	Experiment title: X-ray diffraction studies of the glutamate receptor soluble binding domain	**Experiment number**: LS-1751
Beamline: ID14-4	**Date of experiment**: from: 18.11.2000 to: 20.11.2000	**Date of report**: 8.2.2001
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Report:

NB: This is a preliminary report on measurements conducted in November 2000. The final report will be submitted 1 Sept 2001, as required.

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. Within the large family of ligand-gated ion channels, they are distinguished by the fact that 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 addition, the crystal structures of the truncated S1S2

showed it to be packed in a tight dimer in several crystal forms. The glutamate receptors are thought to be tetramers (or possibly pentamers) with a 4-fold symmetric channel. If the S1S2 dimer is physiologically relevant, the receptor may be a "dimer of dimers", in which case there must be a transition between the S1S2 2-fold symmetry and the channel 4-fold symmetry. This transition would also be mediated by the connecting peptides.

We have crystallized a "full-length" S1S2 construct containing these peptides. In a previous experiment (LS1448), we collected a 2.5 Å resolution native dataset on *P*2 crystals (unit cell: 86.3 x 86.0 x 187.1 Å, β=98.5°) grown from enzymatically deglycosylated protein. These crystals proved very labile in the presence of heavy atoms. Molecular replacement with the published S1S2-kainate structure (2) was unsuccessful. Recently, we identified different crystallization conditions that yielded $P2_12_12$ crystals (unit cell: 96.9 x 176.5 x 87.6 Å) of our protein expressed in the presence of tunicamycin, which inhibits glycosylation during synthesis. These proved more suitable for heavy-atom derivatization. Our goal was to obtain MAD or MIR phases.

Results

Heavy-atom soaked crystals had been screened for diffraction quality at BW7A at DESY. We identified 16 candidate crystals in 9 conditions. In addition, we identified 6 candidate native and bromide-soaked crystals for high-resolution data collection. Datasets were collected from 11 crystals. This included 5 three-wavelength MAD datasets and 5 single-wavelength datasets from atoms whose edge energy was not accessible at ID14-4. Five further crystals that had been screened during the session and looked promising could not be collected due to an optics failure at the end of the experiment. The resolution of the datasets varied from 3.2 – 4.0 Å, with generally > 98% completeness. We are currently reprocessing the data with a new version of XDS. Definitive statistics will be included in the final version of this report (due 1 Sept 2001).

A model of the apo form of the truncated S1S2 was published at the time of these experiments (3). It was used in a molecular replacement search with the best bromide dataset (3.2 Å). A convincing MR solution has recently been obtained, with 4 molecules/a.u. (15-4 Å, CC=0.602). A solution has also been found in the native deglycosylated S1S2 crystals that diffracted to 2.5 Å resolution (8 mol./a.u., 15-4Å, CC=0.598). Refinement of both structures is underway. Exploiting the extensive non-crystallographic symmetry, we have achieved an R_{free} of 30.0% (8-2.5 Å). The dimers are packed such that their local two-fold axes are tilted relative to the crystallographic two-fold. The connecting peptides are oriented toward the crystal two-fold, where they mediate crystal contacts with a symmetry-related molecule. This provides an example of a possible "dimer of dimers" packing for the receptor.

Using phases from the molecular replacement searches, we are in the process of screening all heavy-atom datasets for the presence of derivatives that would provide additional, model-independent phase information. This may prove particularly important in locating the ends of the connecting peptides, which are currently poorly defined.

These data provide the basis for structure solutions of ligand co-crystals, which should reveal how conformational changes propagate into the connecting peptides (and thus to the transmembrane domains). In addition, we know from stopped-flow experiments (7) that ligands dock in the open binding site before a conformational change occurs. The current structures prepare the way for crystallographic analysis of such docking complexes.

References:

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