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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:** Structural dynamics of muscle contraction: a combined mechanical and time-resolved X-ray diffraction study on single muscle fibres	**Experiment number**: LS-1403
Beamline:	**Date of experiment**: from: 6.12.2000 to: 11.12.2000 from: 18.4.2001 to: 23.04.2001	**Date of report**: 20.02.2002
Shifts:	**Local contact(s)**: Peter Boesecke; Narayanan Theyencheri	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Vincenzo Lombardi
*Gabriella Piazzesi
*Marco Linari
*Massimo Reconditi
*Leonardo Lucii

Dipartimento di Scienze Fisiologiche
Viale G.B. Morgagni, 63
50134 Firenze
Italy

*Malcolm Irving
*Yin-Biao Sun

King's College London
26-29 Drury Lane
London WC2B 5RL
UK

Report: The experiments reported here (LS-1403, December 2000 and April 2001) are consequence of the remarkable achievements of the previous experiments planned in the proposal for the Long Term Project (LS-1403, March 1999), which were aimed at proving the tilting lever arm model of the myosin working stroke by measuring the changes in the interference-fine structure of the meridional myosin based X-ray reflections during the quick force recovery that follows step changes in sarcomere length.

The prominent M3 reflection in the small angle X-ray diffraction pattern from a muscle fibre originates from the ~14.5 nm repeat of the globular part of the myosin molecule - the myosin head- protruding from the myosin filament to form cross-bridges with the actin filament. With the vertical collimation of the X-ray beam at ID2 it has been possible to show that during the isometric contraction of a single muscle fibre the M3 reflection is composed of two closely-spaced peaks (at 14.67 nm, LA, and 14.46 nm, HA), due to X-ray interference between the two arrays of myosins in each thick filament (Linari et al., *Proc. Natl. Acad. Sci.* **97**, 7226-7231, 2000). The interference distance changes when the myosin head binds to actin and drives filament sliding by tilting towards the centre of the myosin filament The method has been used to characterise the motions of the myosin heads during the quick recovery of force following a step reduction in sarcomere length which synchronises the 1000/s working stroke (LS-1403, October 99; Piazzesi et al., *Nature* **415**, 659-662, 2002). Shortening steps reduced the ratio of the intensity of the high angle peak over that of the low angle peak (R), as expected for displacement of the myosin heads towards the centre of the myosin filament. R was reduced to about 0.4 at the end (T_1) of a shortening step of 4 nm/half-sarcomere, and did not change significantly during the subsequent rapid force recovery phase (T_1 to T_2 transition). These changes in the interference fine structure of the M3 reflection were explained with models in which (1) force is generated by tilting of the light chain domain (LCD) of one head of each myosin molecule during both the elastic (phase 1) response and the active force redevelopment (the working stroke), (2) the compliances of myosin and actin filaments are taken into account. These results indicate that tilting of the lever arm of the myosin head is the mechanism that drives both isometric force generation and filament sliding and provide evidence for a ~10 nm working stroke in the myosin heads attached to actin. In the series of experiments reported here, we use a load clamp protocol to remove the effects of filament compliance during phase 2.

Experimental protocol: Single fibres from the tibialis anterior muscle of *Rana temporaria* were vertically mounted in a trough containing Ringer solution at 4 °C and at ~2.2 µm sarcomere length between a force transducer and a loudspeaker coil motor as already described (Linari et al., 2000). To have the required spatial resolution, patterns were collected on a storage phosphor image plate detector (IP, A3 size) placed at 9.8 m from the specimen. The adequate time resolution was attained by using two fast shutters (LS 500, ~ 20 µs switching time) in series in front of the preparation. The force in an actively contracting muscle fiber was reduced in ca 150 µs from the isometric value (T_0) to 0.50 or 0.75T_0 and then held at this value. Phase 2 of the length transient is followed by a period of much slower shortening (phase 3) before the final steady state shortening velocity is achieved (phase 4). Load clamp steps were imposed cyclically with a 1 to 5-ms interval between shortening and stretching steps, and 50-ms cycle time. The exposure time was precisely synchronized with the end of the load step (between 150 and 260µs following the step start) or at successive times during phase 2 shortening and was recorded by means of a pin diode stuck on the beam stop. Data from twenty tetani were added up to a total exposure time of 80 ms per step with unattenuated beam. To distribute the radiation damage, the stage with the fibre was vertically shifted by 200 µm after each exposure. IPs were scanned with 100 µm spatial resolution (Molecular Dynamics). Data analysis was performed using Fit2D (by Dr A.P. Hammersley, ESRF) and Peakfit software package (SPSS Inc.).

Results: R in the M3 reflection decreased monotonically with increasing extent of shortening under constant load (Fig.1, filled symbols). The relationship between *R* and extent of shortening was almost independent of the load in the range 0.5 to 0.75 T_0. These results show that the interference fine structure of the M3 reflection and thus the axial motions of the myosin heads in phases 1 and 2 of the length transient depend only on the extent of filament sliding, despite the much slower rate of phase 2 at higher load (about three times slower at 0.75 than 0.5 T_0). The dependence of R on the extent of shortening and on the load were quantitatively reproduced by the tilting LCD model (Fig. 1, open symbols). In this model myosin heads remain attached to actin during phases 1 and 2 of the length transient. Because the load is constant during phase 2, the strain in the myosin and actin filaments is also constant, and all the sarcomere shortening is taken up by distortion of the myosin heads. Thus the centers of mass of the heads continue to move towards the center of the myosin filament during phase 2, and the change in *R* is accounted by a working stroke of ca 10 nm in the attached heads. The IP detector is used for the best spatial resolution but it is very inefficient for its low sensitivity and the necessity to place it *in vacuo*. Extraction/insertion of IPs and subsequent scanning take several tens of minutes. This limited the possibility to collect data for a larger range of loads. This problem will be partly circumvent with the planned development of a more flexible camera. The most efficient solution would be to provide the beamline with a CCD detector with sufficiently high spatial resolution and low background noise.

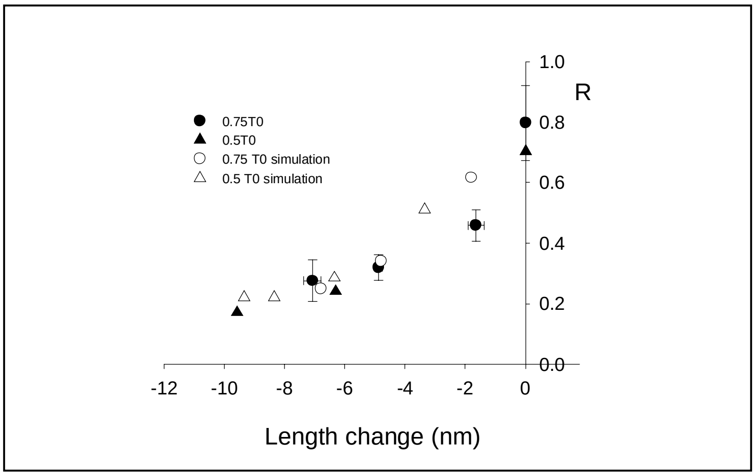

Fig. 1 Ratio between the HA and LA intensities (*R*) vs. the extent of isotonic shortening at 0.5 T_0 (filled triangles) and 0.75 T_0 (filled circles). The point on the ordinate is the control value just before the load step. Mean and SE from 4 fibres. Open symbols are the simulated results for 0.5 T_0, triangles and 0.75 T_0, circles.

Relevant Publications during the Long Term Project LS-1403

- Piazzesi, G., Reconditi, M., Dobbie, I., Linari, M., Bösecke, P., Diat, O., Irving, M., Lombardi, Changes in conformation of myosin heads during the development of isometric contraction and rapid shortening in single frog muscle fibres. *J. Physiol.* **514**, 305-312, 1999.

- Linari, M., Piazzesi, G., Dobbie, I., Koubassova, N., Reconditi, M., Narayanan, T., Diat, O., Irving, M., Lombardi, V. - Interference fine structure and sarcomere length dependence of the axial X-ray pattern from active single muscle fibres. *Proc. Natl. Acad. Sci. USA* **97**, 7226-7231, 2000 (con figura di copertina).
- Piazzesi, G., Reconditi, M., Linari, M., Lucii, L., Sun, Y-B., Narayanan, T., Boesecke, P., Lombardi, V., and Irving, M. - The mechanism of force generation by myosin heads in skeletal muscle. *Nature*, **415**, 659-662, 2002.