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Homology Model of the Closed, Functionally Active, Form of the Amino Terminal Domain of mGluR1

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Abstract—The amino terminal domain (ATD) of metabotropic glutamate receptors (mGluRs) contains the neurotransmitter binding site and is related in sequence to leucine/isoleucine/valine binding proteins (LIVBP). It has been proposed that the ATD of mGluRs shares with periplasmic binding proteins a common mechanism of ligand binding and processing which involves the equilibrium between closed and open forms. The availability of the X-ray structure of LIVBP in its open, unliganded form, has allowed the construction of homology models of the ATD of mGluR1 which have been instrumental in clarifying the mode of binding of agonists and antagonists. We propose in this paper the use of the X-ray structure of AmiC, the controller of transcription antitermination in the amidase operon of *Pseudomonas aeruginosa* as suitable template for the construction of the closed form of the ATD of mGluR1. The resulting model of the closed form of the ATD of mGluR1 indicates that several interdomain hydrogen bonds and salt bridges may be formed upon domain contraction and that the ligand directly participates to this interdomain network. © 2001 Published by Elsevier Science Ltd.

Introduction

Metabotropic glutamate receptors (mGluRs) belong together with $GABA_B$ receptors, the Ca^{2+}-sensing receptor, and pheromone receptors, to family C of the superfamily of G-protein coupled receptors. At least eight mGluR subtypes (mGluR1–mGluR8) have been so far cloned and classified into three groups according to sequence homology and coupling to effector systems (Table 1).[1]

Molecular cloning and sequence analysis demonstrated that mGluRs share with the other members of the family C an array of peculiar structural features, among which the intriguing presence of an unusually extended (more than 500 amino acids) amino terminal domain (ATD). Multiple sequence alignment techniques have then disclosed limited but statistically significant homology between the first 400 amino acids of the amino terminal domain of mGluR1 and leucine/isoleucine/valine binding protein (LIVBP),[2] a member of the family of bacterial periplasmic binding proteins (PBPs). The finding that the ATD of mGlu receptors contains the neurotransmitter binding site and is responsible for agonist and antagonist selectivity between mGlu subtypes[3] has then fostered an intense research activity aimed at understanding the structural and mechanistic significance of the sequence homology with PBPs.

PBPs constitute a class of monomeric soluble proteins that specifically bind and shuttle in the periplasmic space of gram negative bacteria nutrients such as saccharides, vitamins, oxyanions and cations that passively diffuse through non specific pores embedded in the outer membrane.[4] The captured substrate is then delivered to a transport system in the inner bacterial membrane. Despite the wide diversity in size, amino acidic sequence and substrate specificity, all the members of the PBP family of proteins so far crystallized are thought to derive from a common ancestor and appear to fold into two main families, named type I and type II, according to the topological arrangement of β-sheets in their core structure.[5] PBPs are constituted of two globular domains (lobes) connected by a hinge region formed by two or three filaments.[4,6] The two lobes are separated by a deep cleft in which the substrate is accommodated. PBPs exist in an equilibrium between an open form, where the two lobes are separated each other and a closed form, where the two lobes collapse each on the top of the other upon a movement of the hinge region. The substrate binds the open form causing a shift of the equilibrium towards the functionally active closed form (Fig. 1).[4]

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Table 1. Classification of metabotropic glutamate receptors[a,b]

Group	Subtype	Effector system
Group I	mGluR1 mGluR5	+ PLC[a]
Group II	mGluR2 mGluR3	– AC
Group III	mGluR4 mGluR6 mGluR7 mGluR8	– AC

[a]PLC, phospholipase C; AC, adenylyl cylase.
[b]Spliced variants have been characterized for all the subytpes with the exception of mGluR2, mGluR3 and mGluR6.

X-ray crystallography has demonstrated that this peculiar mechanism of ligand binding and processing, referred to as the Venus-Flytrap mechanism,[6] is not limited to the class of PBPs, but is also employed by a number of other, functionally diverse, systems, such as enzymes (for example, glutamate-dehydrogenase, GluDH),[7] control factors (for example, the controller of the amidase operon, AmiC),[8] receptors (for example, the atrial natriuretic peptide receptor, NAPR).[9] The finding that mGluRs share sequence similarity with LIVBP, belonging to type I PBPs, has been interpreted as a clue that a mechanism of ligand binding similar to that of PBPs can be also employed by members of the family C of G-protein coupled receptors. Interestingly, the ligand binding domain of ionotropic glutamate receptors is distantly related to type II PBPs, and the occurrence of a folding similar to that of type II PBPs has been experimentally demonstrated.[10]

The similarity of the ATD of mGluR1 with LIVBP, whose 3D-structure of the open form is known,[11] has been instrumental by O'Hara et al. to construct a conceptual model of the mGluR1′ ATD that allowed to successfully predict amino acids involved in L-Glu binding.[12] Subsequently, we have reported homology models of the open form of the ATD of mGluR1 and mGluR5. In these studies, an explanation for the binding mode of competitive mGluR1 antagonists involving a stabilization of the open form was also given.[13] More recently, working models of the ATD of mGluR4a,[14] mGluR2,[15] and $GABA_B$[16] have been reported.

All these constructs are based on the open form of LIVBP, and are thought to represent models of the inactive form of the ATD of these family C G-protein coupled receptors. The relevance of the availability of a 3D model for the closed form of the glutamate-bound ATD of mGluR1 is associated to the notion that the closed form is the functionally active one. The lack of published data on the structure of the closed form of LIVBP has so far hampered the construction of the analogous closed form for mGluR1 ATD.

In this paper, we report the construction of a homology model of the closed form of the ATD of mGluR1 based on the 3D structure of the Controller of the Amidase Operon (AmiC). AmiC belongs to a family of proteins deputed to the regulation of amide catabolism in *Pseudomonas aeruginosa*.[9] Interestingly, 3D structures of several PBPs in their closed forms are available, for example those of arabinose-binding protein or galactose-binding protein. These proteins, however belong to type II PBP and have a folding motifs significantly different from that of LIVBP. In contrast, the X-ray structure of AmiC showed a folding pattern very similar to that of LIVBP and other type I PBPs. Accordingly, despite the very low sequnce homology, AmiC appears to be the most suitable template for the construction of the closed form of the ATD of mGluR1.

Methods

The closed form of the ATD of mGluR1 was generated by using the homology module as implemented in InsightII software package.[17] The multiple alignment between mGluRs and members of the type I PBP family was generated by using CLUSTALW.[18] The alignment was manually optimized in order to avoid gap insertions in regions with conserved secondary structure as previously described.[13] The only difference is that the first helix of mGluR1 is now shifted in order to match the respective one of PBPs. In this way, although a large loop insertion is generated, Arg78 replaces Arg106 in the binding site according to recently available experimental data.[19] Secondary structure prediction was carried out by using the GOR II[20] method. Figure 2 shows the optimized multiple alignment in the ligand binding domain.

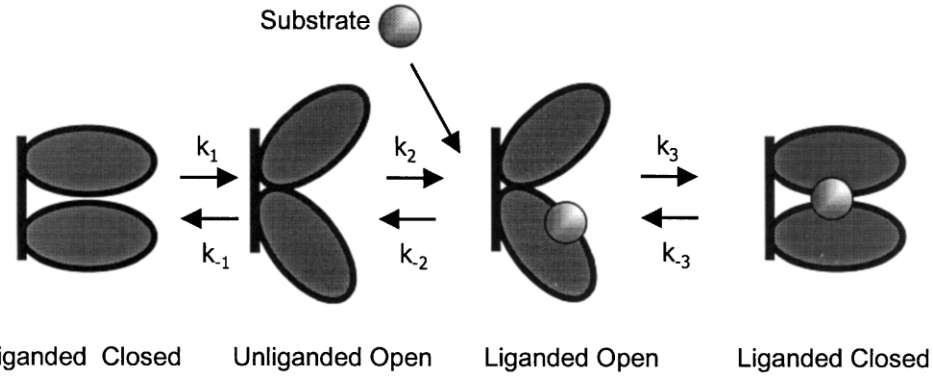

Figure 1. Mechanism of action of periplasmic binding proteins (PBPs). PBPs are in equilibium between open and closed forms.

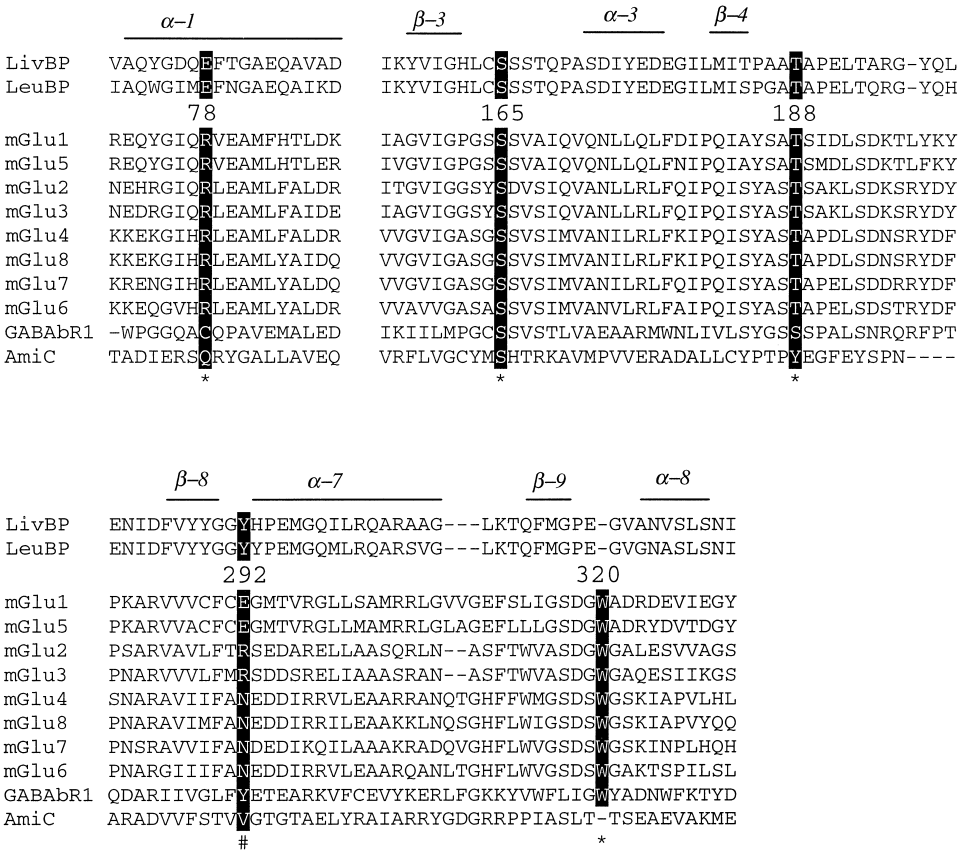

Figure 2. Multiple alignment of mGluRs, $GABA_BRs$, LIVBP, LeuBP and AmiC. Amino acid sequences in the region of putative ligand binding sites are reported. Amino acids relevant for the agonist binding are marked with *.

The atomic coordinates of AmiC were assigned to the respective aligned residues of structural conserved regions (SCRs). Coordinate assignment of variable regions (VRs) were performed by scanning a database of 77 representative proteins from the PBP folding family using the search-loop algorithm implemented in homology module. Since large insertion regions present in the alignment were located far away from the binding pocket, they were not modeled (residues 56–69, 129–155, 350–368, 380–402, 433–445). The end residues of the final model were capped by acetamide at N-terminus and *N*-methylamide at the C-terminus. Ionizable residues localized on the solvent accesible surface were considered charged. Ionizable residues localized in the cleft were considered charged as in the open conformation of the receptor they are exposed to the solvent. Then, the resulting model was submitted to the minimization protocol. This was constituted by a cycle of 200 steps of Steepest Descent minimization with a fixed constraint on the coordinates of backbone atoms, followed by a conjugate gradient minimization with a high harmonic constraint on the backbone atoms and a fixed constraint on the end residues until a RMS of 0.05 was reached. The CHARMM force-field[21] was used for all minimization procedures. The 3D model thus obtained was checked in terms of geometry using PROCHECK[22] and Verify3D.[23] Docking experiments were performed with the Docking module of Insight II. Manual docking was used, and the ligand was kept rigid during the docking procedure. The best complex in terms of binding energy was stored and a sphere of 8 Å was defined around the ligand. Defining a harmonic constant on the atoms outside the sphere region, the atoms inside were minimized until a convergence gradient of 0.05 was reached. The Steepest Descent and the conjugate gradient methods, as implemented in the CHARMM force-field, were used for the minimization protocol with a distance-dependent (1/R) dielectric function. All computations were carried out on a SGI O2 R12000 workstation using InsightII molecular modeling software package distributed by Molecular Simulations Inc.

Results

The inspection of the obtained model of the closed form of ATD of mGluR1 (Fig. 3) reveals interesting structural features. The two lobes are in close contact and the contracted structure appears to be stabilized by a number of inter-lobe interactions as new hydrogen bonds and salt bridges are formed. Since the present model has been energy minimized without explicit solvent, the lobe contraction could be partially seen as a consequence of the minimization conditions. However, the RMS deviation of the backbone atoms of the closed form model thus obtained from those of the X-ray structure of AmiC less than 1, thus demonstrating that the significant contraction between the two lobes is not a minimization artifact.

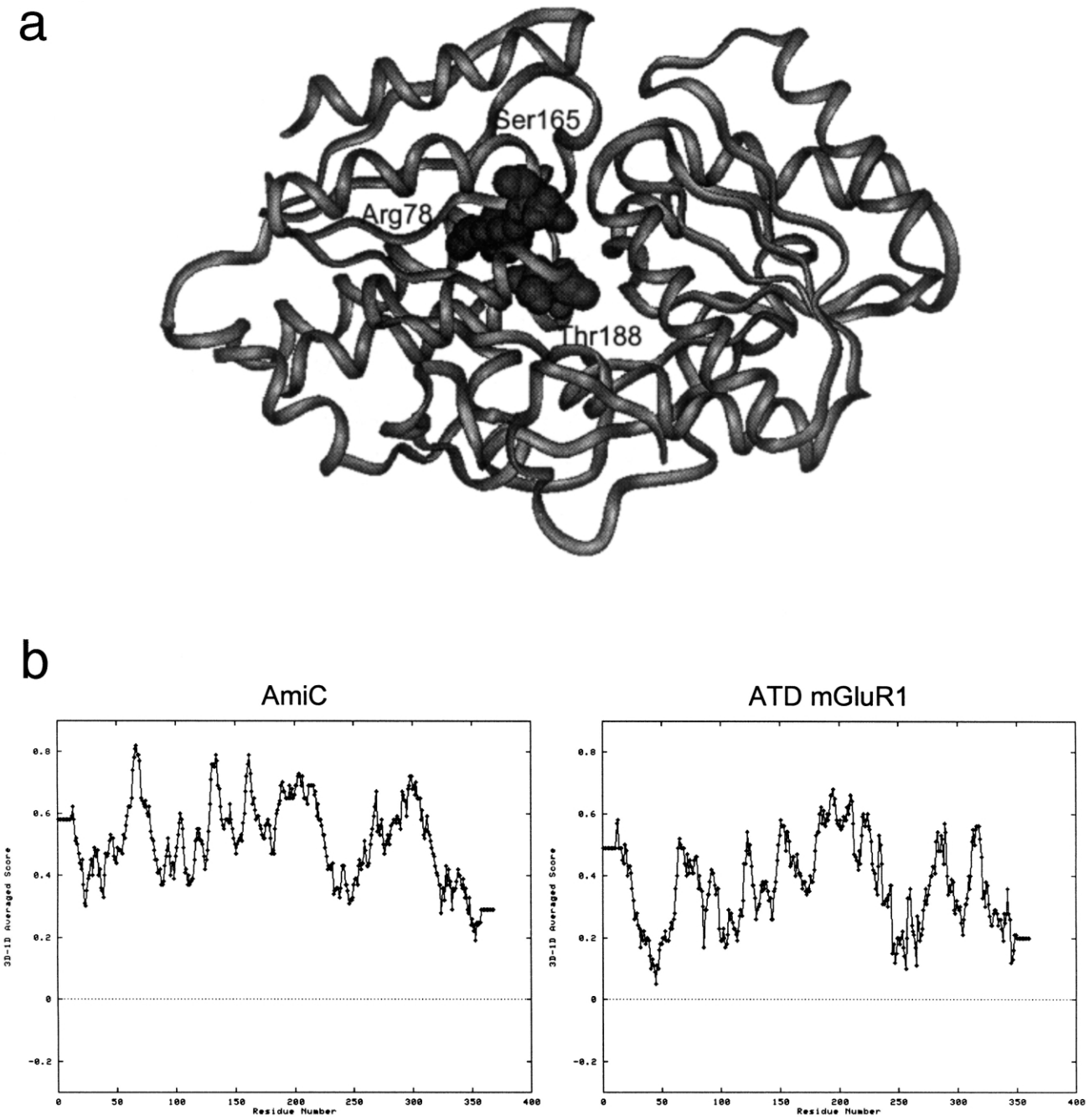

Figure 3. (Top) α-Carbon trace of the model of the closed form of ATD of mgluR1. Ser165, Thr188 and Arg78 are shown as CPK representation. (Bottom) Verify 3D plots of AmiC (experimental structure, left) and ATD of mGluR1 (theoretical model, right). These plots represent a statistical test of the accuracy of the 3D models by comparing the model to its own amino acid sequence using a 3D profile computed from the atomic coordinates of the structure. 3D profiles of correct protein structures match their own sequences with high scores. 3D profiles for protein models known to be wrong score poorly (below zero).

Interestingly, the putative glutamate binding pocket results essentially unaffected by domain contraction. Thus, when docked into the closed form model (Fig. 4), L-Glutamic acid keeps, but it is not limited to, the same interactions noticed in the open form model. Indeed, a noteworthy feature is that the α-amino group of L-Glu, that was exposed towards the cleft in the open form model, is now bound to the side chain of Glu292, positioned on the upper lobe. The amino group of L-Glu thus constitutes the molecular linking between the two lobes. Finally, the domain contraction brought some amino acidic residues localized in the second lobe close to the binding pocket. Among these Trp320, which has been implicated in the binding of agonists. (Fig. 4).[24]

These findings clearly suggests that the binding of the ligands promote and help stabilizing the domain closure. It is also worth noting that inter-domain salt bridge formation has been reported to be one key effect in the stabilization of the closed form in anionic periplasmic binding proteins.[25]

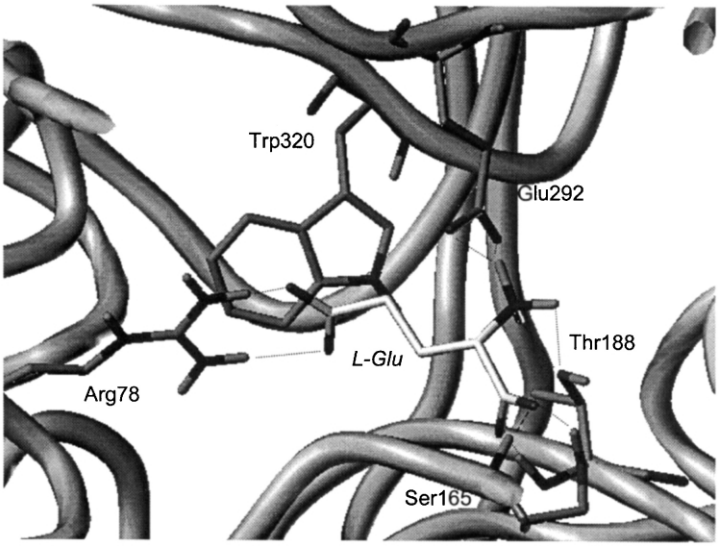

Figure 4. Docking of L-Glu into the proposed active site. The amino acidic moiety interacts with Ser165, Thr188 and Glu292 (upper lobe). The distal carboxylate interacts withArg78. The possible role of Trp320 is also evidenced.

Discussion

Once it is assumed that the ATD of mGluRs is structurally homologous to type I PBPs, a similar mechanism of ligand binding and signal processing may be inferred. The available X-ray structure of the open form of LIVBP has been employed for the construction of the open form of the ATD of mGluR1. The structure of the closed form of LIVBP, that can be considered as the most suitable template for the construction of the closed form of the ATD of mGluR1, is so far unavailable. An alternative template is constituted by the closed form of AmiC, a protein that belongs to the type I PBP folding family, and has a secondary structure pattern very similar to that of LIVBP.

The comparison of the homology models of the open (constructed by using LIVBP as a template) and the closed (constructed by using AmiC as a template) form of the ATD of mGluR1 indicates that a large conformational change is likely to occur upon the ligand binding. It should be mentioned, however, that while this large conformational change is coherent with the Venus flytrap model, it is not always seen in type I or type II PBP-like proteins. For example, the crystallographic structure of a closed form of the Lactose operon repressor (Lac, type I folding) indicates that such a large conformational change does not occur.[26] Moreover, small-angle X ray scattering data point out the absence of a similar large conformational movement in the case of a ionotropic glutamate receptors (structurally homologous to type II proteins), thus suggesting that subtle conformational modifications are responsible for the signal processing.[27] Although these observations should properly be taken into account when the reliability of our model is evaluated, other points are to be considered in favor of the present model. First of all, in the case of mGluR1, the signal transported by the ligand must be transmitted to the cytoplasm through the seven transmembrane domains. It is conceivable that a large conformational movement is needed in order to achieve this long-range transmission. Furthermore, in our model of the closed form, based on the structural homology with AmiC, several interdomain hydrogen bonds and ionic interactions are formed, through highly conserved residues. Importantly, the ligand itself participates to this network by simultaneously connecting both lobes. Finally, the closed form model is in agreement with all the directed-site mutagenesis experiments so far carried out (e.g., Ser 165, Thr188 and Arg78 are simultaneously involved in glutamate binding) and, moreover, allowed to identify in the region of the binding site the presence of a residue, namely Trp320, which is directly involved in the binding of agonists[24] and that was not identified as important in the open form model.

In conclusion, we have reported the construction of the closed form of the ATD of mGluR1 on the basis of the structural homology with AmiC. The proposed model is in agreement with available experimental data and points out that the same template may be used for the construction of other models of receptors belonging to family C of the GPCR superfamily.

Note Added in Proof

After this paper was accepted, a paper by Kunishima et al., *Nature* **2000**, *407*, 971 reported the crystallographic structure of the ligand binding domain of a mGluR1 in the closed form. Our homology model is in excellent agreement with the crystallographically determined structure.

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