Modeling of the Pore Domain of the GLUR1 Channel: Homology with K^+ Channel and Binding of Channel Blockers

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ABSTRACT Molecular models of the M2 segments of the GluR1 channel have been elaborated using a molecular mechanics approach. The models are based on the homology between pore-lining segments of AMPA receptor channels and the KcsA K^+ channel and on cyclic H bonds at the Q/R site of the AMPA receptor channel. The N-terminal region of an M2 segment of the channel is assumed, like that of the K^+ channel, to adopt a helical conformation. Due to a deletion, the C-terminal end of the M2 segment of the AMPA receptor is more stretched than that of the K^+ channel. As a result, only a single oxygen ring may be exposed to the AMPA receptor channel pore. Data on the block of AMPA receptor channels by dicationic adamantane derivatives have been used to select the most relevant model. The model with the oxygen of a Gly residue (position +2 from the Q/R site) exposed to the pore best fits the experimental data. This model also fits experimental data for another class of AMPA receptor antagonists, the polyamine amides. According to the model, the side-chains of the C-terminal residues are involved in intra-receptor interactions that stabilize the structure of the channel rather than in interactions with ions in the pore.

INTRODUCTION

Ionotropic glutamate receptors fall into two major classes: those containing GluR (GluR1–7) and KA (KA1–2) subunits and that respond to AMPA and/or kainate, the non-NMDA receptors, and those that contain NR subunits and that respond to *N*-methyl-D-aspartate, the NMDA receptors. These membrane-bound receptors mediate a majority of the excitatory synaptic events in the central nervous systems of vertebrates. One way of pharmacologically regulating glutamatergic synaptic transmission is by using noncompetitive inhibitors that block the open ion channel. Such compounds may serve as neuroprotective agents against glutamate excitoxicity mediated by non-NMDA and/or NMDA receptors in global ischemia, Parkinson disease, and other neurodegenerative disorders (Pellegrini-Giampietro et al., 1997; Parsons et al., 1999, Zaulyanov et al., 1999). Unlike NMDA receptors, for which there is a long list of noncompetitive antagonists, the list of channel blockers of non-NMDA receptors is rather short. The latter includes polyamines, such as spermine (Isa et al., 1996; Washburn and Dingledine, 1996; Bahring et al., 1997), natural and synthetic polyamine amides, such as philanthotoxin-433 and argiotoxin-636 (Priestley et al., 1989; Jones et al., 1990; Brackley et al., 1993; Herlitze et al., 1993), and dicationic derivatives of adamantane and phenylcyclohexyl (Magazanik et al., 1997; Bolshakov et al., 2000; Tikhonov et al., 2000b). Various monocationic blockers of NMDA receptor channels (MK801, phencyclidine, ketamine, memantine, etc.) are unable to antagonize ion channels of AMPA and kainate receptors (Parsons et al., 1996; Igarashi et al., 1997; Bolshakov et al., 2000).

Ionotropic glutamate receptors and voltage-gated K^+ channels have similar transmembrane topologies and sequence of pore-lining segments (e.g., Wood et al., 1995). In both channel types, the pore-lining segments form reentrant membrane loops. N parts of the segments adopt a helical conformation (pore helix), whereas C parts forming the selective filter have no regular secondary structure (pore loop). Precise structural information on K^+ channels has come from x-ray data on the KcsA K^+ channel (Doyle et al., 1998). Data on the pore-lining segments of ionotropic glutamate receptor channels are indirect, but scanning mutagenesis studies suggest that they are structural similar to K^+ channels (Kuner et al., 1996, 2001; Panchenko et al., 2001). The recent finding of a prokaryotic glutamate receptor with a K^+-selective ion channel, which is related in amino-acid sequence to both eukaryotic glutamate receptors and K^+ channels also supports the idea of structural homology (Chen et al., 1999). Therefore, the x-ray structure of the pore-lining segment of the KcsA K^+ channel (Doyle et al., 1998) may be used as a template for modeling of the M2 segments of eukaryotic ionotropic glutamate receptors. However, great differences in the physiological and pharmacological properties between K^+ channels and glutamate receptors require use of additional sources of information.

The helical parts of the channel-lining M2 segments in ionotropic glutamate receptors terminate at the selective filter of the channel (the so-called N/Q/R site). The N/Q/R site, which may be occupied by either Asn (N), Gln (Q), or Arg (R), is an important determinant of channel permeation and blockade (for review, see Dingledine et al., 1999). In particular, substitution of Asn by Gln in the N/Q/R site of NMDA receptor channels leads to loss of channel sensitivity to MK-801 and Mg^{2+} ions (Burnashev et al., 1992; Mori et al., 1992). According to Ferrer-Montiel et al. (1998),

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introduction of Asn instead of Gln in the Q/R site of AMPA receptor channel significantly improves the blocking activities of MK-801 and phencyclidine. We have previously suggested a cyclic system of intersegmental hydrogen bonds at the N/Q/R site (Tikhonov et al., 1999) to explain experimentally observed differences in the pore diameters and sensitivity to channel blockers of NMDA and non-NMDA receptor channels (Villarroel et al., 1995; Burnashev et al., 1996). Also, we have proposed that the nucleophilic region in the pore of non-NMDA receptor channels involved in the binding of polyamines should be located beyond the N/Q/R site. Although the proposed H bonds are not proved directly, recent x-ray data on the structure of the KcsA K^+ channel in complex with tetrabutylammonium (Zhou et al., 2001) lends support to the hypothesis. The site and binding mode of tetrabutylammonium in the K^+ channel are similar those of monocationic blockers of the NMDA receptor channel according to the intersegment H bonds hypothesis.

Structure-activity data for a series of dicationic derivatives of adamantane and phenylcyclohexyl, which are potent blockers of AMPA receptor channels (Magazanik et al., 1997; Bolshakov et al., 2000; Tikhonov et al., 2000b), provides an additional source of information for modeling an ionotropic glutamate receptor channel. These compounds have a hydrophobic head-group and a charged tail. Because their spatial structures are readily predictable, it is possible to model the topography of their channel binding sites. The relationship between the potency of AMPA receptor channel block by adamantane derivatives and the distance between their hydrophobic adamantane moiety and their terminal ammonium group has a pronounced maximum (Bolshakov et al., 2000). This has led to the suggestion that the distance between their hydrophobic (via head-group) and nucleophilic (via tail) components of their binding site in the AMPA receptor channel is ~6 to 8 Å.

In the study reported herein, we have investigated the spatial structure of the M2 segments of the homo-oligomeric GluR1 receptor using a molecular modeling approach. The main objective was to develop a model of the pore region of the GluR1 receptor channel that could explain the structure-activity relationships of channel blocking antagonists and contribute toward the design of new inhibitors. Special attention has been paid to the identification and three-dimensional localization of the nucleophilic binding site for channel blockers such as adamantine derivatives and polyamine amides.

BACKGROUND OF THE MODEL

The aligned sequences of pore-lining segments of several ionotropic glutamate receptors and the KcsA K^+ channel are presented at Fig. 1. According to x-ray data from the K^+ channel, the helical parts of the M2 segments terminate at Thr-75 (position 16 at Fig. 1), i.e., the N/Q/R site of ionotropic glutamate receptors. The narrowest part of the K^+ channel is lined by main-chain oxygens of C-terminal residues (positions 17–21 at Fig. 1). This array of nucleophilic atoms determines the multi-ion behavior and ion selectivity of K^+-selective channels (Doyle et al., 1998; Shrivastava and Sansom, 2000).

	Relative position																				
Subunit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NR1	L	T	L	S	S	A	M	W	F	S	W	G	V	L	L	N	S	G	I	G	E
NR2A	F	T	I	G	K	A	I	W	L	L	W	G	L	V	F	N	N	S	V	P	I
GluR1	F	G	I	F	N	S	L	W	F	S	L	G	A	F	M	Q	Q	G	C	-	D
GluR2	F	G	I	F	N	S	L	W	F	S	L	G	A	F	M	R	Q	G	C	-	D
GluR6	F	T	L	L	N	S	F	W	F	G	V	G	A	L	M	Q	Q	G	S	-	E
GluR0	E	G	V	Q	N	G	M	W	F	A	L	V	T	L	T	T	V	G	Y	G	D
Shaker	S	S	I	P	D	A	F	W	W	A	V	V	T	M	T	T	V	G	Y	G	D
Kcsa	I	T	Y	P	R	A	L	W	W	S	V	E	T	A	T	T	V	G	Y	G	D

FIGURE 1 Aligned sequences of M2 segments from several subunits of ionotropic glutamate receptors and homologous pore-lining regions from K^+ channels.

The C-terminal ends of pore-lining segments of the KcsA K^+ channel and the K^+-selective channel of the prokaryotic glutamate receptor (GluR0) are highly conserved and differ significantly from equivalent regions of eukaryotic glutamate receptors. This difference may be related to a deletion at the C-terminal ends of the M2 segments in the latter (see Fig. 1). If there is a three-dimensional homology between the pore-lining segments of KcsA K^+ channel, GluR0, and eukaryotic glutamate receptors, then this deletion means that the pore loop is shorter in the latter channel than in the former two channels. As a result, region 17 through 21 of the eukaryotic glutamate receptor channel must be more stretched than its equivalent region in the KcsA K^+ channel and GluR0 with consequential structural and functional implications. Another important difference between the KcsA K^+ channel and an AMPA receptor channel is the diameter of the pore. The effective pore diameter of an AMPA receptor, determined from the dimensions of the largest permeant molecule, is ~7.5 Å (Burnashev et al., 1996). The KcsA K^+ channel is ~4 Å narrower. Because of this, one cannot directly use x-ray data from the K^+ channel for homology modeling the C-terminal ends of the M2 segments of an AMPA receptor. An alternative approach is to impose some constraints on the AMPA receptor model based on independent data. Constraints are also necessary to maintain the general structure of a model channel that does not include the entire polypeptide chain and excludes intrapore water and ions.

Unlike pore helices, which are relatively stable due to internal H bond network, the C-terminal ends of the M2 segments in the model are free, and their conformational freedom must be restricted. Assuming a loop structure for the M2 segments, it is necessary to fix the coordinates of the N- and C-terminal ends of these segments at the same level along the pore axis (Fig. 2 *A*). These plane constraints stretch the C-terminal ends of the segments. Another con-

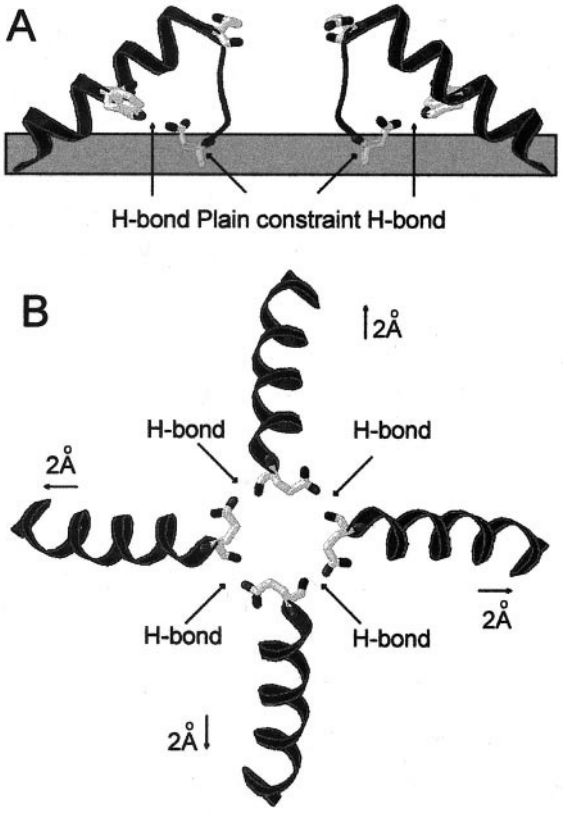

FIGURE 2 Initial geometry of the M2 segments of the GluR1 receptor and constraints used in deriving the conformations of these structures. (*A*) Side view with only nonadjacent segments shown. The helical parts of the M2 segments are presented as ribbons. The slope and orientation of the helices are taken from x-ray data for the KcsA K^+ channel (Doyle et al., 1998). Helical H bonds are clamped by distance constraints. Trp-8, Gln-16, and Asp-21 residues are shown as sticks. Plane constraints were introduced to fix the reentrant loop conformation of the M2 segments. Distance constraints fix the H bonds between Trp-8 and Asp-21 residues. (*B*) Extracellular view. To give a minimum pore diameter compatible with experimental data (Burnashev et al., 1996), the helical parts of the M2 segments were fixed in place 2 Å more distant from the pore axis than in the KcsA channel (Doyle et al., 1998). Distance constraints clamp the intersegmental side-chain to the main chain H bonds at the Q/R site (Tikhonov et al., 1999).

straint is the introduction of H bonds to stabilize the spatial structure of the C-terminal ends of the M2 segments. In the KcsA K^+ channel, the side-chain of Tyr-78 forms an H bond with Trp-68 in the adjacent segment, and the side-chain of Asp-80, which is not exposed to the channel lumen, may form an H bond with Trp-67. In ionotropic glutamate receptors, homologous positions (8 and 21) are also occupied by Trp and Asp residues (Fig. 1). A number of mutagenesis studies suggested the importance of Trp-8 and Asp-21 in defining the channel properties of NMDA and non-NMDA receptors (e.g., Dingledine et al., 1992; Kashiwagi et al., 1997; Williams et al., 1998; Panchenko et al., 1999, 2001). In the pore of the AMPA receptor channel, as in the KcsA K^+ channel, these residues may interact with each other to stabilize the conformation of the C-terminal ends of the M2 segments (Fig. 2 *A*). In the KcsA K^+ channel, the main-chain oxygens of Thr-75 (position 16, N/Q/R site) are exposed to the pore where they form a nucleophilic ring that could bind blocking cations (Zhou et al., 2001). However, in the AMPA receptor channel, which is not sensitive to monocationic blockers, this ring should not be accessible from the pore. Tikhonov et al. (1999) have suggested that the main-chain oxygens contribute to a system of intersegmental hydrogen bonds, i.e., they may be H bonded to side-chain NH_2 groups of homologous residues (Gln at position 16) belonging to adjacent M2 segments (Fig. 2 *B*).

The significant difference in the pore diameter of the KcsA K^+ channel and non-NMDA glutamate receptor channels needs consideration. How does this square with the proposed homology of pore-forming segments? In the KcsA K^+ channel, the small dimension of the pore is determined by the close arrangement of the helical parts of the pore-lining segments. Without changes in this region, it is impossible to provide the pore dimension required for the non-NMDA glutamate receptor channel. The simplest way to enlarge the channel diameter in a model is to place the M2 helices more distant from the pore axis. This change does not affect the internal structure of the segments and the pattern of pore-exposed residues, which are similar in both KcsA K^+ and non-NMDA glutamate receptor channels. With respect to the general structure of the receptor, a small shift does not seem critical for chain packing and may be explained by different interactions of the M2 segments with other parts of a receptor. For instance, the KcsA K^+ channel and GluR0 have two transmembrane segments in each subunit, whereas AMPA receptor subunits have three transmembrane segments.

METHODS

The overall conformational energy of a molecular system is presented as the sum of van-der-Waals interactions, electrostatic interactions, and torsion energies. The AMBER force field (Weiner et al., 1984) with a cutoff distance of 8 Å has been used. Standard values for bond lengths and valent angles were assigned and keep constant. Partial charges for blocking ligands were calculated by CNDO/2 method. The Monte-Carlo with energy minimization (MCM) strategy (Li and Scheraga, 1987) was used to find low-energy equilibrium conformations within internal coordinates (torsion angles and positions of free molecules). The advantage of this approach is that it provides a series of random jumps of a conformation and subsequent gradient minimizations to the nearest energy minimum. Minimizations were terminated when the energy gradient became less than 1 kcal/mol/rad. MCM trajectories were calculated at T = 600 K. Conformations with energies up to 10 kcal/mol from the lowest-energy conformation were counted as possible conformations of a system and stored in stack file for further analysis. An MCM search was terminated when 5000 consecutive minimizations (for calculations of models) or 500 minimizations (for docking of channel blockers) did not lead to a decrease in the global energy minimum or the appearance of new possible conformations.

To restrict the conformational freedom of a system, flat-bottom parabolic constrain penalty functions were introduced. These functions in-

creased the conformational energy of a system if it deviated from specified parameters. Three types of constraint were used. 1) Pin constraints that restricted deviation of the Cartesian coordinates of an atom from a specified point. If the deviation was less than 1 Å, then the energy of the constraint was zero. 2) Distant constraints that were used to fix some H bonds. The energy of the constraint became nonzero if the distance between proton donor and acceptor became more than 2.2 Å. 3) Plane constraints that clamped the distance between a specified atom and a plane passing through three another atoms in a system. In this case, the mobility of the atom in the plane was not restricted. A force constant of 10 kcal/mol/Å was used for the constraints.

Assembly of the models, calculations, and analysis of low-energy conformers were performed using the ZMM-MVM molecular modeling package. ZMM provides basic tools for MCM calculations, various types of constraint, stacking of low-energy conformers, and statistics (Zhorov, 1981; Zhorov and Ananthanarayanan, 1996). MVM is a Windows-based molecular graphics program elaborated by D. Tikhonov (unpublished data). Like Rasmol (Sayle and Milner-White, 1995) and MOLMOL (Koradi et al., 1996) programs, MVM provides various tools for visualization of a molecular system and allows manipulations with fragments and the assembling of new ligands. MVM also serves as an interactive interface for the tuning and running of ZMM jobs.

RESULTS

Model of the pore

Modeling of the pore of the AMPA receptor channel was undertaken using the sequence of the M2 segment (positions 1–21 at Fig. 1) of the GluR1 subunit. The backbone conformation of residues 1 through 15 and the orientation of helices were initially assigned as in the KcsA K^+ channel. The α-helical H bonds in region 1 through 16 were fixed by distance constraints. Pin constraints were imposed to restrict the displacement of 1 through 16 Cα atoms from their initial positions. These constraints maintained the four-fold symmetry of the model. Three types of constraint were imposed to the C-terminal ends of the M2 segments. Cyclic H bonds at the Q/R site were fixed by distance constraints between main-chain oxygens of Gln-16 residues and side-chain amino-groups of homologous residues belonging to adjacent M2 segments (Fig. 2 *B*). Distance constraints were imposed to fix the interaction between side-chains of Trp-8 and Asp-21 (Fig. 2 *A*). Also, Cα atoms of Asp-21 residues were constrained to the plane passing through Cα atoms of Gly-2 residues (Fig. 2 *A*). Homologous residues in KcsA K^+ channel have a similar disposition. All these constraints generate a model of the pore of the GluR1 receptor channel that has a general three-dimensional homology with the pore of the KcsA K^+ channel.

To provide a diameter of the selective filter comparable with the experimental data (Burnashev et al., 1996), each of four M2 helices should be placed more distant from the pore axis than in the KcsA channel (see Background of the Model). We have tested values of shifts from 0 to 3 Å with 0.5-Å step. For each value a local MCM search (10,000 energy minimizations) with torsions of Gln-16 residues randomized was undertaken. The low-energy conformers obtained were tested for ability of their Gln-16 cycle to accommodate the adamantane moiety. The latter has a diameter of 6.9 Å, which is close to the dimensions of *N*-methyl-D-glucamine, the largest weakly permeant organic cation (Burnashev et al., 1996). Tikhonov et al. (2000b) have showed independently that adamantane derivatives can pass through AMPA receptor channels. Models with shifts from 0 to 1.5 Å did not yield conformers accommodating adamantane moiety. For models with shifts 2.0 and 2.5 Å such conformers appeared (18 of 148 and 76 of 134 conformers, respectively). For the model with 3 Å shift, energy of constrains imposing intersegment H bonds at position 16 have became significant, and minimization of low-energy conformations without these constrains has leaded to break of the H bonds between Gln-16 residues. It means that so large shift of M2s does not agree with the proposed H bonds at the Gln-16 site. The 2.0-Å shift was chosen for further work. It corresponds to minimal deviation from K^+ channel structure and agrees with weak permeability of AMPA receptors for adamantane derivatives and other cations of similar dimensions.

For the model described above the long MCM search (30,000 energy minimizations) has been performed. All torsions of the model were varied in energy minimizations but only torsions of the C-terminal end (positions 16–21) were randomized. The search yielded 541 possible conformers. As expected, the conformations of the C-terminal end residues Gln-17, Gly-18, and Cys-19 were most diverse. The importance of residue at position 17 was underlined by Wollmuth et al., (1998) and by Ferrer-Montiel et al., (1998). In the model the side-chain of Gln-17 forms various intra- and intersegmental H bonds, and its NH_2 group can donate the H bonds with main-chain oxygens of residues at positions 16 through 19 of the same M2 segment or with the Ala-13 residue of an adjacent M2 (counter-clockwise direction as viewed from extracellular side). The most interesting interaction of the side-chain of Gln-17 is the formation of an intersegmental H bond with the side-chain oxygen of Gln-16. This H bond turns the side-chain oxygen of Gln-16 away from the pore and, thereby, prevents its interaction with intra-pore cations (Fig. 3). The absence of an accessible nucleophilic group at the Q/R site is an important property of the pore of AMPA receptor channels determining their insensitivity to monocationic blockers.

Distance constraints were imposed to clamp H bonds between Gln-16 residues and between Trp-8 and Asp-21 residues. To test the stability of these H bonds, an additional MCM search without these constraints was performed. With constraints, the lowest-energy conformation was taken as the initial point of trajectory. After 10,000 minimizations without constraints, the conformational energy was decreased by 9.4 kcal/mol. All four H bonds between Trp-8 and Asp- 21 (one in each M2 segment) remained stable in 90% of the conformations. Although H bonds between Gln-16 residues are less stable, the lowest-energy conformation for all of the H bonds was 4.6 kcal/mol higher than

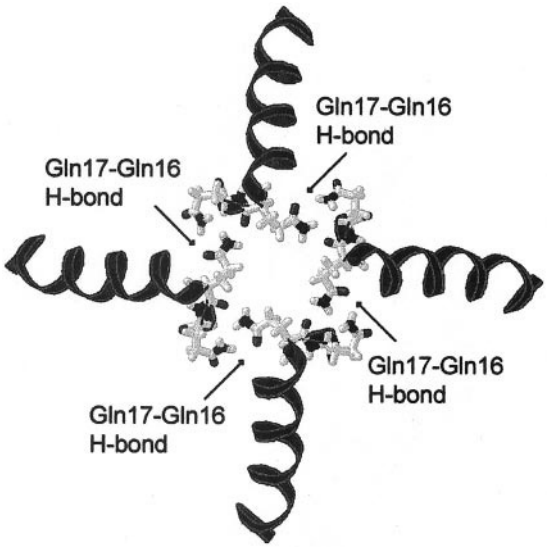

FIGURE 3 Possible role of Gln-17 residues. Extracellular view with the helical parts of the M2 segments shown as ribbons and Gln-16 and Gln-17 residues shown as sticks. In this conformation the side-chain NH_2 groups of the Gln-17 residues form H bonds with side-chain C═O groups of Gln-16 residues belonging to adjacent segments. The main-chain and side-chain oxygens of Gln-16 residues (Q/R site) are not accessible for interaction with intrapore cations, which explains the weak interaction of the GluR1 channel with monocationic blockers.

the global minimum. Also, 95% of the low-energy conformations (including the lowest-energy conformation) exhibited at least two intersegmental H bonds between Gln-16 residues.

Binding of channel blockers

At the C-terminal side, all possible conformations obtained can be divided in three types, which either expose to the pore no oxygens (type I, 39 conformations), the main-chain oxygen of Gly-18 (type II, 32 conformations), or the main-chain oxygen of Cys-19 (type III, 27 conformations). Other possible conformers contained a mixture of these alternatives, i.e., they have significantly different conformations for the four M2 segments. We have not considered such mixed conformers. Type I conformations can be excluded because they are unable to bind intrapore cations. To decide between the type II and type III conformations, we have used structure-activity data on the block of AMPA receptor channels by dicationic derivatives of adamantane. In the homologous series of compounds Ad-CH_2-N^+H_2-$(CH_2)_n$-N^+Me_3 (Ad, adamantane, $n = 2$–7) the compound with $n = 5$ (IEM-1460) is the most active. Prolongation or shortening of the inter-nitrogen chain induces progressive loss of blocking potency (Bolshakov et al., 2000). The hydrophobic adamantane moiety and terminal ammonium group of IEM-1460 probably interact with hydrophobic and nucleophilic regions of the pore of the AMPA receptor channel, respectively. The Gln-16 residues lack accessible nucleophilic atoms and, therefore, are likely to form part of a hydrophobic binding site. Additional evidence that Gln-16 contributes to a hydrophobic part of binding site comes from x-ray data on the KcsA K^+ channel in the presence of the blocking ligand, tetrabutylammonium (Zhou et al., 2001). The blocker binds to the vicinity of Thr-75 residue, which is homologous to Gln-16. In the GluR1 channel model, the nucleophilic site on the C-terminal side of the selectivity filter (Gly-18 or Cys-19) should bind the terminal ammonium group of IEM-1460 with the adamantane moiety binding to the Gln-16.

To discriminate between the type II and III conformations, we searched for the lowest-energy conformations of their complexes with IEM-1460 using MCM docking. The lowest-energy conformations resulting from this approach

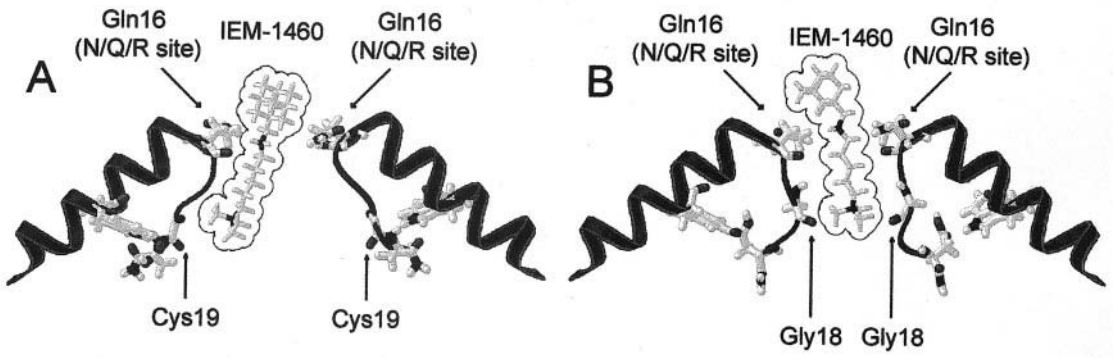

FIGURE 4 Side views of models with either Cys-19 (*A*) or Gly-18 (*B*) main-chain oxygens exposed to the pore, energetically-optimal complexes with IEM-1460. The helical parts of the M2 segments are presented as ribbons, Trp-8, Gln-16, Gly-18 (*B*), Cys-19 (*A*), and Asp-21 residues are shown as sticks. The IEM-1460 molecule is shown as a stick with a van der Waals contour. In *A*, the distance between the Gln-16 residues (N/Q/R site) and the pore-exposed Cys-19 oxygen is too great to allow simultaneous interactions of the adamantane group with Gln-16 and the terminal ammonium group with Cys-19 oxygen. In *B*, the pore-exposed Gly-18 oxygen is closer to the Gln-16 residue, thus allowing interactions of both active moieties of IEM-1460 to their binding sites in the pore.

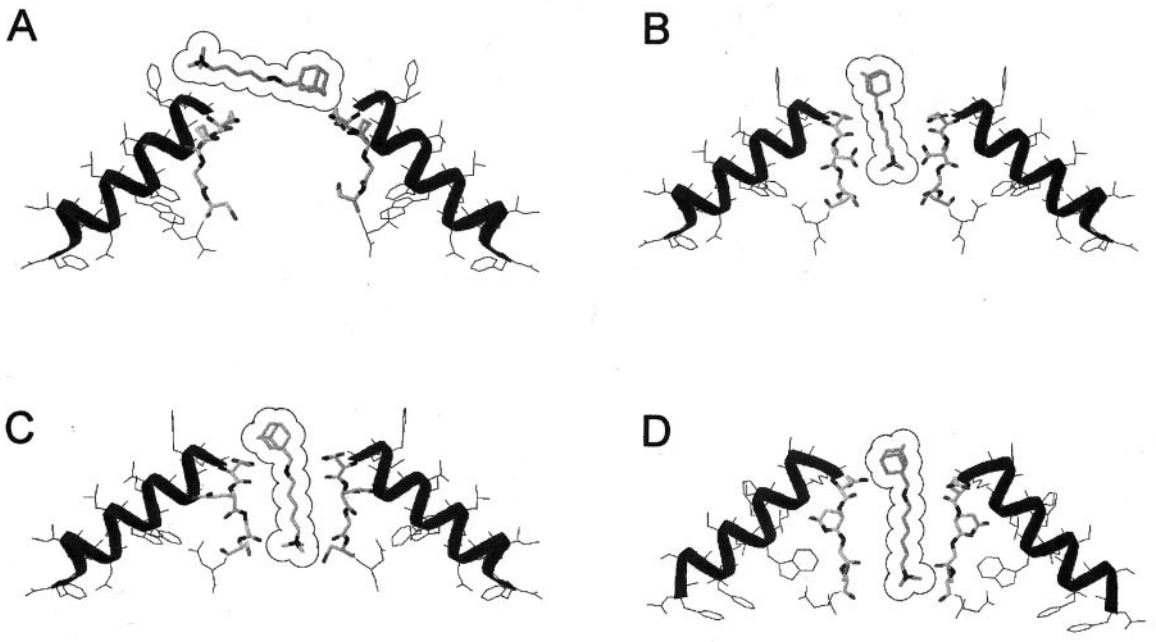

FIGURE 5 Side views of models 2 (*A*), 3 (*B*), 4 (*C*), and 5 (*D*), energetically-optimal complexes with dicationic adamantane derivatives. The helical parts of the M2 segments are presented as ribbons, C-terminal ends are shown as sticks. Ligand molecules are shown as a stick with a van der Waals contour. In *A*, the Gly-18 oxygens are not fully pore-exposed and optimal position of the ligand is outside the narrow part of the pore. In *B*, the pore-exposed side-chain oxygens of Gln-17 provide effective binding of short-chain compound ($n = 4$). In *C*, the pore-exposed Gly-18 oxygens provide the most effective binding of the compound with $n = 5$, which exhibit the strongest blocking activity. In *D*, Cys-19 oxygens are partly exposed to the pore and, as a result, long-chain compound ($n = 6$) bind to the model strongly.

are presented in Fig. 4. In the type III lowest energy conformation, the distance between Gln-16 $C\alpha$ and Cys-19 O is 9.9 to +0.4 Å. This distance is too large to enable an effective interaction of both the adamantane moiety and the terminal ammonium group with their putative binding sites (Fig. 4 *A*). The binding of IEM-1460 to the lowest-energy type II conformation is more plausible (Fig. 4 *B*), i.e., the distance between Gln-16 Cα and Gly-18 O is 7.6 to 8.1 Å. Therefore, type II conformations were tested in more detail. Among 541 possible conformations, five conformers have all four Gly-18 oxygens facing the pore with the side-chain oxygens of Gln-16 projected away from the pore. The conformers have energies of 3.1, 4.3, 4.6, 5.7, and 7.6 kcal/mol above the global minimum found. These conformers will be referred to as models 1 to 5, respectively. MCM docking runs for five adamantane derivatives with the inter-nitrogen chain varying from three to seven methylene groups ($n = 3$–7) to models 1 to 5 were performed.

In model 2, the optimal position of an adamantane derivative is outside the narrow part of the pore (Fig. 5 *A*). Blocking compounds interact mainly with Phe-14, Met-15, and Gln-16 residues. Systematic dependence of energies of the complexes on the length of blocking molecule was not observed. This is probably because Gly-18 oxygens are not fully exposed to the pore and cannot provide an effective interaction with the terminal ammonium group of a blocking molecule. Thus, model 2 belongs to type I rather than to type II of possible conformations. In model 3, the side-chains of Gln-17 residues are exposed to the pore thus providing effective interaction with short-chain compounds (Fig. 5 *B*). In model 5, the Cys-19 oxygens are partly accessible from the pore (Fig. 5 *D*). As a result, long-chain blocking compounds ($n = 6$ and 7) can reach this site and bind as effectively as a blocker with $n = 5$. This model exhibits some properties of type III conformers. Results of docking to the models 1 and 4 agree with experimental data (the highest blocking activity of the compound with $n = 5$) and thus provide explanation for the structure-activity relationships of dicationic adamantane derivatives (Figs. 4 *B* and 5 *C*). These models differ by conformations of side-chains of Gln-17 residues mainly. Although the conformational energy of the model 1 is 2.6 kcal/mol lower than of the model 4, the complexes of model 4 with blockers have an energy that is ~3 kcal/mol lower than the complexes of model 1 with blockers.

Fig. 6 *A* compares experimentally obtained IC_{50} values for the adamantane derivatives (Bolshakov et al., 2000) and calculated parameters of their complexes with model 4. The energy of van-der-Waals ligand-receptor interactions increases monotonically as the length of the inter-nitrogen chain of the blocking molecule is increased. In contrast, the electrostatic component of the interaction energy has min-

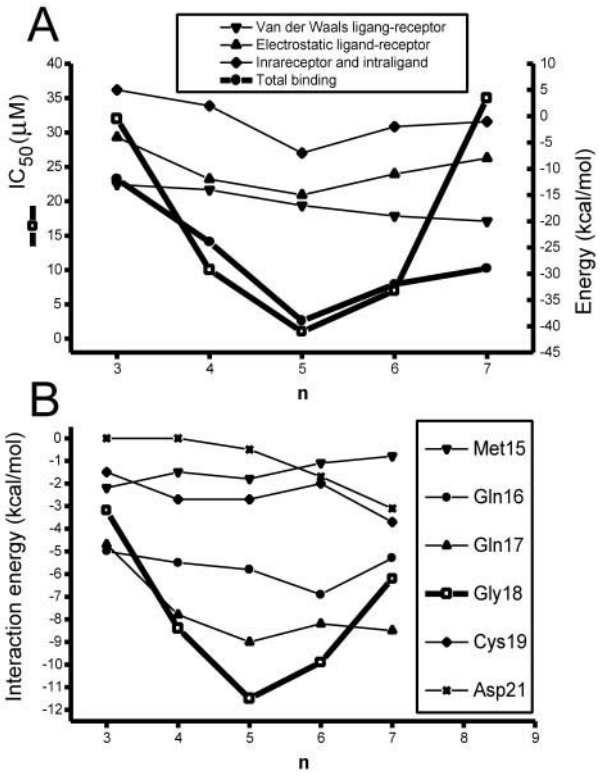

FIGURE 6 Energetics of the binding of dicationic adamantane derivatives (Ad-CH_2-N^+H_2-$(CH_2)_n$-N^+Me_3) with different numbers of inter-nitrogen methylenes to model 4 (with a pore-exposed Gly-18 oxygens). (*A*) Comparison of experimentally measured IC_{50} values (Bolshakov et al., 2000) and different components of conformational binding energy which correlate with IC_{50} data. (*B*) Contributions of different residues to the ligand-receptor interactions. The dependence of Gly-18 energy on n is similar to the experimentally observed activity and to the total electrostatic interactions (see *A*). This indicates that the specificity of AMPA receptor channel block by dicationic adamantane derivatives is due to electrostatic interactions between the terminal ammonium group of the blocking molecule and the pore-exposed Gly-18 oxygens.

imum at $n = 5$. The sum of intra-pore and intra-blocker energy is also lowest for $n = 5$. The latter means that model 4 more easily adopts a conformation favorable for the interaction with IEM-1460 than for binding of other adamantine derivatives. As a result, total conformational binding energy, calculated as difference between the energy of initial state of the complex with remote ligand and the lowest energy found by MCM docking, demonstrates good qualitative agreement with the experimental data. Fig. 6 *B* shows how different rings of homologous M2 residues contribute to the interaction energy (sum of van-der-Waals and electrostatic ligand-receptor interactions). Interactions of the blockers with the residues located at the peripheries of the binding site (Met-15, Cys-19, and Asp-21) are weak. Of the three rings providing essential interactions (Gln-16, Gln-17, and Gly-18), the interaction of IEM-1460 ($n = 5$) with Gly-18 ring is the strongest. The dependencies of total electrostatic energy and of Gly-18 energy on the chain length of an adamantine derivative are similar. This shows that electrostatic interactions between the pore-exposed oxygens of the Gly-18 ring and the terminal ammonium group of the IEM-1460 account for the high blocking activity of this compound found experimentally (Bolshakov et al., 2000). It should be noted that in the presence of IEM-1460 the Gly-18 oxygens project to the pore lumen more directly, indicating that the pore structure may be stabilized by intrapore cations.

Another class of potent noncompetitive antagonists of AMPA receptors comprises natural and synthetic polyamine amides, such as philanthotoxins and argiotoxins. Like dicationic adamantine derivatives, polyamine amides probably interact with the pore of the AMPA receptor channel (Brackley et al., 1993; Bahring and Mayer, 1998). However, unlike the adamantane derivatives, they have donors and acceptors of protons and can adopt a number of conformations. Recently, it has been proposed that, due to intramolecular H bonds, philanthotoxin-343 (PhTX-343) and its derivatives adopt head-and-tail conformations with an extended polyamine chain and a compact head-group exhibiting a hydrophobic surface (Tikhonov et al., 2000a). The stability of such intra-molecular H bonds may be affected by the local environment. To determine the conformation of PhTX-343 that binds to the pore of an AMPA receptor channel and the mode of binding, we have docked this molecule to models 1 and 4. The lowest-energy conformation of the docked complex with the model 1 is shown in Fig. 7 *A*. Despite significant differences in their chemical structures, the binding modes of PhTX-343 and of IEM-1460 are similar (see Figs. 4 *B*, 5 *C*, and 7 *A*). The uncharged part of the PhTX-343 molecule, which has hydrophobic properties, binds to the Q/R site, whereas its terminal ammonium group reaches the Gly-18 oxygen. Residues at positions 17 to 19 are the major contributors to the interaction energy. The large head-group of PhTX-343 also strongly interacts with Met-15 and Ala-13 residues. The optimal conformation of PhTX-343 in the model differs from that calculated in vacuo conditions (Fig. 7 *B*). The latter has two intra-molecular H bonds and a compact head-group. In the complex with the model of AMPA receptor pore one intra-molecular H bond is broken, and the head-group adopts a wider conformation. However, in agreement with previous suggestions, the PhTX-343 molecule keeps its general head-and-tail geometry, and the ammonium group, which is closest to the uncharged part of the molecule, participates in intra-molecular H bond rather than interacting with the pore (see Fig. 7). Docking of PhTX-343 to the model 4 yielded similar results.

DISCUSSION

We have investigated the three-dimensional structure of the pore region of an AMPA receptor channel comprising GluR1 subunits. This investigation has been based on the

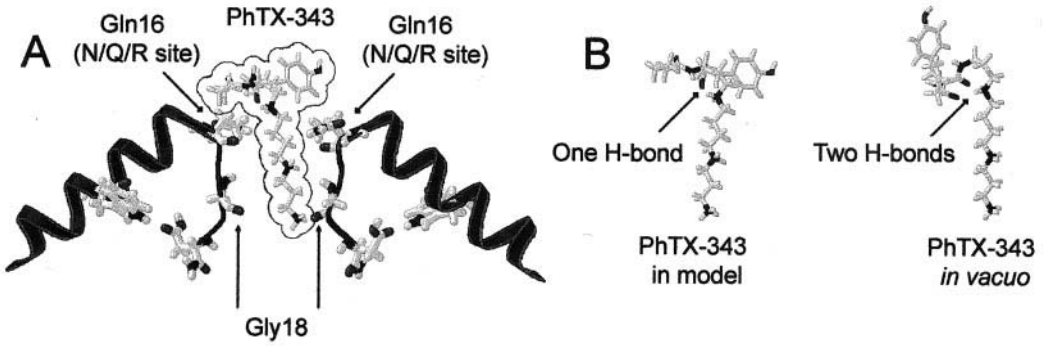

FIGURE 7 Binding of PhTX-343 to the model 1 of the GluR1 channel. (*A*) Side view of the energetically optimal complex of PhTX-343 and the model. Only nonadjacent M2 segments are shown. The helical parts of the M2 segments are presented as ribbons and the Trp-8, Gln-16, Gly-18, and Asp-21 residues are shown as sticks. The PhTX-343 molecule is shown as a stick with a van der Waals contour. The binding mode of PhTX-343 is similar to that of IEM-1460 (see Figs. 4 *B* and 5 *C*). The uncharged part of the PhTX-343 molecule interacts with the Gln-16 residues, whereas the polyamine chain reaches the Gly-18 oxygen of the pore. (*B*) Comparison of the optimal in vacuo PhTX-343 conformation alone and bound to the model pore. In model 1 the head-group of the PhTX-343 molecule adopts a wider conformation with one of intra-molecular H bonds broken. However, a general head-and-tail shape remains and the ammonium group of PhTX-343 that is closest to the uncharged part of the polyamine amide remains involved in an intra-molecular interaction.

homology between pore-lining segments of glutamate receptors and K^+ channels (Wood et al., 1995; Kuner et al., 2001; Panchenko et al., 2001) for which the x-ray structure was recently obtained (Doyle et al., 1998), on new experimental data on the noncompetitive inhibition of AMPA receptors by a homologous series of adamantane derivatives (Bolshakov et al., 2000), and on our previous hypothesis about intersegmental H bonding at the selectivity filter of the glutamate receptor channels (Tikhonov et al., 1999). The use of a molecular modeling approach has allowed to combine experimental data from different sources.

Our models consisted of M2 segments only. By neglecting the remaining part of the receptor molecule, intrapore waters and ions, and membrane lipid, we have not considered all of the components of free energy that may influence the conformation of the M2 segments of GluR1 and the binding of channel blockers. We accept that this is a major limitation, which significantly affects the accuracy of our modeling. In such circumstances it is not possible directly to determine the structure of the channel; we assumed that this can be found among the low-energy conformations that we have discovered.

Docking of a series of dicationic adamantane derivatives has revealed two similar structures (models 1 and 4) providing explanation of structure-activity data. The models also provide a structural explanation for binding of another class of AMPA receptor channel blockers, the polyamine amides and, therefore, may be used for the theoretical design of new channel blocking drugs. In these models, the nucleophilic site of the pore that interacts with a channel-blocking agent is formed, as in the KcsA K^+ channel, by main-chain oxygens of the C-terminal ends of M2 segments. Because this region in the AMPA subunits is more stretched than in the KcsA K^+ channel, the AMPA subunits have only one pore-exposed oxygen ring of Gly-18 residues (Fig. 8). Recent experimental data (Kuner et al., 2001) also emphasize the importance of Gly-18 residues and their contribution to the selective filter of AMPA receptor channels. Tikhonov et al. (1999), among others, have proposed that Asp-21 residues may form a nucleophilic site, which interacts with intrapore cations. The data presented herein provide evidence that the side chains of these residues are involved in intra-receptor H bonding, providing for stabilization of the loop-like conformation of an M2 segment.

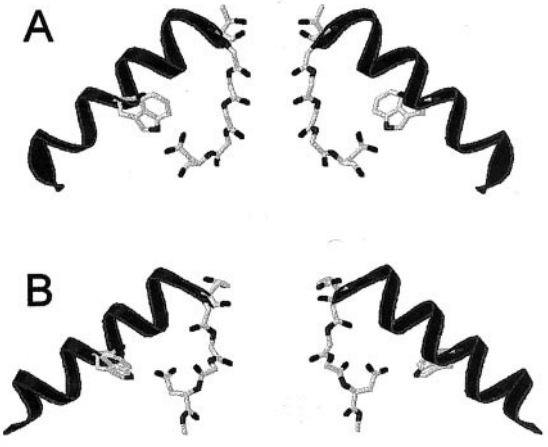

FIGURE 8 Comparison of the spatial structure of the pore-lining segments of the KcsA K^+ channel (Doyle et al., 1998) (*A*) and in the GluR1 channel Model 1 (*B*). In each case a side-view is presented with only two nonadjacent segments shown. The helical parts of the pore-lining segments are presented as ribbons and residues at positions 8, and 16 through 21 are shown as sticks. In the K^+ channel, the narrowest diameter of the pore is formed by four main-chain oxygens at the C-terminal ends of the segments. Due to a deletion, the corresponding region of GluR1 is more stretched with the result that only one main-chain oxygen is exposed to the pore.

According to models 1 and 4, potent channel blockers of non-NMDA glutamate receptors should possess a "head-and-tail" spatial structure with a hydrophobic head group and a positively charged chain. This general structure of a blocking molecule provides simultaneous interaction between a head group and the Q/R site, and between the terminal ammonium group of the blocker and the main-chain oxygen of the Gly-18 residue. Compounds that do not interact with one of the components of the binding site exhibit low channel blocking activity. Potent monocationic blockers of NMDA receptor channels cannot antagonize GluR1 receptors channels because they are too short to reach the Gly-18 site. Because the Q/R site of non-NMDA receptors does not have accessible nucleophilic groups it cannot provide effective binding. Addition of a distant ammonium group to adamantane (as in IEM-1460) or phenylcyclohexyl moiety (an in IEM-1925) produces potent blockers of AMPA receptor channels (Magazanik et al., 1997, Bolshakov et al., 2000; Tikhonov et al., 2000b). Polyamines like spermine are relatively weak blockers and, according to Cu et al. (1998) a significant component of block by polyamines involves hydrophobic binding. Benzyl-polyamines, which are potent NMDA receptor channel blockers, do not block the GluR1 AMPA receptor (Igarashi et al., 1997). In contrast, N1-dansyl-spermine, which has a pronounced head-and-tail structure, strongly antagonizes the channels of both NMDA (Chao et al., 1997) and AMPA (Igarashi et al., 1997) receptors.

Site-directed mutagenesis is a widely used method to investigate the roles of specific amino-acids in macromolecules. It is usually expected that replacements of amino acids in side-chains facing the pore should affect ion permeation and channel block. This approach has been successfully used to investigate the nicotinic acetylcholine receptor (nAChR) channel (see Bouzat and Barrantes, 1997; Arias, 1998). The pore of a nAChR is lined by M2 transmembrane α-helices. Backbone H bonds support the spatial structure of the M2 segments, whereas the side-chains of pore facing residues determine the properties of the ion channel. According to our model, side-chains of some residues in the pore of ionotropic glutamate receptor channels (positions 8, 16, 17, and 21) are involved in the intra-receptor H bonding and participate in the stabilization of the channel structure. Therefore, amino-acid substitutions at these positions (e.g., Dingledine et al., 1992; Kashiwagi et al., 1997; Williams et al., 1998; Panchenko et al., 1999, 2001) should change the conformational properties of the receptor and thus, affect the channel properties indirectly.

This study provides insight into a possible mechanism underlying channel block of a ionotropic glutamate receptor and demonstrates the power and limitations of the molecular modeling approach. When more information on the quaternary structure of the GluR1 receptor becomes available one will be able to extend the analysis beyond the M2 segments and further refine the model. By using such models it will be possible to investigate theoretically the mechanism of ion permeation through an AMPA receptor channel.

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