Titin-Based Modulation of Lattice Spacing and Calcium Sensitivity of Active Tension in Cardiac Muscle

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Introduction

The precise mechanisms by which the heart is able to enhance its contractile performance in response to an increase in end-diastolic volume (the well-known Frank-Starling mechanism) remain to be resolved. The enhanced active force response when muscle is stretched may be explained by the myofilaments moving closer together, thereby increasing the probability of cross-bridge binding to actin.[1] Titin functions as a molecular spring that underlies the passive force of cardiac myocytes. This force is the main contributor to overall passive force of cardiac muscle, except toward the upper limit of the physiological SL range where collagen dominates.[2] We have recently shown that the level of passive force significantly influences the length-dependence of activation.[3] To address the mechanism underlying our findings, we measured the influence of passive tension on interfilament lattice spacing, using x-ray diffraction on mouse myocardium.

Material and Methods

Cardiac muscle strips were isolated from the left ventricular wall of 10-to-12-week-old mice (Balb/C) and bathed in relaxing solution as previously described.[3] The overall experimental arrangement has been described elsewhere.[4] X-ray patterns were collected on the small-angle instrument on the Bio-CAT undulator at beamline 18-ID. X-ray patterns were collected on a CCD-based x-ray detector. Spacings of the 1,0 and 1,1 equatorial reflections were measured and converted to $d_{1,0}$ values, using Bragg's Law. Trypsin was used to specifically degrade titin (0.25 µg trypsin/ml at 25ºC for 25 min).

Results

The SL-dependence of the $d_{1,0}$ spacing before and after trypsin treatment is shown in Fig. 1. Myofilament lattice spacing decreased significantly as SL was increased (Fig. 1), consistent with recent measurements on rat cardiac trabeculae.[4] Degrading titin significantly increased the lattice spacing; this increase was largest at 1.9 µm SL, with a gradual decrease with SL.

Discussion

Our low-angle x-ray diffraction studies on cardiac muscle showed that degradation of titin significantly increases $d_{1,0}$ over the working range of sarcomere lengths in the heart. These findings are consistent with results on mechanically skinned skeletal muscle fibers, where a close correlation is found between titin-based passive tension and $d_{1,0}$ and where, following degradation of titin, $d_{1,0}$ is independent of SL.[5] Thus, titin is a modulator of interfilament lattice spacing in skeletal and cardiac muscle. In cardiac muscle, however, the myofilament lattice was still responsive to SL following titin degradation. This is probably due to large amount of collagen in cardiac muscle.[2] Previous work by our group and others have shown that titin-based passive tension modulates active acto-myosin-based tension.[3,6] Our new findings indicate that the underlying molecular mechanism may involve titin-based radial forces that modulate the myofilament lattice spacing. Thus, titin has the potential to enhance systolic performance as the ventricular volume is increased and is a possible candidate for the molecular length sensor responsible for the Frank-Starling law of the heart.

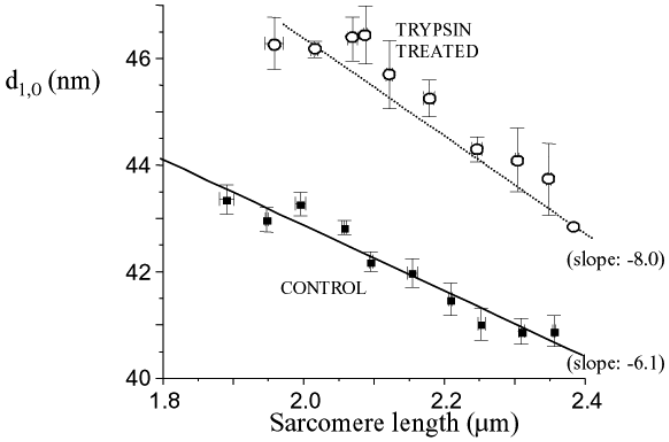

FIG. 1. Effect of trypsin on myofilament lattice spacing of skinned muscle. Results were binned in 0.05-µm SL intervals and mean ± SE of 10 preparation were calculated. Lines are linear regression lines with slopes as indicated. $d_{1,0}$ is the 1,0 spacing of the hexagonal lattice formed by the thick and thin filaments in the sarcomere of cardiac muscle

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