	Experiment title: Crystallographic analysis of the full-length glutamate receptor agonist-binding domain	**Experiment number**: LS-1892
Beamline: ID14-2 ID14-4	**Date of experiment**: from: 08.05.01 to: 09.05.01 27.06.01 28.06.01	**Date of report**: 30.08.2001
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Background

Glutamate receptors located in the post-synaptic membranes of neuronal cells are the primary mediators of excitatory synaptic signals in the central nervous system. A soluble ligand-binding domain (called S1S2) can be separately expressed in recombinant form and retain its full ligand-binding activity and pharmacological selectivity (1). Atomic resolution structures of a truncated version of S1S2 have been reported in the apo form and in complex with various ligands (2, 3). These structures showed a clear conformational change associated with agonist binding. The coupling between these changes and gating of the ion channel is mediated by peptides that connect S1S2 to the receptor's transmembrane domains; these peptides were omitted from the truncated S1S2 construct. The peptides are known to influence activation and desensitization in the intact receptor (4-6) and constitute ca. 20% of the molecular mass of the domain. In a previous experiment (LS1751), we collected a 3.2 Å resolution native dataset on $P2_12_12$ crystals (unit cell: 96.9 x 176.5 x 87.6 Å) of a "full-length" S1S2 construct containing these peptides. These crystals yielded a molecular replacement solution, using the core domain structure as a search model. The domains pack as NCS-related dimers, and the dimers then interact via the connecting peptides (manuscript in preparation). This interaction may mimic the "dimer of dimers" association that has been postulated for the channels (8). To improve the definition of the connecting peptides' electron density, we have sought heavy-atom derivatives of the full-length crystals. In addition, we know from stopped-flow experiments (7) that ligands dock in the open binding site before

conformational change occurs. We have now captured conformational intermediates that reveal the initial docking sites of ligands to the empty binding site.

Results

Measurements were performed on heavy-atom soaked crystals that had been screened for diffraction quality during LS-1751, but could not be collected due to an optics failure. 5 datasets were collected between 2.9 and 3.5 Å resolution, but none were derivatized (see Table). Datasets were also collected from 3 docking intermediate complexes: apo-S1S2 crystals were flash-cooled after brief soaks in saturating concentrations of the antagonists CNQX and NBQX (neither have been structurally characterized) and of the partial agonist kainate. Complexes with the physiological agonist glutamate did not exhibit high-resolution diffraction. Phases were established for all complexes using molecular replacement with the published structure of the GluRB binding domain core (1FTO; ref. 3). The NBQX-soaked crystal form did not reveal difference density at the binding site for ligand, but the soaks with CNQX and kainate did. Refinement of these structures is currently underway.

Table 1: GluRB data sets collected during LS-1892

Data set	**Soak**	**Res. ( )**	**R_{merge} (%)**	**$\langle I \rangle / \langle \sigma_I \rangle$**	**Compl. (%)**	**Aim**	**Result**
443c4ag	10 mM $AgNO_3$ (48h)	3.4	15.3 (53.4)	7.9 (2.5)	79.8 (63.3)	MIR phases	No derivative
440eu	10 mM Eu^{3+} (2 min)	2.9	10.6 (60.4)	10.4 (2.2)	90.7 (94.7)	MIR phases	No derivative
444a1osa	10 mM $OsBr_6$ (5h)	3.2	7.5 (55.5)	12.3 (2.4)	86.7 (97.5)	MIR phases	No derivative
443c4urb	1% UO_2^+ (2h)	3.2	19.0 (91.2)	8.3 (1.9)	85.9 (96.3)	MIR phases	No derivative
430b6xe	3 bars xenon (20 min)	3.5	-	-	-	MIR phases	Poor quality
421kab	1 mM kainate (10 min)	3.3	11.0 (40.5)	9.7 (3.1)	87.5 (68.2)	Intermediate agonist-bound conformation	Under refinement
502b55	0.1 M NBQX (40 min)	3.3	14.3 (50.4)	7.9 (3.11)	88.1 (71.0)	Antagonist-bound structure	No NBQX bound
421cna	1 mM CNQX (25 min)	3.4	13.8 (42.8)	9.9 (3.6)	85.9 (66.6)	Antagonist-bound structure	Under refinement

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