ESRF

Experiment title: Crystal structure of the actin binding domain of utrophin (a dystrophin homologue).	**Experiment number:** LS 789
Beamline: BM14	**Date of experiment:** from: 19/1/98 to: 23/1/98	**Date of report:** 20/2/98
Shifts: 9 awarded, 8 allocated, 7 useable	**Local contact(s):** Dr A. Thompson	*Received at ESRF:*

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Report:

This is a preliminary report in order to apply for follow on beam time. A fuller report will be produced when analysis is complete. Analysis has been delayed by moving institution two weeks after the beam time.

Experiment 1

A small region on the end of a large multiple crystal (stacks of thin crystals) of human utrophin 25-261 containing selenomethionine was found that was single and diffracted to 3.2 Å in space group C2 (a=150 Å, b=55 Å, c=80 Å, β=106°). 180° at three wavelengths (0.9809, 0.9795 and 0.900 A) were collected. Inspection of this indicated that the last 45" of data were weaker either due to less volume in the beam or crystal decay. The worst values for the three 135° data sets were 96 % completeness, 81.5 percent of anomalous pairs measured, 2.7 multiplicity and 15.8 mean I/sigma.

Estimates of the F_A was made with the program REVISE (CCP4). These were fed into SHELXS90, which gave 6 clear sites. Refining these and looking for further site in the anomalous Fouriers gave all 10 Se sites expected. These sites were used to give a map with a 'free R factor' of 0.295 after solvent flattening with DM or a mean figure of merit of 0.908 with SHARP/SOLOMON. I expect to be able to build the structure into this in the next few weeks, particularly as I determined the structure of utrophin 150-258 to 2 Å resolution (roughly half this construct) last year (manuscript in preparation).

Experiment 2

Small crystals of the homologous region of human dystrophin have recently been obtained. A few of these were successfully frozen in the beam. A data set integrated to 3.5 Å was collected off one of these. The space group was P1 (a=60, b=80, c=83 Å, α=61°, β=83°, γ=70°). Rmerge was 0.105. Completeness was 98% and multiplicity 2.9 (270" were collected as this was the last experiment done). This is predicted to have 4 copies in the asymmetric unit. As the sequences are similar, it is expected that the structures will be similar enough to allow molecular replacement.

Experiment 3

3.2 Å data sets were collected at two wavelengths (1.008 Å and 0.829 Å) from methylmercury derivatised crystals of a single site cysteine mutant of utrophin 28-261. This was in the $P2_1$ form previously observed in the laboratory (a=69, b=58, c=144 Å, β=102°. Pseudocentring peaks dominate initial difference and anomalous Pattersons from this data. This awaits further analysis.

Experiment 4

Small crystals of utrophin 28-261 and 28-254 have recently been obtained from two further precipitants. One of these had a very large cell as was very poorly ordered. The other was in space group P422 Cell a=b= 172 Å c=185 Å. These crystals diffracted to a similar resolution of around 3 A. They had a much lower mosaic spread after freezing and the data from the two crystals tried merged much better than the $P2_1$ form does. However there are 6- 10 copies of the molecule in the asymmetric unit so molecular replacement phasing is unlikely. Unless larger crystals of this form can be shown to diffract to higher resolution than the monoclinic forms this will not be pursued.

Biological significance

The absence of dystrophin causes the muscle wasting disease Muscular Dystrophy. Utrophin is an autosomal homologue of dystrophin. They function as linkers between the actin cytoskeleton and the plasma membrane. Both proteins have an actin-binding region at their N-terminus that consists of two calponin homology domains. Point mutations in the actin-binding region of dystrophin cause disease that can be as severe as deletion of the full gene. Expression of utrophin either by upregulation or gene therapy has been suggested as a method of alleviating symptoms and studies in mouse models support this. However in normal cells there are clear differences in localisation of utrophin and dystrophin. Detailed comparison of the actin-binding domains will help to understand the functional differences between these two proteins.