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Molecular dynamics simulation of the ligand binding domain of mGluR1 in response to agonist and antagonist binding

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Summary

The interdomain movements of the ligand binding domain (LBD) of mGluR1 in response to agonist or antagonist binding are studied by 2 ns molecular dynamics (MD) simulations. Our results indicate that MD is able to reproduce many of the experimentally determined features of the open and closed conformations of LBD. Analysis of the ligand behavior over time allows to delineate some of the molecular determinants responsible for the agonist-induced or antagonist-blocked LBD responses.

Introduction

Metabotropic glutamate receptors (mGluRs) [1] constitute a distinct family of G-protein coupled receptors belonging to the subfamily C [2]. After fifteen years since their pharmacological identification, a reasonably clear picture of the architecture and functioning of these receptors has now emerged from molecular cloning studies, alignment of the primary sequences, and construction of chimeric receptors [3, 4, 5]. These studies have revealed the foremost importance of the extracellular amino terminal domain (ATD) of mGluRs which contains the Ligand Binding Domain (LBD) for agonists and competitive antagonists. The LBD of mGluRs shows a distant but significant homology with the Leucine/Isoleucine/Valine Binding Protein (LIVBP) member of the Periplasmic Binding Protein family, and, on the basis of this homology, a mechanism, known as Venus'flytrap model, has been proposed for the functioning of the LBD of mGluR1 (Scheme 1) [5]. Briefly, the LBD of mGluR1 was predicted to have an ellipsoid shape, with two lobes separated by a hinge region. The two lobes undergo an interdomain movement by oscillating between an open and a closed conformation, being the latter the active one. Agonist binding is thought to stabilize the closed, active form, while competitive antagonists should stabilize the open, inactive conformation.

A crucial confirmation for this mechanism came from the recent crystallization of the LBD of mGluR1 [6, 7], made possible by the ability of expressing a soluble portion of the ATD of mGluR1 containing the LBD. These studies have confirmed the existence of the Venus'flytrap modules, in equilibrium between closed and open conformations. Interestingly, a homodimeric assembly was evidentiated. All the homodimers so far crystallized are constituted of two promoters, either in a closed or in an open conformation. Both open and closed conformations have been observed with L-glutamate (L-Glu, **1**, Chart 1) bound, whereas only an antagonist bound open form was detected [7]. Thus, the crystallized complexes clearly confirmed the hypothesis that the LBD functioning is mediated through a Venus'flytrap mechanism operated by the equilibrium between an open and a closed conformation. Furthermore, the crystallization of agonist- and antagonist-bound LBDs supported the notion that agonists act by stabilizing the closed conformation while antagonists should favour the open, inactive form. Although the benefits associated with the availability of X-ray structures of the LBD complexed with ligands are evident in terms of novel opportunities for drug design, the crystal structures of the open and closed

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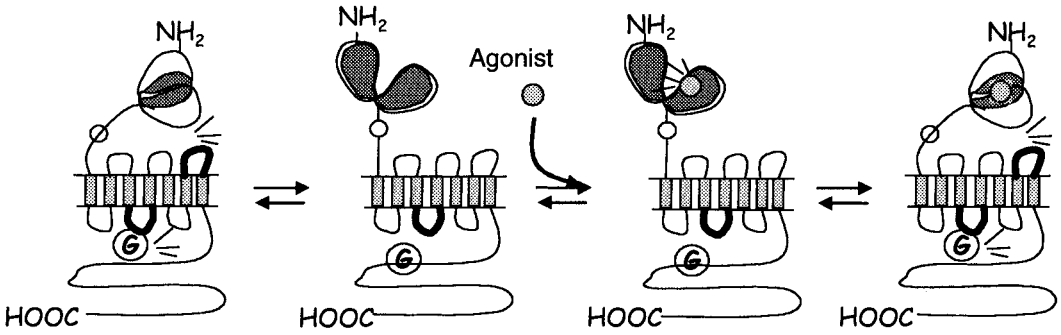


Scheme 1. Schematic representation of the hypothetical agonist-activated Venus'flytrap module of the LBD of mGluR1.

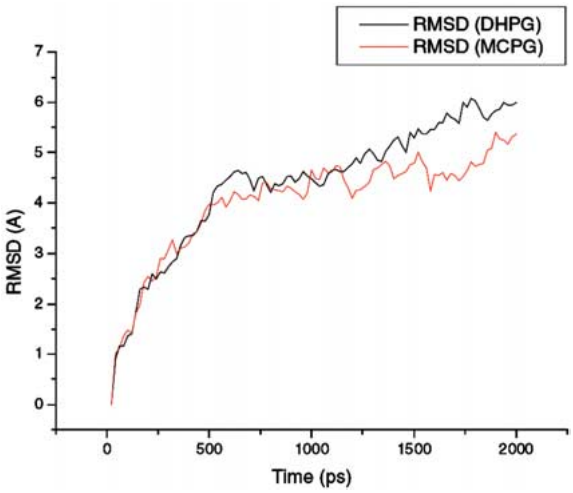


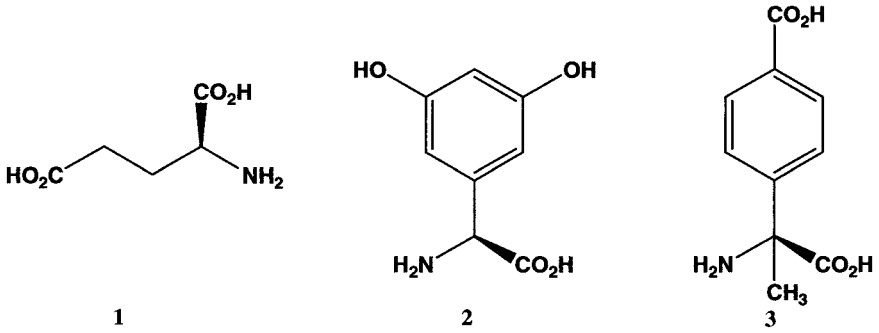
Figure 1. RMS deviation versus time of the Cα trace of the LBD of mGluR1 complexed with an agonist (black) and an antagonist (red).

conformations only represent 'frozen' snapshots of a dynamic and complex process. Indeed, the molecular pathway leading to the inactive open conformation to the active closed one, and the individual role of agonists and antagonists in influencing this process and stabilizing some conformations is still unclear. In this communication, we address this problem by performing molecular dynamics simulations of the conformational movements of the Venus'flytrap modules of the LBD of mGluR1 in response to agonist or antagonist binding. Structurally similar ligands were used, dihydroxyphenylglycine (DHPG, **2**) as mGluR1 agonist and α-methyl-4-carboxyphenylglycine (MCPG, **3**) as a competitive mGluR1 antagonist.

It should be recalled that similar studies [8, 9] have been carried out on the S1-S2 domain of ionotropic glutamate receptors (iGluRs) which are also endowed with a Venus'flytrap architecture.

Methods

The X-ray structure of the agonist-bound form of the LBD of mGluR1 was retrieved from the PDB database (pdb code: 1ewk). Only the promoter in the open conformation was used in the simulations. Agonist (DHPG, **2**) and antagonist (MCPG, **3**) were inserted into the binding pocket by superimposing them to the crystallographically determined coordinates of glutamate and MCPG in the antagonist-bound form of LBD (pdb code: 1ss.pdb), respectively. All the crystallographic water molecules were retained and a shell of 5 Å of TIP3 water was added to both the complexes. The thus obtained systems were energy minimized by using the Charmm-22 force field. The energy minimization was carried out for 1000 steps using the minimization algorithm implemented in NAMD v.2.3b1 [10]. Then, 1 ns simulations were carried out while keeping fix the carbon alpha atoms of the complexes in order to assure a complete equilibration of the side chains, ligands and of the shell of water. Finally, the two complexes were separately simulated for 2ns without any constrains. In each simulation, the system was gradually heated from 0°K to 300°K using 15°K of increment each 500 fs. The dielectric constant was set to 1 and the integration step was 1 fs. Molecular dynamics simulations were carried out with the Charmm-22 force field as implemented in the NAMD v.2.3b1 program on a Beowulf cluster of eight PIII processors at the Perugia Computation Center. NAMD was developed by the Theoretical Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign.



1 2 3

Chart 1.

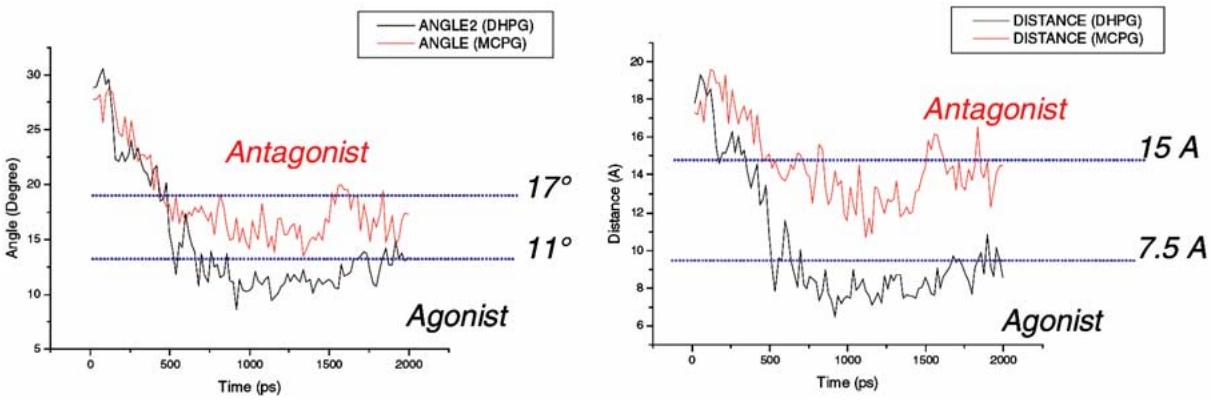


Figure 2. Plots of the variation of the inter-lobe angle (left) and distance (right) versus time for the agonist- and antagonist-bound complexes.

Results and discussion

Interdomain movements

Figure 1 shows, for both complexes, the root mean square deviation (RMSD) of Cα atoms (excluding the ligand and water molecules) from the starting structures over the 2 ns simulations. It can be appreciated the initial drift of both the agonist- and antagonist-bound complexes from the initial structures; then, both complexes plateau at ca. 4.5 and 5 Å, respectively, thus indicating a reasonable structure equilibration.

It should be noted that the drift was expectable, since the starting structure was considerably more opened than the experimentally determined agonist- and antagonist-bound ones. In any case, the RMSD from the starting structure does not give direct information on the direction and the magnitude of the interdomain movements. Thus, we decided to define two geometric features more suited to follow the time-dependent conformational changes. At this aim, we have selected two residues, positioned on the surfaces of the first (Ala59) and the second lobe (Asn264), respectively, and a residue located in the hinge region (Pro476). These three residues define an angle which can be monitored over the simulation, yielding information on the degree of conformational closure of the system. Analogously, the distance between the two residues on the lobes is another descriptor for the degree of closure.

In Figure 2, the variation of the angle and of the distance above definded are shown for both the complexes.

It can be appreciated that DHPG induced a degree of closure significantly more pronunced than that caused by the antagonist MCPG.

It should be noted, however, that in the crystal structure of the LBD complexed with MCPG, the degree of opening induced by the antagonist is wider than that obtained in MD simulation [7]. A reason for this discrepancy can be the attributed to the lack of the dimeric interface in our present MD simulation. Indeed, the notion that simulating only the monomeric promoter may not be sufficient to reproduce correctly the behavior of the LBD is confirmed by the analysis reported in Figure 3.

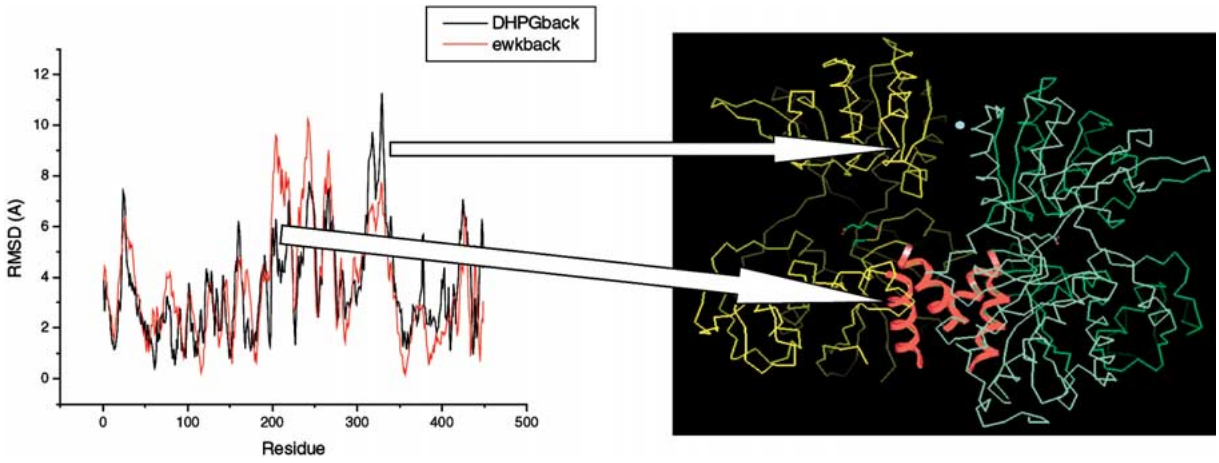


Figure 3. RMSD between the simulated structure complexed with DHPG (black) and the crystal structure of the closed form of the glutamate-bound LBD of mGluR1. Regions of larger deviations correspond to the dimeric interface (right).

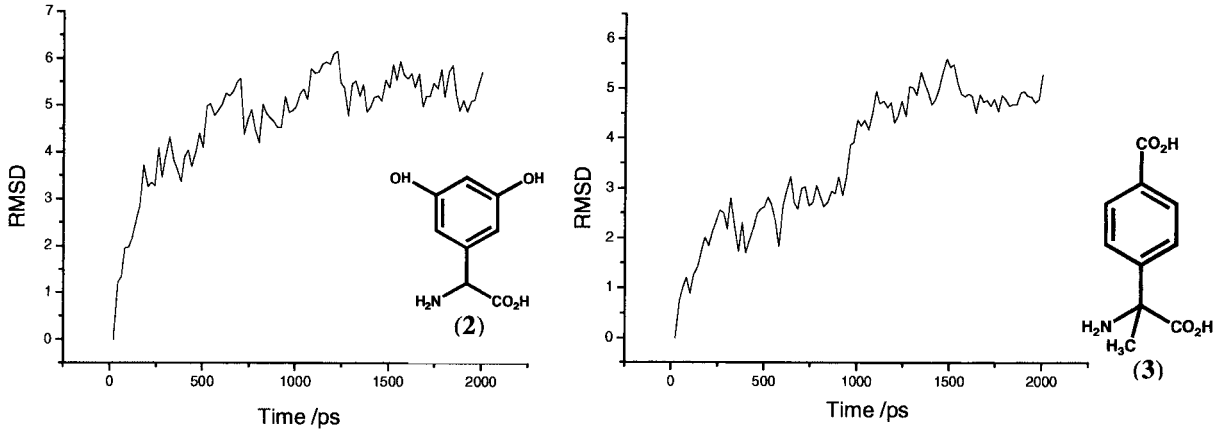


Figure 4. RMS deviation versus time for the agonist (left) and antagonist (right).

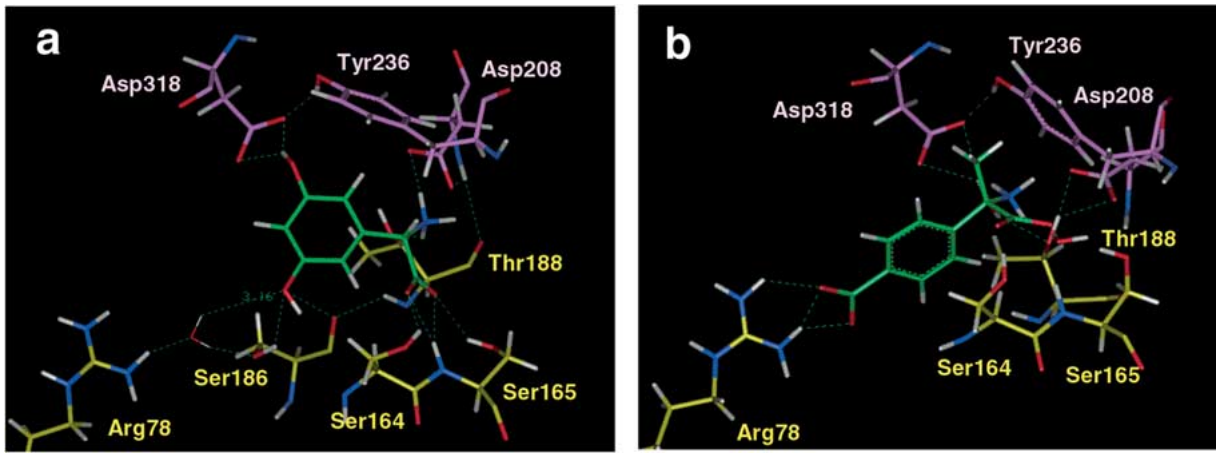


Figure 5a,b. Snapshots of the interaction pattern for agonist (left) and antagonist (right) at the end of the simulation

If the RMSD between the starting structure (open form complexed with glutamate) and the experimentally determined closed form complexed with glutamate (red) or the simulated complex (black) is plotted for each residue, an excellent agreement between simulated and experimental complexes is seen, with the exception of two short sequences. When projected into the 3D structure of the dimeric LBD, these sequences are found exactly at the dimeric interface, thus indicating that the use of a monomeric promoter is not sufficient to reproduce the spatial disposition of the dimeric interface.

Ligand analysis

In Figure 4, the RMSD of DHPG (**2**) or MCPG (**3**) from the starting position is plotted against the time. A significant ligand relocation is observed during the simulations for both the agonist and the antagonists. Examination of the trajectory of the ligands led to the conclusion that, although the extent of the relocation is the same, the behavior of the agonist and the antagonist is different. The aromatic ring of DHPG (**2**) tends to rotate of about 90˚ along the simulation in such a way to simultaneously interact with both lobes, thus providing the molecular linkage between the domains. In particular, one of the hydroxy group of **2** interact with Arg78 through a water molecule while the other tends to form hydrogen bonding with Asp308, localized in the second lobe.

Other key interactions involve the cation-π interaction between the ammonium group of the glycinergic moiety and Tyr236, the productive edge to face aromatic aromatic interaction between DHPG and Tyr236, and the ionic interaction between the proximal nitrogen and Asp208 (Figure 5a-b). MCPG (**3**) also underwent a drift from the initial structure, in such a way to wedge the two lobes. The distal carboxylate seems to displace the structural water and to salt bridge Arg78. The α-methyl group has the effect to mask the interaction between the positively charged nitrogen and the aromatic ring of Tyr236. Another evident effect is the loss of the hydrogen bond between the glycinergic nitrogen and the side chain of Asp208

In summary, the MD simulations carried out on the LBD of mGluR1 in response to agonist or antagonist binding were able to reproduce the interdomain movements which regulate the oscillation between the experimentally determined open and closed conformations. When the behavior of ligands is analyzed, the most interesting feature that emerged from the simulations is the mutual accommodation of ligands on receptor's conformational movements. In particular, even though in the starting position both the agonist and the antagonist were bound to the first lobe only, during the simulations DHPG (**2**) relocated in such a way to link together the two lobes and to stabilize the closed, active form. In contrast, the antagonist MCPG (**3**), also owing to the longer distance of pharmacoporic groups, prevented a complete closure of the two domains, mainly by hindering the key interaction between the positively charged nitrogen of the glycinergic moiety and Asp208 and Tyr236. The observed large interdomain movements and the concomitant ligand relocation may be indicative of an adaptative process required for receptor activation.

Our results put forward long range MD simulations as a suitable tool to analyze the behavior of Venus'flytrap receptor in response to agonist or antagonist binding. The present results, indeed, confirms analogous studies carried out on S1-S2 domain of iGluRs, where a different degree of conformational change of the Venus'flytrap were observed in response to functionally diverse ligands [8, 9]. Nevertheless, some issues still need further clarifications; a first concern is the use of the monomeric promoter instead of the full dimer. It is apparent that the dimeric interface plays a role, among others, in regulating the magnitude of opening of the two promoters. Our results indicated a clear deviation of the RMSD of the simulated structure from the experimentally determined one, extracted from the dimer. The second issue is the length of simulation. While during the 2 ns time clear interdomain movements are observed, it cannot be ruled out that other transitions may occur over longer time. These points are the object of an ongoing research.

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