Biophys. Struct. Mech. 7, 107–124 (1980)

Biophysics of Structure and Mechanism

Cross Bridge Slippage in Skinned Frog Muscle Fibres

P. J. Griffiths*, K. Güth, H. J. Kuhn, and J. C. Rüegg

Department of Physiology II, University of Heidelberg,
Im Neuenheimer Feld 326, D-6900 Heidelberg, Federal Republic of Germany

Abstract. Mechanically skinned single fibres of the semitendinosus muscles of *Rana esculenta* were investigated at ca. 4° C. The fibres were activated by a Ca^{2+} jump technique, which allowed the development of a steady isometric tension within several seconds of entering a calcium rich solution at 4° C. Sequences of length changes of different duration and amplitude were applied to the fibre. It could be demonstrated that the fibre behaved as a Hookean spring in the case of small amplitude length changes (up to 0.5% L_0, ramp duration 0.5 ms) and that a sequence of length changes induced reversible changes in fibre state. In contrast, large stretches (> 1% L_0) induced a muscle "give" if the stretch were not immediately preceded by a release. The data was interpreted on the basis of a strain induced detachment of cross bridges in combination with a rapid reattachment of presumably the same cross bridges in a discharged position. The rates of strain induced detachment and reattachment depended on the stretch amplitude. At amplitudes exceeding 2% L_0 the rates were estimated to be at least several thousands per second.

Key words: Actomyosin interaction – Muscle mechanics – Cross bridge slippage – Contraction mechanism

Introduction

It is now generally accepted that muscular force is generated by cross bridges, which project from the myosin filament and interact periodically with the actin filament (Huxley AF 1957; Huxley HE 1969). Furthermore, it is believed that the stiffness of a sarcomere is a function of the number of attached cross bridges in it. When subjected to a rapid stretch of large amplitude, this elasticity associated with the cross bridge might be extended to such a degree that the

* Present address: c/o Dr. S. R. Taylor's Laboratory, Department of Pharmacology, Mayo Foundation, Rochester, Minnesota 55901, USA

0340-1057/80/0007/0107/$ 03.60

resultant force would cause structural damage at the molecular level. It has been suggested by Sugi (1972) that strained cross bridges can detach rapidly, and thus protect themselves from large forces. His observations on bundles of tetanised frog semitendinosus muscle fibres were extended by Flitney and Hirst (1978a, b), using intact frog sartorius muscle. They described a sudden lengthening of sarcomeres subjected to large stretches, accompanied by a marked reduction in the rate of tension rise during the latter part of the length change.

Experiments conducted on whole muscles are always susceptible to misinterpretation as a result of interfibre variability or changes in the degree of muscle activation. In addition, the presence of a series elastic element in the fibre tendons can lead to a discrepancy between the length change applied to the whole muscle, and that observed at the level of the sarcomere. These difficulties are reduced if single muscle fibres in which the sarcolemma has been destroyed are used, since tendon elasticity and interfibre variability are removed, and chemical conditions can be controlled at the level of the contractile apparatus.

In a previous publication (Güth et al. 1979) evidence was presented indicating that, in glycerol extracted insect flight muscle fibres, rapid detachment of cross bridges accompanying a large stretch ($> 0.5\%$ L_0) was followed by a rapid reattachment. Since insect flight muscle differs structurally from vertebrate skeletal muscle, having a relatively high intrinsic stiffness even in the relaxed state (Machin and Pringle 1959), its behaviour during large stretches may differ from that of frog muscle. In addition it is known that glycerination causes changes in skeletal muscle behaviour and filament structure (Huxley HE 1965; Edman 1966). Further experiments are reported here, performed on mechanically skinned frog muscle fibres, which conform with the data obtained from insect flight muscle. It will be shown that strain induced detachment of cross bridges does not result in a reduction of fibre stiffness, as measured by a subsequent release. It will furthermore be shown that these findings are best accounted for by a strain induced increase in the detachment probability in combination with a rapid attachment of presumably the same bridges.

Methods

Preparation. Single muscle fibres isolated from the semitendinosus muscles of *Rana esculenta* were mechanically skinned using needles under a drop of solution C (Table 1). The fibre was then glued between a length step generator and a force transducer using a rapidly hardening cyanoacrylate glue. The sarcomere length was adjusted to 2.4 μm using the diffraction pattern obtained from a laser light beam directed through the fibre. At this sarcomere length, resting tension in the fibre was zero. Typical fibre lengths after the procedure were 4 mm.

Solutions. Solutions were prepared according to Table 1. Shortly before use, Brij 58 (0.5%), creatine kinase (20 Units $\cdot$ ml^{-1}) and creatine phosphate (10 mM) were added to all solutions, and the final pH was adjusted to 6.70 ±

Table 1

	Solution		
	A	C	D
Potassium	76	76	76
Sodium	10	10	10
Calcium	20	–	–
Magnesium	5.6	5.6	5.6
Imidazole	60	60	60
Chloride	51	51	51
ATP	5	5	5
EGTA	20	20	0.1
HDTA	4	4	24

EGTA (ethylenegcol-bis(B-aminoethylether)NN′-tetraacetic acid); HDTA (hexamethylenediamine NNN′N-tetraacetic acid); ATP (adenosine triphosphate). In addition all solutions contained 10 mM creatine phosphate, 20 Units · ml^{-1} creatine kinase, 0.5% Brij 50. Final pH 6.7, pMg = 3.0, ionic strength 171 mM. All concentrations expressed in mM units

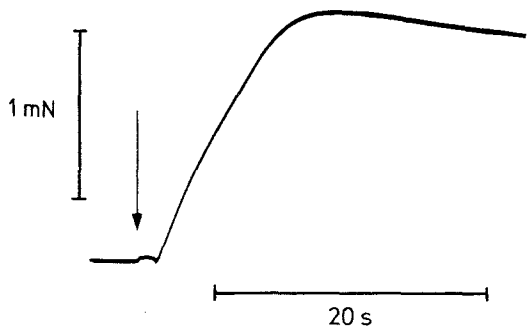


Fig. 1. Activation by a "Ca-jump". A single mechanically skinned frog muscle fibre (semitendinosus of *Rana esculenta)* was activated by a transfer (arrow) from a solution containing low EGTA to a solution containing high CaEGTA solution, see text. Conditions: pCa 5.6; 4° C

0.02. Fibres were exposed to solution C for roughly 30 min before proceeding with the experiment in order to allow sufficient time for Brij to destroy the sarcoplasmic reticulum. Experiments were conducted at a temperature of 4° C.

Activation. Prior to activation, the fibre was transferred to solution D, where it remained for three minutes to allow the EGTA concentration inside the fibre to equilibrate with that of the surrounding medium. The fibre was then rapidly transferred to a calcium rich solution, the pCa of which was selected by mixing solutions C and A in different proportions. On entering the calcium solution, tension developed rapidly, as can be seen in Fig. 1. The point at which the transfer from solution D to the calcium rich solution occurred is indicated by an arrow.

The Mechanical Set-Up. Changes in fibre length were brought about using a Ling Dynamics vibrator (Model 101), driven by a displacement and velocity dependent feedback system (Güth et al. 1979). The change in total fibre length was measured by an integrated field plate system (Siemens, type FP 210), which

detected the movement of a small metal plate mounted on the moving part of the length change generator. The system allowed ramp shaped length changes to be imposed upon the fibre, up to an amplitude of 150 μm. The duration of the ramp could be varied, the fastest length changes having a ramp duration of less than 0.5 ms.

Force was measured using a semiconductor type transducer (Aksjeselskapet Mikro-Elektronikk, type 801). The tip of the transducer was sharpened so as to reduce the mass of the silicon beam, which allowed a resonance frequency of 18 kHz to be obtained with a muscle fibre attached (Güth et al. 1979). For more detailed information on the influence of the limited resonance frequency of the force transducer on the measured force transients and the delay caused by transmission time along the fibre see Güth and Kuhn (1978) and Güth et al. (1979).

Instantaneous Stiffness. Instantaneous stiffness (dF/dL) was measured by displaying force against length change during each ramp, using a digital oscilloscope (Nicolet Explorer 1090A) and a x-y recorder (Hewlett-Packard 7045A). Such records are referred to in the text as force-extension diagrams. As a result, temporal changes in each individual force transient occurring during the ramp could be compared with a continuous record of fibre stiffness obtained during the same transient. This method of stiffness measurement differs from the T1 curves obtained by Huxley and Simmons (1971), which were constructed from the peak force at the end of a length step, but is particularly suitable for the study of fast time course events occurring during the length change. For each length change, fibre stiffness in the relaxed state was also determined.

The length change control feedback system automatically adjusted ramp velocity in proportion to the amplitude of the length change, so that the duration of the ramp remained constant for any series of stretches or releases of different amplitudes.

Results

State of the Preparation After the Skinning Procedure. Owing to the mechanical disturbance of the fibre during the skinning procedure, the mechanical state of skinned fibres was examined before proceeding further with the experimental protocol. Firstly, the uniformity of sarcomere length, an important requirement for the mechanical experiments reported in this paper, could have been distorted by skinning. A non-uniform distribution of sarcomere lengths would be manifested in a diffuse laser pattern. In the relaxed state, however, it was found that skinned fibres had a clear and sharply ordered laser pattern. After maximal calcium activation, the laser pattern rapidly became more diffuse. This effect was less pronounced if the preparation was activated at a calcium concentration evoking less than maximal isometric tension. By using the calcium jump technique, a stable isometric tension could be achieved within 5 s of entering such a calcium solution. Therefore, all experiments reported in this paper were performed on partially activated fibres (pCa = 5.6) at a time after activation when the laser pattern was still fairly prominent.

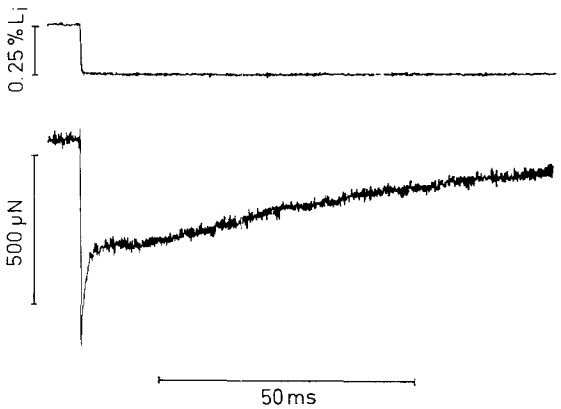


Fig. 2. Force transient of a rapidly released muscle fibre. A single mechanically skinned muscle fibre (semitendinosus of *Rana esculenta*) was released by an amplitude of 0.25% L_0 (L_0 is the fibre length at time 0), using a ramp form length change, ramp duration 0.5 ms. Resting tension was not detectable, isometric force 260 µN. Sarcomere length was 2.4 µm. Release was performed following activation in a calcium rich solution pCa 5.6 at 4° C. No detectable stiffness was measured for a similar length change for this fibre in the relaxed state

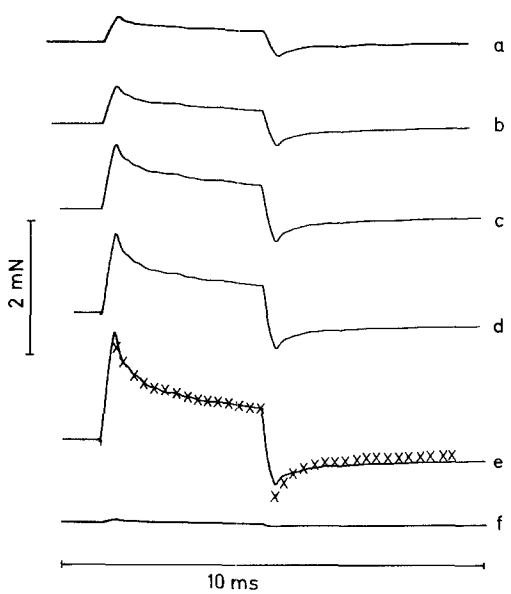


Fig. 3. Stretch release cycles of different amplitudes. A single mechanically skinned frog muscle fibre (semitendinosus of *Rana esculenta*) was stretched and after ca. 4 ms released to the original length. The amplitudes of the length changes were: **a** 0.25% L_0; **b** 0.5% L_0; **c** 0.75% L_0; **d** 1% L_0; and **e** 1.5% L_0. Isometric tension at the time of the stretch was 2.6 mN, 2.15 mN, 2.25 mN, 1.85 mN and 1.75 mN respectively. Crosses in trace e correspond to the 2.5× amplified transient of trace **b.** Trace *f* shows the transient obtained from the relaxed fibre (pCa 8.0, amplitude of length change, 1.5% L_0). All length changes applied as ramps, with ramp duration 0.5 ms. Activating solution pCa 5.6, sarcomere length 2.56 µm. Temperature 2° C

Secondly, skinning might have resulted in damage to the contractile apparatus itself. In this case one would expect that the reaction of the skinned fibre to stretch or release should differ from that of the intact fibre. Huxley and Simmons (1973) have given a detail description of force transients in response to rapid length changes. For length changes of less than 8 nm per half sarcomere, these force transients can be divided into four phases, Phase 1, a linear elastic response during the changing length signal from which stiffness may be calculated; Phase 2, a 'quick recovery' tending to return the tension to the value it had prior to the length change, having a duration of about 2 ms at 4° C; Phase 3, a plateau like region in the force transient lasting about 20 ms; and Phase 4, a gradual return to the original isometric tension. The force transient shown in Fig. 2 was obtained from a skinned fibre during a release of 0.25% L_0. It can clearly be seen that it too contains the four phases observed in the intact fibre. The kinetics of these four phases are also in agreement with those of the living fibre (Huxley and Simmons 1973) about 0.8% release required to reduce tension nearly to zero.

Fibre Force Responses to Different Stretch-Release Amplitudes. Calcium jump activated skinned fibres were subjected to stretch-release cycles with amplitudes between 0.25 and 1.5% L_0. The time between ramp shaped length changes was ca. 4 ms. The duration of the ramp was 0.35 ms. Figure 3 shows typical force transients from one such experiment. During stretch the force increased, and then fell rapidly as soon as the stretch was completed. This rapid fall in tension could be approximated to an exponential decay with a time constant of 0.6 ms. However, it was quite apparent that this fall in tension was composed of more than one exponential in reality, so the time constant quoted here can only be taken as a rough estimate of the velocity of the underlying processes.

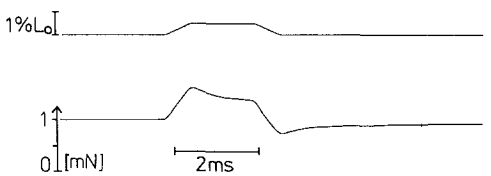
The stretch induced force transients of the different stretch amplitudes are rather similar if properly scaled. This can be seen in Fig. 3, trace e, where crosses show the 2.5× amplified force transient of trace b. The factor of 2.5, which gave the best coincidence of the amplified transient b with transient e, is rather similar to the ratio of the amplitudes of the corresponding length changes. It appears that during the early phase of tension decay following the end of the stretch an additional faster component is present in record e.

On the subsequent release, force fell below the level held prior to the stretch, and then slowly recovered. As the amplitude of the length change was increased, so the force recovery after release became less and less complete.

The Very Early Phases of the Force Transient After a Length Change. Differences between properly scaled force transients induced by different stretch amplitudes were only detected in the very early phase after the stretch. The force-extension diagrams, obtained as described in the methods section, for large and small stretches were then examined. Figure 4 shows the force-extension transient for a stretch-release of 0.5% L_0 with accompanying force transient, Fig. 5 same data for a stretch of 3.0% L_0. It can be seen that the force-extension relationship in Fig. 4 is linear, and that the relationship for the release is parallel to that for the stretch, but simply displaced to a lower level on the force axis. In Fig. 5, the

force-extension relationship is nearly linear during the initial part of the stretch (up to 1.5% L_0) and then declines markedly. The first part of the force-extension diagram corresponding to the release in Fig. 5 is also linear till ca. 2% L_0, becoming then curved. The gradients of the initial parts of the force-extension curves for stretch and release in Fig. 5 are approximately equal.

The force transient in Fig. 5 has a rounded appearance during the stretch, and shows at high time resolution the rapid decay of force which was not present after smaller stretches (Fig. 4).



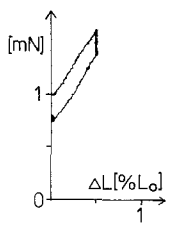
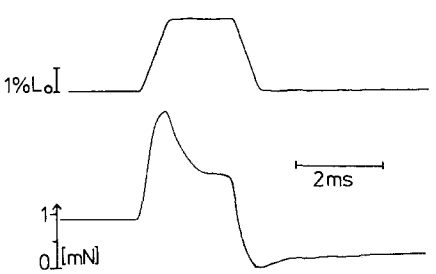


Fig. 4. Stretch-release cycle of low amplitude. The mechanically skinned muscle fibre is stretched and ca. 2 ms later released to the original length. The length signal is shown in the upper trace, force in the lower. The lower part of the figure shows the force-extension diagram for this cycle. Ramp duration 0.5 ms, temperature 5° C, sarcomerelength 2.5 μm, isometric tension, 1.1 mN. Activating calcium concentration 5.6 pCa units



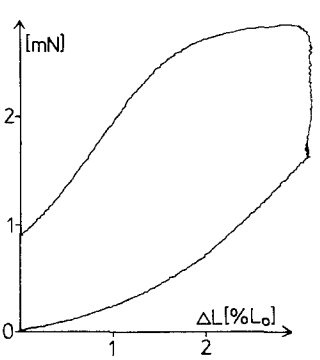


Fig. 5. Stretch-release cycle of high amplitude. The mechanically skinned single muscle fibre was stretched and ca. 2 ms later released to the initial length. The length signal is shown in the upper trace, the force transient in the middle trace. Ramp duration and conditions as in Fig. 4

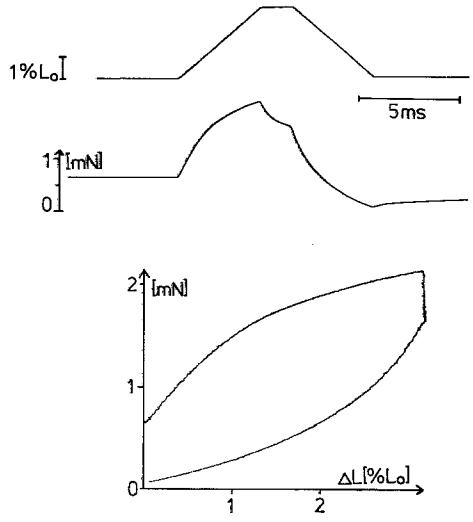


Fig. 6. Stretch-release cycle of high amplitude and low velocity. The mechanically skinned muscle fibre was stretched 3% L_0 using a ramp duration of 3 ms, and then released to the original length. Length signal is shown on the upper trace, force on the lower. The lower part of the figure shows the force-extension diagram. Conditions as in Fig. 4

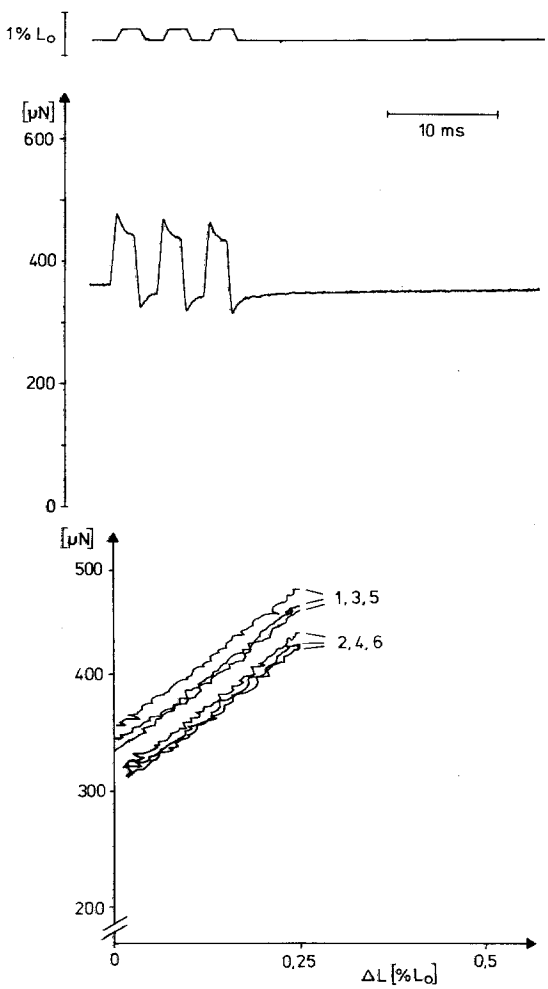


Fig. 7. Sequence of stretch-release cycles of small amplitudes. The mechanically skinned single muscle fibre was repetitively stretched and released to the initial length. The upper trace shows the length signal, the lower trace the force transient. The corresponding length-tension diagram is shown in the lowest part of the figure. The numbers 1–6 indicate the sequence of length changes corresponding to the length-tension plots. Conditions: pCa 5.6; 3° C

In order to determine whether the initial very fast force reducing process induced by the large stretch in Fig. 5 was independent of the stretch velocity, stretch velocity was reduced. As can be seen in Fig. 6, the reduced stretch velocity caused the stretch amplitude at which the slope of the force-extension curve started to decrease to be shifted to the lower amplitude of about 1% L_0.

The stiffness to tension ratio of the initial linear portion of the force-extension curves obtained during stretch shown in Figs. 4, 5, and 6 were found to be identical. If the velocities of the different stretches are expressed in terms of % $L_0 \cdot ms^{-1}$, it can be seen that stretches in Fig. 4 and 6 were delivered to the fibre at the same velocity, despite the difference in stretch amplitude, while the stretch shown in Fig. 5 had a 6× higher velocity. This strongly indicated that viscosity did not influence stiffness measurements to any great degree, even during the most rapid ramps.

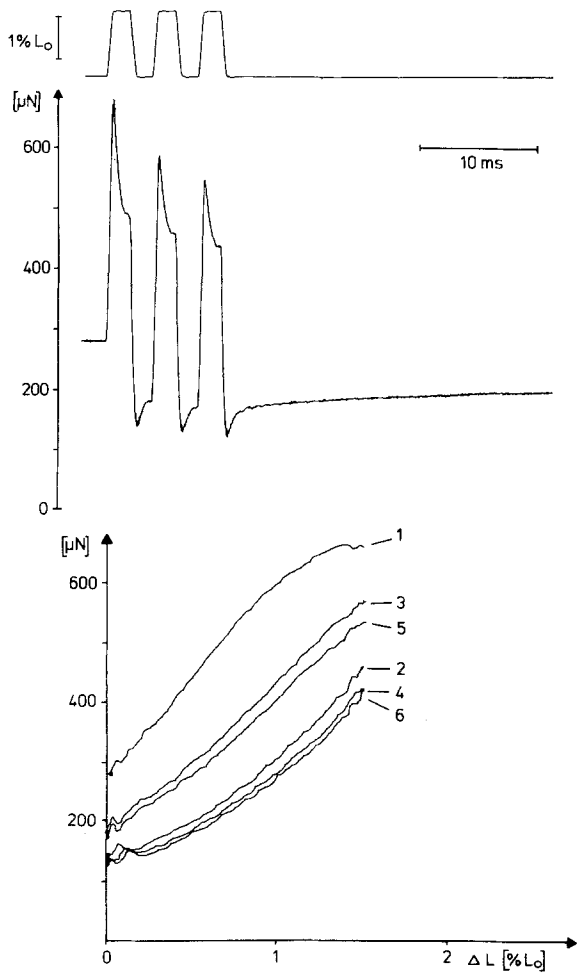


Fig. 8. Sequence of stretch-release cycles of large amplitudes. The mechanically skinned single muscle fibre was repetitively stretched and released to the initial length. The upper trace shows the length signal, the lower trace the force transient. The corresponding length-tension diagram is shown in the lowest part of the figure. The numbers 1–6 indicate the sequence of length changes corresponding to the length-tension plots. Conditions: pCa 5.6; 3° C

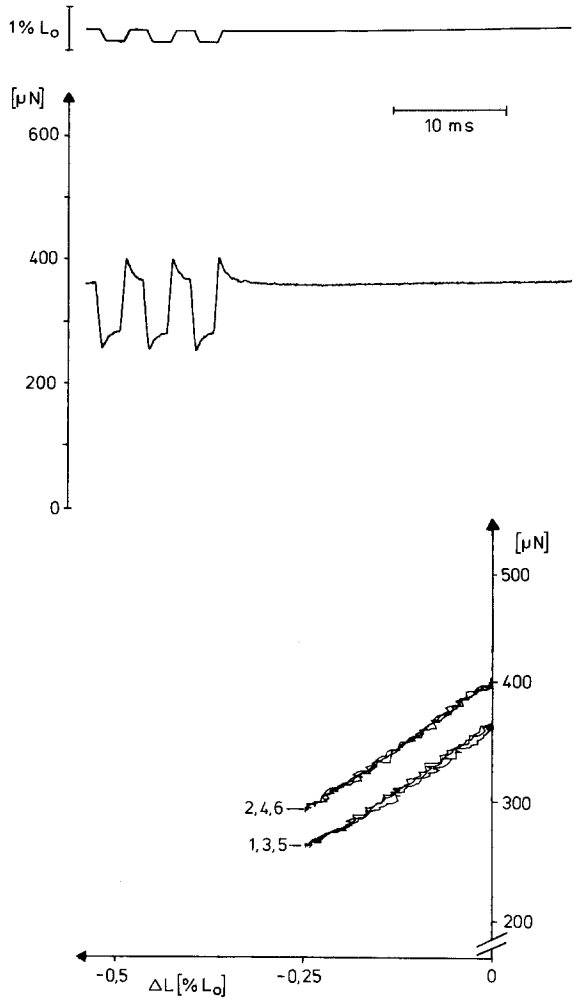


Fig. 9. Sequence of release-restretch cycles of small amplitudes. The mechanically skinned single muscle fibre was repetitively stretched and released to the initial length. The upper trace shows the length signal, the lower trace the force transient. The corresponding length-tension diagram is shown in the lowest part of the figure. The numbers 1–6 indicate the sequence of length changes corresponding to the length-tension plots. Conditions: pCa 5.6; 3° C

Repetitive Cycles of Stretch and Release. The force transients and force-extension diagrams induced by repetitive cycles of brief stretches and releases were investigated, to determine whether the subsequent release following a brief stretch immediately returns the fibre to its initial state. Figures 7 and 8 show the force transients and corresponding force-extension diagrams for two different stretch amplitudes. In the force-extension diagrams the sequence of length changes is indicated by the numbers 1–6. As can be seen from Fig. 7, during a series of low amplitude stretches and releases (0.25% L_0), neither the force transient nor the force-extension diagram of the second and third stretch-release cycle differed markedly from the first, suggesting that following stretch and release the fibre returned to the same state that it was initially in (same force and stiffness at the same fibre length). This is not true for larger amplitude stretch-release cycles (Fig. 8). Whereas the force transient induced by the first cycle was rather similar to that shown in Fig. 5 (stretch amplitude 1.5% L_0), the

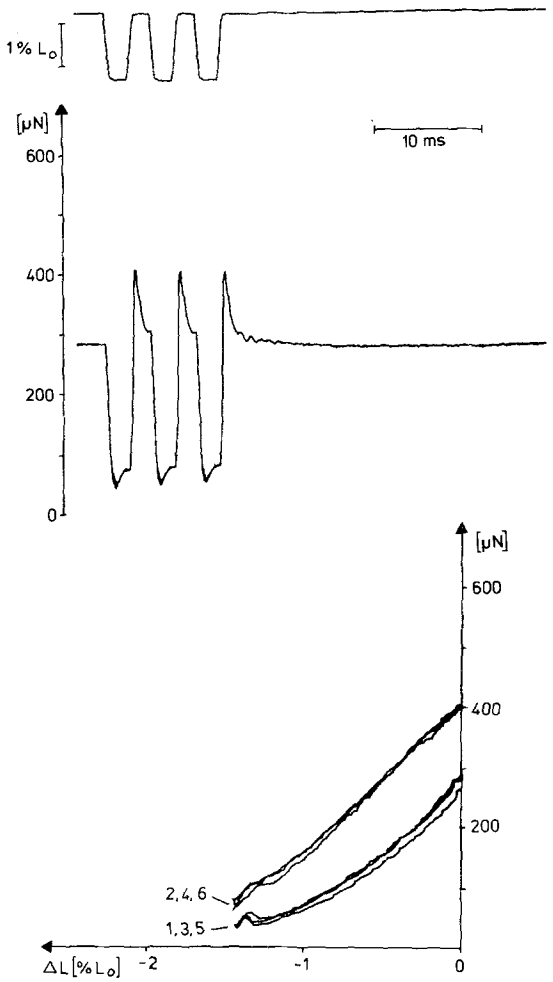


Fig. 10. Sequence of release-restretch cycles of large amplitudes. The mechanically skinned single muscle fibre was repetitively stretched and released to the initial length. The upper trace shows the length signal, the lower trace the force transient. The corresponding length-tension diagram is shown in the lowest part of the figure. The numbers 1–6 indicate the sequence of length changes corresponding to the length-tension plots. Conditions: pCa 5.6; 3° C

transient induced by the second cycle showed a less distinct quick tension decay after stretch. The force-extension diagram of the first stretch-release cycle was also qualitatively different from those of the second and third. Although all three force-extension diagrams resulting from a release appear very similar, the force extension curve produced by the first stretch was steeper than those produced by the second and third stretches. It also showed a marked curvature beyond ca. 1% L_0, while the other two stretch induced force-extension curves were linear throughout the stretch. The slope of the curve at the beginning of the first stretch is very similar to that at the beginning of all three releases.

Repetitive Cycles of Release Restretch. It was shown in the previous section that the first stretch of a sequence of stretches and releases induces an additional fast decay in muscle force. In order to discover whether this process is also induced by a stretch preceded by a release, sequences of length changes were performed as in the previous section, but now starting with a release. In Figs. 9

and 10, force transients are shown in the upper part of the figure, force-extension diagrams in the lower. It can be seen that for low and high amplitudes, the force transients and the force-extension diagrams of the second and third cycles were very similar to those of the first (note that for releases, the first length change occurs in the lower force extension lines). It is furthermore of interest to note that the force-extension diagrams of the high amplitude restretches were curved in a similar way to those of the releases, i.e., the slope of the curve at the *end* of the stretches is similar to that at the *beginning* of the releases. At low amplitude release-restretch cycles, the force-extension curves were straight lines intersecting the base line at about -0.9 to -1.0% L_0 for the releases, and at about -1.0 to -1.1% L_0 for restretches.

Discussion

The Preparation

The frog muscle experiments reported here are in agreement with those reported in insect muscle in a previous publication (Güth et al. 1979), in which an apparent rapid reattachment of cross bridges occurred after rapid detachment during a large stretch. Since the mechanically skinned preparation does not suffer any of the complications that may result from glycerination, the evidence of similar behaviour to large stretches presented in this paper strongly indicates that the observations made in whole muscle and glycerinated muscle preparations do reflect properties of the contractile apparatus, and not artifacts due to series elasticity or damage during glycerol extraction, etc. Since a very much larger parallel elasticity exists in insect muscle fibers than in skeletal muscle, force-extension curves become more difficult to interpret from insect muscle, and assumptions about the behaviour of this parallel elasticity in the muscle must be made from measurements taken from the relaxed fibre. In these experiments, however, resting fibres showed a parallel elasticity of about 5% or less of that measured during activation. In addition, in previous experiments (Griffiths et al. 1979) we have shown that the stiffness of these skinned fibres in the range of length changes $\pm$ 0.3% L_0 was linear and proportional to the degree of activation. This finding, which is in agreement with those of other studies (Goldmann and Simmons 1977; Yamamoto and Herzig 1978), strongly indicates that in the skinned frog muscle preparation neither series elasticity (outside cross bridges) nor parallel elasticity play an important role in influencing the form of force-extension curves. It was also apparent that since changes in the velocity of stretch did not affect the stiffness to tension ratios obtained from Figs. 4–6, fibre viscosity does not greatly affect stiffness measurements in skinned frog fibres at this range of stretch velocities.

It has been shown in the text that the force transient obtained following a quick release of a skinned fibre is both quantitatively and qualitatively similar to that of the intact fibre, as described by Huxley and Simmons (1973). Although our experiments showed that on full activation, tensions developed by the skinned fibre preparation were equal to those obtainable from intact muscle, it

was also found that the laser pattern of fully activated fibres became more diffuse. At pH 6.7, pMg 3.0, it was found that fibres were about 80% activated at a pCa of 5.6, and the laser pattern was still evident though somewhat weakened. Since the laser pattern continued to weaken with time in the activating solution, length changes were administered to the fibre within a few seconds of maximum force being attained. Nevertheless, it must be taken into account that the doubtless weaker laser pattern of the activated skinned fibre preparation may well correspond to a more disordered sarcomere length.

Reversible Processes Induced by Small Repetitive Length Changes

It could be shown that the changes in force resulting from a small length change (less than 0.5% L_0) can be reversed by a subsequent length change returning the fibre to its original length (Fig. 4, 7, and 9) performed within 2 ms, i.e., within the quick phase (Phase II). The force-extension diagrams corresponding to those small repetitive length changes were found to be linear and parallel. This indicates that for rapid length changes below 0.5% L_0, fibre elasticity is linear and not affected by a preceeding small length change. Force-extension curves from the initial length change were extrapolated to zero force, where they crossed the length axis at about -0.9% L_0 to -1.0% L_0. In this respect, force-extension curves from skinned fibres exposed to small stretches or releases are very similar to the T1-curves obtained by Huxley and Simmons (1973). The constancy of stiffness (slope of T1-curve) before and after the quick phase may be taken to mean that the net number of cross bridges does not change during the quick phase (cf. also Ford et al. 1977).

Cross Bridge Slippage Induced by Large Stretches

The character of the force response is markedly changed if the applied length change exceeds 1% L_0. If the first length change in a sequence is a stretch, the slope of the force-extension diagram is approximately linear up to a length of about 1.2% L_0. For this point, stiffness apparently decreased rapidly, till at lengths greater than 2% L_0, the force extension diagram approaches a plateau. If the first length change is a release, the force-extension curve of the subsequent stretch shows a constant slope throughout the length change, i.e., the force does not approach a plateau.

If a sequence begins with a stretch, the force after the first cycle does not return to the original isometric force, but remains somewhat lower (Figs. 3, 5, and 8). If such a sequence begins with a release, the force after the first cycle does return to its original level (Fig. 10).

When such a large stretch is delivered to the muscle fibre, the percentile length change at the sarcomere is the same as that for the whole fibre. Since cross bridges in a sarcomere are arranged in parallel, the length change administered to the half sarcomere results in the same absolute length change for individual attached cross bridges. The overall length of a cross bridge (HMM) is

approximately 30× smaller than the length of the half sarcomere, thus the percentile lengthening of the half sarcomere results in a 30× greater percentile lengthening at the individual cross bridge. Since cross bridges are continually cycling during muscle activation, the average elongation of the cross bridge during a muscle stretch can be calculated by multiplying the stretch velocity by 30 and dividing by the average dwell time of the cross bridge at the actin filament (i.e., the duration of attachment of the individual cross bridge). Unfortunately the dwell time of the cross bridge is not known. Only a rough estimate on the basis of ATPase activity measurements and muscle myosin content can be made. Such an estimation results in a dwell time of ca. 100 ms for both the isometrically contracting and the stretch contracting muscle (the latter because ATPase activity of stretch and nonstretched muscle was found to be nearly the same, cf. Curtin and Davies 1973). It is hard to believe that the average elongation of the cross bridge resulting from a dwell time of this order of magnitude and from physiological stretch velocities (up to several muscle lengths per second) would not result in structural damage. It is therefore not unreasonable to postulate a rapid detachment of strained bridges. Such a phenomenon could account for the apparent fall in stiffness observed during the latter part of large stretches, however, other interpretations of this data are possible. In the following, three possible mechanisms are discussed.

a) The Cross Bridge Elasticity is Non-Hookean for Large Displacement Amplitudes. The plateau of the force-extension diagram in case of large stretches, which are not proceded by a release, could be interpreted as a 'give' of the overstrained elastic element associated with the cross bridge, while it remains attached at the same place on the actin filament. However, if this give is caused by non Hookean behaviour of overstrained cross bridges, it is difficult to understand why only the first stretch and not the later stretches in a sequence of stretches and releases induces such a give in the elastic element (Fig. 8). Furthermore the lower force after the first cycle of stretch and release is difficult to explain by a simple non Hookean elasticity. Nevertheless, a kind of melting of shortened and recrystallising of elongated cross bridge neck regions (Harrington 1971; Holmes 1977; Mason 1978) might account for these results, if the recrystallisation is assumed to be much slower than the melting process.

However, Flitney and Hirst (1978a, b) stretched a muscle slowly but to an extent up to 5% L_0. The result was similar to that reported here: at amplitudes exceeding 1.2% a muscle 'give' was observed. Because no further distinct change in the mechanical behaviour of the muscle occurred during the stretch, it must be assumed that the crystallisation process continues till the neck of the cross bridge is elongated to double its original length. It is difficult to believe that this could occur without cross bridge damage.

b) Detachment of Overstrained Cross Bridges Without Reattachment. An enhanced tendency for cross bridge detachment under strain, as proposed by Huxley HE (1969) and Sugi (1972), likewise explains many of the observed phenomena. Two cases must be distinguished. 1) The cross bridge elasticity is not only linear for small ($< 0.5\%$ L_0) displacements, as indicated by the straight

force-extension diagrams of small length changes (Figs. 4, 7, and 9), but also for the larger displacements discussed here. In this case a net detachment of cross bridges must be manifested in a loss of muscle stiffness, because it is assumed that the main part of the contracting skeletal muscle elasticity is associated with attached cross bridges (Huxley AF 1974). However, it can be seen that the stiffness at the beginning of a stretch is the same as that at the beginning of a release applied a few milliseconds later. 2) The cross bridge elasticity is a function of its displacement, if the displacement exceeds 0.5% L_0. In this case the loss of stiffness caused by cross bridge detachment could be matched by the increased stiffness of the strained cross bridges. Consequently the stiffness at the beginning of the stretch would be the same as at the beginning of the release. Furthermore, the lower force after release observed in large stretch release cycles would result from the reduced number of attached cross bridges on return to the original length. Such a scheme would require, though, that the stiffness should remain constant throughout the stretch, which is not observed. Instead, although the stiffness at the start of the subsequent release is equal to that at the start of the initial stretch, the stiffness in the latter part of the stretch is much reduced, as can be seen in the force-extension diagram for Fig. 5.

c) Rapid Cross Bridge Detachment and Reattachment. The most convincing explanation of the data is to postulate an increased tendency of strained cross bridges to detach coupled with a rapid reattachment of the same cross bridges, the reattachment occurring in such a way as to discharge all force which had been present in the elastic component associated with the cross bridge. Such a detachment-reattachment process has been already proposed (Flitney and Hirst 1978a, b; Güth and Kuhn 1978; Güth et al. 1978, 1979).

In such a scheme, the following 5 phenomena would have to be accounted for:

1) Unchanged Stiffness Before and After Stretch

The reattachment of the cross bridges which detached during stretch because of their overstrained elastic elements would be assumed to reattach very rapidly, with a discharged elastic element. Since the net number of cross bridges would not have changed, no change in fibre stiffness would be expected.

2) The Curved Force-Extension Diagram During Large Stretches

Assuming a linear stiffness is associated with the attached cross bridges, the slippage of strained bridges would appear on the force-extension diagram as a fall in stiffness.

3) Lower Force After a Stretch Release Cycle

During large stretches, overstrained cross bridges would slip, resulting in the loss of force stored in their eleastic elements. If the subsequent release does not

cause a back slippage, the return to the original length would result in the same proportion of attached cross bridges, but with less force stored in their elastic components. The result would be a lower tension at the end of the large amplitude stretch release cycle even though stiffness is unchanged.

4) No drop in the slope of the force-extension diagram obtained during a stretch which was preceded by a release.

Since the attached cross bridges are first relaxed by the release, they would not become overstrained by the subsequent stretch.

5) The release restretch cycles appear identical, whether preceded by a stretch or not.

The slippage during the first stretch would transfer the cross bridges to a new site on the actin filament, at which they would be only moderately strained. During subsequent cycles, there would thus be no need for further slippage. This condition would be similar to that for sequences beginning with a release.

The Rate of Detachment and Reattachment of Slipping Cross Bridges

Cross bridge slippage occurs principally during the ramp. This can be seen in Fig. 5, where the force extension curve becomes very nonlinear during the latter part of the stretch. Since the duration of the ramp was only 0.5 ms, the detachment rate must reach at least several thousand per second.

Slippage as a Function of Cross Bridge Strain

Figure 3 shows that even after a small stretch release cycle, force falls to below the isometric tension level. This may suggest that slippage occurs even during the small amplitude stretches. The force-extension curve in Fig. 5 indicates that large scale slippage only occurs at stretches in excess of 1% L_0. This result is similar to that of Flitney and Hirst (1978a, b), who found a distinct decrease in the slope of the tension response of a stretched intact frog sartorius muscle at ca. 1.2% L_0. For higher stretch velocities, they found that the starting point for slippage was independent of stretch velocity, suggesting that slippage only occurs if bridges are strained beyond a critical force. A sharp onset of slippage was also detected by Güth et al. (1979) in glycerinated insect flight muscle, for stretch amplitudes exceeding 0.5% L_0. The weak muscle slippage observed at smaller stretch amplitudes in these experiments may therefore be due to sarcomere length inhomogeneity (Julian and Morgan 1979a, b) rather than to slippage of weakly strained bridges.

Taking into account the difference in temperature between experiments conducted on glycerinated insect flight muscle (Güth et al. 1979) and the results presented here, it would seem that the force reducing process evident in large amplitude stretches occurs at a much higher rate in frog muscle. If this is taken into account, as well as the high intrinsic stiffness of insect fibres at rest, the behaviour of the two preparations appears very similar.

Increasing Stiffness During Stretch

A slightly increasing stiffness can be detected during the first 1% of larger stretches. Such an observation might be accounted for by a slight nonlinearity in cross bridge elasticity for larger stretches. Where a large amplitude stretch was preceded by a release, the force extension curve became very nonlinear (see Fig. 10). This may be related to nonuniform sarcomere shortening during a large release, as proposed by Sugi (1979), which could result in fibre buckling, and thus nonlinearities in the early part of the restretch. Another possible interpretation would be a rapid cross bridge attachment preceding the detachment induced by cross bridge strain. Such a rapid attachment process as a consequence of muscle elongation may be understood by the action of the cross bridge in the crystalline structure of the actin-myosin lattice as proposed by Wray (1979). This conception also does not interfere with the above interpretation of the slippage phenomenon.

Acknowledgements. This work was supported by the Deutsche Forschungsgemeinschaft, grant Ru 154/13. The technical assistance of Mr. W. D. Busse in the electronic analysis of the data is gratefully acknowledged.

References

Curtin NA, Davies RE (1973) Chemical and mechanical changes during stretching of activated frog skeletal muscle. Cold Spring Harbor Symp Quant Biol 37: 619–626

Edman KAP (1966) The relation between sarcomere length and active tension in isolated semitendinosus fibres of the frog. J Physiol 183: 407–417

Flitney FW, Hirst DG (1978a) Cross bridge detachment and sarcomere "give" during stretch of active frog's muscle. J Physiol 276: 449–465

Flitney FW, Hirst DG (1979b) Filament sliding and energy absorbed by the cross bridges in active muscle subjected to cyclical length changes. J Physiol 276: 467–479

Ford LE, Huxley AF, Simmons RM (1977) Tension responses to sudden length change in stimulated frog muscle fibres near slack length. J Physiol 269: 441–515

Goldmann YE, Simmons RM (1977) Active and rigor muscle stiffness. J Physiol 269: 55–57

Güth K, Kuhn HJ (1978) Stiffness and tension during and after sudden length changes of glycerinated rabbit psoas muscle fibres. Biophys Struct Mech 4: 223–236

Güth K, Kuhn HJ, Herzig JW, Rüegg JC (1978) Evidence for cross bridge slippage in a stretched muscle fibre. Experientia 34: 1183–1184

Güth K, Kuhn HJ, Drexler B, Berberich W, Rüegg JC (1979) Stiffness and tension during and after sudden length changes of glycerinated single insect fibrillar muscle fibres. Biophys Struct Mech 5: 255–276

Griffiths PJ, Kuhn HJ, Güth K, Rüegg JC (1979) Rate of isometric tension development in relation to calcium binding of skinned muscle fibres. Pflügers Arch 382: 165–170

Harrington WF (1971) A mechanochemical mechanism for muscle contraction. Proc Natl Acad Sci USA 68: 685–689
Holmes KC (1977) The myosin cross bridge as revealed by structure studies. In: Riecker G et al. (eds) Int. Symp. Rottach-Egern/Tegernsee, 1976. Springer, Berlin Heidelberg New York
Huxley AF (1957) Muscle structure and theories of contraction. Prog Biophys 7: 255–318
Huxley AF (1974) Muscular contraction. J Physiol 243: 1–43
Huxley AF, Simmons RM (1971) Proposed mechanism of force generation in striated muscle. Nature 233: 535
Huxley AF, Simmons RM (1973) Mechanical transients and the origin of muscular force. Cold Spring Harbor Symp Quant Biol 37: 669–680
Huxley HE (1965) Structural evidence concerning the mechanism of contraction in striated muscle. In: Paul WM et al. (eds) Muscle. Pergamon Press, New York, pp 3–29
Huxley HE (1969) The mechanism of muscular contraction. Science 164: 1356–1366
Julian FJ, Morgan DL (1979a) Intersarcomere dynamics during fixed-end tetanic contractions of frog muscle fibres. J Physiol 293: 365–378
Julian FJ, Morgan DL (1979b) The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. J Physiol 293: 379–392
Machin KE, Pringle JWS (1959) The physiology of insect flight muscle: II. The mechanical properties of a beetle flight muscle. Proc R Soc Biol [Lond] 151: 204–225
Mason P (1978) Dynamic stiffness and cross bridge action in muscle. Biophys Struct Mech 4: 15–25
Sugi H (1972) Tension changes during and after stretch in frog muscle fibres. J Physiol 225: 237–253
Sugi H (1979) The origin of the series elasticity in striated muscle fibres. In: Sugi H, Pollack G (eds) Cross bridge mechanism in muscle contraction. University of Tokyo Press, Tokyo, pp 85–102
Wray JS (1979) Structure of the backbone in myosin filaments of muscle. Nature 277: 37–40
Yamamoto T, Herzig JW (1978) Series elastic properties of skinned muscle fibres in contraction and rigor. Pflügers Arch 373: 21–24

Received August 4, 1980/Accepted October 8, 1980