Properties and Distribution of Fast Twitch and Multiinnervated Slow Fibers in Glycerol-Extracted Extraocular Muscles of the Cat Examined by the Laser Diffraction Method

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ABSTRACT. Mammalian extraocular muscles contain multiinnervated slow fibers. To investigate the properties of these slow fibers, laser diffraction patterns were examined at rest and during contraction. Kittens were anesthetized and the retractor bulbi (RB), the inferior oblique (IO), and the lateral rectus (LR) were isolated from the orbita, and they were treated by 50% glycerol at −20℃ for 4-14 days. These muscle fibers were illuminated by a polarized He-Ne laser beam (632.8 nm) and the intensity of the diffracted light was monitored by moving a small photodiode in the direction perpendicular to the diffraction line. The diffraction patterns of a single fiber prepared from the RB exhibited spatially distinct distribution. The diffraction patterns of the LR and IO, however, were not as sharp as those of the RB. The ratio of peak amplitude to the width at 50% peak of the first order diffraction pattern was taken as an index to describe the sharpness quantitatively. It was observed that most of the muscle fibers from the RB showed high peak/width ratio values, whereas many fibers from the IO and LR exhibted low peak/width ratio values. The muscle fibers with high values were possibly due to fast fibers and those with low values to multiinnervated slow fibers. Based on these results, it was estimated that the slow fibers occupied 30-41% in the IO, 0-11% in the global layer and 36-47% in the orbital layer of the LR. In regard to the contraction, the total light intensity of one side of the first order line decreased remarkably during contraction. This came from structural changes in myosin filaments. It is expected that the decrease in the light intensity during contraction would be different for the two types of muscle fibers.

Key words: extraocular muscles — multiinnervated slow fibers — fast twitch fibers — laser diffraction

It is well known that the mammalian extraocular muscles contain multiinnervated slow fibers[1-5] and their electrical,[3,4,6] mechanical,[7-11] and histological properties[2,12,13] have been extensively investigated. Compared with fast twitch fibers,[12-14] these fibers have sarcomeres of non-uniform length, thick and winding Z-lines, and irregular H-zones. Because of the regular arrangements of A and I bands, striated muscle fibers show diffraction patterns when illuminated by monochromatic light. If the gratings of the slow fibers are

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irregularly arranged, then it is expected that the diffraction patterns of slow fibers should be more dispersed or obscure than those of fast fibers. The aim of the present work was to study the light diffraction patterns of both focally innervated fast twitch fibers and multiinnervated slow fibers of the extraocular muscles and to evaluate their population and distribution. Secondly, changes in the diffraction patterns during the contraction of fast twitch fibers were investigated. Some of the results have been presented previously.[15]

METHODS

Kittens were anesthetized with an intraperitoneal injection of sodium pentobarbiturate, and the eyeballs were removed from their orbits together with the extraocular muscles. These were immersed in an oxygenated Tyrode solution, and the retractor bulbi (RB), inferior oblique (IO), and lateral rectus (LR) were separated from the sclera. The LR was divided into two halves, global and orbital layers. These muscle preparations were further divided into 1 mm thick bundles and then were immersed in a solution containing 145 mM KCl and 6 mM HEPES buffer of pH=7.4. After contracture had ceased, the muscle bundles were tied to glass rods with a silk thread and transferred into 50% glycerol containing 4 mM EGTA. They were preserved in a refrigerator at −20℃ for 4–14 days. The iliopsoas muscle (IP) was prepared to compare eye muscles with body skeletal muscle. A single fiber was prepared from the glycerol treated fibers at room temperatures (21–24℃) in a relaxing solution containing 145 mM KCl, 1 mM $MgCl_2$, 4 mM ATP, and 4 mM EGTA buffered with 10 mM imidazol at pH=7. 0. One end of the fiber was tied to a hook and the other end to a capacitance tension transducer (Model 300, Cambridge Technology, Mass.). The transducer was mounted on a manipulator, so that the sarcomere length could be changed precisely in a range between 2.3 and 4.0 μm. The chamber solution was rapidly exchanged from a relaxing solution to a contraction solution containing 1.0–6.0 mM $CaCl_2$ in addition to the contents in the relaxing solution. The values of pCa were adopted from the calculation of Julian.[16]

The muscle chamber (0.5×0.5×5 cm) was placed on the stage of the microscope and illuminated from the bottom by a polarized He-Ne laser beam (GLG 5350, NEC, Tokyo). The diffracted light was projected to the translucent panel (Elmo, Tokyo) placed above the eye piece lens of the microscope. Thus, the diffraction patterns was monitored on the panel and photographed on 35 m/m film. The light intensity was scanned by moving a small photodiode (0.2 mm×2 mm) in the direction perpendicular to the diffraction line at a height just below the screen, as has been described in our previous study.[17] The sarcomere length was calibrated by control gratings of 1/300, 1/500 and 1/600 mm and was calculated from the equation,

$$s = \frac{\lambda}{\sin\left(\tan^{-1}\frac{x}{y}\right)}$$

where s was the sarcomere length, λ was the wave length of 0.633 μm, x was the distance between the zeroth and the first order line, and y was the distance between the muscle fiber and the photodiode.

RESULTS

1. Diffraction patterns of different muscles

The diffraction patterns of a single fiber prepared from the relaxed IP, RB, and LR were compared. Fig. 1 shows photographic pictures of the diffraction patterns of these muscles projected on a translucent panel and the spatial distribution of the light intensity. The sarcomere length in these fibers was 2.9 -3.1 μm. Only the fibers that exhibited a symmetrical profile and uniformity of the sarcomere length were selected. The diffraction pattern of the IP was most clearly observed, while that of the LR was less sharp than those of the IP and RB.

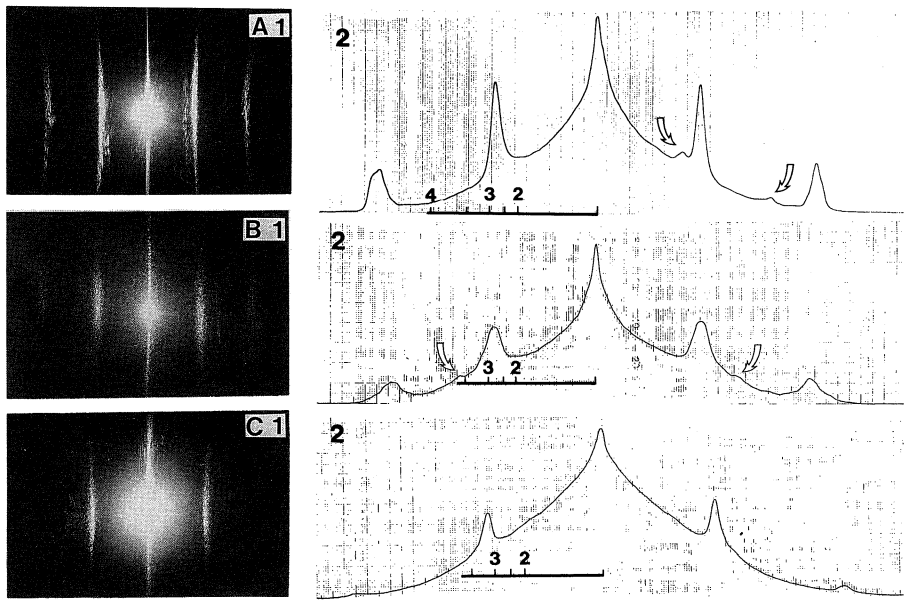

Fig. 1. Photographic pictures and light intensity distribution of the diffraction patterns in relaxing solution obtained from a single fiber of the iliopsoas (A), the retractor bulbi (B) and the global layer of the lateral rectus (C). The light intensity was measured by moving the photodiode along the meridian plane just next to the directly illuminated midpoint. Arrows in A2 and B2 indicate the extra diffraction lines.

Fig. 2 shows one example of the intensity distribution of the diffracted light obtained from a thick fiber in the global layers of the LR, and one from a thin fiber in the orbital layer. The pattern of the thin fiber as compared with thick ones was obscure, irregular and nonuniform along the longitudinal axis of the fiber. Some IO fibers also showed very obscure patterns. As can be seen in Figs. 1 and 2, the first order diffraction patterns of the thick fibers in the global layer were not exactly symmetrical. Moreover, there were extra diffraction lines between the zeroth and first and between the first and second order lines. Certainly, the extra lines were either intensified or attenuated during muscle stretch or rotation. However, they were not investigated

further in the present experiment.

The ratio of the peak amplitude to the width at 50% peak was taken as an index for describing the sharpness of the first order diffraction pattern quantitatively, as illustrated in Fig. 2A. Fig. 3 shows a frequency histogram of the peak/width ratios of the fibers prepared from the IP, RB, IO and LR. In the LR, the measurements were performed on the global and orbital layers separately. Although the classification of the peak/width ratio in Fig. 3 was quite arbitrary, the results indicated that the fibers showing a ratio value below 0.6 were only 4% of the fibers examined in the IP, 15% in the RB, and as much as 40% in the IO. Fibers with a ratio value of more than 2.0 made up 1/3 of the RB but only 4% in IO and are absent in the orbital layers of the LR. The amount of the fibers showing high ratio lay in the order of IP>RB>LR>IO and the global layer > orbital layer in the LR.

Some thin fibers showed clear patterns, while some thick fibers showed obscure ones. Both may be of the intermediate type between fast and slow fibers.

2. Diffraction patterns at different sarcomere lengths

At sarcomere lengths below 2.3 μm, the diffraction patterns of relaxed fast twich fibers were rugged or multipeaked. They were singly-peaked and uniform along the longitudinal axis if stretching and releasing of the fiber was repeated in a range longer than 2.5 μm. The peak/width ratio of the thick fibers from

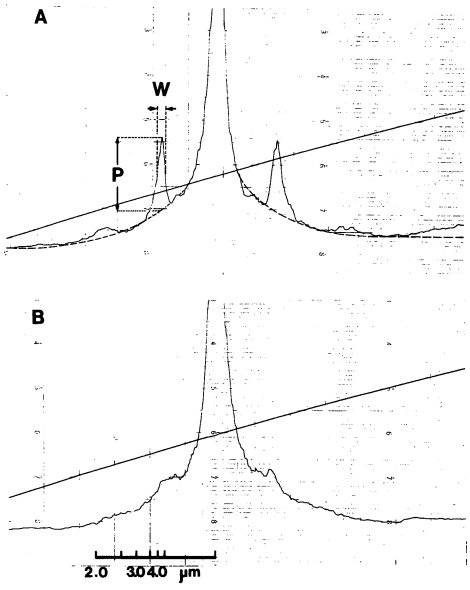

Fig. 2. Light intensity distribution of the diffraction pattern in relaxing solution obtained from one fiber in the global layer (A) and another one in the orbital layer (B) of the same lateral rectus. The broken line in A was drawn by assuming that the base line was the expotential. The amplitude (P) was measured from the base line to the peak of the diffracted line, and the width (W) was measured at the 50% peak. The oblique straight lines in A and B indicate the tracks of the movement of the photodiode.

LR thus measured after trials of stretching and releasing remained nearly constant until the sarcomere length exceeded 2.9 μm, but decreased to 60–80% during further stretching beyond a sarcomere length of 3.3 μm.

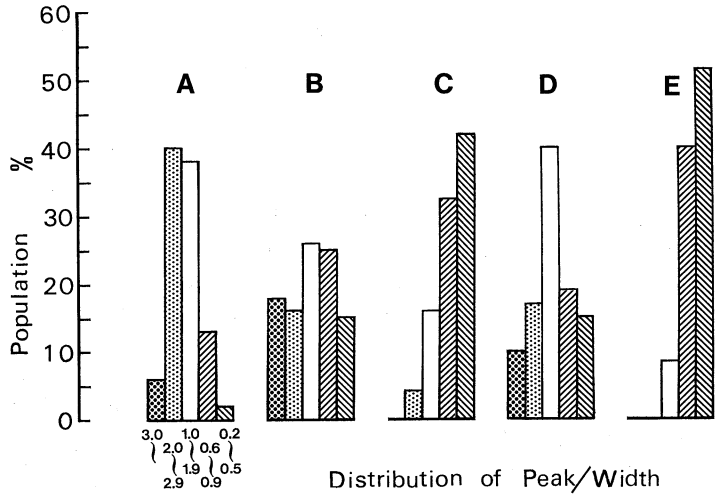

Fig. 3. Histograms showing the frequency distribution of muscle fibers with different peak/width ratio. Frequency was described as % of the total. A: the iliopsoas (n=48), B: the retractor bulbi (n=95), C: the inferior oblique (n=106), D: the global layer (n=81), E: the orbital layer (n=47) of the lateral rectus. Measurements were done on the single muscle fibers prepared from five cats in relaxing solution at sarcomere lengths of 2.6–3.0 μm at a room temperature of 21–24°C.

3. Changes in diffraction patterns during contraction and relaxation

The glycerol extracted fibers contracted in a solution of pCa < 6.0 if the period for the extraction was within three days. Fig. 4 shows the isometric tension and diffraction patterns during the maximum contraction of the RB produced by a solution of pCa=4.5. The diffraction pattern became dispersed at the intial phase of a tension rise and disappeared before the tension attained

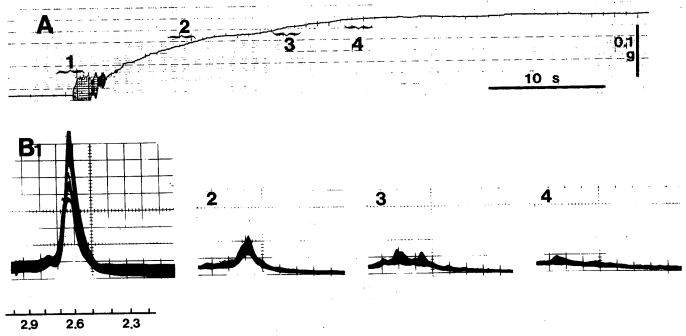

Fig. 4. Diffraction patterns during contraction at a pCa of 4.5 in one fiber of the retractor bulbi. A: 10 ml of the contraction solution was flushed into the muscle chamber of 1.3 ml at the time noted by 1. B: The superimposed trace of diffraction patterns. Records B1 through B4 were obtained at the contraction states noted by 1, 2, 3 and 4 in the tension record A. Calibrations of the sarcomere length in B2, B3 and B4 are the same as in B1 (μm).

the maximal level. The total light intensity of one side of the first order line, which is represented by the area under the diffraction line, also decreased remarkably during contraction. Another finding was that even though the muscle fiber was kept in the isometric condition ; the sarcomere shortened from 2.63 to 2.58 μm. The change in the diffraction pattern was reversible, and a clear pattern appeared again a few minutes after the fiber was relaxed by perfusing the chamber with a relaxing solution of pCa $>$ 7.0.

DISCUSSION

The present study was carried out to compare the diffraction patterns of slow fibers with those of fast twitch fibers. A similar attempt to study how the diffraction patterns are reflected by the fine structure was made by Burton and Baskin,[18] using the crustacean muscle. The intact extraocular muscles contain a large numbers of mitochondriae and glycogen granules,[12] which disturb light diffraction. In addition, obtaining a single fiber is difficult because of the abundant connective tissues. To overcome these problems, glycerol-extracted muscles were used. It was observed in this study that most of the muscle fibers from the IP, RB and the global layer of the LR showed high peak/width ratio values, whereas many fibers from the IO and the orbital layer of the LR exhibited dispersed patterns. Peachey[12] pointed out that slow fibers had thick and winding Z-lines, irregular A-I junctions and nonuniform sarcomere length. These structural properties are altogether the factors that make the diffraction patterns dispersed. Therefore, the fibers with high peak/width ratio values are considered to be fast fibers and those with low peak/width ratio values be slow fibers.

There is doubt, however, as to whether the variations in the diffraction patterns originate completely in structural differences in myofilament arrangements. This is due to the fact that all of the gratings within the myofibrils were not arranged so as to be perpendicular to the incident beam. There were no mitochondriae or glycogen granules in the glycerol-treated muscles used in the present study. Special care was taken not to stretch the muscle fiber excessively during preparation or to remove connective tissue around the fiber. Even though these factors causing artifacts are excluded, there still remain problems involving disturbance of the diffraction patterns. One of these is the angle of laser irradiation. According to Rüdel and Zite-Ferenczy,[19] the incident beam angle and beam direction to the rotational axis altered the intensity of the first order diffraction line of the frog muscle. They also found extra diffraction lines, and they explained that these lines were the consequence of bending of the Z-lines. The fiber examined here showed nearly the same diffraction patterns along the whole length of the longitudinal direction, and the angle of the incident beam to the fiber was finely adjusted to make the pattern clear. Nevertheless, a few of the first order patterns showed asymmetry accompanied by the extra diffraction patterns (Figs. 1, 2), and a low peak/width ratio (Fig. 3). One of the origins of these irregular diffraction patterns probably results in the irregular arrangements of the gratings within the myofibrils or the bending of the Z-lines.[19] The possibility, however, was not completely excluded that these fibers were deteriorated during fiber preparation or other experimental artifacts.

The RB is known to contain only fast fibers similar to body muscles such as the IP.[7,8,20] If it can be assumed that the fibers of a ratio below 1.0 are multiinnervated slow fibers and that 4% of IP fibers and 15% of the RB fibers come not from the existence of slow fibers but from the undefined origin described above, then slow fibers make up 33–44% of the IO. As for the LR, slow fibers make up 37–46% of the orbital layer and about 30% of whole muscle. Previous authors have reported that slow fibers occupied one-third of the cat IO,[7] 25%–30% of the cat IO,[9] one-fourth to one-third of the cat superior oblique (SO),[1] 20–25% of the cat LR,[9] one-third of the cat superior rectus (SR),[4] one fifth of rabbit SR,[2] and 5.3% of the sheep SO.[21] The values obtained in the present study were a little higher than previous ones, probably because of the different experimental method. Kimura[9] reported that the IO contained more slow fibers than the LR. Asmussen and Gauntz[10] also noted that the IO contained more fibers contracting slowly with resistance to fatigue than the LR. The present results also indicated that there were more slow fibers in the IO than in the LR.

The peak/width ratio was observed to be independent of the sarcomere length below 3 μm. In the frog skeletal muscle fiber, Cheung *et al.*[22] examined the peak intensity and width of the first order diffraction pattern. The present results were consistent with theirs. Stretching the fiber beyond 3.3 μm decreased the peak/width ratio, because it probably produced non-uniform distribution of the sarcomere length within myofibrils.

The glycerol fibers treated for a short term of 1–3 days contracted and relaxed with changes in the pCa. The diffraction pattern became dispersed immediately after the exchange from a relaxing solution to a contraction one. Immediately after the exchange of solutions, superficial myofibrils would contract but central myofibrils still remain in the relaxed state. Nonuniform activation along the radial axis may be a main factor that disperses the diffraction pattern. The dispersion of the pattern lasted throughout the full contraction state. Moreover the total light intensity represented by the area under the diffraction line also decreased as the contraction advanced.

At the steady state phase of contraction, nonuniformity along the longitudinal axis may be added to radial nonuniformity. The decrease of light intensity during contraction have been reported on skinned fibers of the frog,[23,24] and this came from structural changes in myosin filaments.[25] Since the turnover rate of cross bridges is different for fast and slow fibers, it is expected that the decrease in the light intensity during contraction would be different for the two types of muscles. Such the difference, however, has not yet been examined because of technical limitations.

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REFERENCES

1) Hess, A. and Pilar, G.: Slow fibres in the extraocular muscles of the cat. J. Physiol. **169**: 780–798, 1963
2) Kern, R.: A comparative pharmacologic-histologic study of slow and twitch fibers in the superior rectus muscle of the rabbit. Invest. Ophthalmol. **4**: 901–910, 1965
3) Bach-y-Rita, P. and Ito, F.: In-vivo microelectrode studies of the cat retractor bulbi fibers. Invest. Ophthalmol. **4**: 338–342, 1965
4) Bach-y-Rita, P. and Ito, F.: In vivo studies on fast and slow muscle fibers in cat extraocular muscles. J. Gen. Physiol. **49**: 1177–1198, 1966
5) Hess, A.: Vertebrate slow muscle fibers. Physiol. Rev. **50**: 40–62, 1970
6) Chiarandini, D.J. and Stefani, E.: Electrophysiological identification of two types of fibres in rat extraocular muscles. J. Physiol. **290**: 453–465, 1979
7) Lennerstrand, G.: Mechanical studies on the retractor bulbi muscle and its motor units in the cat. J. Physiol. **236**: 43–55, 1974
8) Lennerstrand, G. and Hanson, J.: The postnatal development of the inferior oblique muscle of the cat. I. Isometric twitch and tetanic properties. Acta Physiol. Scand. **103**: 132–143, 1978
9) Kimura, H.: Tension and shortening velocity of slow fibers of cat extraocular muscles. J. Physiol. Soc. Jpn. **42**: 151–159, 1980 (in Japanese)
10) Asmussen, G. and Gaunitz, U.: Mechanical properties of the isolated inferior oblique muscle of the rabbit. Pflügers Arch. **392**: 183–190, 1981
11) Stelling, J. and McVean, A.: The contractile properties and movement dynamics of pigeon eye muscle. Pflügers Arch. **412**: 314–321, 1988
12) Peachey, L.D.: The structure of the extraocular muscle fibers of mammals. In the control of eye movements, ed. by Bach-y-Rita, P., Collins, C.C. and Hyde, J.E., New York and London, Acad. Press 1971, pp. 47–66
13) Mayr, R.: Structure and distribution of fibre types in the external eye muscles of the rat. Tissue Cell **3**: 433–462, 1971
14) Peachey, L.D. and Huxley, A.F.: Structural identification of twitch and slow striated muscle fibers of the frog. J. Cell. Biol. **13**: 177–180, 1962
15) Matsumura, M. and Kimura, H.: Light diffraction patterns of fast and slow fibers of cat extraocular muscles. Abstr. Biophys. **18**: 166, 1980 (in Japanese)
16) Julian, F.J.: The effect of calcium on the force-velocity relation of briefly glycerinated frog muscle fibres. J. Physiol. **218**: 117–145, 1971
17) Matsumura, M. and Kimura, H.: Laser diffraction patterns during isometric and auxotonic contractions in frog skeletal muscle. Jpn. J. Physiol. **35**: 343–354, 1985
18) Burton, K. and Baskin, R.J.: Light diffraction patterns and sarcomere length variation in striated muscle fibers of Limulus. Pflügers Arch. **406**: 409–418, 1986
19) Rüdel, R. and Zite-Ferenczy, F.: Interpretation of light diffraction by cross-striated muscle as Bragg reflexion of light by the lattice of contractile proteins. J. Physiol. **290**: 317–330, 1979
20) Bach-y-Rita, P. and Lennerstrand, G.: Structural-functional correlations in eye muscle fibers, eye muscle proprioception. *I*n Basic Mechanisms of Ocular Motility and Their Clinical Implications, ed. by Lennerstrand, G. and Bach-y-Rita, P. New York, Pergamon Press. 1975, pp. 91–109
21) Browne, J.S.: The contractile properties of slow muscle fibres in sheep extraocular muscle. J. Physiol. **254**: 535–550, 1976
22) Cheung, Y.M., Hwang, J.C., Cheung, S.H. and Cheung, S.C.: Changes in diffraction patterns with length in single muscle fibres at rest. Pflügers Arch. **379**: 101–104, 1979
23) Oba, T., Baskin, R.J. and Lieber, R.L.: Light diffraction studies of active muscle fibres as a function of sarcomere length. J. Muscle Res. Cell. Motil. **2**: 215–224, 1981
24) Oba, T. and Hotta, K.: The effect of changing free Ca^{2+} on light diffraction intensity and correlation with tension development in skinned fibers of frog skeletal muscle. Pflügers Arch. **397**: 243–247, 1983
25) Pézolet, M., Pigeon-Gosselin, M., Nadeau, J. and Caillé, J.P.: A molecular probe of the contractile state on intact single muscle fibers. Biophys. J. **31**: 1–8, 1980