Sarcomere length dependence of the force-velocity relation in single frog muscle fibers

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ABSTRACT The force-velocity relation of single frog fibers was measured at sarcomere lengths of 2.15, 2.65, and 3.15 μm. Sarcomere length was obtained on-line with a system that measures the distance between two markers attached to the surface of the fiber, ~800 μm apart.

Maximal shortening velocity, determined by extrapolating the Hill equation, was similar at the three sarcomere lengths: 6.5, 6.0, and 5.7 μm/s at sarcomere lengths of 2.15, 2.65, and 3.15 μm, respectively. For loads not close to zero the shortening velocity decreased with increasing sarcomere length. This was the case when force was expressed as a percentage of the maximal force at optimal fiber length or as a percentage of the sarcomere-isometric force at the respective sarcomere lengths.

The force-velocity relation was discontinuous around zero velocity: load clamps above the level that kept sarcomeres isometric resulted in stretch that was much slower than when the load was decreased below isometric by a similar amount. We fitted the force-velocity relation for slow shortening (<600 nm/s) and for slow stretch (<200 nm/s) with linear regression lines. At a sarcomere length of 2.15 μm the slopes of these lines was 8.6 times higher for shortening than for stretch. At 2.65 and 3.15 μm the values were 21.8 and 14.1, respectively.

At a sarcomere length of 2.15 μm, the velocity of stretch abruptly increased at loads that were 160–170% of the sarcomere isometric load, i.e., the muscle yielded. However, at a sarcomere length of 2.65 and 3.15 μm yield was absent at such loads. Even the highest loads tested (260%) resulted in only slow stretch.

It is concluded that properties of the force generators change with sarcomere length. This is not anticipated by the cross-bridge model of muscle contraction.

INTRODUCTION

In the cross-bridge model of muscle contraction (A.F. Huxley, 1957; H.E. Huxley, 1969; A.F. Huxley and Simmons, 1971), the characteristics of each force generator are identical to one another and independent of sarcomere length. These features have been tested primarily by measuring the isometric length-tension relation (Gordon et al., 1966). Because tetanic plateau force was linearly dependent on the width of the overlap zone (ter Keurs and Elzinga, 1981; Altringham and Bottinelli, 1985; Bagni et al., 1986; Edman and Reggiani, 1987), it has been concluded that characteristics of the force generators are the same at different sarcomere lengths—changing overlap only changes the number of cross-bridges that generate force.

Another test of the independent generator concept is to measure the force-velocity relations at different degrees of overlap. If variations of overlap affect only the number of force generators, force-velocity relations at different sarcomere lengths should differ only in scale; shape should remain invariant. We tested this prediction.

We also studied the sarcomere-length dependence of the discontinuity in the force-velocity relation around zero velocity (Hill, 1938; Katz, 1939) and were able to draw conclusions about the sarcomere length dependence of the load under which yielding (Katz, 1939) occurs.

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METHODS

We studied intact single fibers dissected from the semitendinosus muscle of the frog (*Rana temporaria;* body length 4–5 cm). Frogs were cold adapted (at 4°C) for 1–2 wk before use. Small holes were punched in the tendons, 200–600 μm away from the myotendinous junction. Fibers were mounted horizontally in the experimental chamber between a servomotor and a force transducer (Akers AME 801E, Horten, Norway) which had a resonant frequency of 5–7 KHz. Slack length of the fibers was ~10 mm. Temperature was controlled by blowing cold, predried, air around the chamber. The top of the chamber was covered with a thin piece of glass (after the fiber had been mounted) and cooled by a separate jet of cold air. During experiments temperature did not fluctuate more than ±0.1°C.

Fibers were kept in a physiological salt solution of the following composition (in millimolars): NaCl: 115.5; KCl: 2.0; $CaCl_2$: 1.8; $MgSO_4$: 1.0; Na_2HPO_4: 6.3; NaH_2PO_4: 1; glucose: 5.0. The pH was 7.1 at 2°C. The salt solution was refreshed after each contraction.

Sarcomere length

Sarcomere length was measured using a segment-length-detection system. This method detects the distance between two markers positioned

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along the fiber surface. In most experiments we used black cat hairs as markers. These were 10–20 μm thick and ~100 μm long (cf. Granzier et al., 1987). In some experiments we used 100–150 μm long segments from human black hair (thanks to Miss J. Wong). Markers were dipped in silicone high vacuum grease and then mounted along the upper surface of the fiber, typically 800 μm apart. For mounting, fibers were pre-stretched to a sarcomere length of ~2.9 μm. Care was taken to attach the hairs perpendicular to the fiber axis. Alignment was subsequently checked during slow releases and stretches of the unstimulated fiber, and also during short tetani. If the markers did not remain perpendicular to the fiber axis during any of these maneuvers, their position was corrected. At slack, the marker closest to the tendon was ~2 mm from the myotendinous junction.

The fiber region containing a pair of markers was illuminated with a 3 mm-wide beam of collimated white light, obtained from a 150 W xenon-light source, passed through a heat filter. The image of the two hairs was magnified by an objective lens (N.A: 0.25), split approximately midway between the hairs with a prism, and directed to two identical photodiode arrays (Reticon RL 256 C/17); see Fig. 1. A cylindrical lens positioned in front of the photodiode array compressed the rod-like image of each hair to a dot. Final magnification amounted to 6.4 times, as determined by placing calibration gratings at the level of the fiber.

Marker position was taken as the median of the marker image on the array. This was obtained as described previously (Granzier et al., 1987). Segment length was expressed in terms of sarcomere length by measuring the sarcomere length within the segment by optical microscope; see Fig. 1 *A*. The average length of strings of ~30 sarcomeres within the segment were determined several times at different depths in the fiber. We then assumed that the measured degree of segment length change corresponded to the same degree of sarcomere length change.

The segment length signal had a time resolution of 260 μs. RMS noise, determined from three fibers over a wide range of sarcomere lengths, was 7.2 ± 0.1 Å (SEM, n = 10).

Force control

The servomotor consisted of an electromagnetic puller, which produced linear motions over a range of 6 mm, and a digital control system which allowed us to switch between control of fiber length and control of force at preselected times during the tetanus. The system was characterized as follows. We measured step response as the time from 10 to 90% of the step amplitude. In force control the step response differed somewhat from fiber to fiber since the differences in both tetanic plateau force and length of the fibers resulted in a somewhat different feedback gain. For load steps of ~25% of tetanic plateau force, step response measured for seven fibers was 3.4 ± 0.4 ms (SEM). RMS noise of the force signal in force control was ~0.01 mN. In the case of fiber length, step response for steps of ~100 μm was 0.7 ± 0.1 ms (SEM, n = 10 fibers), while for sinusoidal inputs the 3 dB frequency was ~450 Hz.

Experimental protocol

Fibers were mounted in the chamber and were carefully aligned relative to the photodiode arrays using high-resolution XYZ translators. The markers were then attached to the fiber and the various calibrations made. Before each experiment we measured major and minor fiber diameters with a stereo microscope set at 80X. Cross-sectional area was calculated assuming the fiber had an elliptical cross-section. We then determined the maximal force the fiber could generate, by using short tetani at different lengths around slack (sarcomere length ~2.0 μm). Maximal active stress of all fibers used here, measured at 2.3°C, was 27.2 ± 2.6 N/cm^2(SEM, n = 11).

Experiments were performed at 2.3°C. Fibers were stimulated with two platinum electrodes that ran parallel along the full length of the fiber. Pulses of constant current were used. Frequency was set at ~50 Hz. Successive stimuli of the pulse train had opposite polarities; we found that this prolonged the fiber's life span.

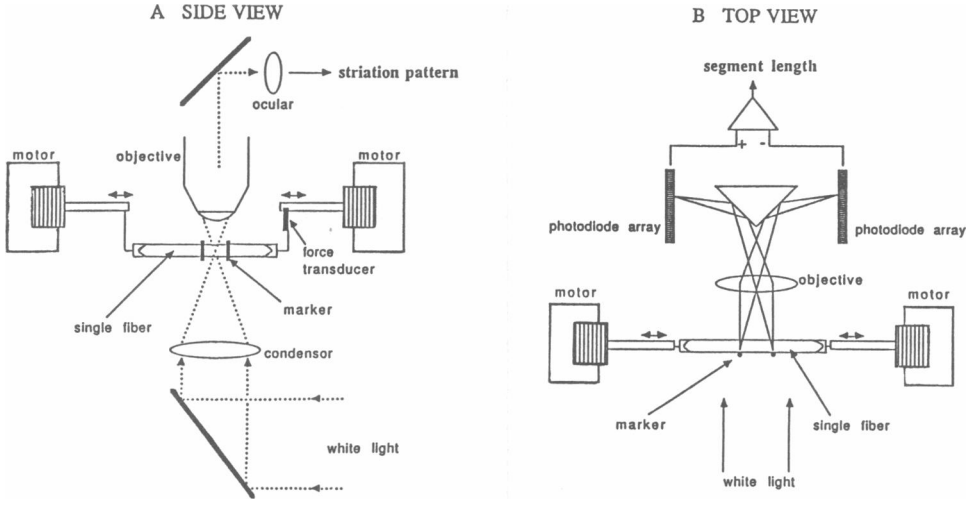

FIGURE 1. Experimental apparatus. (*A*) The fiber was mounted between two servomotors. The left motor was used to control force, the right motor to set fiber length. The right motor had a force transducer mounted between its movable arm and the fiber. This motor was kept stationary during contraction. The striation pattern was visualized with white light, and could be observed continuously via a video camera (not shown) mounted on an ocular of the microscope. N.A. of condensor and objective lens were matched (0.6). (*B*) Segment length detection system. The image of each of the two markers was projected onto a separate photodiode array, and the distance between the markers was measured. See text for additional details.

The objective was to measure the instantaneous velocity at either 2.15, 2.65, or 3.15 μm. First we used short test tetani at different fiber lengths and found by trial and error the fiber length at which sarcomeres were at the desired length during the force plateau before the load clamp. The duration of the subsequent tetanus was then prolonged and when force reached a plateau, load was quickly changed and held constant at a new level. During and immediately after the load step, sarcomere velocity often fluctuated. As soon as the velocity settled to a relatively constant level, its magnitude was measured. The amount of shortening or lengthening that occurred between the start of the load step and the moment when constant velocity was attained varied somewhat with the size of the load step. We accepted the velocity measurement only if the sarcomere length differed from the desired sarcomere length by <50 nm. If necessary, the initial fiber length was adjusted, and the load clamp was repeated.

Force and length signals were digitized with 15 bit analog to digital converters of a four-channel digital oscilloscope (4094/4851, Nicolet Instrument Corp., Fremont, CA). Signals were stored on floppy-discs for subsequent analysis.

RESULTS

Passive force at a sarcomere length of 3.15 μm was 1–2% of maximal active force. This is sufficiently low that effects of passive force on shortening velocity can most likely be ignored at this length (see also Discussion). Sarcomere lengths of 2.15, 2.65, and 3.15 μm were thus chosen as a compromise between broadly varying overlap and minimal interference from passive force.

At a sarcomere length of 2.15 μm, sarcomeres in the region under study were typically isometric during the force plateau before the load step. Occasionally they shortened slowly. On the other hand, at sarcomere lengths of 2.65 μm and 3.15 μm these sarcomeres—in the fiber's central region—always stretched slowly during the force plateau. To keep sarcomeres isometric at those lengths force had to be diminished below the plateau. An example at 2.65 μm is given in Fig. 2. Force during the load clamp was then expressed in two ways. In the first, force was given as a percentage of the maximal force at optimal fiber length. Although this way of expressing force is not essential for the main conclusions of this work, it will allow us to compare our results with those of others (see Discussion). In the second way force was expressed as a percentage of the force that kept sarcomeres isometric; this was done to test whether the shape of the force-velocity relation is independent of sarcomere length. The force traces of Fig. 2 give an example of the two ways in which force was determined.

When force during the load clamp was expressed as a percentage of maximal force at optimal fiber length, relations were obtained as shown in Fig. 3 *A*. At low loads the force-velocity curves at the three sarcomere lengths converged. At other loads the velocity was always highest at 2.15 μm, intermediate at 2.65 μm, and lowest at 3.15 μm. Fig. 3 *B* shows the force-velocity relation around zero velocity on an expanded scale. A discontinuity around zero velocity can clearly be seen: imposing loads higher than isometric results in stretch that is slower than the shortening that occurs when the load is decreased below isometric by a similar amount. The same conclusion can be drawn from the sample records in Fig. 2. At a sarcomere length of 2.15 μm the slope of the fitted regression line in 3B is 8.6 times higher for stretching than for shortening. At 2.65 μm and 3.15 μm the ratios are 21.8 and 14.1 times, respectively. Thus, there is a distinct discontinuity in the slope of the force-velocity relation at zero velocity, and the difference between the slopes for shortening and stretching appears to depend on the sarcomere length.

Results obtained with the load expressed as a percentage of sarcomere isometric (e.g., see loads in parentheses in Fig. 2), are shown in Fig. 4. For loads between 0 and 80%, curves were fitted according to Hill's hyperbolic relation (cf. Edman et al., 1976), while for loads higher than 80% curves were fitted by eye. Fitting according to Hill's hyperbolic relation was done by linearizing the data

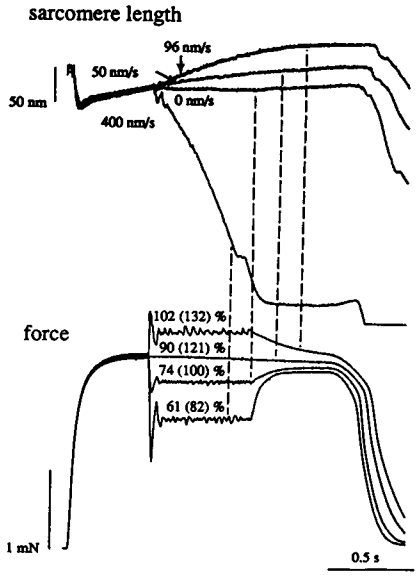

FIGURE 2. Example of representative load clamps. The fiber was first held at a constant length. When force reached a plateau, load was changed to a new level and kept constant at that level. Sarcomere length during the force plateau was 2.65 μm. Second traces from the top in each panel are from a control tetanus in which no load clamp was applied. The first number above each force trace indicates the level of the load clamp as a percentage of the maximal plateau force at optimal fiber length, while the number in parentheses indicates the force level as percentage of sarcomere-isometric force at the actual sarcomere length. Shortening is plotted downwards. Cross-sectional area: 9.2×10^{-3} mm^2; maximal force: 29.4 N/cm^2; temperature: 2.3°C.

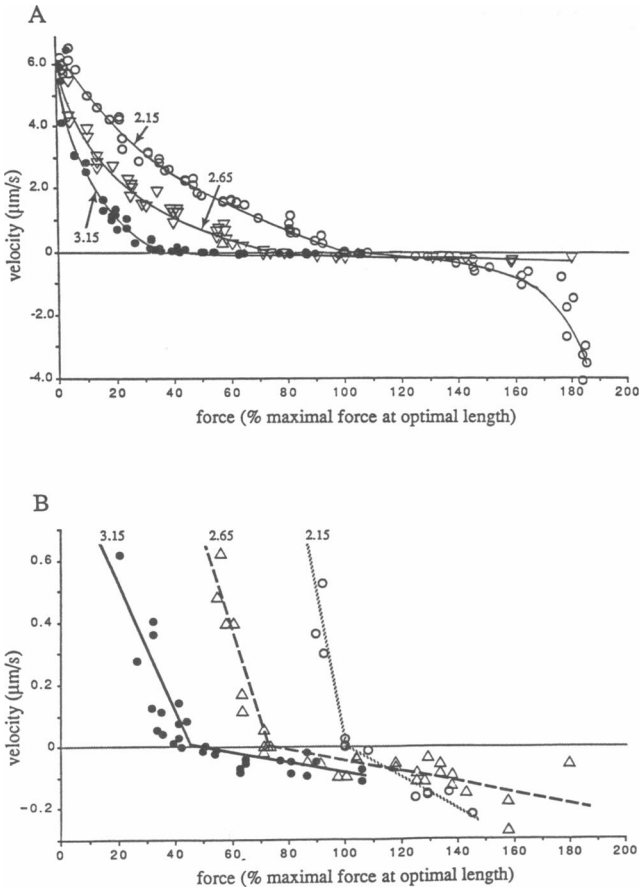

FIGURE 3. (*A*) Force-velocity relations obtained at three different sarcomere lengths. Pooled results from nine experiments. (*B*) Force-velocity relation expanded around zero velocity (same data as in *A*). Linear regression lines for shortening and stretch are shown. The force-velocity relation for shortening is much steeper than for lengthening. Maximal force denotes the plateau force of a fixed-end tetanus at optimal fiber length.

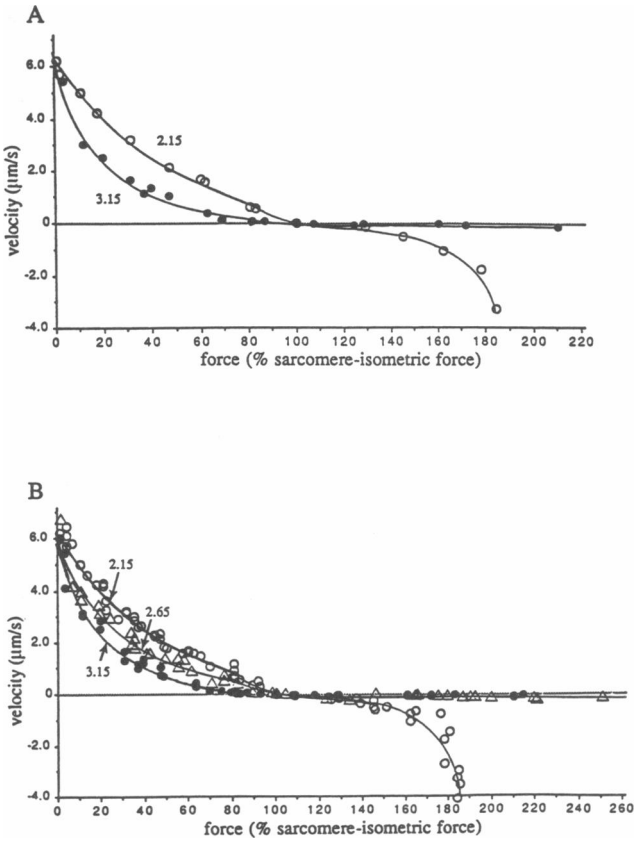

FIGURE 4. Force-velocity relations at different sarcomere lengths. Force is expressed as percentage of sarcomere-isometric force at each length. *A* shows results obtained with one segment, while *B* shows pooled results (one segment per fiber). Force-velocity relation is different at different sarcomere lengths. Note that velocity of stretch at 2.15 μm increases abruptly at a load of ~160–170%, while at the other lengths stretch velocity remains low, even for much higher loads. Curves were fitted by eye for loads higher than 80% and to Hill's hyperbolic equation for loads between 0 and 80%. See text for further details.

and using linear least squares regression (cf. Edman et al., 1976). Obtained values of a/F_0 were 0.55, 0.26, and 0.23 at sarcomere lengths of 2.15, 2.65, and 3.15 μm, respectively. The predicted maximal shortening velocities were 6.5, 6.0, and 5.7 μm/s at sarcomere lengths of 2.15, 2.65, and 3.15 μm, respectively. Thus, maximal shortening velocity appears to be similar at the three sarcomere lengths. For loads higher than zero, however, the curves of Fig. 4 diverge: for a given load relative to sarcomere isometric, the ensuing shortening velocity is lower at longer sarcomere lengths.

Since regional differences in force-velocity properties along single fibers have been found (Edman et al., 1985), it is conceivable that by chance all velocity measurements at short sarcomere length had been obtained from regions that could shorten faster than those regions from which measurements were obtained at long sarcomere length. However, Fig. 4 *A* shows force-velocity curves obtained from the same segment at two different sarcomere lengths. The curves are clearly different. Thus, it is likely that it is the sarcomere length that underlies the differences in the force-velocity relations shown in Fig. 4.

Fig. 4 also shows that at a sarcomere length of 2.15 μm, the velocity of stretch abruptly increases if loads are imposed that are higher than ~160–170% of the isometric load; i.e., the muscle yields. However, at longer lengths sarcomeres are able to hold such high relative loads without yielding. For the highest loads tested, 220 and 260% of the sarcomere-isometric load at 3.15 and 2.65 μm, respectively, the velocity of stretch was still low, and no yielding occurred. Yielding apparently depends on sarcomere length.

We tested whether the lower shortening velocities at long lengths might originate from diminished activation.

Therefore, we added caffeine (0.2 mM) or zinc (50 μm) to the bathing solution. These agents act as potentiators (Lopez et al., 1981; Taylor et al., 1982). We tested whether the shortening velocities at long sarcomere lengths might then be closer to the velocities at 2.15 μm. Although twitch force was much higher at each of the three lengths studied, the potentiators had only a minor effect on either sarcomere behavior or force development during the tetanus. At sarcomere lengths of 2.65 and 3.15 μm both agents resulted in a slightly faster force rise, but the force plateau was unaffected. Furthermore, imposing a load clamp during the plateau resulted in shortening that was the same whether the potentiators were present or absent; an example is given in Fig. 5.

We also tested the effects of caffeine and zinc at a sarcomere length of 2.15 μm. Both potentiators had the same effect. Plateau force was slightly higher than in the normal bathing solution: 2.5 ± 0.9% (SEM, $n = 6$). (Force at 2.65 and 3.15 μm will thus be a lower percentage of force at optimal overlap than found in Fig. 3; i.e., the descending limb measured in the presence of caffeine or zinc will be shifted down somewhat.) In one fiber at this sarcomere length we also imposed load clamps in the presence of caffeine and zinc. Shortening velocity (load 82% of maximal force) was elevated by 8.2% in the presence of caffeine and 7.8% in the presence of zinc. Thus, if anything, velocities in normal saline shown in Fig. 4 may be slightly less than maximal at 2.15 μm, whereas the velocities at 2.65 and 3.15 μm appear to be maximal. In other words, it is unlikely that the difference between the force-velocity relations at the different sarcomere lengths in Fig. 4 results from submaximal activation at a sarcomere length of 2.65 and 3.15 μm.

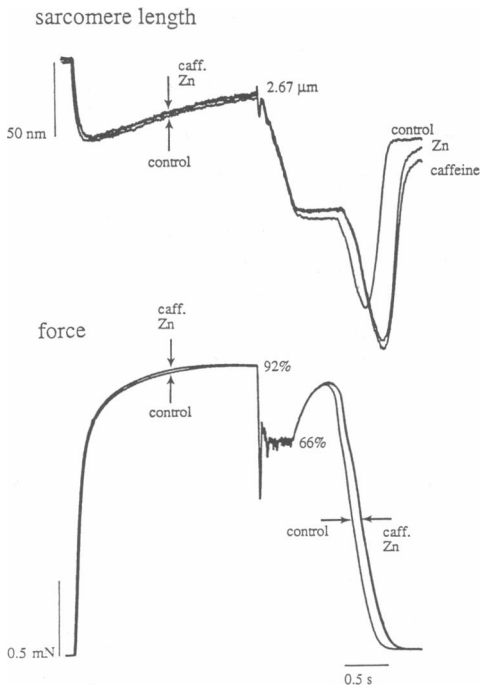

FIGURE 5. Effect of caffeine and zinc on shortening velocity. Three contractions are superimposed: one in normal bathing solution, the other two with zinc or caffeine added. Shortening velocity is 213 nm/s, 202 nm/s, and 200 nm/s for normal saline, caffeine added and zinc added, respectively. Note that the only conspicuous effect of caffeine and zinc is on the relaxation. Cross-sectional area: 7.5×10^{-3} mm^2; maximal force: 27.4 N/cm^2; temperature: 2.3°C.

DISCUSSION

The force-velocity relation, properly scaled, is expected to have the same shape at different sarcomere lengths. We found that this was not the case. The shapes varied with sarcomere length, both for positive and for negative velocities. We consider the implications of these findings vis-a-vis the cross-bridge model.

General features

The force-velocity relation has been studied by many investigators (Hill, 1938; Katz, 1939; Edman et al., 1976; Edman and Hwang, 1977; Edman, 1979; Ferenczi et al., 1984; Edman et al., 1985; Edman, 1988), but generally only at slack length. Most of these studies can therefore be compared only with our results at 2.15 μm. Both the a/F_0 value (a determinant of the curvature of the force-velocity relation) and maximal shortening velocity were higher than reported by others. The a/F_0 value found here was 0.55, while for example Edman et al. (1976) found a value of ~0.3 for intact semitendinosus fibers at similar temperature. Thus, our force-velocity relation at 2.15 μm has less curvature. As for V_{max}, a value of ~6.5 μm/s, or ~3 lengths/s was found here, while Edman et al. (1976) and Edman (1979) reported, again for semitendinosus fibers at ~2.0°C, an unloaded shortening velocity of 2.0–2.5 lengths/s. The reasons for the difference in the a/F_0 value and V_{max} are not immediately obvious. Perhaps there is a difference between the frogs that were used in the two studies. Here we especially selected small frogs (for technical reasons) and it has been found that maximal shortening velocity increases when animal size decreases (McMahon, 1984).

The discontinuity in the force-velocity relation around zero velocity was studied by Katz (1939), using whole muscle. The difference in the slope around zero velocity was a factor of about six, similar to the value of eight found here at a sarcomere length of 2.15 μm. As for yield, Katz (1939) found that muscle lengthened rapidly if the load exceeded ~1.8 times the isometric load. This is

similar to the value of 1.6–1.7 times found here at a sarcomere length of 2.15 μm (Fig. 3).

Thus, the general features of our results are similar to those of other studies in which experimental conditions overlap; only minor quantitative differences were seen.

Effect of sarcomere length on the force-velocity relation

Three effects of sarcomere length on the force-velocity relation were found. (*a*) Shortening velocity against loads between 0 and 100% of the sarcomere isometric load decreased with sarcomere length (Fig. 4). (*b*) The slope discontinuity in the force-velocity relation around zero velocity was larger at 2.65 and 3.15 μm than at 2.15 μm (Fig. 3). (*c*) Yield occurred at a load that was 1.6–1.7 times the sarcomere-isometric load at 2.15 μm, while at 2.65 and 3.15 μm yield was not evident even at loads as high as 2.2 to 2.6 times the sarcomere-isometric load (Fig. 4).

To our knowledge, our study is the first that addresses the effect of sarcomere length on the force-velocity relation in a systematic manner. However, there are several studies in which the effect of sarcomere length was noted as a secondary issue. In a recent paper by Edman (1988), for example, it was shown that the curvature of the force-velocity relation was closer to a true hyperbolic relation at 2.6 μm than at 2.1 μm. We replotted the curves obtained by Edman (shown in Fig. 4 of Edman, 1988) with force expressed as a percentage of the isometric force measured at the different lengths (as in our Fig. 4), and found that at 2.6 μm the velocity was consistently less than at 1.2 μm. Thus, Edman's results are similar to ours in this sense. Hence, the effect of sarcomere length on the force-velocity relation found in our investigation appears to be a genuine property of intact muscle fibers.

Ferenczi et al. (1984) studied the dependence of the force-velocity relation on sarcomere length in skinned fibers. Three different lengths were used: 2.33, 2.45, and 2.74 μm. With force expressed as a percentage of isometric force, as in our Fig. 4, there appeared to be at most a tendency for velocity to be lower at the longer sarcomere lengths. The effect of sarcomere length was much smaller than found in our investigation. Perhaps the sensitivity of the force-velocity relation to changes in sarcomere length is to a large extent lost upon skinning, a possibility that is discussed further in the next section.

Finally, the effect of sarcomere length on yield can be compared with related findings by others. Yield implies that at a certain high load the muscle loses its resistance to stretch. This is probably also why Edman et al. (1978) and Flitney and Hirst (1978) found that during isovelocity stretch of tetanized fibers, force rises rapidly until a high level is reached, whereupon force attains a plateau. Sarcomere length dependence of this plateau force was studied by both aforementioned groups, but with conflicting results. Flitney and Hirst (1978) found that the plateau force during stretch was proportional to the isometric force over the sarcomere length range studied. On the other hand, Edman et al. (1978) found that the plateau force during isovelocity stretches increased with sarcomere length. At a sarcomere length of 2.15 μm, for example, the plateau force was ~1.6 times the isometric force, while at a sarcomere length of 2.65 μm the value increased to 2.1 times (Fig. 4 *A* in Edman et al., 1978). It is not clear why the results obtained by the two groups are different. Perhaps the whole muscle preparation used by Flitney and Hirst (1978) behaves differently from single fibers that were studied by Edman et al. (1978). Thus, at least for single fibers, relative load sustaining ability during isovelocity stretches increases with sarcomere length. This concurs with our finding, also on single fibers, that the relative load under which yield occurs increases with sarcomere length.

In conclusion, we found a clear influence of sarcomere length on the force-velocity relation. Although this issue has not been extensively studied by others, comparison with related results on intact single fibers reported in the literature appears to support our findings.

Origin of sarcomere-length effect

One possible cause of sarcomere-length influence is inhomogeneity. Although the segment comprises only a small fraction of the total length of the fiber, one may nevertheless suppose a degree of sarcomere-length inhomogeneity in the segment. Force generated by the segment might then be determined by the shortest sarcomeres, not by sarcomeres that are at the average length. Force would then be overestimated. If a load step is then imposed and the degree of inhomogeneity were to decrease abruptly, the overestimate of force would decrease, while the opposite would occur if inhomogeneity were to increase. Hence, inhomogeneity could, in theory, affect the shape of the force-velocity relation.

For the above thesis to explain the effect of sarcomere length on the force-velocity relation (Fig. 4), inhomogeneity would have to increase with sarcomere length while to explain the discontinuity in the force-velocity relation around zero velocity (Fig. 3), inhomogeneity upon load step imposition would have to decrease during shortening and increase during stretch. However, Edman et al. (1978, 1982, 1984) concluded that inhomogeneity did not increase during stretch; on the contrary, sarcomeres were more stable. Furthermore, in a related study we imposed load clamps at a wide range of sarcomere lengths and studied the same sarcomere population simultaneously with both the segment-length method and with a laser-

diffraction method (H.L.M. Granzier and G.H. Pollack, manuscript submitted for publication). These two methods are based on different physical principles with different sensitivities to inhomogeneity, so that any substantial change in inhomogeneity would be predicted to give different results. However, the two methods gave essentially identical results. Hence, the origin of the effect of sarcomere-length on the force-velocity relation does not seem to lie in inhomogeneity.

Next we considered whether it might not be the instantaneous sarcomere length itself that affects the force-velocity relation, but sarcomere-length history. Tetani were fixed-end before the load clamp. At extended sarcomere lengths, the sarcomeres under study stretched during this phase. It is known that when stretch is externally imposed during contraction, force-velocity characteristics of the same contraction are affected (Edman et al., 1978; Sugi and Tsuchiya, 1981). After stretch, the force-velocity relation is shifted to the right, towards higher force values. If the same phenomenon were to occur in our fibers as a result of stretch that occurred during the fixed-end phase before the load clamp, velocities at 2.65 and 3.15 μm would be overestimated. Thus, the real velocities at 2.65 and 3.15 μm might be even less than those plotted in Fig. 4, thereby accentuating the sarcomere length effect. Hence it is unlikely that the differences between the force-velocity relations at different sarcomere lengths result from previous stretch.

Alternatively, the source of the differences in force-velocity characteristics could originate from structures responsible for the generation of passive force. However, at a sarcomere length of 3.15 μm, passive force was only 1–2% of active force, while at 2.65 μm it was <0.5%. Thus, the effect of passive force will be small. This is supported by the finding that the maximal shortening velocity at 3.15 and 2.65 μm was similar to the velocity measured at 2.15 μm. If there were an effect of passive force, V_{max} would have been higher at the long sarcomere lengths (Edman, 1979).

In principle, differences of activation could underlie effects of sarcomere length on the force-velocity relation. However, activation appeared to be maximal at 2.65 and 3.15 μm (Fig. 5), while at 2.15 μm it was only slightly submaximal. Thus, the velocities at 2.15 μm might be slightly underestimated. If so, the measured force-velocity relations would underestimate the real effects of sarcomere length.

Finally, since it is unlikely that the contractile proteins gauge sarcomere length directly, there may be some indirect mediating factor. In this respect, the finding of Ferenczi et al. (1984) that sarcomere length does not affect the force-velocity relation in skinned fibers might provide a clue. Skinned preparations differ from intact ones in that the constant-volume relation between sarcomere length and lattice spacing is lost, as shown for example by Magid and Reedy (1980). These investigators found that stretching skinned fibers over the range used by Ferenczi et al. (1984) resulted in a change of interfilament spacing that was small compared with that of the intact fiber. Thus, variation of interfilament spacing might mediate the effect of sarcomere length on the force-velocity relation.

Interfilament spacing

That interfilament spacing might play a role in determining the shape of the force-velocity relation is supported by recent investigations on skinned fibers in which sarcomere length was kept constant while interfilament spacing was reduced by osmotic compression. Reduction of filament spacing below that found in intact fibers resulted in both a decrease of the isotonic shortening velocity and, to a lesser extent, decrease of isometric force (April and Maughan, 1986; Metzger and Moss, 1987; Tsuchiya, 1988). Furthermore, Kawai and Schulman (1985) and Tsuchiya (1988) found that compression resulted in increased stiffness. Thus, it appears that filament spacing indeed affects the mechanical performance of muscle.

How interfilament spacing affects isometric force and isotonic shortening is unclear. It has been suggested that reduced interfilament spacing leads to steric hindrance which slows down actomyosin interaction (April and Maughan, 1986) or, alternatively, results in a passive interaction between thin and thick filaments (Tsuchiya, 1988). Although this might underlie the reduction of shortening and stretch velocity with sarcomere length as found in our study, the ability of sarcomeres to hold much higher forces before yield occurs (Fig. 4) still remains to be explained. Perhaps there are multiple effects of the decrease of filament spacing. One effect could be steric hindrance, while another might be, e.g., an increase in the actin-myosin bond strength, allowing the cross-bridge to hold larger forces before yield occurs. The latter might result from the charge redistribution that is expected to occur during the encroachment of the thin and thick filaments, since both filaments are negatively charged (Elliot et al., 1986). Clearly, further work will be required to clarify the relation between interfilament spacing and mechanical performance of muscle.

CONCLUSION

If the characteristics of the force generators are independent of sarcomere length, as in the cross-bridge model (A.F. Huxley, 1957), the shape of the force-velocity relations at different sarcomere length should be identi-

cal. We found the opposite: the shape depends on sarcomere length for both positive and negative velocities. A consequence is that the characteristics of the force generators are not fixed, but vary with sarcomere length.

How the characteristics would have to vary with sarcomere length in the cross-bridge model can be determined as follows. In this model (A.F. Huxley, 1957), both the ratio between the slope of the force-velocity curve for slow shortening and slow stretch, and the load under which yield occurs are proportional to $f1/g1$ ($f1$: rate constant for attachment; $g1$: rate constant for detachment). Thus, our findings suggest that the $f1/g1$ ratio would have to change with sarcomere length. The implication is that the percentage of the cross-bridges in the overlap zone that are attached during isometric contraction would change with sarcomere length. (The number of cross-bridges under isometric conditions varies with $a/(a + 1)$; $a = f1/g1$). Furthermore, since the generated force for shortening velocities less than V_{max} decreases with sarcomere length (Fig. 4), the rate constant of detachment of cross-bridges that generate negative force, $g2$, would have to decrease under those circumstances with sarcomere length. Thus, within the framework of the cross-bridge model our results imply that the kinetics of the cross-bridge cycle would change thoroughly with sarcomere length or, maybe more fundamentally, with myofilament lattice spacing.

In conclusion, although recent length-tension studies (ter Keurs and Elzinga, 1981; Altringham and Bottinelli, 1985; Bagni et al., 1986; Edman and Reggiani, 1987) all suggest that changing sarcomere length only changes the number of cross-bridges that generate force while the characteristics of the cross-bridges remain the same, the results presented here show that such a mechanism does not hold under conditions in which sarcomeres are either shortening or lengthening.

Received for publication 31 May 1988 and in final form 16 September 1988.

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