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Journal of Structural Biology 145 (2004) 205–215

Journal of Structural Biology

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Model structures of the *N*-methyl-D-aspartate receptor subunit NR1 explain the molecular recognition of agonist and antagonist ligands

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Received 5 May 2003, and in revised form 13 October 2003

Abstract

Molecular models of the ligand-binding domain of *N*-methyl-D-aspartate subunit R1 (NR1) were made using the published crystal structures of rat glutamate receptor B (GluRB), the bacterial glutamate receptor (GluR0), and the glutamine-binding protein (QBP) of *Escherichia coli*. Separate models of NR1 were built to represent the ligand-binding conformation for agonist (glycine, D- and L-isomers of serine and alanine, and the partial agonist ligand D-cycloserine) and antagonist (5,7-dichloro-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (DCKA) and *E*-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1-*H*-indole-2-carboxylic acid (MDL 105,519)) ligands. Side-chain conformations of residues within the NR1 ligand-binding site were selected that optimized the hydrophobic packing and hydrogen bonding among residues, while taking into account published data comparing receptor mutants with wild-type NR1. Ligands docked to the model structures provide a rational explanation for the observed differences in binding affinity and receptor activation among agonist and antagonist ligands. NR1 prefers smaller ligands (glycine, serine, and alanine) in comparison with GluRB and GluR0 that bind L-glutamate: the bulky side chain of W731 in NR1 dramatically reduces the size of the ligand-binding site, functioning to selectively restrict recognition to glycine and the D-isomers of serine and alanine. Nevertheless, many of the interactions seen for ligands bound to GluRB, GluR0, and periplasmic-binding proteins are present for the ligands docked to the model structures of NR1.
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Keywords: NMDA receptor; NR1 subunit; Ionotropic glutamate receptors; Model structures; Agonist binding; Antagonist binding

1. Introduction

N-Methyl-D-aspartate (NMDA) receptors fulfill an important role in many central nervous system (CNS) activities, including physiological processes such as memory and learning. NMDA receptors are also involved in the progression of ischaemic damage and neuronal apoptosis. Together with the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors, NMDA receptors belong to the family of *ionotropic* glutamate receptors (iGluRs). iGluRs are ligand-gated ion channels and, when activated, the pore formed by iGluR subunits is permeable to cations (i.e., Na^+, K^+, and Ca^{2+}). All iGluRs bind L-glutamate as the endogenous agonist, although, NMDA receptors simultaneously require two different agonist ligands: L-glutamate and glycine (JW Johnson and Ascher, 1987), but D-serine can replace glycine (Berger et al., 1998; Mothet et al., 2000).

Three subfamilies of NMDA subunits have been identified: NR1 (with 8 splice variants; (Anantharam et al., 1992; Sugihara et al., 1992)), NR2 subunits A–D (Monyer et al., 1992), and NR3 subunits A and B (Chatterton et al., 2002; Ciabarra et al., 1995; Sucher et al., 1995). The binding site for glutamate is located within NR2, while the binding site for glycine/serine is located within NR1 (Kuryatov et al., 1994). The NMDA receptor is present in nature as a heteromeric receptor, and some studies suggest it is tetrameric (Chen et al., 1999; Rosenmund et al., 1998; Schorge and

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doi:10.1016/j.jsb.2003.10.016

Colquhoun, 2003). The topology of NMDA receptor subunits is similar to that of the other iGluRs: an extracellular amino-terminal domain, two extracellular domains (S1 and S2) that form the ligand-binding site, four transmembrane segments (M1–M4), and a carboxyl-terminal domain. The S1 domain of the ligand-binding site is located between the amino-terminal domain and the first transmembrane segment (M1); the S2 domain is located between M3 and M4.

Representative crystal structures have been determined for engineered S1–S2 constructs (missing the amino-terminal domain, carboxyl-terminal domain, and M1–4) of two iGluR subunits: rat GluRB (Armstrong and Gouaux, 2000; Armstrong et al., 1998) and the prokaryotic glutamate receptor ion channel (GluR0) of *Synechocystis* (Mayer et al., 2001); the soluble periplasmic glutamine-binding protein (QBP) of *Escherichia coli* (Sun et al., 1998) corresponds to the S1–S2 structure. The S1 and S2 lobes of the ligand-binding domain are formed from a β-pleated sheet surrounded by α-helices. Two strands forming the hinge region connect the S1 and S2 domains and a large conformational change takes place on ligand binding. For GluRB, structures have been solved of the apo form as well as for S1–S2 bound to agonist and antagonist ligands (Armstrong and Gouaux, 2000; Armstrong et al., 1998; Hogner et al., 2003), supporting the proposed "Venus fly-trap" model (Sack et al., 1989) of receptor activation. In iGluRs these conformational changes function as a gating mechanism controlling ion flow through the channel.

In the present study we have constructed models of the ligand-binding site (S1–S2) of the NR1 subunit for the agonist- and antagonist-bound conformations, based on X-ray structures representative of the S1–S2 domain with bound ligands. We have carefully considered the known structures and their reported complexes with agonist and antagonist ligands, as well as the results on ligand binding to site directed mutants, valuable information that can be extrapolated to the NR1 subunit. Furthermore, our studies of agonist and antagonist interactions with GluRD (Jouppila et al., 2002; Lampinen et al., 1998; Lampinen et al., 2002) together with the modeling of the full set of human AMPA and kainate receptors and correlations of docked ligands with experimental studies on homomeric receptors (Pentikäinen et al., 2003) provides detailed knowledge on the types of interactions likely to be present in the NR1 subunit too. Published experimental data exist for agonists and antagonists of the NR1 subunit, including receptor activation studies using functional receptors, ligand affinity data using the isolated ligand-binding domain, and the effects of site-directed mutants. For the agonists glycine, D- and L-serine, D- and L-alanine, and the partial agonist D-cycloserine, as well as two antagonists, DCKA and MDL 105,519, where experimental data have been reported, we have docked the ligands to model structures representing the agonist-bound conformation or the antagonist-bound conformation, as appropriate. The similarities and differences among the different complexes lead to a rational explanation of the effects of different ligands on NR1.

2. Materials and methods

2.1. Structural modeling of the agonist and antagonist-binding conformations of the NR1 ligand-binding domain

Three-dimensional structures were obtained from the Protein Data Bank (PDB; (Berman et al., 2000)), including the ligand-binding extracellular domain S1–S2 of rat GluRB in complex with L-glutamate (PDB code: 1ftj; (Armstrong and Gouaux, 2000)) and the antagonist DNQX (1ftl; (Armstrong and Gouaux, 2000)), GluR0 of *Synechocystis* in complex with L-serine (1iit; (Mayer et al., 2001)) and QBP, the glutamine-binding protein in complex with L-glutamine from *E. coli* (1wdn; (Sun et al., 1998)). The amino acid sequences of all available NMDA, AMPA, and kainate receptor subunits from different organisms were downloaded from SWISS-PROT (Boeckmann et al., 2003).

The structural superposition of the known structures was made with VERTAA (Johnson and Lehtonen, 2000). This structure-based alignment was then aligned with sequences of the NMDA, AMPA, and kainate receptor subunits using MALIGN (Johnson and Overington, 1993) and a structure-based sequence comparison matrix (Johnson et al., 1996). MALIGN and VERTAA are implemented within the Bodil Modeling Environment (http://www.abo.fi/fak/mnf/bkf/research/johnson/bodil.html; J.V. Lehtonen, D.-J. Still, V.-V. Rantanen, M. Gyllenberg, and M.S. Johnson, unpublished).

Within the S1–S2 domain, human NR1 (and splice variants 1 and 2), rat NR1 (and splice variants A–G), and mouse NR1 differ at only two positions, 415 and 460, which are not located near the ligand-binding site. (The correspondence between the NR1 numbering scheme and the known structures is shown in Table 1.)

The model structure of the NMDA receptor NR1 subunit was built using the rat NR1 sequence. The S1–S2 domain of NR1 is 32% (GluRB), 21% (QBP), and 18% (GluR0) identical in sequence with the three template X-ray structures. NR1 has a 31 residue long sequence extending from a loop in the known structures near the beginning of the S1 domain. In NR1 this loop contains four cysteine residues, most likely forming two disulfide bonds and stabilizing the structure that buds-out from this loop region. The binding of glycine to the rat NMDA receptor was not affected when these cysteines were mutated in the NR1 subunit (Laube et al., 1993), suggesting that the loop does not directly

Table 1
Correspondence between the ligand-binding residues in the three-dimensional structures of GluRB (1ftj/1ftl), GluR0, and QBP and residues in the NR1 ligand-binding domain

NR1	GluRB	QBP	GluR0
Phe408	Tyr405	Phe16	Phe54
Phe484	Tyr450	Phe50	Ile92
Pro516	Pro478	Gly68	Pro110
Thr518	Thr480	Thr70	Ser112
Asn520	Thr482	Thr72	Thr114
Arg523	Arg485	Arg75	Arg117
Gln536	Leu498	Ser88	Ser132
Val684	Leu650	Lys115	Val274
Ser687	Gly653	Thr118	Thr277
Ser688	Ser654	Gly119	Thr278
Val689	Thr655	Ser120	Ala279
Tyr692	Phe658	Tyr123	Trp282
Trp731	Leu704	His156	Phe313
Asp732	Glu705	Asp157	Asp314
Val735	Met708	Asp160	Ala317
Ser756	Lys730	Gln183	Glu340
Phe758	Tyr732	Tyr185	Tyr342

participate in ligand binding. In the rat GluRB structures, the loop where this insertion occurs in NR1 extends away from the ligand-binding region (arrows in Fig. 1).

Model structures of NR1 were built based on the sequence alignment (Fig. 2, part of a larger alignment of AMPA, kainate, and NMDA sequences). The model of the agonist-binding conformation of NR1 was made using the structures 1ftj, 1iit, and 1wdn, and separate models were produced using the programs MODELLER 6.1 (Sali and Blundell, 1993) and NEST (Z. Xiang and B. Honig, unpublished; http://trantor.bioc.columbia.edu/~xiang/jackal/). These models were superimposed and the individual side-chain conformations were selected from the models where the hydrophobic packing and hydrogen bonding was optimal. Where amino acid conformations were not reasonable in either model, a side-chain rotamer library (Lovell et al., 2000), implemented within Bodil, was used to identify optimal conformations. The model of the antagonist-bound conformation of NR1 was built with NEST using the crystal structure of the rat GluRB–DNQX complex (1ftl). The side-chain conformations of three residues in the ligand-binding site (Trp731, Asp732, and Gln536) were altered to optimize their intramolecular interactions.

2.2. Ligand minimization and docking to NR1

Ligands were built with the program Sybyl 6.7 (Tripos, St. Louis, MO, USA). Each ligand structure was energy minimized prior to docking to the modeled receptor structure using the Tripos force field and conjugate gradient method until the energy gradient was less than 0.05 kcal/mol. Protonization of polar groups was checked by comparison of the final three-dimensional structures of the ligands with similar (sub)structures obtained from the Cambridge Structural Data Bank (Allen and Kennard, 1993).

The program GOLD 1.2 (Jones et al., 1995; Jones et al., 1997), which allows for full ligand flexibility and partial protein flexibility, was used to dock ligands to the structural models of NR1. The search volume was limited to a 15 Å radius sphere centered at the binding site.

3. Results and discussion

Agonist (glycine, D-and L-serine, D- and L-alanine, and the partial agonist D-cycloserine) and the antagonist ligands DCKA and MDL 105,519, for which experimental data have been reported for NR1, have been docked to model structures built for the S1–S2 ligand-binding domain of NR1, taking into account the differences in domain closure seen in the X-ray structures of agonist versus antagonist complexes of GluRB, as well as the agonist-bound complexes of GluR0 and QBP. In order to explain the experimental data available for NR1, we have compared the similarities and differences in the interactions seen among the ligand complexes of the related known structures with the corresponding residues found in the NR1 ligand-binding domain. We will show that the models of S1–S2 of NR1, which also reflect our knowledge of the key aspects of agonist and antagonist ligand interactions shared by AMPA and kainate ionotropic glutamate receptors (Pentikäinen et al., 2003), do provide a rationale structural explanation of the published experimental data on NR1.

3.1. Arg523 and Asp732 bind the α-amino and α-carboxylate of agonists with the assistance of Pro516, Thr518, and Ser688

Agonist ligands seen in the template crystal structures share two features important for binding to S1–S2: (a) the α-carboxylate group of the ligand forms a salt bridge with the guanido group of arginine located at position 523 (NR1 numbering; Fig. 2), the carboxylate also accepts hydrogen bonds from the main-chain nitrogen atom from the residues at positions 518 and 688 and (b) the α-amino group of the ligand donates hydrogen bonds to the carboxylate group of either aspartate or glutamate at position 732, to the main-chain oxygen atom at position 516, and to the side-chain hydroxyl group at position 518 (Table 1). Arg523 is completely conserved and Asp/Glu732 is conservatively varied within bacterial periplasmic amino acid-binding proteins (e.g., lysine–arginine–ornithine (Oh et al., 1993), leucine–isoleucine–valine (Sack et al., 1989), and glutamine- (Sun et al., 1998) binding proteins) as well as in

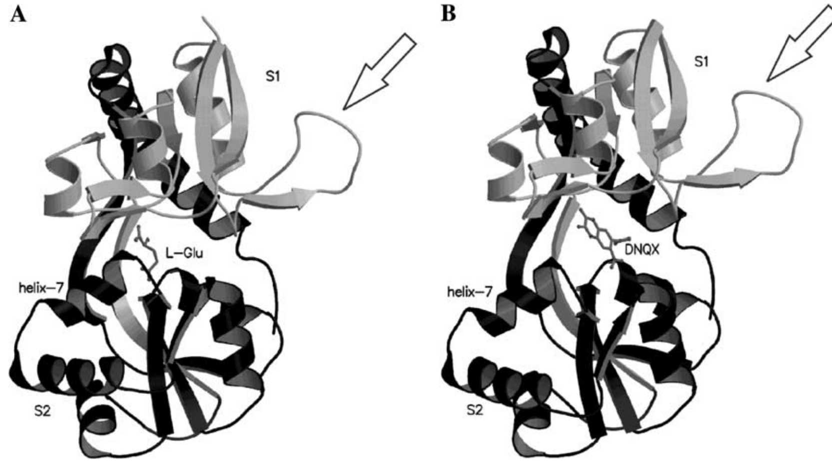

Fig. 1. Differences in domain closure with bound agonist and antagonist ligands can be seen by careful examination of the two structures. Ribbon model of the S1 (gray)–S2 (black) construct of the ligand-binding domain of rat GluRB (A) with the bound agonist L-glutamate (PDB code 1ftj) and (B) the bound antagonist DNQX (PDB code 1ftl). The loop corresponding to the long insertion in NR1 is indicated with arrows. Ligands are presented as ball-and-stick figures. Figs. 1 and 3–5 were prepared with MOLSCRIPT (Kraulis, 1991) and rendered using RASTER3D (Merritt and David, 1997).

the iGluRs, including the NR1 subunit of the NMDA receptor. Thus, it is very likely that similar interactions with agonist ligands are also present in NR1 (Table 1 and Fig. 3).

In addition to the structural evidence for specific interactions obtained from crystal structures of related proteins, the importance of these interactions has also been verified by mutations made to NR1 itself and the

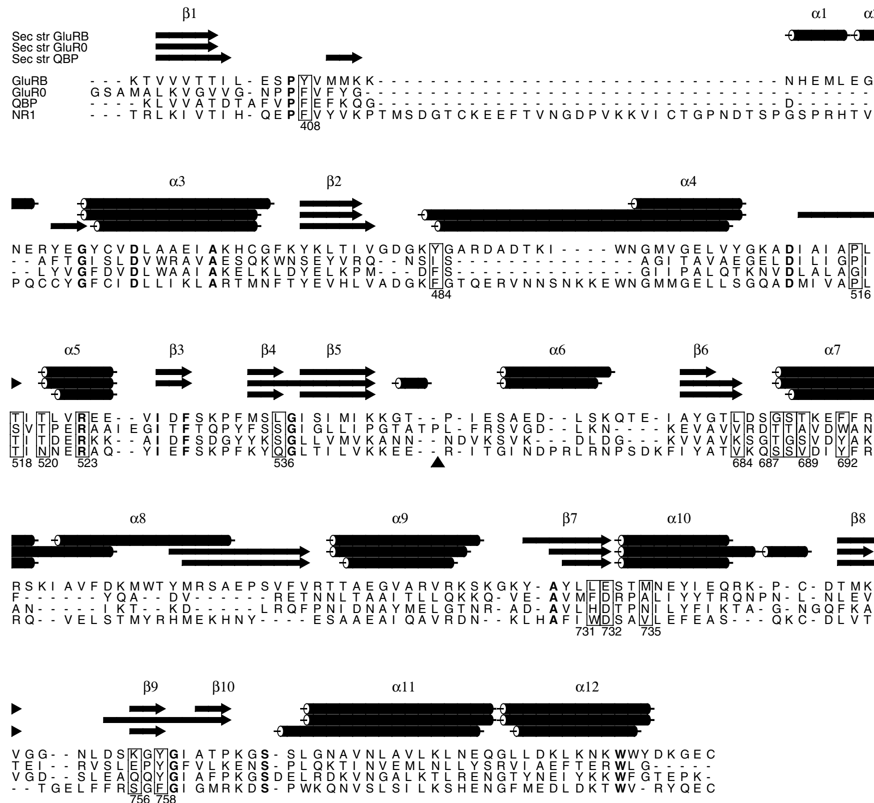

Fig. 2. Structure-based sequence alignment. The sequences of the known structures of GluRB (1ftj), GluR0 (1ii5), and QBP (1wdn) are shown together with the NR1 sequence; the black triangle marks the S1–S2 junction in the engineered S1–S2 constructs of GluRB and GluR0, and the corresponding sequences in NR1. The secondary structure assignments (labels from GluRB only) for the known structures are shown above the alignment. Residues forming the ligand-binding site are boxed (NR1 numbering); identities in all four proteins are in bold. The alignment was produced using VERTAA and MALIGN in Bodil and formatted using ALSCRIPT (Barton, 1993).

effect of these mutations on ligand binding. Arginine at position 523 has been mutated to asparagine and lysine (Hirai et al., 1996) and to leucine (Wafford et al., 1995). In each case, the expression of the NMDA receptor, containing both the mutated NR1 and wild-type NR2 subunits, produced non-functional receptor channels. The mutation of Asp732 in NR1 to glutamate, asparagine, and glycine, has been shown to change the efficacy of ligand binding as measured by EC_{50} values: wild-type NR1, 0.2 μM; Asp732Glu, 849 μM; Asp732Gly, 1736 μM; Asp732Asn, 2892 μM (Williams et al., 1996). The effect of the Asp732Glu mutation is much smaller than for the mutation of Asp732 to either glycine or asparagine, because, the side-chain carboxylate of glutamate can maintain similar interactions with ligands via the longer side chain, but the additional methylene group negates the interaction of the side chain with Gln536. When Asp732 is mutated to glycine, the stabilizing interactions both to the receptor itself via Gln536 and to the α-amino group of the ligand are lost, resulting in a dramatic decrease in the efficacy of bound glycine. With the Asp732Asn mutant, the side-chain amide oxygen atom may be able to interact with both Gln536 and the α-amino group of ligands (Fig. 4), but then one key interaction seen in each of the known structures would not take place. In the crystal structures a water molecule (W1, W2, and W4 in Fig. 3) donates a hydrogen bond to the second oxygen atom of aspartate/glutamate at position 732. In NR1 the amide nitrogen in Asp732Asn would not be able to accept a hydrogen bond from this water molecule (W5 in Fig. 4A) while at the same time maintaining interactions with the ligand and Gln536.

3.2. The residue at position 484 packs against the hydrophobic surface of agonist ligands

In the template structures, hydrophobic residues (tyrosine, phenylalanine or isoleucine) are found at the position equivalent to 484 in NR1, functioning to shield hydrophobic parts of bound ligands from the solvent. In NR1, phenylalanine is present and its conformation is based on that seen for Tyr450 in GluRB (1ftj) (Fig. 3A). Consequently, the C^{α} atom of the bound agonist ligands packs against the phenyl ring of Phe484 in NR1 (Fig. 4). (Note that the conformation of Phe484 was not modeled by using the conformation of Phe50 from QBP (Fig. 3B) because the conformation of the side chain and of the bound ligand (L-glutamine) differs from the other template structures (Figs. 3A and C), and it is likely that the ligand-binding conformation in NR1 is more similar to other glutamate receptors than to QBP.)

In NR1, the effects of mutations on ligand-induced receptor activation by glycine (Kuryatov et al., 1994) support the importance of position 484 for ligand binding. Phe484Ala (3300 μM) and Phe484His (1100 μM) clearly affect the EC_{50} in comparison to wild-type receptor (0.52 μM). Loss of the large aromatic side chain in the Phe484Ala mutant would disturb the hydrophobic packing of the ligand with the receptor and, most likely, the extra space provided by the mutation is occupied by polar water molecules, resulting in reduced binding. When Phe484 is mutated to histidine, the $N^{\varepsilon 2}$ atom of histidine could form a hydrogen bond with the main-chain oxygen atom of Pro516, potentially blocking the interaction between Pro516 and the α-amino group of the ligand.

3.3. Ser687 and Trp731 interact with each other and line the binding pocket

In the template structures various amino acids (Table 1) with varying side-chain conformations are present at position 731 (NR1 numbering). In GluRB (1ftj) the corresponding residue is leucine (Leu704), whose side chain points away from the ligand-binding site and does not have any role in ligand binding (Fig. 3A). In QBP where histidine (His156) is present, the side chain does have an important role in ligand binding: the $N^{\varepsilon 1}$ atom accepts a hydrogen bond from the hydroxyl group of Ser120, while, the $N^{\varepsilon 2}$ atom donates a hydrogen bond to the carbonyl oxygen atom of the γ-group of the ligand (Fig. 3B). Likewise, phenylalanine (Phe313) in GluR0 points towards the ligand-binding site too (Fig. 3C). In the model structures of NR1, the bulky side chain of Trp731 points towards the ligand-binding pocket as well, placing limits on the size of the ligand that can bind to NR1. The side chain of Trp731 is fixed in place by several interactions: a hydrogen bond between its side-chain nitrogen atom ($N^{\varepsilon 1}$) and the side-chain hydroxyl group of Ser687, and hydrophobic packing against Phe484, Val684, Val689, and Ala714 (Fig. 4). In addition, mutation of Ala714 to nearly any residue type, except threonine leads to decreased efficacy (higher values of EC_{50}) with agonist ligands (Wood et al., 1997). In the model structure, the replacement of the residue at position 714 with a bulky side chain would alter the conformation of the Trp731 side chain and interfere with ligand binding.

As seen in the crystal structures of GluR0 and QBP, the side-chain hydroxyl group of threonine at the position corresponding to Ser687 in NR1 (Table 1 and Figs. 3B and C) donates a hydrogen bond to the main-chain oxygen atom of Val274 (GluR0) or Lys115 (QBP). A similar interaction is likely to occur in NR1 between the side-chain hydroxyl group of Ser687 and the main-chain oxygen atom of Val684 (Fig. 4). In NR1 the mutation of serine to glycine only has a marginal effect on receptor activation; the EC_{50} is 13 μM for the mutant in comparison to 0.52 μM for the wild-type receptor (Kuryatov et al., 1994). Based on our model structure, the empty space resulting from this mutation is likely to be filled by a water molecule that could mimic the interactions made by the serine hydroxyl group with Trp731 and Val684.

3.4. Hydrophobic interactions and hydrogen-bonding networks locate around bound ligands

In each of the crystal structures, the position corresponding to 758 in NR1 donates a hydrogen bond to the side-chain carboxylate group of aspartate/glutamate corresponding to Asp732 in NR1 (Table 1). Moreover, the side chain of residue 758 is sandwiched between the residue at position 516 (hydrophobic packing) and the amino acid at position 536 (hydrogen bonding interactions in the case of QBP and GluR0, hydrophobic interactions in the case of GluRB (1ftj)) (see Table 1 and Fig. 3).

In NR1 the side-chain conformation of Asn520, Gln536, Ser688, Tyr692, and Phe758 was selected in order to maximize the hydrogen-bonding network within the ligand-binding site and at the same time to maintain interactions similar to those seen in the template crystal structures (Fig. 3). The residues in NR1 can form similar interactions: the side chain of Gln536 can donate a hydrogen bond to Asp732, and Phe758 is sandwiched in between Pro516 and Gln536. In addition, the side chain of Gln536 is fixed in place by an additional hydrogen bond formed with Thr518 (Fig. 4).

When Phe758 in NR1 is mutated to serine, the NMDA receptor is not functional (Hirai et al., 1996). One plausible explanation is that in the absence of the alanine ligand, Phe758Ser can donate a hydrogen bond to the carbonyl oxygen atom of Gln536 forcing the Gln536 side chain to change its conformation and, consequently, the conformation of Asp732 is altered, preventing the side chain of Asp732 from interacting with the ligand. This assertion is supported by results from the Phe758Ala mutant in which the presence of the methyl side chain, which cannot hydrogen bond to Gln536, has a negligible effect on the EC_{50}: 0.8 μM for the wild-type is reduced to 1 μM for the mutant (Hirai et al., 1996). It is a reasonable suggestion that the empty space provided by the Phe758Ala mutation is filled with one or two water molecule; their presence is not expected to change the orientation of the nearby side chains relative to the wild-type receptor.

In the model structures of NR1 Asn520, Ser688, and Tyr692 form a second hydrogen-bonding network. The side-chain hydroxyl group of Ser688 accepts a hydrogen bond from the side-chain amino group of Asn520 and the side-chain carbonyl oxygen atom of Asn520 accepts a hydrogen bond from the hydroxyl group of Tyr692 (Fig. 4). In each of the template structures, threonine is present at position 520 (Table 1), and the shorter side chain is unable to participate in the hydrogen-bonding network.

3.5. Water molecules play essential roles in ligand binding

The GluRB structure (1ftj) has a water molecule near Gly653, Ser654, and Thr655 at the amino-terminal end of helix-7. Helix-7 in GluRB corresponds to helix-4 in QBP and helix-6 in GluR0. This water molecule (W1 in Fig. 3A) is hydrogen bonded to Lys730, Thr655, Ser654, and Glu705. In QBP a water molecule, W2, is found near Thr118, Gly119, and Ser120 at the amino-terminal end of helix-4 (Fig. 3B). W2 is hydrogen bonded to Ser120, to Asp157 and to a second water molecule, W3. Due to the presence of Gly119 in QBP, W3 occupies the position equivalent to the side-chain hydroxyl group of Ser654 in GluRB (1ftj) and Thr278 in GluR0. In the crystal structure of GluR0 a water molecule (W4 in Fig. 3C) is located near Thr277, Thr278, and Ala279 at the amino-terminal end of the helix-6. Water W4 is hydrogen bonded with Asp314 and Thr278 in a similar way as seen in GluRB and QBP (Fig. 3). These water

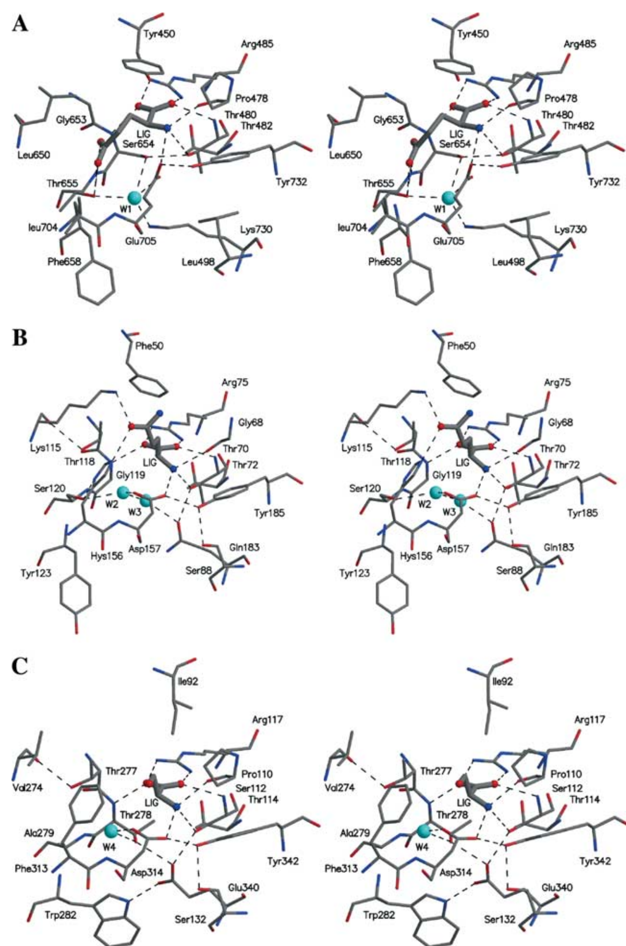

Fig. 3. Binding of agonist ligands in the crystal structures of (A) GluRB with bound L-glutamate (1ftj), (B) QBP with bound L-glutamine, and (C) GluR0 with bound L-serine (in stereo). Residues of the binding sites are numbered according to Table 1 for the individual structures. The ligands (LIG) are shown as ball-and-stick figures. W1–W4 (cyan spheres) refer to water molecules present in the crystal structures. Hydrogen bonds are shown with dotted lines. In (B), the side-chain amide group of Gln183 was flipped by 180° in order to optimize the hydrogen bonding in the structure, and the missing oxygen atom of the α-carboxylate group of the ligand (L-glutamine) was added.

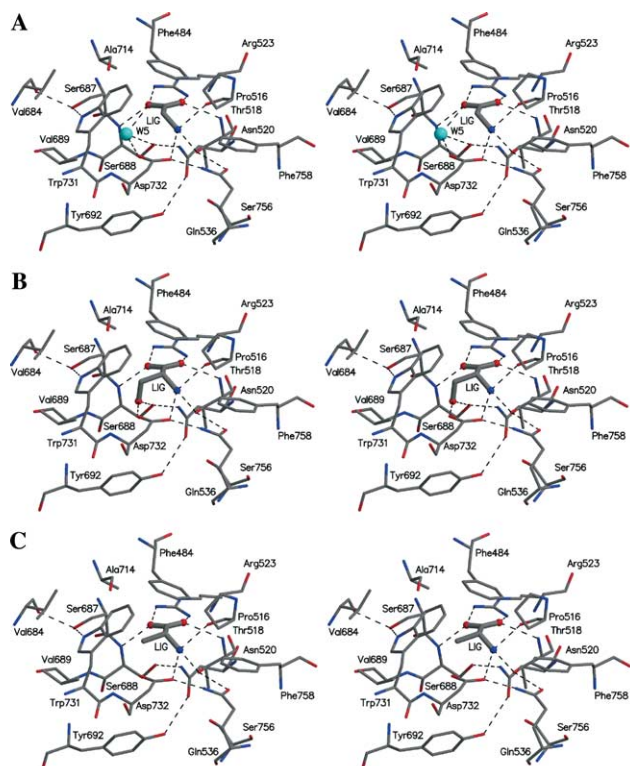

Fig. 4. Agonist ligands docked to the NR1 model structure, agonist-binding conformation (in stereo). (A) Glycine, (B) D-serine, and (C) D-alanine. W5 (cyan sphere), a water molecule likely to be present when glycine is bound, corresponding to the hydroxyl group of D-serine in (B).

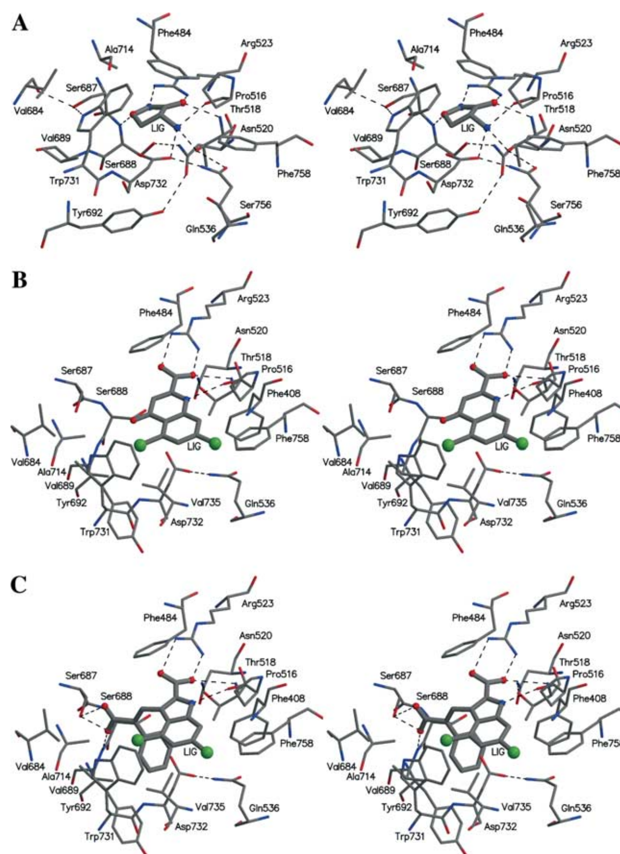

Fig. 5. Partial agonist and antagonist ligands docked to the NR1 model structures (in stereo). In (A) the partial agonist D-cycloserine is docked to the NR1 model, agonist-binding conformation. The antagonists, (B) DCKA, and (C) MDL 105,519, are docked to the model structure of NR1 in the more open antagonist-bound conformation. Chlorine atoms are shown as green spheres.z

molecules in the template structures (W1, W2, and W4 in Fig. 3) have very low crystallographic temperature factors (1ftj: 18.77, 1iit: 19.54, and 1wdn: 14.50), are involved in similar interactions, thus supporting their importance in the hydrogen-bonding network.

Similar to GluRB and GluR0, in NR1 position 688 is occupied by serine. Thus, in the NR1–glycine complex we predict that only one water molecule (W5 in Fig. 4A) would be present having interactions similar to those seen for W1 in GluRB (1ftj), W2 in QBP, and W4 in GluR0. When D-serine is bound to NR1, the hydroxyl group would displace W5 of the glycine–NR1 complex and interact with the amino-terminus of the helix (residues 687–689) corresponding to helix-7 in GluRB and with the side chain of Asp732 and Ser688 of the helix (Fig. 4B). In addition, the longer side chain of D-serine, when compared to glycine, would improve the hydrophobic packing of the ligand with Phe484 and Trp731.

3.6. Agonist binding to NR1

Affinity data for NR1, as measured by IC_{50} values, have only been reported for expressed S1–S2 constructs ((Ivanovic et al., 1998), S1–S2 domain; (Miyazaki et al., 1999), amino-terminal and S1–S2 domains): D-serine (10.7; 4.95 μM) was found to be a better ligand than glycine (27; 13.8 μM). Based on the model structures for the agonist complexes, the hydroxyl group of D-serine (Fig. 4B) would locate to the same position as water molecule W5 would in the case of bound glycine (water W5 in Fig. 4A), and the differences in affinity can be explained by the entropic cost of immobilizing the water molecule when NR1 binds glycine.

Agonist binding to functional NMDA receptor channels containing both the NR1 and NR2 subunits, as measured by EC_{50} values, ranks the following ligands in terms of their ability to activate the receptor: glycine ⩾ D-serine > D-alanine > D-cycloserine > L-serine and L-alanine (Hess et al., 1996; Matsui et al., 1995; McBain et al., 1989; Mothet et al., 2000; Pace et al., 1992; Priestley et al., 1995). D-Alanine should interact with NR1 (Fig. 4C) in similar way as glycine does (Fig. 4A), however, the methyl side-chain of D-alanine would displace water molecule W5 from its position in the glycine complex, affecting the hydrogen-bond network, resulting in a higher EC_{50}. L-Alanine and L-serine are weaker agonists than their D-isomers, reflecting the

steric clashes that would take place between the side chains of the L-isomers and both Phe484 and Trp731 in comparison with models of NR1 in complex with the D-isomers.

3.7. D-Cycloserine, a partial agonist ligand of NR1

4-Amino-isoxazolin-3-one (D-cycloserine) is a partial agonist of NR1 (Priestley et al., 1995; Sheinin et al., 2001). The model structure for the NR1 agonist ligand-binding form was used to study D-cycloserine binding because only small differences in domain closure are seen in the agonist (glutamate) (PDB-code: 1ftj; (Armstrong and Gouaux, 2000)) and partial agonist (kainate) (1gr2, 1ftk; (Armstrong and Gouaux, 2000; Armstrong et al., 1998)) complexes with the S1–S2 construct of GluRB. D-Cycloserine exists substantially as a zwitterion at physiological pH (Lee et al., 1998; McBain et al., 1989); in this protonization state, the amide of the ring is negatively charged and would interact with Arg523 in a similar way as the α-carboxylate group of the agonist ligands does (Fig. 5A). The positive charge of the zwitterion is localized on the amino group at position 4 that, like the α-amino group of the agonist ligands, donates three hydrogen bonds to Pro516, Thr518, and Asp732. The carbon atoms of D-cycloserine can pack against the side chains of Trp731 and Phe484. As in the case of D-alanine discussed above, the methylene group at position 5 in D-cycloserine (Fig. 5A) would displace water molecule W5 proposed for the NR1 complex with glycine (Fig. 4A). Although we have used the agonist-bound model of the NR1 S1–S2 construct, it is clear that the isoxazoline ring of D-cycloserine would form ideal interactions with the binding domain (hydrogen bond from the main-chain nitrogen atom of Ser688 to the isoxazoline oxygen atom and hydrophobic packing of the ring with the side chains of Trp731 and Phe484) in a slightly more open form than is proposed for the agonist ligands.

3.8. Antagonist binding to NR1: DCKA and MDL 105,519

In the structures of the GluRB complexes with the antagonist ligands DNQX (1ftl, (Armstrong and Gouaux, 2000)) and ATPO (Hogner et al., 2003), the receptor remains in much more open form than is seen when binding the partial agonist ligand kainate. Thus, in order to model the binding of antagonist ligands to NR1, a separate structural model for the NR1 S1–S2 construct was built based on the available DNQX complex structure (Fig. 1B).

The NR1 ligand 5,7-dichloro-4-oxo-1,4-dihydroquinoline-2-carboxylic acid (5,7-dichlorokynurenate, DCKA) is an antagonist (Baron et al., 1991). DCKA is a derivative of kynurenic acid, which is an endogenous antagonist of NR1 (Danysz et al., 1989; Moroni et al., 1988). DCKA contains a quinoline ring with a carboxylate group and two chlorine atoms; the carboxylate group would ideally form a salt bridge with Arg523. In the docked complex (Fig. 5B) the nitrogen atom of the quinoline ring donates a hydrogen bond to the main-chain oxygen atom of Pro516, the quinoline ring of DCKA stacks with the side chain of Phe484 (π–π interactions), and the chlorine atom at position 7 is hydrophobic and packs with Phe408, Val735, and Phe758. The chlorine atom at position 5 packs against the indole ring of Trp731 and the methylene group of Asp732.

The antagonist ligand, *E*-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1-*H*-indole-2-carboxylic acid (MDL 105,519; (Baron et al., 1996) binds with much higher affinity to NR1 (0.0039 μM) than is reported for glycine (13.82 μM), D-serine (4.95 μM), and DCKA (0.554 μM) (Miyazaki et al., 1999). MDL 105,519 has a structure similar to DCKA, but an indole ring replaces the quinoline ring. The carboxylate group and two chlorine atoms are attached as in DCKA and participate in equivalent interactions in the docked complex, as does the nitrogen atom in the indole ring and in the quinoline ring, both of which are hydrogen bonded to the main-chain oxygen atom of Pro516 in the models (Figs. 5B and C). MDL 105,519 differs from DCKA by having a bulky 2-phenyl-2-carboxyethenyl substituent at position 3. The carboxylate group of this substituent would interact favorably with the positive dipole at the amino-terminus of the helix corresponding to helix-7 in GluRB (Fig. 5C). The phenyl group of this substituent would pack with the methyl group of Thr518, the C^{β} atom of Ser688, the side chain of Val689, and with the planar hydrophobic surface of the carboxylate group of Asp732. Together, these additional interactions present in MDL 105,519 explain its higher affinity in comparison with DCKA.

4. Conclusions

In order to provide a rational explanation for agonist and antagonist binding to the NR1 subunit of the NMDA receptor it was necessary to produce model structures that reflect both the agonist-binding conformation and the more open antagonist-binding conformation. While GluRB is presently the most closely related protein to NR1 whose structure is known, we have included two other structures in this study. Together with GluRB, solved in complex with a variety of agonist ligands and several antagonists, the structures of QBP and GluR0 in complex with their natural ligands give added insight into the structural features important for ligand binding related to the similarities and differences of the amino acid residue in the vicinity of the ligand-binding site.

The sequence of the NR1 subunit ligand-binding domain shares many features in common with these

defined structures, including the conservation of key residues involved in direct interactions with the α-amino group and the α-carboxylate group of bound ligands. The amino-terminus of helix-F in AMPA receptors like GluRB plays an important role, also fulfilled in GluR0 and QBP, and very likely present and serving the same role in NR1 too. A conserved water molecule is present near the amino-terminus of the helix in GluRB, GluR0, and QBP, taking part in forming an important hydrogen-bonding network at the binding site. In the NR1 model structure, a water molecule is suggested to be located near the helix but only when glycine is the bound ligand, as the hydroxyl-group of D-serine, and possible the methyl group of D-alanine, would occupy this space when these ligands are bound.

NR1 differs from GluRB, GluR0, and QBP in that the preferred natural agonist ligands of NR1 are the small amino acids glycine and D-serine (and D-alanine) and that the L-isomers are discriminated against. The comparative analysis of the binding sites of the iGluRs and QBP with NR1 attributes the large reduction in the size of the binding pocket in NR1 to the presence of Trp731. The indole ring of tryptophan is much more bulky than the corresponding amino acids present in GluRB, GluR0, and QBP, and in the model structures the position of the ring is held in place by hydrogen bonding to the ring nitrogen and by hydrophobic packing with other residues, effectively serving to limit the size of the binding site to glycine plus a water molecule and to D-serine and D-alanine. The L-isomers of serine and alanine bind only poorly to NR1; in their modeled complexes the side chains of the L-isomers would clash with Trp731 and Phe484. The similarities and differences among the different complexes lead to a rational explanation of the effects of different agonist ligands on NR1.

Larger ligands of NR1 are partial agonists, D-cycloserine, or antagonists, DCKA and MDL 105,519, effects that are generally thought to result from holding the ligand-binding domain in a more open form, leading to lower levels of activated receptor. In the case of D-cycloserine the model based on the agonist-bound conformations of the known structures did serve to elucidate the likely structural interactions that take place, in agreement with the available experimental data, but it was also clear that this ligand would form ideal interactions in the modeled complex if the model reflected a slightly more open form of the domain, similar to that seen for the structure of the partial agonist kainate in complex with GluRB. Model structures of NR1 based on the antagonist-bound DNQX–GluRB complex structure provide an appropriate degree of receptor closure for the docked antagonists DCKA and MDL 105,519. The ring structures of DCKA (quinoline), but especially MDL 105,519 (indole), with their attached carboxylate group and two chlorine atoms, can form a larger number of interactions with the S1–S2 domain of NR1 than can D-cycloserine or any of the agonist ligands, explaining the much higher affinity of the antagonists in comparison with the agonist ligands.

After the submission of this work for publication, crystal structures of NR1 in complex with D-serine, glycine, D-cycloserine, and DCKA were published (Furukawa and Gouaux, 2003). We have thus compared the X-ray structures of the complexes with the modeled interactions reported here. Despite the presence of numerous differences between our model and the X-ray structures, the size, and shape of the ligand-binding site and the types of interactions are very similar. Consequently, the predicted ligand-binding conformations and the interactions between ligands and NR1 are also highly similar to those seen in the crystal structures of the ligand complexes. Moreover, the conformations of the ligands were essentially identical in the NR1 structures and in the model structures.

In our model structures of NR1 a large number of intramolecular hydrogen bonds, mainly involving side-chain interactions, were predicted to stabilize the receptor structure and these features were based on the closest known structure, GluR-2. In the crystal structures of NR1 these direct intramolecular interactions are often replaced by indirect links via structural water molecules. As a result, the conformations of the polar side chains in NR1 model structures are different from that in the NR1 X-ray structures. In contrast, the conformations of the hydrophobic side chains were predicted with high accuracy even though the main chain trace differed between the models and the NR1 X-ray structures. The largest difference was seen for Trp731 where the ring is flipped by 180°, but the side chain occupies the same relative location in the model structures and in the NR1 structures.

The main features of ligand binding to NR1 as revealed by the X-ray structures are very similar to what was predicted in the model structures. Thus, key interactions were predicted correctly between the ligands and Arg523, Asp732, Ser688, and Thr518 of the binding pocket. However, the orientation of the side-chain carboxylate group of Asp732, which is hydrogen bonded to the α-amino group of agonist ligands, is rotated 90° in comparison with the predicted structures as well as with respect to the structures of GluRB, GluR0, and QBP. We also had predicted the involvement of a water molecule in binding the Asp732 side chain near helix-7 where there was space available in the model structure (see Fig. 4A); this water molecule is not seen in the NR1 structure.

While model structures are very useful starting points for investigating receptor–ligand interactions and in the design of new ligands when the authentic structures are not available, X-ray structures of ligand complexes provide solid evidence for modes of binding since even

common features present in known related structures can and do differ. The structures of GluRB, GluR0, and QBP share from 18 to 32% sequence identity with NR1. Nonetheless, despite the relatively low sequence similarity between NR1 and the template structures and the resulting errors found in the modeled complexes, essential features of the binding cavity and key interactions with the ligands are reflected in the model structures and they would still have served a useful role in helping us understanding the binding function of the NR1 receptor in the absence of the authentic structures.

Acknowledgments

This study was supported by the Academy of Finland, the Technology Development Center of Finland, the National Graduate School in Informational and Structural Biology, the University of Padova—Italy—(Post-Lauream scholarship), CSC Finnish IT Center for Science and Fatman Bioinformational Designs Ltd.

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