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**: IV from: 15.03.06 to: 21.03.06	**Date of report**: 12.1.2007
Shifts: 18	**Local contact(s)**: Pierre Panine, Theyencheri Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

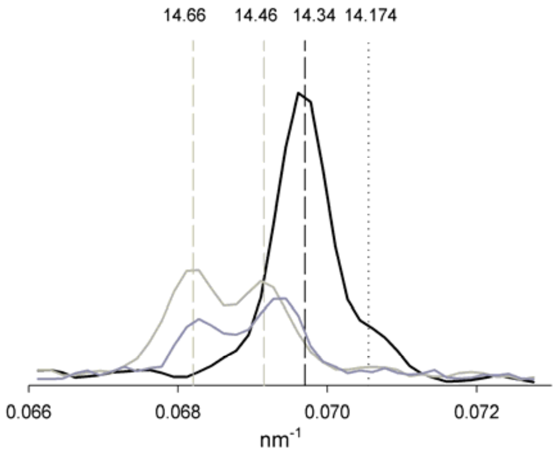
The aim of this project is to investigate the structural dynamics of the molecular motor of muscle, the myosin heads that cross-link the myosin and the actin filaments, responsible for the generation of force/shortening in muscle and for the braking action of muscle during forcible lengthening. The investigation is made combining fast mechanics and X-ray diffraction in single fibres isolated from 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 were 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. In the experiments of previous allocation periods (I and II), we completed experiments concerning the 1st and 3rd question of this LTP and started to address the 2nd question, the discrimination of structural changes in the myofilaments and myosin heads during activation and rise of isometric force. We separated the effects of activation from those of force generation by comparing 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 (V_0). In the 3rd allocation period we focussed on the changes of meridional and layer line reflections during the rise of isometric force and force redevelopment after unloaded shortening of amplitude 50 or 100 nm per half-sarcomere. In the 4th allocation period we recorded X-ray interference changes in the M3 reflection by placing the FReLoN CCD detector at 10 m distance from the preparation.

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). 5 ms time frames were collected using a CCD FReLoN detector (ESRF, Grenoble, France) and a 10 m camera length (so as to collect the fine structure of the meridional reflections up to the M3) during the tetanus rise, during unloaded shortening (V_0= 2.55 ± 0.06 μm/s/hs) of 5% and 10% the fibre length (l_0) imposed on the isometric tetanic tension (T_0), and during the tension redevelopment following the end of unloaded shortening. Data analysis was performed using SAXS Package (P. Boesecke, ESRF), Fit2D (A. Hammersley, ESRF) and Igor Pro (WaveMetrix, Inc.). Mechanical data were collected and analysed with LabVIEW software.

Results:

We could describe the time course of the changes in spacing and fine structure of the M3 reflection during the development of the isometric tetanus, during the 5% and 10% shortening at V_0 imposed at the isometric tetanus plateau and during tension redevelopment after the end of shortening. The Figure shows example profiles of the M3, collected at rest (black), at the isometric tetanus (light grey) and during the development of the isometric tetanus, when the force was about half of its plateau value (dark grey). The vertical lines and the corresponding numbers indicate the position (nm) of the component peaks. The centroid of the M3 reflection in a resting fibre is at 14.34 nm, corresponding to the axial repeat of myosin molecules along the filament in these conditions. During active isometric contraction, the centroid shifts to 14.56 nm, mainly as a result of a change in the underlying periodicity of the filament backbone, and the reflection is split into two peaks due to interference between the two arrays of myosin heads in each sarcomere (Linari et al., 2000). The profile recorded during force development contains contributions from both actin-attached and detached myosin heads (Brunello et al., 2006). The challenge for the analysis and interpretation of these data is to isolate the conformations of the attached and detached heads using the X-ray data for this and other reflections.



Publications from these experiments:

E. Brunello, P. Bianco, G. Piazzesi, M. Linari, M. Reconditi, P. Panine, T. Narayanan, W. Helsby, M. Irving and V. Lombardi - Structural changes in the myosin filament and cross-bridges during active force development in single intact frog muscle fibres: stiffness and X-ray diffraction measurements. *J. Physiol.,* **577.3**, 971-984, 2006.

M. Reconditi – Recent improvements in small angle X-ray diffraction for the study of muscle physiology. *Rep. Prog. Phys.* **69**, 2709-2759, 2006.

E. Brunello, M. Reconditi, M. Linari, R. Elangovan, P. Panine, T. Narayanan, G. Piazzesi, V. Lombardi and M. Irving Spacing changes in the myosin based X-ray reflections during isometric force development. *Biophys. J.* **90**:427a, 2073-Pos, 2006.

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.

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 Motility* **25**, 246, 2004.