EUROPEAN SYNCHROTRON RADIATION FACILITY

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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: 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**: from: 30.01.02 to: 05.02.02	**Date of report**: 14.10.02
Shifts:	**Local contact(s)**: Peter Boesecke	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Vincenzo Lombardi
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Report: The experiments reported here (SC-885, 1st allocation period) are related to the first point of the experimental programme in the Long Term Project proposed by Vincenzo Lombardi: the measurement of the size of the axial motion of myosin heads (the working stroke) that drives force generation and filament sliding in intact fibres from skeletal muscle. During the previous LS-1403, thanks to 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, due to axial repeat of myosin heads at about 14.5 nm, 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. Nat. Acad. Sci.* **97**, 7226-7231, 2000). The interference distance changes when the myosin head binds to actin and drives sliding of actin filament towards the centre of the myosin filament. The method has been used to characterise the motions of the myosin heads during the elastic response and the quick recovery of force following a step change in sarcomere length (Piazzesi et al., *Nature* **415**, 659-662, 2002). Shortening steps reduced the ratio of the intensity of the HA peak over that of the LA peak, as expected for displacement of the myosin heads towards the centre of the myosin filament. However, we found that the reduction in interference distance was smaller than that expected from the imposed filament sliding and was mainly related to the elastic response. On the contrary, the increase in the interference distance induced by a lengthening step mainly occurred during the quick force recovery. These results can only partly be explained by taking into account the effect of filament compliance and indicate that the partner head of the same myosin molecule with one head attached contributes to the M3 reflection and rapidly attaches on stretch. The spatial resolution necessary for interference measurements was only possible with the Image Plate detector and this limited the precision of measurements of changes of intensity of the M3 reflection, since the time frames of different phases of the force transient had to be collected in different exposures separated by the long delay required for changing the Image Plate. In the experiments reported here we used a short camera (3 m) and the CCD detector for recording the intensity changes of the M3 reflection and of the weak actin-based layer line reflections during the quick recovery following shortening and lengthening steps.

Experimental protocol: Single fibres from the tibialis anterior muscle of *Rana temporaria* were horizontally 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 a CCD detector placed at the end of a 3 m camera at the plateau of the isometric tetanus (force T0), just before the length step (Ti) and between 1 and 3 ms following the step (T2). Adequate time resolution (2 ms) was attained by using two fast shutters (LS 500, ~20 μs switching time) in series in front of the preparation. Data were collected from nine fibres for either a step stretch or a step release of 4-5 nm, for which the T2 recoveries were 1.3 T0 and 0.8 T0 respectively. For each step, trains of 40 steps were imposed at the plateau of isometric tetanic contractions. The repriming protocol that made possible signal averaging from the train of steps provided that each step was followed after 4 ms by a similar step in the opposite direction and the cycle was repeated every 50 ms. To distribute the radiation damage, the fibre and the stage were shifted along the fibre axis by 200 μm after each tetanus by using a remote controlled motor. For each fibre, data from ten-thirty tetani per fibre were added up to a total exposure time of 10 s for the stretch and 6.2 s for the release. Data analysis was performed using Fit2D (by Dr A.P. Hammersley, ESRF) and Peakfit software package (SPSS Inc.). The radial integration limits were: for the M3 reflections ± 60 nm, for the M6 reflection ± 60 nm, for the 1st actin layer line 26-7 nm, for the 6th and 7th actin layer line 60-10 nm.

Results: At the end of quick recovery following the stretch the intensity of the M3 reflection relative to the intensity before the step (I_{M3}) was 0.64 ± 0.06 (mean and S.D.), the intensity of M6 reflection (I_{M6}) was 0.66 ± 0.06, that of the 1st actin layer line (I_{1st}) was 0.93 ± 0.12. At the end of quick recovery following the release I_{M3} was 0.69 ± 0.02, I_{M6} was 1.31 ± 0.15, I_{1st} was 1.28 ± 0.03. There was no significant stretch/release-dependent change in the intensity of 6th and 7th actin layer lines. In terms of the crystallographic model of the myosin head, these results indicate that (1) in isometric conditions the orientation of the light-chain binding domains of both the attached and the partner myosin heads are near the perpendicular to the filament axis (Piazzesi et al., 2002), since both the execution of the working stroke elicited by a release and the reversal elicited by a stretch reduce I_{M3}, (2) the partner head of each myosin molecule that has one head attached is has enough order to contribute to both I_{M3} and the actin layer lines, so that its attachment on stretch, as suggested on the basis of mechanical and X-ray interference results, does not increase the contribution of myosin to the actin-based helical symmetry.

Recent relevant publications on ESRF experiments:

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).

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

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.