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-1388
Beamline: ID02	**Date of experiments**: from: 10.11.2004 to: 16.11.2004	**Date of report**: 27.2.2006
Shifts: **18**	**Local contact(s)**: Pierre Panine	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The aim of this project is to investigate the structural dynamics of the molecular motor of muscle, the myosin heads that cross-link the myosin and the actin filament, responsible for the generation of force/shortening in muscle and for the braking action of muscle during forcible lengthening. The investigation is made combining fast mechanics and X-ray diffraction in single fibres isolated from the frog skeletal muscle, where the molecular mechanism of contraction can be studied in the native system. Thanks to the collimation of the X-ray beam at ID2, ESRF, we can exploit the X-ray interference between the two arrays of myosin heads in the thick filament, to measure the motion of the myosin heads with sub-nanometre resolution. The experiments during SC-1388 are aimed at defining structural changes in the myosin heads and in the myosin and actin filaments (1) during the synchronous execution of the isotonic working stroke following step reduction in force superimposed on isometric contractions; (2) during the process of activation and force development in the isometric contraction; (3) during the force enhancement by stretch that is responsible for the efficient braking action of the active muscle in eccentric contractions. During previous LTP (SC-885) and the first allocation period of this LTP, by using the interference X-ray diffraction method, we showed that the working stroke in the myosin head domain can be synchronised with step reduction in load imposed on the isometric contraction. We demonstrated that the working stroke is ca 11 nm at low load and is smaller and slower as the load is increased. These results are important to define the molecular determinant of the high efficiency in energy conversion by muscle and are published in a paper in Nature (Reconditi at al., Nature, 428:578, 2004).
We dedicated the 2nd allocation period of LTP SC-1338 to complete the experiments devoted to study the structural changes accompanying the force enhancement by stretch and we started to address the other fundamental question of this LTP, the discrimination of structural changes in the myofilaments and myosin heads during activation and rise of force in an isometric contraction.

Methods:

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., PNAS 97:7226, 2000). 2D X-ray patterns were collected on the FReLoN CCD detector placed at 3 or 10 m from the specimen. Adequate time resolution to isolate the short lived transients following step perturbations in length was attained by generating short time windows (up to 100 μs) with two fast shutters (LS 500, ~ 20 μs switching time) in series in front of the preparation. For each step, trains of steps were imposed at the plateau of isometric tetanic contractions to increase the signal to noise ratio by adding corresponding time windows. 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 5-10 s. 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:

The X-ray interference responses to stretches of 2-6 nm were collected at the end of the elastic response and at the end of the quick recovery. A model simulation of these responses confirms the mechanical evidences of a very fast attachment of the detached head of the same myosin molecule that has one head already attached. The results and the model have been presented at the Biophysical Society conference and a paper is in preparation to be submitted to PNAS.

As far as the investigation of structural changes at the beginning of the contraction, to separate the effects of activation from those of force generation we compared meridional and layer line reflections collected during the isometric force development with those collected while preventing force generation with a ramp shortening at the unloaded shortening velocity (V0). Shortening at V0 just after the end of latency relaxation reveals the recruitment, or the stiffening, of an elastic component in parallel to myosin cross-bridges. Unfortunately the structural responses revealed inconsistent in subsequent tetani and X-ray exposures as if the structure responsible for this elasticity (very likely titin) were much more fragile than the other components of the sarcomere. In this respect the preliminary experiments, done with the RAPID detector during a previous visit (SC-885), resulted much more productive as the dynamics of the response could be defined by the series of frames collected in only one contraction. Under any other respect the FReLoN CCD detector available at the beamline was adequate. The spatial resolution necessary for interference measurements was obtained with a 10 m distance between detector and preparation. The productivity of the high brilliance beamline ID2 for time-resolved X-ray diffraction/interference measurements on single muscle fibres improved as the FRe-LoN CCD detector with adequate spatial resolution replaced imaging plates. For the structural studies during the activation and the development of the isometric force we took advantage of the 30 Hz framing rate made possible by binning in the direction not critical for spatial resolution.

Publications from these experiments:

E. Brunello, G. Piazzesi, M. Linari, P. Bianco, M. Reconditi, P. Panine, W. Helsby, M. Irving and V. Lombardi. Time course of formation of myosin cross-bridges in tetanized single fibres from frog muscle measured by X-ray diffraction. *Biophys. J.* **86(1)**:564a 2925-Pos, 2004

M. Reconditi, L. Lucii, E. Brunello, M. Linari, Y.-B. Sun, T. Narayanan, P. Panine, G. Piazzesi, M. Irving and V. Lombardi. X-ray interference study of stretch-induced cross-bridge attachment in active single fibres from frog muscle. *J. Muscle Res. Cell Motil.* **25**, 246, 2004.

E. Brunello, M. Reconditi, P. Bianco, M. Linari, P. Panine, T. Narayanan, W. Helsby, G. Piazzesi, M. Irving and V. Lombardi. A parallel elasticity observed by x-ray diffraction in tetanized single muscle fibres. *J. Muscle Res. Cell Motil.* **25**, 246, 2004.

M. Reconditi, E. Brunello, R. Elangovan, P. Panine, T. Narayanan, M. Irving, M. Linari, G. Piazzesi and V. Lombardi. Myosin cross-bridge recruitment by stretch occurs on the submillisecond timescale. *Biophys. J.* **90**, 106a, 493-Pos., 2006