Articles

Molecular Modeling of the GABA/$GABA_B$ Receptor Complex

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A three-dimensional model of the extracellular domain of the $GABA_B$ receptor has been built by homology with the leucine/isoleucine/valine-binding protein. The complete putative GABA-binding site in the extracellular domain is described in both the open and closed states. The dynamics of the "Venus flytrap" mechanism has been studied, suggesting that the molecular dipole moments play a key role in GABA binding and receptor activation. Important residues putatively implicated either in ligand binding or in the dynamics of the receptor are pinpointed, thus highlighting target residues for mutagenesis experiments and model validation.

Introduction

GABA (γ-aminobutyric acid)[1] is the well-known inhibitory neurotransmitter in the mammalian central nervous system where it exerts its effects through ionotropic ($GABA_{A/C}$) receptors, to produce fast synaptic inhibition, and metabotropic ($GABA_B$) receptors, to produce slow, prolonged inhibitory signals.[2] The physiological role of classical $GABA_A$ receptors has been characterized for a long time and compared to that of the $GABA_B$ receptors, which were not discovered until 1981.[3,4] The characterization of several potent $GABA_B$ receptor antagonists allowed to reveal in more depth the role of the $GABA_B$ receptor system. In general, presynaptic $GABA_B$ receptors modulate synaptic transmission by depressing neurotransmitter release, including that of GABA itself, through autoreceptors,[5] while postsynaptic $GABA_B$ receptors contribute to the inhibitory control of overall neuronal excitability. Thus, $GABA_B$ receptors play a critical role in fine-tuning the central nervous system synaptic transmission and are attractive targets for the treatment of epilepsy, anxiety, depression, cognitive deficits, sclerosis, and nociceptive disorders.[6,7] As a result, the development of $GABA_B$ receptor agonists and antagonists is of great therapeutic interest.

Until now, although the first agonist, baclofen,[8] was synthesized in 1962, few $GABA_B$ receptor agonists and antagonists have been discovered.[9−13] All these ligands present very similar structures deriving from the GABA structure. Moreover, the discrimination between agonist and antagonist structures still remains unclear, and structure−activity relationship (SAR) data are very poor. In addition, the crystallographic structures of the $GABA_B$ receptors are not available; thus, design of new ligands based on direct docking or on an experimental structure is not yet possible. Research for new ligands modulating $GABA_B$ receptor activity seems to be a difficult issue. To solve this problem, the current approaches consist in modeling the three-dimensional (3D) molecular structure of these $GABA_B$ receptors, their dynamics, and their interactions with ligands.

Cloning and photoaffinity labeling experiments of the $GABA_B$ receptor demonstrated two isoforms, designated GBR1a and GBR1b, which only differ in their N-terminal regions.[14] In addition, a GBR1-related protein, GBR2, was shown to be coexpressed with GBR1 in many brain regions and to interact with it through a short domain in the C-terminal cytoplasmic tail. This interaction produces a fully functional $GABA_B$ receptor at the cell surface. The authors concluded a new alternative approach in the modulation of the $GABA_B$ receptor system.[15,16]

Sequence homology analysis allowed to determine some structural features of these $GABA_B$ receptors. They possess seven putative transmembrane domains. They belong to the family of G-protein-coupled receptors (GPCRs) and more particularly to the class III subfamily which comprises the metabotropic glutamate receptors (mGluR),[17,18] the Ca^{2+}-sensing receptor,[19] and a recently discovered new group of putative pheromone receptors.[20−22] Like other class III subfamily receptors, the $GABA_B$ receptors possess a large extracellular domain that shares significant similarity with periplasmic binding proteins (PBPs) such as the leucine/isoleucine/valine-binding protein LIVBP (PDB code: 2LIV).[23] Moreover, this domain has been shown to play a critical role in ligand recognition in metabotropic glutamate receptors.[24] This statement is reinforced by the expression of soluble proteins corresponding to the N-terminal domain of $mGlu_1R$, $mGlu_4R$, and $GABA_B$ receptors, which are able to bind their respective ligands.[25−27]

Recently, mutagenesis and modeling of the extracellular domain of the $GABA_{B1a}$ receptor by homology with the leucine-binding protein allowed the identification of

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MLLLLLLAPLFLRPPGAGGAHTPNATSEGCQIIHPPWEGGIRYRGLTRDQVKAINFLPVDYE
IEYVCRGEREVVGPKVRKCLANGSWTDMDTPSRCVRICSKSYLTLENGKVFLTGGDLPALDG
ARADFRCDPDFHLVGSSRSICSQGQWSTPKPHCQVNRTPHSER**RAVYIGALFPMSGGWPGGQ**
ACQPAVEMALEDVNSRRDILPDYELKLIHHDSKCDPGQATKYLYELLYNDPIKIILMPGCSS
VSTLVAEAARMWNLIVLSYGSSSPALSNRQRFPTFFRTHPSATLHNPTRVKLFEKWGWKKIA
TIQQTTEVFTSTLDDLEERVKEAGIEITFRQSFFSDPAVPVKNLKRQDARIIVGLFYETEAR
KVFCEVYKERLFGKKYVWFLIGWYADNWFKIYDPSINCTVDEMTEAVEGHITTEIVMLNPAN
TRSISNMTSQEFVEKLTKRLKRHPEETGGFQEAPLAYDAIWALALALNKTSGGGGRSGVRLE
DFNYNNQTITDQIYRAMNSSSFEGVSGHVVFDASGSRMAWTLIEQLQGGSYKKIGYYDSTKD
DLSWSKTDKWIGGSPPADQTLV*IKTFRFLSQKLFISVSVLSSLGIVLAVVCLSFNIYNSHVR*
YIQNSQPNLNNLTAVGCSLALAAVFPLGLDGYHIGRNQFPFVCQARLWLLGLGFSLGYGSMF
TKIWWVHTVFTKKEEKKEWRKTLEPWKLYATVGLLVGMDVLTLAIWQIVDPLHRTIETFAKE
EPKEDIDVSILPQLEHCSSRKMNTWLGIFYGYKGLLLLLGIFLAYETKSVSTEKINDHRAVG
*MAIYNVAVLCLITAPVTMILSSQQDAAFAFASLAIVFSSYITLVVLFVPKMRRLITR*GEWQS
EAQDTMKTGSSTNNNEEEKSRLLEKENRELEKIIAEKEERVSELRHQLQSRQQLRSRRHPPT
PPEPSGGLPRGPPEPPDRLSCDGSRVHLLYK

Figure 1. Complete sequence of the $GABA_{B1a}$ subtype receptor and its two domains: the extracellular domain in bold characters and the transmembrane domain in italic gray characters.

some important residues such as Ser246, implicated in antagonist binding.[28] However, the other binding residues were not identified, especially in the homologous region corresponding to the hydrophobic side chain of leucine in the 2LIV binding site. In addition, authors suggested that this $GABA_B$ extracellular domain functions as a Venus flytrap because some mutations, such as Gln312, far from the binding site, modify the binding of ligands. This observation also derived from crystallographic studies of several other similar binding proteins which bind L-arabinose (PDB code: 1ABE), D-galactose, and sulfate. Indeed, Sack et al.[29] have observed three structural forms of PBPs: an unliganded open form,[29] an open form liganded with leucine,[29] and a closed form liganded with L-arabinose.[30] However, crystallographic structures allowing to observe one PBP in both forms, unliganded open form and liganded closed form, are not yet available.

In the present modeling study, we report (i) the generation of a model for the $GABA_B$ receptor extracellular domain and (ii) the docking of the GABA into the open and closed states of the extracellular domain binding site. Some aspects of the dynamics of the extracellular domain have also been studied.

Computational Details

Receptor. Sequence alignment: The multiple alignment of extracellular domain was generated using the ClustalW program.[31] The default options were used; only gap penalty values from 3 to 30 were tested. All sequences were extracted from the National Center for Biotechnology Information (NCBI) pubMed database (http://www.ncbi.nlm.nih.gov). The metabotropic glutamate receptor sequences (human mGluR1−8), the Ca^{2+}-sensing receptor sequence, and the sequences of the $GABA_{B1}$ and $GABA_{B2}$ receptor subtypes were aligned with the sequences of the bacterial periplasmic proteins that bind leucine/isoleucine/valine (PDB code: 2LIV) and leucine (PDB code: 2LBP). The alignment was then manually modified in order to eliminate gaps into the sequence that aligned with the known secondary structure elements of 2LIV and 2LBP. The resulting alignments were then used to generate the 3D model of the $GABA_{B1}$ receptor extracellular domain, which was taken as reference.

Molecular modeling: The 3D model of the $GABA_{B1}$ receptor extracellular domain was constructed by following the sequence alignment and the 2D homology using the coordinates of 2LIV for the extracellular domain in its open unliganded form. The closed liganded form of the extracellular domain of the $GABA_{B1}$ receptor was constructed by fitting the two lobes of 2LIV on the two lobes of the L-arabinose-binding protein crystal structure (PDB code: 1ABE). Indeed, these proteins have a remarkable similarity in tertiary structures, despite the small degree of amino acid sequence identity in the ligand-binding site. Thus, the sequence alignment of the 1ABE was not easily usable to construct by sequence homology the closed liganded form of the extracellular domain of the $GABA_{B1}$ receptor. Different options of the Sybyl software,[32] such as the Biopolymer module with Mutate Monomer or Search Loop algorithms, were used to construct the two 3D structures, the extracellular domain in its both open and closed forms.

The two generated structures were then optimized using molecular dynamics with a constraint on the backbone, allowing the side chains to adjust. The same simulation was then repeated without the constraint on the backbone. The molecular dynamics optimization was performed at constant temperature with an integration step of 0.5 fs. The structures were initialized at 300 K starting from a Boltzmann distribution set followed by 200 ps of simulation, also at 300 K. The conformations were recorded every 200 fs. Conformations generated during the equilibration period have not been retained for analysis. The Tripos force field was used with a dielectric constant equal to 1. The Kollman charges, implemented in Sybyl, were preferred as electrostatic contribution to other charge calculation methods. At this level of investigation, we focused on the optimization of local interactions in order to obtain an acceptable template for discussion. For instance, water molecules were not explicitly taken into consideration.

For each of the two structures, a statistical evaluation of all the recorded conformations was performed using the Verify 3D algorithm[33] obtained through the Verify 3D structure evaluation server (www.doe-mbi.ucla.edu/services/verify3D.html). For each structure, the conformation giving the best 3D/1D score was selected.

Finally, the two selected structures were subjected to energy minimization using the Tripos force field with a convergence criterion of 0.01 kcal/mol. The Kollman charges were used as electrostatic contribution.

Ligand. In this report, only the putative GABA-binding mode will be presented, since very poor information is available concerning the binding mode of other $GABA_B$ receptor ligands. GABA was considered in its zwitterionic form. Binding data on different mutants of the $GABA_B$ receptor have been reported[28] and are taken into account. The ligand was modeled using Sybyl 6.4 on a Silicon Graphics O2 R10000 station. The starting conformations were optimized by molecular mechanics algorithm using the Tripos force field. The lowest-energy conformations were found by means of the Sybyl/search option and then used as initial conformations for docking. The AM1 semiempirical method was used to calculate the electrostatic

(a)

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MGR4_HUMAN    GVIGASGSSVSIMVANILRLFKIPQISYASTAPDLSDNSRYDFFSRVVPSDTYQAQAMVD
MGR8_HUMAN    GVIGAAASSVSIMVANILRLFKIPQISYASTAPELSDNTRYDFFSRVVPPDSYQAQAMVD
MGR7_HUMAN    GVIGASGSSVSIMVANILRLFQIPQISYASTAPELSDDRRYDFFSRVVPPDSFQAQAMVD
MGR6_HUMAN    AVVGASASSVSIMVANVLRLFAIPQISYASTAPELSDSTRYDFFSRVVPPDSYQAQAMVD
MGR2_HUMAN    GVIGGSYSDVSIQVANLLRLFQIPQISYASTSAKLSDKSRYDYFARTVPPDFFQAKAMAE
MGR3_HUMAN    GVIGGSYSSVSIQVANLLRLFQIPQISYASTSAKLSDKSRYDYFARTVPPDFYQAKAMAE
MGR1_HUMAN    GVIGPGSSSVAIQVQNLLQLFDIPQIAYSATSIDLSDKTLYKYFLRVVPSDTLQARAMLD
MGR5_HUMAN    GVIGPGSSSVAIQVQNLLQLFNIPQIAYSATSMDLSDKTLFKYFMRVVPSDAQQARAMVD
CASR_HUMAN    AVVGATGSGVSTAVANLLGLFYIPQVSYASSSRLLSNKNQFKSFLRTIPNDEHQATAMAD
LIVK_ECOLI    YVIGHLCSSSTQPASDIYEDEGILMISPGATAPELTQRG-YQHIMRTAGLDSSQGPTAAK
LIVJ_ECOLI    YVIGHLCSSSTQPASDIYEDEGILMITPAATAPELTARG-YQLILRTTGLDSDQGPTAAK
ARAF_ECOLI    GFV--ICTPDPKLG--------------SAIVAKARGYD-MKVIAVDDQFVNAKGKPMDT
GABAB1a       IILMPGCSSVSTLVAEAARMWNLIVLSYGSSSPALSNRQRFPTFFRTHPSATLHNPTRVK
GABAB2        MVFGGVCPSVTSIIAESLQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAILK
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MGR corresponds to the different subtypes of Metabotropic Glutamate Receptors.

CASR corresponds to the Calcium-Sensing Receptor.

LIVK, LIVJ and ARAF correspond to the Leucine, Leucine/Isoleucine/Valine and L-Arabinose Binding Protein, respectively.

The bold characters indicate the regions implicated in the interactions with the ligands.

(b)

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                         β1              α1              β2
2LIV_ECOLI          1   EDIKVAVVGAMSGPVAQYGDQEFTGAEQAVADINAKGGIKGN-KLQIAKYDDACDPKQAV
ABP_ECOLI               ENLKLGFL--VKQPEEPWFQTEWKFADKAGKDL--G--FEVI-KIAVPDGEKTLNAIDSL
GABA-B1A_HUMAN      167 RAVYIGALFPMSGGWPG-GQACQPAVEMALEDVNSRRDILPDYELKLIHHDSKCDPGQAT

                         α2       β3        α3        β4      α4      β5
2LIV_ECOLI          60  AVANKVV-NDGIKYVIGHLCSSSTQPASDIYEDEGILMITPAATAPELTARG-YQLILRT
ABP_ECOLI               AAS-------GAKGFV--ICTPDPKLG--------------SAIVAKARGYD-MKVIAVD
GABA-B1A_HUMAN      226 KYLYELLYNDPIKIILMPGCSSVSTLVAEAARMWNLIVLSYGSSSPALSNRQRFPTFFRT

                               α5           β6        α6            β7
2LIV_ECOLI          118 TGLDSDQGPTAAKYILEKVKPQRIAIVHDKQQYGEGLARAVQDGLKKGNANVVFFDGITA
ABP_ECOLI               DQFVNAKGKPMDTVPLVMMAATKIGERQGQELYKEMQKRGWD--VKESAVMAITANELDT
GABA-B1A_HUMAN      286 HPSATLHNPTRVK-LFEKWGWKKIATIQQTTEVFTSTLDDLEERVKEAGIEITFRQSFFS

                              α7        β8        α8               β9
2LIV_ECOLI          178 GEKDFSTLVARLKKENIDFVYYGGYHPEMGQILRQARAA---GLKTQFMGPEGVANVSLS
ABP_ECOLI               ARRRTTGSMDALKAAGFPEKQIY-QVPTKSNDIPGAFDA---ANSMLVQHPEVKHWLIVG
GABA-B1A_HUMAN      345 ---DPAVPVKNLKRQDARIIVGLFYETEARKVFCEVYKERLFGKKYVWFLIGWYADNWFK

                         α9            β10                  α10
2LIV_ECOLI          235 NIAG----------ESAEGLLVTK-----PKNYDQV--PANKPIVDAIKAK-----KQDP
ABP_ECOLI               MNDS----------TVLGGVRATE-----GQGFKAA--DIIGIGINGVDAV-----SELS
GABA-B1A_HUMAN      402 IYDPSINCTVDEMTEAVEGHITTEIVMLNPANTRSISNMTSQEFVEKLTKRLKRHPEETG

                              α11                              α12
2LIV_ECOLI          273 SGAFVWTTYAALQSLQAGLNQS-------------------DDPAEIAKYLKANSVDTVM
ABP_ECOLI               KAQATGFYGSLLPSPDVHGYK------------------------SSE--MLYNWVAKDV
GABA-B1A_HUMAN      462 GFQEAPLAYDAIWALALALNKTSGGGGRSGVRLEDFNYNNQTITDQIYRAMNSSSFEGVS

                          β11           β12      β13
2LIV_ECOLI          314 GPLTWDEKGDLKG-FEFGVFDWHANGTATDAK  344
ABP_ECOLI               EPPKFTEVTDVVL-ITRDNFKEELEKKGLGGK
GABA-B1A_HUMAN      522 GHVVFDASGS---RMAWTLIEQLQGGSYKKIG  550
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Figure 2. (a) Sequence alignment of the binding site of the class III subfamily of GPCRs. (b) Complete sequence alignment of the extracellular domain of the GABA$_{B1a}$ receptor compared to the sequence of 2LIV and ABP.

term. Indeed, this method is more suitable for ligand than the Kollman charges.

Ligand−Receptor Complex. Docking: The docking was studied for GABA on the GABA$_B$ receptor extracellular domain. The procedure was carried out in the same way as previously published.[34] Briefly, the docking was performed by taking into account available structural constraints derived from previous experiments, thus minimizing computational time. At this level of confidence in the model, we preferred this biased docking to a random docking procedure. The first step was to define an anchor point for GABA into the GABA$_B$ receptor. Since the GABA$_B$ receptor binding site and the PBP binding site present a high homology, one of the GABA carboxylate oxygen atoms was anchored in the position corresponding to one leucine carboxylate oxygen atom complexed to the LIVBP. The position of this leucine oxygen atom, interacting with Ser79 of 2LIV, was derived from crystallographic data.[29] Moreover, a mutagenesis study on the GABA$_B$ receptor confirmed the existence of an interaction between GABA and residue Ser247 of its receptor. Ser247 aligned with

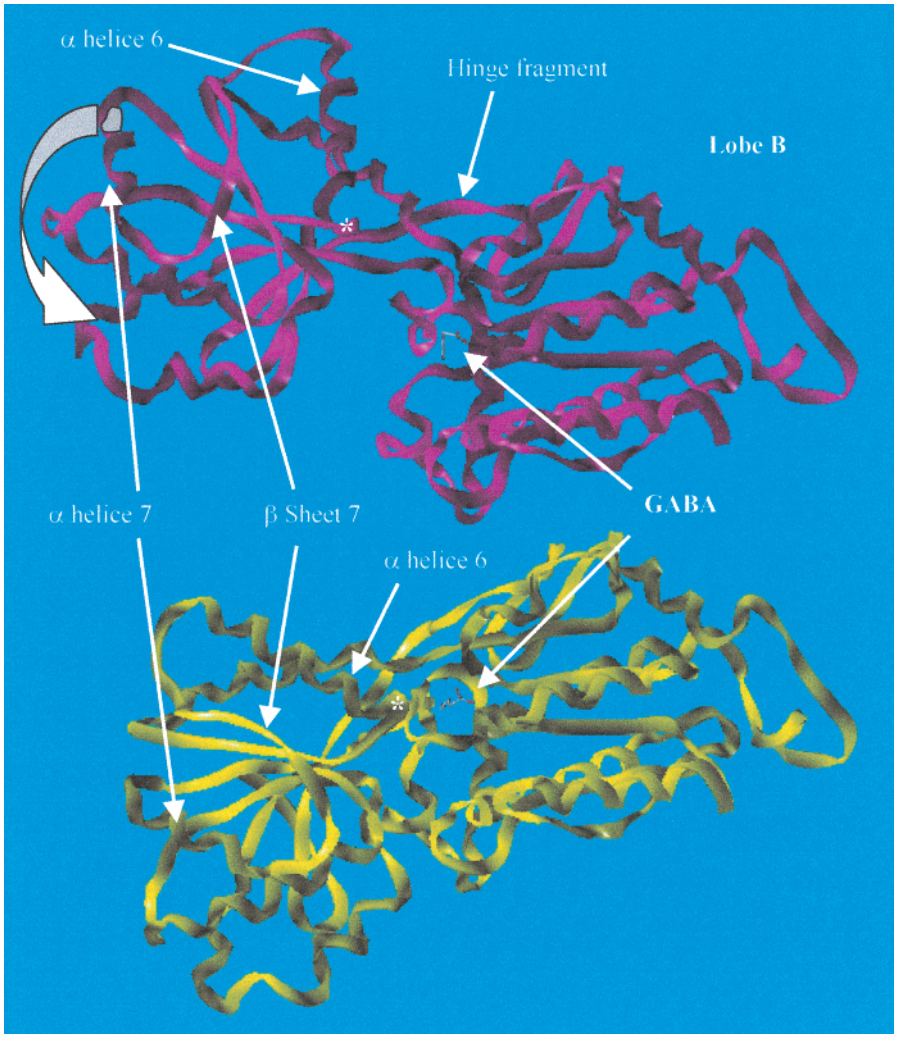

Figure 3. Open form of the $GABA_B$ receptor extracellular domain, in magenta, compared to its closed form, in yellow. The large arrow indicates the movement of lobe A compared to lobe B. GABA is shown in its binding site. The asterisk (*) indicates the position of the loop comprising α-helix 6 and *β*-sheet 6.

Ser79 of 2LIV.[28] Other putative anchor points were tested, such as the GABA ammonium group anchored near Ser246 (see the Discussion section). The semi-automated docking procedure we used consisted in reorienting the ligand inside the frozen receptor while simultaneously twisting all rotatable bonds. The ligand was reoriented relative to three coordinate axes around the anchor point with an angle step of 10°. Sybyl systematic search option was performed for each orientation of the ligand. The angle step for the search was 10° and the energy of the entire receptor−ligand complex was calculated at each step.

The lowest-energy complexes of GABA/$GABA_B$ receptor obtained in the course of the semi-automated docking procedure were optimized using the Tripos force field with the Kollman charges for the protein and the AM1 charges for the ligand, as the best compromise. The use of Gasteiger−Hückel or Gasteiger−Marsili charges for both protein and ligand did not affect significantly the results. A dielectric constant equals to 1 was applied.

Molecular Dynamics. A molecular dynamics study was performed on the complexes in order to optimize the relative positioning of each residues surrounding the ligand. At this stage of the study, water molecules were not explicitly taken into account, though it might affect the docking, more particularly in the open state of the extracellular domain. When the dynamic simulation significantly affected the position of an anchor fragment, another docking study with a new anchor point was performed.

The molecular dynamics studies, applied to each complex, were performed at constant temperature with an integration step of 0.5 fs and a coupling of 50 fs. The structures were initialized at 300 K starting from a Boltzmann distribution set followed by 200 ps of simulation at the same temperature. The conformations were recorded every 100 fs. The molecular dynamics procedure was only applied to the ligand and the residues around this ligand within a radius of 8 Å. The Tripos force field was used with the Kollman charges for the protein and the AM1 charges for the ligand. Since the dielectric constant is difficult to evaluate as a function of the dynamics and location, it was taken equal to 1. Moreover, trials with a dielectric constant of 4, for example, did not affect significantly the results. The average structure, implemented in Sybyl, was selected as a valid structure for each model. This average structure corresponds to the average position of each atom, which is calculated from the time series of positions stored in the file. The complexes were finally minimized with the Tripos force field, as described above.

Results and Discussion

The complete sequence of the $GABA_{B1a}$ receptor, shown in Figure 1, contains 961 amino acid residues. The multiple sequence alignment analysis allows to determine the location of the extracellular and transmembrane domains. The 383 bold characters indicate the position of the extracellular $GABA_B$ receptor domain, whereas the italic gray characters indicate the position of the transmembrane domain. Each domain is described in more detail in the next paragraphs. Since

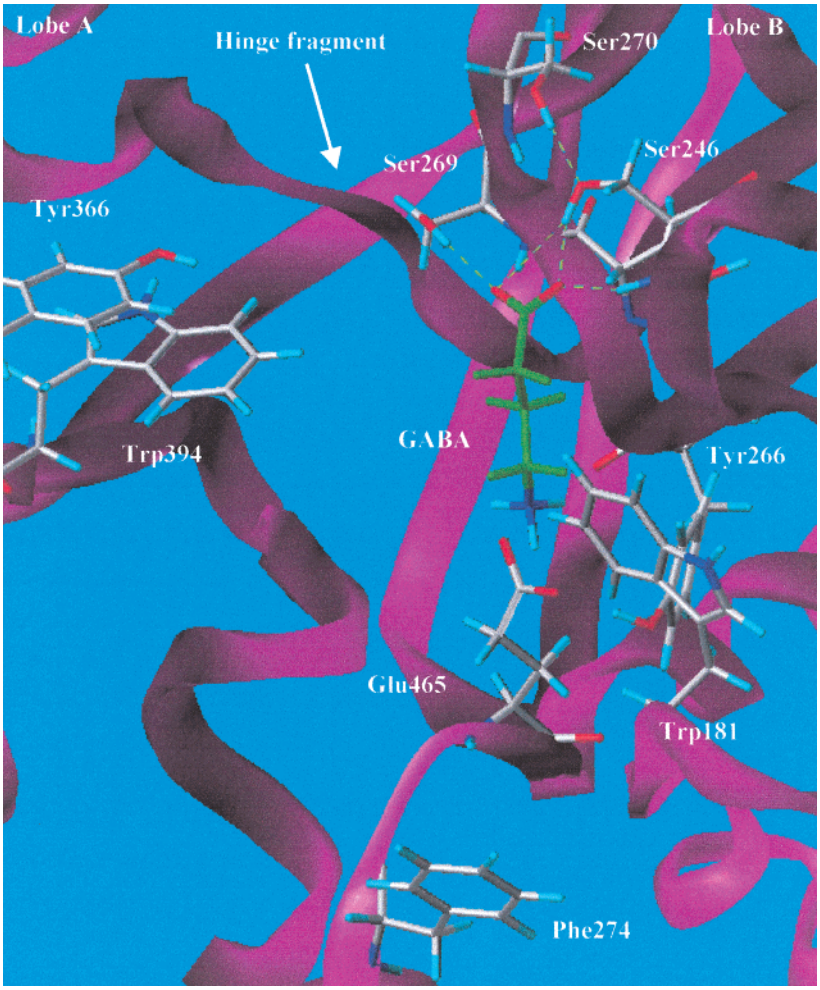

Figure 4. Docking of GABA into the binding site of the GABA$_{B1a}$ receptor extracellular domain (open form). Important residues and hydrogen bonds are shown.

the extracellular domain is most probably submitted to a large dynamic rearrangement named the Venus flytrap mechanism, this domain will be studied in its open and closed states.

Sequence Alignment. 1. Open State Form. The sequence alignment proposed for the extracellular domain is presented in Figure 2. The alignment of sequences comprised between residues 70 and 130 of 2LIV (Figure 2a) reveals a very high identity (39−45% identity with the FASTA program[35]) between the members of the class III subfamily of GPCRs. For each receptor, this region corresponds to the binding site of its endogenous ligand: L-glutamate with Ser165 in subtype 1 or with Ser159 in subtype 4 of the metabotropic glutamate receptors;[23,36−38] calcium with Ser147 in the calcium-sensing receptor;[39] leucine with Ser79 for 2LIV and 2LBP[29] receptors and GABA with Ser246 for the GABA$_{B1}$ receptor.[28] In this region, the alignment was directly predicted by the program and is conserved whatever the gap penalty.

In the other regions, manual alignment was performed in preserving the secondary structures and including the gap into the loop structures. The final and global alignment used to construct the extracellular domain of the GABA$_{B1}$ receptor is shown in Figure 2b. This alignment corresponds, with the FASTA program, to a sequence identity of about 21% for a 371-amino acid residue overlap. The secondary structure of the 2LIV and its two major residues Ser79 and Thr102 (in bold) implicated in leucine binding are indicated. In addition, and according to ref 25, the residues modifying the binding properties of the GABA$_B$ receptor are indicated in bold. This alignment reveals two major insertions: one between α9 and β10 and one between α11 and α12. These two insertions are located one on each lobe and far from the ligand-binding site. Thus, the use of the loop search option of Sybyl to approximate the 3D topology of these two domains should not alter the ligand-binding site. The loops with the best root-mean square and homology with PBP fragments were selected.

2. Closed State Form. The observation of the 3D structures of both arabinose-binding protein (1ABE) and 2LIV reveals that each lobe is constituted of four α-helices which alternate with five β-sheets. Thanks to the high degree of homology between the 3D structures of the two lobes of the 1ABE and 2LIV, 1ABE was selected as a template to build the closed state form of the GABA$_{B1a}$ receptor extracellular domain. The sequence alignment between the two PBPs, the GABA$_{B1a}$ receptor, and 1ABE indicates, whatever the gap penalty, either a large gap into the sequence corresponding to the ligand-binding site (Figure 2a, PBPs with 1ABE or GABA$_{B1a}$ with 1ABE) or a bad position of the crucial residues implicated in ligand binding (PBPs with GABA$_{B1a}$ and with 1ABE). This problem is essentially

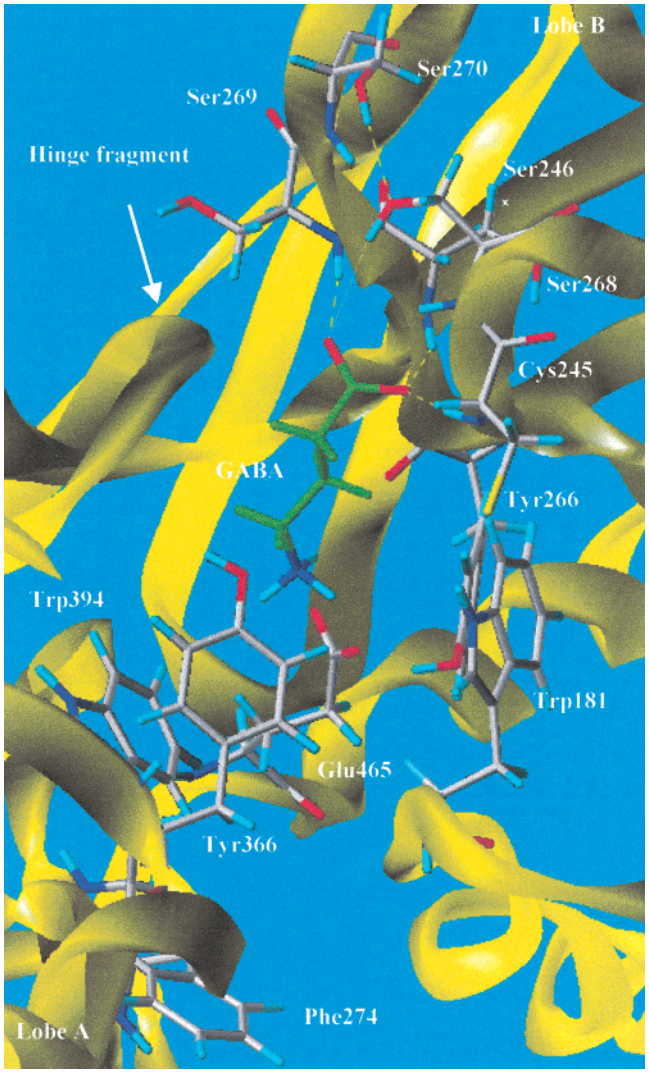

Figure 5. Docking of GABA into the binding site of the $GABA_{B1a}$ receptor extracellular domain (closed form). Important residues and hydrogen bonds are shown.

due to the fact that the residues implicated in ligand binding are localized in loop domains, sensitive to the dynamics of the protein. Thus, a direct sequence homology modeling was not achievable.

Alternatively, the generation of the 3D model of the closed extracellular domain of the $GABA_{B1a}$ receptor was directly undertaken by molecular modeling on a 3D template (see below).

Molecular Modeling. 1. Open State Form. The extracellular $GABA_B$ domain has been modeled in its open state by homology with the crystal structure of 2LIV. This model is presented in magenta in Figure 3. The two lobes are linked together by three segments. The C- and N-terminal ends indicate the direction of the polypeptide chain. The N-terminal region is preceded by the region allowing the distinction between the two subtypes of $GABA_{B1}$, a and b, whereas the C-terminal region is followed by the transmembrane domain. In addition, Galvez et al.[28] suggested that Cys219 and Cys245 are implicated in a disulfide bond, which would allow the correct folding of the protein and the stabilization of this domain of the receptor. Accordingly, the model includes this disulfide bond which is located near the ligand-binding site.

The GABA-binding site is localized on lobe B facing lobe A. Ser246 is likely to bind directly with GABA since it has been proposed, from sequence analysis,[28] that this residue is the homologue of Ser79 of 2LIV, which is implicated in the binding of leucine.[29] In addition, the Ser246Ala mutation totally suppresses the binding of an antagonist which might bind similarly to GABA to this residue.[28]

2. Closed State Form. The closed state form of the extracellular domain of the $GABA_{B1a}$ receptor was constructed by fitting the two lobes of 2LIV on the two lobes of 1ABE. Indeed, the comparison of the structure of each lobe in the closed form of 1ABE with the structure of each lobe in the open form of 2LIV reveals that the tertiary structure of these subdomains remains unaffected upon closing. Only the "hinge" fragment between them is twisted. Each lobe of the $GABA_{B1a}$ receptor was built and the three fragments linking the two lobes of the closed $GABA_B$ receptor were connected. The adjustment of these connections was performed based on the crystallographic data of 1ABE, since the two lobes of 1ABE are also linked by three segments. These results are in agreement with the model of the closed form of the metabotropic glutamate receptor type 4 extracellular domain.[38] This model was constructed by using as template the model of the 2LIV closed form, established by small-angle X-ray scattering and computer modeling studies.[40]

Figure 3 presents the closed $GABA_B$ extracellular domain in yellow compared to the open state in magenta. This comparison allows to imagine the dynamic process between the open and closed forms. This figure reveals a large dynamic process between the step corresponding to the binding of the ligands and the final step of the dynamic process. During the movement, after fixation of the ligand on lobe B, the two lobes come closer together with a rotation around the hinge fragment. The large arrow in Figure 3 indicates this motion of the $\alpha 7-$loop$-\beta 7$ fragment. In addition, a loop region, indicated by an asterisk, presents its residues far from the binding site in the open state, whereas in the closed state these residues are located near the ligand. For this fragment (QQTTE), the average distance C_α lobe A$-C_\alpha$ lobe B is about 9 Å in the closed state and 19 Å in the open state. Gln312 belongs to these residues, and Galvez et al.[28] have shown that the mutation of this residue modifies the binding of GABA. This observation is in agreement with the fact that this segment could be implicated in the dynamic process. This model is also in agreement with the Venus flytrap mechanism,[41] where the bound ligand is trapped into the protein. In addition, no steric hindrance and repulsion were observed during the minimization of the closed form, indicating that this conformation could be possible.

Ligand−Receptor Complexes. The binding site of GABA into the $GABA_B$ receptor extracellular domain is not yet well defined. Only some putative binding residues were identified by sequence homology analysis and mutagenesis, especially Ser246.[28] The authors also tried to determine the residues implicated in the binding of the ammonium region of the ligand, without reported success. To understand the binding mode of the ligands, GABA was docked into the open and closed forms of the $GABA_{B1a}$ receptor extracellular domain.

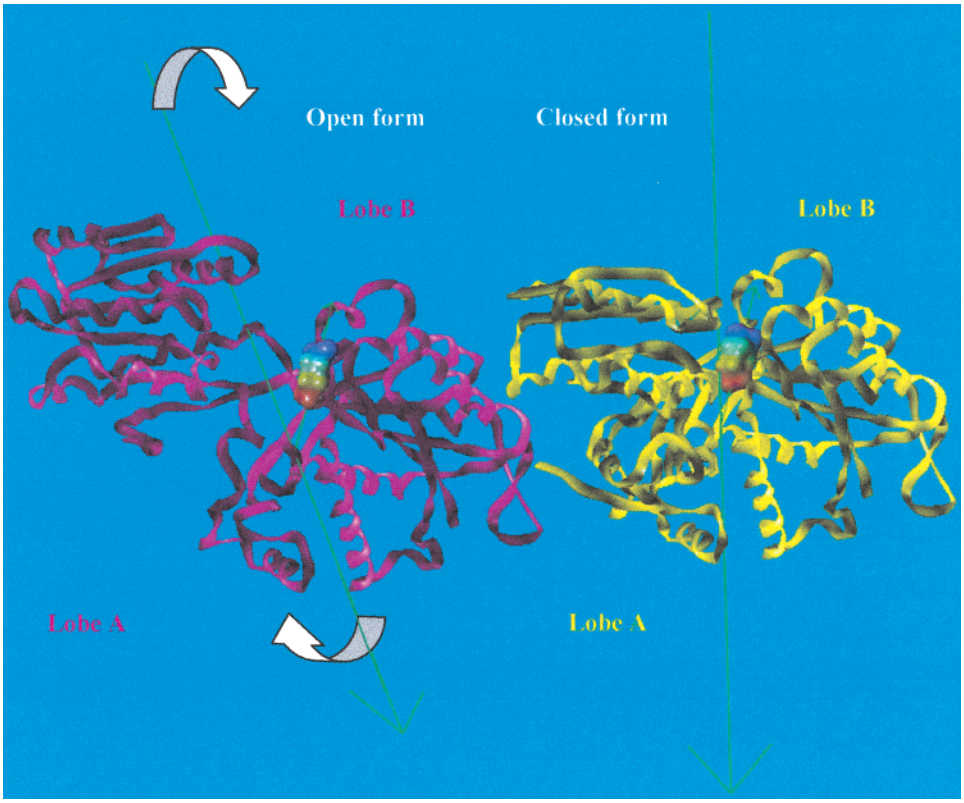

Figure 6. Comparison of the dipole moments between the open and closed forms of the GABA$_{B1a}$ receptor extracellular domain. The green arrows indicate the dipole moments of the receptor and of the ligand. GABA is represented by the electrostatic potential on its Connoly surface. Blue areas indicate regions with negative charges, whereas brown areas indicate regions with positive charges. Lobes B of the two forms are aligned, and the white arrows indicate the movement necessary to align the dipole of the open form with the dipole of the closed form.

Taking into account experimental data (crystallography[29] and mutagenesis[28]) and computational data (sequence alignment and molecular modeling[28]), the GABA anchor fragment was positioned near residue Ser246. The major problem was to decide whether GABA interacts with this residue via its carboxylic moiety or its ammonium moiety. Since GABA derives from the decarboxylation of the L-glutamate and taking into account the analogy between the L-leucine/2LIV binding site and the GABA/GABA$_B$ receptor binding site, it was tempting to assume that the ammonium moiety of GABA would be located near residue Ser246. However, the two possibilities were tested, and it was found that Ser246 most probably interacts with the carboxylate moiety of GABA. The protein−ligand interaction enthalpy is about −100 kcal/mol when the carboxylate is placed near Ser246 and about +10 kcal/mol when the ammonium is placed near Ser246. If the GABA ammonium group was anchored near Ser246, the carboxylate group could only interact with residue Gln464, with a low protein−ligand interaction enthalpy (+20 kcal/mol). All these interaction enthalpy evaluations must only be taken as relative values since, for instance, the solvation term is not taken into account. Interestingly, mutagenesis studies revealed that the mutation of this residue does not affect the binding of GABA.[28] In addition, molecular dynamic simulations reject GABA from the binding site when the ammonium group is placed near Ser246 while the complex remains stable in the other case. Other anchor positions, 1 Å around Ser146 or 1 Å around Glu465, have been tested. It allowed to determine all the active sites and led to similar docking.

Figure 4 shows the docked GABA into the open form with a relative protein−ligand interaction enthalpy of −101 kcal/mol. This docking reveals a hydrogen bond network around the carboxylate of GABA, implicating Ser246, Ser269, and Ser270. Ser270 allows the stabilization of the Ser246 hydroxyl group. This fact is in agreement with mutagenesis data.[28] Noteworthy, another similar solution with the same relative protein−ligand interaction enthalpy (−96 kcal/mol) was found when the GABA carboxylate forms a hydrogen bond with the Ser269 NH-amide group instead of the Ser269 hydroxyl group. This could explain the moderate effect of the mutation Ser269Ala[28] on GABA binding. In the closed form (Figure 5), the GABA carboxylate group interacts with the same residues as in the open form except for Ser269. In this closed form, the relative protein−ligand interaction enthalpy is about −130 kcal/mol. This result suggests that the binding of GABA is more favorable in the closed form than in the open form.

In both the open and closed states, the GABA ammonium moiety was found to make a strong ionic interaction with the carboxylate of residue Glu465. The distance COO^-−NH_3^+ is about 3.5 Å (see Figures 4 and 5). In Figure 4, Glu465 is surrounded by several aromatic residues such as Tyr266 and Phe274. The distance COO^-Glu465−aromatic is about 4 Å. These residues belong to lobe B. On lobe A, several aromatic residues are also found, especially Trp394. In the open state, Trp394 is distant from the GABA ammonium

group by about 10 Å. In the closed form (Figure 5), Trp394 is now located just beside the GABA ammonium group at about 4 Å. It results that the GABA ammonium is encaged in an aromatic cluster stabilizing the complex by π−cation interactions. Furthermore, this electron-rich hydrophobic environment increases the electrostatic interaction between the Glu465 carboxylate group and the GABA ammonium group. This proposed docking mode is consistent with the importance attributed to residue Glu465 in the binding of GABA.

The study of the dipole moment related to the open and closed forms is another argument which could support the proposed location of GABA into the binding site and might provide a clue on the dynamics of the extracellular domain of the $GABA_B$ receptor. In Figure 6 the dipole moments of GABA and of the receptor in both the open and closed states are shown. The $GABA_B$ extracellular domain presents a strong dipole moment in both the open and closed states. The global dipole moment of the receptor is 20 times higher than that of GABA. Very interestingly, we observed that the superimposition and orientation of the receptor and GABA dipole moments are optimal in the closed state. Indeed, in the closed state, the dipole of the receptor crosses the binding site between lobes A and B and is approximately aligned with the GABA dipole moment, but in the opposite direction. In the open state, the two dipoles are not aligned. The relative movement of the two lobes upon closing allows or results from the alignment of dipole moments (see Figures 3 and 6). The dipole moment could be interpreted as a preponderant factor governing the dynamics of the $GABA_B$ receptor extracellular domain.

In summary, our model suggests that residues, such as Trp394 and Glu465, might be implicated in GABA binding and/or in the dynamics of the $GABA_B$ extracellular domain. Our model is supported by the existence of a dipole moment which might be implicated in the reorganization of the two lobes.

Conclusion

Because of the lack of a crystallographic structure, a combination of most available experimental data and modeling tools, such as sequence alignment, homology modeling, and docking, was used to better understand the 3D structure of the $GABA_B$ receptor, its interactions with GABA, and its dynamics. A better understanding of this receptor might allow the design of new ligands able to modulate the $GABA_B$ activity.

This study proposes a detailed binding mode of GABA into the modeled receptor extracellular binding site (open and closed states). Several arguments, such as dipole moment alignment, contribute to support "in silico" our model.

Obviously, this model relies on many hypotheses and remains speculative. However, the coherence of many observations on sequences and on the 3D models might not be fortuitous, as demonstrated on other GPCR subfamily.[42] The model leads to predictions that could be easily tested, in particular by mutagenesis. We predict for instance that Glu465 and Trp394 might be involved in GABA binding and efficacy.

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