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Mechanical dissociation of the M-band titin/obscurin complex is directionally dependent

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ABSTRACT

Titin and obscurin, two giant muscle proteins, bind to each other in an antiparallel Ig–Ig fashion at the M-band. This interaction must be able to withstand the mechanical strain that the M-band typically experiences and remain intact. The mechanical force on these domains is likely exerted along one of two axes: a longitudinal axis, resulting in a 'shearing' force, or a lateral axis, resulting in a 'peeling' force. Here we present molecular dynamics data suggesting that these forces result in distinct unraveling pathways of the titin/obscurin complex and that peeling the domains apart requires less work and force.

1. Introduction

The sarcomere, the smallest contractile unit in muscle, drives virtually all bodily motion. In order for the sarcomere to work effectively, actin and myosin filaments, along with other peripheral members of the contractile apparatus, must be properly positioned [1]. Skeletal muscles accomplish this complex organizational task through an intricate web of scaffolding proteins that must be simultaneously pliable enough to accommodate motion yet sturdy enough for force propagation [2–3]. The most obvious of these sarcomeric macromolecular scaffolds are the Z-disk and the M-band [3–4]. While the Z-disk is largely inflexible, the M-band distorts significantly upon the application of force yet regains its original structure upon muscle relaxation [3,5].

Many proteins in the M-band, including M protein, myomesin, obscurin, and titin, are organized as a series of Ig-like and FnIII-like domains, arranged in tandem and connected via semi-flexible peptide linkers [2,6]. Proteins containing such structural elements likely provide elastic stability by acting as long flexible fibers that are crosslinked extensively [3,7]. Implicit in this organization is that the forces holding the M-band together, at least in the aggregate, must be strong; weak protein–protein interactions would break with force, which in turn would unravel the M-band.

Two of the proteins anchored in the M-band, titin and obscurin, are critical for global muscle cell organization [8–10]. Titin (3–4 MDa) performs multiple roles in the sarcomere including setting the overall sarcomere length and acting as a stretch sensor [2,11–14]. Obscurin (750–900kDa) is the only known protein to link cytoskeletal elements with the surrounding sarcoplasmic reticulum membrane and transverse tubule structures [15]. The extreme N-terminus of obscurin (Ig1) binds directly to the extreme C-terminus of titin (M10) at the M-band [16]. The high resolution structures of titin bound to a close cousin of obscurin, obscurin-like Ig1 (OL1), reveal the M10/OL1 complex exists in an antiparallel Ig–Ig formation [17–18]. NMR and more recent X-ray studies show that Ig1 also binds to M10 in this same manner [19–20]. Given (a) the head-to-tail structure of the M10/OL1 complex, (b) the long, filamentous overall architecture of both obscurin and titin, and (c) that mechanical force exerted on this complex must be initiated distally, we reasoned that there are two ways in which the domains can be separated. If other molecules do not significantly influence the orientation of the complex one would expect a pulling force to peel the two domains apart from each other (Fig. 1A, top). This has been experimentally tested on M10/OL1 via AFM [17]. Alternatively, one or both domains may be held rigidly in place requiring shear force to separate the domains (Fig. 1A, bottom). Thus, a detailed understanding of how titin and obscurin

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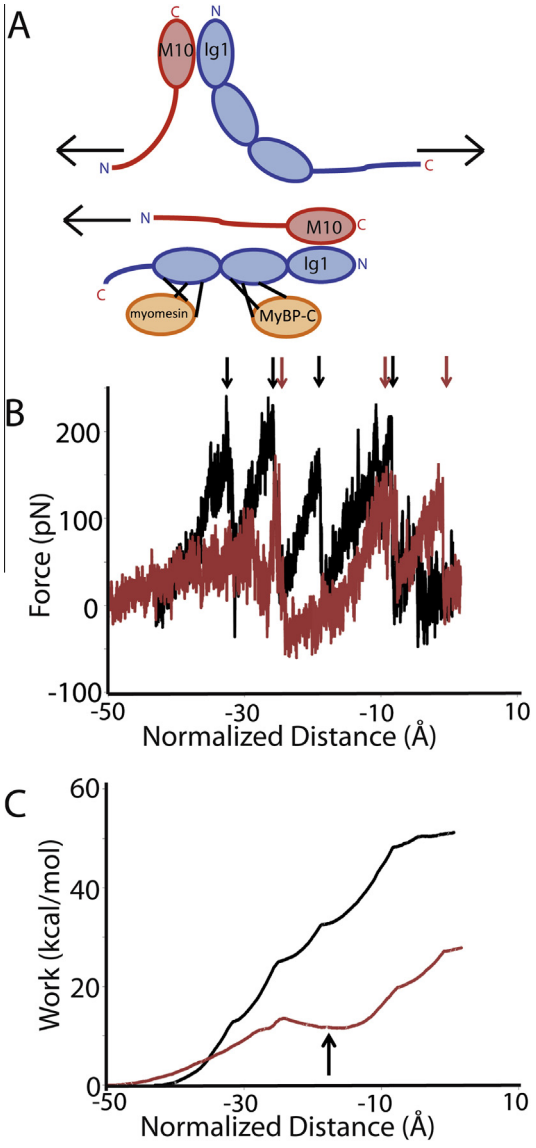

Fig. 1. (A) Schematic of the two models by which the M10 domain can be separated from the OL1 structure. Top is a peeling model, while the bottom is a shearing model. (B) Force–distance trace of the shearing model (black) and the peeling model (red). Small arrows correspond to H-bond rupture points. (C) Work–distance trace of the shearing model (black) and the peeling model (red). In (B) and (C) the '0' position is the point of domain separation and the arrow in (C) highlights the presence of a metastable, molten globule-like state.

behave when pulled from different directions may give insight into how this complex is oriented within the M-band.

2. Results and discussion

We performed steered molecular dynamic (SMD) [21,22] simulations on the M10/OL1 system and found that the shearing model produces multiple closely-spaced spikes of near-equal force. In contrast, there are not as many force spikes in the peeling simulation. Furthermore, the maximum force required to shear this complex apart approaches 250 pN while the force required to peel the domains is roughly 75 pN less (Fig. 1B). As is typical for SMD simulations, our calculated peeling force is an order of magnitude higher than that measured with AFM. This occurs because of the different pulling speeds between the two techniques. However, it has been demonstrated that the mechanical insights gained from SMD are valid [17,23–24].

To examine whether the molecular mechanism of domain separation could explain the differences in the maximum force, we plotted work vs. distance (Fig. 1C). While this comparison is normally used to calculate free energy, it can also provide insight into how many energy-requiring events are necessary to break OL1 away from M10. This analysis shows shearing is accomplished in multiple closely spaced events while peeling happens in two distinct steps. The peeling steps are interrupted by a significant intermediate period where the domain ends can be moved away from each other without the requirement of a significant amount of work (Fig. 1C, arrow). Additionally, the total amount of work to separate the domains is much less in the peeling model, and a longer distance is required to separate the domains.

Next, we examined the relationship of the energy steps in both models to molecular events. Backbone hydrogen bonds between Glu92, Tyr94, and Ala96 of OL1 and Val21, Thr23, and Ala25 of M10 initially hold M10/OL1 together (in fuchsia, Fig. 2A). These bonds form an inter-protein antiparallel beta sheet, and are surrounded by extensive hydrophobic interactions consisting of residues Pro11, Pro12, Phe14, Phe17, Ala93, Tyr94, Ala95, and Ala96 of OL1 and Pro11, Val21, Leu22, Thr23, Val24, Ala25, and Ala27 of M10 (spheres, Fig. 2A–C). In both the shearing and peeling simulations, these native hydrogen bonds are broken early in the simulation (Fig. 2D–E, first arrow). In the shearing model, new transient backbone hydrogen bonds then re-form with residues further down the opposite beta strand. This rupture/reformation pattern repeats in a predictable pattern, and coincides with the high force peaks in the shearing force/distance graph (Fig. 1B). Having to break multiple rounds of hydrogen bonds explains the large amount of work required to shear the M10/OL1 domains apart. The second round of hydrogen bond breaking (Fig. 2D; second arrow) coincides with a rapid loss of hydrophobic contacts between the two subunits. Since this event does not require more force than breaking the first round of hydrogen bonds, it seems that hydrophobic interactions make a smaller contribution to mechanical stability than might have been expected. During the remainder of the shearing simulation, hydrogen bond breakage always corresponds to increased force and work.

The peeling model initially follows the same pattern as the shearing model. However after an initial decline in the number of hydrophobic contacts and backbone hydrogen bonds, these values stabilize during a period in which no work is being done on the system (Figs. 1C, 2C and E). Here, this intermediate complex is metastable (see arrow in Fig. 1C) and resembles a molten globule with extensive hydrophobic contacts. At this point the OL1 and M10 domains have pivoted around the interdomain hydrophobic region and the two Ig structures are perpendicular (compare Fig. 2A and C, and the Supplemental movies). This twisting motion precludes reformation of backbone interdomain hydrogen bonds and continues until the domains are parallel before they fully separate. Several side chain-side chain and side chain-backbone hydrogen bonds form and then break during this time. Unlike in the shearing model, these hydrogen bonds do not form in a predictable repeating pattern. The breaking of these transient hydrogen bonds corresponds to a broad force increase around −10 Å and another around −5 Å (Fig. 1B, red arrows). Hydrophobic contacts rupture at the later stages of domain separation, and once again do not contribute strongly to the amount of force or work required for domain separation.

In both models, the force required to break hydrogen bonds dominates the energy landscape. Analysis of both trajectories provides an excellent study into the limitations of hydrophobic interactions to resist mechanical stress. While such interactions resist force, they clearly play an ancillary role here. Without specific bonds, hydrophobic interactions can glide over other hydrophobic surfaces. This creates a more malleable interaction surface,

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