In-situ Extension of Titin in Skeletal Muscle Myofibrils Shows Simultaneous Segmental Elongation

Chris Tiessen, Armaan Sekhon, Walter Herzog
Human Performance Lab, Department of Kinesiology, University of Calgary, Alberta Canada
Email: chris.tiessen@ucalgary.ca

Summary

Passive force in skeletal muscle can be attributed to the giant protein titin. Titin has three extensible segments, the tandem-proximal immunoglobulin (Ig) domains, the PEVK and the distal Ig domains. Here, for the first time, we use real-time immunofluorescent tracking of titin in isolated myofibrils to show the extension of these three segments occur much more simultaneously *in situ* than shown in isolated titin constructs or single filaments. Thus, we implicate immunoglobulin domain unfolding as potentially relevant to passive force generation at long, yet physiological, sarcomere lengths.

Introduction

Titin is a giant spring-like protein anchoring myosin thick filaments to the Z-disk in skeletal and cardiac muscle. It is the primary contributor of passive force at the sarcomere level. Titin produces tensile force in response to segmental elongation of extensible domains, notably its unique PEVK motif, its tandem-proximal immunoglobulin (Ig) domains, and distal Ig domains. The generally accepted sequence of extension is first, unstructured inter-Ig domain regions align at low forces; second, titin's PEVK extends elastically, contributing most of the physiological force production; and third, Ig domain unfolding occurs at exceptionally long sarcomere lengths, particularly in the tandem-proximal region of titin (near the Z-disk) [1]. This sequence of extension of titin's segments was derived largely from isolated molecular constructs or single titin filaments [1]. Until now, it has not been possible to delineate the real-time position of titin's segments (PEVK, tandem-proximal, and distal Ig domains) in intact sarcomeres. Here, we show that although the accepted sequence of titin extension is applicable, segmental extension occurs much more simultaneously *in situ*.

Methods

We have developed a novel immunofluorescent tracking system for titin permitting real-time tracking of titin's extensible regions (tandem-proximal Ig domains, PEVK, and distal Ig domains). This model produces identical passive and active forces to unlabeled myofibrils, indicating labelling did not affect sarcomere integrity. Labels were placed on the N2A region, separating the tandem-proximal and PEVK regions, and the distal end of the PEVK region (F146) (figure 1).

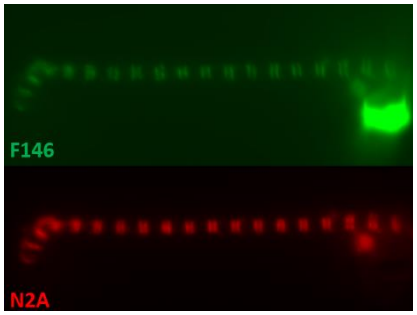

Figure 2: Fluorescent image of a single myofibril, showing the N2A and F146 via AF647 and AF488 secondary channels, respectively. Shown at 100x magnification.

We isolated individual myofibrils from the psoas of female New Zealand white rabbits. Myofibrils were stretched from 2.7μm/sarcomere to 4.5μm/sarcomere using a piezoelectric motor. We measured tensile force using optical nano-levers calibrated to a known stiffness. We measured the length of titin's tandem-proximal Ig domains, PEVK, and distal Ig domains, and considered Ig domains 'unfolded' if the length was greater than the theoretical contour length when all Ig domains were folded for rabbit psoas (225nm and 117nm for the tandem-proximal and distal Ig domains, respectively) [2].

Results and Discussion

Titin's tandem-proximal Ig domains, PEVK region, and distal Ig domains all elongated upon myofibril extension. However, we found that both Ig domain regions showed unfolding *before* the PEVK region reached full extension. Moreover, Ig domain unfolding seems to occur starting at sarcomere lengths of ~3.0μm, within the limit of physiological extension (figure 2).

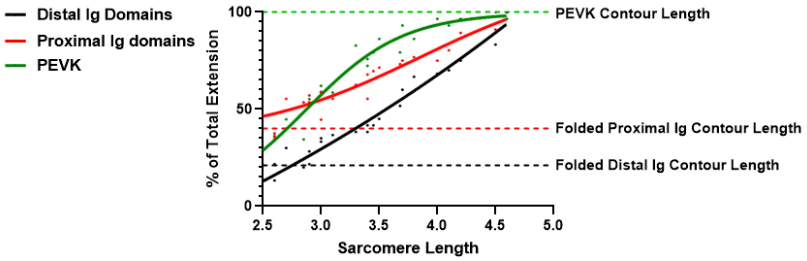

Figure 1: Normalized extension of titin segments at various sarcomere lengths. Data obtained from n=7 myofibrils. Theoretical contour lengths [2] are shown as a percentage of final length.

We believe that the *in-situ* environment of intact sarcomeres may alter forces required for unfolding, thus explaining the decreased Ig domain region stiffness *in-situ*. This effect may be enhanced during strenuous exercise due to recent evidence from titin-constructs indicating that lowered muscle pH may decrease titin stiffness [3].

Conclusions

We show that titin's extensible segments extend much more simultaneously *in situ* using immunofluorescent real-time tracking in intact myofibrils. This implicates immunoglobulin domain mechanics as physiologically relevant at long physiological lengths. This effect may be enhanced by additional physiological changes during intense exercise [3].

References

[1] Trombitas et al. (1998). *J. Cell Biol.*, **140**: 853-859.
[2] Schappacher-Tilp et al. (2015) *PLOS one, 10:* e0141188.
[3] Mudiyanselage et al. (2022), *Int J Mol Sci*, **9**: 4779