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Crystal structure of the kainate receptor GluR5 ligand-binding core in complex with (*S*)-glutamate

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Abstract The X-ray structure of the ligand-binding core of the kainate receptor GluR5 (GluR5-S1S2) in complex with (*S*)-glutamate was determined to 1.95 Å resolution. The overall GluR5-S1S2 structure comprises two domains and is similar to the related AMPA receptor GluR2-S1S2J. (*S*)-glutamate binds as in GluR2-S1S2J. Distinct features are observed for Ser741, which stabilizes a highly coordinated network of water molecules and forms an interdomain bridge. The GluR5 complex exhibits a high degree of domain closure (26°) relative to *apo* GluR2-S1S2J. In addition, GluR5-S1S2 forms a novel dimer interface with a different arrangement of the two protomers compared to GluR2-S1S2J.

Keywords: Ionotropic glutamate receptor; Kainate receptor; GluR5 ligand-binding core; Crystal structure

1. Introduction

Ionotropic glutamate receptors (iGluRs) form a family of ligand-gated ion channels that play a central role in rapid neuronal signaling in the central nervous system. The complex roles of the iGluRs are far from being understood in detail but it is generally accepted that these receptors are implicated in a number of psychiatric and neurological disorders such as Alzheimer's, Parkinson's and Huntington's diseases, schizophrenia and epilepsy [1–3]. Hence, the iGluRs are being pursued as obvious drug targets.

The iGluRs are membrane-bound and form tetramers assembled as dimers-of-dimers [4–8]. One receptor subunit is composed of an extracellular N-terminal domain, a ligand-binding core made of segments S1 and S2 forming two domains (D1 and D2), three transmembrane spanning regions (M1–M3) and a re-entrant loop between M1 and M2 as well as a cytoplasmic region. The current picture on iGluR function implies a "Venus flytrap" mechanism of the bilobular ligand-binding core that in the resting state (without ligand bound) is in an open form and upon binding of agonist, it adopts a closed form with concomitant opening of the channel pore. The extent of domain closure is correlated to the activation and desensitization of the receptor [1,9–13].

The iGluRs have been subdivided into three heterogeneous classes based on sequence identity and their affinities for the selective agonists 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate (AMPA), kainate and *N*-methyl-D-aspartate (NMDA). A soluble construct of the ligand-binding core of the AMPA receptor GluR2 (GluR2-S1S2J) [9] and of the NMDA receptor NR1 [14] have previously been expressed in *Escherichia coli* and both were shown to have pharmacological binding profiles resembling those of the full-length receptors. The subsequent crystallization and structure determinations of these two constructs in complex with different agonists [9–11,14–16] and antagonists [9,17] have provided a wealth of information on binding modes and mechanisms of action of the AMPA and NMDA classes of receptors. Until now, however, this knowledge has been missing for the kainate class.

Here, we report the X-ray structure of the ligand-binding core of GluR5 (GluR5-S1S2) in complex with the endogenous neurotransmitter (*S*)-glutamate. The GluR5 receptor belongs to the kainate class of iGluRs. Hence, the present structure provides detailed information on hitherto unknown modes of interaction of (*S*)-glutamate with the first representative of this class of iGluRs.

2. Materials and methods

2.1. Expression and purification

The construct for expression of the ligand-binding core of GluR5 was made as follows. The S1 and S2 segments constituting the ligand-binding core were polymerase chain reaction (PCR)-amplified from full-length GluR5 using the primers: S1-forward: ATTTGAATTCCGTGGTGCTAACCGCACACTCATTG; S1-reverse: ATTAGGTACCCTTCCGGTAAAGGATGC; S2-forward: ATTAGGTACCCCCATCGATTCCGCAGACG and S2-reverse: AATTCTCGAGTTAAGGGCAGCCATTCCCC. PCR-products were digested with restriction enzymes and ligated into pET-28a(+) (Novagen, WI, USA) in a three-point ligation. The expressed protein contains an N-terminal His-tag, a trypsin cleavage site and the GluR5 sequence [430-ANRTLI...SILYRK-544], [667-PIDSAD...WRGNGC-805] separated by a Gly-Thr linker. An N-terminal glycine in the

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Abbreviations: 4-AHCP, 2-amino-3-(3-hydroxy-7,8-dihydro-6*H*-cyclohepta[*d*]-4-isoxazolyl)propionate; AMPA, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate; ATPA, 2-amino-3-(5-*tert*-butyl-3-hydroxy-4-isoxazolyl)propionate; *E. coli*, *Escherichia coli*; EDTA, ethylenediaminetetraacetate; GluR2-S1S2J, ligand-binding core construct of GluR2; GluR5-S1S2, ligand-binding core construct of GluR5; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate; iGluR, ionotropic glutamate receptor; IPTG, isopropyl-β-D-thiogalactopyranoside; M1–M3, three transmembrane spanning regions; NMDA, *N*-methyl-D-aspartate; NR1-S1S2, ligand-binding core construct of NR1; r.m.s.d., root mean square deviation; PCR, polymerase chain reaction; PEG, polyethylene glycol; vdW, van der Waals

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crystallized GluR5-S1S2 construct is a reminiscence of the trypsin cleavage site. The numbering corresponds to GluR5-2. The sequence was verified by sequencing.

The GluR5 ligand-binding core expression construct was transformed into the *E. coli* cell line Origami 2 (Novagen). Two liters of Hyper Broth (Athenaes, MD, USA) containing 30 μg/ml kanamycin and 12.5 μg/ml tetracycline were inoculated with an overnight culture and grown to an OD_{600} of ca. 1.8 at 37 °C, 120 rpm before induction with isopropyl-β-D-thiogalactopyranoside (IPTG) to 250 μM. Protein was expressed overnight at 25 °C, 120 rpm shaking.

After cell harvesting and centrifugation, the protein was purified by Ni^{2+} affinity chromatography using Chelating Sepharose (Amersham Pharmacia, Uppsala, Sweden) and eluted with a buffer containing 20 mM Tris–HCl, pH 7.5, 500 mM NaCl and 200 mM imidazole. Following dialysis against 100 volumes of buffer containing 20 mM NaOAc, pH 5.5, 1 mM (*S*)-glutamate and 10 mM ethylenediaminetetraacetate (EDTA), the His-tag was cleaved of by trypsin digestion. The protein was applied to a Mono-S cation exchange column (Amersham Pharmacia) in 20 mM NaOAc, pH 5.5, 1 mM (*S*)-glutamate and 10 mM EDTA, and eluted with a gradient of 0–200 mM NaCl. A final gel filtration step was done on a Superdex 75 column (Amersham Pharmacia).

2.2. Crystallization and data collection

For crystallization of the complex, a 5.2 mg/ml GluR5-S1S2 solution (10 mM HEPES, pH 7.0, 30 mM NaCl and 1 mM EDTA) containing 20 mM (*S*)-glutamate was used, giving a protein to ligand ratio of 1:110. Crystals were obtained at 6 °C by the hanging drop vapor diffusion method. Drops of 1 + 1 μl protein and reservoir solutions were applied; the reservoir solution consisting of 17% polyethylene glycol (PEG) 4000, 0.1 M phosphate-citrate, pH 4.0 and 0.4 M lithium sulfate. Crystals were transferred through a cryo-protectant with 20% glycerol in reservoir solution prior to flash-cooling in liquid nitrogen.

The X-ray diffraction data were collected at cryogenic temperature with wavelength of 0.8128 Å using a MAR CCD detector at beamline X11 (DESY, Hamburg, Germany). The crystal diffracted to 1.94 Å. The HKL package (Denzo and Scalepack) [18] was used for autoindexing and data processing; for statistics see Table 1.

2.3. Structure determination and refinement

The GluR5-S1S2:(*S*)-glutamate structure was determined by the molecular replacement method using CaspR [19]. The crystal structure of GluR2-S1S2J in complex with (*S*)-glutamate (MolA, pdb code 1FTJ) was used as the input structure. A solution comprising two molecules was obtained. Automated model building was performed with the program ARP/wARP [20], resulting in the tracing of 83% of the residues. The missing residues were built manually using the program O [21]. Refinement of the structure was performed with the program REFMAC 5 [22] as implemented in the CCP4 suite of programs [23]. For statistics on refinement, see Table 1.

2.4. Structure analysis and figure preparation

The HINGEFIND script [24] implemented in the program VMD [25] was used to calculate the ligand-induced domain closure relative to the *apo* GluR2-S1S2J structure (pdb code 1FTO, MolA). The CCP4 program CONTACTS was used in the analysis of protein–ligand and protein–protein interactions. The interface accessible surface area was generated by the Protein–Protein Interaction Server [26]. The programs Pymol [27] and Molscript [28] were employed in the preparation of figures.

2.4.1. Protein data bank accession number. The atomic coordinates and structure factors of GluR5-S1S2:(*S*)-glutamate have been deposited with the RCSB Protein Data Bank under the Accession code 1YCJ.

3. Results and discussion

3.1. The crystal structure of GluR5-S1S2

The structure of the ligand-binding core of GluR5 is shown in Fig. 1. Two similar molecules (MolA and MolB) are observed in the asymmetric unit of the crystal (with root mean square deviation (r.m.s.d.) on 250 Cα atoms of 0.57 Å). All residues of MolB (the S1 residues 430–544, a Gly-Thr linker and the S2 residues 667–805) were traced. The C-terminal residues Arg800-Pro805 of MolA could not be modelled.

Table 1
Crystal data, data collection and refinement statistics

Space group	$P2_1$
Unit cell parameters	
a (Å)	51.7
b (Å)	57.9
c (Å)	88.8
α (°)	90.0
β (°)	102.5
γ (°)	90.0
Molecules (a.u.)	2
Mosaicity (°)	0.26
Resolution range (Å)[a]	25.00–1.94 [2.01–1.94]
Unique reflections	37 489
Average redundancy	3.1
Completeness (%)	99.7 [100]
R_{sym} (%)	5.0 [31.4]
$I/\sigma(I)$	18.5 [3.9]
Refinement range (Å)	20.3–1.95
Non-hydrogen atoms	4492
Amino-acid residues	507
(*S*)-glutamate	2
Sulfate ions	7
Water molecules	398
R_{work} (%)[b]	19.9
R_{free} (%)[c]	26.9
R.m.s.d. bond lengths (Å)/angles (°)	0.021/1.9
Residues in allowed regions of Ramachandran plot (%)[d]	98.7
Average *B*-values (Å^2) for protein/Glu[e]/water/sulfate atoms	28.5/16.1/36.9/65.0

[a]The values in brackets correspond to the outermost resolution shell.
[b]$R_{work} = \sum_{hkl}(||F_{o,hkl}| - |F_{c,hkl}||)/|F_{o,hkl}|$, where $|F_{o,hkl}|$ and $|F_{c,hkl}|$ are the observed and calculated structure factor amplitudes.
[c]R_{free} is equivalent to the R_{work}, but calculated with reflections omitted from the refinement process (5% of reflections omitted).
[d]The Ramachandran plot was calculated according to [36].
[e]Glu denotes (*S*)-glutamate.

As expected from sequence alignments, the structure of the ligand-binding core of GluR5 is very similar to that of the AMPA receptor GluR2 and structurally less related to the glycine preferring NMDA receptor NR1 (Fig. 1). From a structural alignment of GluR5-S1S2 and GluR2-S1S2J it is evident that the main overall differences are located to five surface loops, especially in the vicinity of residues 449 and 711. Also, variations are found next to Asn493, Thr730 and Gln747, where deletions have occurred in GluR5, and at the N- and C-terminal regions (see Fig. 2). Furthermore, the positions of the α-helices from residues 774–787 (GluR5 numbering) are slightly shifted.

3.2. Interactions of (*S*)-glutamate with GluR5-S1S2

(*S*)-glutamate binds in a very similar manner to the ligand-binding cleft in both molecules. The interactions between ligand and protein include hydrogen bonds, ionic interactions and van der Waals (vdW) interactions. The α-carboxylate group interacts with Thr518 and Arg523 of D1, and with Ser689 of D2 (Table 2 and Fig. 3). The α-ammonium group forms a tetrahedral network of interactions with protein residues Pro516 and Thr518 of D1, and Glu738 of D2. The γ-carboxylate is involved in hydrogen bonding to Ser689 and Thr690. Four areas in both D1 (residues 489–490, 516–518,