

Muscle diffraction at CHESSE

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The modern concept of muscle contraction is based on structural, mechanical and biochemical studies, all indicating that relative sliding between the two filament types in the structural sarcomere unit depends upon cyclic ATPase-coupled interactions between so-called cross-bridges. These bridges join thick filaments, made up primarily of the protein myosin, to thin filaments consisting primarily of the protein actin. These filaments, 30 nm and 10 nm in diameter, respectively, form an interdigitating hexagonal lattice. The prevailing "swinging cross bridge model" envisions a 90 to 45 degree "power stroke" or rowing motion of the cross-bridges that translocates thin actin-containing filaments relative to thick myosin-containing filaments, leading to sarcomere shortening.

There have been a number of exciting results in recent years leading toward understanding the structural basis for this process, including *in-vitro* motility assays which have shown that isolated myosin S1 cross-bridges can move isolated actin filaments in fruit flies. But the exact nature of the force-generating myosin-

actin interaction, the ATP-driven power stroke, remains unclear.

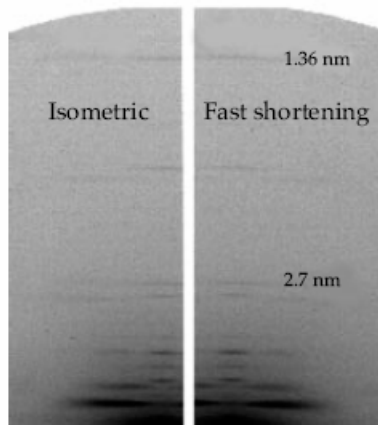
There is no doubt that structural studies of *intact* muscle fibers are indispensable to unraveling these mechanisms, since actin or myosin in crystals cannot generate force. Fiber diffraction of muscle has a key role in such studies because of its ability to study muscle fibers under hydrated, physiological conditions, in fact even in the living state. Furthermore, it has the ability to detect global changes in sarcomere structure at the physiologically relevant millisecond and sub-millisecond time scale.

Measuring the extensibility of muscle filaments

In the last CHESSE Activity Report we described a small angle camera designed¹ to fit in the high flux F-1 station. We have used this setup to obtain very accurate spacing measurements of certain wide-angle meridional reflections from contracting muscles. The intent of these measurements was to test the assumption that the flexibility of the filaments is essentially negligible, and that there is no measurable change in spacing when the muscle goes from the re-

laxed state to isometrically contracting. This is the central assumption of many influential theories of muscle contraction based on mechanical experiments. This assumption turns out to be false.

To show this, we (H. E. Huxley, A. Stewart, H. Sosa and myself) studied the behavior of the first actin meridional reflection (at a spacing of approximately



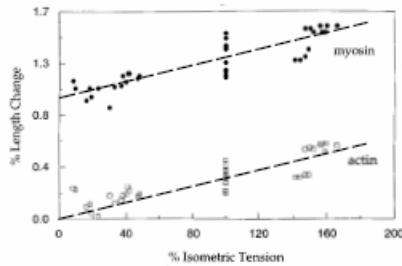
(Figure 1) A side-by-side comparison, on a single image plate, of diffraction from living frog sartorius muscles either relaxed (left) or electrically stimulated to contract. In the contracted state (right), the 1.36 and 2.7 nm layer lines are seen to move to higher angle.

2.73 nm), the second order of this reflection (1.365 nm) and the 15th order myosin meridional reflection at approximately 2.86 nm (Fig. 1) from living frog sartorius muscles either relaxed or electrically stimulated to contract. These reflections are very weak and require the high intensity available at the F-1 station to collect enough signal before the muscle fatigues.

These reflections also undergo significant changes in spacing which can be measured with great accuracy in an arrangement which allows patterns at different levels of tension during a single experimental series to be compared side by side on the same image plate (Fig. 1). We found that when the muscle changes from the relaxed state to that of isometric contraction, the actin 2.73 nm reflection increases in spacing by between 0.3 and 0.35%. The force exerted on the filaments can be augmented or diminished by applying moderate speed length changes to a contracting muscle essentially allowing the compliance of the filaments to be measured.

Analysis of these data is still proceeding, but a preliminary look (Figure 2) shows an approximately linear relation of filament length change to the level of force exerted by the muscle. More sophisticated analysis of the paired data suggest some nonlinearity at low tension levels. Notice that the curve for thick filaments has a non-zero intercept. This corresponds to a spacing change associated with activation or "turning on" the muscle. The thin filaments do not appear to show such a change.

In the experiments described so far, we were looking at fairly long lived states (large fraction of a second), much longer than the time scale of the basic force producing process (~ ms). We were also able to show an actin spacing change of 0.25 - 0.3% during a 2 ms time frame immediately following a quick release,



(Figure 2) Filament length as a function of force exerted by the muscle. Data were extracted from x-ray images like those in figure 1.

duлятор source (which is also small to start with) can be very helpful for single fiber experiments, since for a given focus size one can deliver more flux to the sample than for a wiggler source delivering the same total flux. To get

around the count rate limitations of delay-line type detectors we used a quantum-limited large format (1Kx1K) CCD detector operating as a streak camera, in which one dimension of the detector recorded time with a sampling interval of 1 ms, while the other dimension recorded distance along the equator in the conventional manner.

The CCD image in Figure 3 gives a direct visualization of the evolution with time of the equatorial x-ray pattern. The equatorial (10) and (11) reflection from single fibers of *Rana Temporaria tibialis anterior* muscle were recorded. During fixed-end tetanus rise, the previously reported

transient increase in equatorial spacing was clearly defined. The excellent spatial resolution of the CCD detector allowed following diffraction peak widths to the same time resolution, showing a marked transient increase in disorder (of the second kind) which gives information on the cooperative movement of groups of fibril components. The physiological significance of this effect is being investigated.

The CCD detector used in this study is the same as that used to record crystallographic data with exceptionally high precision ($R_{\text{sym}} = 2\%$) (see CCD article on page 36). Only a software selection was required to switch between the streak camera and conventional modes of operation. Thus this kind of detector shows great promise for investigating transient phenomena at the very high flux beam lines that are becoming available.

showing that this elastic behavior is rapid and is relevant to the basic force producing event. These experiments² also show that very detailed X-ray diffraction patterns can be obtained to the required millisecond level time scale on the F-1 beam line. Until small angle facilities at third generation synchrotron sources such as the ESRF and APS become available, these kinds of experiments will be possible only at CHESS.

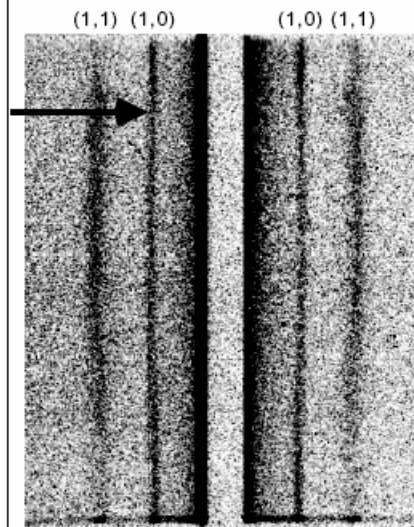
Single cells are a much better mechanical preparation than whole muscle; but because they are so much smaller, they give correspondingly less diffraction signal. They are also very labor intensive to prepare. Cecchi *et al.* have reported³ time resolved x-ray diffraction studies of single muscle cells (fibers ~3mm x 200 μm) during the rise of tetanic tension. This study used a one-dimensional wire counter with a sampling time of 10 ms to record equatorial x-ray intensity during tetanus rise, while simultaneously monitoring sarcomere length and tension. It would be very desirable to extend these studies to the millisecond or sub-millisecond time resolution which is the time scale of the mechanical events we are studying.

A truly international collaboration of Eric F. Eikenberry (Robert Wood Johnson Medical School), Fredrik Osterberg (Princeton University), G. Cecchi, M. A. Bagni, (Florence), C. C. Ashley, and P.J. Griffiths (Oxford) and myself collected time resolved, two-dimensional x-ray diffraction patterns during the CHESS undulator run last October. The low beam divergence of an un-

[1] Camera designed by H.E. Huxley, Brandeis, J. Bordas, Daresbury, and T. Irving, CHESS. See a descriptive article in "Synchrotron Radiation and Biophysics," Irving, T. and Huxley, H. E., (Oxford University Press) 1984.

[2] These observations of filament extensibility complement and extend studies done by Dr K. Wakabayashi (Osaka) and colleagues at the Photon Factory who studied the changes in spacing upon contraction as a function of muscle length. The two groups will submit their results together in the near future.

[3] Cecchi, *et al.*, *Biophys. J.* 59, 1273-1283 (1991)



(Figure 3) CCD streak camera image of x-rays diffracted from single fibers of *Rana Temporaria* muscle during fixed-end tetanus rise. Each horizontal row is a one-dimensional pattern recorded during 1 millisecond. The arrow shows the onset of a transient increase in lattice spacing. The (10) and (11) hexagonal lattice reflections are as indicated. Note the varying width of the (11) reflection.