X-ray Diffraction Pattern Recorded from a Single Myofibril of Muscle

Muscle contraction is caused by the interaction between two major contractile proteins, myosin and actin, within muscle cells. In striated muscle, these proteins are polymerized and form filaments with regular helical repeats, and these filaments are in turn arranged in a regular hexagonal lattice. Because of this architecture, a striated muscle gives rise to a number of strong reflections when irradiated with X-ray beam, making X-ray diffraction method a powerful tool to study the molecular mechanism of muscle contraction.

The structure of a striated muscle is shown in Fig. 1. A striated muscle is made of many muscle cells (diameter, 50 - 100 μm; length, millimeters to centimeters). Each muscle cell contains thousands of myofibrils (diameter, 1 - 3 μm). A myofibril is made of 2 - 3 μm-long sarcomeres (minimal functional unit of muscle) connected in series. A sarcomere contains a single hexagonal lattice of protein filaments as described above.

The strongest reflections of muscle, called equatorial reflections, originate from the hexagonal arrangement of filaments. Since a muscle contains an enormous number of myofibrils with random lattice orientations, the recorded diffraction pattern is basically a rotary-averaged "powder diffraction pattern (a diffraction pattern that would be recorded from ground powder of mineral crystals)", from which information about the lattice plane orientations is lost. This problem cannot be solved by the use of a single muscle cell, since it still contains a large number of myofibrils. The only solution to preserve that crucial information is to record diffraction patterns from a single myofibril, which is only 1/1000 by volume of a muscle cell, the smallest muscle specimen which has been used for X-ray diffraction studies.

The strategy was not to isolate a single myofibril from a muscle cell, but to generate X-ray microbeams by using a pair of pinholes (diameter, 2 μm) and to shoot at a single myofibril within a muscle cell without isolating it (Fig. 2). Unlike in conventional recording, the muscle cell was irradiated end-on so that the beam would pass through only one myofibril if ideally aligned. As a material, the flight muscle of an insect (bumblebee) was chosen because its myofibrils were well separated from each other and its filament lattices were of crystal quality.

Figure 3 shows representative diffraction patterns from the insect flight muscle [1] recorded in this way at beamline **BL45XU** [2]. The pattern obtained with 50-μm pinholes consists of a number of concentric circles typical of powder diffraction, since there are still many myofibrils in the beam-path

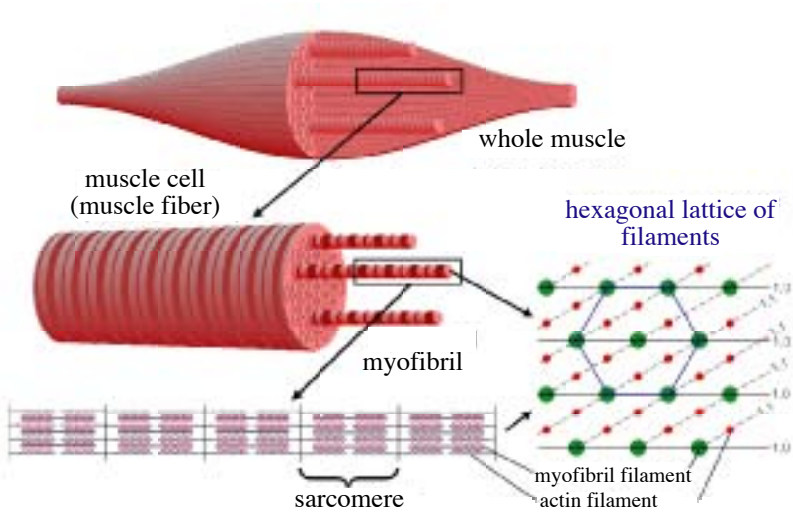

Fig. 1. Structure of vertebrate striated muscle.

(Fig. 3(a)). If, on the other hand, 2-μm pinholes are used, the reflections are spot-like and arranged in a hexagonally symmetrical fashion. These patterns are what is expected from a single hexagonal lattice. The lattice plane from which each reflection originates can be readily identified.

The muscle cells used to record the diffraction patterns were ~3 mm long, and the well-defined spot-like reflections mean that the lattices of more than 1000 sarcomeres were exactly in register; a 0.1° twist in every sarcomere would reduce the pattern to a powder diffraction as in Fig. 3(a). This extraordinary register of filament lattice could be beneficial in transmitting force effectively in the flight muscle of the insect, which exhibits sophisticated flight maneuver.

The present results represent the first example of X-ray diffraction recording from micrometer-sized hydrated, functional protein assemblies within a cell. Cells contain a variety of regular protein assemblies other than myosin-actin contractile machinery, and the success of the present recording opens the possibility that X-ray diffraction technique can also be applied to study the structure of these minute cellular protein assemblies.

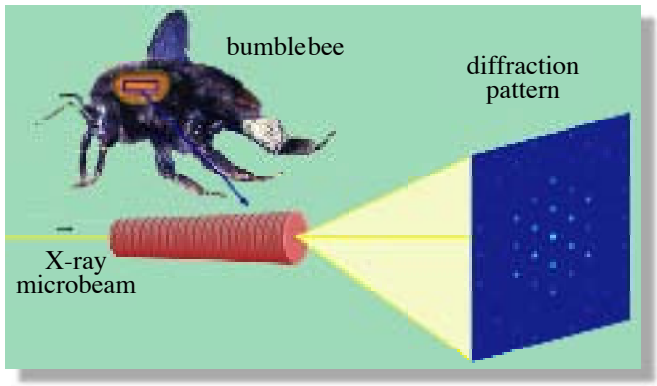

Fig. 2. Schematic diagram of recording an end-on diffraction pattern from a single myofibril in a flight muscle cell of a bumblebee.

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References

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[2] T. Fujisawa *et al.*, J. Appl. Cryst. **33** (2000) 797.

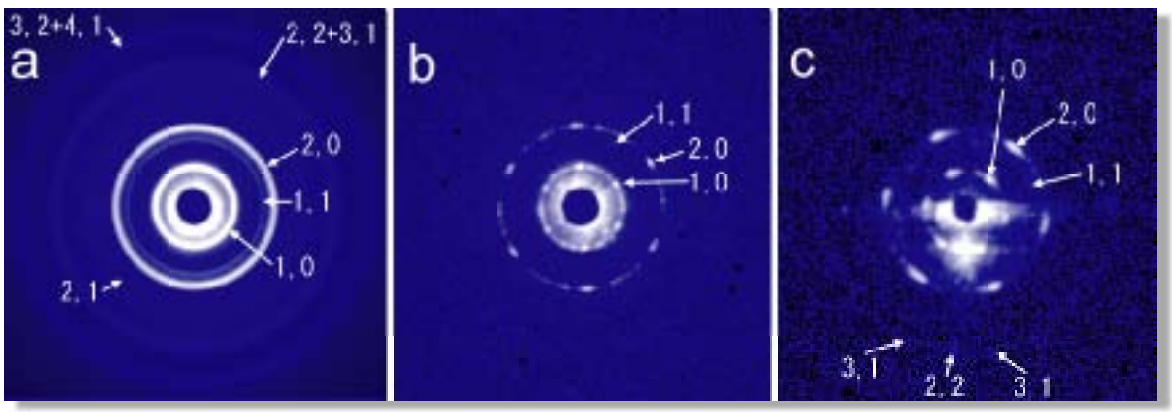

*Fig. 3. End-on diffraction patterns recorded from bumblebee flight muscle. **(a)** A pattern recorded with 50 μm pinholes. **(b, c)** Patterns recorded with 2-μm pinholes. The numbers indicate the crystallographic indices of lattice planes.*