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Decrease in light diffraction intensity of contracting muscle fibres

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Abstract. Single fibres from the semitendinosus muscle of frog were illuminated normally with a He–Ne laser. The intensity transient and fine structure pattern of light diffracted from the fibre undergoing isometric twitches were measured. During fibre shortening, the intensity decreased rapidly and the fine structure pattern preserved its shape and moved swiftly away from the undiffracted laser beam. The fine structure patterns of the contracting and resting fibre were nearly identical. The ratio of intensities of the contracting and resting fibre of the same sarcomere length was determined as a function of the time elapsed after fibre stimulation. The time-resolved intensity ratio increased with sarcomere length and became unity when sarcomere length was between 3.5 μm and 3.7 μm. A diffraction theory based on the sarcomere unit was developed. It contained a parameter describing the strength of filament interaction. The comparison between the theory and data shows that the initial intensity drop during contraction is primarily due to filament interactions. At a later stage of contraction, sarcomere disorder becomes the major component causing the intensity to decrease. Diffraction models which use the Debye-Waller formalism to explain the intensity decrease are discussed. The sarcomere-unit diffraction model is applied to previously reported intensity measurements from active fibres.

Key words: Light diffraction intensity, muscle fibres

Introduction

The diffraction pattern of a striated muscle fibre illuminated normally by a laser beam shows a series of bright lines. It is believed that the bright lines are the result of light diffraction by the sarcomeres because the sarcomeres form a semi-crystalline lattice. Consequently, the intensity of each line is affected by changes in the optical properties and arrangement of the sarcomeres. After the fibre is activated, the intensity of each line decreases. The exact cause of the intensity drop is uncertain. Thus far, the decrease in intensity of active fibres has been interpreted solely in terms of an increase in sarcomere disorder (Fujime 1975; Rüdel and Zite-Ferenczy 1980; Oba et al. 1981, 1983). However, the amount of sarcomere disorder has not been determined precisely to provide support for this interpretation (Umazume and Yoshioka 1980).

The diffraction intensity falls fastest during the early phase of an isometric contraction. During this phase, the increase in sarcomere disorder is expected to be small. Then, the early sharp drop in intensity could be caused by a factor not related to sarcomere disorder. This factor is likely to be associated with contractile filament interactions. Since the fine structure pattern of a diffraction order is dependent on the arrangement of the sarcomeres within the fibre (Judy et al. 1982; Leung 1982a, b, 1984; Sundell et al. 1986), its variation during a twitch of the fibre indicates a change in sarcomere arrangement or sarcomere disorder (Leung 1983). Therefore, a diffractometer has been constructed to monitor simultaneously both the diffraction fine structure pattern and the intensity during an isometric twitch. The aim of this study is to demonstrate that the intensity decrease in active fibres is related to filament interactions and sarcomere disorder.

Materials and methods

Single fibres from the semitendinosus muscle of *Rana tigrina regulosa* were isolated and mounted horizontally in chambers filled with Ringer's solution at room temperature. One end of the fibre was attached to a micromanipulator by a fine stainless steel hook impaling the tendon. The other end was fastened similarly to

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a force transducer (Cambridge, Massachusetts, Model 404) for tension measurement. A He–Ne laser was placed about 1.5 m from the fibre so that its beam diameter at the fibre was about 6 mm. The enlarged beam passed through a (3 mm × 5 mm) window placed just beneath the experimental chamber and illuminated a 3 mm section of the fibre at normal incidence. Illuminating a relatively large fibre section with a more uniform beam would lessen unwanted intensity changes due to slight movements of the fibre during isometric contractions. The fibre was stimulated by an electrical pulse through two long platinum electrodes lying parallel to the fibre.

Optical detection system

The left first-order diffraction from the active fibre divided into two components after it had passed through a beam splitter. These two components were analysed simultaneously by two different light detection systems (Fig. 1). One system employed a photomultiplier to measure the intensity of the central portion of the diffraction line. The other system used an array of silicon photocells to track the fine structure of the same diffraction line and to measure the sarcomere length of the fibre.

The photomultiplier detection system received the diffraction reflected from the beam splitter (Fig. 1). After the beam splitter, the diffracted light passed through a (2 cm × 3 cm) aperture and a convex lens and then converged on a diffuser. The diffuser placed just in front of the photomultiplier (Hamamatsu, R928) made the photomultiplier insensitive to any small lateral displacement of the diffracted light. To test the photomultiplier system the sarcomere length of the fibre at rest was adjusted to 2.7 μm. The photomultiplier was driven by an electric motor to sweep across the width of the diffraction line. Its output showed a broad band with a flat top. The centre and the width of flat top corresponded to the diffraction angle $\Theta = 13.55°$ and $\Delta\Theta = 2.8°$, respectively. In terms of sarcomere length (S), $\Theta = 13.55° \pm 1.4°$ corresponded to 2.45 μm $\leqq S \leqq$ 3.00 μm. When the detection system was aligned at $\Theta = 13.55°$, the diffraction from the fibre with its sarcomere length set to any value between 2.45 and 3.00 μm was detected by the system without distortion. The detection system was always moved to a position such that the photomultiplier could detect continuously without distortion the diffraction from the fibre during an isometric contraction even though the diffraction was moving. The output of the photomultiplier was recorded by a storage oscilloscope (Hitachi, VC-6015) and displayed by an $x-y$ recorder.

The second light detection system contained 15 rectangular silicon photocells (Hamamatsu, S875–16R)

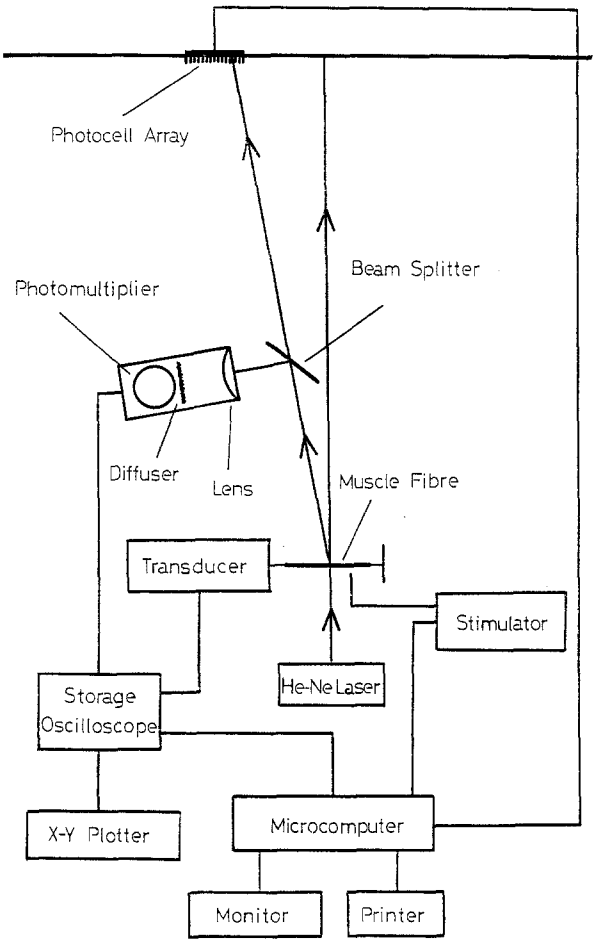


Fig. 1. Schematic diagram of the experimental setup

lying contiguously and parallel to one another on a plane 70 cm above the muscle fibre. Each photocell had a sensitive area of 1.1 mm × 5.9 mm. The separation between the photocell was 2.7 mm. The width of each photocell was slightly smaller than the width of the fine structure within the first-order diffraction line. The photoarray was aligned so that the diffraction line was centred on the first photocell. In general, the width of the diffraction line covered three photocells. According to the grating equation, the position of each photocell corresponded to a specific sarcomere length. During the contraction cycle of the fibre the output of each photocell was measured every millisecond, amplified, digitized, and then stored in a microcomputer. The variation of each photocell's output with time was then displayed by the microcomputer on a printer. From the outputs of the photocells the variations of the fine structure pattern and sarcomere length with time were obtained.

Experimental protocol

The principal factor that determined useful data for analysis was the diffraction fine structure pattern. Af-

ter the fibre was stimulated to undergo a fixed-end twitch, the movement of the fine structures belonging to the first-order diffraction line was monitored by the photocell array. If the fine structure pattern varied substantially such that each fine structure could not be tracked during the early phase of fibre shortening, no further measurements would be taken. The variation of the fine structure pattern was sometimes caused by a twist in the fibre or motion of the fibre relative to the illumination. The twist caused the diffraction pattern to move obliquely across the photoarray axis. The oblique movement introduced new structures into the detection zone and gave rise to a new diffraction pattern. In this case, the fibre was untwisted slowly until the pattern preserved its integrity during the early phase of contraction. During twitch, the fibre might swing away from the illumination. This motion also affected the fine structure pattern. Usually, motion artefacts were reduced by rotating the fibre around its length so that the direction of unwanted motion was along the incident laser beam. If the fine structure pattern still varied substantially during shortening after various manipulations of the fibre, the fibre would be discarded. The criterion for intensity measurement was the constancy of the fine structure pattern during the early phase of shortening. Among 15 preparations only four fibres satisfied the constancy criterion. Intensity measurements were recorded for these four fibres.

Theory

A striated muscle fibre acts like a three-dimensional optical diffraction grating because its sarcomeres form a quasi-crystalline ensemble. Its Fraunhofer diffraction intensity (I) is interpreted as being related to the Fourier transform of the sarcomeres (Judy et al. 1982; Leung 1982b, 1987; Roos and Leung 1987). As an approximation,

$$I = c E^2 G^2 K, \tag{1}$$

where c is a constant; E and G are the equatorial and meridional diffraction amplitudes of the sarcomere, respectively; and K is the interference of light waves scattered from all the illuminated sarcomeres (Leung 1982b). The ratio (R) between the intensity of the active fibre and the intensity of the same fibre at rest is

$$R = \frac{I_a}{I_r} = \frac{G_a^2 K_a}{G_r^2 K_r}. \tag{2}$$

The subscripts a and r represent active and resting components, respectively. According to Fujime (1975),

$$G_r = \frac{S}{\pi n}\left[F \sin(n\pi L/S) + (-1)^n f \sin(2n\pi l/S)\right], \tag{3}$$

where F and f are the scattering densities, and L and l are the lengths of the thick and thin filaments, respectively. After the fibre is stimulated, it is believed that the interaction between the contractile filaments causes shortening. This interaction can effect the scattering efficiency of the overlapping contractile filaments. Its strength is described by an additional scattering density (δ) in the filament overlap region only. Then,

$$G_a = G_r + \frac{S\delta}{n\pi}\left[\sin(n\pi L/S) + (-1)^n \sin(2n\pi l/S)\right]. \tag{4}$$

Consequently, the ratio of the first-order ($n=1$) diffraction intensities can be expressed as

$$R = \left|1 + \frac{(\delta/f)\left[\sin(\pi L/S) - \sin(2\pi l/S)\right]}{(F/f)\sin(\pi L/S) - \sin(2\pi l/S)}\right|^2 \frac{K_a}{K_r}. \tag{5}$$

When the filament overlap just disappears, $S = 2l + L$ and the bracket term of Eq. (5) becomes unity and $R = K_a/K_r$. When $S > 2l + L$, $\delta = 0$ and $R = K_a/K_r$ also. The relative scattering density of the thick and thin filaments, (F/f), has been estimated to be about 3 (Huxley and Hanson 1957). Generally, $l = 1\,\mu m$ and $L = 1.6\,\mu m$.

Results

Fibres were selected for measurements of diffraction intensity only when the diffraction fine structure pattern recorded with the photoarray persisted during active shortening of the fibre. An example of the variation of the diffraction pattern with shortening is shown in Figs. 2 and 3. During passive shortening, the two

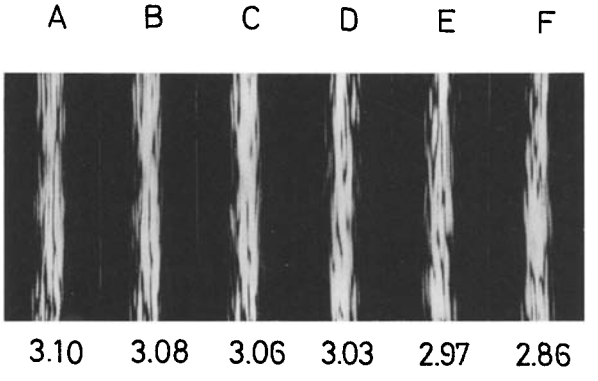


Fig. 2. First-order diffraction pattern of a fibre which was allowed to shorten passively from an average sarcomere length of 3.10 μm (A) to that of 2.86 μm (F). Pattern (B) taken at 3.08 μm, (C) 3.06 μm, (D) 3.03 μm and (E) 2.97 μm. The position of the fine structure on the right side of Pattern (A) is equivalent to a sarcomere length 3.13 μm, and that of the left 3.08 μm. The pattern show that the two broader fine structures persisted during a small change in the length of the fibre. The two bars on the left show the size and separation of two adjacent photocells relative to the size of the fine structures

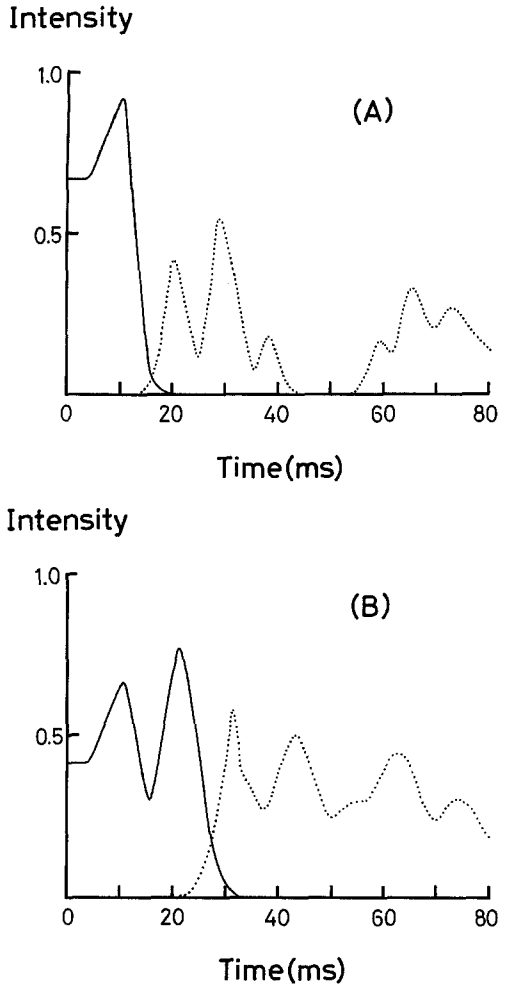


Fig. 3 A and B. The time course of the two broader fine structures measured with the photoarray during a fixed-end twitch. The diffraction pattern of the resting fibre is shown in Fig. 2 A. The position of the first photocell corresponded to a sarcomere length of 3.12 μm, that of the second 3.07 μm, the third 3.01 μm and the fourth 2.96 μm. In (**A**) the *solid line* shows the output of the first photocell and the *dotted line* the output of the third photocell. In (**B**) the *solid line* shows the output of the second photocell and the *dotted line* the output of the fourth photocell

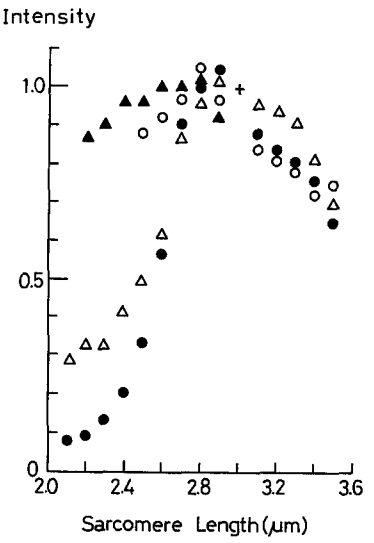
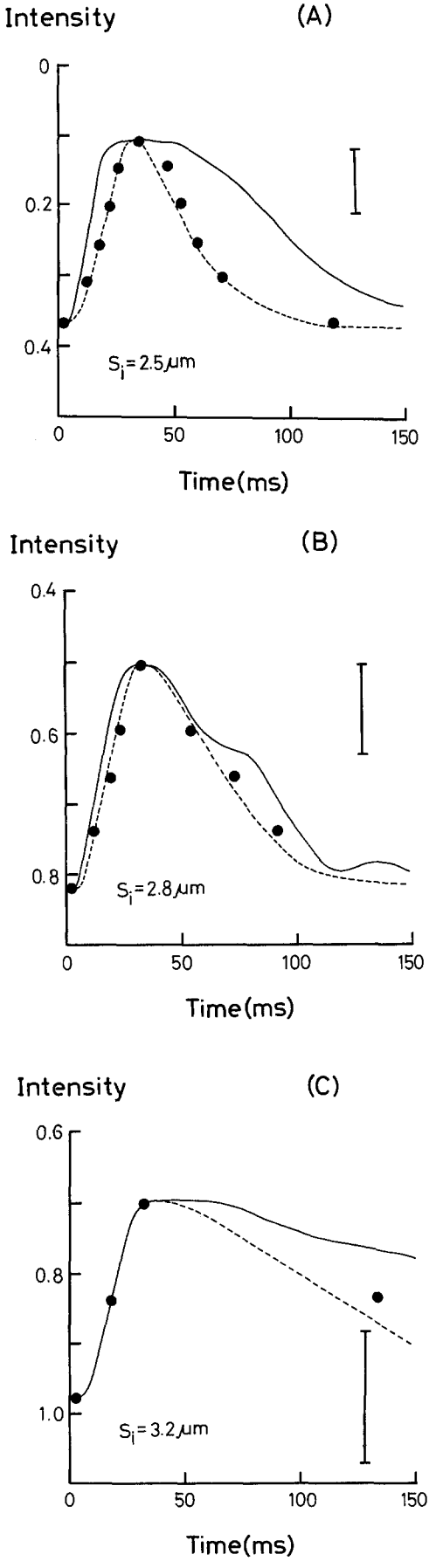


Fig. 4. Variation of the diffraction intensity of resting fibres with sarcomere length. The intensity was arbitrarily normalized to unity at a sarcomere length of 3.0 μm, each symbol represents data from one fibre

wider fine structures persisted and finally coalesced after a shortening of about 8% (Fig. 2). The outputs of the photoarray (Fig. 3) show that the two wider fine structures also persisted throughout the shortening phase of a fixed-end twitch. Initially, one of the fine structures illuminated the first photocell while the other illuminated the second photocell. After the fibre was stimulated, the fine structures remained stationary during the latent period. During this period, the first two photocells had constant outputs. Then, the first fine structure moved across and away from the first photocell. Because nothing else illuminated this photocell its output had only one peak (Fig. 3 A). The first peak of the output of the second photocell (Fig. 3 B) was due to the second fine structure moving away and across this photocell. The second peak was due to the first fine structure illuminating the photocell at 21 ms after simulation. The output of the third photocell shows two groups of peaks. The first group of three peaks which occurs around 30 ms describes the fine structure pattern during contraction. The second group near 70 ms represents the pattern during relaxation. The two original fine structures still persisted 30 ms after stimulation. The position of the third photocell corresponded to a sarcomere length of 3.02 μm. The appearance of the third peak in the output of the third photocell is consistent with the pattern of the resting fibre. The output of the fourth photocell indicates the overlapping of the patterns associated with contraction and relaxation or a state near the peak of shortening. Nevertheless, the original two fine structures could still be identified.

The diffraction intensity of the selected fibre remained constant during the latent period. Then, it decreased rapidly and reached a value close to the minimum within 25 ms after stimulation. Near the peak of shortening the intensity varied slowly around the minimum value. During relaxation, it increased and fluctuated randomly in returning to its initial value of the resting fibre. A set of intensity transients was recorded for the same illuminated section of a fibre stretched to different resting lengths. This set of data and additional measurements on the resting fibre provided the intensity (I_r) of the resting fibre as a function of sarcomere length (Fig. 4). Three typical intensity transients with tension and sarcomere length measurements are shown in Figs. 5 A–C. The time course of sarcomere length of the active fibre was determined by analyzing



the outputs of the photocells. The position of each photocell corresponded to a sarcomere length. The time necessary for the diffraction line to reach a particular photocell after the fibre was stimulated was determined by calculating the centroid of the output of that photocell. Therefore, each photocell illuminated by the diffraction line yielded a time value and a sarcomere length. The corresponding pairs of data sets provided the time course of sarcomere length of the active fibre and they are tabulated as dots in Fig. 5A–C. From the corresponding time course of sarcomere length each intensity transient was converted to a plot showing the intensity of the active fibre vs. sarcomere length. Then, the ratio (R) between the diffraction intensity of the active fibre (I_a) and the intensity of the resting fibre (I_r) at the same sarcomere length (S) was calculated. The intensity ratios were tabulated according to the time (t_1) elapsed after the application of the stimulus. For example, Fig. 6A shows the R data at $t_1 = 10$ ms. The data from the four fibres were least-squared fitted to a straight line. The fit provided $R = 0.16\,S + 0.41$ with a standard deviation of 0.005. The intensity ratio data associated with $t_1 = 15$, 20 and 30 ms and with the intensity minimum which occurred at $50\text{ ms} \leqq t_1 \leqq 60$ ms are illustrated in Fig. 6B–E. The data were also fitted to straight lines. The result of the fit is illustrated in Fig. 7.

Discussion

Origin of the intensity decrease

It is well known that light diffraction intensity decreases when the muscle fibre is activated. Thus far, this decrease has been attributed primarily to an increase in disorder of the diffraction elements or in sarcomere disorder. The pattern of fine structures of a diffraction order is very sensitive to the sarcomere arrangement within the fibre (Leung 1984; Sundell et al. 1986; Roos and Leung 1987). Then, the preservation of the fine structure pattern during the early phase of fibre shortening suggests that there is little increase in sarcomere disorder during that time. Therefore, to account for the early sharp drop in intensity it is proposed that there is another factor. This factor is related to the physiological events occurring in active fibres.

Several chemical events take place in the myofilaments after the fibre is electrically stimulated. Not all

Fig. 5A–C. Diffraction intensity (*solid line*), tension development (*broken line*), and sarcomere length (*dots*) of a fibre during fixed-end twitches. S_i is the resting sarcomere length, the vertical line represents a tension of 100 mg. In (**A**) the sarcomere length at peak of shortening was 2.34 μm, in (**B**) 2.63 μm, and in (**C**) 3.09 μm.

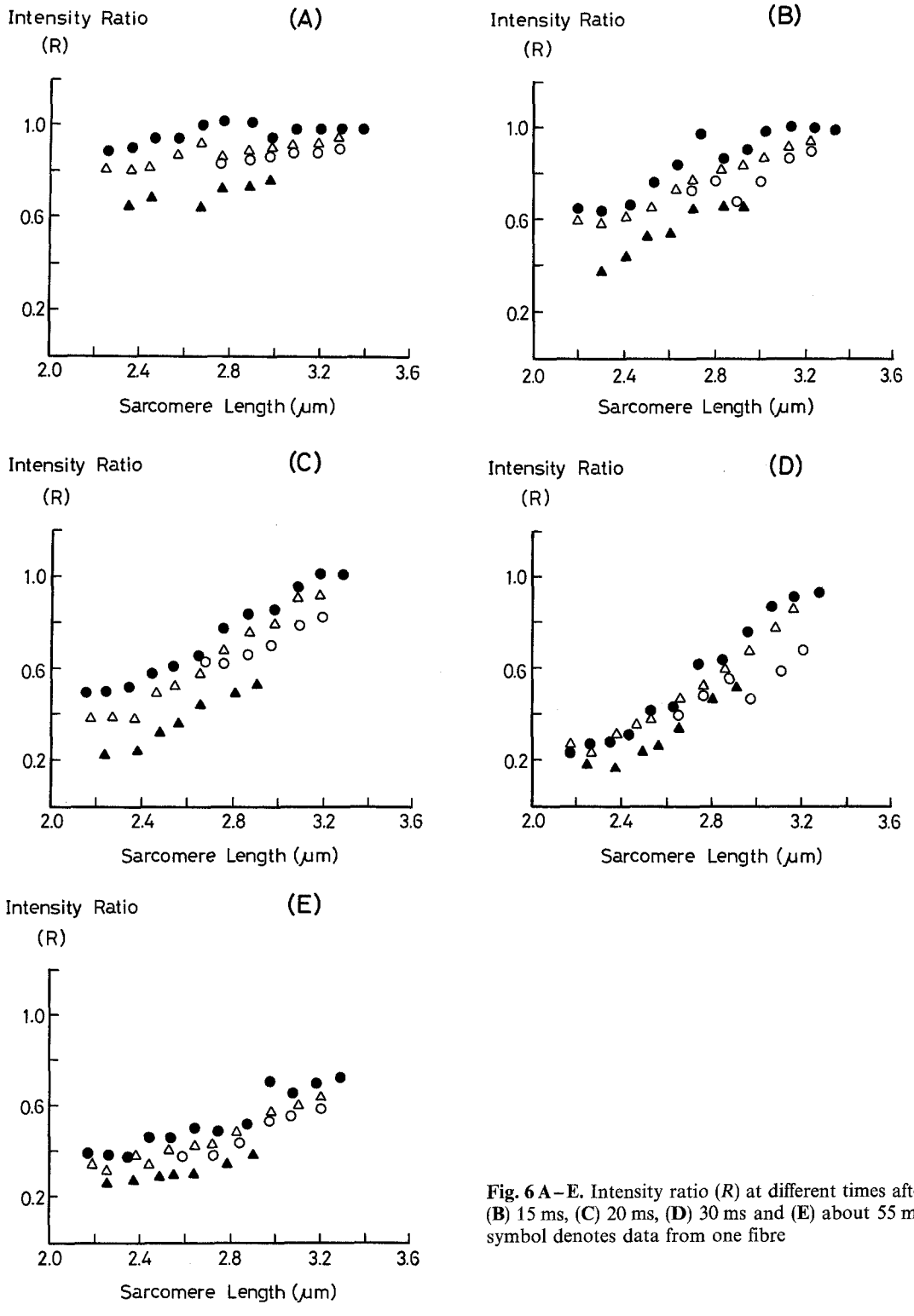


Fig. 6 A–E. Intensity ratio (R) at different times after stimulus. (**A**) 10 ms, (**B**) 15 ms, (**C**) 20 ms, (**D**) 30 ms and (**E**) about 55 ms after stimulus. Each symbol denotes data from one fibre

events affect the diffraction intensity appreciably. The pH changes due to ATP hydrolysis and rephosphorylation of ADP are about 1% during an isometric twitch of a fibre (MacDonald and Jöbsis 1976). To a first approximation, they would cause the diffraction intensity to drop by about 6% (Sabbadini et al. 1979). Calcium ions released from the sarcoplasmic reticulum reduce the optical contrast between the contractile filaments and the myoplasm. This small reduction in contrast can only result in a minor drop in intensity. The interaction between the thick and thin contractile filaments is probably the dominant event in active fibres. It could alter the scattering efficiencies of these filaments and consequently cause a large change in the

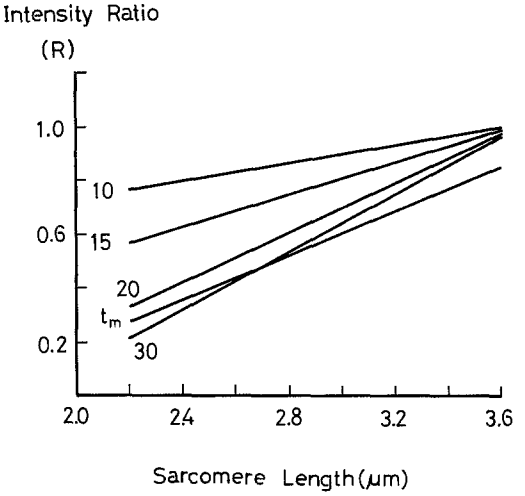


Fig. 7. Straight lines from least-squares fits of the intensity ratio data tabulated in Fig. 6 A–E. The number next to the line is the time elapsed after stimulation, and t_m is about 55 ms. The standard deviation of the fit for the R data taken at 10 ms was 0.005, at 15 ms was 0.08, at 20 ms was 0.013, at 30 ms was 0.005, and at about 55 ms was 0.007

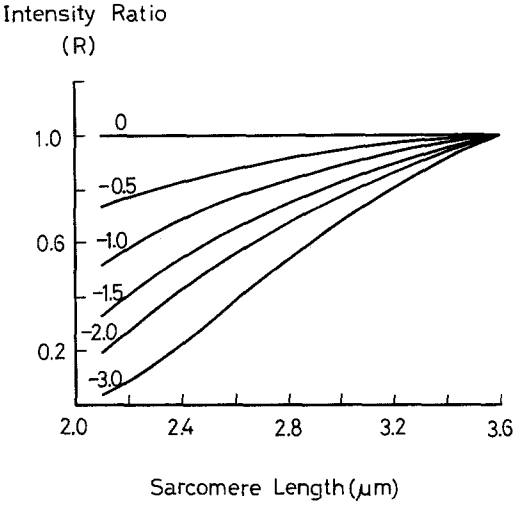


Fig. 8. Theoretical curves for the intensity ratio (R). They were calculated using Eq. (5) with $K_a/K_r = 1$. The number next to each curve is the value of δ/f

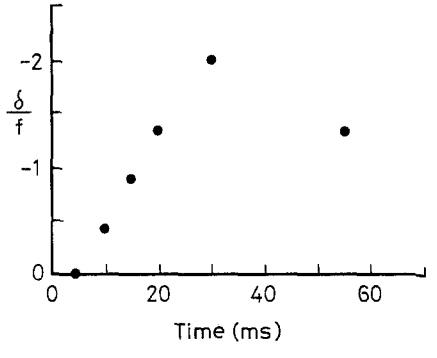


Fig. 9. Variation of the filament interaction parameter δ/f with time

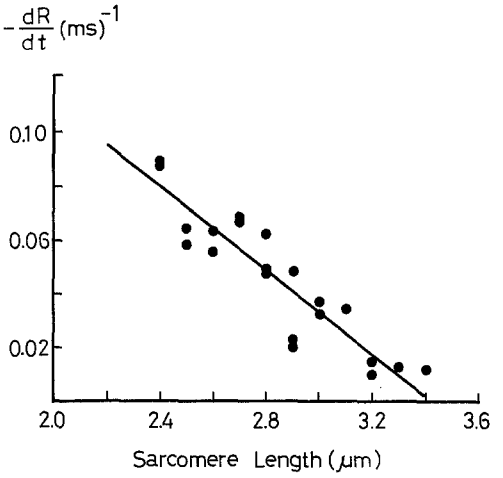


Fig. 10. Rate of decrease of R at different sarcomere length. The straight line was the result from a least-squares fit of the data

diffraction intensity. The interaction between the thick and thin filaments can be described by an additional scattering density (δ) in the overlap of the thick and thin filaments. This additional density represents the amount of filament interactions per unit length. Then, the intensity ratio R follows Eq. (5). The factor K_a/K_r is the fraction of intensity decrease due to sarcomere disorder. If disorder is not significant, the factor is close to unity. In this case, the theoretical R varies with δ/f and sarcomere length as shown in Fig. 8. The theoretical curves agree with the experimental result illustrated in Fig. 7. The agreement indicates that the amount of change in δ/f is dependent only on the time after stimulation and not on sarcomere length. The comparison between the average slopes of the theoretical curves and those shown in Fig. 7 provides a rough estimate of the time course of δ/f (Fig. 9). The analysis suggests that filament interactions can explain the intensity drop and these interactions peack between 20 and 50 ms after stimulation.

It is suggested that the early fall in the diffraction intensity is primarily caused by filament interactions. If this is the case, the strength of filament interaction would be proportional to the filament overlap and consequently would decrease with sarcomere length. The theory suggests that the rate of intensity decrease or $-\mathrm{d}R/\mathrm{d}t$ is an indication of the strength of filament interaction. From the intensity transient the maximum $\mathrm{d}R/\mathrm{d}t$ and the corresponding sarcomere length were determined. The result is shown in Fig. 10. It indicates that the rate is higher at shorter sarcomere lengths or larger filament overlaps and approaches zero near a sarcomere length of 3.4 μm. This phenomenon is consistent with the notion that the intensity decrease in active fibres is related to filament interactions.

The negative value of δ indicates that filament interactions decrease the scattering efficiencies of the thick and thin filaments at the overlap. This decrease

in the scattering efficiencies is primarily caused by a drop in the optical polarizability in the overlap region because scattering intensity is proportional to polarizability of the scatterers. A reduction in the polarizability of a skinned fibre going from a relaxed state to a rigor state was observed (Yeh et al. 1983) using optical ellipsometry. Electrochemical measurements on barnacle fibres show that contraction causes a reduction of 30%–50% of the myoplasmic effective charge density (Caillé 1979). The decrease in polarizability could come from changes in molecular conformation during fibre activation.

Sarcomere disorder

The pattern of diffraction fine structures is the result of light scattering by the illuminated sarcomeres. Identical patterns of the resting and active fibre imply that the resting and active sarcomere arrays have identical geometry. The fine structure pattern of a contracting intact and unattached myocardial cell retains its profile until the cell is near the peak of shortening (Leung 1983). During the early stage of contraction of the cell, the sarcomeres shortened freely and independently because they were not under the influence of any external force. Therefore, a constant fine structure pattern suggests independent sarcomere shortening without any significant increase in sarcomere disorder. During the initial stage of a fixed-end contraction, a skeletal fibre shortens primarily by lengthening its tendons. The shortening implies that each sarcomere generates an internal force larger than its external force and subsequently would shorten independently of other sarcomeres. This independent and uniform shortening would not lead to a large increase in sarcomere disorder. Light micrographs show that the striation pattern is clear during the early phase of shortening and becomes diffuse at the peak of shortening (Julian et al. 1978). Clarity of the striation pattern suggests little sarcomere disorder. Measurements here can provide a general trend of sarcomere disorder after the fibre has been stimulated. The theory indicates that at $S = 3.6$ μm there is no filament overlap and $R = K_a/K_r$, which decreases with sarcomere disorder. The R value of the least-squares fitted line (Fig. 7) at $S = 3.6$ μm decreases with time. Its small decrease at the beginning is consistent with the interpretation that there is little increase in sarcomere disorder the early phase of contraction. Furthermore, using the slope of the fitted line to determine δ/f or filament activation reduces the contribution from sarcomere disorder.

Debye-Waller formalism

Diffraction models which assume that there is only an increase in sarcomere disorder upon activation of the fibre are capable of explaining the decrease in diffraction intensity. Fujime (1975) proposed that the intensity drop was due to an increase in random fluctuations of the positions of thick filaments along the fibre axis. His proposal is analogous to the Debye-Waller factor in X-ray diffraction where the decrease in X-ray diffraction intensity is due to thermal vibrations or random fluctuations of the atoms. It was assumed that the amount of random fluctuations of the positions of A-bands along the longitudinal axis of the fibre did not necessarily alter the arrangement of sarcomeres as individual units. The arrangements of sarcomere units in the active and resting fibre of the same sarcomere length were taken to be the same.

Another diffraction model which assumes that the intermyofibrillar misalignment becomes more serious during muscular activity can also account for the intensity decrease (Yeh et al. 1980; Oba et al. 1981, 1983). Yeh et al. (1980) showed that the diffraction intensity is equal to $U \exp(-\sigma^2)$. The factor U is related to the structural factor and positions of the myofibrils, and σ is the standard deviation of the random misalignments. If the intensity decrease is solely due to this type of disordering, R would be equal to $\exp(\sigma_r^2 - \sigma_a^2)$. If $(\sigma_r^2 - \sigma_a^2)$ is much smaller than one, R would approximate $1 - (\sigma_a^2 - \sigma_r^2)$. Presumably, $(\sigma_a^2 - \sigma_r^2)$ is proportional to the active force and therefore proportional to $(3.6 - S)$. Then, $R = 1 -$ constant $(3.6 - S)$. This theoretical R agrees with the R measurements shown in Fig. 6. Rüdel and Zite-Ferenczy (1980) also used a disorder model similar to the one proposed by Yeh et al. (1980) to explain their intensity data. They applied the Debye-Waller formalism to the diffraction units and their R was equal to $\exp[2(M_r - M_a)]$, where M is the Debye-Waller factor. Basically, the disorder models proposed by Yeh et al. (1980) and Rüdel and Zite-Ferenczy (1980) are equivalent. It appears that a model which relates an excess disorder in the myofilaments (Fujime 1975), myofibrillar alignment (Yeh et al. 1980), or Bragg planes (Rüdel and Zite-Ferenczy 1980) to active force would explain the decrease in the diffraction intensity with sarcomere length. However, measurements show that intensity falls sharply before active force becomes appreciable. This early fall is probably related to filament activation. For the first 20 or 30 ms after fibre stimulation, filament interactions play an important role in causing the intensity to drop. Beyond this period, disorder in the diffraction units becomes increasingly more dominant. It may be concluded that the intensity decrease is primarily due to the activation and disorder of the diffraction units. These two processes, filament activation and sarcomere disorder have different time courses.

Previous measurements

There is abundant evidence demonstrating that diffraction intensity falls upon stimulation of the fibre

(Paolini and Roos 1975; Table 4, Baskin et al. 1979; Rüdel and Zite-Ferenczy 1980; Oba et al. 1981, 1983). However, the relation between the size of the intensity drop and sarcomere length appears to be uncertain. Kawai and Kuntz (1973) found that the intensity change peaked at a sarcomere length of 2.8 μm and approached zero at a sarcomere length of 2.2 μm or 3.6 μm. Similar conclusions were reached by Rüdel and Zite-Ferenczy (1980) in their study on the diffraction efficiency of active fibres. Paolini and Roos (1975) showed that the ratio between the intensities of the fibre under tetanic contraction and the resting fibre increases with sarcomere length. The ratio approaches unity around a sarcomere length of 3.1 μm. Baskin et al. (1979) observed that the intensity ratio was about 0.4 for a sarcomere length between 2.6 μm and 3.6 μm and approached unity at a sarcomere length of about 2.2 μm. More recently, Oba et al. (1981) measured the intensity ratio for intact and skinned single fibres from frog semitendinosus and concluded that the intensity ratio was 0.3 for sarcomere lengths below 3.6 μm and near unity for lengths near and above 3.6 μm. With the exception of the measurements presented by Baskin et al. (1979), all other measurements including those reported here show little or no intensity drop when the thick and thin filaments have small or zero overlap. The correspondence between the intensity ratio of active and resting fibre and the amount of filament overlap at long sarcomere lengths indicates that a component of the intensity drop is likely to be related to filament interactions.

Most reported intensity measurements on active fibres were taken at the plateau of isometric tetanus. The sarcomere array at the plateau is much more disordered than the resting array (Julian et al. 1978). Then, K_a/K_r would be less than unity and intensity would decrease. Many investigators have mentioned that disordering in the diffracting arrays is the major cause of the intensity decrease. Oba et al. (1981) made many measurements on intact and skinned single fibres during full tetanic activation and calculated the ratio of intensities of the active and resting fibre. Their intensity ratio data points are highly scattered, indicating the variability of the interference ratio, K_a/K_r. Furthermore, their intensity ratio is independent of sarcomere length from 2.2 μm to 3.6 μm. This independency suggests that the major cause for the intensity drop in fibres during tetanus is due to a large increase in sarcomere disorder, this disorder would decrease K_a/K_r and consequently R. When a system such as a multifibre preparation is sufficiently random, K_a would be similar to K_r. Then, R obtained from multifibres would illustrate the effects of filament interactions more clearly than that obtained from a single fibre. The R values calculated from the data reported by Paolini and Roos (1975) on the whole sartorius muscle of frog agree with the intensity ratio tabulated here (Fig. 5). It seems clear that the optical interference factor is a crucial component in determining the intensity ratio.

The measurements of Oba et al. (1981) set the lower limit for the intensity decrease in active fibres. During the plateau of tetanus both the filament activation and sarcomere disorder are strongest and both processes decrease the diffraction intensity. Therefore, the lower limit of the intensity ratio is about 0.2. All intensity ratios reported previously and in this work are larger than 0.2.

The present theory treats the contractile filament interaction as a scattering parameter. It appears to be able to account for a major portion of the diverse measurements reported on the puzzling sharp drop in diffraction intensity of fibres following activation. However, it can only be semi-quantitative because sarcomere disorder and the absolute polarizabilities of the contractile filaments in intact fibres have not been precisely determined. Under controlled situations, it can be very useful for investigating physiological events of active fibre.

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