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Neil J. Harrison · Sarah C.R. Lummis

Molecular modeling of the $GABA_C$ receptor ligand-binding domain

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Abstract We have constructed a molecular model of the ligand-binding domain of the $GABA_C$ receptor, which is a member of the Cys-loop ligand-gated ion channel family. The extracellular domains of these receptors share similar sequence homology (20%) with *Limnaea* acetylcholine-binding protein for which an X-ray crystal structure is available. We used this structure as a template for homology modeling of the $GABA_C$ receptor extracellular domain using FUGUE and MODELLER software. FlexX was then used to dock GABA into the receptor ligand-binding site, resulting in three alternative energetically favorable orientations. Residues located no more than 5 Å from the docked GABA were identified for each model; of these, three were found to be common to all models with 14 others present only in certain models. Using data from experimental studies, we propose that the most likely orientation of GABA is with its amine close to Y198, and its carboxylate close to R104. These studies have therefore provided a model of the ligand-binding domain, which will be useful for both $GABA_C$ and $GABA_A$ receptor studies, and have also yielded an experimentally testable hypothesis of the location of GABA in the binding pocket.

Keywords $GABA_\rho$ receptor · Rho receptor · Ligand-gated ion channel · Cys-loop receptor · Neurotransmitterbinding site · Ligand docking

Abbreviations LGIC: Ligand-gated ion channel · ACh: Acetylcholine · AChBP: Acetylcholine-binding protein · nAChR: Nicotinic acetylcholine receptor · SCAM: Substituted cysteine accessibility method

N. J. Harrison · S. C.R. Lummis (✉)
Department of Biochemistry, University of Cambridge,
Tennis Court Road, Cambridge, CB2 1QW UK
E-mail: sl120@mole.bio.cam.ac.uk
Tel.: +44-1223-765950
Fax: +44-1223-333345

Introduction

The $GABA_C$ receptor is a member of the Cys-loop family of ligand-gated ion channels (LGIC), which includes nicotinic acetylcholine (nACh), $GABA_A$ and glycine receptors [1]. These receptors function as a pentameric arrangement of heteromeric subunits, although some examples of homomeric receptors are known. Each subunit has an extracellular domain, which binds ligands, and a transmembrane domain, which consists of four transmembrane crossing segments M1–M4; M2 lines the ion pore. The proteins are important therapeutic targets. Valium, for example, which is one of the most widely prescribed drugs in the developed world, acts at $GABA_A$ receptors, and drugs that target neuronal nACh receptors are important for Alzheimer patients. To understand their mechanism of action, and for the rational design of novel compounds, it is important to understand the molecular details of the binding sites of these proteins. To date, however, no LGIC structures have been resolved to atomic level. Nevertheless, the structure of a protein homologous to the extracellular domain of the nACh receptor—the acetylcholine-binding protein (AChBP)—has been resolved to 2.1 Å [2]. While there are probably some important differences between this protein and the nACh receptor ligand-binding domain, it has proved to be a useful model for nACh, $GABA_A$, and 5-HT_3 receptor extracellular domains [3–7]. Recently, a chimeric protein consisting of the AChBP extracellular domain and the 5-HT_3 receptor transmembrane domain was shown to be functional, thus providing further justification for the construction of homology-based models [8].

$GABA_C$ receptors are also sometimes referred to as $GABA_\rho$ receptors, as the first subunit cloned from this family, ρ1, was located in the retina [9]. This subunit was originally placed in the $GABA_A$ family, but as the receptors are insensitive to the classic $GABA_A$ antagonist bicuculline, they were subsequently considered to be

part of a distinct receptor family [10]. There are a number of other structural and functional differences to $GABA_A$ receptors, but perhaps the most interesting from the modeling viewpoint is that the $GABA_C$ receptor can function as a homopentamer. Hence, like AChBP, it contains five identical binding sites, and thus may serve as an easier model candidate than nACh and $GABA_A$ receptors. Another significant difference, not only from $GABA_A$ receptors but also from most other Cys-loop receptors is that the $GABA_C$ BC_{50} value (GABA concentration required for half maximal binding; 0.65 μM) is similar to its EC_{50} value (concentration required for half maximal response; 0.81 μM), and that the receptors show little desensitization [11, 12]. Tentative interpretation of this data suggests that the $GABA_C$ receptor exists in only one ligand-induced conformational state. As AChBP was crystallized in what is considered to be an activated, ligand-bound state [13], it is probably a highly appropriate model to study the ligand-binding domain of this receptor.

Here, we describe the use of alignment and modeling software to create a model of the extracellular domain of the $GABA_C$ receptor, and examine the possible orientations of GABA when docked into the binding pocket defined by this model. Another model of this receptor was published during the course of this work; it differs from our approach in that a manual method was used: AChBP residues were mutated in silico to match those in the $GABA_C$ ρ1 sequence, followed by a series of energy minimizations. The strategy we used was similar to that of Reeves et al. [7], where the model was built from an alignment that considers structure as well as sequence. Following creation of an appropriate model, GABA was computationally docked into the binding region. This revealed a number of potential orientations of the ligand that are evaluated using published experimental data, and indicate that the most likely orientation of GABA is with its amine close to Y198, and its carboxylate close to R104.

Materials and methods

Sequence alignment

The human $GABA_C$ ρ1 subunit sequence was aligned with the sequence of a monomer of the AChBP using FUGUE [14]. FUGUE assesses sequence similarity, but then it quantifies this in the context of three-dimensional (3D) structure; it defines a structural environment in terms of main-chain conformation, secondary structure, solvent accessibility, and also H-bonding status. FUGUE uses environmentally specific substitution tables and structure-dependent gap penalities, where scores for amino acid matching and insertions/deletions are evaluated depending on the local environment of each amino acid residue in a known structure [14].

Modeling

Three-dimensional models of the extracellular domain of the $GABA_C$ ρ1 subunit were created using MODELLER [15], based on the crystal structure of the AChBP monomer. The models (30 were generated) were then analyzed by means of a Ramachandran plot (RAMPAGE, de Bakker and Lovell, http://raven.bioc.cam.ac.uk/rampage.php). The best model was considered to be the one with a single amino acid in an unfavorable region of the plot; this was subsequently remodeled using CODA [16]. CODA predicts the structure of a short peptide using two algorithms, FREAD and PETRA. FREAD selects from a database of fragments whose structures have been solved, while PETRA uses a database of computer-generated structures, and a final prediction is made by CODA.

A pentamer was then generated by superimposing the modified monomer onto each monomer of the pentameric AChBP. The $GABA_C$ pentamer was then energy minimized in SYBYL using the AMBER force field [17], which is able to remove steric clashes at the subunit interfaces, and then reanalyzed using RAMPAGE.

Ligand docking

GABA was docked into the binding region of the $GABA_C$ receptor using FlexX (Tripos Inc., St. Louis, MO, USA). FlexX employs an incremental construction algorithm, in which the ligand is broken down into components (by cutting at each acyclic non-terminal single bond), and then gradually rebuilt into the binding site based on interactions of these components with the receptor. Connected components can be grouped into fragments, and these fragments are joined together to create the complete ligand. The models generated by FlexX were then used by Swiss-PDB viewer to extract all amino acids that possess at least one atom within 5 Å of the ligand. Potential H-bonds were also identified using Swiss-PDB viewer.

Results

Alignment of AChBP and the $GABA_C$ ρ1 extracellular domain

The sequence identity between the $GABA_C$ ρ1 extracellular domain and the AChBP monomer is less than 20%, yet the structural identity shared across the Cys-loop family, and hence between these two proteins of interest, is estimated as closer to 80% [4]. For this reason, FUGUE, which quantifies sequence alignment in the context of 3D structure, was used to create the alignment. These data are shown in Fig. 1a, and, for comparative purposes, a ClustalX alignment of these sequences, and those from two other related receptors,

are shown in Fig. 1b [18]. FUGUE has been shown to outperform this type of alignment [19]. Both alignments show that residues in the $GABA_C$ receptor-binding loops that have been shown to be important for function, such as Y198 and Y247 [20], align with similarly important residues in the other proteins. These are discussed in more detail below.

The region of the ρ1 sequence between the N-terminus and the first binding loop is longer by approximately 20 amino acids than other family members (Fig. 1b). The region of the ρ1 sequence that aligns with the α-helix of AChBP appears especially far from the N-terminus when compared to the other Cys-loop family models, and so further secondary structural analysis of this region was performed using PROF [21]. PROF predicted an α-helical region from residues 53 to 63, supporting the FUGUE alignment (Fig. 2).

The Cys-loop region does not align well in FUGUE. This is to be expected, as this region of the AChBP differs from all Cys-loop receptors. In the region preceding loop D, the alignment is also relatively poor, but this small stretch of amino acids has no associated functional significance. In most other regions, however, the two sequences align well, with only small, infrequent gaps. The predicted binding loop regions align well between the $GABA_C$ and AChBP sequences, as do many functionally important residues in these regions (Fig. 1), suggesting that the FUGUE alignment is indeed satisfactory.

Creation of $GABA_C$ ρ1 extracellular domain model

Following the alignment, a 3D model of the $GABA_C$ extracellular domain was chosen from a selection created using MODELLER. The quality of the model was analyzed using a Ramachandran plot, which revealed only one of the residues was in the unfavored region: L169, which had unfavorable torsion angles. L169 is located in loop E, and may be of importance to the

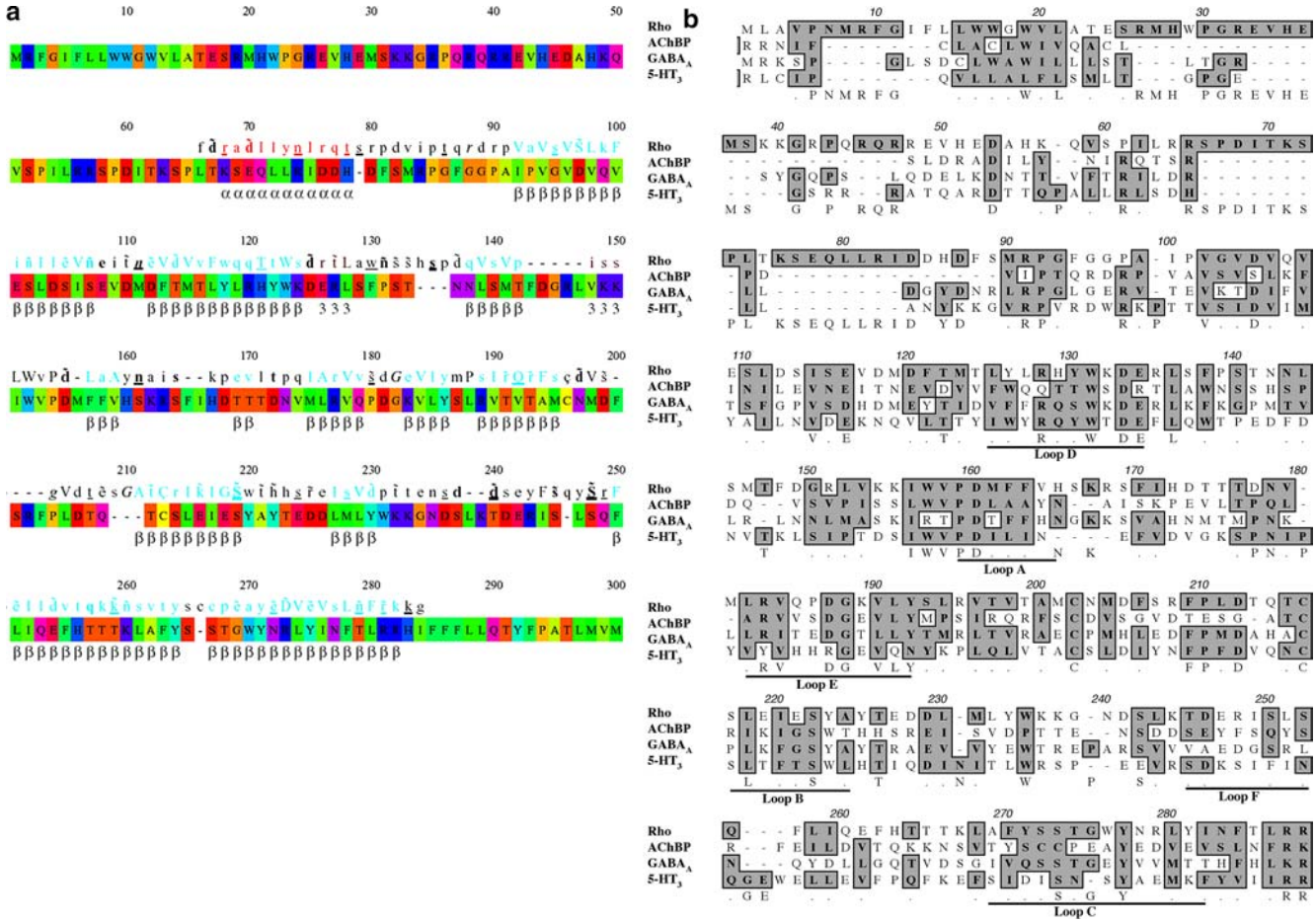

Fig. 1 Alignments of the $GABA_C$ receptor ρ1 subunit sequence. **a** Alignment with AChBP using FUGUE. The structural details of AChBP (*top line*) are shown using the program JOY (Mizuguchi et al. 1998), and amino acids in the $GABA_C$ receptor ρ1 subunit (*lower line*) are colored according to their properties. Key to JOY: α-helix, *red x*; β-strand, *blue x*; 3^{10} helix, *maroon x*; solvent accessible, *lower case x*; solvent inaccessible, *upper case x*; H-bond to main-chain amide, *boldface x*; H-bond to main-chain carbonyl, *underlined x*; disulphide bond, *cedilla* ç; positive ø-torsion angle, *italic x*. **b** ClustalW alignment with the $GABA_C$ ρ1 receptor subunit, AChBP, $GABA_A$ α1, and $5\text{-}HT_{3A}$ receptor subunits. Areas of similarity are shaded and the regions that form the binding loops A–F are indicated

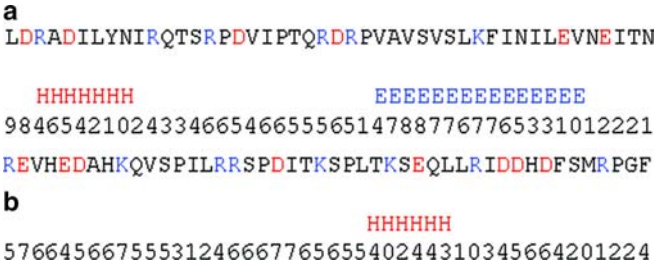

Fig. 2 Secondary structure prediction using PROF. **a** Predicted secondary structure of AChBP, showing α-helical region in correct position. **b** Predicted secondary structure of $GABA_C$ ρ1, showing position of α-helix matches that predicted by FUGUE alignment. *Top row* of figure represents sequence. *Second row* represents predicted structure: H, α-helix; E, β-sheet. *Bottom row* defines confidence in prediction: 1–10, where 10 is the complete confidence in prediction

structure of the binding site. A five amino acid stretch including L169 was therefore remodeled using CODA, which negated the unfavorable positioning of this residue. A 3D subunit-dimer indicating the binding loops is shown in Fig. 3a.

The pentamer of the $GABA_C$ ρ1 extracellular domain was created by superimposing the monomer onto each monomer of the AChBP, followed by subsequent energy minimization. The pentamer is shown in Fig. 3b. GABA was then docked into the binding site of the $GABA_C$ model using FlexX. The binding site was located to a pocket defined by residues Y198 and Y247, residues known to be important in binding from previous studies of the $GABA_C$ receptor [20]. The ligand-binding site was located at the interface between two adjacent subunits, about midway down the pentamer (Fig. 3a). The position is comparable to that of HEPES, nicotine, and carbachol in the AChBP structure [2, 13].

Thirty models were generated by FlexX. These could be divided into three groups based on the residues that were within 5 Å of the ligand. The residues associated with each orientation (1–3) are shown in Table 1, and a pictorial representation of GABA in the binding site has been generated for each orientation (Fig. 4).

Orientation 1

Here GABA is orientated toward the residues of loop E: M156, L157, R158, L166, Y167, S168 form a cavity-like region into which the GABA molecule is directed by its amine group. The amine group is stabilized by three H-bonds: two to backbone carbonyl groups (of M156 and L166), and one to the side chain hydroxyl of S168. The carboxylate group of GABA faces away from this loop E cavity, and toward the loop C residues Y247 and S243. The hydroxyl of S243 is able to H-bond to GABA, stabilizing its position. The hydroxyl group of Y247 is located within 3 Å of the carboxylic acid group, but its orientation does not favor the formation of a H-bond. Reaching up toward the negative carboxyl group is the side chain of R158, which may help to stabilize this moiety.

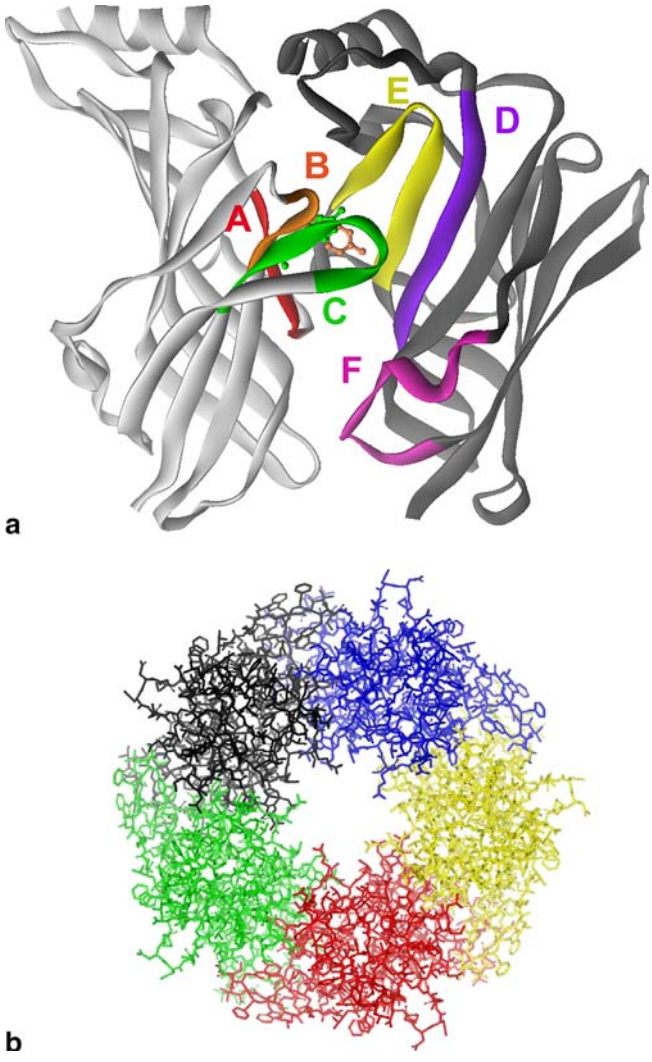

Fig. 3 The 3D dimer, and subsequent pentamer, model of the $GABA_C$ ρ1 extracellular domain. **a** Dimer model showing backbone structure, with binding region outlined at the subunit interface: loops A (*red*), B (*orange*), C (*green*), D (*purple*), E (*yellow*), F (*pink*) are shown, as are the residues (Y198, Y247) that defined the binding cavity into which GABA was docked. **b** Pentamer model, with each of the five subunits illustrated in a different color

Loop B residues Y198 and A199 are located with 5 Å of GABA, though no particular interactions are implicated between these residues and the ligand. The amino acids appear to form a "V"-shaped bend around the central bonds of GABA, just above the loop E cavity. In this model, no residues from loops A, D, and F are within 5 Å of the ligand.

Orientation 2

Here the location of GABA molecule is reversed compared to orientation 1, with the amine group facing toward loops B and C, and the carboxylate group pointing toward loops D and E. Compared to orientation 1, the ligand has shifted approximately 3 Å in the direction of

Table 1 Residues within 5 Å of GABA in the three orientations identified by FlexX

Binding loop	Residue (ρ1 receptor)	**1**	2	3	$GABA_AR$ equivalent residues
D	**Tyr 102**	-[a]	+[b]	-	αF64
D	Arg 104	-	-	+	αR66
D	His 105	-	-	+	αQ67
A	Phe 138	-	+	-	βY97
E	Met 156	+	-	-	αL118
E	Leu 157	+	-	-	αL119
E	Arg 158	+	-	+	αR120
E	Leu 166	+	-	+	αL128
E	**Tyr 167**	+	-	+	αY129
E	Ser 168	+	+	+	αT130
B	Ser 197	-	+	-	βS156
B	**Tyr 198**	+	+	+	βY157
B	Ala 199	+	+	-	βG158
C	**Tyr 241**	-	+	-	βF200
C	Ser 243	+	+	+	βT201
C	**Tyr 247**	+	+	-	βY205
C	Arg 249	-	+	-	βR207

Tyr residues, which dominate the binding pocket, are highlighted

[a] "-" represents > 5 Å from GABA

[b] "+" represents ≤ 5 Å from GABA

loop C, which brings residues Y241 and R249 closer to the ligand, with only one residue from loop E, S168, within 5 Å of GABA. The repositioning of GABA means that S197 (loop B), F138 (loop A), and Y102 (loop D) are now within 5 Å of the ligand.

The close proximity of the amine group to loops B and C would allow H-bonds to be formed here with the backbone carbonyls of S197 and Y198, which may stabilize the position of this group. Y247 is close to the positive amine, and is positioned such that a cation–π interaction is possible here. Indeed, this model is the most aromatic in nature, with Y102, F138, Y241, Y198, and Y247 surrounding the ligand. GABA's carboxylate group is close to the hydroxyl groups of Y198 and S168, and may be stabilized by an H-bond to the serine. The only positive residue within 5 Å of the ligand is R249, which is located at the opposite side of the cleft.

Orientation 3

Here the carboxylic acid group of GABA has swung toward loop D, which relocates the amine group away from the residues of loops B and C. R104 from loop D is now within 5 Å of the ligand and could form two H-bonds with the carboxylate group of GABA. This is the only model in which the H-bonding capacity of this moiety is satisfied, and R104 and the adjacent H105 also provide basic groups, which would help to stabilize the charged carboxylate. The amine group of GABA is orientated toward the loop E residues; it could be stabilized by H-bonds to the backbone carbonyl of L166 and also to the side chain hydroxyl of S168. Y167, of loop E, and Y198, of loop B, are in the vicinity of the amine, and have the potential to make cation–π interactions here. GABA, however, has noticeably little interaction with loop C, with only S243 being positioned within 5 Å of the ligand. The absence of Y247 in this orientation is also quite striking, as it is located in close proximity to GABA in orientations 1 and 2.

Discussion

The crystallization of the AChBP yielded a new dawn for comparative modeling studies in the Cys-loop family, as understanding the molecular mechanisms of these neurologically important proteins has been considerably hampered by the lack of structural information. AChBP is homologous to the extracellular domain of the nACh receptor, and thus by association to all the other members of this family. AChBP does, of course, lack many features of the complete receptor, but if its limitations are appreciated, it offers a unique source of structural information that can provide testable structural and functional hypotheses for all Cys-loop receptors. Here we have used the structure of AChBP to model the extracellular domain of the $GABA_C$ receptor. This region contains the binding sites for GABA, which lie at the subunit interfaces. Docking of GABA into the binding site revealed three energetically favorable positions of GABA. Examination of the residues within 5 Å of GABA showed that they fall in regions of the sequence (loops A–F, see Fig. 1b) that have been proposed to constitute the binding site. As described in more detail below, comparing the different orientations reveals that orientation 1 seems unlikely, but both orientations 2 and 3 are supported by experimental evidence, although, as discussed below, the current evidence best supports orientation 3.

The $GABA_C$ receptor-binding pocket

Identification of residues in the $GABA_C$ receptor-binding pocket reveals that it is rich in tyrosine residues. This is to be expected as aromatic residues, and in particular tyrosine, have been shown to be important in all Cys-loop receptor-binding pockets. What is unusual, however, is the complete lack of tryptophan residues, which, because of their intense aromaticity, play critical roles in

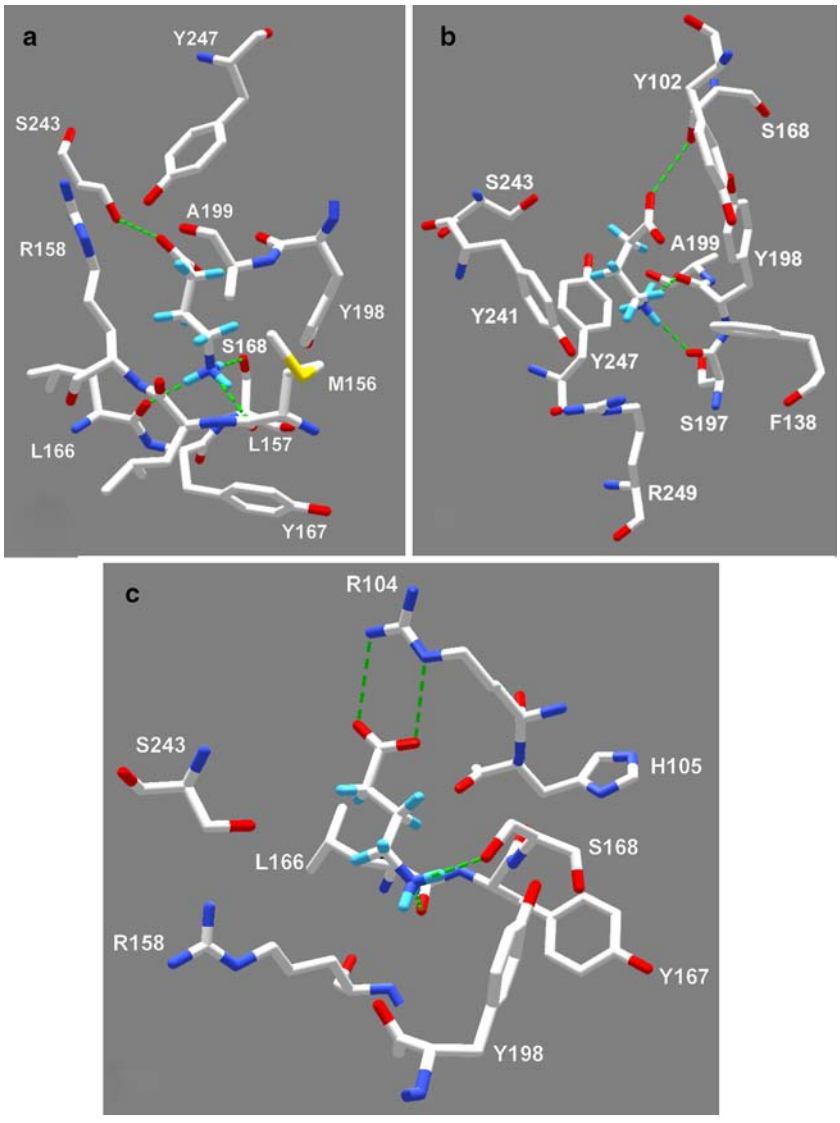

Fig. 4 GABA docked into the binding site using FlexX. **a** orientation 1, **b** orientation 2, and **c** orientation 3. Residues shown are those within 5 Å of GABA. H-bonds are shown as green dashed lines

ligand binding in at least three members of this family [8]. The docking data revealed that three residues are common to all three orientations of GABA: S168, Y198, and S243. S168 aligns with M114 in AChBP, and S243 with C187, both of which have been shown to be involved in ligand binding. S168 has also been shown experimentally to play an important role in the function of the $GABA_C$ receptor—changing this residue to cysteine resulted in a 300-fold increase in EC_{50} [22], and the resulting cysteine mutant could be protected from modification by GABA. Our data provide an explanation for this critical role as S168 has the potential to form two H-bonds: one with GABA (either with the amine as in orientations 1 and 3, or with the carboxylate, as in model 2) and the other with the hydroxyl of Y198.

An aromatic residue is highly conserved at the position equivalent to Y198 throughout the Cys-loop family, where it has been shown to be critically important in ligand binding. Molecular details of its role are beginning to emerge and tryptophan at the equivalent position in both nACh and 5-HT_3 receptors is known to form a cation–π interaction [23, 24]. GABA possesses a similar positively charged amine group, and therefore has the potential to form a similar interaction. This could occur with GABA in orientation 3 and possibly, although less likely, in orientation 2. It is not completely clear from the docking studies, however, if such a bond does form, as FlexX (and indeed all currently available docking programs) has limited ability to recognize cation–π bonds.

Orientation 1

Residues located close to GABA in orientation 1 are mostly those of loop E—six of the ten residues identified as being within 5 Å of GABA are in this loop. The E loop does not contribute such a high proportion of ligand-binding residues in other Cys-loop receptors, suggesting that this is an unlikely orientation of the ligand. In support of this, SCAM studies have shown that, of

the residues we have identified (M156, L157, R158, L166, Y167, and S168), only cysteine mutants of M156 and S168 are protected from modification by GABA, although the R158C mutant expressed poorly, yielding insufficient data [22]. M156 aligns with γL109 in the Torpedo nACh receptor, which has been implicated in the binding region by photolabeling studies [11]. Similarly, the equivalent residue in the 5-HT_{3A} receptor (Y143) has been located in the binding pocket, but appears to play a role in gating rather than binding [25].

Y247 is close to the carboxyl group of GABA in orientation 1, although no specific interaction is obvious. This residue is conserved as an aromatic residue throughout the Cys-loop family, and studies on $GABA_A$ [26], 5-HT_3 [27], and nACh receptors [28] have shown that the equivalent residue is involved in binding and/or gating. Indeed in MOD-1 receptors, the equivalent residue (W226) forms a cation–π bond with the agonist [29]. Y247 is also important for the function of the $GABA_C$ receptor: changing it to phenylalanine caused a 50-fold increase in EC_{50} and replacing it with serine resulted in non-functional receptors [20]. These large changes indicate an important role for this residue, which is not implicated in orientation 1.

Thus overall the experimental evidence does not support this orientation of GABA.

Orientation 2

Here GABA is closest to loops B and C, although it is also within 5 Å of residues in loops A (F138), D (Y102), and E (S168). F138 has been identified by SCAM protection studies as a binding site residue [22] and an aromatic residue is conserved at this position in the $GABA_A$ and $GABA_C$ receptor families. Equivalent loop A residues have been shown to be important for function in 5-HT_3 [30], $GABA_A$ [31], and nACh [32] receptors. Three residues in loop B are in close proximity to GABA: S197, Y198, and A199. Y198 has been discussed above and may form a cation–π bond here, although the location of the amine group is not optimal for this type of interaction. GABA could, however, H-bond to Y198 through its backbone carbonyl. The adjacent upstream residue, S197, could also H-bond to GABA through its backbone carbonyl, although the equivalent residue in the $GABA_A$ γ2-subunit (S171) is not involved in ligand binding, but is instead predicted to play a role in subunit–subunit interactions [33].

Four loop C residues are close to GABA: Y241, S243, Y247, and R249. Mutations of Y241 have been shown to affect GABA-mediated activation of $GABA_C$ receptors [20], and Y241 is equivalent to αY190 in Torpedo nACh receptors, which is critical for binding and gating [32, 34]. In AChBP, the hydroxyl from the equivalent tyrosine forms an H-bond with K139, which may be critical for gating [2]. Y241 may play a similar role in $GABA_C$ receptors, in which case its proximity to GABA would not be a good indicator of this orientation of the ligand. Y247 has the potential to form a cation–π interaction with GABA in orientation 2, although changing this residue to phenylalanine, which can also form a cation–π bond, caused a much larger increase in EC_{50} (50-fold, [24]) than would be expected. Thus, either there is no such bond here, or the hydroxyl of Y247 is involved in another significant interaction. Our model would support the latter, as the hydroxyl of Y247 has the potential to H-bond with the carbonyl backbone of A199.

The role of R249 is currently unclear but recent studies in $GABA_A$ receptors [35] have strongly implicated the aligning residue, also an arginine, in the binding and unbinding of GABA. In orientation 2, R249 is not suitably placed to act similarly, thus either it has a different role or this orientation is not correct.

Y102 in loop D is close to GABA in orientation 2. This residue has previously been indicated to be part of the binding domain in this receptor, though it was proposed to be more important for gating than ligand binding [36]. An aromatic at the position equivalent to Y102 is conserved throughout the Cys-loop family, and the equivalent residue in the AChBP has been shown to contact agonist ligands in crystallographic studies [2]. Mutagenesis in the $GABA_A$ receptor has also implicated the homologous αF64 to form part of the ligand-binding site [37]. Thus, while this residue is clearly important and present in the binding site, its role has not yet been determined.

Thus overall there is some experimental evidence in favor of orientation 2, but some that is contradictory.

Orientation 3

This orientation is the only one where both charged groups of GABA are energetically satisfied: the positively charged amine group is close to Y198, where there is the potential for a cation–π interaction, and the carboxyl group is close to R104, where both charge and H-bonding capacity would be satisfied. In the $GABA_A$ receptor, the residue equivalent to R104 (αR66) has been shown to be important in receptor function [37, 38], and it has been proposed to form part of a crown of arginines in the $GABA_A$ receptor which stabilize the carboxylate group [35]. In addition, mutation of the equivalent residue, γQ57, in the Torpedo nACh receptor affects agonist affinity [39]. These data suggest this is the correct orientation of GABA.

Not all the evidence, however, is as supportive. Other residues close to GABA include the loop E residues R158, L166, Y167, and S168. Of these, as discussed above, there is currently only evidence for a role of S168 in receptor function, although changing L166 to cysteine did cause an increased EC_{50}, suggesting it has some role here [22]. It should, however, be borne in mind that the amino acids in the binding pocket were not permitted to move during the docking procedure, which is unlike the situation in vivo, and it is also not yet clear how accurate the homology model will prove to be. Thus, while we

favor this location of GABA in the binding site, it is probable that in detail it may not be completely accurate. We believe, however, that the model we have derived yields a sufficiently accurate estimation of the agonist location to provide a useful experimentally testable hypothesis.

Conclusions

We have used the structure of AChBP to generate a model of the $GABA_C$ ρ1 extracellular domain; this is broadly similar to extracellular domain models for other Cys-loop receptors. The binding site is located between adjacent subunits, and the residues which contribute to this region are in accordance with experimental data from the $GABA_C$ receptor, and also from other members of the Cys-loop family.

The computational docking of GABA has revealed three possible orientations of this ligand in the binding site. Comparing these with data from experimental studies suggest that orientation 1 is unlikely but that there is some evidence to support both orientations 2 and 3. Orientation 3, however, currently seems to be best supported by the available data, and we propose that GABA in this orientation binds to and activates the receptor. We await further high-resolution studies to test our hypothesis.

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