Investigating the Effects of Calcium on Titin in Myofibrils

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Summary

Titin's role in active muscle contraction remains unclear, but recent evidence suggests it contributes to active force by increasing stiffness during muscle contraction, though the underlying molecular mechanisms are unknown. One theory suggests that titin binds to actin, thereby shortening the spring-like protein [3,5], with calcium proposed to regulate this binding [5]. This study assessed calcium's role in titin-actin binding by tracking the PEVK segment of titin throughout myofibril stretches under various conditions: (i) passive, (ii) active with inhibited cross-bridge formation, (iii) active isometric contractions, and (iv) active isometric contractions preceded by active stretching. We found that passive and active stretching with cross-bridge inhibition showed similar PEVK elongations, while active isometric contractions, with or without stretching, significantly increased PEVK length, suggesting that titin-actin binding occurs only when cross-bridge binding is allowed, but not with calcium activation alone, as had been proposed previously.

Introduction

Muscle contraction has been associated throughout history with the sliding of two contractile filaments, actin and myosin [1]. However, this theory is unable to explain several well-acknowledged mechanical properties of skeletal muscle, including the so-called residual force enhancement (rFE) property. Although debated, titin is now recognized as a molecular spring that modulates its stiffness based on contractile conditions [3]. The molecular mechanisms of how titin increases its stiffness remain speculative with the primary theory suggesting that titin binds its proximal, extendable I-band segment to actin, thereby shortening its spring-like I-band segments [3,5]. Calcium, which enters the contractile region during activation, has been thought by many to regulate this interaction [e.g., 5]. The purpose of this study was to determine calcium's role in titin-actin binding by following the antibody labelled distal I-band section of titin called PEVK while stretching single myofibrils and sarcomeres under various conditions including: (i) passive, (ii) active condition while inhibiting cross-bridge formation using 2,3-Butanedione monoxime (BDM), [4], (iii) active isometric contractions, and (iv) active isometric contractions preceded by active stretching. We hypothesized that calcium causes a proximal portion of titin to bind to actin, thereby increasing the elongation of the more distal PEVK region upon calcium activation.

Methods

New Zealand White rabbit psoas myofibrils were isolated and incubated with two distinct antibodies that labelled the proximal and distal ends of titin's PEVK region. Myofibrils were stored initially in rigor solution, then placed in a bath with relaxing solution devoid of calcium. Suitable myofibrils were picked up with a needle-nano lever system and observed under optical microscopy. The myofibrils were then stretched from an average sarcomere length of 2.7 µm to 3.5 µm under the four conditions described above.

Results and Discussion

Passive stretching and stretching with cross-bridge attachment inhibited resulted in similar PEVK segment elongations, while active isometric contractions with and without stretching resulted in significant increases of the PEVK segment length (Figure 1). We concluded from these results that titin-actin binding does not occur when sarcomeres are stretched passively or actively when cross-bridge attachment is inhibited but only occurs when active muscles are stretched, and cross-bridge binding is allowed. Therefore, our results suggest that calcium is not the regulator of titin-actin interactions as had been proposed previously, but strong cross-bridge binding is required to allow for titin-actin interaction that contributes to rFE in skeletal muscle.

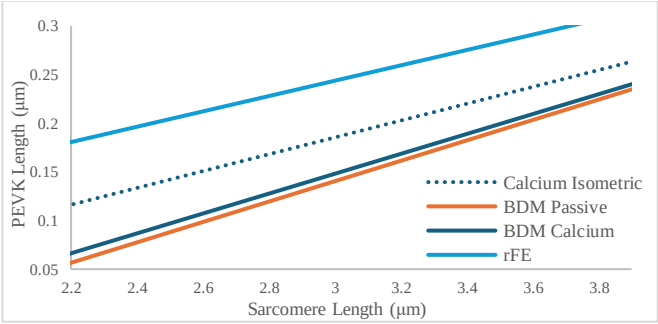

Figure 1: PEVK lengths at a given sarcomere length in myofibrils in various conditions (rFE collected by Armaan Sekhon).

Conclusions

Our results challenge previous theories that calcium activation regulates titin binding to actin. Rather, we provide strong evidence that cross-bridge binding allows titin to bind to actin. We tentatively propose that the displacement of tropomyosin on actin upon strong cross-bridge binding frees up attachment sites for titin to bind to actin.

References

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