



	Experiment title: Experimental determination of the meridional phases in X-ray diffraction diagrams of live contracting muscles.	Experiment number: LS-1559
Beamline: ID2,BL4	Date of experiment: from: 1, March 2000 to: 6, March 2000	Date of report: 17-08-00
Shifts: 15	Local contact(s): T. Narayanan	<i>Received at ESRF:</i>

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Report: High resolution X-ray diffraction patterns from muscles at rest, as well as undergoing contraction in isometric, isotonic and under stretch conditions were recorded in a range from ca.1200 to 7 nm using the CCD camera available at station ID2, BL4. Excellent angular resolution was obtained and it was found that all meridional diffraction features, for muscles in these major reference states, exhibit pronounced interference effects. The interference patterns stem from the bi-polar disposition of the myosin diffracting units on either side of the M-line. As predicted in our previous application these results demonstrate that the necessary phase information needed to determine the axial disposition of the myosin heads throughout the contractile cycle can be obtained. Phasing of the isometrically contracting pattern has been completed and a manuscript is submitted to Biophys. J. The title and abstract of this manuscript reads:

Meridional X-ray diffraction diagrams, recorded with high angular resolution, from muscles contracting at the plateau of isometric tension show that the myosin diffraction orders are clusters of peaks. These clusters are due to pronounced interference effects between the myosin diffracting units on either side of the M-line. A

theoretical analysis based on the polarity of the myosin (and actin) filaments, shows that it is possible to extract phase information from which the axial disposition of the myosin heads can be determined. The results show that each head in a crown pair has a distinct structural disposition. It appears that only one of the heads in the pair stereospecifically interacts with the thin filament at any one time.

Note that the conclusion that only one of the heads in the pair stereospecifically interacts with the thin filament is something that was previously predicted by Diaz-Baños et al [1]. The phasing of resting and isotonically contracting muscles is practically completed and a manuscript is in preparation.

Other interesting results that have emerged from this work are: i) the spacing, c , of myosin heads which at isometric plateau of tension, P_0 , is ~ 14.54 nm increases during active stretching, which generates a steady $2P_0$, by 0.2-0.3 % (i.e. c is 14.57–14.58 nm). However, after fixing the length at the end of the stretch the tension generated is still $2P_0$ but the spacing reverts back to the original 14.54 nm. This suggests that in addition to thin filament elasticity [2] there may well be substantial elasticity in the thick filament or, alternatively, as the myosin heads are attached to the thin filament one sees a further extensibility of F-actin under actively stretching conditions; ii) experiments carried out under various degrees of overlap have shown how to separate the various contributions to the meridional pattern by structures other than myosin, e.g. C-protein and troponin. This latter point we propose to explore in future sessions of beam time.

Other previous work is now published [2] or submitted [3], and reprints/preprints are included with this report.

- [1] Diaz-Baños, Bordas, Lowy and Svensson. 1996, *Biophys. J.* **71**, 5s76–589.
- [2] Bordas J., Svensson A., Rothery M., Lowy J., Diakun G. P., and Boesecke P. 1999. *Biophys. J.*, **77**, 3197-3207.
- [3] ‘Axial disposition of myosin heads in isometrically contracting muscles’ J. Juanhuix, J. Bordas, J. Campmany, A. Svensson, M. L. Bassford and T. Narayanan. 2000, *Biophysical Journal*, (submitted).