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Electrostatic forces or structural scaffolding: What stabilizes the lattice spacing of relaxed skinned muscle fibers?

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HIGHLIGHTS

- Electrostatics predicts that the lattice collapses to 60% beyond overlap.
- This collapse is not observed or prevented by titin filaments.
- Collapse is prevented by very weak radial M-band or Z-line elasticity.
- Fitting spacing-length data also requires electrostatic interactions.
- Electrostatic interactions also explain temperature-dependent spacings.

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ABSTRACT

The filament lattice in relaxed striated muscle is thought to be stabilized by electrostatic forces between charged filaments; electrostatic theories based on known filament charge densities do predict that the lattice spacing drops slightly with sarcomere length when actin and myosin filaments overlap. However, at sarcomere lengths with no overlap, electrostatic forces are reduced to a very low level and electrostatic models predict that the lattice collapses to a much smaller spacing. This collapse is not observed, which suggests that the A-band and I-band lattices are stabilized mechanically by the M-band and Z-line. To determine which mechanisms operate, consider a model where charged-filament interactions are supplemented by elastic titin filaments and radially elastic M-bands and Z-lines. To make progress, this model is simplified by assuming that the areas of A-band and Z-line unit cells are equal. Published data for the length-dependence of the lattice spacing, in and out of overlap, can be fitted to a mechanical model with known titin elasticity and very weak M-band or Z-line stiffness (≈ 0.15 pN/nm per unit cell), which implies that electrostatic interactions cannot be ignored. A better fit is obtained when electrostatic interactions are restored. Electrostatic interactions also explain why the lattice spacing of relaxed muscle is a decreasing function of temperature.

1. Introduction

It has long been thought that the stability of the filament lattice in striated muscle is controlled by a balance of electrostatic forces between charged filaments, namely Coulombic repulsion and van-der-Waals attraction (Elliott, 1968, 1980; Millman and Nickel, 1980; Millman and Irving, 1988; Millman, 1998); this concept is supported by recent modeling (Smith and Stephenson, 2011). The dominant repulsive interaction is between negative charges on actin and the light chains of S1 heads on myosin filaments; the net strength of this interaction is strictly proportional to the length of filaments in overlap. However, purely electrostatic models always fail when the sarcomeres are stretched beyond zero overlap; actin–myosin charge interactions are reduced to zero, leaving very small electrostatic actin–actin and myosin–myosin interactions which are independent of sarcomere length. Such models predict that the lattice suddenly collapses to about 60% of its spacing at full overlap, which is not observed.

Experimentally, the lattice spacing of relaxed skinned fibers decreases by about 10% on stretching from full overlap to beyond the zero overlap length (3.6 μm per sarcomere in frog muscle). Most of this decrease occurs in the region of partial overlap, and at very large lengths the spacing decreases more slowly (Higuchi and Umazume, 1986; Higuchi, 1987). The authors suggested that the filament lattice of skinned fibers is stabilized solely by mechanical means, namely by radial elastic structures at the M-band or Z-line (Fig. 1A). The A-band lattice, whose spacing is best determined from X-ray diffraction, cannot be stabilized solely by M-band

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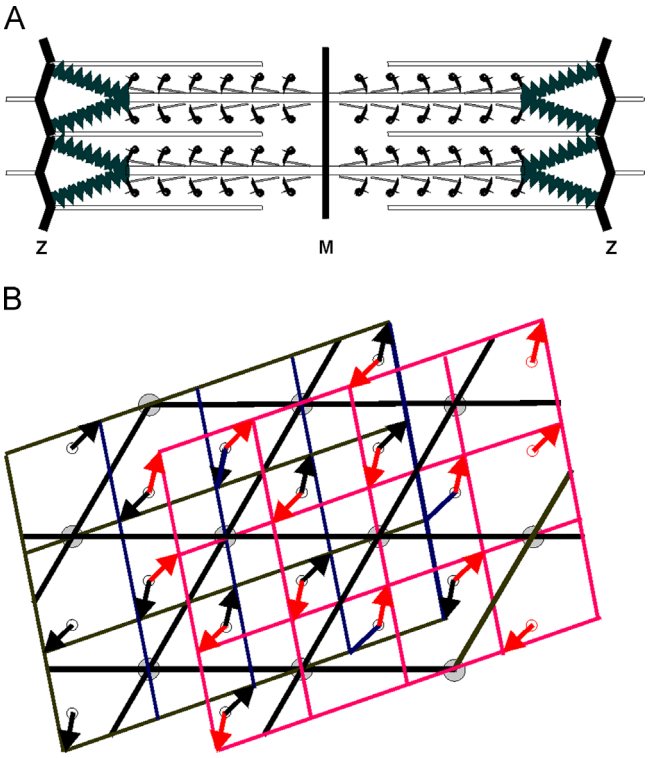

Fig. 1. (A) Schematic representation of filaments in one sarcomere of striated muscle, showing myosin filaments decorated with S1 heads and centered on the M-band lattice, and actin filaments in each half-sarcomere tethered to their Z-line lattices. The A-band is the region occupied by myosin filaments and I-bands are the regions of actin filaments not in overlap with the A-band. Titin filaments run the length of each half-sarcomere and connect each F-myosin to vertices of the Z-line lattice; they are drawn (as heavy folded lines) only in the I-bands. There are six titin filaments per F-myosin in each half-sarcomere. (B) Cross-sectional geometries of the A-band and Z-line lattices, shown superimposed for two half-sarcomeres above and below a common Z-line structure. A-band unit cells (heavy black lines) are rhomboid with F-myosin vertices (large gray circles), and occupy the same positions above and below the Z-line. The front and back faces of the Z-line define laterally displaced rhomboid unit cells (light lines), shown in black and red respectively. The front and back faces are physically linked by alpha-actinins, which are not shown. Small open circles show the A-band positions of actin filaments, which are the same in adjacent half-sarcomeres. At the Z-line, actin filaments are laterally displaced to vertices of the Z-line, shown by black and red arrows for the half-sarcomeres above and below it. There are four different displacement vectors in each half-sarcomere, so the true A–Z unit cell is a supercell of two rhomboidal A-band unit cells (Knupp et al., 2002). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stiffness, which would produce a length-independent spacing. In longer sarcomeres, titin filaments are stretched and their lateral displacement between A-band and Z-line produces a radially inward force which acts to compress the A-band and the Z-line (Fig. 1A). Thus in cardiac muscle, the lattice expanded when titin filaments were removed with trypsin, and the decrease in lattice spacing with sarcomere length was less when the fiber was osmotically compressed (Cazorla et al., 2001). The existence of such filaments was postulated by Higuchi and Umazume (1986), so that the lattice spacing would be a decreasing function of sarcomere length.

A mechanical mechanism for lattice stability should also include the radial elastic properties of the Z-line lattice, shown in Fig. 1B. Evidence for elastic stiffness in the Z-line comes from electron microscopic observations of bunched actin filaments at Z-lines (Bergman, 1983; Trombitas et al., 1995; Irving et al., 1998), and X-ray observations which show that the ratio of Z-line and A-band spacings falls linearly with titin tension (Irving et al., 2011). Elastic stiffness in the Z-line is necessary to maintain the Z-line spacing at a different value from the A-band spacing, so that titin filaments generate equilibrium spacings which lie between the resting spacings of the isolated M-band and Z-line lattices.

With this model, a purely mechanical formulation of the forces responsible for lattice stability can be constructed, using the elastic properties of titin filaments and adjustable parameters for the M-band and Z-line lattices (Higuchi, 1987); the simplest model requires the radial elastic stiffnesses κ_M and κ_Z and resting spacings d_{M0}, d_{Z0} of their unit cells. The values of κ_M and κ_Z are unknown. Although the stiffness of the relaxed fiber has been measured by osmotic compression (Maughan and Godt, 1980; Umazume and Kasuya, 1984; Millman and Irving, 1988) and atomic force microscopy (Yoshikawa et al., 1999; Nyland and Maughan, 2000; Akiyama et al., 2006), it is not possible to use these measurements to separate the component stiffnesses generated by the M-band, the Z-line or (in the overlap region) electrostatic interactions. Titin filaments alone do not generate radial stiffness about a finite equilibrium spacing; if no other forces were involved, the radial components of titin tension would shrink the lattice to the point where filaments touch each other. In the presence of other radial forces, titin filaments merely act to link the lattice spacings of the A-band and Z-line. Published values of passive tension as a function of sarcomere length in relaxed fibers can be used to fix the strength and characteristic exponent of titin tension.

With long sarcomeres where titin tension is significant, there is a strong argument in the favor of minimal differences in A-band and Z-line spacings. Each unit cell of the Z-line is associated with one actin filament, which in the A-band is associated with the triangular subcell of the A-band lattice with a myosin filament at each vertex (Fig. 1B). If the area of each Z-line unit cell differs from that of the (triangular) A-band unit cell, Z-line cells will be progressively displaced relative to their corresponding A-band cells as one scans over the cross-section of the fibril. This mismatch will be resisted by titin tension, increasingly so as sarcomere length is increased. For the purposes of this paper, it is sufficient to ignore any lateral mismatch between A-band and Z-line by assuming that the areas of the A-band and Z-line unit cells remain equal at all times. Then the equilibrium lattice spacings d_M and d_Z are related by the geometry of their unit cells, so the combined elastic energies of expansion/compression for the M-band and Z-line lattices can be written in terms of a single stiffness parameter κ and a resting spacing d_0. The number of unknown parameters is reduced from four to two.

With this mechanical model, we find that the observed length dependence of the A-band lattice spacing can be fitted with a very small value of κ, namely 0.15 pN/nm. As a function of d_M, the combined elastic energy of the M-band and Z-line is very small when compared with the repulsive part of the electrostatic energy at maximum filament overlap (2.2 μm for frog). Hence the entire calculation should be re-run to include electrostatic interactions, including actin–myosin interactions in the overlap region and actin–actin interactions at all sarcomere lengths. When this is done, a better fit to the spacing data is achieved with a similar value of κ (0.18 pN/nm). Hence electrostatic interactions cannot be neglected in determining the lattice spacing, and are dominant in almost all the overlap region of sarcomere lengths.

2. Computational methods

The model considered here is defined by the potential energy V per A-band unit cell of a single half-sarcomere of the relaxed muscle fiber, which is a function of the A-band spacing d_M and half-sarcomere length L. Let

$$V = V_E + V_P + V_M + V_Z \quad (1)$$