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THREE-DIMENSIONAL RECONSTRUCTION OF A FULL-SIZE $GABA_B$ RECEPTOR

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The three-dimensional (3D) pattern of a full-size $GABA_B$ receptor has been reconstructed using computer techniques. To simulate a real microenvironment for the $GABA_B$ receptor, the latter was embedded in the bilipidic membrane with the corresponding salt-water environment. Since homology modeling of the $GABA_B$ receptor is among the computational methods allowing one to predict 3D coordinates when experimental data are not available, we reconstructed the structure of a full-size $GABA_B$ receptor by stepwise homology modeling of individual subunit parts. The stability of receptor subunits was evaluated by calculating the molecular dynamics. It has been found that C-terminal domains of the intracellular receptor show a tendency toward compaction, and coiled-coil areas form a structure almost identical to that specified by crystallization of these fragments. The structure obtained can be applied for further examination of the structural mechanisms of $GABA_B$ receptor interaction with GABA agonists and antagonists. It is quite evident that molecular dynamics computations might be a valuable tool in probing details of the receptor function.

Keywords: $GABA_B$ receptor, subunit composition, 3D structure, bilipidic membrane, molecular dynamics.

INTRODUCTION

γ-Aminobutyric acid (GABA) is, probably, the most important inhibitory neurotransmitter in the mammalian CNS. This transmitter is extensively distributed in the brain and plays a crucial role in reducing the neuronal excitability throughout the nervous system. It has been reported [1] that 30-40% of all CNS neurons utilize GABA as the primary neurotransmitter. Since there are about 40% of all synapses in the brain working with GABA and, therefore, having GABA receptors, the latter are believed to be the most common in the mammalian CNS.

The presence of two types of postsynaptic receptors in the brain that recognize GABA, namely $GABA_A$ and $GABA_B$ ones, has been well documented. $GABA_A$ receptor-mediated tonic inhibition plays an important role in the CNS functioning. These receptors, linked directly to binding sites of ion channels, are located in the cell membrane and contain two functional domains, an extracellular one that binds the neurotransmitter and a membrane-spanning domain that forms an ion channel. Since $GABA_A$ receptors combine transmitter-binding and channel functions into a single molecular entity, they are also frequently qualified as ligand-gated ion channels. It has been reported that ionotropic $GABA_A$ receptors contribute to an increase in the conductance for chloride ions. In electrophysiological studies using voltage-clamp and single-channel recording techniques, the operation of a $GABA_A$ receptor-gated Cl^- ion channel has been described in detail [2, 3]. Activation of such a channel results in hyperpolarization of the neuronal membrane, and this increases the threshold for generation of an action potential (AP) in the case of action of excitatory transmitters that depolarize the membrane. Shunting of the cell membrane (a drop in its resistance) accompanying activation of GABA receptors also decreases the excitability of postsynaptic neurons.

$GABA_A$ receptors are multimers formed by at least four or five individual protein subunits. It is believed that the subunit compositions of most $GABA_A$ receptors in various brain regions and even in various neurons within a given region may be dissimilar.

$GABA_B$ receptors were identified when it became clear that GABA can potently inhibit depolarization-

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induced neurotransmitter (norepinephrine) release in brain slices, but a number of $GABA_A$ receptor agonists were found to be unable to mimic GABA-induced inhibition of such neurotransmitter release [4]. Now it has become obvious that $GABA_B$ receptors are located on both post- and pre-synaptic membranes, and these receptors do not include ion channels as a part of their structure. Instead, they affect channels by activation of intermediate molecules called G proteins. $GABA_B$ metabotropic receptors are also called G protein-coupled ones. Although there is a considerable body of evidence that a large proportion of $GABA_B$ receptors are coupled to G proteins, it has been also reported that some presynaptic $GABA_B$ receptors may be directly linked to K^+ channels, since activation of these receptors in many brain regions results in an increase in the K^+ channel conductance, with a resultant hyperpolarization of the neuronal membrane [2, 4]. In the postsynaptic membrane, $GABA_B$ receptors trigger, through G proteins, a cascade of intracellular reactions leading to the opening of potassium channels in the postsynaptic membrane [5]. Due to this event, inhibitory postsynaptic potentials (IPSPs) lasting hundreds of milliseconds develop. According to the respective IPSP kinetics, $GABA_B$ receptors are easily distinguished from $GABA_A$ receptors [6]. It has been also reported that $GABA_B$ receptors regulate the function of extrasynaptic $GABA_A$ receptors via a postsynaptic mechanism [7], mediate slow inhibitory synaptic neurotransmission, and play a key role in long-term synaptic plasticity [8, 9]. There is evidence that $GABA_B$ receptors are involved in neuronal migration and positioning [10, 11]. Disruption of $GABA_B$ receptor-mediated synaptic pathways is implicated in many diseases, including neuropathic pain, spasticity, drug addictions, hyperalgesia, memory disorders, muscle spasticity, schizophrenia, and epilepsy [12-16].

The $GABA_B$ receptor is a heterodimer, with an extracellular domain containing a neurotransmitter binding site and an intracellular domain that binds to G proteins. This receptor is composed of two subunits, $GABA_B1$ (R1) and $GABA_B2$ (R2), which differ from each other in their N-terminal amino acid sequences and arise due to alternative splicing [17, 18]. Each subunit consists of a large extracellular module called Venus flytrap (VFT), seven transmembrane domains, and an intracellular C-terminal domain. $GABA_B1$ and $GABA_B2$ subunits demonstrate a 54% similarity in their amino acid sequence, but only the extracellular domain of $GABA_B$ R1 can bind ligands, such as GABA, baclofen, and orthosteric antagonists (CGP54626, CGP64213, etc.) [19–21]. It is believed that the formation of fully functional $GABA_B$ receptors requires co-assembling of both R1 and R2 subunits of the $GABA_B$ receptor [17, 22-26]. In the membrane, $GABA_B$ receptors bind to G protein composed of α, β, and γ subunits [27]. This is G protein that ensues interaction of $GABA_B$ receptors with presynaptic voltage-gated N- and P/Q-type calcium channels [28, 29]. Multiple isoforms of human $GABA_B$ R1 subunit ($GABA_B$ R1a, $GABA_B$ R1b, $GABA_B$ R1c, and $GABA_B$ R1e) have been described, but only $GABA_B$ R2 has been adequately identified [30]. It should be noted that, at present, the structural basis for interaction between GABA and the $GABA_B$ receptor has still not been elucidated. It is obvious that computational methods for predicting the 3D coordinates can be beneficial for such a biomedical research, as well for homology modeling. This technique is applied in the situations where experimental structural data are not available but needed. Molecular dynamics simulation has also become relevant in the studies of such biological systems.

In our study, we reconstructed the 3D structure of a full-size $GABA_B$ receptor in a real microenvironment using computer-based techniques.

METHODS

The amino acid sequences of the human $GABA_B$ receptor subunits were retrieved from the international database UniProt (http://www.uniprot.org/ [32]; the access number for $GABA_B$R1 is Q9UBS5, and that for $GABA_B$R2 is O75899).

To start the operations with protein 3D structures, we used the atomic coordinate data deposed in Protein Data Bank (PDB, http://www.rcsb.org/pdb/home/home.do) [33]. The respective data are based on the results of X-ray crystallographic analysis of the extracellular domain of the $GABA_B$ R2 subunit at a 0.238 nm resolution (R-factor, 0.202), which has been stored in the 4F11 record. This record is not complete, as amino acid residues 42-466 of the extracellular domain of the $GABA_B$ R2 subunit are enclosed in the crystal under study. Structural data have been deciphered for the receptor fragment 52-466.

The results of X-ray crystallographic analysis of an intracellular coiled-coil heterodimer of the $GABA_B$ receptor at a 0.162 nm resolution (R-factor, 0.217) are stored in the 4PAS record. In the crystal under

study, amino acid residues 884-918 of the $GABA_B$ R1 receptor subunit and residues 779-817 of the $GABA_B$ R2 subunit are enclosed.

Since homology modeling of the $GABA_B$ receptor is among the computational methods to predict 3D coordinates when experimental structural data are not available [34], we reconstructed the structure of a full-size $GABA_B$ receptor by stepwise homology modeling of individual subunit parts.

The choice of optimal fold templates was based on the structure integrity, percentage of identity, percentage of similarity, and qualitative criteria of spatial models [35]. The quality of the modular architecture of protein structures was estimated using a web server MolProbity as a general-purpose web service, which can calculate and display the H-bond and van der Waals contacts in the interfaces between components, offering qualitative validation for the 3D structures of proteins [36]. The modules were integrated in the overall 3D structure using home-made software FlexBones.

Optimization for geometric reconstruction of the patterns was performed using Amber3 force field [37] and a method of the conjugate gradient [34, 38, 39]. The 3D structure and character of protein styling of the chain were analyzed using Swis-PdbViewer 1.9.1 [40]. Visualization and analysis of the contact surfaces and potential dimerization interfaces were handled through desktop-based DS Visualizer software, versions 2.0 and 3.5.

We used a SymmDock web-tool [41] for prediction of spatial structure of complete receptor by geometrically based molecular docking. To simulate the real microenvironment for a $GABA_B$ receptor, the latter was embedded in the bilipidic membrane with the corresponding salt-water environment. The next optimization of the receptor spatial structure was performed with GROMACS software (version 4.5.3) [42] using a Charmm27 force field [43, 44].

The stability of receptor subunits in complex biological membranes was evaluated by calculating the molecular dynamics using GROMACS software. The results of calculation of such dynamics for a $GABA_B$ receptor/biological membranes complex were evaluated basing on the root-mean-square deviations (RMSD) between atoms, the root-mean-square fluctuations (RMSF), and energy of non-valent interactions. Visualization of complex behavior during the molecular dynamics was performed using Visual Molecular Dynamics 1.6.1 software [45].

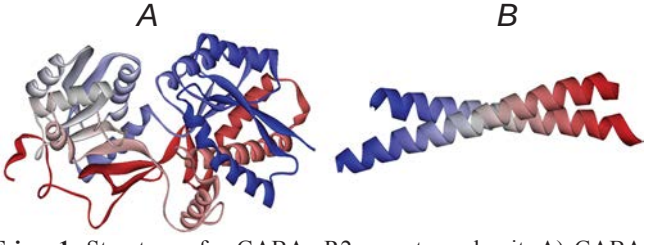
RESULTS AND DISCUSSION

Despite the evident functional significance of $GABA_A$ and $GABA_B$ receptors, the question of structural interactions between GABA and these receptors still remains open. It is known that interaction of GABA with $GABA_B$ receptors is provided by molecular conformation of the latter. The position of nitrogen and oxygen atoms, as well as the distance between these atoms causes the formation of a transmitter-receptor complex that alters the membrane conductivity due to the formation of pores in the membrane.

A model of the $GABA_B$ receptor was built according to amino acid sequences obtained from the RCSB.PDB (Protein Data Bank) database using the specialized program Deep View – TheSwissPdbViewerv3.7.

To build the 3D model of the $GABA_B$ structure, we used a crystallized fragment of the extracellular domain of the R2 subunit (amino acid residues 52-466) [20]. The corresponding entry in the PDB database has a number 4F11 (Fig. 1). This structure shows a high quality according to the MolProbity criteria. A coiled-coil heterodimer of the intracellular domain of the $GABA_B$ receptor containing 884-918 amino acid residues of subunit R1 and 779-817 residues of subunit R2 [31] has an appropriate entry in the PDB database (number 4PAS) (Fig. 1.2).

Correct prediction of the protein structure by the amino acid sequence may be achieved in two ways, namely superposition of the known spatial structure of the homologous protein and a method of "threading" with step-by-step addition of short fragments and iterative optimization of the energy systems. Since the $GABA_B$ receptor does not have homologs with the fully deciphered spatial structure, we reconstructed the latter for the $GABA_B$ R1 and R2 subunits by stepwise modeling of some homologous parts of these subunits using Robetta web-based tools. To perform these operations, the amino acid sequence of the each



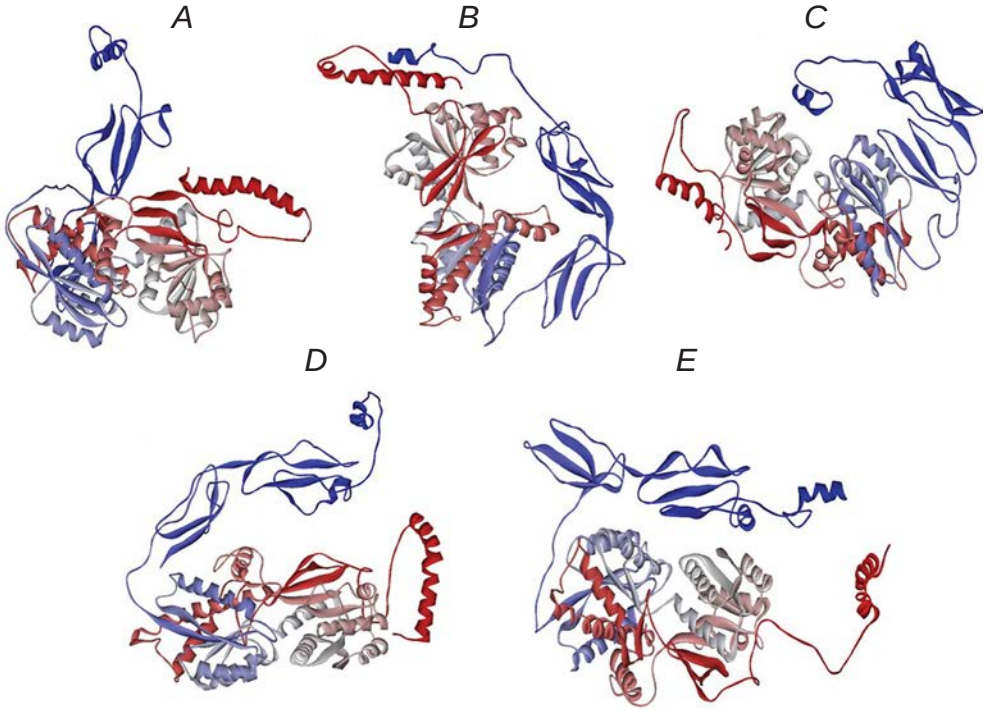
F i g. 1. Structure of a $GABA_B$ R2 receptor subunit. A) $GABA_B$ R2 receptor subunit (aminoacid residues 52-466, record 4F11 in the PDB database); B) intracellular coiled-coil heterodimer of the $GABA_B$ receptor (record 4PAS in the PDB database).

Р и с. 1. Структура R2-субодиниці $ГАМК_B$-рецептора.

subunit was divided into three parts, the extracellular domain, transmembrane one, and intracellular domain, loaded separately in the Robetta web server. The latter individually estimated the presence of homologous fragments and selected an optimal algorithm for prediction of the protein structure from the ROSETTA software package implemented in the cluster (in the absence of homologs, the protein structure is predicted by the "threading" method). The results obtained from this server were carefully analyzed in terms of the consistency with the available data on the structure and functioning of the $GABA_B$ receptor. First of all, we rejected the models that did not contain a compact spatial convolution *per se*, and we also admitted false those models that contained any signs of incorrect server operating with the sequence sent (for example, building the model of a point-symmetric monomer, lack of the modular protein organization, packing of the latter in a globule that hardly contains regular secondary structural elements, and the presence of a disordered C-terminal "tail" having more than two hundred amino acid residues). After preliminary sorting, the models were analyzed more carefully with respect to clear differences of domains. The extracellular one had a characteristic "claw" structure and seven transmembrane helices forming a transmembrane hydrophobic substitution in the receptor subunit. The possibility of formation of a coiled-coil structure in the C-terminal domain was tested. The structural variants with N- and C-termini of the protein housed close to each other (according to the known structural organization of the $GABA_B$ receptor subunits) and those containing more or fewer transmembrane helices were considered incorrect.

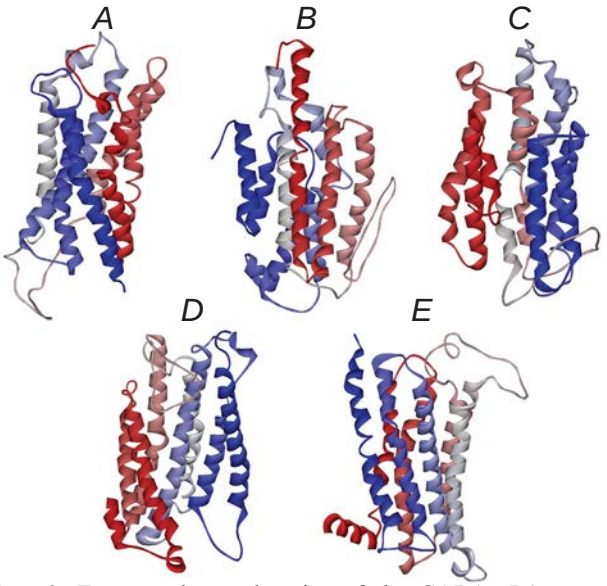
Extracellular Domains of the $GABA_B$ Receptor. Due to the fact that the $GABA_B$ R1 and R2 subunits have a modular architecture, it was possible to predict the structure of each domain separately and to optimize the domain geometry irrespectively of the others. The server operation resulted in making five variants of the spatial convolution of the extracellular domains of the R1 and R2 subunits with a given amino acid sequence that differed in positions of the C- and N-termini and in convolution of the N-terminus. Thereafter, to determine the most energetically favorable structure of the extracellular R1 and R2 subunits for further modeling, the geometry optimization of those models was performed, and option A was found to be the most energetically favorable (Fig. 2.).

Transmembrane Domains of the $GABA_B$ Receptor. Five variants offered by the server differed from each other in the number of alpha helices. Similarly to what was performed in modeling of the extracellular domain of the $GABA_B$ R1 and R2 subunits, we geometrically optimized the obtained models of the transmembrane domain of the R1 and R2 subunits trying to choose among them the most energetically favorable one. Option A turned out



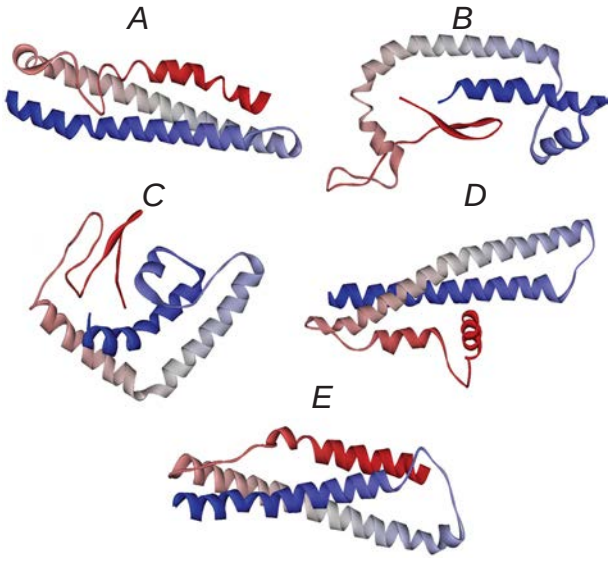
F i g. 2. Extracellular domains of the $GABA_B$ R1 receptor subunit (calculated using the Robetta server). E are energies of the models after minimization (optimization of geometry); for panels A to D, E= –10248.727123, –9089.999205, –9096.350844, and –9022.325294 kcal/mol, respectively.

Р и с. 2. Зовнішньоклітинні домени R1-субодиниці $ГАМК_B$-рецептора.



F i g. 3. Transmembrane domains of the GABA$_B$ R1 receptor subunit, calculated using the Robetta server. E is a local minimum of the model energy; for panels A to E, E = –4515.786627, –4515.786627, –3743.081283, –3865.567809, and –3671.730337 kcal/mol, respectively.

Р и с. 3. Трансмембранні домени R1-субодиниці ГАМК$_B$-рецептора.



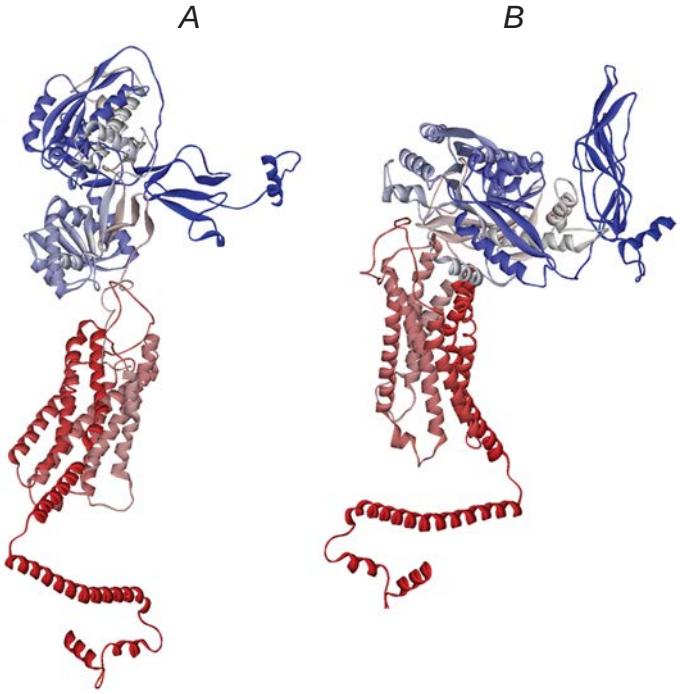
F i g. 4. Options for the C-terminal domain of the GABA$_B$ R1 receptor subunit predicted by Robetta server. E are energies of the models after minimization (optimization of geometry) for panels A to E, E = –1711.511183, –1717.739975, –1837.595221, –2139.205012, and –1789.278895 kcal/mol, respectively.

Р и с. 4. Опції для С-термінального домена R1-субодиниці ГАМК$_B$-рецептора.

to be the most energetically favorable (Fig. 3). In addition to model A, model F also corresponded to a possible spatial convolution of the transmembrane receptor (based on the structural criteria), and it had a less compact arrangement of the helices. However, it turned out after geometry optimization that the energy of model F was the greatest among the models studied. Therefore, the option was rejected.

Intracellular Domains of the GABA$_B$ Receptor. We have also obtained five variants of spatial convolution of the intracellular domain of the GABA$_B$ R1 and R2 subunits having a given amino acid sequence. The models differed from each other in their spatial structure. Models A, D, and F were inspected to be involved in the formation of the coiled-coil structure. To determine the most energetically favorable variant of the model, geometry optimization was carried out. As a result, option D was defined as the most energetically favorable (Fig. 4).

Integration of the Modules into the Overall 3D Structure. The latter was processed using the home-made FlexBones software. In the models used, GABA$_B$ receptor subunits were dissimilar in the layout of their extracellular domain. Geometry optimization of the obtained models was performed to select the most energetically favorable one for further dimerization (Fig. 5). The model consists of three domains. In



F i g. 5. GABA$_B$ R1 receptor subunits integrated into the overall 3D structure. E is energy of models after minimization (optimization of geometry) for panels A and B, E = –17385.679688 and –12768.388016 kcal/mol, respectively.

Р и с. 5. R1-субодиниці ГАМК$_B$-рецептора, інтегровані в просторову структуру.

particular, the extracellular domain contains α-helices and β-elements. The latter sequentially alternate with α-helices (known as a convolution termed by Rossmann), thus forming a kind of "claws" linked to a β-barrel structure. Based on the published data, we can assume that the neurotransmitter enters precisely the "claws" site [21]. The transmembrane domain (amino acid residues 593-854) consists of seven sequentially connected α-helices. External interfaces of these α-helices are mainly composed of glycine, leucine, isoleucine, tryptophan, alanine, serine, and valine residues, while internal interfaces are composed of leucine, valine, alanine, serine, cysteine, isoleucine, tyrosine, asparagine, and glycine. The intracellular C-terminal domain contains a long helix. The spiral area (see Table 2) forming a coiled-coil structure is responsible for dimerization of the receptor subunits.

As the $GABA_B$ receptor exists as a dimer, we decided, after modeling the subunits of this receptor, to search possible sites of dimerization and spatial structures of the dimer obtained. Modeling of the dimeric $GABA_B$ R1/R2 subunits was performed using a SymmDock web server. The latter proposes several variants of a dimeric structure of the $GABA_B$ receptor, which reflect specific criteria for estimation and "sorting" of the variants. For example, we may re-estimate the role of hydrophobic interactions, electrostatic interactions, balance of hydrophobic and electrostatic interactions, etc. Using dimerization servers, we have estimated the type of dimeric organization of any proposed variant. In particular, the variants that are basically similar to each other were ranged, to optimize the number of oncoming calculations and to choose the one being the most representative among possible dimeric interfaces. For the model of the $GABA_B$ R1 and R2 subunits selected, dimeric models have been constructed (Fig. 6).

From a large number of the structures analyzed, only one structure corresponding well to the data on the spatial dimeric organization of $GABA_B$ receptor subunits has been selected, and the contact surfaces between the subunits in the dimer were studied (Table 1). Afterwards, the selected dimeric model was embedded into the biological membrane.

Simulation of the molecular dynamics of the dimeric complex of the $GABA_B$ receptor with the membrane was analyzed using the Gromass 4.5.3 package. At that, we observed compaction of the intracellular domains of the receptor subunits and convergence of the transmembrane domains on the 18th nsec of molecular dynamics simulation, as well as interaction between the extracellular domains of the receptor subunits on the 36th nsec (Fig. 7). The analysis of the molecular dynamics over 36 nsec (relative to the starting geometry) using a *g_rms* module of the GROMACS software allowed us to get the values of RMSD of the dimer geometry of the simulated complex of the $GABA_B$ receptor/bilipidic membrane. As is shown in Fig. 8.A, the geometry of the model undergoes major rearrangements within the first 5 nsec of molecular dynamics assessment, and the value of the latter is 0.2 nm on the 5th nsec compared to the original model geometry. Then, the changes occur gradually.

The RMSF of amino acid residues in the dimer were assessed using the *g_rmsf* module of the GROMACS software. It has been shown that the simulated fluctuations of amino acid residues are virtually synchronous in both dimeric monomers (Fig. 8.B), although there are some differences in the RMSF values for the monomers within the regions having 5-30, 120-145, and 900-925 amino acid residues. The data obtained suggest that the highest RMSF values for amino acid residues occur in the N- and C-terminal regions of the dimeric complex of the $GABA_B$ receptor and the membrane, namely within regions corresponding to 1-150 and 850-961 amino acid residues. These regions are the most flexible and responsible for the N-terminal formation in extracellular and intracellular domains of the subunit. In contrast, a region corresponding to 615-850 amino acid residues is the least flexible, and it is responsible for the formation of the transmembrane domain.

In addition, we have estimated non-valent interaction energies for the dimeric models using simulation of the molecular dynamics (36 nsec). As is shown in Fig. 8.C, the 5-nsec molecular dynamics simulation and calculated energy were significantly reduced as compared to the original value, although these indices somewhat increased afterwards.

Thus, we reconstructed for the first time the full-size structure of the $GABA_B$ receptor and assessed its behavior under realistic conditions (the $GABA_B$ receptor has been embedded in the bilipidic membrane with the corresponding salt-water environment). It was shown that the receptor C-terminal (intracellular) domains demonstrate a tendency toward compaction, and coiled-coil areas form a structure almost identical to that specified by crystallization of these fragments. It was revealed that extracelullar domains form asymetric contact interfaces between subunits

T a b l e 1. Amino acid residues of $GABA_B$ subunits

Т а б л и ц я 1. Амінокислотні залишки в субодиницях $ГАМК_B$-рецептора

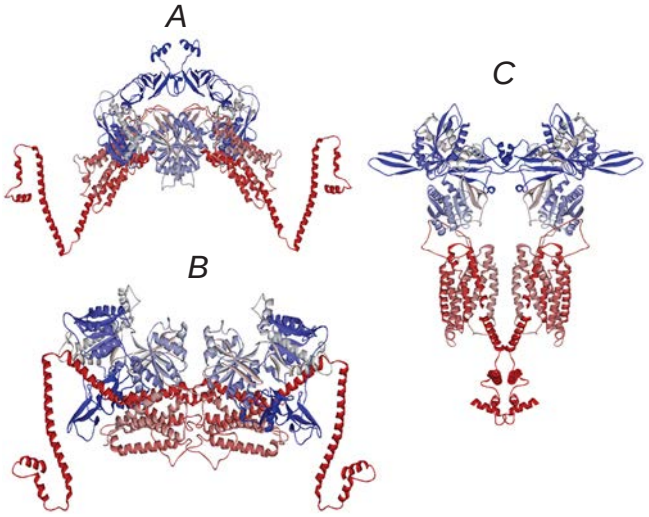
Amino acid residues of the R1 subunit forming a contact surface	Amino acid residues of the R2 subunit forming a contact surface
Intracellular domain	
Arg889	Arg787
Glu892	Gln792
Lys893	Asn795
Asn895	His796
Arg896	Arg799
Glu897	Met800
Glu899	Thr803
Lys900	Glu804
Ile901	Asp806
Ile902	Lys807
Ala903	Glu810
Glu904	Glu811
Lys905	Met814
Glu906	Gln815
Glu907	Gln817
Arg908	Asp818
Ser910	Glu821
Glu911	Thr824
Arg913	
His914	
Gln917	
Gln920	
Gln921	
Arg923	

T a b l e 2. Amino acid residues involved in forming of contact interfaces between R1 and R2 subunits after 36 nsec molecular dynamics

Т а б л и ц я 2. Залишки амінокислот, залучені у формування контактів між субодиницями R1 та R2 через 36 нс молекулярної динаміки

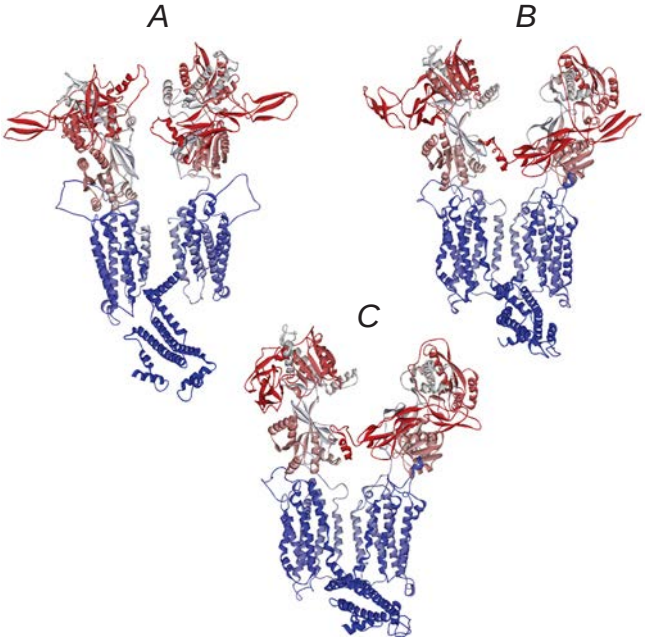
R1 subunit	R2 subunit
Exstracellular domain	
Met1	Ser288
Leu2	Gln292
Leu3	Val293
Leu6	His294
Leu7	Thr295
Leu10	Glu296
Phe11	Asn298
Gln21	Ser299
Pro23	Ser300
Asn24	Arg301
Ala25	Cys302

Thr26	Leu303
Ser27	Arg304
Glu28	Lys305
Ile654	Ile490
	Met493
Transmembrane domain	
Lys590	Leu481
Val597	Ala487
Ser600	Ile490
Leu601	Leu491
Val604	Ile494
Leu605	Ser497
Val607	Ala498
Val608	Phe501
Cys609	Phe502
Ser611	Lys505
Phe612	Asn506
Val854	Arg507
Met857	Asn508
Arg858	Phe762
Leu860	Asn765
Ile861	Gln766
Ser868	Lys510
	Lys593
Intracellular domain	
Tyr615	Ala783
Asn616	Ser784
Ser617	Ser786
Hsd618	Arg787
Val619	Gln792
Leu860	His796
Arg863	Asp806
Gln867	Lys807
Ala870	Glu810
Gln871	Thr813
Met874	Met814
Asn882	Leu816
Glu885	Gln817
Arg889	Pro820
Glu892	Glu821
Lys893	His830
Arg896	Tyr831
Ala904	Asn839
Hsd914	Glu845
Gln917	Ser846
Ser918	Thr847
Gln921	Asp848
Leu922	Lys851
Glu933	
Arg948	
Leu949	
Asp952	
Arg955	



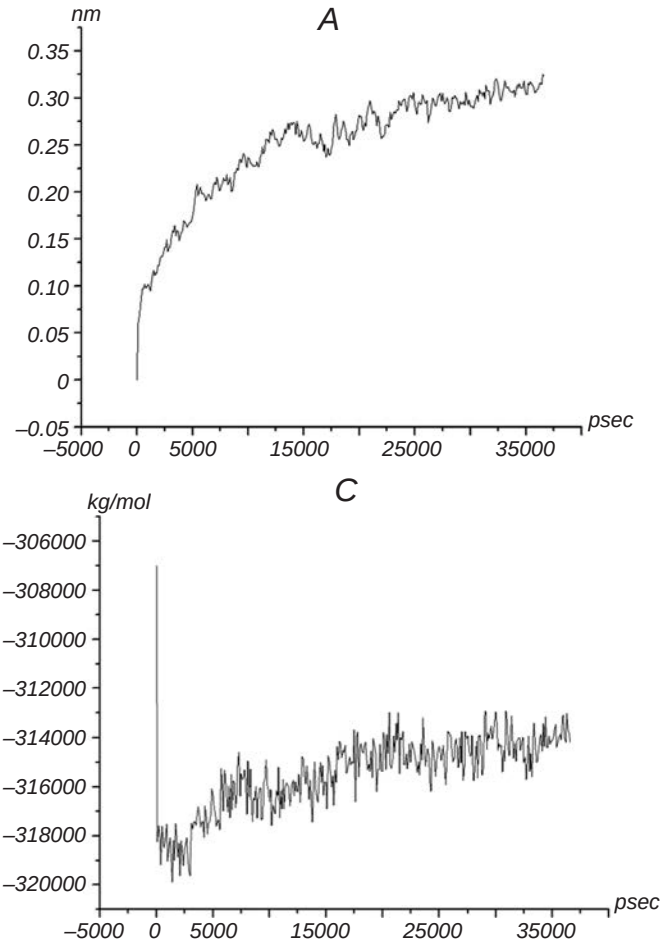
F i g. 6. Dimer models for the GABA$_B$ R1 receptor subunit

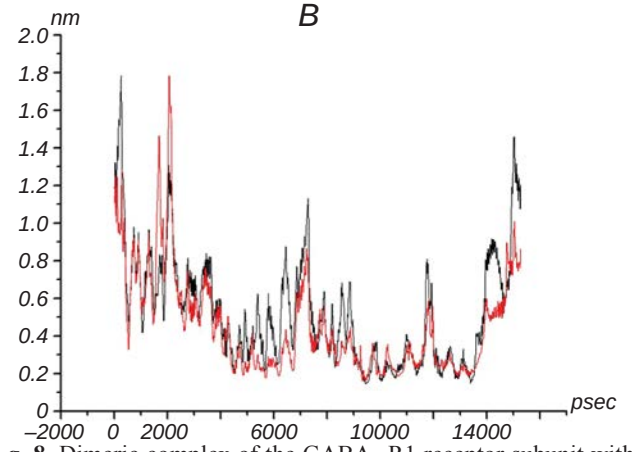
Р и с. 6. Моделі димерів для R1-субодиниці ГАМК$_B$-рецептора.



F i g. 7. Changes in the simulated dimer of the GABA$_B$ R1 subunit resulting from the calculation of the molecular dynamics; A, B, and C) structures before calculation of the molecular dynamics (A), after 18 nsec (B), and after 36 nsec (C).

Р и с. 7. Зміни в модельованих димерах R1-субодиниці ГАМК$_B$-рецептора після розрахунку молекулярної динаміки.





F i g. 8. Dimeric complex of the GABA$_B$ R1 receptor subunit with the bilipid membrane in the process of calculation of the molecular dynamics. A) root-mean-square deviations (RMSD) of alpha-carbon atoms within 36 nsec relative to the starting geometries of the latter in the modeled dimeric complex of the GABA$_B$ R1 receptor subunit with the bilipid membrane in the process of calculation of the molecular dynamics; B) root-mean-square deviation fluctuations (RMSF) of amino acid residues in the simulated dimeric complex of the GABA$_B$ R1 receptor subunit with the bilipid membrane; C) non-valent interaction of energies in model dimers of the GABA$_B$ R1 receptor subunit with the bilipid membrane at simulation of the molecular dynamics for 36 nsec. In B, black and gray lines correspond to the data for chains A and B, respectively.

Р и с. 8. Комплекс R1-субодиниці ГАМК$_B$-рецептора з біліпідною мембраною в процесі розрахунку молекулярної динаміки.

R1 and R2 over the 36 nsec molecular dynamics (Table 2). The structure obtained can be useful for further examination of the structural mechanisms of $GABA_B$ receptor interaction with GABA agonists and antagonists, e.g., the contribution of the R2 subunit to stabilization of the active receptor conformation and to interaction of the latter with G protein.

It is quite evident that calculation of the molecular dynamics might be a valuable tool in probing details of the receptor structure and dynamics. The method of molecular modeling enables researchers to construct complete spatial models of any receptor and to meet challenges in drug discovery and development. For example, calculations of the molecular dynamics of ligand-receptor complexes make it possible to predict and explain the agonist/antagonist location in ligand-receptor binding sites and to estimate the important functional significance of amino-terminal domain dimerization. It also allows one to simulate the processes of closing and opening of the amino-terminal domain and propose an alternative explanation for the functional role of agonists, which consists in changing the conformations of side chains of the amino acid residues.

Thus, we succeeded in reconstruction of the 3D structure of a full-length $GABA_B$ receptor in the real microenvironment. It was shown that a subunit of the simulated $GABA_B$ receptor consists of the extracellular, transmembrane, and intracellular domains. The extracellular domain is represented by β-elements consistently alternating with α-helices (Rossmann's convolution) and forming a kind of "claws" connected by a β-cylinder structure. The transmembrane domain contains seven concatenated α-helices, and the intracellular domain consists of α-helices and a site that serves to form the coiled-coil structure.

Simulation of the molecular dynamics for 18 nsec results in compaction of the intracellular domains of the receptor subunits and in convergence of the transmembrane domains, whereas simulation of the molecular dynamics for 36 nsec results in interaction between the extracellular domains of the receptor subunits.

Calculations of the RMSF values of simulated amino acid residues indicate that such fluctuations are practically synchronous for both monomers of the dimer. The greatest RMSF values for amino acid residues are observed in the N- and C-terminal domains of the simulated dimer complex of the $GABA_B$ R1/R2 subunits with the bilipid membrane. The least flexible site is that located between amino acid residues 615 to 850, and it is responsible for the formation of the transmembrane domain.

The reconstructed 3D structure of the full-length $GABA_B$ receptor seems to be adequate; in future it will allow us to simulate the interaction of an agonist with the examined receptor.

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Since our study dealt exclusively with computer modeling, it was not necessary to confirm its compliance with the statements of the International Convention (Strasbourg, 1986, and later versions).

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РЕКОНСТРУКЦІЯ ПРОСТОРОВОЇ СТРУКТУРИ ПОВНОРОЗМІРНОГО ГАМК$_B$- РЕЦЕПТОРА

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Р е з ю м е

Проведена реконструкція просторової структури повнорозмірного ГАМК$_B$-рецептора. Для імітації реального мікросередовища ГАМК$_B$-рецептор був вбудований у біліпідну мембрану з відповідним водно-сольовим мікрооточенням. Оскільки гомологічне моделювання ГАМК$_B$-рецептора є важливим обчислювальним методом прогнозування просторових координат, коли експериментальні дані щодо структури не є доступними, ми реконструювали структуру повнорозмірного ГАМК$_B$-рецептора з використанням ступінчастого гомологічного моделювання окремих частин субодиниць. Стабільність субодиниць рецептора оцінювали, розраховуючи молекулярну динаміку. Було показано, що субодиниця модельованого ГАМК$_B$-рецептора складається з позаклітинного, трансмембранного і внутрішньоклітинного доменів. Встановлено, що внутрішньоклітинні С-термінальні домени рецептора мають тенденцію до компактизації, а надспіралізовані ділянки утворюють структуру, майже ідентичну до тої, що зумовлена кристалізаці-

єю цих фрагментів. Проведена реконструкція просторової структури повнорозмірного рецептора $ГАМК_B$ є адекватною і такою, що може бути корисною для подальшого дослідження структурних механізмів взаємодії даного рецептора з агоністами і антагоністами ГАМК. Моделювання молекулярної динаміки може бути важливим інструментом вивчення деталей структури і динаміки рецептора.

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