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Synchronous oscillations of length and stiffness during loaded shortening of frog muscle fibres

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1. A study was made of the damped oscillations in fibre length that are observed when isolated muscle fibres from the frog are released during the plateau of an isometric tetanus to shorten against a constant load (force clamp recording) near the isometric level (temperature, 1.0–11.0 °C; initial sarcomere length, 2.25 μm).

2. The oscillatory length changes of the whole fibre were associated with similar length changes of marked consecutive segments along the fibre. The segmental length changes were initially in synchrony with the whole-fibre movements but became gradually more disordered. At the same time the length oscillation of the whole fibre was progressively damped.

3. The fast length step that normally occurs at the outset of the load-clamp manoeuvre was essential for initiating the oscillatory behaviour. Accordingly, no length oscillation occurred when the load clamp was arranged to start as soon as the selected tension level was reached during the rising phase of the tetanus.

4. The instantaneous stiffness was measured as the change in force that occurred in response to a high-frequency (2–4 kHz) length oscillation of the fibre. During the load-clamp manoeuvre, when the tension was kept constant, the stiffness underwent periodic changes that correlated well in time with the damped oscillatory changes in fibre length. However, there was a phase shift between the stiffness oscillation and the oscillation of shortening velocity, the latter being in the lead of the stiffness response by 21.4 $\pm$ 0.8 ms ($n = 19$) at 1.8 $\pm$ 0.1 °C .

5. A mechanism is proposed to explain the oscillatory behaviour of the muscle fibre based on the idea that the quick length step at the outset of the load clamp leads to synchronous activity of the myosin cross-bridges along the length of the fibre.

A muscle fibre that is released to shorten against a constant high load during tetanic stimulation generally exhibits a series of low-frequency length oscillations that are progressively damped during the course of shortening (Armstrong *et al.* 1966; Sugi & Tsuchiya, 1981; Edman, 1988; Granzier *et al.* 1990). The same phenomenon appears when the fibre is released against a load that is slightly higher than the tetanic force (Armstrong *et al.* 1966; Sugi & Tsuchiya, 1981). In either case the cyclic length changes have the character of a damped oscillation that dies away within 200–300 ms at low temperature. There has been no thorough investigation of this phenomenon in the past, and the nature of the oscillations remains unclear. It has previously been noted that both the amplitude and duration of the length oscillation are quite variable among different fibres and there are fibres in which the oscillatory behaviour does not appear at all during loaded shortening or lengthening (see Edman, 1988). This variability suggests that there are one or more uncontrolled factors that are essential for the emergence of the phenomenon.

The present study was performed to establish what conditions are required for the oscillatory behaviour to appear during loaded shortening and to gain insight into the underlying mechanism. The study was performed on intact single muscle fibres from frog using techniques that enabled measurements of length changes both from the fibre as a whole and from small consecutive segments along the preparation. In order to evaluate possible changes in the number of attached cross-bridges during the length oscillations the instantaneous stiffness of the fibre was continuously monitored in some experiments. The results provide evidence that the oscillatory behaviour of the muscle fibres during loaded shortening reflects synchronous activity of the cross-bridges initiated by the quick length step at the onset of the load clamp. Some of the experiments have been presented in a preliminary form (Edman & Curtin, 1993).

METHODS

Preparation and mounting

The experiments were performed on single fibres dissected from the anterior tibialis muscle of *Rana temporaria*. The experimental procedures used were approved by the Animal Ethics Committee of the University of Lund. The frogs, which had been stored at +4 °C for at least 7 days before use, were killed by decapitation followed by destruction of the spinal cord. The fibres were normally dissected the afternoon before the experiment and kept in Ringer solution at +4 °C overnight.

For the experiment the fibres were mounted between a force transducer and a servo-controlled electromagnetic puller (motor No. 1). The tendons were held by aluminium clips as described previously (Edman & Reggiani, 1984). In experiments in which stiffness was measured a second puller (motor No. 2) was used as well, the latter serving to produce a high-frequency, sinusoidal length oscillation of the fibre (see below). In these experiments the force transducer was mounted on the arm extending from motor No. 1. The tendon attached to motor No. 2, i.e. the puller producing sinusoidal length changes, was tied by nylon threads to the free end of the puller arm. To ensure firm attachment of the tendon throughout the experiment two layers of Parafilm were wound on the outside of the tendon around the puller arm. The Parafilm strip was held in place by attaching its ends to a small hook on the puller arm. Tension was recorded by means of a semiconductor strain-gauge element (AE 801, Aksjeselskapet Mikroelektronikk, Horten, Norway). The transducer element had been modified to increase the frequency response of the transducer as described by Edman & Lou (1990). The resonant frequency of the force transducer was 19 kHz when the transducer was submerged in the bathing fluid.

The standard Ringer solution had the following composition (mM): NaCl, 115.5; KCl, 2.0; $CaCl_2$, 1.8; $Na_2HPO_4 + NaH_2PO_4$ (total concentration), 2.0; pH 7.0. In experiments where intracellular acidification was produced bicarbonate-buffered solutions were used containing (mM): $NaHCO_3$, 10; NaCl, 108.0; KCl, 2.0; $CaCl_2$, 1.8; the solutions were equilibrated with a mixture of O_2 and CO_2 to give pH 7.0 or 6.57 near 0 °C. The proportion of CO_2 in the gas mixture was 2.82 or 8.8% (calculated using $pK = 6.319$ and CO_2 solubility = 0.0737 mol l^{-1} at 1 atm CO_2; Edsall & Wyman, 1958). The temperature of the bathing solution was constant to within 0.2 °C in a given experiment but ranged from 1.0 to 3.2 °C for experiments on different fibres. In one series of experiments, to be described separately, the temperature was varied between 1.5 and 11.0 °C.

Stimulation

The fibres were stimulated with 0.2 ms current pulses passed between two platinum plate electrodes that were placed symmetrically on either side of the fibre approximately 2 mm from it. The stimulus strength was set approximately 15% above the threshold and a train of pulses was given to produce a fused tetanus of 1 s duration at 2 min intervals. The frequency of the pulses (usually within the range 16–22 s^{-1} at 1–3 °C) was adjusted as appropriate to give complete mechanical fusion under the various conditions studied. In the experiments with intracellular acidification a lower pulse frequency had to be used since the refractory period of the fibre membrane is increased under these conditions. However, the tetanic force remains fused because fibres relax more slowly when acidified under the conditions used here (Curtin & Edman, 1989). The period of data collection was preceded by 10–20 tetani at 2 min intervals.

In some experiments, to be described separately, the fibre was moderately fatigued by reducing the interval between the tetani from 2 min (control) to 15 or 30 s. As the tetanization frequency was increased, the isometric force was steadily reduced and levelled off at approximately 75% of its original (control) value after 25–30 contractions at the high frequency (for further information, see Edman & Lou, 1990).

Recording of signals

Force and puller positions and, in some experiments, stiffness and segment length measurements were recorded on a digital oscilloscope and the signals stored on disk.

Fibre length, sarcomere length and cross-sectional area were determined as described by Edman & Reggiani (1984).

Segment length recording

Opaque markers of letterpress (mass about 0.1 μg) were firmly attached onto the upper surface of the muscle fibre to delineate discrete segments of about 0.5 mm (measured in each case) along the entire length of the preparation. The outermost markers were placed approximately 0.2 mm from the edge of the tendons, i.e. 0.3–0.4 mm from the fibre–tendon junctions. The optical system which detected changes in segment length was similar to that described by Edman & Reggiani (1984). Briefly, laser light was shone through the fibre and the magnified image of the fibre projected onto a photodiode array (Fairchild CCD 133, time resolution 40 μs). An analogue circuit converted the output from the array to a signal proportional to the percentage change in segment length as the fibre went through a contraction (see Edman & Reggiani, 1984). The system detected and recorded (with an accuracy better than 0.2% of the segment's length) the length of only one segment during a given contraction, so the tetanus and interval pattern was repeated until records were made from all segments (8–9 in number) along the fibre. The separation between the markers was measured in the resting fibre using the ocular micrometer of a stereomicroscope at ×40 magnification.

Measurement of fibre stiffness

Full details of methods and apparatus used to measure fibre stiffness are given by Edman & Lou (1990) and only an outline is presented here. In these experiments the fibres were mounted between two electromagnetic pullers (motors No. 1 and 2) as described earlier. Motor No. 1 (which had the force transducer mounted on its shaft) was used to clamp force to the pre-set level. For stiffness measurements motor No. 2 produced a sinusoidal length oscillation of constant amplitude throughout the tetanus period. The frequency of the oscillation was generally 2–4 kHz, in a few experiments 1 kHz, and the peak-to-peak amplitude was 10–11 μm corresponding to approximately 2 nm per half-sarcomere (h.s.). Stiffness was measured by recording the changes in force that occurred in response to the imposed length oscillation. A stiffness signal was formed by first passing the signal from the force transducer through a narrow bandpass filter (Q value 5.5), the optimum frequency of which was set to the actual frequency of length oscillation used. The filtered signal was thereafter rectified by means of a precision rectifier circuit to provide a direct read-out of the stiffness during the course of the tetanus. The bandwidth of the rectified signal was DC–2.3 kHz. The force signal was recorded without the superimposed force oscillation by using a notch filter, which produced maximum attenuation at the frequency employed for the length oscillation. The signal from the position transducer of the oscillating puller (motor No. 2) was treated in the same way to provide a measurement of the peak-to-peak amplitude of the length oscillation.

Curve fitting

For analysis of the oscillations of fibre length and stiffness the following function describing a damped harmonic movement was fitted to the length and stiffness traces:

$$y = Kx + L + A\sin(2\pi fx + \phi)\exp(-x/\tau). \quad (1)$$

In this equation the constants K and L define the overall slope and intercept of the record. A denotes the initial amplitude of oscillation,

f the frequency and τ the decay time constant of the oscillation. ϕ denotes the phase angle of the oscillatory movement. The numerical values of the above constants with their corresponding 95% confidence intervals, and the correlation coefficient, were calculated by using an iterative computer program, based on non-linear regression analysis. The curve fitting was done using routines available in Excel and Prism. The correlation coefficient of the fitted curves was generally high being approximately 0.99 for the puller movements and approximately 0.60 for the stiffness oscillations. Measurements were carried out from the fitted curves.

Statistics

Unless stated otherwise, the probability values are based on Student's t test (paired). All statistics are given as means ± S.E.M. A probability of 0.05 was taken as indicating statistical significance.

RESULTS

Characteristics of length oscillations during loaded shortening

Figure 1*A* illustrates load-clamp recordings from a single muscle fibre that was released to shorten against a high load (0.94 of the isometric force, P_0) during the plateau of a fused tetanus. The length signal of the fibre had an initial rapid phase, which coincided with a steep change in tension at the onset of force clamp. This initial length change, which is attributable to recoil of elastic elements in series with the contractile machinery, was followed by a second shortening phase, the velocity of which was adjusted by feedback control to keep the tension constant at the pre-set level throughout the clamp period. The shortening trace can be seen to have a superimposed damped oscillation, i.e. the fibre length change was periodically accelerated and decelerated around a mean velocity with the amplitude of oscillations decreasing exponentially (see further below). During the first few cycles of oscillation the amplitude of the length changes was large enough to lead to an increase in fibre length during the deceleration phase.

The length oscillations during loaded shortening only appeared when the load was greater than approximately $0.8P_0$ and was most pronounced at clamp tensions close to $0.9P_0$. As will be described later, length oscillations like those illustrated in Fig. 1*A* require a fair amount of uniformity of the contractile behaviour along the muscle fibre. Preparations originally showing distinct length oscillations during loaded shortening lost their oscillatory behaviour as an early sign of damage arising somewhere along the fibre, which eventually leads to grossly non-uniform behaviour.

Figure 1*B* illustrates that no length oscillations of the type observed at high loads appeared at loads appreciably below $0.8P_0$. Under these conditions there was a smooth shortening of the fibre after the initial transient at the onset of the load clamp.

Fibres exhibiting damped length oscillations during high-load shortening also showed a similar behaviour when the load was raised *above* the isometric level under load-clamp conditions (Fig. 2). In this case there was an initial steep increase in fibre length that rapidly raised the force to the desired level followed by slow stretching of the fibre. The damped length oscillation during the slow stretch phase can be seen to mirror the pattern of movements observed during slow shortening (cf. Fig. 1*A*). The amplitude of the length oscillation during slow stretching increased as the load was raised to approximately $1.10P_0$. Higher clamp levels were not pursued in order to avoid a large initial quick stretch that would reduce the uniformity of the contractile behaviour along the fibre.

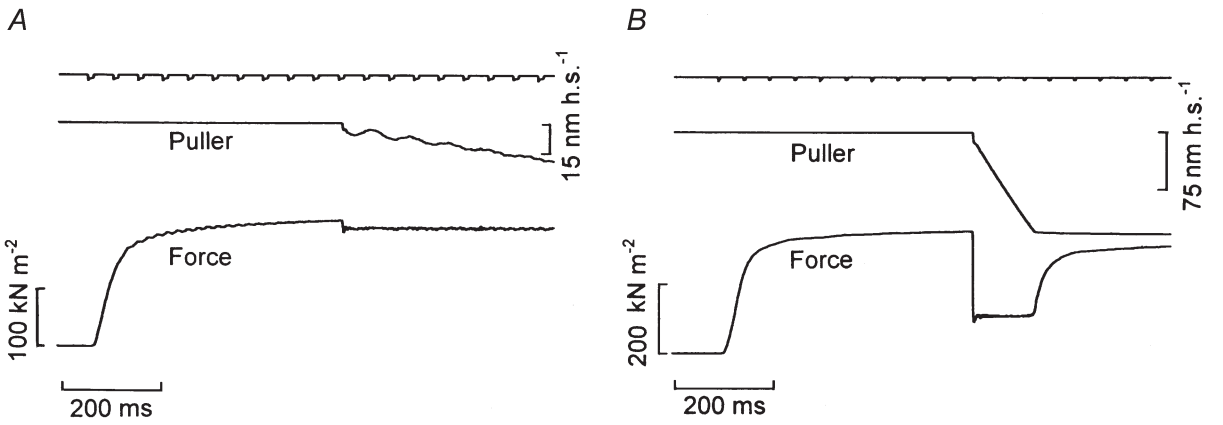

Figure 1. Example records of load-clamp recordings performed at two different tension levels during the plateau of an isometric tetanus

A, load-clamp level 94% of maximum tetanic force (P_0). Note a small initial length step, coincident with the abrupt fall in tension, followed by a sequence of damped length oscillations. *B*, load-clamp level 31% of maximum tetanic force. Except for an initial transient due to a slight underdamping of the puller no oscillatory length changes can be seen in this case. The stimulus markers (top) indicate that the length oscillations in *A* were unrelated to the stimulation frequency.

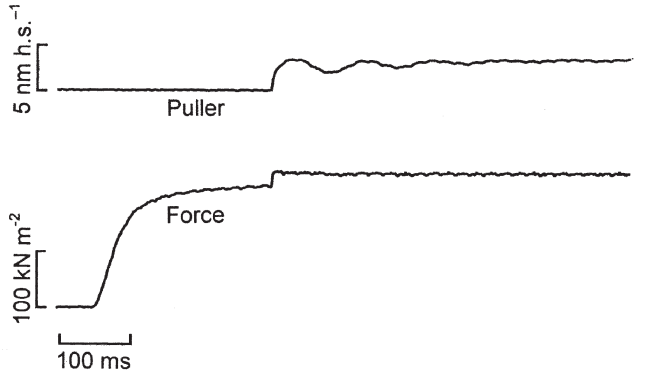

Figure 2. Damped length oscillations during load-clamp recording above the isometric force level

The initial length step (stretch), coincident with the rapid rise of tension, is followed by oscillatory length changes analogous to those observed during load-clamp recording below the isometric force.

The oscillatory behaviour of the fibre during loaded shortening did not occur unless there was an initial fast length change at the onset of the load clamp. This is illustrated in Fig. 3 which shows load-clamp recordings at high load (approximately $0.9P_0$) in the absence (*A*) and in the presence (*B*) of an initial length step. In Fig. 3*A*, the load clamp was arranged to start as soon as the isometric tension reached the selected level ('afterloaded contraction'). The ensuing shortening can be seen to proceed quite smoothly under these conditions with no length oscillation. By contrast, damped length oscillations did occur at the same tension level when the load clamp started with a quick length step during the tetanus plateau (Fig. 3*B*).

Oscillations in segment length

In experiments on five fibres, the length of short, marked segments was recorded to establish how each part of the fibre behaved as the length of the entire fibre oscillated during force clamp. Since the detector system recorded only one segment at a time (see Methods), a series of tetani was required to obtain a complete set of length records from the segments along the fibre. As pointed out earlier (Edman & Reggiani, 1984), the mechanical response of any particular segment of a frog muscle fibre is remarkably constant during repeated contractions. A series of length records obtained from the various segments therefore depicts, in effect, the complete pattern of length changes along the fibre during any given contraction.

Although the shortening pattern varied to some extent between the different fibres, the following general features were found in every fibre tested and can be seen in the example records in Fig. 4. Most segments along the fibre exhibited length oscillations of varying amplitude. The oscillations were in phase with those in other segments during the first two to three cycles and coincided well with the damped oscillations in the puller position. With continuing shortening the amplitude of oscillation of the different segments was reduced and the movements became less synchronous. At the same time the length oscillation recorded from the whole fibre was steadily reduced and finally disappeared. These results

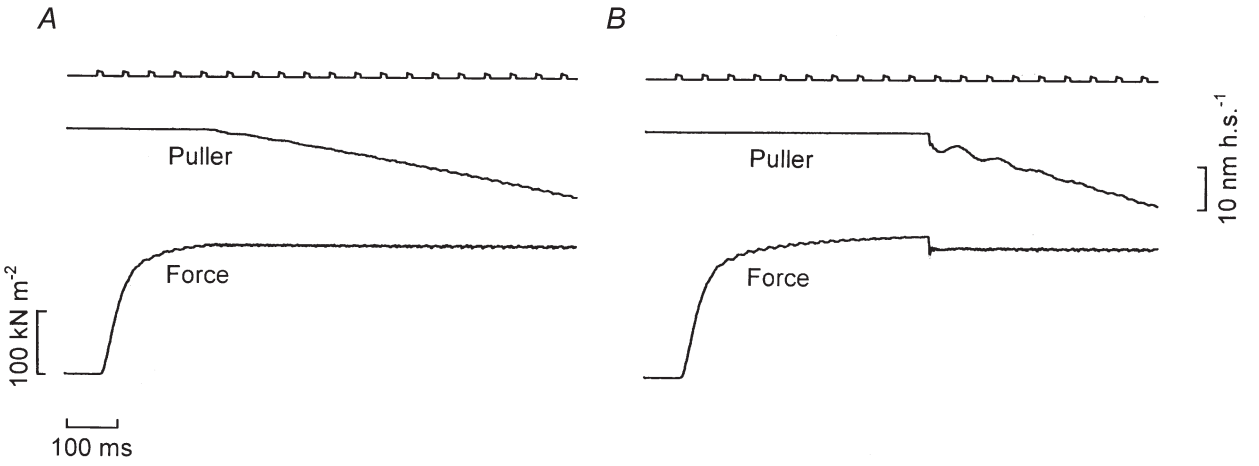

Figure 3. Requirement of an initial length step during the load-clamp manoeuvre for the occurrence of length oscillations

Load-clamp recording at a given load ($0.91P_0$) in the absence (*A*) and in the presence (*B*) of an initial length step at the onset of the clamp. In *A*, the load clamp was arranged to start when the selected force level was reached during the rising phase of the tetanus. In *B*, illustrating a conventional load-clamp manoeuvre, the same clamp level was attained by a quick release from the tetanus plateau. Note that in the absence of a distinct length step there was virtually no length oscillation during the load-clamp recording.

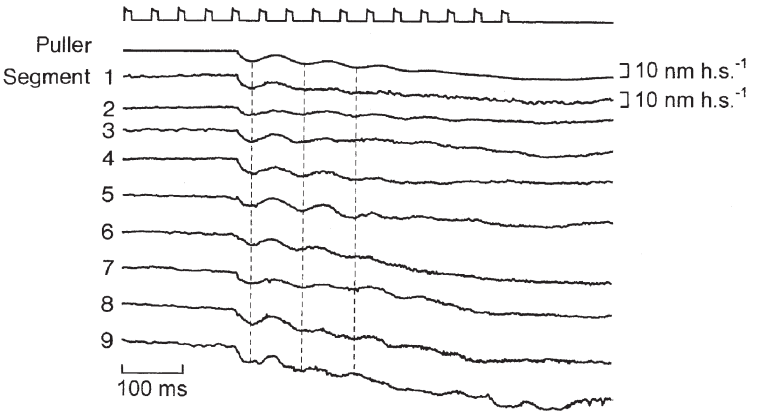

Figure 4. Length changes recorded in short consecutive segments along a single muscle fibre

Movements of nine consecutive, marked segments along an isolated muscle fibre during load-clamp recording at a high ($0.92P_0$) load. The movement of the whole fibre is indicated by the puller signal (top trace). The dashed vertical lines, which are positioned at three consecutive minima of the puller signal, indicate quite synchronous length oscillations along the fibre at first, but the synchrony and also the amplitude of the segmental movements were successively reduced by continued shortening.

thus provide evidence that the oscillatory length changes recorded at the whole-fibre level are the sum of changes in individual segments along the preparation. The degree of synchrony among the segments determines the amplitude of the length oscillation of the whole fibre and, also, how quickly the length oscillation declines. The records shown in Fig. 4 also confirm that the overall rate of loaded shortening, like the maximum speed of shortening, varies along the length of a muscle fibre (Edman *et al.* 1985, 1988).

Oscillations in fibre stiffness

In a series of 13 experiments fibre stiffness was measured (see Methods) during tetani in which length oscillations occurred during force-clamp recording at high loads. Records from such an experiment are illustrated in Fig. 5. The results show that while the tension was kept constant during the load-clamp manoeuvre, the instantaneous stiffness underwent periodic changes that correlated well with the oscillatory changes in fibre length (see below).

For the analysis of the length and stiffness oscillations a mathematical function of a damped harmonic movement was fitted to the experimental data using an iterative computer routine as described in Methods. Examples of such curve fittings are shown in Fig. 6, both curves starting at the end of the initial length step after release. The fitted length and stiffness curves are superimposed on the experimental data in Fig. 6*A* and *C* and are shown separately, for comparison, in Fig. 6*B* and *D* after rescaling the oscillation amplitude appropriately and reducing the overall slope of the two curves to zero. Since the instantaneous stiffness of active muscle is inversely related to the speed of shortening (Julian & Sollins, 1975; Ford *et al.* 1985; Edman *et al.* 1997), it is of relevance to compare the stiffness oscillations with the *velocity* of the puller movements. Such a comparison is illustrated in Fig. 7 in which the first derivative of the length curve shown in Fig. 6*B* and the corresponding stiffness curve (Fig. 6*D*) are displayed together.

Measurements from records like those illustrated in Figs 6 and 7 are summarized in Table 1 for experiments in which the fibres were suddenly released to move against a constant load within the range $0.90–1.12P_0$. The peak-to-peak amplitude of the length oscillation, estimated from the value of A in eqn (1) at the time of release, amounted to 2.7 ± 0.3 nm h.s.$^{-1}$. The corresponding amplitude of the stiffness oscillation was $7.3 \pm 1.2\%$ of maximum tetanic stiffness. The measured periods of the length and stiffness oscillations were 86.8 ± 2.2 and 88.8 ± 2.4 ms, respectively, and were not statistically different ($P > 0.6$).

As illustrated in Fig. 7 there was a clear difference in phase between the stiffness oscillation and the *velocity* of the length oscillation, the latter being in the lead by approximately one-fourth of a cycle. Thus, as is stated in Table 1, the first trough of the stiffness oscillation occurred 21.4 ± 0.8 ms later than the preceding peak of the velocity oscillation ($P < 0.0001$). This lag of the stiffness response relative to the speed of the length change is likely to reflect the time required to re-adjust

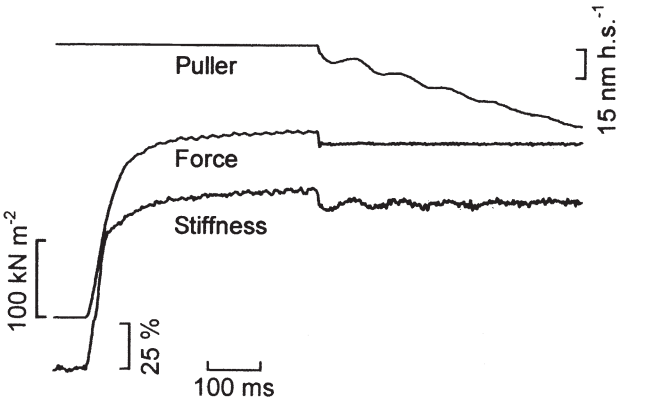

Figure 5. Oscillatory changes in fibre stiffness during load-clamp recording

Records of fibre length (puller movement), force and instantaneous stiffness during load clamp initiated on the plateau of a fused tetanus. Note the simultaneous oscillations of stiffness and fibre length while the force stays at a constant level.

Table 1. Characteristics of length and stiffness oscillations at 1.8 ± 0.1 °C

Load-clamp level	0.90–1.12 P_0	n
Peak-to-peak amplitude of length oscillation (nm h.s.$^{-1}$)	2.7 ± 0.3	13
Peak-to-peak amplitude of stiffness oscillation (% of maximal tetanic stiffness)	7.3 ± 1.2	13
Period of length oscillation (ms)	86.8 ± 2.2	19
Period of stiffness oscillation (ms)	88.8 ± 2.4	19
Phase shift between oscillations in *shortening velocity* and stiffness (ms)	21.4 ± 0.8	19

the number of attached cross-bridges in response to a velocity change in the high-force range (see Discussion).

Influence of fatigue and intracellular acidification on length oscillations

Intracellular acidification and moderate fatigue both enhance the damped length oscillation that occurs after the muscle fibre is released to shorten against a constant high load. Two representative experiments illustrating these effects are presented in Fig. 8. The records in *A* and *C* (controls) were obtained as the fibres were immersed in standard Ringer solution (pH 7.0) and stimulated to produce a 1 s isometric tetanus at 2 min intervals. Figure 8*B* and *D* shows corresponding records after a period of fatiguing stimulation and intracellular acidification (see Methods), respectively, in both cases leading to a moderate (approximately 20–25 %) decrease in tetanic force. These two interventions can be seen to markedly increase the duration of the damped oscillation. This enhancement of the oscillatory behaviour during moderate fatigue and intracellular acidification is most probably associated with improved synchrony of the segmental movements along the fibre in accordance with the results presented in Fig. 4. Results from an experiment (data not shown) similar to that shown in Fig. 4 support this view by showing synchronous length oscillations in different segments along the fibre over a substantially longer time during intracellular acidification than in the control.

The frequency of the length oscillations was determined under control conditions and after fatiguing stimulation and acidification in two series of experiments of seven and six fibres, respectively. The measurements were made from curves fitted to the experimental data according to eqn (1) and the data are summarized in Table 2. The results show that neither moderate fatigue nor acidification caused any significant change of the oscillation frequency during loaded shortening.

Temperature dependence of length and stiffness oscillations

The temperature dependence of the oscillations in fibre length and stiffness during loaded shortening was studied in the same fibres at temperatures varying between

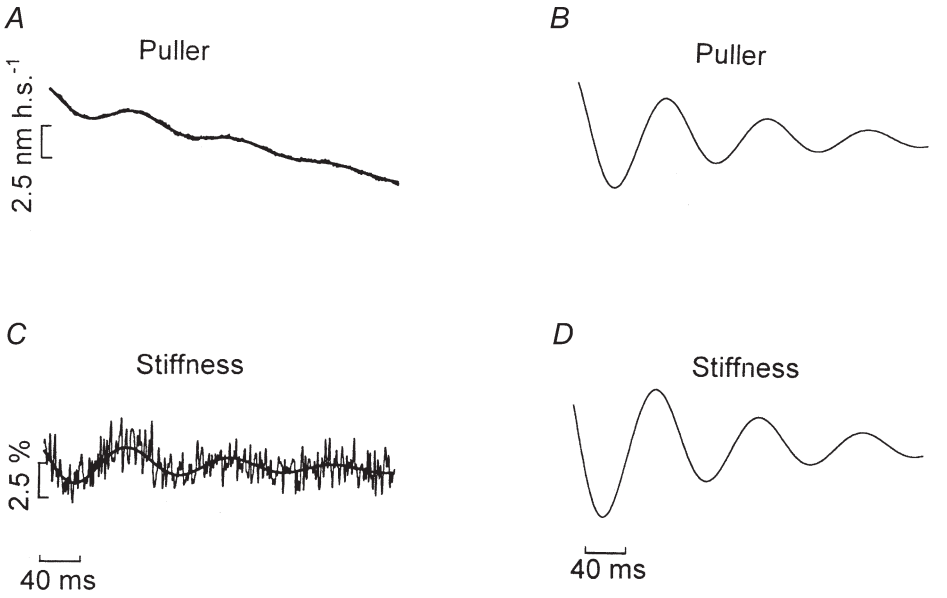

Figure 6. Mathematical function of a damped harmonic oscillation fitted to length and stiffness signals recorded during load-clamp at a high load

The initial step change of the length and stiffness signals at the onset of the load clamp omitted. *A* and *C*, the calculated curves superimposed on the length (Puller) and stiffness records. *B* and *D*, the same curves as in *A* and *C* with overall slopes reduced to zero.

1.5 ± 0.2 and 11.0 ± 0.3 °C. The frequency of oscillation was determined from the mathematical function of a damped harmonic movement that was fitted to the experimental data as described earlier (see Fig. 6). The results derived from four experiments are presented in Table 3. The numerical values of the frequencies of oscillation in length and stiffness can be seen to agree well at the two temperatures, the temperature coefficient (Q_{10}) being 2.84 ± 0.15 and 2.85 ± 0.22, respectively. The results also show that the phase shift between the oscillations in shortening velocity and stiffness has a corresponding temperature dependence in that the phase shift at 11.0 °C was less than half the value recorded at 1.5 °C ($Q_{10} = 0.45 \pm 0.04$).

Table 2. Influence of moderate fatigue and acidification on the frequency of length oscillations during loaded shortening

	Oscillation frequency (Hz)	Deviation from control (Hz)	n*
Control	11.6 ± 0.8	—	7
Moderate fatigue	13.3 ± 0.2	1.7 ± 0.9	
		$P > 0.05$	
Control	12.0 ± 0.9	—	6
Acidification	12.9 ± 0.3	0.8 ± 0.9	
		$P > 0.05$	

*Data are based on one calculated value per fibre including 1–6 recordings in each fibre under control conditions and during fatigue and acidification, respectively.

DISCUSSION

The emergence of length oscillations in a muscle fibre that is released to shorten against a high load during a tetanus has been documented before (Armstrong *et al.* 1966; Sugi & Tsuchiya, 1981; Edman, 1988; Granzier *et al.* 1990), but there has been no systematic investigation of this phenomenon in the past and the underlying mechanism has remained obscure. The present study provides evidence that the oscillatory behaviour during loaded shortening represents a unique situation where the myosin cross-bridges in a major part of the fibre are acting in synchrony over a relatively long time.

Characteristics of length oscillations

The length oscillations appear when the fibre is suddenly released during a tetanus to allow the fibre to shorten, or elongate, while the load is kept constant within the range 0.9–1.1 P_0. The force level at which the phenomenon occurs is thus related to the maximum tetanic force and is independent of the numerical value of that force. Our results demonstrate that the quick length step (release or stretch), occurring at the onset of the load-clamp, is the actual trigger of the subsequent length oscillation. Accordingly, no length oscillation occurs when the load clamp is arranged to start as soon as the pre-set force is reached during the rising phase of the tetanus (see Fig. 3). The quick length step that normally introduces the load-clamp can be presumed to synchronize the contractile system along the fibre and initiate the length oscillation (see Proposed mechanism of length oscillations). A further requirement is that the synchrony along the fibre is maintained over a sufficiently long time for the oscillation to develop. As the shortening (or lengthening) proceeds, however, the synchronous behaviour of the fibre gradually disappears (see below). At the same time, there is a progressive decline, and final disappearance, of the oscillation in fibre length. The fact that the appearance of length oscillations during shortening is confined to the high-force range (loads greater than approximately 0.8 P_0) suggests that the initial synchronization of the mechanical activity along the fibre vanishes quickly as the load-clamp level is reduced and the speed of shortening is increased.

The initial amplitude and the lifetime of the length oscillation can thus be presumed to reflect the degree of uniformity of the contractile properties along the muscle fibre. As previously demonstrated, individual segments along frog muscle fibres may vary greatly with respect to their maximum speed of shortening, active force and power output (Edman *et al.* 1985). The ability of the fibre to shorten against a given load may therefore differ substantially from one region to another. Only fibres

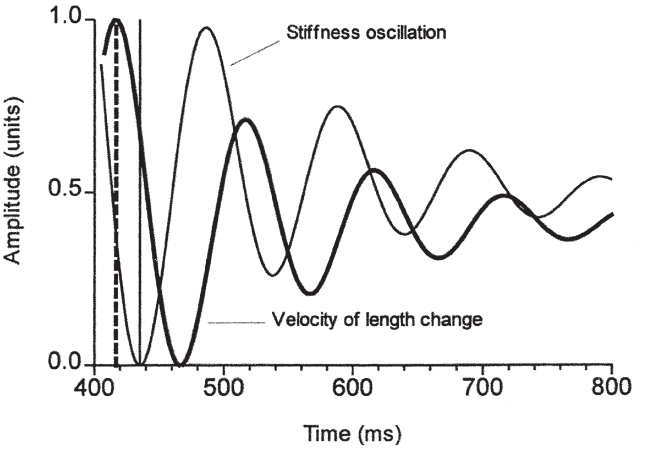

Figure 7. Time lag of the stiffness response relative to the velocity of the oscillatory length change

The velocity of the oscillatory length changes during length-clamp recording (thick line) and the corresponding changes in fibre stiffness (thin line) shown on the same time base. The amplitude of the first cycle in each curve is put as unity. The thick line is the first derivative of the trace shown in Fig. 6*B*; the thin line (fibre stiffness) is a replotting of the trace shown in Fig. 6*D*. The dashed vertical line indicates the time at which peak velocity of shortening is attained during the first cycle; the thin vertical line marks the nearest trough of the stiffness oscillation. Note that the stiffness response (which is inversely related to the speed of shortening, see text) is not instantaneous but lags behind the shortening velocity by approximately 20 ms.

Table 3. Influence of temperature on length and stiffness oscillations

Temperature (°C)	1.5 ± 0.2	11.0 ± 0.3
Frequency of length oscillation (Hz)	10.53 ± 0.23	29.06 ± 0.98
Frequency of stiffness oscillation (Hz)	10.28 ± 0.21	28.34 ± 1.63
Phase shift between oscillations in *shortening velocity* and stiffness (ms)	23.3 ± 1.9	10.7 ± 1.1
Q_{10} of length oscillation frequency	2.84 ± 0.15	—
Q_{10} of stiffness oscillation frequency	2.85 ± 0.22	—
Q_{10} of phase shift between oscillations in shortening velocity and stiffness	0.45 ± 0.04	—
Number of fibres *	4	4

* Data are based on one calculated value per fibre including 1–4 recordings at respective temperature in each fibre.

with fairly uniform contractile properties may thus be expected to maintain the initial synchrony of the cross-bridges within the fibre long enough to enable length oscillations to occur during load-clamp recording. Any differences in the contractile properties along the fibre will be enhanced during the course of the shortening, or lengthening, due to redistribution of sarcomere length. The weaker regions will damp the oscillatory movement and finally quench the oscillation altogether. In a minority of fibres there were virtually no length oscillations detectable after the initial transient during load-clamp recording, suggesting that the mechanical properties of these fibres were markedly non-uniform. It is essential to point out in this connection, however, that fibres with marked segmental differences in force and shortening velocity are normal variants in a population of frog striated muscle fibres (see Edman *et al.* 1985). Such preparations show the same stable performance over a

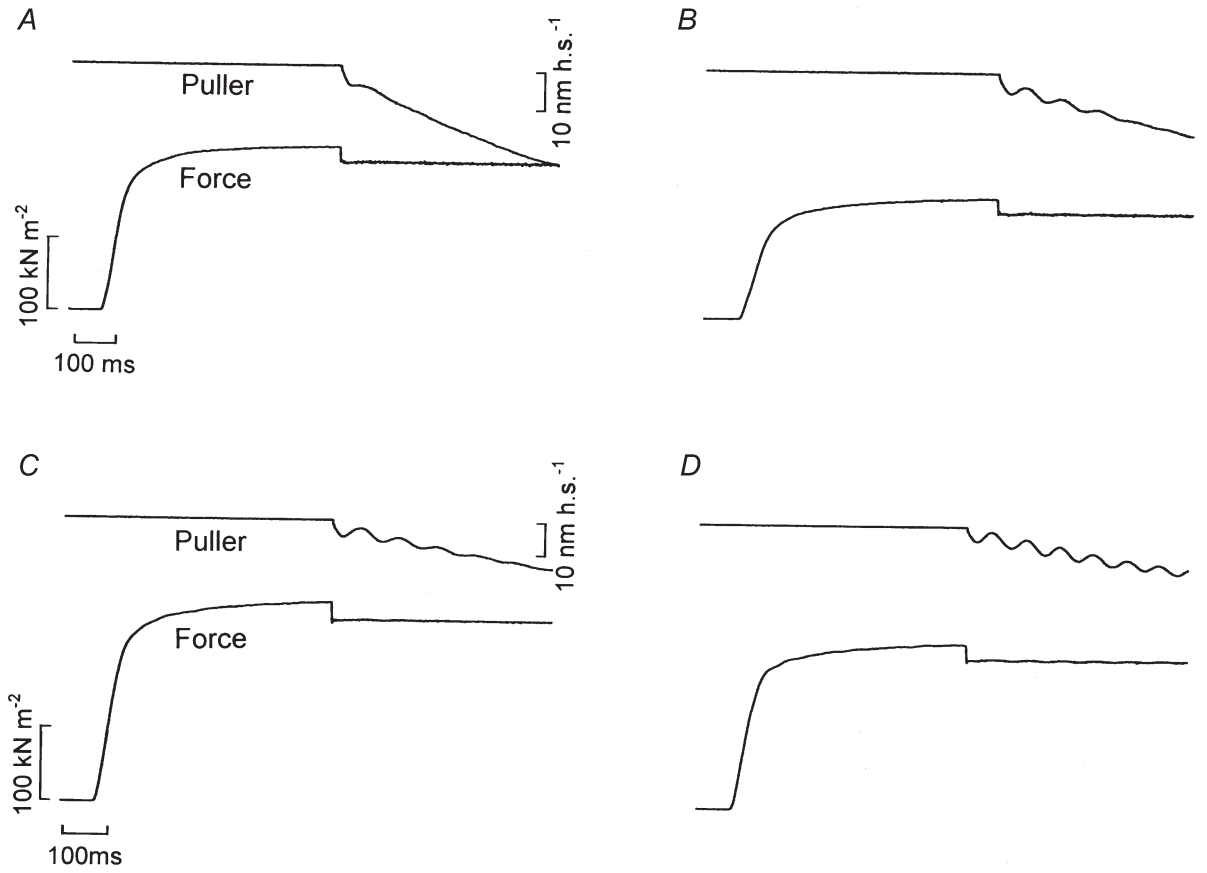

Figure 8. Influence of fatigue and intracellular acidification on length oscillations

Load-clamp recordings performed during the plateau of an isometric tetanus. *A* and *C*, control conditions: ordinary Ringer solution of pH 7.0, 2 min intervals between tetani. *B*, moderate fatigue after a period of tetanization at 15 s intervals, pH 7.0. *D*, intracellular acidification after immersion of the fibre in bicarbonate/CO_2-buffered solution of pH 6.6, 2 min stimulation intervals. Note that both fatiguing stimulation and acidification result in more pronounced oscillatory movements during load clamp.

long experiment as do fibres in which the contractile properties are more uniform.

It is of interest to note that both amplitude and duration of the length oscillation during loaded shortening are markedly enhanced during moderate fatigue and intracellular acidification. The most likely explanation of these effects is that the two interventions tend to equalize the contractile strength along the fibre, both during the tetanus plateau and during tetanus relaxation (see Curtin & Edman, 1989). As a consequence, the synchrony of the oscillatory process along the preparation is improved and the amplitude of the length changes recorded from the fibre therefore enhanced.

The period of the oscillations in fibre length and fibre stiffness agreed exceedingly well, being 86.8 ± 2.2 and 88.8 ± 2.4 ms (1.8 ± 0.1 °C), respectively, suggesting that the two kinds of oscillation are tightly coupled. The temperature dependence of the two oscillatory processes (Q_{10} ~2.85) was also very similar (Table 3) and agreed with the Q_{10} value of 2.67 previously found for the velocity of unloaded shortening in frog muscle fibres under similar conditions (Edman, 1979). Taken together, these findings support the view that the oscillatory length and stiffness changes dealt with in the present study are based on changes in cross-bridge activity as proposed below.

Proposed mechanism of length oscillations

During load-clamp recording any tendency of the fibre to increase or decrease its active force is counteracted by allowing the fibre to alter its speed and direction of movement appropriately. The measured shortening velocity thus reflects the fibre's ability to produce force relative to the clamp level and is a function of the number of attached cross-bridges and the force generated by these bridges. Thus when the force producing capability of the fibre is greater than required to carry the load, the servo system keeps the velocity of the movement above average (> 0.5 in Fig. 7) in an attempt to reduce the number of attached bridges so as to match the load. The opposite is true when more cross-bridges need to be recruited, in which case the servo system has to decelerate the movement. The oscillation of stiffness provides evidence that the number of attached cross-bridges does vary with the cyclic changes in shortening speed. Periodic changes in stiffness of elastic components outside the cross-bridge domain are probably unimportant in this measurement. The force is kept constant during the load-clamp manoeuvre and the stiffness residing in structures acting in series with the myofilament system will therefore remain constant during the measurement. Furthermore, the oscillatory changes in myofilament overlap are very small (< 3 nm h.s.$^{-1}$) during shortening at the low velocities studied, and a change in myofilament compliance (Huxley *et al.* 1994; Kojima *et al.* 1994; Wakabayashi *et al.* 1994; Higuchi *et al.* 1995) would therefore be negligible during an oscillation cycle. A detailed analysis of the force–stiffness–velocity relationships during shortening at high loads has been presented earlier (Edman *et al.* 1997) using a four-state cross-bridge model. As suggested by this model an increase in shortening velocity within the range P_0–$0.85P_0$, i.e. at loads where length and stiffness oscillations occur, is attributable in part to a decrease in the number of attached cross-bridges and in part to lower force output per bridge. The opposite holds true when the shortening speed is reduced, in which case the number of attached cross-bridges is raised and the force output per bridge is increased.

The following sequence of events may be thought to underlie the oscillations in velocity and stiffness as the muscle fibre is released to shorten against a high load under force-clamp conditions. After release the fibre shortens rapidly to bring the tension to the selected level. This is achieved by reducing the number of attached cross-bridges as indicated by the simultaneous fall in stiffness. During the length step a fraction of new cross-bridges will be formed, the 'new' bridges being attached in a configuration where they are ready to begin a power stroke (cf. Josephson & Edman, 1998). As the newly formed cross-bridges go through their power stroke, the force generated by the fibre will tend to exceed the clamp level and the servo system therefore has to accelerate the fibre so as to reduce the number of cross-bridge connections and reduce the force production per bridge (cf. above). This will continue, with a concomitant decline in fibre stiffness, until the total force produced by the bridges becomes inadequate to uphold the pre-set clamp level. In this situation the fibre has to decelerate to increase the number of cross-bridge connections and their force producing capability once again. These cycling changes in shortening velocity and cross-bridge number, the latter reflected by the periodic changes in fibre stiffness, will continue as long as the various parts of the fibre remain in synchrony (see earlier). A requirement for the above mechanism to occur is that the force response to a change in shortening velocity is not instantaneous but needs a finite time to be completed. In accordance with this view our experiments showed that the stiffness oscillation lagged behind the oscillation in shortening velocity by 21.4 ± 0.8 ms thus creating a phase shift corresponding to one-fourth of an oscillation cycle. To this should be added the time needed to complete the power stroke after attachment of the bridge.

The mechanism underlying the length oscillations during loaded shortening has recently been dealt with in a model of the mechanochemical cycle of myosin by Duke (1999, 2000). The model predicts that the myosin power strokes become synchronized when the muscle shortens against a high load and the number of active cross-bridges therefore is large. The synchronized activity of the cross-bridges will, according to Duke's model, lead to oscillatory movements whenever the muscle is free to shorten under a load near the isometric force. However, in contrast to the experimental findings reported in the present paper, the model proposed by Duke gives no indication that the

initial length step during load clamp is crucial for initiating the oscillatory length changes.

The mechanism underlying the oscillatory movements during *stretching* can be presumed to be a mirror image of that described above for loaded shortening. In both situations the initial length step (release or stretch) has a key role in leading to synchronization of cross-bridges along the fibre. The ensuing changes in speed of shortening or lengthening are in both cases an attempt to maintain the tension at the pre-set level by appropriately varying the number of attached cross-bridges and the force output per bridge.

Comparison with 'asynchronous' insect flight muscles

Length oscillations occur as a physiological mechanism in 'asynchronous' insect flight muscles based on cyclic changes in activation of the contractile system as the length of the muscle is altered. Stretching the insect flight muscle increases myofilament activation and so increases the contractile strength, whereas shortening has the opposite effect. As a result, stimulation will set in motion a mechanically resonant system in which the muscle, initially deactivated by shortening, is being reactivated by elastic rebound. Repeated shortening–stretch cycles arise in this way and the muscle continues to oscillate for as long as it receives electrical stimuli (e.g. Jewell & Rüegg, 1966; Pringle, 1967; Rüegg, 1968). A mechanism of this kind can probably be ruled out as a cause of the length and stiffness oscillations described in the present paper. In fact, the behaviour of the fibre after the initial length step during load-clamp recording is precisely opposite to what would be expected from the mechanism proposed for the insect flight muscles. According to this mechanism the fibre would be partially deactivated by the initial length step during load-clamp recording below the isometric level. This would temporarily decrease the shortening speed whereas, in reality, the shortening speed is increased. Conversely, the rapid increase in length that initiates a load-clamp manoeuvre above isometric force would increase the contractile strength and therefore momentarily decrease the stretch velocity which is contrary to what actually occurs.

Conclusion

This study has demonstrated that the slow oscillations in length that occur as a muscle fibre is released to shorten, or elongate, against a constant load near the tetanic force level are associated with cyclic changes in fibre stiffness, indicating cyclic changes in the number of cross-bridge connections. The length and stiffness oscillations represent a situation in which the myosin cross-bridges have been brought into synchronous activity in response to the small length step (release or stretch) that occurs at the transition from isometric to load-clamp recording. The occurrence of the length and stiffness oscillations is explainable by the fact that a finite time (> 22 ms) is required for the force to fully adapt to a change in shortening velocity in the high-force range. This creates a phase shift between the two variables, stiffness and shortening velocity, during load-clamp recording.

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