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Molecular Dynamics Simulations of the Conformational Changes of the Glutamate Receptor Ligand-Binding Core in the Presence of Glutamate and Kainate

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ABSTRACT **Excitatory synaptic transmission is mediated by ionotropic glutamate receptors (iGluRs) through the induced transient opening of transmembrane ion channels. The three-dimensional structure of the extracellular ligand-binding core of iGluRs shares the overall features of bacterial periplasmic binding proteins (PBPs). In both families of proteins, the ligand-binding site is arranged in two domains separated by a cleft and connected by two peptide stretches. PBPs undergo a typical hinge motion of the two domains associated with ligand binding that leads to a conformational change from an open to a closed form. The common architecture suggests a similar closing mechanism in the ligand-binding core of iGluRs induced by the binding of specific agonists. Starting from the experimentally determined kainate-bound closed form of the S1S2 GluR2 construct, we have studied by means of molecular dynamics simulations the opening motion of the ligand-binding core in the presence and in the absence of both glutamate and kainate. Our results suggest that the opening/closing interdomain hinge motions are coupled to conformational changes in the insertion region of the transmembrane segments. These changes are triggered by the interaction of the agonists with the essential Glu 209 residue. A plausible mechanism for the coupling of agonist binding to channel gating is discussed.** **Proteins 2001;44:460–469.** **© 2001 Wiley-Liss, Inc.**

Key words: activated molecular dynamics; glutamate receptors; molecular modeling

INTRODUCTION

Glutamic acid (Glu) is the main excitatory neurotransmitter in the vertebrate central nervous system. Fast excitatory synaptic transmission is mediated by ionotropic glutamate receptors (iGluRs) by means of the ligand-induced opening of transmembrane ion channels.[1,2] Flux of monovalent and divalent cations through the postsynaptic membrane depolarizes the cell and propagates the electrical impulse.[3–6] iGluRs have been classified according to their sensitivity to several agonists: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainic acid (KA), and *N*-methyl-D-aspartic acid (NMDA).[7,8]

The ionotropic glutamate receptor has been described as a tetrameric complex.[9,10] Each subunit contributes to the cation-permeable channel with three transmembrane segments and a membrane-embedded re-entrant loop.[11,12] The extracellular part of the subunit is shaped by the ligand-binding core, which is formed by two segments, usually named S1 and S2, separated by two of the transmembrane portions and the re-entrant loop. An N-terminal domain (~400 amino acids), of unknown function, is also present in the extracellular part of the subunit.[13] The ligand-binding core of iGluRs shows sequence similarity with bacterial periplasmic binding proteins (PBPs).[14] PBPs are components of high-affinity transport systems for a variety of ligands, including amino acids.[15] Metabotropic glutamate receptors (mGluRs) present about 20% amino acid sequence identity with PBPs. Despite the fact that this homology is on the border of questionable biological significance, a multiple sequence alignment of mGluRs and several members of the PBP family revealed that the identity is indeed biologically meaningful.[16] As alignments of sequences of iGluRs with mGluRs have been reported,[17,18] the similarity of PBPs with mGluRs can be extended to iGluRs. The three-dimensional structures of a number of PBPs have been determined, and all of them contain two domains connected by two or three peptide stretches and separated by a cleft in which the binding site is located.[19] In the ligand-bound form, the domains are placed close to each other and the ligand is completely buried.[20] The conformational changes from the open to the closed form must involve a large-scale, rigid-body movement of one domain relative to the other.[21,22]

The structural analogy of the ligand-binding core of iGluRs with PBPs has been confirmed not long ago.

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Constructs in which the S1 and S2 regions of iGluRs are coupled by short linkers (5–13 residues) have pharmacological properties that are similar to those of the wildtype membrane-bound receptors.[13] Some of these S1S2 constructs form well-ordered crystals,[23] and the three-dimensional structure of one of them (GluR2) has been solved by X-ray crystallographic techniques.[24] The overall features of the ligand-binding core strikingly resemble the structure of the glutamine-binding protein (QBP),[25] a member of the PBP family. The kainate binding site is located inside the cleft and includes several residues from both domains.[24] The 2-carboxyl group of kainate forms essential interactions with the guanidinium group of Arg 108 and the main-chain NH group of Thr 103, both of them located on domain 1. The amino group of kainate is bound by the carbonyl oxygen of Pro 101 and the side-chain hydroxyl of Thr 103 (also on domain 1), and establishes a strong interaction with the carboxylate of the essential Glu 209 located on domain 2. The carboxymethyl group forms hydrogen bonds with NH groups of Ser 158 and Thr 159 and with the hydroxyl group of Thr 159 placed on the N-terminus of the F helix of domain 2. These interactions stabilize the closed form in the presence of the ligand.

The three-dimensional resolution of a kainate/GluR2 ligand-binding core complex is a step of great importance for the structure-based design of ligands of pharmacological interest in relation to several neurological disorders, such as schizophrenia or epilepsy.[26] However, the lack of information about the structure of the apoform of the iGluR ligand-binding core hampers the design of effective antagonists that might bind to an open form of the S1S2 core. The structural analogy and the few, but significant, sequence relationships between the ligand-binding core of iGluRs and PBPs support the idea that both proteins not only have a common architecture, but also share a similar closing mechanism, gated by the binding of specific ligands. The aim of this work is to take advantage of this analogy to simulate the opening of the ligand-binding core and to obtain an open form that could be used as a template for the development of novel iGluRs antagonists.

METHODS

Crystallographic Data

Three-dimensional structures of the ligand-binding core of GluR2[24] and glutamine-binding protein in both the ligand-bound form[25] and the ligand-free form[27] were obtained from the Protein Data Bank (PDB codes: 1GR2, 1GGG, and 1WDN, respectively). Residue numbers referred to in this work correspond to those in the S1S2 GluR2 construct (1GR2.pdb file). Two loops (residues 31–45 and 129–143) of the ligand-binding core of GluR2 were model-built since no electron density data was available for them in the crystallographic structure. Positive and negative values for the differences in dihedral angles between the Cα traces of open and closed forms represent relative clockwise and anticlockwise increments, respectively.

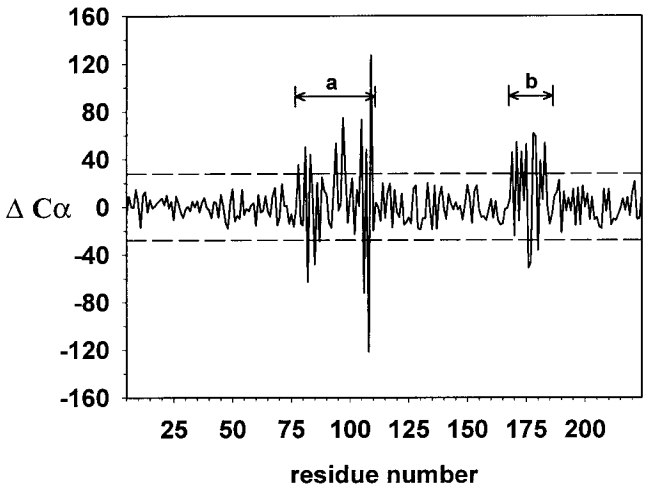

Fig. 1. Differences in Cα dihedral angles (ΔCα) along the peptide backbone between the open and closed forms of glutamine-binding protein (QBP). The two high-mobility segments (a and b) correspond to residues 75 to 110 and 172 to 193, respectively. The horizontal dashed lines represent the mean of the difference for all residues ± 2σ.

Energy Refinement

In order for the crystal structures to adapt to the AMBER force field,[28] the proteins were gradually energy refined using the SANDER module in AMBER[29] and a cutoff of 10.0 Å. First, only the model-built loops were allowed to move; then the ligand (if present) was included in the energy minimization; subsequently, the amino acid side chains were also included in the energy refinement, and finally the whole structure was energy-minimized although protein backbone atoms were restrained to their initial positions with a force constant of 2 Kcal mol^{-1} $Å^{-2}$. No explicit water molecules were included in the calculations but a distance-dependent dielectric constant ($\epsilon = 4r_{ij}$) was used to dampen electrostatic interactions.

Molecular Dynamics Simulations

The refined structures were used as input for the subsequent molecular dynamics (MD) simulations under the same dielectric conditions as above. In a 6-ps heating phase, the temperature was raised from 0 to 298°K, and velocities were reassigned at each new temperature according to a Maxwell–Boltzmann distribution. The dihedral angles of the Cα trace were restrained to those of the initial structure by means of a harmonic potential with a force constant of 300 kcal mol^{-1} rad^{-2}, except for the built-in loops. In addition, the relative position of the Cα atoms of the two residues preceding (−2 and −1) and the two residues following these loops (+1 and +2) was maintained by means of $Cα_{-1}$—$Cα_{+1}$ and $Cα_{-2}$—$Cα_{+2}$ distance restraints (50 kcal mol^{-1} $Å^{-2}$) and a $Cα_{-2}$—$Cα_{-1}$—$Cα_{+1}$—$Cα_{+2}$ dihedral angle restraint (300 kcal mol^{-1} rad^{-2}). The ligand (if present) was completely free to move. For the rest of the simulation at 298 K the dihedral restraints involving only the hinge regions were removed and a "partial template forcing" approach was

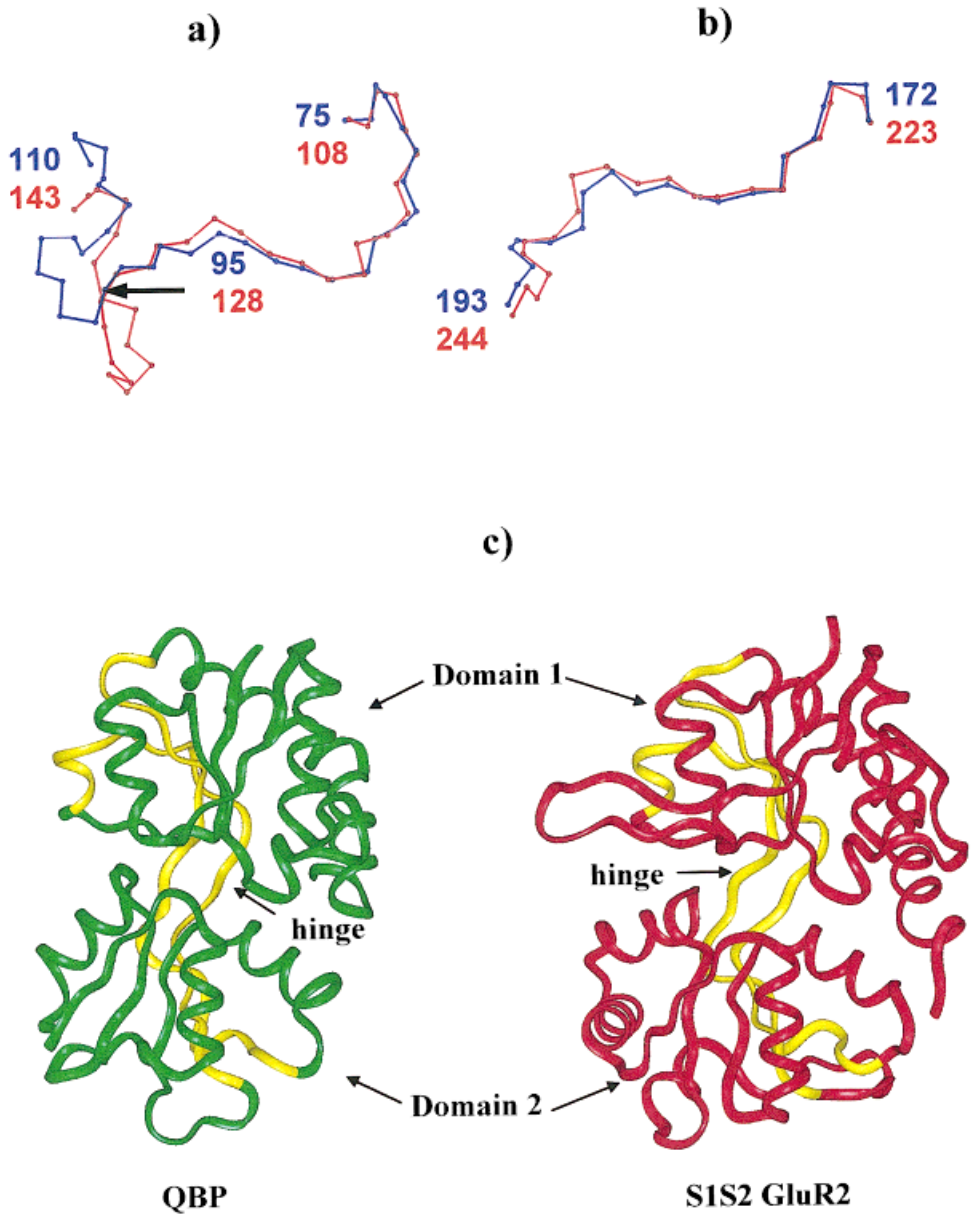

Fig. 2. **a:** Superimposition of the first hinge region (residues 75–110) of QBP (in blue) onto the corresponding stretch (residues 108–143) in the S1S2 GluR2 construct (in red). **b:** Superimposition of the second hinge region (residues 172–193) of QBP onto the corresponding stretch (residues 223–244) in the S1S2 GluR2 construct (color as above). **c:** Overall structural analogy between QBP and the S1S2 GluR2 construct (the hinge regions are shown in yellow).

used to study the motion of the domains. To this end the Cα atoms of the hinge regions in the "closed" structure (41 atoms in all) were restrained to the crystallographic positions of equivalent atoms in the "open" structure by means of a harmonic potential with a force constant that was progressively increased (0.25 kcal mol^{-1} $Å^{-2}$ per run) during 10 consecutive runs of 60 ps each. To check for possible protocol dependencies, two additional simulations of the kainate–GluR2 complex were carried out in which the time span was doubled or quadruplicated whereas the force constant was concomitantly halved or quartered, respectively. The SHAKE algorithm was applied to constrain all bonds to their equilibrium values and an integration time step of 2 fs was used throughout. The list of nonbonded pairs was updated every 25 steps and coordinates were saved every 2 ps.

RESULTS

Based on its similarity with PBPs, the apo form of the GluR2 ligand-binding core can be assumed to exist in an open conformation in which the separation between domains is larger than that observed in the closed form

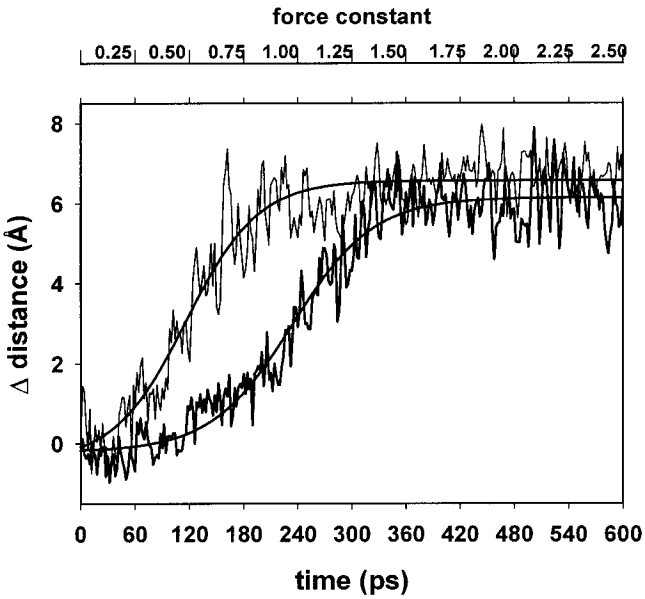

Fig. 3. Degree of closure of the GluR2 ligand-binding core domains expressed as the increment in the distance between Cα atoms of residues Ser 158 and Arg 108 over the simulation time. Thin and thick lines correspond to the MD simulations in the absence and in the presence of glutamate, respectively. The upper axis shows the value of the template force constant applied at different times during the simulations.

stabilized by kainate. This agonist-induced rearrangement or "closing" of the ligand-binding core could be transmitted to the transmembrane segments of the whole receptor, resulting in opening of the cation channel. This kind of closing motion is common in many enzymes and other protein molecules,[30] including PBPs,[22] which present two or more domains connected by a few strands of polypeptide chains that may be considered as hinges. Conformational changes are usually limited to the hinge region in so far as the domains behave virtually as rigid bodies. The final structure depends on the displacement of the domains with respect to each other, not only in terms of the degree of domain closure, but also in terms of the pivotal positioning of the domains.

Identification of the Hinge Region

The first issue in the analysis of a hinge motion in proteins is the identification of the hinge region, which can be achieved by comparison of the open and closed structures. In the case of the ligand-binding core of iGluRs, the lack of information about the open form precludes direct determination of the hinge. However, the structures of the open and closed forms of QBP have been solved.[25,27] In order to determine the residues undergoing the main conformational changes during the opening/closing transition of QBP, dihedral angles of the α-carbon (Cα) trace were measured in both the open and closed structures. Figure 1 plots the difference between these dihedrals versus the residue number. The more significant conformational changes are apparent in two segments [Fig. 1 (a, b)], corresponding to residues 75 to 110 and 172 to 193. These segments include two peptide stretches that connect the two domains forming a short antiparallel β sheet, while the remaining residues are organized as distorted β strands. Similar hinges have been described in other PBPs[20,21] and also in lactoferrin.[31,32]

Despite the fact that the structural homology between the S1S2 ligand-binding core of GluR2 and the closed form of QBP is higher than their sequence identity, some of the structural elements in the S1S2 construct are absent in QBP. Figure 2 shows a superimposition of the Cα trace of the two hinge strands previously identified in QBP with the analogous segments in the S1S2 GluR2 construct, corresponding to residues 108 to 143 and 223 to 244. The first segment [Fig. 2(a)] displays a high structural homology in the stretch comprised between residues 75 and 95 (108 to 128 in S1S2 GluR2). On the other hand, the conformation of the loop made up of residues 96 to 110 (129 to 143 in S1S2 GluR2) is different in both proteins. In the GluR2 construct, this stretch corresponds to the linker joining S1 and S2 that replaces the transmembrane region, and was model-built (see Methods) since no electron density was available for it in the X-ray diffraction data.[24] The second hinge segment in QBP [Fig. 2(b)] presents a high structural homology in the whole stretch. In the light of the comparisons between the structures of the open and closed forms of QBP, and between the latter and the closed form of the ligand-binding core of GluR2, we can assume that the hinge region in the S1S2 core is formed by two peptide segments (residues 108–128 and 223–244). The position of the hinge region in both proteins is shown in Figure 2(c).

Molecular Dynamics Simulations

When a detailed atomic picture of large-scale motions in proteins is inaccessible to experimental means, MD simulation methods offer an easily affordable alternative. Since protein domains involved in hinge motions are characterized by behaving essentially as rigid bodies,[30] the overall shape of the S1S2 GluR2 domains can be maintained during the MD simulation by restraining the dihedrals of their Cα trace to the values found in the closed form. Two loops (residues 31–45 and 128–143) were not constrained due to the absence of electron density in the crystallographic structure. As mentioned above, one of these loops (residues 128–143) corresponds to the site of insertion of the transmembrane segment that forms the cation channel (i.e., the region of the linker added in the S1S2 construct).

The hinge motion was induced by forcing just the Cα atoms of the hinge region residues to adopt the conformation of these stretches in the open structure of QBP (Fig. 1, Supplementary Material). This approach has been used in the simulation of large-scale motions in proteins and is usually known as template forcing, or activated molecular dynamics.[33] In this method, an extra term is added to the molecular potential energy function in order to bias the trajectory of a selected part of the moving structure so that it finally adopts the conformation of the template. The

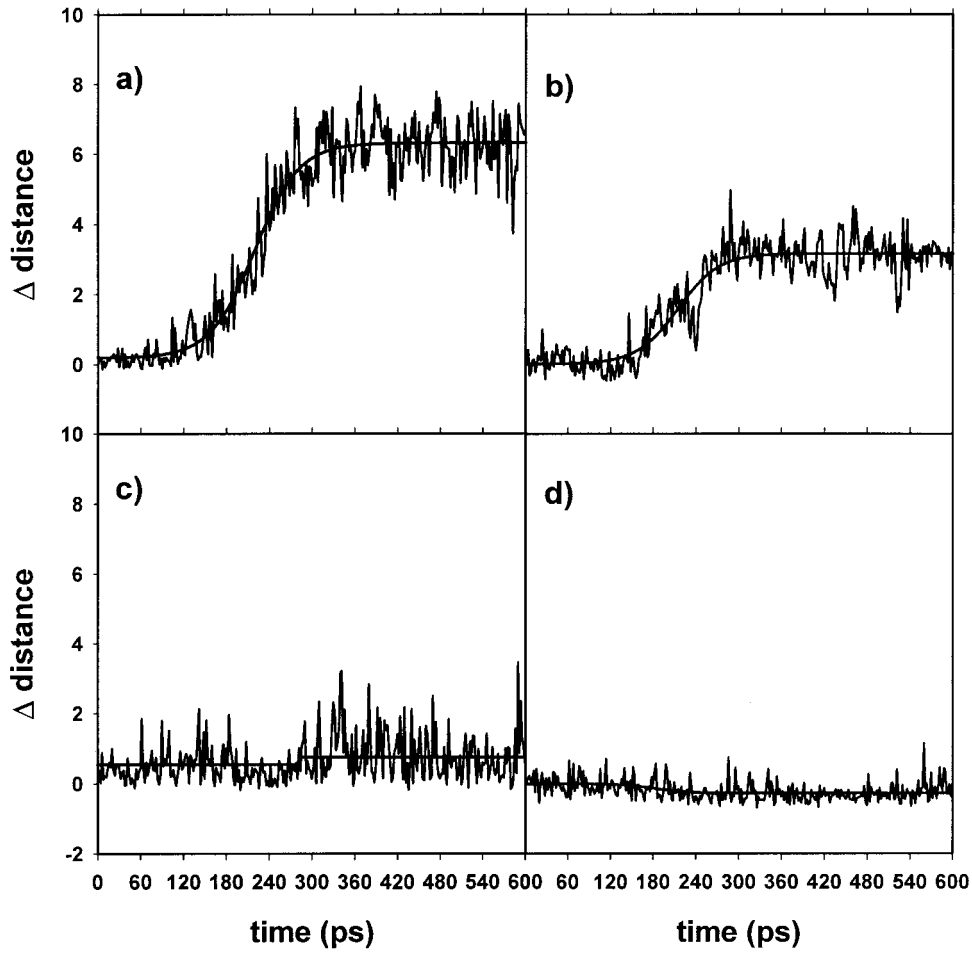

Fig. 4. Time evolution of the distances (Å) between the glutamate ligand and several key residues making up the binding site relative to the starting structure. Distances were measured between: (**a**) the carboxylate group of Glu 209 and the amino group of glutamate, (**b**) the NH group of Ser 158 and the side chain carboxyl group of glutamate, (**c**) the carbonyl oxygen of Pro 101 and the amino group of glutamate, and (**d**) the guanidinium group of Arg 108 and the α-carboxyl group of glutamate.

choice of the force constant can dramatically affect the results of the simulation: a very large force constant could arbitrarily superimpose the moving stretches to the template, artificially overcoming energy barriers and depriving the simulation of any physical sense; on the contrary, a small force constant may not supply sufficient extra energy to overcome local energy barriers. To circumvent this problem, a progressively increasing force constant was used during the simulation. Under these conditions, any difference in the trajectories in the presence or absence of ligand can be attributed to stabilization of the closed form by the ligand. Since the domain motions are activated by the extra energy supplied to the system, the time scale is obviously very different from the real situation. By using the kainate–GluR2 complex as a test system, we found that the different conditions tested (increments in force constant from 0 to 2.5 kcal · mol^{-1} in 0.6, 1.2, and 2.4 ns; see Methods) yielded comparable results in terms of both the shape of the curve and the force constant necessary for opening to take place (Fig. 2, Supplementary Material). Thus, domain separation depends on the value of the force constant applied independently of the time employed to reach that value. In view of these results, for the remaining molecular systems only the shortest simulation was carried out.

MD simulation in the absence of ligand

The degree of domain closure along the simulation time can be assessed by monitoring the increase in distance between the Cα atoms of residues Ser 158 and Arg 108, which are located at the domain interface (these residues were chosen because they are also involved in agonist binding). In the absence of ligand, the two domains of the S1S2 GluR2 construct rapidly adopted an open conformation, corresponding to a very low template force constant value (Fig. 3). In order to check the influence of the nonconstrained loops, MD simulations were performed in which the dihedrals of the Cα trace corresponding to each loop were restrained either separately or simultaneously. The first loop (residues 31–45) had no influence on the

degree of closure between domains. The linker loop (residues 129–143), however, was found to be essential for the opening mechanism. When the dihedrals defining the Cα trace of this loop were constrained to their initial values, opening of the domains could not be achieved during the simulation time (Fig. 3, Supplementary Material). Large conformational differences are apparent in the analogous loop in QBP (residues 95–110) when open and closed forms are compared (see Fig. 1).

The fact that only a very small force constant was necessary and the demonstration that opening takes place when the domains are considered as essentially rigid bodies strongly suggest that no conformational changes in the polypeptide chain are required for domain separation other than those in the hinges and the insertion region of the transmembrane segments. From a thermodynamic point of view, the small force constant at which opening takes place can be attributed to the absence of significant energy barriers between open and closed forms, which is in agreement with the spontaneous channel opening of iGluRs observed in the absence of agonists.[34]

MD simulation in the presence of glutamate

As expected, opening of the domains in the presence of glutamate takes longer in comparison with the apo form (Fig. 3). Obviously, the interactions between the ligand and the different residues involved in its binding to the protein give rise to local energy barriers that must be overcome before opening takes place. The relative position of the ligand with respect to some binding site residues during the simulation is shown in Figure 4. The evolution of the distance between the carboxylate of the essential Glu 209 and the amino group of the glutamate ligand [Fig. 4(a)] is closely similar to that representing domain separation (Fig. 3). Domain opening is also correlated with the distance between the hydroxyl group of Thr 159 and one of the oxygens of the glutamate's side chain carboxylate [Fig. 4(b)]. Both residues, Glu 209 and Thr 159, are located in domain 2, as well as Ser 158, whose main chain NH group initially forms a hydrogen bond with the other carboxylate oxygen of the glutamate side chain. This bond is also similarly disrupted upon domain separation (data not shown). On the contrary, ligand interactions with domain 1 residues making up the binding site, such as Thr 103 [Fig. 4(c)], Arg 108 [Fig. 4(d)], and Pro 101 (data not shown) are maintained during the whole simulation. This distinct behavior can be interpreted in the sense that interactions involving domain 2 are weaker than those involving domain 1, and may suggest that the primary interactions between the ligand and the open form are established with this latter domain.

Figure 5 shows the increase in distance between Cα atoms of Gly 132 (located in the transmembrane segment insertion region) and Cys 278 (at the N terminal region, where the polypeptide chain continues into the last transmembrane segment of the GluR2) during the simulation time. The motion of this loop is almost simultaneous with the opening of the ligand-binding core, suggesting that

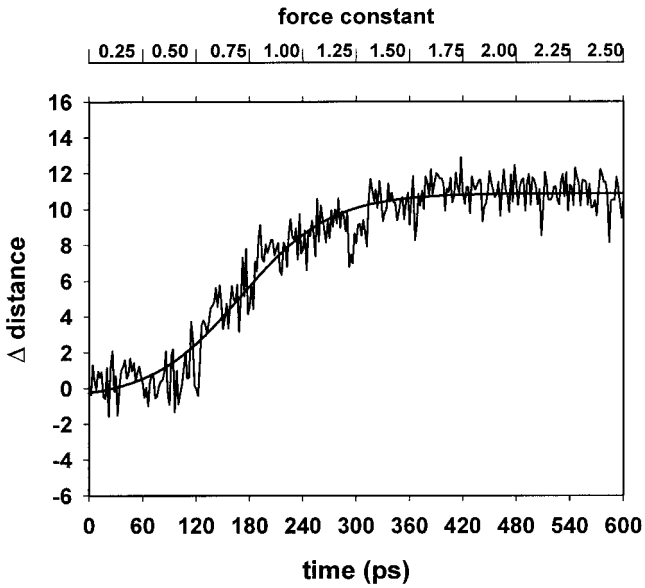

Fig. 5. Motion of the transmembrane segment insertion loop during the simulation in the presence of glutamate. The motion is expressed as the increment in the distance (Å) between Cα atoms of Gly 132 and Cys 278. The upper axis shows the value of the template force constant applied at different times during the simulation.

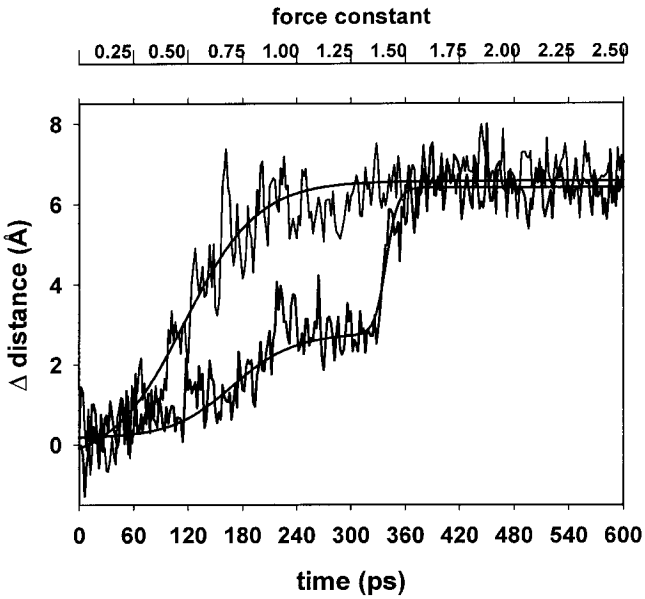

Fig. 6. Degree of closure of the ligand-binding core domains expressed as the increment in the distance between Cα atoms corresponding to residues Ser 158 and Arg 108 over the simulation time. Thin and thick lines correspond to the MD simulations in the absence and in the presence of kainate, respectively. The upper axis shows the value of the template force constant applied at different times during the simulations.

domain opening is coupled to conformational changes in the insertion regions of the transmembrane segments. As all the motions during the opening transition are concerted, it is not possible to correlate a particular ligand-residue interaction with a predominant role in channel gating.

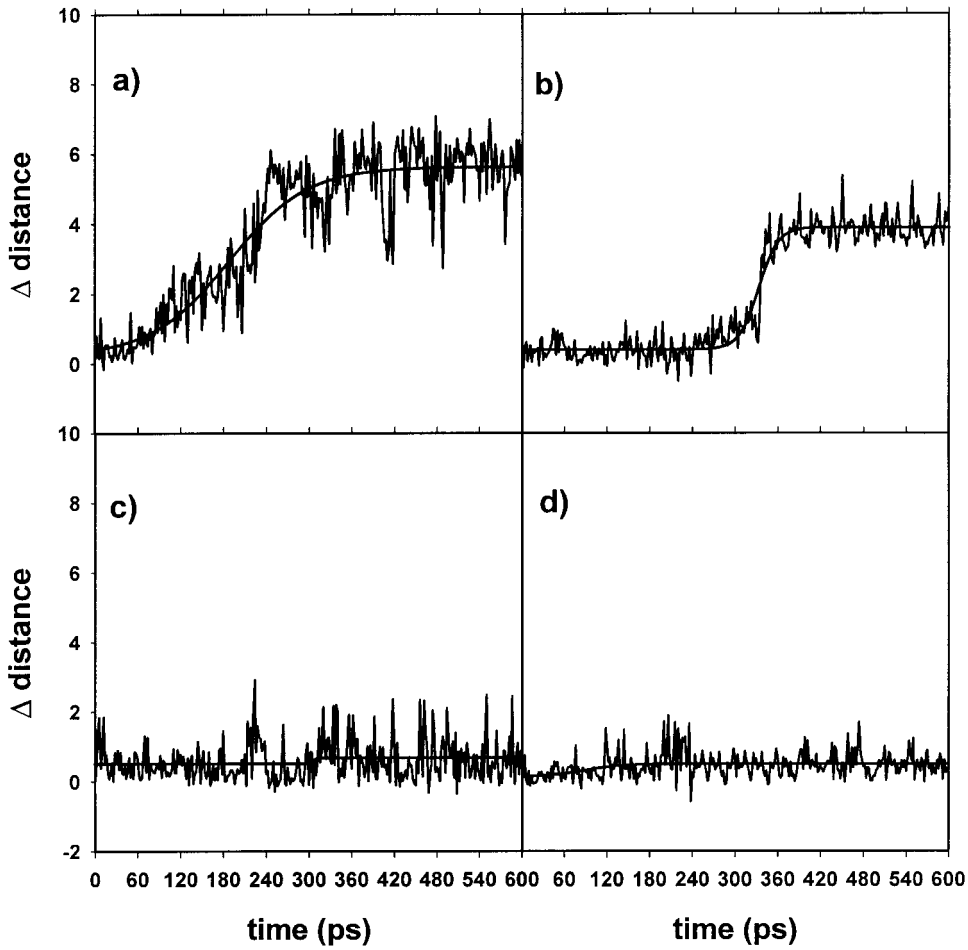

Fig. 7. Time evolution of the distances Å between the kainate ligand and several key residues making up the binding site relative to the starting structure. Distances were measured between: (**a**) the carboxylate group of Glu 209 and the amino group of kainate, (**b**) the NH group of Ser 158 and the side chain carboxyl group of kainate, (**c**) the carbonyl oxygen of Pro 101 and the amino group of kainate, and (**d**) the guanidinium group of Arg 108 and the α-carboxyl group of kainate.

MD simulation in the presence of kainate

The opening of the ligand-binding core in the presence of kainate shows very different features from those observed in the presence of glutamate. Two well-defined phases are observed during domain separation (Fig. 6). In a first phase, opening is initiated but then a conformation with a high degree of closure is stabilized. This first phase is similar to the single one observed in the presence of glutamate (see Fig. 3), but the opening motion is stopped at a force constant of 1 kcal · mol^{-1}, suggesting the presence of a local energy barrier which needs extra energy to be overcome. When the force constant reaches a value of 1.5 kcal · mol^{-1}, the ligand-binding core attains the completely open conformation. The analysis of the position of kainate relative to the residues making up the binding site during this simulation (Fig. 7) shows that the first phase in the domain opening motion is concurrent with disruption of the interaction between the carboxylate of the essential Glu 209, located in domain 2, and the amino group of kainate [Fig. 7(a)]. Adoption of the completely open conformation is achieved only after the interactions of kainate with residues present at the N-terminus of the F helix from domain 2, such as Thr 159 [Fig. 7(b)] and Ser 158 (data not shown), are disrupted. As reported above for glutamate, interactions of kainate with residues located in domain 1 are undisturbed during the simulation time [Fig. 7(c,d)].

The motion of the linker loop takes place when the ion pair between the ligand and Glu 209 is broken apart (Fig. 8). The sudden displacement of this loop suggests that conformational changes in residues involved in the motion occur in a co-operative fashion. These changes take place while the ligand-binding core still presents a high degree of closure. This semi-closed structure is stabilized by the interaction of the side-chain carboxylate group of kainate with the residues present at the N-terminus of the F helix from domain 2, and also by interactions between kainate and binding site residues from domain 1. Therefore, the

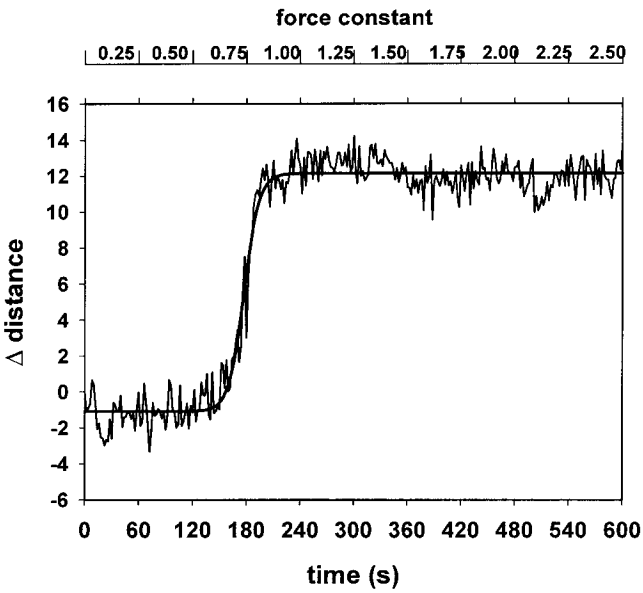

Fig. 8. Motion of the transmembrane segment insertion loop during the simulation in the presence of kainate. The motion is expressed as the increment in distance (Å) between Cα atoms of Gly 132 and Cys 278. The upper axis shows the value of the template force constant applied at different times during the simulation.

two phases observed during domain separation are due to the presence of an intermediate form of kainate/GluR2 ligand-binding core complex in which the motion of the stretch that replaces the transmembrane segment has already been triggered by disruption of the interaction between the agonist and Glu 209, but kainate is still buried within the cleft [Fig. 9(a)].

DISCUSSION

Molecular modeling based on the structural analogy between PBPs and the ligand-binding core of iGluRs was used to predict, with remarkable success,[35] theoretical models of the latter proteins before the crystal structure of a construct of this region of the GluR2 receptor was solved.[24] PBPs are well-known examples of proteins undergoing ligand-triggered, large-scale hinge motions.[30] In this work, we have carried out MD simulations in an attempt to model the opening of the domains that shape the ligand-binding core of GluR2 in the absence and in the presence of glutamate and kainate. The hinge regions of the GluR2 ligand-binding core were identified by comparing to those found in the structurally homologous QBP. This homology also allowed us to assume that the binding of specific agonists to the extracellular core of iGluRs might give rise to a rearrangement of the ligand-binding core that could be transmitted to the transmembrane segments of the whole receptor, resulting in opening of the cation channel.

Little is known about how the conformational changes associated with agonist binding to the extracellular core of iGluRs are transduced into channel pore gating. Electrophysiological studies of cells expressing chimeric NMDA receptors suggest that a short stretch preceding the first transmembrane segment (TM1) serves as a dynamic link between ligand binding and channel gating.[36,37] More evidence for the implication of this region in channel gating has been provided by use of the substituted cysteine accessibility method. The accessibility of the pre-TM1 stretch in a cysteine-substituted channel changes after glutamate binding.[38] This region is a short segment (around 15 amino acids), without structural homology with PBPs, which is presumed to be located on (or close to) the extracellular vestibule of the NMDA receptor channel pore.[34,36] This stretch was replaced by the linker in the S1S2 GluR2 construct used in this work. The corresponding segment in QBP shows important conformational changes in the open form, relative to the closed form (Fig. 1), and is immediately adjacent to one of the hinge stretches identified for the ligand-binding core of GluR2 by structural analogy with QBP (Fig. 2).

The linker loop must be necessarily free (unconstrained) to allow the opening of the ligand-binding core domains during the simulations. The motion of this loop during the MD simulation in the presence of glutamate is concerted with the wide hinge motion of the domains (Fig. 5). Besides, from the MD simulation in the presence of kainate the motion of this loop has shown to be concurrent with the disruption of the interaction between the carboxylate of the essential Glu 209 and the amino group of the ligand (Fig. 8). In the primary sequence, Glu 209 is distant from the transmembrane insertion segment region and from the hinge regions. However, the stretch containing this residue folds together with these regions in a strongly packed structural element in the complex with kainate. Residue Glu 209 is located at the junction between a helix (usually named helix I[24]) and a β-strand making up a β-sheet with two other short β-strands belonging to the hinge regions [Fig. 9(c), closed form]. Following disruption of the interaction between Glu 209 and the agonist, the structure of this region becomes more relaxed. The β-sheet becomes disordered and the strand connecting the pre-TM1 stretch moves away [Fig. 9(c), open or intermediate forms].

In the light of the conformational changes found during our MD simulations, a plausible mechanism for the coupling of agonist binding to channel gating can be proposed: in the unliganded state of iGluRs, the ligand-binding core region containing both the essential Glu 209 residue and the stretch connecting the preTM1 segment adopt a lax structure that allows organization of TM1 in the closed channel pore configuration. Upon agonist ligand binding, its interaction with Glu 209 induces conformational changes in this region, which exert a tension on the TM1 segment through the pre-TM1 stretch. The tension transmitted by the rearrangement of the ligand-binding core to the TM1 segment could be responsible for the changes in the organization of transmembrane segments leading to channel opening.

The present work supports the validity of using activated MD simulations with an incremental force constant in the study of large-scale hinge motions in proteins.

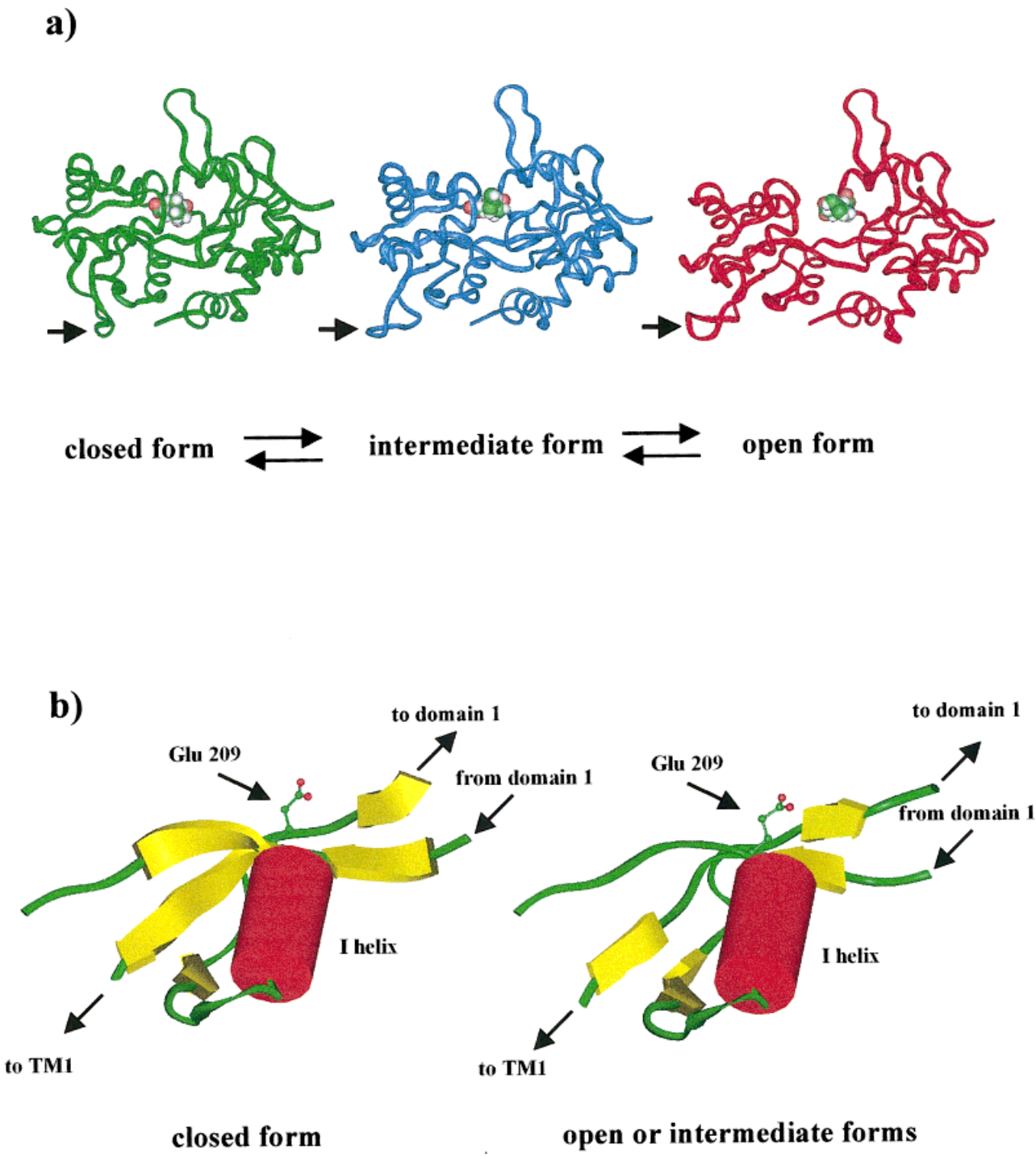

Fig. 9. **a:** Three representative conformations of the GluR2 ligand-binding core found during the molecular dynamics simulation in the presence of kainate. The arrow indicates the transmembrane segment insertion loop. **b:** Conformational changes observed in the structure of the Glu 209 region of the ligand-binding core before and after disruption of the interaction between this residue and the ligand. Secondary structural elements were determined using the Kabsch–Sander algorithm.

Ideally, the calculations should have been done in the presence of explicit water molecules, but the computational cost is currently beyond our capabilities. Besides, given that the domains behave as essentially rigid bodies, the outcome in the presence of water is very likely to be the same with respect to the structural changes involved although the magnitude of the force constant applied and the time scale necessary for these changes to take place

will probably expand due to the cohesive properties of water and the damping of electrostatic interactions.

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