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**: 4th All. Period, from: 22.04.03 to: 29.04.03	**Date of report**: 12.08.03
Shifts:	**Local contact(s)**: Pierre Panine	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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

Laboratorio di Fisiologia, DBAG
c/o Dipartimento di Scienze Fisiologiche
Viale G.B. Morgagni, 63 50134 Firenze
Italy

*Malcolm Irving
Yin-Biao Sun

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

Report: The experiments reported here (SC-885, 4th allocation period) are related to experiments selected to exploit the performances of RAPID detector developed by Daresbury detector group, temporarily installed at ID2 to test the characteristics of the most advanced gas filled detector for time resolved SAXS measurements with the beam of a 3rd generation synchrotron. We pursued the task repeating an experiment just started with the FReLoN CCD detector during SC-885 3rd allocation period, namely the study of the structural dynamics of the filaments and the fraction of force generating heads during the transition from resting to active tetanic contraction (T0). The effect of the activation was isolated from that of increase in force by comparing the data from isometric tetani with those from tetani where the initial rise of force was prevented by imposing shortenings at the unloaded shortening velocity (V0) (Fig. 1). Due to the limited spatial resolution of RAPID with respect to FreLoN (PSF ~400 µm FWHM with respect to 220 µm), only the intensity and spacing of the reflections are compared here, by choosing a specimen-detector distance of ca 3.5m (Fig 2). With the CCD detector and the unattenuated flux obtained with the second undulator ($6*10^{13}$ ph/s/100mA), the M3 and M6 reflections could be recorded with 5 ms resolution during the development of the tetanus. One 5 ms frame was collected per tetanus, and 24 tetani were necessary to describe the 120 ms of tetanus development. There was no sign of radiation damage up to the end of the experiment, as the fibre was shifted along after each tetanus. The 1st actin layer line could be resolved by adding data from two fibres (10-20 ms total exposure). With the RAPID detector (Fig. 1) a whole sequence of 30 frames of 4.8 ms was collected during the first 144 ms of the tetanus development and the sequence could be repeated in successive tetani. The radiation damage could be reduced by shifting the fibre after each tetanus (144 ms exposure). Unfortunately, the efficiency of RAPID detector was severely reduced by the different beam energy at ID2 (12 KeV) with respect to that at beamline 16.1 at Daresbury (7.5 KeV), and this, in conjunction with the small diffracting mass of our single fibre preparation, compromised the quality of the results. However we could use the maximum flux available through the by-pass camera ($3*10^{13}$ ph/s/100mA with the second undulator) without reaching the count rate saturation. For each protocol data from 8 fibres for a total of ca 70 ms per frame were sufficient to resolve the M3 and M6, but not the 1st actin layer line. The data show that the ca 1.6% increase in spacing of both M3 and M6 from resting to T0 is delayed in parallel with force when the initial rise of tetanic force is prevented by shortening at V0. The spacing of M3, but not that of M6, reduces by 0.2% during the time the force is kept to zero by the shortening ramp. These results give a direct evidence that there are large scale changes in the thick filament structure related to both activation and force.

Methods: Single intact fibres dissected from the tibialis anterior muscle of *Rana temporaria* were 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 (Piazzesi et al., J. Physiol. 514:305, 1999). Patterns were collected on the RAPID gas-filled detector placed at 3.5 m (to collect the intensities of the higher order meridional reflections, up to M6, and of the actin layer lines). Data analysis was performed using SAXS Macros (P. Boesecke, ESRF) and Peakfit software (SPSS Science). The radial integration limits were: ± 37 nm for the M3 and M6 reflections.

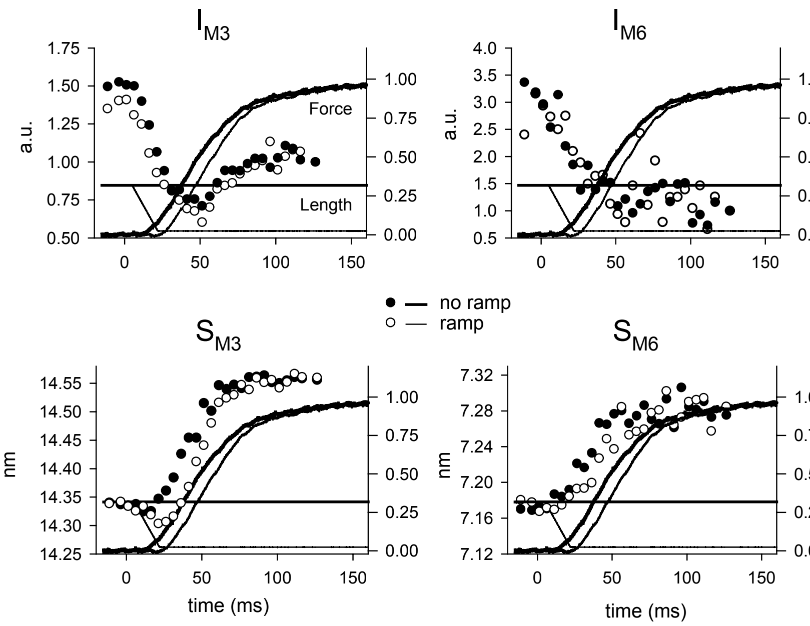

Fig. 1

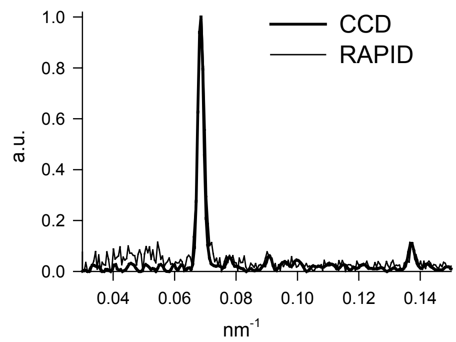

Fig. 2

Figure 1. Time course of force, intensity (I, normalised for the value at T0, upper panels) and spacing (S, lower panels) of M3 (left panels) and M6 (right panels) in isometric conditions (no ramp, thick lines and filled circles) and after a shortening at V_0 (ramp, thin lines and open circles) imposed just before isometric force development. The right ordinate in all panels is force relative to T0; the left ordinate in the upper panels is I, in the lower panels is S. X-ray points are 5ms time frames for a total exposure of 70 ms per frame. Data from 8 fibres.

Figure 2. Comparison between meridional intensity profiles collected from single fibres at T0 with the FReLoN CCD detector (thick line, 20 ms exp. time) and the RAPID gas-filled detector (thin line, 92 ms exp. time).

Recent papers on ESRF experiments:

1. 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

2. 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.

3. M. Reconditi, N. Koubassova, M. Linari, I. Dobbie, T. Narayanan, O. Diat, G. Piazzesi, V. Lombardi and M. Irving. The conformation of myosin head domains in rigor muscle determined by X-ray interference. *Biophys. J.*, **85**, 1098-1110, 2003.