

X-ray diffraction studies of cardiac muscle

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Introduction

Heart disease is the number one killer in the United States today. In order to understand various aspects of heart disease, we need to understand not only the details of cardiac muscle physiology but also the underlying molecular-level changes in the structures that make up cardiac muscle that lead to these macroscopic phenomena.

The so-called Frank Starling relationship is the most important regulatory mechanism in the heart of adult humans. It describes an enhancement of contractile function of the heart in response to an increase in end-diastolic ventricular volume. Increased volume implies an increase in muscle length. It has been known for some time that this phenomenon has, as its basis, an increase in the activation of myofibrils by calcium as muscle length increases [1, 2, 3]. This behavior is exhibited by both skeletal and cardiac muscle, but the phenomenon is much more pronounced in cardiac muscle.

The underlying molecular mechanisms for this increase in Ca^{2+} sensitivity are as yet unclear. It has been suggested by MacDonald and Moss [4] that the enhanced contractile response of cardiac muscle when it is stretched occurs (at least partly) because the myofilaments move closer together, thereby enhancing the probability of favorable acto-myosin interaction. This, in turn, results in increased sensitivity to calcium by cooperative activation of the thin filaments. This is an attractive hypothesis since it proposes a simple, physical basis for a very important feature of cardiac muscle physiology.

Cardiac muscle has been difficult to study because of the generally poorer quality of diffraction patterns as compared to skeletal and insect flight muscle [5]. Many experiments in the past used preparations from relatively large, expensive animals such as cats, dogs, and even goats. There is relatively little literature on rat and mouse muscle (however, see [6]). Development of a viable preparation of cardiac muscle from small, relatively inexpensive animals would allow a larger range of problems to be addressed.

Here we describe x-ray diffraction apparatus and techniques that have allowed us to obtain high-quality x-ray fiber diffraction patterns from rat trabeculae muscle.

Methods and Materials

Thin, uniform, and unbranched trabeculae were carefully dissected from the free wall of the right ventricles from rats (LBNF-1, 200–250 grams) and then skinned with Triton X-100. Typical dimensions of these preparations are 3–5 mm long, 80–150 μm wide, and 50–80 μm thick. Trabeculae were attached to aluminum T-clips prior to mounting in the experimental setup. Intact trabeculae were dissected similarly

but were maintained in Krebs- Hansleit solution after dissection with 20 mm added 2,3-butanedione monoxime (BDM) to keep the samples quiescent during the experiment [7].

Small-angle x-ray diffraction measurements used the Bio-CAT undulator-based beamline at the Advanced Photon Source, Argonne National Laboratory, Argonne, IL. Figure 1 shows the overall experimental setup. The x-ray diffractometer had a 3 m distance between the sample and the detector. The x-ray wavelength used was 1.03 \AA . All flight paths were evacuated except for a small gap around the sample chamber itself (~1 mm downstream, 2 mm upstream of the sample). The beam size at the sample position was collimated to about 0.4 x 0.8 mm, and about 0.040 x 1 mm (vertical x horizontal) at the detector and contained a maximum incident flux of $\sim 3 \times 10^{12}$ photons/s. (Approximately 10 times this flux could be made available by using the doubly focused beam, but this wasn't necessary for these experiments). X-ray diffraction patterns were collected with a CCD-based x-ray detector (1024 x 1024 pixels, 60 mm x 60 mm active area).

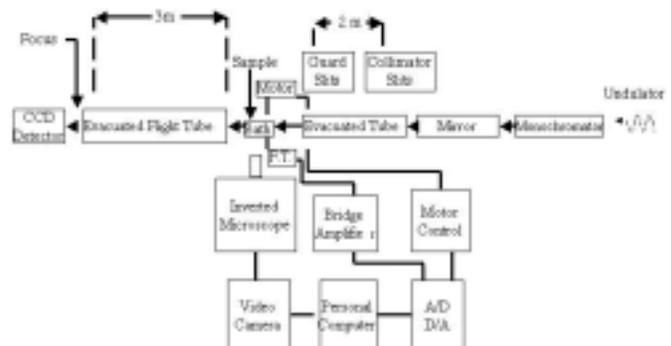


Figure 1: Overall experiment setup.

Samples were mounted in a small trough with dimensions of 0.8 mm wide x 40 mm long x 5 mm deep with windows allowing simultaneous collection of the x-ray patterns and viewing of the striation pattern using a long working distance (~4 mm) 40X objective of a video-equipped inverted microscope. The fiber was held via the aluminum clips on hooks between a force transducer (Kulite BG10) and a servomotor (Cambridge Technologies Model 308B) held on micromanipulators. During the experiment, ~20 ml of bathing solution was continuously perfused through the chamber using a peristaltic pump except during digitization of the striation images. All experiments were done at 23°C.

Sarcomere length was measured both immediately before and after the x-ray exposure by FFT-based analysis of digitized striation images as described [7]. Physiological parameters (force, sarcomere length, and muscle length) were recorded continuously using routines written in the LabView graphical data acquisition language (LabView V, National Instruments) and a National Instruments PCI1200 data acquisition board [7].

Results

Figure 2 shows a two-dimensional x-ray pattern taken from a membrane-intact rat trabeculae specimen. A smoothly varying background has been subtracted for clarity. As has been previously noted [5], x-ray patterns from cardiac muscle are generally poorer than those from skeletal muscle, probably because the constituent myocytes of the trabeculae are less well aligned than myofibrils in a muscle fiber and also because of the large amount of collagen present, which contributes to the relatively high background. Nevertheless, we can observe clearly resolved 1,0 and 1,1 equatorial reflections, the 14.3 nm equatorial reflection and myosin layer lines and the 5.9 nm actin-based reflection.

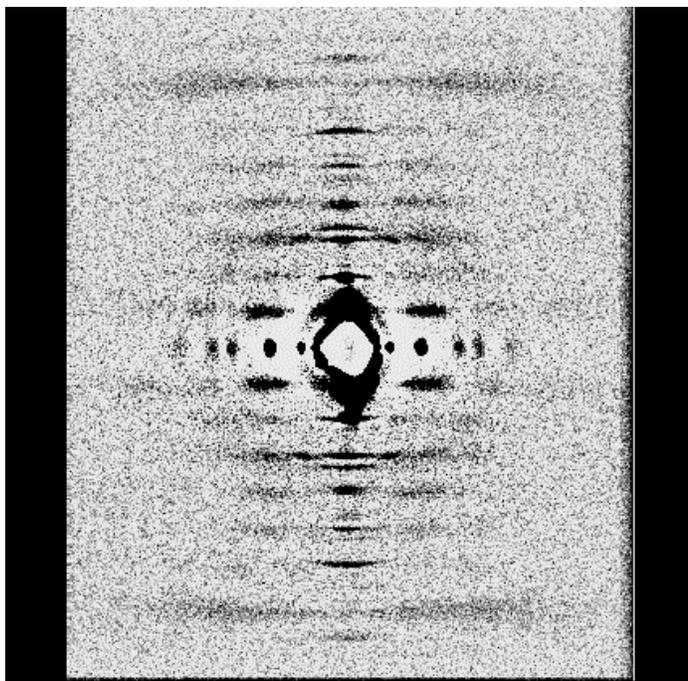


Figure 2: A two dimensional x-ray pattern taken from a membrane-intact rat trabeculae specimen.

We have also used this apparatus to collect lattice spacing data from both skinned and intact rat trabeculae that show conclusively that the lattice spacing in these preparations are a sensitive function of the sarcomere length, consistent with the MacDonal and Moss hypothesis. These results will appear elsewhere.

Discussion

We have shown that by using appropriately designed apparatus with the Bio-CAT undulator beamline, it is possible to obtain high-quality x-ray patterns from rat cardiac muscle with simultaneous collection of physiological parameters. We are in the process of extending these studies to mouse myocardium so that we can take advantage of the many transgenic model systems for various human pathologies now available.

Acknowledgments

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