ESRF

Experiment title: A FIBRE DIFFRACTION STUDY OF THE ACTOMYOSIN COMPLEX.

Experiment number: LS-135

Beamline: 4 /ID2

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Report:

This was our first visit to the small angle, undulator beamline and the main aims of our initial experiments were the following:

1. To assess the longevity of a skinned muscle fibre preparation in such an intense beam as part of a feasibility trial for future time resolved experiments.
2. To find the optimum x-ray flux for our present steady state experiments.
3. To assess the detection systems available on BL4 for our present experimental purposes, i.e gas detector vs.imaging plate system.
4. To take exposures of the actin filament pattern from non-overlapped muscle fibres, with sufficient signal to noise extending out to ea. 10Angström, before and after decoration with myosin head motor protein.

We were able to answer the first three questions and obtained data from actin filaments which are unfortunately not good enough for accurate analysis but that convince us that the experiment is possible. The findings are summarised below:

1. The lifetime of skinned rabbit psoas fibres in the full flux beam of $2*10^{13}$ photons/sec on BL4 was <lsec at 20°C in the relaxed state! Not encouraging, but at 0°C this was significantly improved and ME-action patterns showed no sign of deterioration for the first 4sec, although free radical damage is clearly a slower process and fibres surviving during the few seconds of irradiation decay within a few minutes of the exposure. This is very encouraging indeed as it means that a

number of "one-shot" time resolved experiments should be possible following reaction initiation using caged-calcium ions and caged nucleotides to yield more detailed information than hitherto possible. We must await the development of high count rate detectors.

2. The optimum x-ray flux for steady state experiments in which skinned muscle fibres have to survive several x-ray exposures for comparative purposes was found to be ca. 10^{11} photons/sec on BL4. This means reducing maximum flux ca. 50-1 100X . This was found by exposing different pieces of the same muscle fibre to a constant dose of x-rays of differing intensities. Diffraction patterns were examined and the fibre birefringence of the exposed regions was monitored as a function of time. Fibres survived the chosen dose at the highest fluxes but showed subsequent decay monitored by the birefringence. The extent of this decay reduced as intensity was reduced. However, it appeared that lowering the flux beyond the above value, and using longer exposure times, ca. 5 reins, produced patterns with some evidence of filament lattice disordering. There are clearly different rate processes involved in radiation-induced muscle fibre damage.

3.Detectors for steady state experiments. Not surprisingly the imaging plate system was found to be the better system for steady state exposures where we are interested in relatively fine spatial resolution of overlapping layer lines and of rather sharp meridional features of the pattern. Detectors are mounted in the evacuated flight tube on BL4, obviously advantageous for an on line detector, but unfortunately this means that introducing a plate and exchanging it is rather time consuming.

4.Actin diffraction patterns. A very attractive feature of BL4 for our steady state experiments is the constancy of the focal size over several metres distance. We were able to resovle the 1st actin layer line at ~36nm from the equatorial features and then move to shorter camera length to collect out to 1.3nm. Unfortunately, when we came to look at the actin features of stronger x-ray exposures on the Molecular Dynamics Imaging Plate system did we notice non-uniformities in the detection system. We concluded that the source of the highly non-uniform low angle background in our pictures, showing a patchy 20°/0 intensity variation, was not a function of the plate but was characteristic of a particular scanner (we tried 2, both showed similar sensitivity variations but in different regions of the scanned image). The problem was not resolved in the short time we had available and the test data we took are therefore difficult to analyse! We look forward to using the Fuji scanner which has been purchased recently.