

Binding Site Flexibility: Molecular Simulation of Partial and Full Agonists within a Glutamate Receptor^[S]

Yalini Arinaminpathy, Mark S. P. Sansom, and Philip C. Biggin

Structural Bioinformatics and Computational Biochemistry, Department of Biochemistry, The University of Oxford, Oxford, United Kingdom

Received July 14, 2005; accepted October 11, 2005

ABSTRACT

Ionotropic glutamate receptors mediate fast synaptic transmission in the mammalian central nervous system and play an important role in many different functions, including memory and learning. They have also been implicated in a variety of neuropathologies and as such have generated widespread interest in their structure and function. Molecular Dynamics simulations (5×20 ns) of the ligand-binding core of the GluR2 glutamate receptor were performed. Through simulations of both wild type and the L650T mutant, we show that the degree of protein flexibility can be correlated with the extent to which the binding cleft is open. In agreement with recent experiments, the simulations of kainate with the wild-type construct show a slight increase in β -sheet content that we are able to localize to

two specific regions. During one simulation, the protein made a transition from an open-cleft conformation to a closed-cleft conformation. This closed cleft conformation closely resembles the closed-cleft crystal structure, thus indicating a potential pathway for conformational change associated with receptor activation. Analysis of the binding pocket suggests that partial agonists possess a greater degree of flexibility within the pocket that may help to explain why they are less efficient at opening the channel than full agonists. Examination of water molecules surrounding the ligands reveals that mobility in distinct subsites can be a discriminator between full and partial agonism and will be an important consideration in the design of drugs against these receptors.

The ionotropic glutamate receptors (iGluR) receptors are a family of ligand-gated ion-channels that open in response to the binding of glutamate and are responsible for fast excitatory neurotransmission in vertebrate central nervous systems. In humans, dysfunction of iGluRs has been implicated in a variety of neuropathologies (Dingledine et al., 1999). iGluRs have been classified according to their sequences and the pharmacology of their responses to a variety of ligands (Holman and Heinemann, 1994). Those receptors (GluR1–4) that show greatest sensitivity to the agonist α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) are termed AMPA receptors (Borges and Dingledine, 1998). Likewise, those that show greatest sensitivity to kainate (GluR5–7, KA1–2) are referred to as kainate receptors (Lerma et al., 1997; Chittajallu et al., 1999). Receptors that are activated

by *N*-methyl-D-aspartate (NMDAR1, NMDAR2a-d) are called NMDA receptors (Yamakura and Shimoji, 1999). In vivo, NMDA receptors require both glutamate and glycine to bind for activation.

The overall architecture of iGluR consists of two extracellular domains and a transmembrane (TM) domain. The ligand-binding domain, the crystal structure of which is known (Armstrong et al., 1998), forms the second extracellular domain. It is made up of two polypeptide segments (called S1 and S2) that discontinuously form the two subdomains (or lobes) referred to as D1 and D2 (Fig. 1). The transmembrane domain is inserted between the S1 and S2 segments. Thus, the polypeptide chains exit from the D2 subdomain as the S1 segment to form the two transmembrane helices (M1 and M2) plus a P-loop [a transmembrane architecture reminiscent of that of the potassium channel KcsA (Kuner et al., 2003; Pang et al., 2003)], then forming the S2 peptide segment, which re-enters the D2 subdomain. The chain then crosses the membrane to form a third TM helix, M3, followed by a short intracellular C-terminal region. In addition to the similarity in the transmembrane domain to potassium channels, functional evidence (Mano and Teichberg, 1998; Rosen-

This work was funded by grants from The Wellcome Trust, the Biotechnology and Biological Sciences Research Council, and the EPSRC. Y.A. is a Medical Research Council research student.

[S] The online version of this article (available at <http://molpharm.aspetjournals.org>) contains supplemental material.

Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>.
doi:10.1124/mol.105.016691.

ABBREVIATIONS: iGluR, ionotropic glutamate receptor; GluR, glutamate receptor; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDA, *N*-methyl-D-aspartate; PDB, Protein Data Bank; RMSD, root mean square deviation; RMSF, root mean square fluctuation; WT, wild-type.

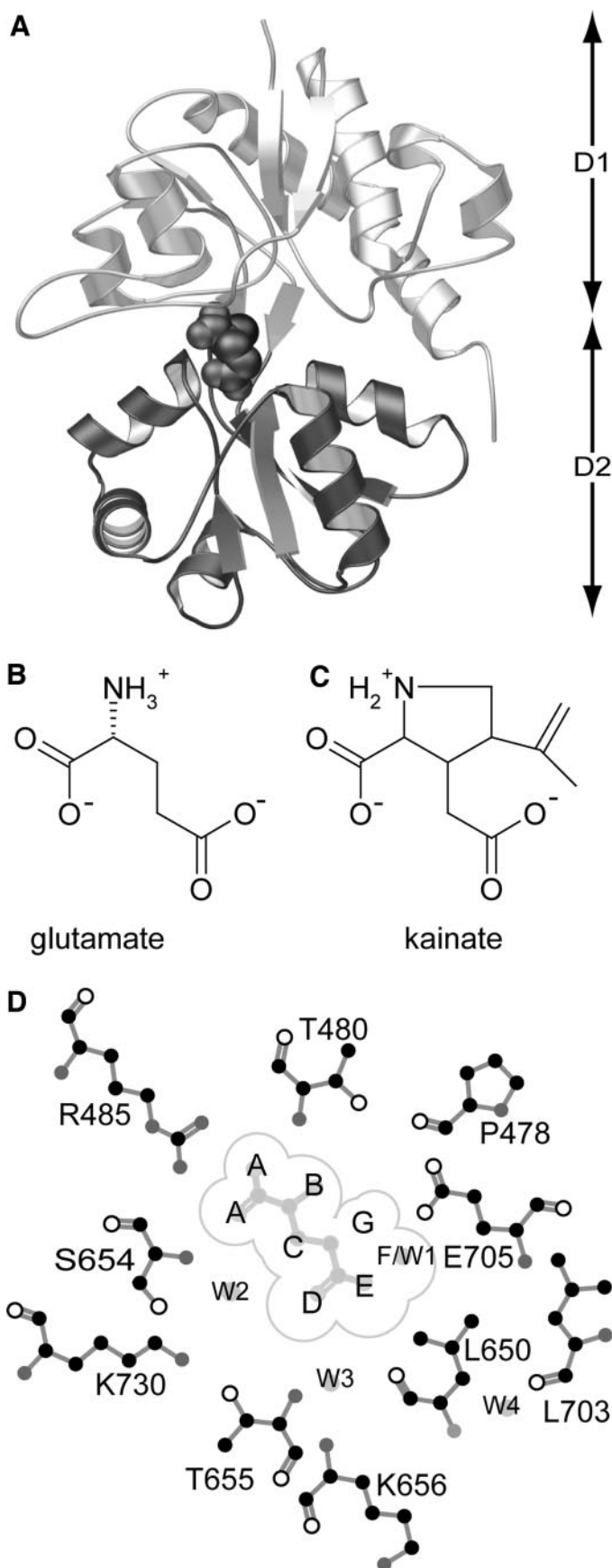


Fig. 1. A, ribbon diagram of the crystal structure of the ligand-binding core of GluR2 in complex with glutamate. The two subdomains are indicated as D1 and D2. Glutamate is shown in spacefill format. The

mund et al., 1998) and electron microscopic images (Saffering et al., 2001) indicate that glutamate receptors are tetrameric assemblies.

In each of the subunits, the ligand binding site is situated in the cleft between the D1 and D2 subdomains with differences in the binding pocket reflecting the different agonist specificity (Armstrong and Gouaux, 2000). The proposed mode of action for this receptor family is indeed one in which the two halves of the ligand-binding domain behave as two fairly rigid subdomains that open and close in a “clam-shell” fashion. (Mano et al., 1996). The natural agonist glutamate helps to stabilize the closed-cleft conformation of the ligand-binding domain, thus favoring the open-state of the channel.

Kainate (Fig. 1C) is a partial agonist in that it elicits currents (from homomeric GluR4 receptors) that are ~8-fold smaller than those elicited by AMPA or glutamate (Swanson et al., 1997). The kainate-bound crystal structure showed an intermediate degree of domain closure compared with the open-apo-state and glutamate-bound structures. This partial closure of the ligand-binding domain seems to be caused by steric hindrance from the isoprenyl group of the kainate molecule (Fig. 1B). As a consequence, two key residues that line the binding pocket, Lys650 and Tyr450, are held further apart than in structures with full agonists bound. This has been addressed by mutagenesis and subsequent crystallography studies, where it was shown an L650T mutation could induce a larger degree of domain closure consistent with that of a full agonist (Armstrong et al., 2003).

It has been proposed that the degree of cleft separation is a determinant of the efficacy of the agonist (Armstrong and Gouaux, 2000). This proposal, however, is complicated by the fact that partial agonists have also been shown to give single conductances similar to full agonist but with a reduced open probability (Jin et al., 2003). We were thus interested to see to what extent flexibility and dynamics might affect the behavior of the ligand-binding cleft. We report here multiple long simulations (20 ns) of both full and partial agonist-bound systems. We find that the kainate-bound simulation displays much higher protein fluctuation, as indicated by somewhat shorter simulations performed previously (Arinaminpathy et al., 2002). We also observe ligand flexibility such that interactions within the binding site can be mediated by water molecules. We are also able to report on the stability of waters within subsites around the ligand (Fig. 1D), which may have important consequences for drug-design. Finally, we examine the effect of the L650T mutation and compare the changes in dynamics with respect to the wild-type receptor.

Materials and Methods

The simulations described use a similar protocol to that described by us in previous simulations (Arinaminpathy et al.,

chemical structures of glutamate and kainate are shown in B and C, respectively. A two-dimensional representation of the binding pocket from the LigPlot program (Wallace et al., 1995) is shown in D. Carbon atoms are dark-gray circles, oxygen atoms are white circles, nitrogen atoms are light-gray circles, and water molecules are light-gray circles. Subsites are overlaid onto the glutamate binding position (shown also in light gray). Waters found within 4 Å of bound glutamate are indicated by a W. W1 corresponds to subsite F, whereas waters W2, W3, and W4 sit near subsites C, D, and E.

2002). In brief, crystal structures (Armstrong and Gouaux, 2000) for the glutamate [Protein Data Bank (PDB) code 1FTJ], kainate-bound (PDB code 1P1N and 1FW0) and the apo states (PDB code 1FT0) of the S1S2 construct were downloaded from <http://www.rcsb.org> (Berman et al., 2000) (Table 1). Sequence positions are numbered according to the predicted mature polypeptide. Missing atoms were added in Quanta (Accelrys, San Diego, CA), and the N and C termini were acetylated and amidated, respectively, to mimic the continuation of the protein chain. Residues modeled in their default ionization states as pK_a calculations (Adcock et al., 1998) did not reveal any unusual protonation states for a system at pH 7. Crystallographic water molecules within 4 Å of the protein were retained, and each system was placed in a box of water of approximately 11,000 simple point charge waters (Hermans et al., 1984) and the appropriate number of counter ions needed to ensure neutrality. Counter-ions were positioned such that they were in bulk solvent. From previous comparative studies (Pang et al., 2005) in which several similar proteins from this fold family were simulated and compared, we are confident that positioning in this way is not critical to the overall dynamics of the protein. All systems were energy minimized and subjected to a short (200 ps) restrained molecular dynamics run in which the protein (and ligand if present) heavy atoms were restrained by an harmonic force of $1000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$. After this period, all restraints were removed, except for bond lengths, which were restrained with the LINCS (Hess et al., 1997) algorithm, and the simulations ran for 20 ns. For analysis that was dependent on an average property, such as root-mean-square fluctuation (RMSF), the first two nanoseconds were discarded and treated as equilibration based on analysis of root-mean-square deviation (RMSD) plots of individual subdomains. Electrostatics were evaluated with Particle Mesh Ewald (Darden et al., 1993; Essman et al., 1995) and a 10-Å cut-off. The temperature was coupled with the Berendsen thermostat (Berendsen et al., 1984) at 310 K and a τ_T of 0.1 ps. The integration time step was 2 fs, and coordinates were saved every 5 ps. All simulations were performed with GROMACS (<http://www.gromacs.org>; Berendsen et al., 1995; Lindahl et al., 2001) with the GROMACS forcefield. Cluster analysis was performed using the linkage algorithm within the GROMACS codes. Molecular figures were generated with Molscrip (Kraulis, 1991) and rendered with Raster3D (Merritt and Bacon, 1997).

Results and Discussion

Simulation Stability and Conformational Drift. A first-pass measure of the stability and conformational drift of a protein in a simulation is provided by the RMSD of the protein coordinates from their initial values as a function of time (Fig. 2A). It can be seen that the RMSD of protein in the presence of glutamate tends to a plateau within 3 to 4 ns with small fluctuations around a value of ~ 2 Å. In contrast, the protein with kainate present exhibits a much larger degree of fluctuation around a slightly higher value (2.3 Å). This is in agreement with earlier, shorter simulations (Arinaminpathy et al., 2002). By plotting the RMSD of the $C\alpha$ atoms of secondary structure elements of indi-

vidual subdomains (i.e., D1 with respect D1), we found that this increased fluctuation was attributable to the D1 domain (Fig. 2, B and C). The fluctuation for the partial agonist kainate in the D2 domain is indistinguishable from the fluctuations observed for the full agonist glutamate. This supports the hypothesis that the D2 lobe may simply act as a rigid domain to pull the channel region open (Armstrong et al., 2003). The RMSF provides another way in which to examine protein flexibility (Fig. 2D). The general trend is well preserved across the simulations, with the largest fluctuations occurring at loop regions. Of the two strands that connect the D1 and D2 subdomains (residues 493–500 and residues 727–734), the second strand exhibits larger fluctuations in the Open-Apo simulation consistent with NMR experiments, in which these regions are shown to be in one of the more mobile regions of the GluR2 construct (McFeeters and Oswald, 2002).

Domain Closure and Intersubdomain Motions. Comparison of the crystal structures of the ligand-binding domain constructs with and without agonists suggested that the degree of domain closure between the two subdomains could be responsible for differences in the effectiveness of the ligands as agonists. In addition, it was hypothesized (Armstrong et al., 2003) that the effectiveness of kainate could be increased by mutation of the “doorkeeper” residue Leu650 to a smaller residue to enable greater domain closure. These aspects were explored further in these simulations in terms of intersubdomain motions (i.e., how the motion of the domains is influenced by the presence and type of ligand). In this analysis, the intersubdomain separation is defined as the distance between the centers of mass of the two subdomains (Fig. 3). At the beginning of the simulation, the proteins can be ordered in terms of decreasing intersubdomain distance as Open-Apo > WT-Kai \sim L650T-Kai > Closed-Apo \sim Glu, as expected.

The separation in the Closed-Apo and Glu simulations remains reasonably constant at ~ 24 Å, with more fluctuation apparent in the Closed-Apo simulation, confirming that these undergo the least amount of conformational fluctuations (as observed in the RMSD analysis) and remain “closed” over the duration of the simulations. At 0 ns, both the L650T-Kai and WT-Kai simulations exhibit comparable intersubdomain separations of ~ 25.3 and ~ 25.0 Å, respectively. Over 20 ns, a small increase in the separation between the subdomains is seen in the WT-Kai simulation, resulting in a separation of ~ 25.9 Å at 20 ns. Although larger fluctuations are exhibited in the L650T-Kai simulation over the duration of the simulation, the mean (averaged over the last 10 ns of the simulation) separation of $\sim 25.1 \pm 2.7$ Å is significantly smaller than that between the subdomains in the WT-Kai system. In comparison, for the Glu simulation, the separation remains constant at ~ 24 Å. It is noteworthy that there is a difference of ~ 1 Å in intersubdomain separations in the order Glu < WT-Kai < L650T-Kai.

The Open-Apo simulation undergoes the most change in intersubdomain separation (Fig. 3A). In the first ~ 5 ns, the protein undergoes substantial fluctuations and the separation drops by ~ 2 Å to adopt a separation close to that of the glutamate-bound structure; i.e., the Open-Apo structure had switched to a closed conformation. From this analysis and from visual inspection of the Open-Apo trajectory, it seems that the two subdomains move together to generate a struc-

TABLE 1
Summary of simulations

Simulation	Protein	Resolution	Ligand	Time
		Å		ns
Glu	1FTJ	1.9	Glutamate	20
Open-Apo	1FT0	2.0	—	20
Closed-Apo	1FTJ	1.9	—	20
WT-Kai	1FW0	2.0	Kainate	20
L650T-Kai	1P1N	1.6	Kainate	20

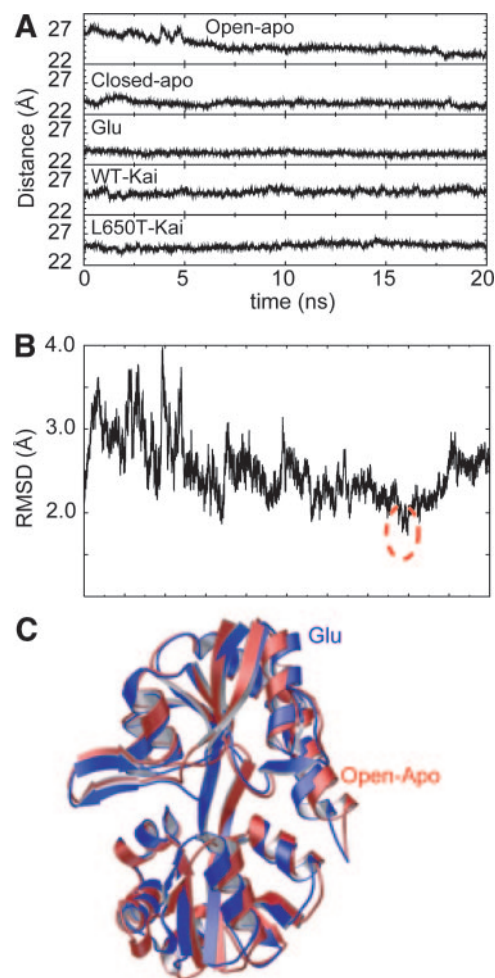
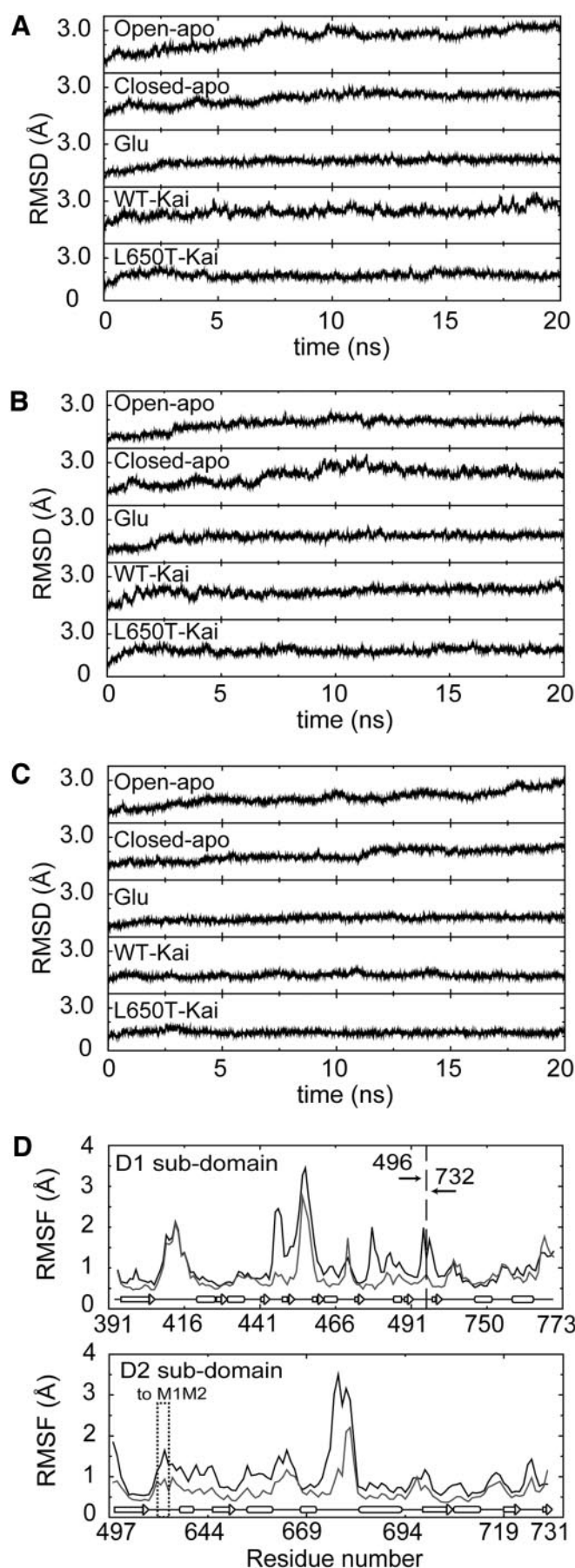


Fig. 3. A, separation between the centers-of-mass of the D1 and D2 subdomains as a function of time for the Open-Apo, Closed-Apo, Glu, Kai, and L650T-Kai simulations. B, comparison of the Open-Apo simulation with the glutamate-bound GluR2 crystal structure. $C\alpha$ RMSD of the Open-Apo simulation compared with the glutamate-bound crystal structure. The red dashed circle indicates the time frame from which the snapshot shown in C was taken. C, the Open-Apo structure (red) fitted to the closed (glutamate-bound) crystal structure (blue) at $t = 15.96$ ns.

ture resembling the closed form of the ligand-binding domain similar to the Glu bound structure. The “most closed” form of the Open-Apo simulation was found to be at ~ 16 ns. The $C\alpha$ RMSD at 15.96 ns between the Open-Apo and glutamate-bound crystal structure was 1.8 Å (Fig. 3B), which indicates that the simulation is able to move between the two observed

Fig. 2. A, root mean square deviation (RMSD) from the initial structure (at $t = 0$ ns) as a function of time for all $C\alpha$ atoms, shown for the Open-Apo, Closed-Apo, Glu, Kai, and L650T-Kai simulations over the 20-ns duration of the simulations. B, conformational stability of the D1 subdomain. $C\alpha$ RMSD of the core secondary structure elements (α -helix and β -strand regions only as defined by DSSP (Kabsch and Sander, 1983)) of the D1 subdomain fitted onto the initial co-ordinates of D1). C, conformational stability of the D2 subdomain. $C\alpha$ RMSD of the core secondary structure elements (α -helix and β -strand regions only as defined by DSSP (Kabsch and Sander, 1983)) of the D2 subdomain fitted onto the initial co-ordinates of D2). D, root mean square fluctuations (RMSF) of the D1 and D2 subdomains (calculated from 2 to 20 ns). All simulations followed a similar pattern, but only Open-apo (black line) and Glu (gray line) are shown for clarity. The Open-apo simulation exhibits the largest degree of fluctuation. Secondary structure elements are indicated at the base of each plot (helices are shown as oblongs and sheets as arrows). It can be seen that the regions of higher fluctuation correspond to the loop regions.

states of the protein. An overlay of the two conformations is shown in Fig. 3C. The change in the radius of gyration between these two extremes is 0.8 Å, which is consistent with the difference between the radius of gyration reported for the Open-Apo and Glu-bound crystal structures (Armstrong and Gouaux, 2000). We also used the program hingeind (Wriggers and Schulten, 1997) to compare the degree of subdomain closure using only secondary structural elements (to remove the influence of loop motions). The difference in the extent of domain-closure between the Open-Apo crystal configuration and the snapshot at 15.96 ns was 9°, which is very similar to the extent of domain-closure between the Open-Apo and glutamate-bound crystal structures (7°). Thus, we are confident that we are moving between the two crystallographically observed extremes.

Essential dynamics analysis was carried out on the trajectories of all the simulations. From the total fluctuations of a molecule, it is possible to extract the dominant dynamical modes responsible for the principal conformational transitions. Analysis of the Open-Apo simulation revealed that 81% of the motion was encompassed by the first 10 Eigenvectors. For the Closed-Apo, WT-Kai, L650T-Kai, and Glu simulations, 73, 64, 65, and 58% of the total motion, respectively, was encompassed by the first 10 Eigenvectors. The first two Eigenvectors of the Open-Apo simulation corresponded to very distinct motions. Eigenvector1 was a hinge-bending motion between the D1 and D2 subdomains, and Eigenvector 2 was a twisting motion of D1 relative to D2. Thus, the simulation has provided details of a *pathway* for subdomain closure within the GluR2 ligand-binding domain. Although this motion occurs on a relatively fast timescale (on the order of nanoseconds) our observations of similar motions across a range of proteins possessing this fold (Pang et al., 2003, 2005) gives us confidence that this motion is functionally relevant.

Secondary Structure Changes upon Ligand-Binding.

We examined the secondary structure content throughout the simulation, which in general was very well preserved. However, we did observe a subtle and interesting difference between the WT-Kai and the L650T-Kai simulations. We found that kainate binding in the wild-type complex (WT-Kai) promotes the formation of β -sheet structure. This finding is supported by Fourier transform infrared spectroscopic studies on the ligand-binding domain of GluR4 (Jayaraman et al., 2000), where an increase in the β -sheet content of the protein was observed as a result of kainate binding to the wild-type receptor. In our simulations, this increase in β -sheet content can be localized to two regions in the D1 subdomain (Fig. 4, A and C). The first region corresponds to residues 453 to 460 (Fig. 4, A and C, region I) and the second region corresponds to loop 2 of this receptor (Fig. 4, A and C, region ii). We note here that the regions that tend toward β -sheet formation encompass residues Thr480 and Pro478 (Fig. 4C), which form part of the binding pocket. It is noteworthy that no such sheet formation was observed for the L650T-WT simulation (Fig. 4B).

Behavior of the Binding Pocket. In all simulations, the ligand displayed little movement with respect to the binding pocket. For the glutamate and L650T-Kai simula-

tions, the distributions for all three dihedrals ($C_\alpha-C_\beta-C_\gamma$, $C_\alpha-C_\beta-C_\gamma-C_\delta$, and $N-C_\alpha-C-O$) examined were unimodal, indicating one stable bound conformation over the duration of the 25-ns simulation. In contrast, WT-Kai exhibited carboxyl group flipping with respect to the dihedral $N-C_\alpha-C-O$, indicating a little more flexibility but not changing the binding mode per se. Cluster analysis (cut-off = 0.4 Å) revealed only a single cluster found for bound ligand in the Glu simulation, whereas kainate in the WT-Kai simulation gave eight clusters and only three in the L650T-Kai simulation. These results support the suggestions that the partial agonist kainate in the WT complex is less rigidly bound than glutamate and that kainate in the L650T mutant will behave with intermediate properties.

Given the difference in the apparent flexibility of the protein, we wanted to investigate the behavior of the ligands in the binding pocket. Glutamate exhibits very little change in its orientation and conformation within the binding pocket. Kainate seems to possess slightly more freedom, perhaps because of the more open nature of the

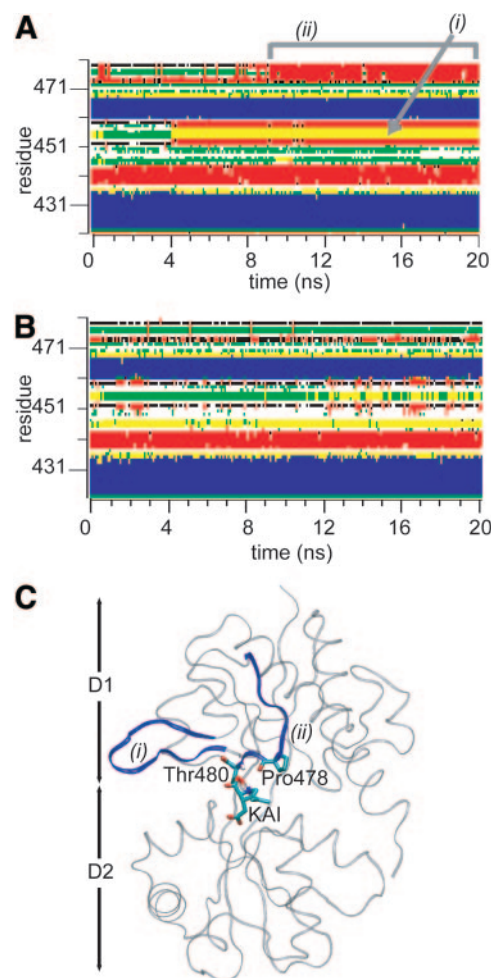


Fig. 4. Changes in secondary structure for the WT-Kai simulation (A) and the L650T-Kai simulation (B). Only the change in secondary structure for a part of the D1 subdomain, in particular residues 421 to 481, is shown. Helices B (residues 421–432) and C (residues 461–469) are well maintained (indicated in blue in A and B) in both simulations. C, schematic representation of the WT simulation at time $t = 0$ ns. The two regions in subdomain D1 observed to preferentially form β -sheets are highlighted in blue. Residues Thr480 and Pro478 are thought to be primarily responsible for the conformational change in this region.

cleft, but all the key interactions reported in the crystal structure complex are maintained throughout the simulation. Because the positions of water molecules within this binding pocket are believed to play a central role in agonist binding, we investigated their behavior. We were particularly interested in the mobility of the water within and around the subsites surrounding the ligand (as described by Armstrong and Gouaux, 2000). Through the course of the simulations, we noted that water molecules were able to penetrate into the binding pocket and interact with the ligand and protein within and around these subsites (Fig. 5). To broadly quantify the mobility between each simulation, we simply summed the number of different waters making similar contacts (between ligand and protein) within each of the subsite regions. The results (Table 2) are very clear-cut and are able to discriminate between a full agonist (glutamate) and a partial agonist (kainate) purely on the rate of water exchange within the binding pocket. Furthermore, an intermediate level of exchange is observed for the L650T mutant, which behaves more like a full agonist than a partial agonist (Armstrong et al., 2003). In the case of the Glu and L650T-Kai simulations, subsite E had at least one water that persisted for more than 50% of the time. This site in the WT-Kai simulation was a site of frequent exchange, as was the case for subsite D. The increased amount of flexibility within the binding pocket

and increased cleft opening in the WT-KAI simulation also manifests itself at the A and B subsites, where solvent is able to frequently penetrate and interrupt the direct protein-ligand interactions made by Arg485 and Pro478 (Fig. 5B). This suggests that there is room for expansion within the binding pocket such that larger ligands might be able to at least fit into the binding pocket. We would anticipate that such ligands would be even weaker agonists than kainate. The behavior of water surrounding subsite D principally reflects a key water identified in a series of crystallographic studies (Armstrong and Gouaux, 2000) and more recently has been suggested to play a central role in GluR1/GluR2 versus GluR3/GluR4 selectivity (Hogner et al., 2002; Frandsen et al., 2005).

A further interesting conformational change is observed at 8.78 ns, where the conformation of the protein and the position of the kainate change slightly, such that a water bridging the NH group of Glu705 with the Kai- γ -CO₂ group is forced out of the binding pocket and the Glu705-NH makes a direct interaction with the ligand instead (Fig. 5B).

The glutamate ligand within the protein is extremely stable; although the waters freely swap interaction sites within the binding pocket, the fact that crystallographically positioned waters are still within the binding pocket reflects the closed-cleft nature of this form of the protein. Despite this stability, there is some flexibility with the pocket. We observed that Arg485 could occasionally flip out for periods of time ranging from tens of picoseconds to more than one nanosecond. During the flip, water molecules were able to penetrate and bind with the α -carboxyl moiety of the glutamate. Arg485 would then swing back in, pushing the water out such that the originally observed direct interaction was regained (see Supplemental Fig. 1 for snapshots of this process).

We also examined the behavior of the Thr650 in the Kai-L650T mutation. We found that the threonine could flip (around its C α -C β bond) such that it would push a water (in a position corresponding to W2 in the crystal structures) out and form a direct interaction with the Kai- γ -CO₂ group (see Supplemental Fig. 2 for snapshots). When the threonine returned to its starting conformation, water was again able to return to that region to make an interaction with the Kai- γ -CO₂ group.

We also examined the behavior of the waters within the binding pocket of the apo simulations. Water moved relatively freely within the pocket, indicating that the presence of ligands is necessary to localize water to specific sites.

Critical Evaluation of Method. It should be remembered here that this study as with other recent studies

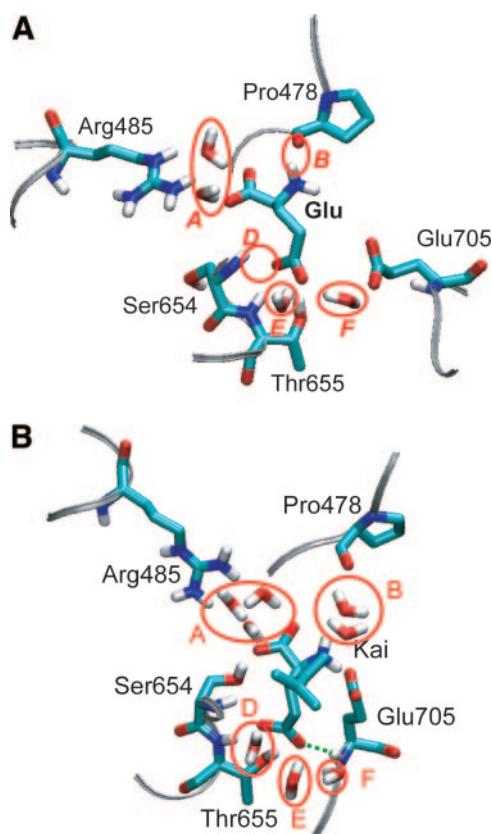


Fig. 5. The role of water molecules in the binding site of the Glu simulation (taken at time $t = 10.1$ ns) (A) and the Kai simulation (at time $t = 17.1$ ns) (B). Subsites A and B are formed by residues from subdomain D1, and subsites D, E, and F are formed by residues from subdomain D2. Sites A, B, D, E, and F, in which water molecules were found over the duration of the simulations, are labeled. In the Kai simulation, the majority of protein ligand interactions are water-mediated, in stark contrast to those in the Glu simulation, which are direct.

TABLE 2

Water exchange rates in the binding pocket

The sites are regions in close proximity to the sub-sites defined by (Armstrong and Gouaux, 2000). The values reported are the total number of times that a water in a subsite is replaced by a different water.

Subsite	Glutamate	Kai	Kai-L650T
A	20	253	55
B ^a	0	2	0
D	3	14	3
E	4	9	3
F	1 ^b	13	12

^a No exchange because of no intermediate water.

^b Persists for 2 ns and is subsequently not replaced.

(Armstrong and Gouaux, 2000; Arinaminpathy et al., 2002; McFeeters and Oswald, 2002) is based upon the artificial construct that corresponds to the ligand-binding domain of the full-length protein. However, given that the ligand-binding affinities for the construct mirror those observed in the full-length receptor (Chen et al., 1995; Kuusinen et al., 1995), we have some confidence that ligand-protein interactions in the single domain construct accurately reflect those in the intact receptor. Further support for this is provided by visible absorption spectroscopy studies (Deming et al., 2003) that demonstrate the same electronic environment for the antagonist 6-cyano-2,3-dihydroxy-7-nitroquinoxaline in the isolated ligand-binding domain as in homomeric GluR4 receptors transiently expressed in human embryonic kidney 293 cells. We should also recall that the current simulation studies are on the monomeric ligand-binding domain. It should be pointed out here that the current simulations are short relative to the time-scale of physiological activation of GluR channel (recent laser-pulse photolysis experiments with caged glutamate suggest the fastest opening time for GluR2 and GluR1 channels to be 17 μ s (Li et al., 2003) and \sim 35 μ s (Li and Niu, 2004), respectively, and similar microsecond time-scales for opening were reported for experiments performed on rat hippocampal neurons, where a mixture of non-NMDA subunits are expressed (Li et al., 2002).

Conclusions

We have shown here how the flexibility of the protein seems to depend on the agonist present and also the degree of domain closure. That the structures maybe more flexible than perhaps first suggested has been recently supported by the observation that AMPA seems to adopt multiple-binding modes to the L650T mutation (Armstrong et al., 2003). We have also shown how the protein may move between an open and a closed state. Furthermore, we have shown how water mobility within the binding pocket can be correlated with degree of domain closure and the agonist present. Knowledge of the behavior of these waters should help to improve the rational design of drugs against these proteins. This will be even more important in the kainate-selective receptors, where the cavity is larger (Mayer, 2005; Nanao et al., 2005; Naur et al., 2005), and thus consideration of the water behavior will be critical.

Acknowledgments

We thank the Oxford Supercomputing Centre for computer time.

References

- Adcock C, Smith GR, and Sansom MSP (1998) Electrostatics and the selectivity of ligand-gated ion channels. *Biophys J* **75**:1211–1222.
- Arinaminpathy Y, Sansom MSP, and Biggin PC (2002) Molecular dynamics simulations of the ligand-binding domain of the ionotropic glutamate receptor GluR2. *Biophys J* **82**:676–683.
- Armstrong N and Gouaux E (2000) Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. *Neuron* **28**:165–181.
- Armstrong N, Mayer ML, and Gouaux E (2003) Tuning activation of the AMPA-sensitive GluR2 ion channel by genetic adjustment of agonist-induced conformational changes. *Proc Natl Acad Sci USA* **100**:5736–5741.
- Armstrong N, Sun Y, Chen G-Q, and Gouaux E (1998) Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature (Lond)* **395**:913–917.
- Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, and Haak JR (1984) Molecular dynamics with coupling to an external bath. *J Chem Phys* **81**:3684–3690.
- Berendsen HJC, van der Spoel D, and van Drunen R (1995) GROMACS: a message-passing parallel molecular dynamics implementation. *Comput Physics Commun* **95**:43–56.
- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, and Bourne PE (2000) The protein data bank. *Nucleic Acids Res* **28**:235–242.
- Borges K and Dingledine R (1998) AMPA receptors: molecular and functional diversity. *Prog Brain Res* **116**:153–170.
- Chen C-C, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN (1995) A P2X purinoceptor expressed by a subset of sensory neurons. *Nature (Lond)* **377**:428–431.
- Chittajallu R, Braithwaite SP, Clarke VR, and Henley JM (1999) Kainate receptors: subunits, synaptic localization and function. *Trends Pharmacol Sci* **20**:26–35.
- Darden T, York D, and Pedersen L (1993) Particle mesh Ewald—an N.log(N) method for Ewald sums in large systems. *J Chem Phys* **98**:10089–10092.
- Deming D, Cheng Q, and Jayaraman V (2003) Is the isolated ligand binding domain a good model of the domain in the native receptor? *J Biol Chem* **278**:17589–17592.
- Dingledine R, Borges K, Bowie D, and Traynelis SF (1999) The glutamate receptor ion channels. *Pharmacol Rev* **51**:7–61.
- Essman U, Perera L, Berkowitz ML, Darden T, Lee H, and Pedersen LG (1995) A smooth particle mesh Ewald method. *J Chem Phys* **103**:8577–8593.
- Frandsen A, Pickering DS, Vestergaard B, Kasper C, Nielsen BB, Greenwood JR, Campiani G, Fattorusso C, Gajhede M, Schousboe A, et al. (2005) Tyr702 is an important determinant of agonist binding and domain closure of the ligand-binding core of GluR2. *Mol Pharmacol* **67**:703–713.
- Hermans J, Berendsen HJC, van Gunsteren WF, and Postma JPM (1984) A consistent empirical potential for water-protein interactions. *Biopolymers* **23**:1513–1518.
- Hess B, Bekker J, Berendsen HJC, and Fraaije JGEM (1997) LINC: a linear constraint solver for molecular simulations. *J Comput Chem* **18**:1463–1472.
- Hogner A, Kasturup JS, Jin R, Liljefors T, Mayer ML, Egebjerg J, Larsen IK, and Gouaux E (2002) Structural basis for AMPA receptor activation and ligand selectivity: crystal structures of five agonist complexes with the GluR2 ligand-binding core. *J Mol Biol* **322**:93–109.
- Holman M and Heinemann SF (1994) Cloned glutamate receptors. *Annu Rev Neurosci* **17**:31–108.
- Jayaraman V, Keesey R, and Madden DR (2000) Ligand-protein interactions in the glutamate receptor. *Biochemistry* **39**:8693–8697.
- Jin R, Banke TG, Mayer ML, Traynelis SF, and Gouaux E (2003) Structural basis for partial agonist action at ionotropic glutamate receptors. *Nat Neurosci* **6**:803–810.
- Kabsch W and Sander C (1983) Dictionary of protein secondary structure: pattern-recognition of hydrogen-bonded and geometrical features. *Biopolymers* **22**:2577–2637.
- Kraulis PJ (1991) MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J Appl Crystallogr* **24**:946–950.
- Kuner T, Seeburg PH, and Guy HR (2003) A common architecture for K⁺ channels and ionotropic glutamate receptors. *Trends Neurosci* **26**:27–32.
- Kuusinen A, Arvola M, and Keinänen K (1995) Molecular dissection of the agonist binding site of an AMPA receptor. *EMBO (Eur Mol Biol Organ)* **14**:6327–6332.
- Lerma J, Morales M, Vicente MA, and Herreras O (1997) Glutamate receptors of the kainate type and synaptic transmission. *Trends Neurosci* **20**:9–12.
- Li G and Niu L (2004) How fast does the GluR1Q flip AMPA receptor channel open? *J Biol Chem* **279**:3990–3997.
- Li G, Pei W, and Niu L (2003) Channel-opening kinetics of GluR2Q flip AMPA receptor: a laser-pulse photolysis study. *Biochemistry* **42**:12358–12366.
- Li H, Nowak LM, Gee KR, and Hess GP (2002) Mechanism of glutamate receptor-channel function in rat hippocampal neurons investigated using the laser-pulse photolysis (LaPP) technique. *Biochemistry* **41**:4753–4759.
- Lindahl E, Hess B, and van der Spoel D (2001) GROMACS 3.0: a package for molecular simulation and trajectory analysis. *J Mol Model* **7**:306–317.
- Mano I, Lamed Y, and Teichberg VI (1996) A Venus flytrap mechanism for activation and desensitization of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors. *J Biol Chem* **271**:15299–15302.
- Mano I and Teichberg VI (1998) A tetrameric subunit stoichiometry for a glutamate receptor channel complex. *Neuroreport* **9**:327–331.
- Mayer ML (2005) Crystal structures of the GluR5 and GluR6 ligand binding cores: molecular mechanisms underlying kainate receptor selectivity. *Neuron* **45**:539–552.
- McFeeters RL and Oswald RE (2002) Structural mobility of the extracellular ligand-binding core of an ionotropic glutamate receptor. Analysis of NMR relaxation dynamics. *Biochemistry* **41**:10472–10481.
- Merritt EA and Bacon J (1997) Raster3D: photorealistic molecular graphics. *Methods Enzymol* **277**:505–524.
- Nanao MH, Green T, Stern-Bach Y, Heinemann SF, and Choe S (2005) Structure of the kainate receptor subunit GluR6 agonist-binding domain complexed with domoic acid. *Proc Natl Acad Sci USA* **102**:1708–1713.
- Naur P, Vestergaard B, Skov LK, Egebjerg J, Gajhede M, and Kasturup JS (2005) Crystal structure of the kainate receptor GluR5 ligand-binding core in complex with (S)-glutamate. *FEBS Lett* **579**:1154–1160.
- Pang A, Arinaminpathy Y, Sansom MSP, and Biggin PC (2003) Interdomain dynamics and ligand binding: molecular dynamics simulations of glutamine binding protein. *FEBS Lett* **550**:168–174.
- Pang A, Arinaminpathy Y, Sansom MSP, and Biggin PC (2005) Comparative molecular dynamics: similar folds and similar motions? *Proteins Struct Funct Bioinf*, in press.
- Rosenmund C, Stern-Bach Y, and Stevens CF (1998) The tetrameric structure of a glutamate receptor channel. *Science (Wash DC)* **280**:1596–1599.

- Safferling M, Tichelaar W, Kummerle G, Jouppila A, Kuusinen A, Keinänen K, and Madden DR (2001) First images of a glutamate receptor ion channel: oligomeric state and molecular dimensions of GluRB homomers. *Biochemistry* **40**:13948–13953.
- Swanson GT, Kamboj SK, and Cull-Candy SG (1997) Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation and subunit composition. *J Neurosci* **17**:58–69.
- Wallace AC, Laskowski RA, and Thornton JM (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Prot Eng* **8**:127–134.
- Wriggers W and Schulten K (1997) Protein domain movements: detection of rigid

domains and visualization of hinges in comparison of atomic coordinates. *Proteins Struct Funct Genet* **29**:1–14.

Yamakura T and Shimoji K (1999) Subunit and site specific pharmacology of the NMDA receptor channel. *Prog Neurobiol* **59**:279–298.

Address correspondence to: Dr. Philip Biggin, Structural Bioinformatics and Computational Biochemistry, Department of Biochemistry, The University of Oxford, South Parks Road, Oxford OX1 3QU, UK. E-mail: phil@biop.ox.ac.uk
