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Topology of interaction between titin and myosin thick filaments☆

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ABSTRACT

Titin is a giant protein spanning between the Z- and M-lines of the sarcomere. In the A-band titin is associated with the myosin thick filament. It has been speculated that titin may serve as a blueprint for thick-filament formation due to the super-repeat structure of its A-band domains. Accordingly, titin might provide a template that determines the length and structural periodicity of the thick filament. Here we tested the titin ruler hypothesis by mixing titin and myosin at *in situ* stoichiometric ratios (300 myosins per 12 titins) in buffers of different ionic strength (KCl concentration range 100–300 mM). The topology of the filamentous complexes was investigated with atomic force microscopy. We found that the samples contained distinct, segregated populations of titin molecules and myosin thick filaments. We were unable to identify complexes in which myosin molecules were regularly associated to either mono- or oligomeric titin in either relaxed or stretched states of the titin filaments. Thus, the electrostatically driven self-association is stronger in both myosin and titin than their binding to each other, and it is unlikely that titin functions as a geometrical template for thick-filament formation. However, when allowed to equilibrate configurationally, long myosin thick filaments appeared with titin oligomers attached to their surface. The titin meshwork formed on the thick-filament surface may play a role in controlling thick-filament length by regulating the structural dynamics of myosin molecules and placing a mechanical limit on the filament length.

1. Introduction

Titin is a giant filamentous polypeptide that stretches between the Z- and M-lines of the striated-muscle sarcomere (Granzier and Labeit, 2006; Henderson et al., 2017; Linke and Krüger, 2010). Whereas the I-band section of titin functions as a molecular spring that defines sarcomeric elasticity, the Z-line, A-band and M-line regions of the molecule are tightly associated with the local sarcomeric protein components. Hence, in the A-band titin is associated with the myosin thick filaments. Because super-repeat sequences appear in the A-band section of titin (Bang et al., 2001; Labeit and Kolmerer, 1995) which apparently match the spatial periodicity of the thick filament (Henderson et al., 2017; Squire, 1997), it has been suggested that titin functions as a molecular blueprint for A-band organization by providing a ruler that defines the length of the thick filament (Trinick, 1996a; 1994; 1992; Tskhovrebova and Trinick, 2012). Although the titin ruler hypothesis has been coined as soon as the titin sequence was revealed (Labeit and Kolmerer, 1995), the binding of A-band-specific anti-titin antibodies (Whiting et al., 1989) and high-resolution electron microscopic analysis (AL-Khayat et al., 2013) placed titin on the surface of the thick filament which cast uncertainties on a simple geometric layout feature of titin. Furthermore, conflicting and hotly debated evidence emerged that raised doubts about a directly templating function (Granzier, 2015; Granzier et al., 2014; Tskhovrebova et al., 2015). Recently, however, it was found that the deletion of two super-repeats from the C-zone of titin resulted in an exactly corresponding reduction of the A-band width (Tonino et al., 2017), thereby causing a re-emergence of the titin-ruler hypothesis (Tskhovrebova and Trinick, 2017). The mechanisms of how the length of the myosin thick filament is regulated by the A-band titin sequences, however, are not known.

In the present work we carried out a simple, direct test of titin's potential myosin-templating function. It has long been known that titin is able to bind myosin (Murayama et al., 1989; Soteriou et al., 1993; Trinick, 1996b), although the exact topology of the interaction has not been resolved. Considering that under *in vitro* conditions it is possible to generate bipolar myosin thick filaments resembling the native form by reducing the ionic strength, we tested whether and how such a thick-filament formation might occur in the presence of full-length titin. We found that myosin molecules do not line up along titin but form thick filaments independently. By contrast, when allowed to equilibrate

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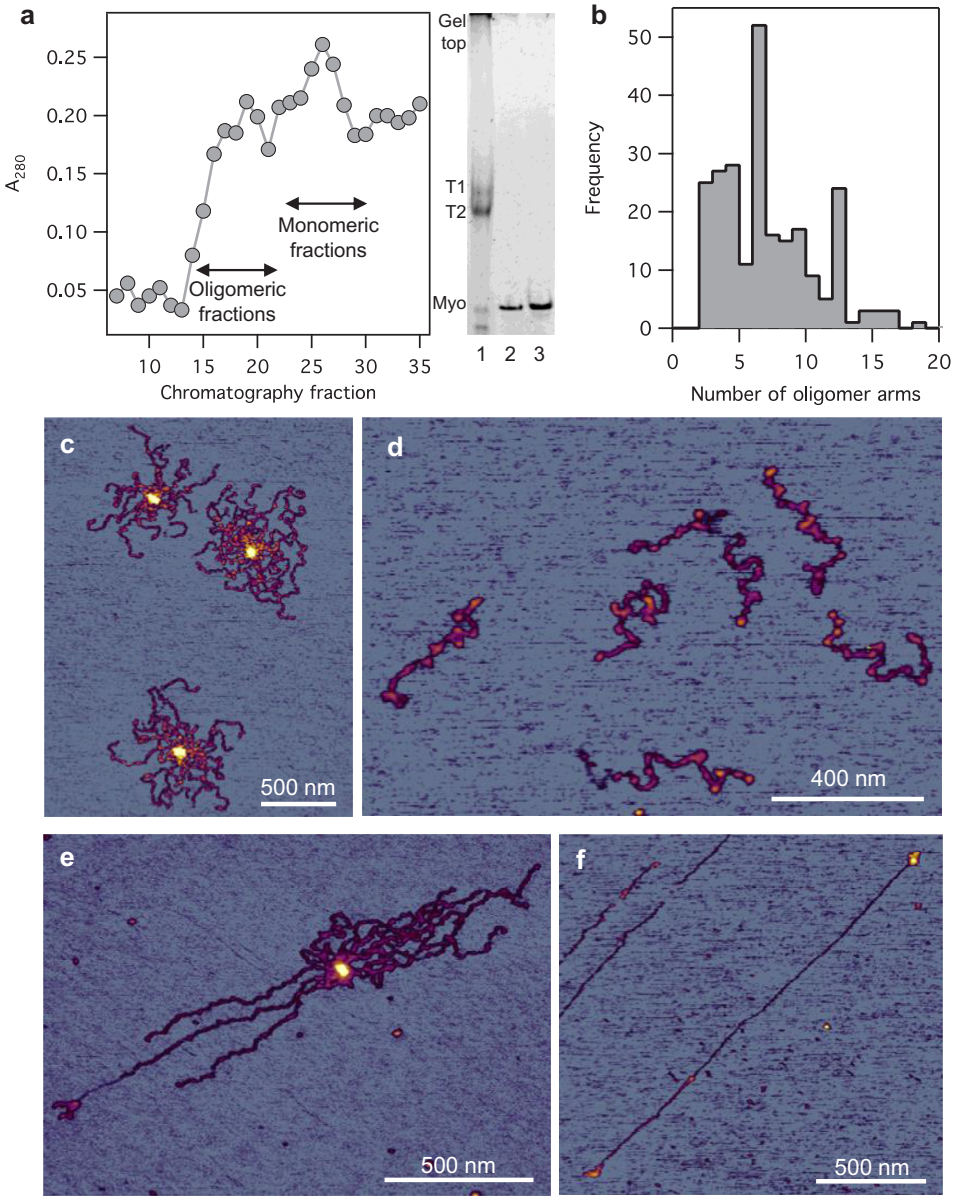

Fig. 1. AFM morphology of purified skeletal-muscle titin. a. Chromatographic (Sepharose CL-2B) elution profile of titin. Inset, ProSieve QuadColor-stained 1% agarose gel profile of the samples used. Lane 1, 50 μg titin, monomeric fraction. Lanes 2 and 3, myosin 40 and 90 μg, respectively. b. Distribution of the number of titin monomers, called here as "oligomer arms" resolved in oligomers of titin. c. AFM image of a sample from the oligomeric titin fractions. The characteristic "Medusa-head-like" appearance of titin oligomers, formed by the head-to-head binding of monomers, emerges in which the monomers are referred to here as oligomer arms. d. AFM image of a sample from the monomeric titin fractions. The random-chain appearance of the titin molecules is well visible. e. Titin oligomer combed and straightened by using meniscus force. f. Titin monomer straightened and extended by meniscus force.

configurationally, titin oligomers formed a meshwork on the surface of myosin thick filaments. Thus, titin is not a simple geometric template for thick-filament formation but is likely to regulate thick-filament length by forming a surface wrapping that places an upper limit on the filament length.

2. Materials and methods

2.1. Purification of titin

Skeletal-muscle titin was prepared from rabbit *m. longissimus dorsi* by using previously published protocols (Mártonfalvi et al., 2017; Soteriou et al., 1993). Muscle samples were obtained from male New Zealand white rabbits by using a procedure (Approval number: XIV-I-001/29-7/2012) approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics and by the Directorate for Food-chain Safety and Animal Health of the Government of Pest County with reference to the Hungarian Law on the Protection and Humane Treatment of Animals (XXVIII/1998). Purified titin samples were stored on ice in the presence of protease inhibitors (40 μg/ml leupeptin, 20 μM E64) until further use. Typically, samples were used within two weeks of purification. Sample purity was analyzed with vertical agarose gel electrophoresis (1%) according to established methods (Warren et al., 2003). The purified titin samples contained trace amounts of myosin (see Fig. 1a inset).

2.2. Purification of skeletal-muscle myosin II

Myosin was purified from rabbit *m. longissimus dorsi* by using previously published protocols (Margossian and Lowey, 1982) that involve cycles of alternating precipitation and dissolution at low and high ionic strength, respectively. High-concentration (typically ~30 mg/ml) monomeric samples were stored in 50% glycerol at −20 °C until further use. Prior to using the myosin sample, glycerol was removed by either a precipitation-dissolution cycle or by dialysis. According to electrophoretic analysis (Fig. 1a inset) the myosin sample was devoid of titin contamination.

2.3. Preparation of thick filaments

Thick filaments were prepared by either a direct dilution into or a dialysis against a buffer containing KCl at a concentration ranging