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Binding modes of noncompetitive AMPA antagonists: a computational approach

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Abstract

The activity of functional AMPA receptors (AMPARs) is modulated by noncompetitive antagonists. So far, no information about the molecular mechanism of action and the localization of the binding pocket(s) is available. We speculated that the leucine/isoleucine/valine binding protein (LIVBP)-like domain of AMPAR, localized at the extracellular N-terminus of the receptor, might be involved in the binding of noncompetitive antagonists and we tested this hypothesis through a computational approach involving the comparison with NMDA and metabotropic glutamate receptors and the generation of a 3D homology model of the LIVBP-like domain of AMPAR. The results suggest that the interdomain cleft of the LIVBP-like domain of AMPAR may contain the noncompetitive antagonist binding pocket.

Keywords: AMPA; LIVBP; Homology modeling; Uncompetitive antagonists

1. Introduction

Most of the excitatory transmission in the central nervous system (CNS) of vertebrates is mediated by two main families of glutamate receptors (GluR), namely ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). iGluRs constitute a family of membrane spanning, cation specific, ion channels composed of several subunits, classified as NR1, NR2A–D, NR3A–B; GluR1–4; GluR5–7, KA1 and KA2 which assemble with variable stoichiometry to give functional NMDA, AMPA and KA receptors, respectively [1]. The family of the G-protein coupled mGluRs is composed by at least eight subtypes, termed mGluR1–8, which have been operatively classified into three groups according to sequence homology, agonist and antagonist selectivity and neuronal localization [2].

Both iGluRs and mGluRs share a modular architecture in which the amino terminal, extracellular domain, plays a fundamental role (Fig. 1).

Sensitive sequence alignment techniques have disclosed limited but significant homology relationships between portions of the extracellular domain of GluR and members of the family of bacterial periplasmic binding proteins (PBPs). Particularly, it was shown that the first two thirds of the amino terminal domain (ATD) of mGluRs share sequence homology with leucine/isoleucine/valine binding protein (LIVBP) [3], belonging to the type I folding motif of PBP [4]. Interestingly, it was also reported that the same homology is present between LIVBP and the ATD of the $GABA_B$ receptor [5] and that iGlurs have also a LIVBP-like region localized at beginning of the extracellular ATD [6]. On the other hand, the segment (S1) located immediately before the first putative transmembrane domain in iGluRs as well as the segment (S2) located between the putative third and fourth transmembrane domains displays moderate homology with lysine/ornithine/arginine binding protein (LAOBP) [6], belonging to type II folding motif of PBP.

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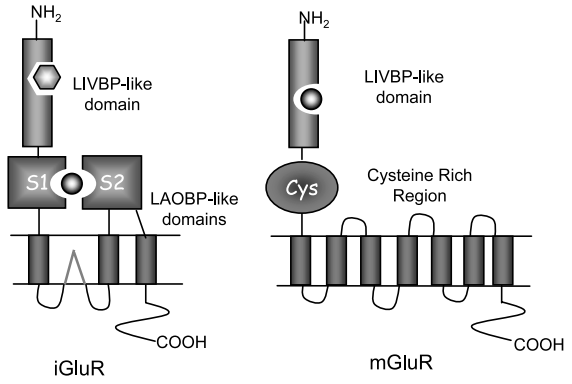


Fig. 1. Modular architecture of ionotropic (left) and metabotropic (right) glutamate receptors. The N-terminal domain of the extracellular portion of both receptor families is homologous to LIVBP.

Following these first discoveries, several experiments, including the recent crystallization of both the LIVBP-like domain of mGluRs [7] and the LAOBP-like domain of iGluRs [8], have provided conclusive evidence that these domains contain the binding sites for glutamate, other agonists and competitive antagonists. Furthermore, the large conformational movements associated with the functioning of these PBP like proteins, which consist in the interlobe closure after ligand binding, are responsible for the opening and desensitization of the channel (iGluRs) or for the signal transduction to G-proteins (mGluRs). The biological significance of the LIVBP-like domain of iGluRs has instead remained for long time much more elusive. Indeed, engineered constructs containing the S1–S2 domain of NMDA but lacking the LIVBP portion displayed an unchanged ability to bind glutamate, thus demonstrating that this domain does not influence the glutamate binding [9]. However, it has been recently demonstrated that deletion of the LIVBP-like domain in NR2A subunit of NMDA receptor eliminated the high-affinity, voltage dependent Zn^{2+}-inhibition [10]. Furthermore, molecular determinants of Zn^{2+} binding were identified and, according to the bilobar structure of LIVBP domain, a mechanism for Zn^{2+} binding and NMDA inhibition was proposed. Site-directed mutagenesis experiments has supported the interesting proposal according to which the noncompetitive NMDA antagonist ifenprodil binds the LIVBP-like domain of NMDA, thus pointing out the possibility of achieving pharmacological control of NMDA by acting at the LIVBP-like site [11].

AMPA receptors (AMPARs), constituted by assembly of GluR1–4 subunits, have also a LIVBP-like region, known as X domain, localized at the N-terminal region of the receptor. Analogous to the NMDA receptors, the LIVBP-like domain of AMPAR does not bind glutamate or competitive antagonists, and so far only a structural role in the dimerization process has been proposed for it [12].

Given the growing evidences on the peculiar role that the LIVBP-like domains play in the regulation and modulation of the function of both ionotropic and metabotropic glutamate receptors, in this paper we speculated that the LIVBP-like domain of AMPAR might contain the binding site for noncompetitive antagonists. This hypothesis was preliminarily tested by a combined homology modeling/docking studies approach aimed at understanding the binding mode of some known noncompetitive AMPAR antagonists (Scheme 1), for which a variety of electrophysiological data exist but no binding information is currently available. The results of this computational study are herein reported and discussed.

2. Computational approach

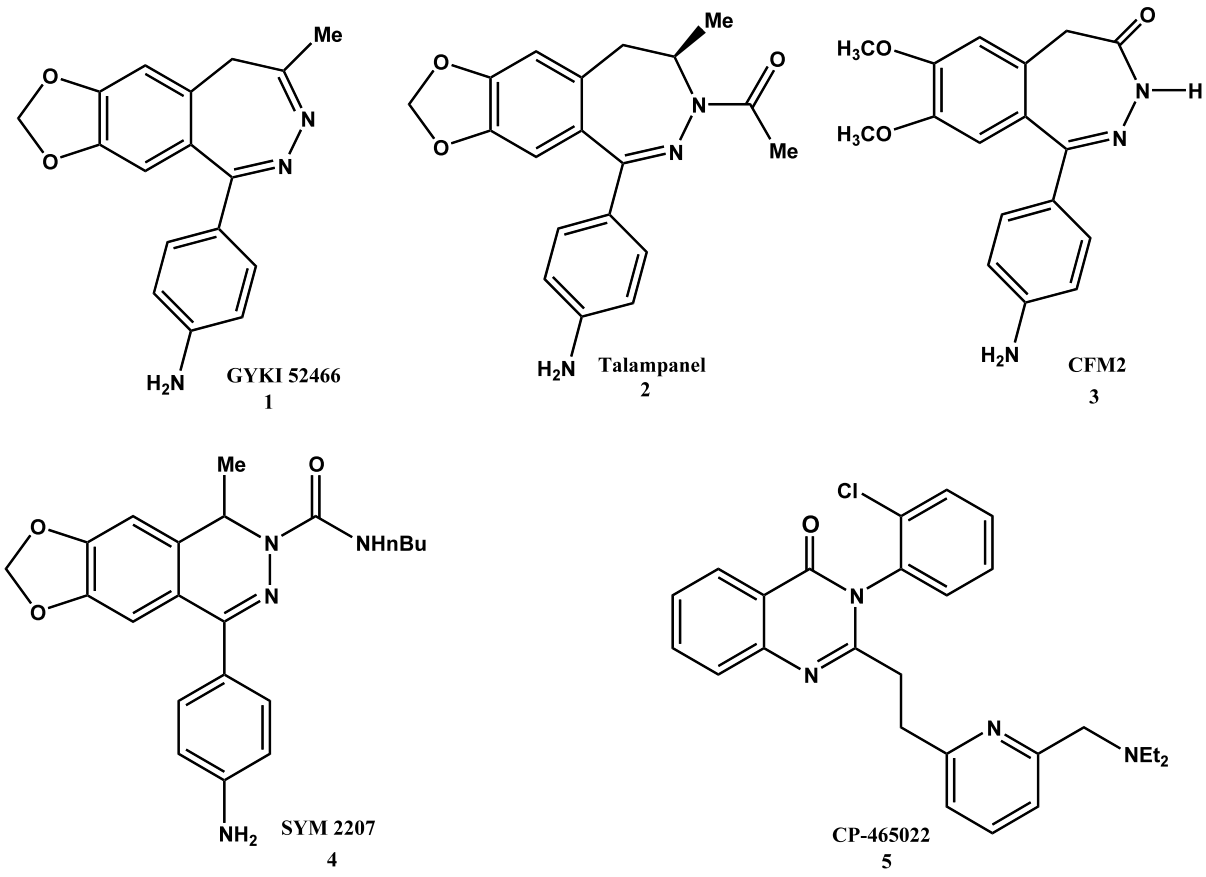
The computational approach that we have used is based on the following steps: (i) generation of a multiple alignment among the sequences of the LIVBP-like regions of GluR1 and the sequences of the analogous regions of NR2B, and mGluR1; (ii) construction of a homology model for the LIVBP-like region of AMPAR; (iii) identification of a putative binding site for non-competitive antagonists also on the basis of available mutagenesis experiments carried out on the LIVBP-like domain of NR2B; (iv) docking experiments performed with selected noncompetitive antagonists and critical discussion of the results.

Details on the computational protocol are given in the Section 3.

3. Methods

The sequences of the ATDs of AMPAR (LIVBP-like region), mGluR1 and NMDAR (NR2B, LIVBP-like region) were aligned using the Align123 module of INSIGHTII software package (see Fig. 2).

The Blosum-62 matrix was used with a gap insertion penalty of 11 and a gap extension penalty of 1. Where missed, secondary structures were predicted using the PHD server. The alignment was carefully checked to avoid gap insertion where conserved secondary structure motifs were present and to improve the overall folding quality as suggested by the Verify3D server. MODELER module within INSIGHTII was used with the default setting to build a 3D model of the LIVBP-like domain of the AMPA receptor. The recently reported crystal structure of the closed functionally active conformation of mGluR1 in complex with glutamate (pdb code: 1ewk) was used as template [13]. The atomic coordinates of the 3D model thus obtained were submitted to a minimization protocol using the Charmm22 force-field. During the minimization, a harmonic restrain of 20 kcal/mol



Scheme 1.

was applied on Cα atoms. The energy minimization was performed using a cycle of 2000 steps of Powell algorithm until a gradient of 0.05 kcal/mol was reached. A statistical validation of the structure was carried out by using the Verify3D server [14].

The docking protocol was carried out using the Lamarckian genetic algorithm (LGA) of AUTODOCK program v.3.0 [15]. The putative binding site was defined in the cleft formed by the lobes of the LIVBP-like domain. It was identified by a grid of 60 points along the x, y and z directions and a grid spacing of 0.375. The coordinates of the center of the grid were placed in the cleft of the LIVBP-like domain ($x = 0.05$; $y = 7.66$; $z = 3.24$). A distance dependent function of the dielectric constant was used for the calculation of the electrostatic maps. All docked compounds were submitted to 100 runs of AUTODOCK search. Each run was constituted by the evolution of a population of 50 individuals in 27×10^3 generations. The maximum number of energy evaluation was set to 15×10^5. Other parameters controlling the LGA were set to their respective default values. A cluster analysis was performed on the results obtained from the 100 runs using an RMS tolerance of 1.0 Å. All calculations were carried out on a SGI O2 R10000 workstation.

4. Results and discussion

The result of the multiple alignment among the ATDs of AMPAR (LIVBP-like region), mGluR1 and of NMDA (NR2B, LIVBP-like region) are reported in Fig. 2.

Based on this alignment and using the known crystal structure of the ligand binding domain (LBD) of mGluR1 as template [13], a homology model of the LIVBP-like domain of AMPAR was obtained.

The globular shape of the LIVBP-like domain, characterized by two lobes, connected by a hinge region, can be appreciated in Fig. 3.

Once having generated a reliable model of the LIVBP-like region of AMPAR, putative amino acids possibly involved in the binding of modulators were identified. Our search was driven by two information. The first one is that all the PBP like proteins so far crystallized have the binding pocket for their ligands localized in the cleft region separating the two lobes. The second one is that individual amino acids involved in the binding of ifenprodil to NR2B isoform of the NMDA receptor have been identified [11].

Given the evolutionary similarity between ionotropic glutamate receptors, we felt to hypothesize that a

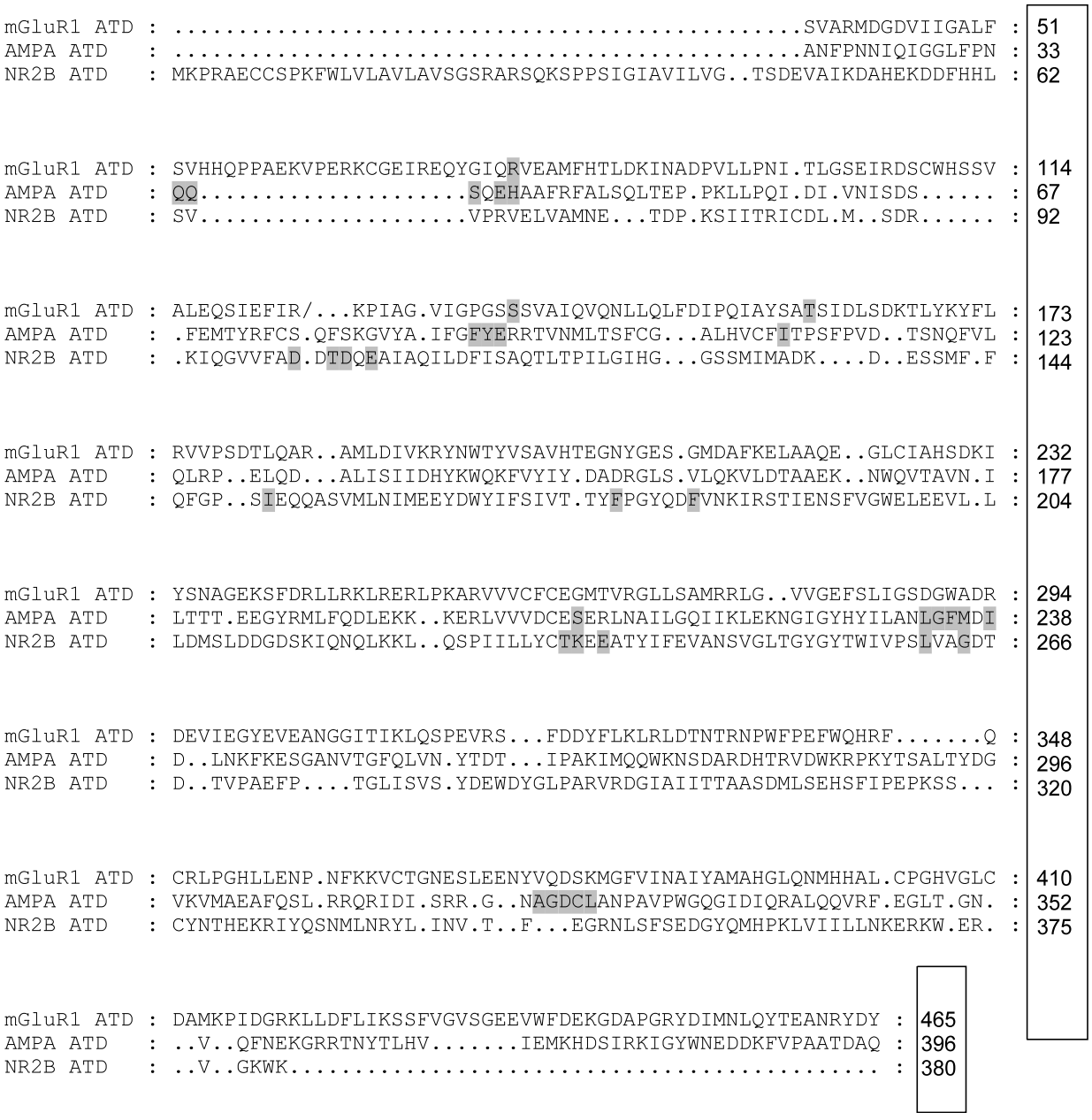


Fig. 2. Alignment of the ATDs of AMPAR, mGluR1 and NR2B. The residues involved in the binding of glutamate (mGluR1) and ifenprodil (NR2B) are shaded.

putative binding pocket in the LIVBP-like region of AMPAR would be conserved with the binding pocket of the analogous region of NR2B.

Thus, the set of amino acids in the AMPAR conserved with the analogous residues of NR2B involved in the recognition of ifenprodil and identified by Perin-Dureau [11] (Asp101, Thr103, Asp104, Glu106, Ile150, Phe176, Phe182, Thr233, Lys234, Glu236, Leu261, Gly261) were localized in the 3D model of the LIVBP-like region of AMPAR and found in the cleft region of the domain. This putative binding pocket region, which encompasses residues belonging to both lobes and is lined-up by residues 34–36, 38, 39, 88–90, 109, 206, 233–236, 238, 320–324 (GluR1-rat numbering), was therefore chosen to perform docking studies. With the objective to identify the correct binding mode of noncompetitive AMPAR antagonists, we selected the most representative compounds of each class so far identified, i.e. benzodiazepines (GYKI 52466, **1**; talampanel, **2**; CFM-2, **3**) 16a–c phthalazines (SYM 2207, **4**) 16d, and quinazolines (CP-465022, **5**) 16e (Scheme 1).

Table 1 summarizes the results of the docking procedure carried out using AUTODOCK 3.0.5.

All the studied compounds had a single preferred disposition in the identified binding site and presented a common binding mode (Fig. 4).

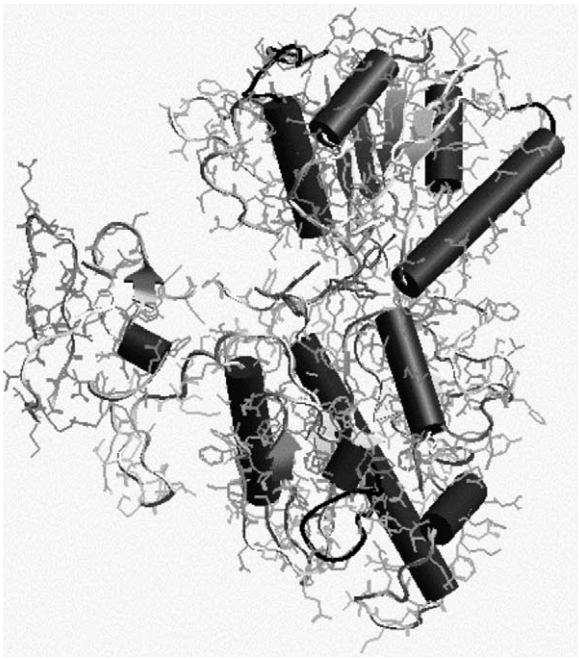


Fig. 3. 3D model of the LIVBP-like domain of AMPAR. The putative binding pocket is localized in the cleft separating the two lobes.

Table 1
Results of the docking experiments

Comp.	Cluster number	Dock energy	Population	Hbond
1	3	−7.76	74	Gln34,His39
2	1	−8.09	45	Gln34,His39
3	2	−7.75	29	Gln34,Ala320
4	5	−9.88	23	Gln34,Asp322
5	2	−10.42	15	null

The putative binding pocket consists of Gln34, Gln35, Glu38, His39, Tyr89, Ile109, Ser206, Leu233-Ile236, Ala320-Leu324. Both van der Waals and hydrogen-bonding interactions appeared to contribute largely to the stability of the compounds into the binding site. In particular, compounds **1** and **2** were involved in hydrogen-bonds with Gln34 and His39, whereas **3** and **4** were hydrogen-bonded to Gln34, Ala320 and Asp322. Fig. 4b shows the docking of **3** into the putative binding pocket. The 4-amino substituent forms a hydrogen-bond with the backbone of Ala320, while the endocyclic NH of the diazepine nucleus interacts with the Gln34. Another interesting feature emerging from the docking studies is the possibility of a π–π interaction between the aromatic ring of the 2,3-benzodiazepine system and the side-chain of Tyr89. The two benzene rings were in fact almost perpendicular, that is in a disposition particularly suitable for a edge-to-face aromatic–aromatic interaction.

The modular architecture of glutamate receptors, of both metabotropic and ionotropic type, is currently receiving a growing interest, as new information about the possible role of individual domains become available. In particular, glutamate receptors have adopted a strategy finalized at the fine control of their functions based on the molecular mechanisms owed by the class of PBPs. Thus, metabotropic glutamate receptors have a dimeric extracellular domain which is homologous to LIVBP and contains the binding pocket for glutamate and competitive antagonists. A molecular model for mGluR activation and blockade could be proposed on the basis of the large conformational movements associated with the functioning of the LIVBP domain.

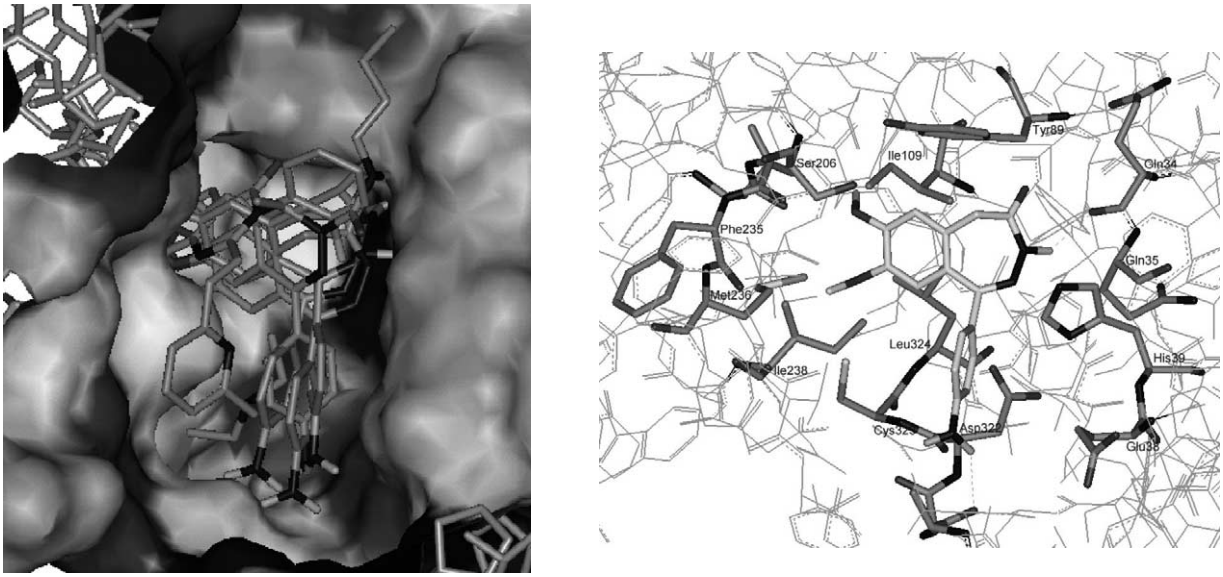


Fig. 4. (a) Superimposition of the best AUTODOCK solutions for compounds **1**–**5** into the putative binding pocket of AMPAR; (b) docking of CFM2 (**3**) into the putative binding pocket.

Analogously, the channel properties of the iGluRs can be interpreted on the basis of the concerted opening/closing processes of the LAOBP-like S1–S2 domains of the tetrameric arrangement which constitutes the functional receptors. Ionotropic receptors have also a LIVBP-like domain whose function has remained elusive until the discovery that in functional NMDA receptors this domain has allosteric modulatory properties and contains the binding site for Zn^{2+} and for the noncompetitive antagonist ifenprodil.

In this paper, we present the speculative hypothesis that the LIVBP-like domain of AMPA receptors contains the binding pocket for noncompetitive antagonists. Indeed, from sequence alignment it could be demonstrated that the AMPAR-forming GluR1–GluR4 subunits have a N-terminal domain homologous to LIVBP. This domain is not involved in binding of glutamate or competitive antagonists and so far only a structural role in dimerization process has been proposed.

Our approach has been based on the following steps: construction, by comparative modeling and minimization, of the LIVBP domain of the GluR2 subunit; identification of a putative binding pocket for modulators, also on the basis of existing data on mutagenesis experiments carried out on NMDA receptor; docking of selected known noncompetitive AMPAR antagonists (**1**–**5**).

The results thus obtained support the hypothesis that the chosen antagonists may bind the LIVBP-domain. We are aware that, in the absence of direct experimental proofs (such as site-directed mutagenesis), a strong bias towards the expected results is introduced in the computational protocol. The following points should, however, be considered: (i) So far, no indication is available as far as a possible binding site for noncompetitive antagonists. Likewise, no displacement binding assays have to date developed for these ligands. Studies on chimeric receptors have demonstrated that AMPAR negative modulators do not bind the glutamate binding domain and glutamate does not bind the LIVBP-like domain. (ii) The LIVBP-like, ATD, is present in all the known iGluRs. A cooperativity between the glutamate binding site and the LIVBP-like domain has been demonstrated for the NMDA receptor and hypothesized to be present in other ionotropic receptors [17] as a result of the evolutionary pressure leading to the modular architecture of this receptor family. (iii) If a positive allosteric modulatory function is accepted for the LIVBP-like domain of functional AMPA receptors, then inhibition of the cooperativity would result in the pharmacologically observed noncompetitive antagonism such as that elicited by **1**–**5** studied derivatives.

In conclusion, our computational results indicate the possibility that the LIVBP domain of AMPA receptors contains the binding site for noncompetitive antagonists and suggest individual residues for site directed mutagenesis experiments.

Acknowledgements

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