonds with Glu194 and Glu196 (chain A). Carboxy group of GABA interacts with Arg104 (chain B) and Tyr198 (chain A). Tyr247 (chain A) participates in the hydrophobic contact with the GABA molecule.

is a hypothesis by Cromer et al. [13] that glutamate residues located in the loop B of the binding site of $GABA_A$ and $GABA_C$ receptors may participate in GABA binding. This hypothesis is supported by mutation studies of β_2Glu155 in $GABA_A$ receptor [36] which have shown that the mutation of this residue produces a spontaneously open channel. This residue is homologous to Glu196 in $GABA_C$ receptor.

The main goal of our work was to find a reasonable position of the ligand in a rather big binding site. There is no structural reason to prefer one docked orientation of a ligand in the binding site to another; tyrosine and arginine residues were shown to be important for channel opening [9,32], and there were no mutational studies of glutamate residues in the $GABA_C$ receptor. So we used molecular dynamics in partially solvated and partially restrained system to rationalize the docking results. Thorough investigation of the loop C dynamics and the aromatic box formation was not performed because it required much more complex system treatment.

Interpretation of the GABA position in the binding site after docking seems somewhat embarrassing. Cation–π interactions which determine the orientation of a protonated amino group [12] are not treated by the DOCK force field, nor by other usual force fields [37,38]. Consequently, correct docking of the ligand may be performed only with the help of an advanced force field or scoring function which takes cation–π interaction into account. Therefore, we have tried to build an alternative hypothesis of the ligand-binding based on simpler assumptions. Namely, glutamate residues located deep in the binding site may form salt bridges with the cationic center of the ligand. Despite these bridges are not revealed by GABA docking, the docked position of TPMPA in the binding site is preferentially defined by the interaction with the glutamate residues. In this case hydrophobic interactions of TPMPA carbon atoms and Tyr residues appear to be more probable than cation–π. Moreover, the phosphinic group of TPMPA remains free of interactions that means that the salt bridge and the hydrophobic interaction are strong enough to define the preferred orientation of TPMPA.

Molecular dynamics simulation has shown that when the cation–π interaction is not taken into account, predicted position and conformation of GABA is unstable, and a GABA molecule moves from the starting position into the cavity between Arg104 and Arg158 (Fig. 7). The salt bridge with Arg104 changes into weak H-bond, and two hydrogen bonds with Tyr198 and Ser168 do appear in the average structure. This observation is in good agreement with the data from previous modeling studies [10,11] and mutation data [9,33], despite of different template used for the receptor modeling.

Aromatic residues of the binding site move to form a hydrophobic cluster which can be associated with the aromatic box. Participation of histidine residue in this cluster may help to explain the mechanism of zinc inhibition [39] through steric clashes between the Zn–His complex and other aromatic residues.

The dynamics of the ligand–receptor complex model based on the apo form of the receptor can be used to formulate an assumption about initial stages of the agonist binding. We propose that the first act of binding is GABA carboxyl trapping by the formation of a salt bridge with Arg104. This position may be initially stabilized also by the formation of a hydrogen bond between the amino group of GABA and Ser243, which can be found at the end of the loop C turn, and the 8 Å distance between Ser243 and Arg104 is rather favorable for the GABA molecule, which is about 5 Å long. Nevertheless, this proposition is rather questionable. Interaction of the amino group of GABA with Tyr198 initiates the move of the loop C and the formation of aromatic box. The equilibrium pose of the ligand is outside the aromatic box, which is inconsistent with other models built based on the structures of

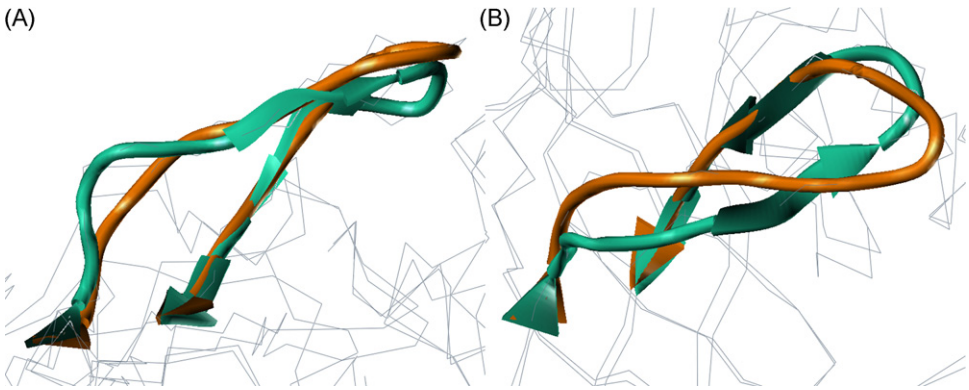

Fig. 12. Spatial alignments of the loop C for the initial (orange) and averaged over the secondary dynamics simulation (turquoise) structures. (A) Bottom view. A rather small rotation of the loop C compared to the initial GABA simulation (Fig. 8A) is observed. (B) The same alignment, side view. The deformation of the loop turn is notable.

agonist-bound templates. The main reason of such inconsistency is the dynamic character of our model and the use of the apo template for its building. Most previous models of ligand–receptor interactions of this system have not mentioned dynamics of the system, but only docking and molecular mechanics or QM/MM optimization. In [34] a MD simulation was performed, but the agonist-bound conformation of the receptor model was used as the starting point of the simulation. Consequently, starting geometry used in that study is much more stable than one used in our work, but possible features of the ligand binding may not be revealed by that model because there are no reasons to suppose that agonist-bound conformation of the AChBP is very similar to the agonist-bound conformation of the $GABA_C$ receptor. We have observed the main conformational change during the receptor activation: the movement of the loop C during agonist and antagonist trapping with amino acids involved, movement of the ligand and binding site rearrangement using the apo form of the receptor as the starting point.

Our simulation of TPMPA dynamics gave opposite results: the docked position of the ligand was not perturbed and was additionally stabilized by a salt bridge between the ligand and Arg104 (Fig. 9). Aromatic residues did not form a cluster similar to that formed during GABA dynamics. Consequently, stabilization of TPMPA position by salt bridges and hydrophobic interactions does not allow the molecule to leave the binding site during the dynamics. Conformation of the binding site is much more stable during dynamics compared with the GABA simulation due to a lower conformational flexibility of the ligand. Moreover, such orientation of the molecule prevents the aromatic box formation and may be used for rationalization of antagonistic properties of TPMPA.

The main difference between our model of the receptor–TPMPA complex and the previous model by Abdel-Halim et al. [11] is the salt bridge between Glu196 and the ammonium group of TPMPA. This difference appears due to the usage of different templates: whereas in the model based on agonist-bound template [11] Glu residues from the loop B are buried under the loop C, in the apo form-based model they are more accessible for the ligand.

We proposed that equilibrium configuration of the receptor–TPMPA complex may better reveal the structure of the binding site in the apo-form than the static model obtained by straight modeling. To check this assumption a GABA molecule was docked into this structure of the binding site (Fig. 11). Interestingly, orientation of the ligand molecule in this case was determined by the salt bridges with Glu196 and Arg104 and hydrophobic interactions with aromatic residues. Despite of higher conformational flexibility of the GABA carbon skeleton compared to that of TPMPA, the position of the ligand during the dynamics is rather stable and the salt bridges remain present during all the simulation. Smaller conformational changes of the C-loop in this simulation compared to the initial simulation (Fig. 12) should be the consequence of the GABA orientation inside the binding site which is more characteristic for an antagonist together with the deformation of the C-loop which appeared during the antagonist dynamics simulation. Moreover, this conformation of the loop may be less suitable for the aromatic box formation.

The main conclusion of these simulations is that the glutamate residues Glu194 and Glu196 should play a significant role in the ligand binding, especially antagonist binding. They are located very deep in the binding site and consequently are almost inaccessible for water molecules when the ligand is bound. When bound, the cationic center of the ligand should prefer a salt bridge formation with acidic residues rather than the cation–π interactions, which are energetically less favorable than the salt bridge [38]. Further investigation of the GABA binding site should include mutation studies of these residues.

5. Conclusions

We have built a homology model of the ligand-binding domain of the ρ_1 $GABA_C$ receptor based on the AChBP template. This model reveals the structure of the receptor in the apo form. The well-known ligands of the $GABA_C$ receptor, GABA and TPMPA, were docked into the binding site and the resulting structures of the complexes were optimized by molecular dynamics. It is proposed that residues Glu194 and Glu196 play a major role in ligand binding and receptor activation along with well-known Arg and Tyr residues. The obtained binding mode may be a guide to further mutational studies and structure-based search of potential $GABA_C$ receptor ligands.

Acknowledgements

We acknowledge Alex A. Oliferenko for careful reading the manuscript.

This work was supported by the Grant NSh-2552.2006.3 of the President of Russian Federation and the Grant 08-03-00783 of the Russian Foundation for Basic Research.

References

[1] M. Chebib, G.A.R. Johnston, GABA-activated ligand gated ion channels: medicinal chemistry and molecular biology, J. Med. Chem. 43 (2000) 1427–1447.
[2] H. Qian, H. Ripps, Response kinetics and pharmacological properties of heteromeric receptors formed by coassembly of GABA ρ- and γ_2-subunits, Proc. R. Soc. Lond. B 266 (1999) 2419–2425.
[3] R. Enz, G.R. Cutting, $GABA_C$ receptor ρ subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties, Eur. J. Neurosci. 11 (1999) 41–50.

[4] C. Arnaud, P. Gauthier, C. Gottesmann, Study of a GABAC receptor antagonist on sleep-waking behavior in rats, Psychopharmacology 154 (2001) 415–419.
[5] K. Brejc, W.J. van Dijk, R.V. Klaassen, M. Schuurmans, J. van der Oost, A.B. Smit, T.K. Sixma, Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, Nature 411 (2001) 269–276.
[6] P.H.N. Celie, S.E. van Rossum-Fikkert, W.J. van Dijk, K. Brejc, A.B. Smit, T.K. Sixma, Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures, Neuron 41 (2004) 907–914.
[7] S.B. Hansen, G. Sulzenbacher, T. Huxford, P. Marchot, P. Taylor, Y. Bourne, Structures of *Aplysia* AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations, EMBO J. 24 (2005) 3635–3646.
[8] T. Grutter, L. Prado de Carvalho, V. Dufresne, A. Taly, M. Fischer, J.-P. Changeux, A chimera encoding the fusion of an acetylcholine-binding protein to an ion channel is stabilized in a state close to the desensitized form of ligand-gated ion channels, C. R. Biol. 328 (2005) 223–234.
[9] A. Sedelnikova, C.D. Smith, S.O. Zakharkin, D. Davis, D.S. Weiss, Y. Chang, Mapping the ρ_1 $GABA_C$ receptor agonist binding pocket, J. Biol. Chem. 280 (2005) 1535–1542.
[10] N.J. Harrison, S.C.R. Lummis, Molecular modeling of the GABAC receptor ligand-binding domain, J. Mol. Model. 12 (2005) 317–324.
[11] H. Abdel-Halim, J.R. Hanrahan, D.E. Hibbs, G.A.R. Johnston, M. Chebib, A Molecular basis for agonist and antagonist actions at $GABA_C$ receptors, Chem. Biol. Drug Des. 71 (2008) 306–327.
[12] S.C.R. Lummis, D.L. Beene, N.J. Harrison, H.A. Lester, D.A. Dougherty, A cation–π binding interaction with a tyrosine in the binding site of the $GABA_C$ receptor, Chem. Biol. 12 (2005) 993–997.
[13] B.A. Cromer, C.J. Morton, M.W. Parker, Anxiety over $GABA_A$ receptor structure relieved by AChBP, Trends Biochem Sci. 27 (2002) 280–287.
[14] P.-J. Corringer, N. Le Novère, J.-P. Changeux, Nicotinic receptors at the amino acids level, Annu. Rev. Pharmacol. Toxicol. 40 (2000) 431–458.
[15] N. Unwin, Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution, J. Mol. Biol. 346 (2005) 967–989.
[16] N. Unwin, A. Miyazawa, J. Li, Y. Fujiyoshi, Activation of the nicotinic acetylcholine receptor involves a switch in conformation of the α subunits, J. Mol. Biol. 319 (2002) 1165–1176.
[17] V.I. Chupakhin, V.A. Palyulin, N.S. Zefirov, Modeling the open and closed forms of $GABA_A$ receptor: analysis of ligand–receptor interactions for the GABA-binding site, Dokl. Biochem. Biophys. 408 (2006) 169–174.
[18] Y. Murata, R.M. Woodward, R. Miledi, L.E. Overman, The first selective antagonist for a $GABA_C$ receptor, Bioorg. Med. Chem. Lett. 6 (1996) 2073–2076.
[19] D.I. Osolodkin, V.I. Chupakhin, V.A. Palyulin, N.S. Zefirov, Modeling and analysis of ligand–receptor interactions in the $GABA_C$ receptor, Dokl. Biochem. Biophys. 412 (2007) 25–28.
[20] G.R. Cutting, L. Lu, B.F. O'Hara, L.M. Kasch, C. Montrose-Rafizadeh, D.M. Donovan, S. Shimada, S.E. Antonarakis, W.B. Guggino, G.R. Uhl, H.H. Kazazian Jr., Cloning of the γ-aminobutyric acid (GABA) ρ_1 cDNA: a GABA receptor subunit highly expressed in the retina, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 2673–2677.
[21] UniProt Consortium, The Universal Protein Resource (UniProt), Nucleic Acids Res. 35 (Database issue) (2007) D193–D197.
[22] J. Shi, T.L. Blundell, K. Mizuguchi, FUGUE: sequence-structure homology recognition using environment-specific substitution tables and structure-dependent gap penalties, J. Mol. Biol. 310 (2001) 243–257.
[23] A. Šali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[24] R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Cryst. 26 (1993) 283–291.
[25] I.D. Kuntz, J.M. Blaney, S.J. Oatley, R. Langridge, T.E. Ferrin, A geometric approach to macromolecule–ligand interactions, J. Mol. Biol. 161 (1982) 269–288.
[26] D.A. Case, T.E. Cheatham III, T. Darden, H. Gohlke, R. Luo, K.M. Merz Jr., A. Onufriev, C. Simmerling, B. Wang, R. Woods, The Amber biomolecular simulation programs, J. Comp. Chem. 26 (2005) 1668–1688.
[27] J. Wang, R.M. Wolf, J.W. Caldwell, P.A. Kollman, D.A. Case, Development and testing of a general amber force field, J. Comput. Chem. 25 (2004) 1157–1174.
[28] A. Jakalian, D.B. Jack, C.I. Bayly, Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation, J. Comput. Chem. 23 (2002) 1623–1641.
[29] W. Humphrey, A. Dalke, K. Schulten, VMD: visual molecular dynamics, J. Mol. Graph. 14 (1996) 33–38.
[30] S. Miyazaki, Y. Kuroda, S. Yokoyama, Characterization and prediction of linker sequences of multi-domain proteins by a neural network, J. Struct. Funct. Genom. 15 (2002) 37–51.
[31] A. Demuro, A. Martínez-Torres, R. Miledi, Functional and pharmacological properties of GABAρ1Δ51 receptors, Neurosci. Res. 36 (2000) 141–146.
[32] J. Amin, D.S. Weiss, Homomeric ρ_1 GABA channels: activation properties and domains, Recept. Channels 2 (1994) 227–236.
[33] K.C. Chou, Modelling extracellular domains of GABA-A receptors: subtypes 1, 2, 3 and 5, Biochem. Biophys. Res. Commun. 316 (2004) 636–642.
[34] C. Melis, S.C.R. Lummis, C. Molteni, Molecular dynamics simulations of GABA binding to $GABA_C$ receptor: the role of Arg^{104}, Biophys. J. 95 (2008) 4115–4123.
[35] N.J. Harrison, S.C.R. Lummis, Locating the carboxylate group of GABA in the homomeric rho $GABA_A$ receptor ligand-binding pocket, J. Biol. Chem. 284 (2006) 24455–24461.
[36] J.G. Newell, R.A. McDevitt, C. Czajkowski, Mutation of glutamate 155 of the $GABA_A$ receptor β_2 subunit produces a spontaneously open channel: a trigger for channel activation, J. Neurosci. 24 (2004) 11226–11235.
[37] A.D. Mackerell Jr., Empirical force fields for biological macromolecules: overview and issues, J. Comput. Chem. 25 (2004) 1584–1604.
[38] J.P. Gallivan, D.A. Dougherty, A computational study of cation–π Interactions vs salt bridges in aqueous media: implications for protein engineering, J. Am. Chem. Soc. 122 (2000) 870–874.
[39] T.-L. Wang, A. Hackam, W.B. Guggino, G.R. Cutting, A single histidine residue is essential for zinc inhibition of GABA ρ1 receptors, J. Neurosci. 15 (1995) 7684–7691.