Journal of Physiology (1988), **407**, *pp.* 231–241
With 5 text figures
Printed in Great Britain

TIME-RESOLVED X-RAY DIFFRACTION STUDIES ON THE EFFECT OF SLOW LENGTH CHANGES ON TETANIZED FROG SKELETAL MUSCLE

BY Y. AMEMIYA*, H. IWAMOTO†, T. KOBAYASHI†, H. SUGI†‡
H. TANAKA† AND K. WAKABAYASHI§

*From the * Photon Factory, National Laboratory for High Energy Physics, Tsukuba, Ibaraki, Japan 305, the † Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo, Japan 173 and the § Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka, Japan 560*

(*Received 1 March 1988*)

SUMMARY

1. The mechanism of the enhancement and the deficit of isometric force by slow length changes in frog skeletal muscle was studied with the time-resolved X-ray diffraction technique, using intense X-rays of synchrotron radiation.

2. When a tetanized muscle was slowly stretched by 4% from sarcomere lengths 2·3–2·4 μm, the force rose to a peak during stretch and then decreased to a steady level 10–15% higher than that immediately before stretch.

3. The intensity of the 1,1 equatorial reflection decreased nearly linearly during stretch and then again increased after the completion of stretch, reaching a steady level $12 \pm 5\%$ (mean $\pm$ S.D., $n = 11$) lower than that immediately before stretch. The above 1,1 intensity change was roughly a mirror image of the force change.

4. The intensity of the 1,0 equatorial reflection showed no marked changes in response to a slow stretch, except for an initial transient increase observed occasionally.

5. If a tetanized muscle was slowly released by 4% from sarcomere lengths 2·3–2·4 μm, the steady force attained after the completion of release was lower than that immediately before release.

6. The 1,1 intensity increased slightly during release, while the 1,0 intensity did not change significantly.

7. The half-width of both the 1,0 and the 1,1 reflections did not change appreciably in response to slow length changes.

8. Slow length changes always produced changes in the spacing between the reflections as expected from the constant-volume behaviour of the myofilament lattice.

9. These results indicate that a slow stretch produces disordering of the myofilament lattice in such a way that the thin filaments are displaced from trigonal positions in the thick filament lattice. The resulting increase in the overall repulsion

* Where this work was carried out.
‡ To whom correspondence should be addressed.

forces between the filaments may lead to the enhanced isometric force after stretch.

INTRODUCTION

In the preceding paper (Sugi & Tsuchiya, 1988), the force and the stiffness changes in response to slow length changes were studied on tetanized single frog skeletal muscle fibres to give information about the mechanism of the enhancement and the deficit of isometric force after slow length changes (Abbott & Aubert, 1952; Délèze, 1961; Edman, Elzinga & Noble, 1978; Maréchal & Plaghki, 1979). The most striking result obtained in the above work was that, despite a marked enhancement of isometric force after a slow stretch, the steady stiffness value attained after the stretch was definitely smaller than that before the stretch, reflecting a decrease in the myofilament overlap at the stretched fibre length. Since the stiffness of tetanized muscle is generally taken as a measure of the number of the cross-bridges attached to the thin filaments (Huxley & Simmons, 1973; Julian & Sollins, 1975), the above result suggests that the enhanced isometric force is associated either with an increased force exerted by each cross-bridge or with a recruitment of some force-bearing mechanism other than the cross-bridges. On the other hand, the deficit of isometric force after a slow release appeared to be associated with a decrease in the force-generating capacity of individual fibre segments irrespective of whether they shortened or remained isometric during the applied length changes.

The experiments to be described in this paper were undertaken to explore the mechanism of the enhancement and the deficit of isometric force in more detail by studying the effect of slow length changes on the force and the X-ray diffraction pattern of tetanized frog skeletal muscle by the time-resolved X-ray diffraction technique. By the use of intense X-rays of synchrotron radiation, it was possible to record the intensity changes of the equatorial reflections with a time resolution of 50 ms. The results suggest that the force enhancement after a slow stretch is associated with disordering of the myofilament lattice. Preliminary accounts of this work have already appeared (Sugi, Tanaka, Kobayashi & Amemiya, 1984; Tanaka, Kobayashi, Amemiya, Wakabayashi, Iwamoto & Sugi, 1986).

METHODS

Preparation

The sartorius muscle isolated from the bull-frog (*Rana catesbeiana*) was mounted vertically in an experimental chamber with two Mylar windows to pass X-rays through the middle of the muscle. The pelvic end of the muscle was clamped to a steel rod extending from a force transducer (type UT, Shinkoh Co.; compliance, 1 μm/g; natural frequency of oscillation, 150 Hz), while the tibial end was connected to an electromagnetic vibrator (type 200, Ling Dynamic Systems Ltd) with a jeweller's chain. Muscles were continuously perfused with oxygenated Ringer solution (115 mM-NaCl, 2·5 mM-KCl, 1·8 mM-$CaCl_2$, pH adjusted to 7·2 by $NaHCO_3$), the temperature of the solution being kept at $5 \pm 0{\cdot}1$ °C with a thermo-electric device (Coolnix, Yamato Kagaku Co.). The initial sarcomere length of the muscle was adjusted to 2·3–2·4 μm by optical diffraction of He–Ne laser light. The chamber contained a multielectrode assembly consisting of eight platinum wires connected as alternate anodes and cathodes.

Time-resolved X-ray diffraction technique

A focused and monochromatized X-ray beam of wavelength 0·155 nm was produced by a camera designed by Amemiya, Wakabayashi, Hamanaka, Wakabayashi, Matsushita & Hashizume (1983)

from the synchrotron radiation of the storage ring operated at 2·5 GeV and 65–105 mA. The camera consisted of seven 20 cm long segments of totally reflecting curved silica mirror and a bent triangular-shaped silicon crystal monochromator cut at 7·8 deg to the 111 planes (Wakabayashi, Hamanaka, Amemiya, Tanaka, Wakabayashi & Hashizume, 1983). The specimen-to-detector distance was 240 cm, and the beam sizes at the specimen and at the detector plane were 2×5 and $1{\cdot}3 \times 2{\cdot}6$ mm (vertical × horizontal) respectively. The intensity distribution of the X-rays scattered perpendicular to the muscle long axis, i.e. the equatorial diffraction pattern was recorded by a 20 cm long, linear position-sensitive detector using a 400 ns internal delay line (Rigaku Denki Co.) and an encoding digitizer (type 4202, LeCroy Research Systems) for position read-out. The signals of one-dimensional patterns were stored as a function of time in a CAMAC memory (type 3588, LeCroy Research Systems) linked to a computer (Micro-11/F23, Automation System Research). Unless otherwise stated, the memory was divided into 100 time segments of 256 channels, each corresponding to a successive 50 ms time period of data collection.

General procedure

The muscle was tetanized with a 2·5–4 s train of 1 ms supramaximal rectangular current pulses at 20 Hz. The muscle was first kept isometric, and when the full isometric force was developed, slow constant-velocity stretches or releases (4% of the initial muscle length at 0·08–0·32 length/s) were applied with the vibrator, the movement of which was sensed by a light beam–photodiode system and controlled with a feed-back circuit. With the initial sarcomere length of 2·3–2·4 μm, the development of resting force by a 4% stretch only amounted to less than 2–3% of the maximum tetanic force (2·3–2·8 kg/cm^2). The length and force changes were observed with a storage oscilloscope (type 5110, Tektronix Inc.), and also stored in the CAMAC memory together with the X-ray data. Each muscle was tetanized 20 times at intervals of 30 s, and the fall in tetanic force between the first and the last tetani was less than 20–25%. Data over twenty tetani for each muscle were accumulated in the relevant memory segments to obtain reasonable photon statistics in the diffraction pattern. In some cases, such data from two to five different muscles were added and averaged.

Data analysis

The integrated intensities of the 1,0 and 1,1 equatorial reflections for each of the 100 time segments were obtained by measuring the area under the peaks of a diffraction pattern (see Fig. 2). The background level under each peak was drawn by a second-order polynomial fitting; the data points out of any reflection slopes were smoothed by calculating the 1, 2, 1 running average (Podolsky, Onge, Yu & Lymn, 1976). The intensity of these reflections was taken as the mean value of the intensities on the left and right sides of the diffraction pattern.

RESULTS

Effects of slow length changes on the equatorial reflections of tetanized muscle

Intensity changes of the equatorial reflections during the enhancement of isometric force by slow stretches. Figure 1 shows the effect of a slow stretch (4% of the initial muscle length at 0·16 length/s) applied to a tetanized muscle (initial sarcomere length, 2·3 μm) on the force and on the intensity and the half-width of the 1,0 and 1,1 equatorial reflections. The initial isometric force development was associated with a decrease in the 1,0 intensity and an increase in the 1,1 intensity as reported by many authors (Huxley & Brown, 1967; Elliott, Lowy & Millman, 1967; Haselgrove & Huxley, 1973; Amemiya, Sugi & Hashizume, 1979), and both intensities reached a steady level during the plateau of isometric tetanic force. In response to the subsequent application of slow stretch, the force rose to a peak during stretch and then started to decay after the completion of stretch, approaching a steady level higher than the steady force immediately before stretch. The above increase in isometric force by a slow stretch despite a decrease in the myofilament overlap is

known as the force enhancement by stretch (Abbott & Aubert, 1952; Délèze, 1961; Edman *et al.* 1978; Sugi & Tsuchiya, 1988). The 1,1 intensity was found to decrease nearly linearly during stretch and then started to increase again after the completion of stretch, reaching a steady level lower than that immediately before stretch.

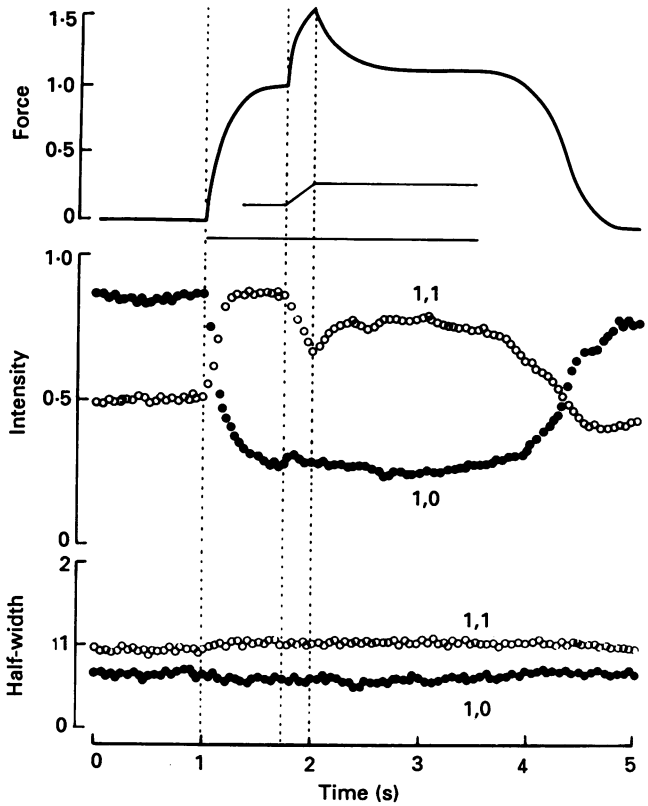

Fig. 1. Effect of a slow stretch (4 % of the initial length at 0·16 length/s) on the force and on the intensity and the half-width of the 1,0 (●) and the 1,1 (○) equatorial reflections. The muscle was tetanized for 2·5 s at 2·3 μm sarcomere length. In this and subsequent figures, the force is expressed as fraction or multiple of the steady isometric tetanic force, and the intensity and the half-width of the equatorial reflections are expressed in arbitrary units. Each plot represents an average of twenty tetani. Time course of stretch and the period of stimulation are indicated under the force trace.

Examples of equatorial X-ray diffraction patterns from a tetanized muscle before and during a slow stretch (4 % at 0·08 length/s) are presented in Fig. 2. As shown in Figs 1 and 2, the half-width of the 1,1 reflection did not change appreciably during the changes in the 1,1 intensity in response to the applied stretch. The above characteristic decrease in the 1,1 intensity followed by a partial recovery was roughly a mirror image of the force changes during and after stretch. On the other hand, the 1,0 reflection exhibited no significant changes in response to stretch, except that it occasionally showed a small transient increase in the intensity at the beginning of stretch.

Similar results were obtained on ten other muscles examined. The decrease in the steady 1,1 intensity level after 4% stretches in a tetanized muscle was $12 \pm 5\%$ (mean $\pm$ S.D., $n = 11$), while the increase in the steady isometric force after 4% stretches ranged from 10 to 15%. These values appeared to be independent of the stretch velocity at least in the range of velocities used (0·08–0·32 length/s).

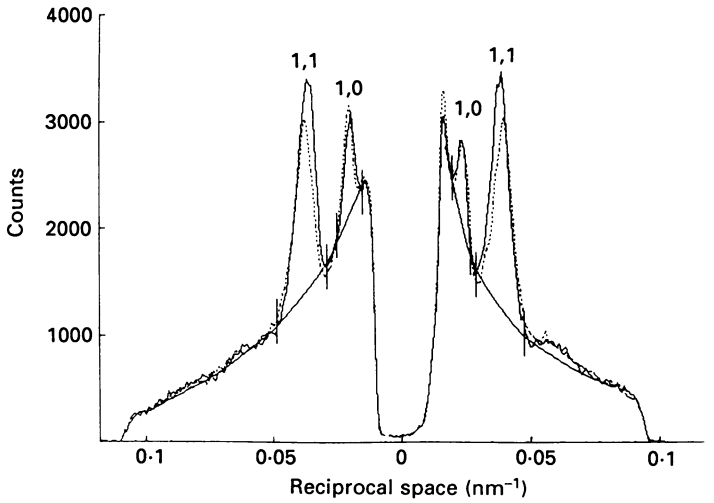

Fig. 2. Equatorial X-ray diffraction patterns of a tetanized bull-frog sartorius muscle before (continuous line) and during (dashed line) a slow stretch (4% of the initial length at 0·16 length/s). The former pattern was recorded at the plateau of isometric tetanus (within 0·15 s before the beginning of stretch), while the latter pattern was recorded during stretch (within 0·15 s before the completion of stretch). Each pattern is an average of twenty tetani. The stretch was complete in 0·25 s. Vertical lines crossing the background level indicate the margin of the 1,0 and 1,1 reflections.

Intensity changes of the equatorial reflections during the deficit of isometric force by slow releases. Figure 3 shows the effect of a slow release (4% at 0·16 length/s) applied to a tetanized muscle (initial sarcomere length, 2·4 μm) on the force and on the intensity and the half-width of the 1,0 and the 1,1 reflections. The force decreased during release and started to increase after the completion of release to reach a steady level lower than that immediately before release. The above decrease in isometric force by a slow release despite an increase in the myofilament overlap is known as the force deficit by release (Abbott & Aubert, 1952; Maréchal & Plaghki, 1979; Sugi & Tsuchiya, 1988). The 1,1 intensity was observed to increase slightly during release, though the increase was just barely perceptible in some cases. After the completion of release, the 1,1 intensity tended to decrease with time to make it difficult to determine the steady 1,1 intensity level. No significant change in half-width of the 1,1 intensity was seen during the above changes in the 1,1 intensity. As with slow stretches, the 1,0 reflection showed no appreciable changes in response to slow releases, except that it tended to increase after the completion of release. Similar results were obtained on seven other muscles.

Intensity changes of the equatorial reflections during isometric tetanus at different

muscle lengths. Since the amount of overlap between the myofilaments changes as a result of slow length changes, the effect of changing the initial sarcomere length on the steady level of the 1,0 and the 1,1 intensities during an ordinary isometric tetanus was examined by tetanizing a muscle isometrically at two different lengths, one at 2·3 μm sarcomere length and the other at 2·4 μm sarcomere length. The muscle was

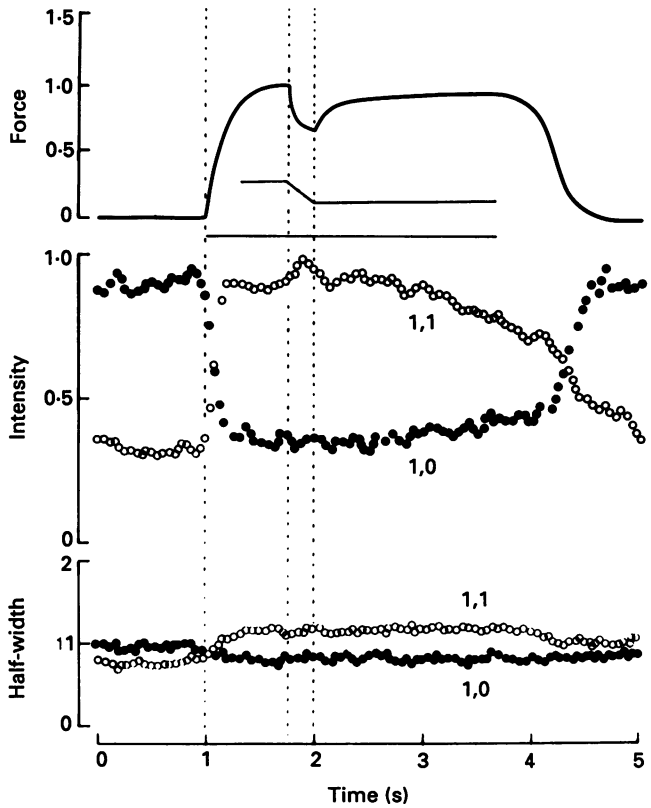

Fig. 3. Effect of a slow release (4% of the initial length at 0·16 length/s) on the force and on the intensity and the half-width of the 1,0 (●) and the 1,1 (○) equatorial reflections. The muscle was tetanized for 2·5 s at 2·4 μm sarcomere length. Each plot is an average of twenty tetani. Time course of release and the period of stimulation are indicated under the force trace.

tetanized 5 times at each length, and the intensity changes of the 1,0 and the 1,1 reflections were recorded with a time resolution of 200 ms. As shown in Fig. 4, the steady level of both the 1,0 and the 1,1 intensities during an isometric tetanus did not differ appreciably between the two different muscle lengths. This may be compatible with the reports that the intensity ratio of the 1,0 and the 1,1 reflections changes only slightly with small changes of the sarcomere length (Podolsky *et al.* 1976; Amemiya *et al.* 1979). Similar results were obtained on two other muscles examined. These results may be taken to indicate that the intensity changes of the equatorial reflections in response to slow length changes cannot be simply explained as being due to sarcomere length changes.

Changes in the interfilament spacing during slow length changes. As can be seen in Fig. 2, the position of both the 1,0 and the 1,1 reflections changed as a result of the applied muscle length change, indicating the change in the interfilament spacing. Slow stretches always produced an increase in the spacing between the reflections, i.e. a decrease in the interfilament spacing, whereas slow releases always caused a

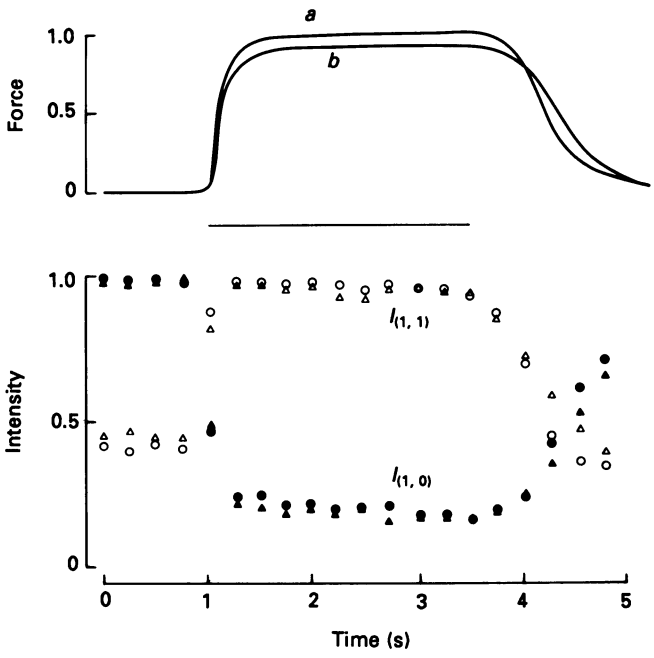

Fig. 4. Effect of changing the initial sarcomere length on the intensity (I) changes of the 1,0 and the 1,1 reflections during the ordinary isometric tetanus at 2·3 μm (△, ▲) and at 2·4 μm (○, ●) sarcomere length. The muscle was tetanized for 2·5 s. Each plot represents an average of five tetani. Force records at 2·3 μm (*a*) and 2·4 μm (*b*) sarcomere lengths are shown in the upper part.

decrease in the spacing between the reflections, i.e. an increase in the interfilament spacing. These results are consistent with the fact that the myofilament lattice volume remains constant when the sarcomere length in living relaxed muscle or actively contracting muscle is varied (Huxley, 1953; Elliott, Lowy & Worthington, 1963; Amemiya *et al.* 1979).

DISCUSSION

Changes in the myofilament lattice caused by slow length changes

The equatorial X-ray diffraction pattern of frog skeletal muscle arises from the hexagonal lattice of the thick and thin filaments, and has been studied intensively to obtain information about the behaviour of the cross-bridges during contraction. During isometric force development, the 1,0 intensity decreases while the 1,1 intensity increases (Figs 1 and 3, Elliott *et al.* 1967; Huxley & Brown, 1967; Haselgrove & Huxley, 1973; Amemiya *et al.* 1979). As the 1,0 plane consists only of the thick filaments while the 1,1 plane consists of both the thick and thin filaments

in a ratio of 1:2, the above 1,0 and 1,1 intensity changes have been interpreted as being due to the movement of X-ray scattering mass, i.e. the cross-bridges, from the vicinity of the thick filaments to the vicinity of the thin filaments (Huxley, 1968).

The present experiments have shown that, in response to a slow stretch applied to an isometrically tetanized muscle, the 1,1 intensity showed a marked decrease which was followed by a partial recovery, whereas the 1,0 intensity did not change

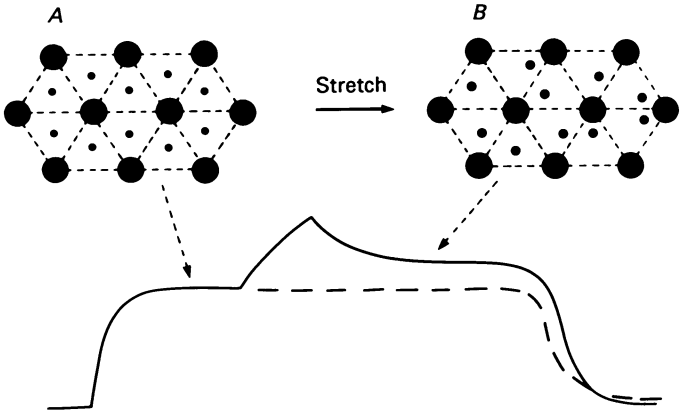

Fig. 5. Diagram showing the disordering of the myofilament lattice by stretch as a possible cause of the enhancement of isometric force after stretch.

appreciably except for an initial small transient increase in the intensity observed occasionally (Fig. 1). Since the marked intensity changes took place in the 1,1 intensity but not in the 1,0 intensity, the above effect of slow stretch cannot be explained as being due to the movement of mass between the thick and thin filaments or to the change in number of the cross-bridges attached to the thin filaments. A characteristic feature of the 1,1 intensity change was that the half-width of the 1,1 reflection did not change significantly during and after the applied stretch (Figs 1 and 2).

A most plausible explanation for the 1,1 intensity changes based on the present results is that a slow stretch applied to a tetanized muscle causes disordering of the 1,1 myofilament lattice without significant changes in the mean interfilament spacing as shown diagrammatically in Fig. 5. Before stretch, the hexagonal myofilament lattice is well ordered so that the thin filaments are located in trigonal positions in the hexagonally packed thick filaments (*A*). When the muscle is stretched, the thin filaments are displaced from their initial position without significant change in their overall mean position in the thick filament lattice (*B*). The above stretch-induced disordering of the myofilament lattice persists for the rest of the tetanus, as indicated by the low 1,1 intensity level attained after the completion of stretch (Fig. 1).

The effects of slow releases on the equatorial reflections, on the other hand, were much less marked than those of slow stretches, though the 1,1 intensity occasionally increased slightly in response to the applied release (Fig. 3), suggesting that the position of the thin filaments in the thick filament lattice tends to be more ordered.

At present, it is difficult to correlate this result with the decrease in the force-generating capacity of the contractile system suggested in the preceding paper (Sugi & Tsuchiya, 1988).

Possible involvement of the disordering of the myofilament lattice in the force enhancement by stretch

Matsuda & Podolsky (1986) showed that the 1,1 intensity of relaxed skinned rabbit psoas fibres decreased markedly at pH below 5·5, while the 1,0 intensity remained constant, indicating that the thick filament lattice is more stable than the thin filament lattice, in agreement with our interpretation of the present results (Fig. 5). Their results also indicate that the thin filaments are positioned within the thick filament lattice by electrostatic repulsion forces between the filaments. Since the electrostatic force is inversely proportional to the square of the interfilament distance, the stretch-induced displacement of the thin filaments from the trigonal position in the thick filament lattice (Fig. 5) should result in an increase in the overall repulsion forces within the whole myofilament lattice. As mentioned previously, the myofilament lattice in a living muscle behaves as a constant-volume system. In the constant-volume myofilament lattice system, the increase in the overall repulsion forces between the filaments would result not only in an increase in the force required to get them closer together (i.e. increased resistance to stretch), but also in an increase in the force required just to maintain them in position (i.e. increased isometric force). Thus, the increased lateral repulsion forces in the myofilament lattice would be converted into an increment of steady isometric force, showing up as the enhancement of isometric force after stretch. As discussed in the preceding paper (Sugi & Tsuchiya, 1988), the above idea that the stretch-induced disordering of the myofilament lattice causes the force enhancement after stretch can also explain the enhancement of isometric force that occurs with decreased stiffness (Sugi & Tsuchiya, 1988).

Edman (1979) showed that the velocity of unloaded shortening in tetanized frog muscle fibres increased more than twofold at sarcomere lengths where the resting force amounted to about 7% of the maximum tetanic force, indicating a marked effect of prestretched parallel elastic component in accelerating the shortening velocity. When the tetanized fibres were allowed to shorten under zero load after they had been stretched or lengthened isotonically, the shortening velocity did not differ significantly from that when they were allowed to shorten during an isometric tetanus (Edman *et al.* 1978; Sugi & Tsuchiya, 1981). This implies that the increased lateral repulsion forces in the myofilament lattice may tend to restore the thin filament positions but tend not to cause an expansion of the myofilament lattice, which leads to an active force development or active shortening when coupled with the constant lattice volume mechanism. On this basis, the stretch-induced increment of isometric force is passively maintained, and in a sense resembles the catch state taking place in molluscan smooth muscle.

The isovolumic behaviour of the myofilament lattice no longer exists after mechanical or chemical skinning of muscle fibres (e.g. Matsubara & Elliott, 1972). Tanaka, Tanaka & Sugi (1979) measured the rate of ATP splitting in glycerinated muscle fibres, and found that, over a wide range of sarcomere lengths studied, the

rate of ATP splitting in Ca^{2+}-activated fibres was higher after slow stretch than before stretch, unlike living muscle fibres which show a markedly reduced ATP splitting rate after stretch (Curtin & Davies, 1973). This seems to indicate that the mechanism of force enhancement by stretch in living fibres is different from that in skinned or glycerinated fibres.

As to the cause of the disordering of the myofilament lattice responsible for the enhancement of isometric force after stretch, it may be that the strain on the thin filaments produces non-uniformity of charge distribution along the thin filaments, and this in turn disturbs the electrostatic equilibrium of the regular array of the myofilaments. Matsubara & Yagi (1985) observed that the intensity of the 14·3 nm meridional reflection of a tetanized muscle decreases by a slow stretch, suggesting that the myosin heads spread out along the thick filaments. This might be attributed, at least in part, to the disordering of the 1,1 lattice, since the heads attached to the thin filaments would spread out in various directions as the thin filaments go out of the trigonal positions in the thick filament lattice.

REFERENCES

ABBOTT, B. C. & AUBERT, X. M. (1952). The force exerted by active striated muscle during and after change of length. *Journal of Physiology* **117**, 77–86.

AMEMIYA, Y., SUGI, H. & HASHIZUME, H. (1979). X-ray diffraction studies on the dynamic properties of cross-bridges in skeletal muscle. In *Cross-bridge Mechanism in Muscle Contraction*, ed. SUGI, H. & POLLACK, G. H., pp. 425–433. Baltimore: University Park Press and Tokyo: University of Tokyo Press.

AMEMIYA, Y., WAKABAYASHI, K., HAMANAKA, T., WAKABAYASHI, T., MATSUSHITA, T. & HASHIZUME, H. (1983). Design of a small-angle X-ray diffractometer using synchrotron radiation at the Photon Factory. *Nuclear Instruments and Methods* **208**, 471–477.

CURTIN, N. A. & DAVIES, R. E. (1973). Chemical and mechanical changes during stretching of activated frog skeletal muscle. *Cold Spring Harbor Symposia on Quantitative Biology* **37**, 619–626.

DÉLÈZE, J. B. (1961). The mechanical properties of the semitendinosus muscle at lengths greater than its length in the body. *Journal of Physiology* **158**, 154–164.

EDMAN, K. A. P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *Journal of Physiology* **291**, 143–159.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *Journal of Physiology* **281**, 139–155.

ELLIOTT, G. F., LOWY, J. & MILLMAN, B. M. (1967). Low angle X-ray diffraction studies of living striated muscle during contraction. *Journal of Molecular Biology* **25**, 31–42.

ELLIOTT, G. F., LOWY, J. & WORTHINGTON, C. R. (1963). An X-ray and light diffraction study of the filament lattice of striated muscle in the living state and in rigor. *Journal of Molecular Biology* **6**, 295–305.

HASELGROVE, J. C. & HUXLEY, H. E. (1973). X-ray evidence for radial cross-bridge movement and for the sliding filament model in actively contracting skeletal muscle. *Journal of Molecular Biology* **77**, 549–568.

HUXLEY, A. F. & SIMMONS, R. M. (1973). Mechanical transients and the origin of muscular force. *Cold Spring Harbor Symposia on Quantitative Biology* **37**, 669–680.

HUXLEY, H. E. (1953). X-ray analysis and the problem of muscle. *Proceedings of the Royal Society* B **141**, 59–62.

HUXLEY, H. E. (1968). Structural difference between resting and rigor muscle: evidence from intensity changes in the low-angle equatorial X-ray diagram. *Journal of Molecular Biology* **37**, 507–520.

HUXLEY, H. E. & BROWN, W. (1967). The low-angle X-ray diagram of vertebrate striated muscle and its behaviour during contraction and rigor. *Journal of Molecular Biology* **30**, 384–434.

JULIAN, F. J. & SOLLINS, M. R. (1975). Variation of muscle stiffness with force at increasing speeds of shortening. *Journal of General Physiology* **66**, 287–302.

MARÉCHAL, G. & PLAGHKI, L. (1979). The deficit of the isometric tetanic tension redeveloped after a release of frog muscle at a constant velocity. *Journal of General Physiology* **73**, 453–467.

MATSUBARA, I. & ELLIOTT, G. F. (1972). X-ray diffraction studies on skinned single fibres of frog skeletal muscle. *Journal of Molecular Biology* **72**, 657–669.

MATSUBARA, I. & YAGI, N. (1985). Movements of cross-bridges during and after slow length changes in active frog skeletal muscle. *Journal of Physiology* **361**, 151–163.

MATSUDA, T. & PODOLSKY, R. J. (1986). Ordering of the myofilament lattice in muscle fibres. *Journal of Molecular Biology* **189**, 361–365.

PODOLSKY, R. J., ONGE, R. ST, YU, L. & LYMN, R. W. (1976). X-ray diffraction of actively shortening muscle. *Proceedings of the National Academy of Sciences of the U.S.A.* **73**, 813–817.

SUGI, H., TANAKA, H., KOBAYASHI, T. & AMEMIYA, Y. (1984). Changes in the equatorial X-ray diffraction pattern from tetanized frog skeletal muscle during and after a slow stretch. *8th International Biophysics Congress*, p. 207. Petersfield: Meon Conference Services.

SUGI, H. & TSUCHIYA, T. (1981). Enhancement of mechanical performance in frog muscle fibres after quick increases in load. *Journal of Physiology* **319**, 239–252.

SUGI, H. & TSUCHIYA, T. (1988). Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. *Journal of Physiology* **407**, 215–229.

TANAKA, H., KOBAYASHI, T., AMEMIYA, Y., WAKABAYASHI, K., IWAMOTO, H. & SUGI, H. (1986). Time-resolved X-ray diffraction studies of tetanized frog skeletal muscle during and after slow length changes using synchrotron radiation. *Journal of Muscle Research and Cell Motility* **7**, 278.

TANAKA, H., TANAKA, M. & SUGI, H. (1979). The effect of sarcomere length and stretching on the rate of ATP splitting in glycerinated rabbit psoas muscle fibers. *Journal of Biochemistry* **86**, 1587–1593.

WAKABAYASHI, K., HAMANAKA, T., AMEMIYA, Y., TANAKA, H., WAKABAYASHI, T. & HASHIZUME, H. (1983). Performance of the muscle diffractometer. In *Photon Factory Activity Report 1982/1983*. Tsukuba V188–90, Tsukuba: National Laboratory for High Energy Physics.