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: Structure-function relation of the molecular motor in muscle: a time-resolved X-ray diffraction study on single muscle fibres**	**Experiment number**: SC-885
Beamline:	**Date of experiment**: 2nd All. Period, from: 17.04.02 to: 22.04.02 3rd All. Period, from: 27.11.02 to: 3.12.02	**Date of report**: 22.02.03
Shifts:	**Local contact(s)**: Peter Boesecke (2nd a.p.), Pierre Panine (3rd a.p.)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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

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 (SC-885, 2nd and 3rd allocation periods) are related to point 4 of the experimental programme in the Long Term Project SC-885: force generation in muscle is driven by transition between states with different degree of tilting of the light chain domain of the myosin head (Rayment *et al.*, *Science* **261**:58-65, 1993). To test this idea we collected the X-ray diffraction pattern during isometric contractions at different temperatures (0-17 °C). In fact, the recent mechanical demonstration with unprecedented precision that, in an intact muscle fibre, the temperature changes the force per myosin head without affecting the fraction of attached force-generating heads (Linari *et al. Pflügers Arch.* **442**:R65/38, 2001), makes this procedure ideal for the structural test. During the previous LS-1403, the vertical collimation of the X-ray beam at ID2 allowed changes in the fine structure of the M3 reflection, due to X-ray interference between the two arrays of myosins in each thick filament to be measured (Linari et al., *Proc. Nat. Acad. Sci.* **97**, 7226-7231, 2000). Thus we determined the axial movement of the heads during the execution of the working stroke that drives the actin filament towards the centre of the myosin filament (Piazzesi et al., *Nature*, **415**, 659-662, 2002). Interference data collected during LS-1403 showed that an increase of the temperature from 0 °C to 11 °C, increasing the isometric tetanic force (T_0) by 36%, decreased the ratio (R) of the intensities of HA over LA peak of the M3 reflection by 20%. Reliable data on the temperature dependence of the intensity of the M3 reflection (IM3) could not be collected during LS-1403, because the spatial resolution necessary for interference measurements was only possible with the Image Plate detector and frames at different temperatures were separated by the long delay required for changing the Image Plate. In the experiments reported here we used the new CCD detector and could collect the intensity and fine structure of the meridional reflections as well as the intensity of the actin layer line reflections during isometric tetani at four different temperatures (0, 4, 10 and 17 °C).

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). 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) and Peakfit software package (SPSS Inc.). The radial integration limits were: ± 120 nm for the M3 and M6 reflections and from 21 to 4.8 nm for the 1st actin layer line.

Results: Increasing the temperature from 0 °C to 17 °C (that produced an increase in T_0 by 44%) (1) reduced R monotonically from 0.90 ± 0.02 (0 °C) to 0.75 ± 0.01 (17 °C) (mean ± SEM, 12 fibres, see Fig. 1); (2) slightly increased IM3, that attained a maximum of 1.11 ± 0.08 at 10 °C (10 fibres); (3) increased the spacing of the M3 reflection (SM3, 14.568 nm at 0 °C) in proportion to the isometric force with a slope that was twice that estimated during the elastic response to rapid length changes (Piazzesi et al., 2002); (4) did not change significantly the intensity of M6 reflection; (5) increased the intensity of the 1st actin layer line by 56 ± 18% (6 fibres).

Changes in the fine structure of M3 reflection was interpreted using the tilting lever arm model of the myosin head and a structural model of the sarcomere with distributed filament compliance (Piazzesi et al., 2002). The tilt of the light chain domain with increase in isometric force was equal to that expected from the increased strain in the filaments, provided that only half of the axially ordered heads are exerting the isometric force, as assumed previously (Piazzesi et al., 2002).

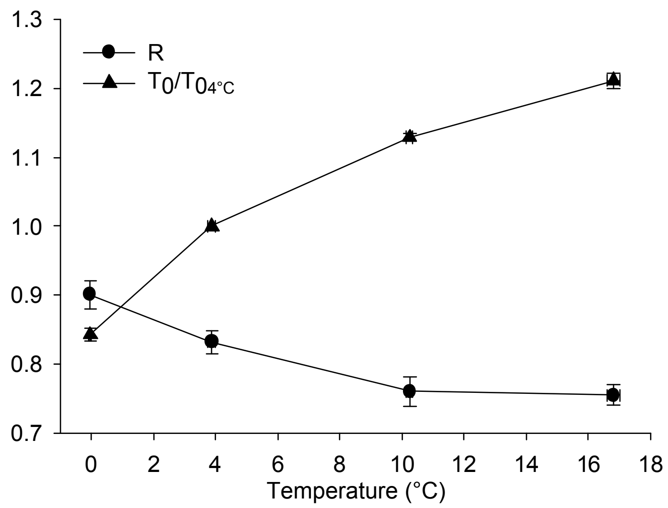

Fig. 1. Relations of isometric force relative to the force at 4 °C ($T0/T0_{4°C}$) and R versus temperature. Bars are SEM

The productivity of ID2 for time-resolved X-ray diffraction/interference measurements on single muscle fibres increased by about ten times in 2002 due to the replacement of imaging plates by the FReLoN CCD detector. Consequently in the 3rd allocation period we were able to complete the temperature experiments and collect also preliminary data on another question at point 4 of the programme: the changes in myosin filament structure between resting and active muscle. Thanks to the increase in flux due to the second undulator, 2D patterns could be recorded with 5 ms resolution during the development of force and relaxation in the isometric tetanus. The effect of the activation was isolated from that of increase in force by imposing shortenings at the maximum velocity at different times after the beginning of stimulation. Further improvements in CCD design at ID2 are expected to allow even faster time framing in future experiments.

Recent papers on ESRF experiments:

1. M. Linari, G. Piazzesi, I. Dobbie, N. Koubassova, M. Reconditi, T. Narayanan, O. Diat, M. Irving, V. Lombardi. 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 (with cover figure).

2. G. Piazzesi, M. Reconditi, M. Linari, L. Lucii, Y-B. Sun, T. Narayanan, P. Boesecke, V. Lombardi, and M. Irving. The mechanism of force generation by myosin heads in skeletal muscle. *Nature*, **415**, 659-662, 2002

3. M. Reconditi, G. Piazzesi, M. Linari, L. Lucii, , Y.-B. Sun, P. Boesecke, T. Narayanan, M. Irving and V. Lombardi. X-ray interference measures the structural changes of the myosin motor in muscle with Å resolution. *Notiziario Neutroni e Luce di Sincrotrone* **7** (2), 19-29, 2002.