

Structural States in the Z Band of Skeletal Muscle Correlate with States of Active and Passive Tension

MARGARET A. GOLDSTEIN, LLOYD H. MICHAEL,
JOHN P. SCHROETER, and RONALD L. SASS

From the Section of Cardiovascular Sciences, Department of Medicine, and the Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030, and the Department of Biology, Rice University, Houston, Texas 77251

ABSTRACT In skeletal muscle Z bands, the ends of the thin contractile filaments interdigitate in a tetragonal array of axial filaments held together by periodically cross-connecting Z filaments. Changes in these two sets of filaments are responsible for two distinct structural states observed in cross section, the small-square and basketweave forms. We have examined Z bands and A bands in relaxed, tetanized, stretched, and stretched and tetanized rat soleus muscles by electron microscopy and optical diffraction. In relaxed muscle, the A-band spacing decreases with increasing load and sarcomere length, but the Z lattice remains in the small-square form and the Z spacing changes only slightly. In tetanized muscle at sarcomere lengths up to $2.7 \mu\text{m}$, the Z lattice assumes the basketweave form and the Z spacing is increased. The increased Z spacing is not the result of sarcomere shortening. Further, passive tension is not sufficient to cause this change in the Z lattice; active tension is necessary.

INTRODUCTION

The Z band is an essential part of the sarcomere and is required for tension transmission along the myofibril. Therefore, the relationship between Z spacing and A spacing in adjacent sarcomeres has been explored. As a muscle shortens, the sarcomere length decreases and the A spacing (d_{10} of the hexagonal lattice) increases (Elliott et al., 1967; Yu et al., 1977). X-ray diffraction studies of living muscle also shows that a reflection attributable to the Z band moves in conjunction with the A spacing (Elliott et al., 1967; Yu et al., 1977). Furthermore, Davey (1976) has shown by electron microscopy that Z spacing changes with A spacing. The interpretation has been that the Z spacing varies as a function of sarcomere length (Squire, 1981). However, in none of these studies was the contractile state of the muscle correlated with the structural state of the Z band. The assumption was that the Z lattice had a single structural state.

In the Z band, the ends of the thin contractile filaments interdigitate in a te-

Address reprint requests to Dr. M. A. Goldstein, Section of Cardiovascular Sciences, Dept. of Medicine, Baylor College of Medicine, Houston, TX 77030.

tragonal array of axial filaments held together periodically by cross-connecting Z filaments (Knappes and Carlsen, 1962; Reedy, 1964; Kelly and Cahill, 1972; Franzini-Armstrong, 1973; Goldstein et al., 1977; Yamaguchi et al., 1985). We have shown that the Z band can exist in two different structural states, each having a characteristic optical diffraction pattern (Goldstein et al., 1979, 1982). Individual subunits and/or regions within a single Z-band lattice can exhibit two different structural appearances in cross section owing to the arrangement of the cross-connecting filaments. Using two-dimensional projections of a three-dimensional model, we showed how movement of the cross-connecting Z filaments could explain the two lattice forms (Goldstein et al., 1982). We predicted that if muscles were fixed in a completely relaxed or a maximally activated state, we would observe one form predominating in each case. We have shown that in relaxed muscle, the Z band is in the small-square form, and in tetanized muscle, it is in the basketweave form (Goldstein et al., 1986). A 20% increase in the Z spacing for the basketweave form compared with the small-square form was observed for sarcomeres near rest length (Goldstein et al., 1986).

The purpose of the present study is to question what happens to the form and dimensions of the Z band as the sarcomere length changes. We use an experimental situation where we know the contractile state of the muscle and the structural state of the Z band. We have examined stretched muscle by increasing load: (a) under conditions that allow the muscle to resist stretch, (b) under conditions where the muscle cannot resist stretch, and (c) under tetanizing conditions after the muscle is stretched. We have documented Z spacing, A spacing, and the lattice form in cross sections and sarcomere lengths in longitudinal sections for each of these three experimental groups.

MATERIAL AND METHODS

Dissected fragments 6–8 mm in length and 500–1,000 μm in diameter were placed in double-jacketed Plexiglas baths and force was monitored on a polygraph (Park et al., 1982). Each bath contained Krebs-Ringer bicarbonate buffer at 29°C and was oxygenated by bubbling with 95% O₂/5% CO₂. A muscle from each rat ($n = 7$) was preloaded with 0.25 g, field-stimulated 6 times/min by pulses 1 ms in duration and 20% above threshold voltage for an equilibration period of 30 min, and then stretched until maximum active force was observed. At this length or at longer lengths ($n = 8$) for stretched and tetanized preparations, the muscles were tetanized (1,000–1,800 stimuli/min) and fixed within 10–30 s by addition of pure 50% glutaraldehyde to allow a 2% final concentration in the bubbling bath. Control muscles were placed in the bath, equilibrated for 30 min as noted, and fixed at rest length.

We used different stretched preparations to obtain a variety of long sarcomere lengths and to test the effect of preparation on the small-square form. The stretched muscles were equilibrated and then (a) stretched by a 6-g load and maintained for 60 min before fixation ($n = 2$); (b) placed in 100 mM PIPES buffer, instead of Krebs-Ringer bicarbonate, equilibrated, stretched to different loads (3–12 g), and maintained 30 min before fixation ($n = 8$); (c) placed in the bath in Krebs-Ringer bicarbonate, field-stimulated 6 times/min by pulses 1 ms in duration and 20% above threshold voltage continually while stretching the muscle by increasing increments of load up to 12 g, and stabilized at the maximum load before fixation ($n = 8$).

The final length and tension were recorded at fixation. Muscle strips were removed after 30 min and fixed for an additional 30 min in the same fixative, weighed, minced into small

cubes, immersed in 2% glutaraldehyde in 100 mM PIPES buffer for 1 h, and processed for electron microscopy (Goldstein et al., 1977, 1986).

Blocks of muscle in longitudinal orientation were selected at random from the same rat muscles selected for optical diffraction measurements and were oriented with the long axis of the myofibers parallel to the knife edge. Measurements of sarcomere length (one per myofibril made with dial calipers on prints at $\times 14,000$) were therefore minimally affected by compression. A mean length \pm SEM of each muscle (one block per muscle) was determined.

Electron micrographs from at least one block in cross section from every muscle were examined to show the Z-band lattice form. These and/or additional blocks were selected at random for suitable orientation. Then optical diffraction analysis was carried out as previously described (Goldstein et al., 1977). Observations (260) from 34 muscles (5 control, 10 stretched, 6 stretched with EGTA, 5 tetanized, and 8 stretched and tetanized) were then pooled to determine whether the small-square form of the Z band has the same range of spacings irrespective of the sarcomere length or type of preparation and whether the basketweave form has consistently larger spacings.

Means and standard deviations for Z spacings in each lattice form were computed. A least-squares linear fit was used to express the relationship between Z spacing and sarcomere length for each Z-band lattice form in each data set.

RESULTS AND DISCUSSION

A Z band from relaxed muscle in cross section exhibits the small-square pattern. The electron micrograph in Fig. 1 A resembles those taken in a previous *in situ* study from muscles not tetanized and muscles tetanized but allowed to relax for 10 min before fixation (Goldstein et al., 1986). A maximally activated state (tetanized muscle) consistently promotes a basketweave pattern in the Z-band lattice (Fig. 1 B) at many different sarcomere lengths ranging from 1.8 to 2.7 μm . The micrograph of *in vitro* tetanized muscle in Fig. 1 B resembles others taken at different sarcomere lengths in this study and those shown previously for preparations kept at rest length (average sarcomere length, $2.18 \pm 0.17 \mu\text{m}$ [$n = 20$ muscles]; Goldstein et al., 1986). The transition from small square to basketweave is always accompanied by an expansion of the Z lattice as determined by measurement of optical diffraction patterns. The Z spacing (defined as the distance of the side of a tetragonal array formed by the cross-cut ends of the thin filaments from one sarcomere; see Fig. 1 C) consistently increases by $\sim 20\%$ from the small-square form to the basketweave form independently of sarcomere length.

Fig. 2 is a graph of Z spacing as a function of sarcomere length for both structural forms of the Z band. The small-square form of the Z band has the same range of spacings regardless of sarcomere length and type of preparation. The basketweave form of the Z band has consistently larger Z spacings. Note that the basketweave values cluster at the shorter sarcomere lengths. The average Z spacing for the small-square form is $19.96 \pm 1.01 \text{ nm}$ ($n = 196$) for sarcomere lengths ranging from 1.8 to 3.7 μm (see inset of Fig. 2). Furthermore, the average Z spacing for the basketweave form is $24.15 \pm 1.09 \text{ nm}$ ($n = 54$) for sarcomere lengths up to 2.7 μm . A linear least-squares fit to all Z spacing data, pooled with no distinction made between structural states, reveals an apparent decrease in Z spacing with sarcomere length, and the slope is similar to that reported in x-ray diffraction studies (Elliott et al., 1967; Yu et al., 1977) and an electron-microscope study by Davey (1976). The

inset in Fig. 2 shows the best-fit least-squares line for each of the two distinct structural states. In each structural state, the Z spacing is essentially independent of sarcomere length. Note that at a given sarcomere length, the transition from small square to basketweave is invariably accompanied by a 20% increase in Z spacing. Thus, tetanus must induce a force with a component perpendicular to the myofibril. We agree with Davey (1976) that the Z band may contribute to the series elastic force. However, the structural constraints defined in this study require a more complex mechanism than expansion caused by sarcomere shortening.

Passive tension along the direction of the myofibril axis is not sufficient to change the Z lattice from small square to basketweave. Regardless of the amount of load

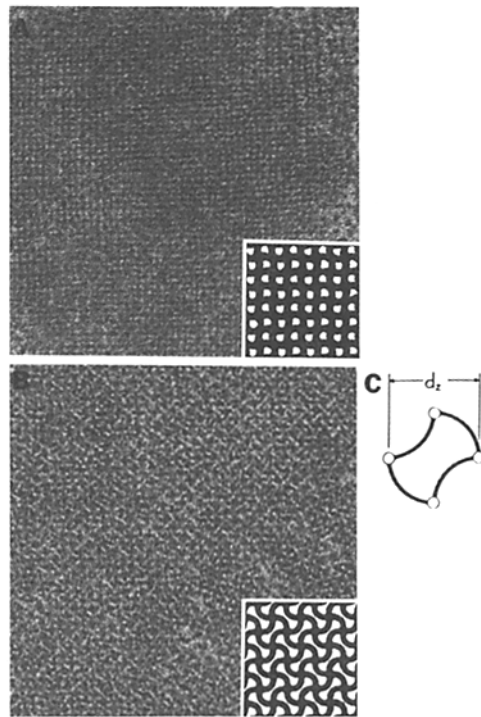


FIGURE 1. Electron micrographs of cross-sections of rat soleus muscle, $\times 150,000$. (A) Z band in the small-square form typical of control and stretched rat soleus muscles. Muscle of sarcomere length = $2.5 \mu\text{m} \pm 0.01$ ($n = 50$) and force per cross-sectional area = $14 \text{ mN}/\text{mm}^2$. (B) Z band in the basketweave form typical of soleus muscles fixed during tetanic contraction. Muscle of sarcomere length = $2.3 \mu\text{m} \pm 0.01$ ($n = 50$) and force = $24 \text{ mN}/\text{mm}^2$. The insets in both figures are computer-generated, two-dimensional projections from the three-dimensional model based on experimentally derived dimensions. (C) The diagram indicates the Z spacing recorded by optical diffraction.

applied to the end of the muscle strip and the type of preparation as sarcomere lengths increase, the Z lattice stays in the small-square form (see Fig. 3). These data suggest that the Z band imparts mechanical stability to the sarcomere during passive stretch. That is, if the ends of the thin filaments in the small-square Z lattice are initially at a minimum separation, the small-square form would persist at longer sarcomere lengths, regardless of passive tension. This constancy of the small-square Z spacing does not interfere with the continuing decrease in A spacing as the sarcomere lengthens. The observed constant Z spacing in the small-square form at rest length and shorter sarcomere lengths shows that sarcomere shortening also does not change the form and dimension of the Z band.

Values for Z spacing in the basketweave form (Fig. 2) are from stretched and tetanized muscle and from tetanized, unstretched in vitro preparations reported in this study pooled together with values from tetanized in situ preparations near rest length taken from a previous study (Goldstein et al., 1986). At sarcomere lengths near rest length and shorter, a plateau phase of tetany is easy to maintain and the basketweave form is very consistently observed. It is increasingly difficult to induce the basketweave form under tetanizing conditions at sarcomere lengths $>2.7 \mu\text{m}$. When the muscle is stretched by increasing load before tetanizing conditions, the

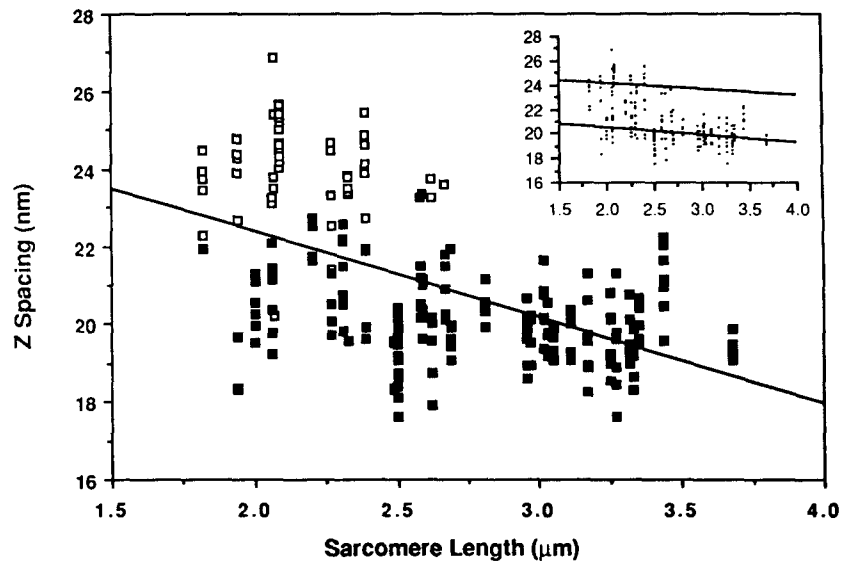


FIGURE 2. Z spacing for each Z structure, the small-square form (■) and the basketweave form (□). Each observation is an average of two to four measurements of the $[2, 0]$ and $[4, 0]$ or $[1, 1]$ spacings in an optical diffraction pattern. The line is a linear least-squares fit of the pooled data with a slope of $-2.23 \pm 0.22 \text{ nm}/\mu\text{m}$ and an intercept at $26.86 \pm 0.58 \text{ nm}$. The inset shows a linear least-squares fit for each of the Z structures: the fit for the basketweave data has a slope of $-0.47 \pm 0.76 \text{ nm}/\mu\text{m}$ and an intercept at $25.12 \pm 1.64 \text{ nm}$; the fit for the small-square data has a slope of $-0.59 \pm 0.17 \text{ nm}/\mu\text{m}$ and an intercept at $21.73 \pm 0.47 \text{ nm}$.

decline in active tension is rapid (the plateau phase of tetany is hard to maintain and few muscles contain Z bands with the basketweave form). These observations are apparent in the clustering of basketweave values for sarcomere lengths $<2.7 \mu\text{m}$ (see Fig. 2) and the observation of the small-square form of the Z lattice in stretched and tetanized muscles at longer sarcomere lengths (Goldstein et al., 1987). The Z spacing in this data group resembles all other stretched and relaxed preparations shown in Figs. 2 and 3.

We have measured the corresponding A-band d_{10} values under all these conditions. A decrease in A spacing with increasing sarcomere length was observed for both Z-band data sets: stretched muscle in the small-square form and tetanized muscles in basketweave form. Thus, A spacing changes as a function of sarcomere

length, as shown previously by others in contracting muscle (Elliott et al., 1967; Yu et al., 1977; Zappe and Maeda, 1985). Unlike the A spacing, Z spacing for a particular structural state varies only slightly with sarcomere length. One would predict then that the Z/A ratio would vary directly with sarcomere length, unlike the constant Z/A ratio reported in x-ray diffraction studies (Elliott et al., 1967; Yu et al., 1977). We have presented a detailed discussion of this point elsewhere (Goldstein et al., 1987).

We conclude that the Z band can have two states: a relaxed small-square form with Z spacing of 20 nm and a maximally activated expanded basketweave form of Z spacing 24 nm at sarcomere lengths between 1.8 and 2.7 μm . The basketweave form is consistently associated with the tetanized or activated state. At all sarcomere

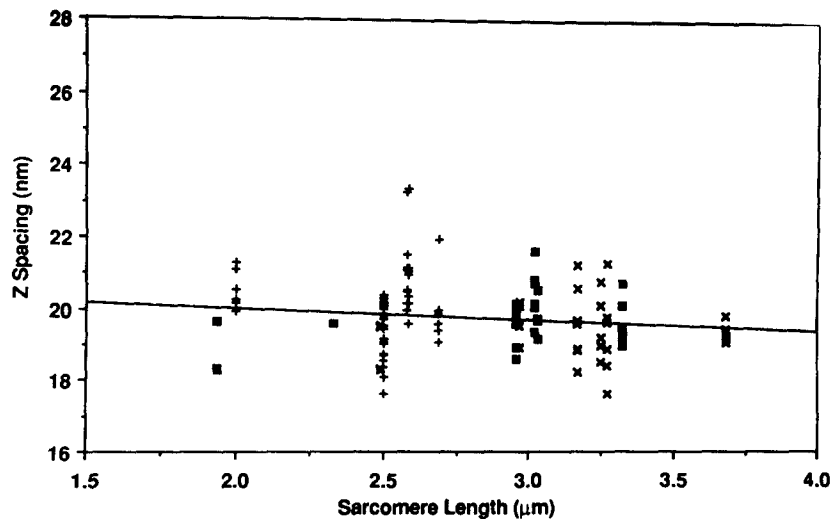


FIGURE 3. Z spacing vs. sarcomere length. Each observation is an average of two to four measurements of the [2, 0] and [4, 0] or [1, 1] spacings in an optical diffraction pattern. EGTA-treated muscles in PIPES (■), unstimulated stretched muscles in PIPES (+), and stimulated stretched muscles in Krebs-Ringer (×) are shown. The line is a linear least-squares fit to all the data with a slope of $-0.3 \pm 0.21 \text{ nm}/\mu\text{m}$ and an intercept at $20.63 \pm 0.59 \text{ nm}$. These values are comparable to those for all small-square data shown in Fig. 2.

lengths, expansion of the Z spacing occurs in the transition from small square to basketweave. No change in sarcomere length, such as sarcomere shortening, is required to induce the increased Z spacing.

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