doi:10.1016/j.jmb.2006.08.075 J. Mol. Biol. (2006) 363, 743–761

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

X-ray Interference Studies of Crossbridge Action in Muscle Contraction: Evidence from Quick Releases

Hugh Huxley[1]*, Massimo Reconditi[2], Alex Stewart[1] and Tom Irving[3]

[1]*Rosenstiel Center, Brandeis University, Waltham, MA 02454, USA*

[2]*Department of Animal Biology & Genetics, University of Florence, Florence, Italy*

[3]*Department of Biological Chemical Physical Science Illinois Institute of Technology Chicago, Ill 60616, USA*

We have used a high-resolution small angle X-ray scattering system, together with a high-performance CCD camera, on the BioCAT beamline at the APS synchrotron radiation facility at the Argonne National Laboratory, to study X-ray interference effects in the meridional reflections generated by the arrays of myosin crossbridges in contracting muscle. These give information about axial movements of the myosin heads during contraction with sub-nanometer resolution. Using whole intact muscle preparations (frog sartorius) we have been able to record the detailed behavior of M3 (the first order meridional reflection from the myosin crossbridges, at 14.56 nm) at each of a number of quick releases of increasing magnitude, on the same specimen, and at the same time make similar measurements on higher order myosin meridional reflections, particularly M6. The latter provides information about the dispersion of lever arm angles of the actin-attached myosin heads. The observations show that in isometric contraction the lever arm angles are dispersed through ±20–25° on either side of a mean orientation that is about 60° away from their orientation at the end of the working stroke: and that they move towards that orientation in synchronized fashion, with constant dispersion, during quick releases.

The relationship between the shift in the interference fringes (which measures the shift of the myosin heads scattering mass towards the center of the sarcomere, and the changes in the total intensity of the reflections, which measures the changes in the axial profile of the heads, is consistent with the tilting lever arm mechanism of muscle contraction. Significant fixed contributions to the meridional reflections come from unattached myosin heads and from backbone components of the myosin filaments, and the interaction of these with the contributions from actin-attached myosin heads determines the behavior of these reflections.

*Corresponding author

Keywords: muscle; myosin; crossbridges; X-ray; quick releases

Introduction

Recent developments in X-ray diffraction have made it possible to obtain much more detailed information about how the myosin crossbridges produce force and movement in muscle contraction. Experiments on single fibers have been described in detail by Lombardi and his colleagues.[1–5] We have been carrying out analogous experiments on whole intact muscle, so far described only in abstract form.[6–12] Such intact preparations do not provide the same very high temporal and mechanical resolution that can be obtained from single fibers, but they give much stronger X-ray diffraction patterns, so that many comparative experiments can be performed on the same specimen, and the patterns can be recorded to higher spatial resolution. This has provided significant additional information about crossbridge configurations and behavior in active muscle.

In part 1 of this study we will describe evidence obtained from the behavior of X-ray patterns during quick releases. In the following paper this will be extended to the interpretation of the patterns given by muscles during normal steady shortening.

Earlier work

A key feature of early versions of the sliding filament hypothesis was that a cyclical structural

E-mail address of the corresponding author: huxley@brandeis.edu; alex@brandeis.edu

0022-2836/$ - see front matter © 2006 Published by Elsevier Ltd.

change should occur in myosin crossbridges attached to actin, during the force-generating part of their cyclical action.[13,14] The exact nature of the change remained unclear for many years, though a convenient model, which accommodated a great deal of data, involved an elongated crossbridge either tilting about its site of attachment to actin, or undergoing an equivalent change of shape[15]. The crossbridge then detached from actin, and the change in orientation or configuration was reversed before the next attachment took place. However, direct experimental evidence to support this model was slow in appearing.

X-ray diffraction studies at 1 ms time resolution during mechanical transients showed that a large decrease in the intensity of the 14.5 nm meridional reflection (M3) generated by the axial repeat of the crossbridges took place almost synchronously when small (1% of muscle length), rapid (<1 ms) length changes were applied to an otherwise isometrically contracting muscle.[16,17] This provided direct evidence that myosin heads undergo some major change in orientation or configuration during their working stroke, as they drive the actin and myosin filaments past each other. However, although the evidence was consistent with the tilting model, since the axial density profile of an elongated crossbridge would be broadened and reduced in amplitude by tilting to an orientation or configuration less perpendicular to the fiber axis, and would give a weaker X-ray reflection, other explanations, such as disordering, were also possible. A strong inference from this experiment is that in a contracting muscle, the M3 reflection arises primarily from force-producing myosin heads attached to actin, since the reflection was observed to decrease to only one fifth of its original isometric intensity during the releases. Unattached heads were presumably too disordered to contribute strongly to the reflection.

Recent studies

During the 1990s, Irving, Lombardi, and their colleagues[18–21] succeeded in making X-ray measurements at even higher time resolution on single muscle fibers under a variety of protocols in which the mechanical state of the muscle was very accurately defined; they showed that all the detailed intensity changes observed were accounted for very accurately by a tilting model.

Such a model became even more plausible when the high resolution X-ray crystallographic structure of myosin subfragment 1 (S1), which forms the crossbridges, was obtained by Rayment and his colleagues.[22,23] They showed that the S1 consisted of two major domains. The more globular domain (catalytic domain) contained the actin-binding site and the ATP-binding site. The second domain (light chain domain) was a remarkable elongated structure, in which the C-terminal portion of the myosin heavy chain formed a single α-helical structure about 9 nm long, with the two myosin light chains wound around it, presumably giving it structural stability. When the myosin S1 was attached to actin, this elongated structure pointed outwards, towards where the myosin filament backbone would be located in the intact muscle, and to which it would be attached.

Such a remarkable structure naturally suggested that the elongated domain functioned as a lever arm, to amplify smaller structural changes occurring in the catalytic domain during ATP hydrolysis and actin binding, and so provide the 5 or 10 nm of axial movement required by the tilting model. Subsequent crystallographic studies[24–28] suggesting or showing different orientations of the lever arm dependent on the exact nucleotide species bound, and other types of evidence about the behavior of the neck region of myosin (for example Uyeda *et al*.[29] and Burgess *et al*.[30]) have strongly supported that view.

However, it still remains essential to obtain further evidence to show that the lever arm motion is actually what produces force and filament sliding in a contracting muscle, to ascertain whether any additional processes may also be taking place, and to ascertain all the structural details of the mechanism within the highly specialized environment of an intact striated muscle fiber.

A new X-ray technique

A powerful new way to obtain such evidence was recently recognized by Lombardi and his colleagues.[1,2] When the 14.5 nm meridional reflection (M3) from contracting muscle is recorded at the very high spatial resolution made possible by current synchrotron X-ray sources, the reflection can be seen to consist of two close-spaced peaks of somewhat unequal intensity.[31] It was suggested that this splitting was caused by interference effects between the diffractions from the arrays of crossbridges in the two halves of each thick filament.[32] Analogous phenomena had been noted previously in resting muscle,[33–36] but technical limitations at that time did not allow adequate resolution for corresponding observations to be made on contracting specimens.

Lombardi and his colleagues realized that the position of the interference fringes where they sample the envelope of the M3 reflection, and hence the relative heights of the peaks so generated, provide an extremely sensitive measure of the average axial position of the center of scattering mass of the crossbridges. If a synchronized movement of the attached myosin heads takes place, such as would occur during a very rapid release or stretch, then the fringes should shift and their relative intensities change by calculable amounts. This would provide new and very accurate information about configurational changes in the crossbridges during the working stroke.

Basic principles of interference

The 14.5 nm meridional X-ray reflection is generated by the axial repeat of sets of myosin