ESRF	**Experiment title:** X-ray diffraction tudies of microcrystals of the glutamate receptor soluble binding domain.	**Experiment number:** LS1448
Beamline: ID14-4	**Date of experiment:** from: 26.11.99 to: 28.11.99	**Date of report:** 29.02.00
Shifts: 3	**Local contact(s):** Steffi ARZT	*Received at ESRF:* - 3 MAR. 2000

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Report: ***Introduciton*** Glutamate receptors located at the surface of neuronal cells are the primary mediators of the excitatory synaptic response in the central nervous system. Within the large family of ligand-gated ion channels, they are the only channel-receptor for which a soluble binding fragment (called S1S2) can be isolated recombinantly and retain its full binding activity and selectivity (Kuusinen *et al.,* 1995). This subdomain of the glutamate receptor shares some homology with bacterial periplasmic binding proteins (Nakanishi *et al.,* 1990), and it was proposed to function in a similar way, i.e. a cleft closure of S1S2 induced by binding of glutamate which would in turn trigger channel opening, and ion flux. However our recent SAXS measurements on the GluR-B S1S2 domain indicate that there might be some differences (Abele *et al.,* 1999). An atomic resolution structure of a truncated ligand binding domain S1S2 of the glutamate receptor-B in complex with kainate was reported (Armstrong *et al.*, 1998). However, in order to completely understand the conformational changes associated with the ligand binding and channel opening triggering step, the structure of an unliganded form of the receptor is a high priority. We have obtained crystals of the S1S2 domain of the GluR-B in the absence of ligand; in addition our molecule includes peptides (=25% of total MW) omitted from the reported kainate complex structure

that link the core of the domain to the channel and that couple ligand binding to channel opening and desensitization.

Result Typically, crystals of S1S2 have a size of 100 x 100 x 50 micron, do not diffract to high resolution on a lab source, and show important variation in diffraction quality between crystals from the same drop with some showing partial twinning. Initial freezing conditions were established from work at ESRF and DESY. However, it was observed that a large amount of crystals needed to be screened in order to find a good diffracting one, and the mosaic spread of the crystals remained very high. Most of these diffracted to 5-7 Å and were partially twinned, and showed high variability in the unit cell parameters. From these data, the space group could be determined. The GluR-B S1S2 crystals are of space group P2 with cell parameters of 87.0 Å x 87.0 Å x 230.0 Å, ß=100° and four molecule in the asymetric unit. In order to improve the diffraction quality of the crystals two approaches were taken: new freezing conditions were investigated, and the protein was chemically deglycosylated prior to crystallization. It was observed that the combination of new cryoprotection conditions and deglycosylation of the protein significantly improved the resolution of the crystals. However variability in the crystals still remain. We were able to collect a complete native dataset at 2.8Å resolution on ID14-3; this crystal showed partial merohedral twining. Like the original low diffracting crystals, these are also of space group P2 but with a contracted cell; we observed a 20% reduction in the long c axis. Using this high resolution dataset, attempts to solve the structure by molecular replacement using the available kainate complex structure have failed. More recently we have collected a 2.8Å dataset from a crystal that showed no twinning (on LS1150). Structure determination will be carried out with MIRAS and/or MAD experiments. Initial heavy atom screening has been done on native low resolution diffracting crystals. This has allowed us to reduce the number of potential heavy atom candidates to try with the deglycosylated high resolution diffracting crystals. **The currently reported experiment at ESRF allowed us to verify the effect of chemical deglycosylation and new freezing conditions on the quality of our crystals, and thus obtain our first high resolution dataset of the GluR-B S1S2 binding domain. The crystal diffracts to 2.5 Å resolution. However the data is usable only to 2.8 Å with an R-factor of 22% and 98.5% completeness in the last resolution shell. The overall R-factor is 8.0% with 97% completeness**.