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Muscle specimens. Fiber bundles were dissected from psoas muscle of 6 months old male rabbits (New Zealand White) followed by overnight skinning in 1% Triton X-100 in relaxing solution, washing with relaxing solution and storage in relaxing solution on ice until use within 36 hours. Relaxing solution composition (in mM): 40 Imidazole; 10 EGTA; 6.4 Mg-acetate; Na-ATP; Na-Azide; DTT; 70 potassium proprionate and 10 creatine phosphate (pH 7.0 at room temperature). The following protease inhibitors were present in all solutions. leupeptin 20 μM; E-64 30 μM; PMSF 2.5 μM. All mechanical experiments were carried out in relaxing solution, some of which contained 1.5 % Dextran T-500. This was motivated by the report of Farman et al (Farman and others 2006) that demonstrated that adding a small amount of Dextran T500 (~1%) to the bathing solution in cardiac muscle yielded a significant increase in calcium sensitivity and in $I_{1,1}/I_{1,0}$ that was then stable up to 6% Dextran. This was seen as an indication that when the lattice spacings swelled upon skinning there were structural changes that affected calcium sensitivity that was reversable when myofilament lattice spacings were moved closer to their physiological values. 1.5% was chosen as a minimal level to bring the lattice spacing into this stable range. For the combined mechanical/X-ray experiments, the ends of the fiber bundles were attached to small aluminum clips that were used to mount the fiber bundles to the tension transducer and motor (see below).

X-ray diffraction. Small-angle x-ray diffraction experiments were performed at the BioCAT beamline 18 ID at the Advanced Photon Source, Argonne National Laboratory (Fischetti and others 2004) using the apparatus described previously (Irving and others 2000). Experiments were done using a 2 or 3 m distance between the sample and the detector and with the X-ray beam energy set to 12 keV (1.03 Å wavelength). 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 by 0.8 mm, and about 0.040 by 1 mm (vertical x horizontal) at the detector and contained a maximum incident flux of ~3 x 10^{12} photons/s. Muscle bundles were mounted horizontally in a small trough with dimensions of 0.8 mm wide x 32 mm long x 5 mm deep with windows allowing simultaneous collection of the X-ray patterns and viewing of the striation pattern using a 40 X long working distance objective with a video-equipped inverted microscope (Nikon TMS). The slides of the sample chamber were hollowed out allowing the x-ray beam to pass through the fiber via 0.001" thick Kapton™ windows. The fiber was held via the aluminum clips on hooks between a tension transducer (Kulite BG10) and a servo-motor (Cambridge Technologies Model 308B). During the experiment, ~20 ml of bathing solution (18 ºC) was continuously pumped through the chamber using a peristaltic pump, except during the digitization of the striation images, which takes 2 seconds. X-ray exposure times were ~0.5 seconds and the diffraction patterns were collected using a CCD-based X-ray detector (active area 55 x 88 mm, 1798 x 1027 pixels (Phillips and others 2002)). Prior to analysis, diffraction patterns were corrected for dark current, flat field and spatial distortions.

Data Acquisition and Sarcomere Length Measurement. Sarcomere length was measured immediately before and after the X-ray exposure by a FFT analysis technique of digitized striation images as described previously (Fan and others 1997; Irving and others 2000). Sarcomere length was monitored and found to not change during stress relaxation. Tension, muscle length and X-ray beam intensity were digitized by an A/D converter (National instruments model PCI-1200) installed in the same computer used for digitizing the striation images.

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