EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** THE MOLECULAR MOTOR IN MUSCLE STUDIED WITH TIME-RESOLVED X-RAY DIFFRACTION ON SINGLE MUSCLE FIBRES	**Experiment number**: SC-1257
Beamline: ID02	**Date of experiment**: from: 17 September 2003 to: 23 September 2003	**Date of report**: 27 February 2006
Shifts: 18	**Local contact(s)**: Pierre Panine	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Vincenzo Lombardi
*Gabriella Piazzesi
*Marco Linari
*Massimo Reconditi
*Pasquale Bianco
*Elisabetta Brunello

Laboratorio di Fisiologia, DBAG
c/o Dipartimento di Fisica
Via G. Sansone, 1
50019 Sesto Fiorentino (FI)
Italy

*Malcolm Irving

King's College London
New Hunt's House
Guy's Campus
London SE1 1UL
UK

Report:

The aim of this project was to determine the structural change in the myosin head responsible for the generation of force. This is obtained with combined mechanical and X-ray diffraction experiments on single muscle fibres from the frog, where the molecular mechanism of contraction can be studied in the unperturbed system. Thanks to the collimation of the X-ray beam at ID2, ESRF, we have previously shown that we can use the X-ray interference between the two arrays of myosin heads (Linari et al. PNAS 97:7226, 2000; LS-1403) in the thick filament to measure, with subnanometre resolution, the structural changes associated with the working stroke in the myosin heads (Piazzesi et al., Nature 415:659, 2002; SC885). During SC-1257 we completed the experiments dedicated to characterise the structural changes associated with the enhanced force production at higher temperature. We measured the intensities of the axial X-ray reflections from the periodic repeat of the myosin heads along the filaments, of the actin-based layer line reflections, corresponding to the helical periodicities of the actin filament, and of the equatorial reflections, related to the mass distribution in the plane perpendicular to the filaments, during active isometric contraction in the temperature range 0ºC to 17ºC. We also used the new X-ray interference technique to give a precise measure of the axial motions of the myosin heads towards the centre of the sarcomere when isometric force is modulated by temperature.

Methods: Single fibres from the tibialis anterior muscle of *Rana temporaria* were vertically mounted in a trough containing Ringer solution, at ~2.2 μm sarcomere length between a force transducer and a loudspeaker coil motor as already described (Linari et al., 2000). The temperature of the bathing solution was controlled by feedback to a thermoelectric module. Fibres were stimulated via electrodes mounted on mica windows, on either side of the fibre and 600 μm apart. Patterns were collected on the image intensified FReLoN CCD detector placed at either 10 m (to collect intensity and fine structure of the low order meridional reflections) or 3 m (to collect intensity of the higher order meridional reflections, up to M6, and of the actin layer lines). Data analysis was performed using Fit2D (by Dr A.P. Hammersley, ESRF), SAXS Package (by Dr P. Boesecke) and Peakfit software package (SPSS Inc.).

Results: Two dimensional X-ray patterns were collected during isometric tetani at 0, 4, 10 and 17°C. Isometric force was 43 ± 2% (mean ± SE, n=8) higher at 17ºC than 0ºC. The intensity of the first actin layer line increased by ~60%, and the ratio of the intensities of the equatorial 1,1 and 1,0 reflections by 20%. The M3 X-ray reflection from the axial repeat of the heads along the filaments was ~30% more intense at 17ºC, suggesting that the heads became more perpendicular to the filaments. The ratio of the intensities of the higher and lower angle peaks of the M3 reflection (R_{M3}) was 0.93 ± 0.02 (n=5) at 0ºC and 0.77 ± 0.02 at 17ºC. Simulation of the interference changes with a crystallographic model of the heads indicates that their light-chain domains tilt by 9º, producing an axial displacement of 1.4 nm, which is equal to that required to strain the actin and myosin filaments under the increased force. A paper has been published in the Journal of Physiology. The spatial resolution necessary for interference measurements was obtained with the new FRe-LoN CCD detector placed at 10 m from the preparation. The productivity of high brilliance beamline ID2 for time-resolved X-ray diffraction/interference measurements on single muscle fibres increased by a factor of ten when the FRe-LoN CCD detector with adequate spatial resolution replaced imaging plates. ID2 is now competitive with the APS BioCAT beamline for these experiments, becoming the best facility dedicated to muscle research. Further improvements in CCD design at ID2 are expected to allow faster time framing.

Publications from this experiment:

M. Linari, E. Brunello, M. Reconditi, Y.-B. Sun, P. Panine, T. Narayanan, G. Piazzesi, V. Lombardi and M. Irving. The structural basis of the increase in isometric force production with temperature in frog skeletal muscle. *J. Physiol.,* **567.2**, 459-469, 2005.

M. Linari, E. Brunello, M. Reconditi, L. Lucii, Y.-B. Sun, T. Narayanan, P. Panine, G. Piazzesi, V. Lombardi and M. Irving. The structural basis of increased force per myosin head at high temperature. *J. Muscle Res. Cell Motil.* **25**, 246, 2004.